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DISSERTATION

ENVIRONMENTAL PROTEOME PROFILING APPLIED TO THE STUDY OF POLYBACTERIAL METAL RESISTANCE AND ADAPTATION

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2008

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CARLA M. R. LACERDA ENTITLED ENVIRONMENTAL PROTEOME PROFILING APPLIED TO THE STUDY OF POLYBACTERIAL METAL RESISTANCE AND ADAPTATION BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

ENVIRONMENTAL PROTEOME PROFILING APPLIED TO THE STUDY OF POLYBACTERIAL METAL RESISTANCE AND ADAPTATION

Environmental biotechnology can be defined as the use of biotechnology to solve environmental engineering problems, frequently involving bacterial communities and unsequenced species. Here we define environmental proteomics as the proteomic profiling of microorganisms of environmental relevance, targeting the improvement of environmental bioprocesses. This study demonstrates our ability to obtain proteomic data for communities of microorganisms and for environmental isolates, providing unique insights into the physiology and ecology of these systems. A combination of qualitative and quantitative proteomics methods (two-dimensional electrophoresis and/or chromatography followed by tandem mass spectrometry) was used to investigate the proteome of a sequenced mixed culture, an unsequenced mixed culture, and a bacterial isolate from the original unsequenced mixed culture. In the first case study, two soil organisms were grown in co-culture in an attempt to observe proteins induced as a response to the presence of another organism. Many proteome changes were detected and quantified, with proteins involved in protein and DNA metabolism being the most largely modulated. In the second case study, an unsequenced mixed culture was exposed to cadmium and had its dynamic response analyzed. While the community responded significantly to all shock durations, the greatest amount of change was observed in the first fifteen minutes of shock. The main groups of differentially expressed proteins identified were transport proteins, showing that the main method for cadmium tolerance was active efflux. In the study of the adaptation of a pure culture, the most cadmium-tolerant organism in the original unsequenced community was isolated and cultivated in different

concentrations of cadmium. In the last case study of metal resistance, the proteomes of this isolate were compared as it responded to short-term exposures to chromium, iron and cadmium. Metals induced proteome responses in both short- and long-term exposures, meaning that the mechanisms for adaptation and resistance are different. This project demonstrates the potential of environmental proteomics and its intricacies as different proteomic workflows are employed. This is also one of the first evaluations of metaproteomic changes due to the metal response of mixed bacterial cultures, revealing the large potential of environmental proteomics to uncover unique insights into systems-level bacterial functions.

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I. Introduction

I.I. Background on environmental proteomics

Environmental biotechnology can be defined as the use of biotechnology to solve engineering problems, frequently involving microbial communities, complex and heterogeneous environments, and unsequenced, little studied species. It targets the use of microorganisms for remediation of soil and water and for the development of environment-friendly processes. Within this large scope of applications, tools are constantly developed to better describe microbial physiology, metabolism and other aspects associated with improving the performance of these organisms. Proteomics is defined as the large-scale study of protein expression, where protein activities are viewed as parts of a whole, and little interpretation is made based only on single proteins. The combination of proteomics and environmental biotechnology gives rise to environmental proteomics, here defined as the proteomic profiling of microorganisms of environmental relevance, enabling the acquisition of functional information to allow for the design of more efficient environmental bioprocesses.

Over the past 20 years, the use of genomics methods has grown rapidly in environmental biotechnology research and has yielded fascinating results[1-3]. The majority of these methods rely on the 16S small subunit ribosomal DNA (rDNA), which is variable among species but conserved over generations. Analysis of the 16S rDNA can thus identify bacterial species. However, no functional information can be concluded about function from rDNA analysis. An alternative would be a transcriptomic approach, which is the global analysis of gene expression at the mRNA level. However, the main challenge is the impossibility of studying many genes due to the lack of genome sequences for most environmentally-relevant microorganisms, an issue that

can be overcome in a proteomics study. The weakness of these nucleic acid-based methods is that they only reveal genotypic information and potential function, which does not necessarily lead to useful information on metabolic capacity, and physiological responses to variable environmental conditions. Proteomics can provide this information, but there are major challenges associated with extracting proteins from different matrices, complexity of environments, and unavailability of protein sequences for relevant bacterial species.

The characterization of bacterial communities is of great importance due to their central role in environmental bioprocesses. However, communities are highly complex, which makes it very difficult to appropriately design, control, and troubleshoot those processes. As a consequence, most environmental biotechnology studies consider only axenic cultures, which is a simplification of real processes. Lately, we have seen some studies using bacterial community genomics – metagenomics - based on DNA extraction from specific environments, e.g., biofilms[4], sea water[5], soil[6] and waste treatment[7]. The metagenomics concept makes use of the genomics definition, which is the determination of the total genetic content (DNA sequences) of an organism, and applies it to a metaorganism, which can be understood as a collection of organisms living in community. Along with the metagenomics development, we can also see the establishment of bacterial community proteomics field[8-12] – metaproteomics.

I.II. Proteomics tools and general workflows

Proteomic approaches can be qualitative or quantitative. So far, most studies are qualitative, and the efforts towards quantification are very recent. A few papers are summarized here that use microorganisms of environmental relevance to improve proteomics methods. In the field of environmental proteomics, experimental efforts are of particular interest, mainly alternative

protein separation and identification techniques. Each of these contributes to the separation and identification of proteins from unsequenced organisms.

In quantitative proteomics, labeling approaches are used with the purpose of marking samples with a label whose abundance can be measured. Stable isotope labeling can be used in culture, where labeled nitrogen or carbon, or even amino acids, are incorporated in the growth medium and all newly-synthesized proteins are equally labeled. This method can be used for relative and absolute quantification, depending upon the standards that are used[33, 34]. Another labeling option is during the extraction step, where proteins can be labeled with isobaric tags. Choe et al.[35] presented a comparison of the quantification potential of 2DE and isobaric tagging. They found that both methods demonstrated consistent measurements and that quantification was generally similar. An alternative option is shotgun label-free quantification, an approach used successfully by Zhang et al.[36]. They demonstrated that label-free shotgun proteomics can detect both differential and conserved protein expression. They also showed that spectral and peptide counts are highly reproducible between technical replicates and that sequence coverage is relatively nonreproducible. Different labeling techniques can be applied according to the project goals and examples of this can be seen in future chapters.

In Figure 1 we represent the traditional proteomics workflows, 2DE or shotgun-based, starting from sample preparation. Protein sample quality is probably the most important step in proteomic studies, and several techniques are employed for purification such as dialysis, ultrafiltration and precipitation. Fractionation methods are also used depending on the objective of the experiment, and rely on different protein properties, such as isoelectric point and molecular weight, cellular location, post-translational modifications, or ligand-binding interactions. More on prefractionation methods and applications can be found on a methods paper written by Cordwell[37]. Another reference article for 2DE methods, also by Cordwell, covers the whole

process from sample preparation to mass spectrometric analysis[38]. It describes in detail methods for bacterial protein extraction, including sequential extraction of proteins from both Gram-positive and Gram-negative bacteria, and extraction and enrichment of membrane and extracellular proteins.

The separation methods used by Cordwell[38] are protein-based and accomplished by two-dimensional gel electrophoresis. Detailed descriptions of isoelectric focusing (IEF), 2DE, and image analysis can be found in the same paper. After image analysis, protein spots need to be excised prior to in-gel tryptic digestion for peptide mass spectrometry. Shotgun separation, on the other hand, can be accomplished at the protein or peptide level, usually involving an electrophoretic and a chromatographic step, but different combinations can be used. As an example, Gan et al.[39] used six different workflows, including protein and peptide IEF, protein IEF followed by peptide strong cation exchange (SCX) liquid chromatography, protein 2DE followed by peptide SCX, protein weak anion exchange and peptide SCX, peptide SCX only, and finally, peptide IEF only. Because different approaches favor distinct protein properties, this study showed that different proteins are identified depending on the separation method used. This makes it clear that different sample categories (cells, tissues, etc.) require an optimization of the separation protocol to obtain better results.

The bottom part of Figure 1 is focused on the identification steps, for which new mass spectrometry technologies have been essential. Different types of classic and tandem mass spectrometers are used to determine peptide masses and that is coupled with database searching aiming to find protein identifications. Zhu et al.[40] applied a standard separation workflow using native and denaturing 2DE to present a fully-automated high-throughput LC-MS/MS. Peptides are automatically introduced from the in-gel digestion workstation into a capillary column by an autosampler connected to an LC pump. Another pump was then used to deliver the gradient and

elute the peptides from the capillary column directly into the mass spectrometer. This study represents a classic mass spectrometric approach, based on electrospray ionization and tandem ion trap mass analysis. Other frequently used setups are MALDI-TOF/TOF and ESI-Q/TOF. Great emphasis is put on the tandem part, where we can obtain amino acid mass information, which facilitates peptide sequencing. Database searches are accomplished by translating databases, such as NCBInr, digesting and fragmenting them in silico. Experimental MS and MS/MS data are then compared to the database and significant protein and peptide matches are considered for protein identifications. An alternative to tandem mass spectrometry is the use of Fourier-transform ion cyclotron resonance mass spectrometry as a part of an accurate mass and time (AMT) tag strategy. The AMT tag strategy uses the high accuracy of the FTICR-MS to measure peptide masses and elution times so that they are considered unique tags among all other peptides, based only on these two properties. Advantages of the AMT approach include high sensitivity and high confidence for protein identification as well as extensive proteome coverage. Mohan et al.[41] used a multidimensional separation and concentration platform coupled with FTICR-MS to obtain a high number of protein identifications.

Database searching can be very challenging, especially for unsequenced organisms, and requires some additional strategies such as cross-species identification or de novo sequencing. In the case of cross-species identification, significant peptide matches are considered as identifications even if they come from a species different from the original one in the experiment, as described by Cordwell et al.[42, 43] and Habermann et al.[44]. In the case of de novo sequencing, where peptide sequences are reconstructed in silico from tandem data, a sequence homology search is required. Shevchenko et al.[45] developed MS-BLAST, a search engine that performs BLAST searches on de novo-created peptides. Basically, de novo sequencing is performed on MS/MS data, and a collection of peptide sequences from the same protein are searched on a

protein BLAST. Currently, another topic of interest has been the annotation of hypothetical proteins – proteins whose genetic code have been described, but were never previously observed in a living organism. Around 40% of the total gene products predicted in bacterial genomes are annotated as hypothetical. As proteomic data acquisition progresses and more of these predicted gene products are observed, research groups have developed schemes for annotation and functional classification of these predicted, newly-observed proteins.

I.III. Comparison with other global technologies – "-omics"

Regardless of these challenges, proteomics can still provide useful information about the functions of microorganisms of environmental relevance that cannot be obtained by any other -omics approach. Proteomics can also complement other -omics technologies in several aspects, including confirming the existence of gene products predicted from DNA sequences. HumpherySmith *et al.*[13] evaluated the complementarity of proteomic research to nucleic acid-based techniques. In this paper, they assess the need for improvement in other areas such as molecular half-life and functional competence of biomolecules, among others. By putting together all the information generated in these complementary approaches, one would achieve a holistic cellular biology view, most commonly known as systems biology.

A few interesting studies have been published on the use of proteomics for the classification of bacteria. Dworzanski and Snyder[14] used mass spectrometry to identify peptides and correlate them with a bacterial species. Basically, peptide sequences identified from fragment ion information were assigned to bacterial species according to the initial organismal affiliation of the identified peptide. Histograms were then constructed for each species in order to reveal the closest bacterial relatives. The bacterial sample was finally identified according to the highest number of

confidently identified peptides. Proteomics was also used for phylogenetic classification of closely-related bacterial species as described by Dopson *et al.*[15]. In their study, 2DE-based protein expression similarities were used to construct phylogenetic trees. This approach is only valid for highly similar species cultivated under the same conditions; otherwise it can be complicated to compare 2DE gel differences or even associate protein spots to the same proteins in the cases compared. This method can overcome common pitfalls faced in phylogeny, particularly, the need for accurate DNA sequences and their alignment, and the reliance on one gene (usually 16S rDNA), thereby overlooking important information such as lateral gene transfer. A similar study was conducted by Francisco *et al.*[16], in which they used fatty acid methyl esters and 2DE to cluster isolates from a bacterial community under chromium stress. Forty-eight bacterial species were identified by numerical analysis of fatty acid profiles and 10 clusters were formed by comparing the protein profiles of each species. They were able to demonstrate that the mechanisms of Cr(VI) resistance and reduction differed from group to group suggesting that both Cr(VI) resistance and reduction are shared abilities and not an exclusive characteristic of a single group. A similar comparison of protein profiles to assist phylogenetic classification was accomplished by Coenye *et al.*[17]. Their study reclassifies strain AC1100 of *Burkholderia* as a new species, based on genotypic and phenotypic differentiation. These three examples show that proteomic approaches (either 2DE- or shotgun-based) can be associated with taxonomic information, independently of DNA sequences.

An -omics technology of interest in the analysis of bacterial function is transcriptomics. Singh and Nagaraj[18] reviewed the applications of transcriptomics and proteomics to bioremediation. Microarray-based transcriptomics is restricted to the analysis of sequenced organisms given that gene probes are necessary for hybridization and measurement of gene expression. In addition, this

approach only evaluates potential function at the mRNA level, which does not fully correlate to the post-translation of proteins[18]. Since the analysis is done at the mRNA level, all the information related to protein translation and post-translation is not taken into consideration. Thus, the mRNA analysis only views the potential for gene expression, but ignores other events where mRNA might be degraded before translation occurs, or simply a shut down of translation to save energy. Among all the advantages and disadvantages, proteomics still holds a unique position since mRNA only transmits information from genes to ribosomes. Proteins, on the other hand, are the key players of *in situ* bioremediation, participating in all cellular reactions and mechanisms. The *Desulfovibrio vulgaris* transcriptome and proteome were used as models to develop statistical methods that improve their correlation[18]. The studies comparing transcriptomics and proteomics conclude that there is not a very good correlation between the two data types[18-24] (as explained above). Possible reasons for that are the differences in dynamic range and rates of production and degradation of both proteins and mRNA, or even false positives in either case. Nie *et al.* worked on models to predict the abundance of undetected proteins[25], multiple regressions to identify sources of variations[26], and sequence features that affect translational efficiency[27]. Another combined transcriptome and proteome study of *D. vulgaris* describes the response to oxidative conditions[28]. A number of differentially expressed gene products were identified in response to oxygen exposure, including thiol-specific peroxidases. Other products also identified had the functional categories of nucleic acid and protein biosynthesis, detoxification and cell division. Xia *et al.*[29] studied the proteome of *Methanococcus maripaludis* and evaluated its correlation with mRNA microarrays. They used metabolic labeling and multidimensional LC coupled with a quadrupole ion trap mass spectrometer to find 55% of the total proteins of that organism. They compared a wild and a mutant strain to support the duplex experiment and validated it through microarrays, and

concluded that shotgun proteomics and microarrays can provide similar measurements of global gene expression.

The combination of proteomics and transcriptomics can be valuable. The combination of both approaches provides the capability of finding genes and/or proteins that are neglected by the other approach, possibly due to their physical properties. Monchy *et al.*[23] studied the proteome response to copper in *Cupriavidous metallidurans* CH34. In the presence of a high copper concentration, a total of 10 proteins were induced, while only one protein was repressed. They observed that both at the transcript and protein levels most of the induced proteins were plasmid-encoded, but chromosomal proteins also had their expression modulated by copper. They identified a number of proteins involved in copper resistance and other stress-related proteins. Tomás-Gallardo *et al.*[30] investigated aromatic metabolism in *Rhodococcus sp.* strain TFB. The combination of transcriptomics and proteomics made it possible to observe the induction of specific catabolic pathways by the corresponding aromatics, the evidence of a stress response during growth on aromatics, and the absence of catabolite repression by glucose suggesting a metabolic advantage of this organism. Nunez *et al.*[31] compared the proteomics and transcriptomics of a specific regulon in *Geobacter sulfurreducens*. They found significant differences between the wild and the mutant proteomes based on the activity of the regulon of interest. They also learned that several cellular processes can be regulated by genes present in this regulon and that some of the gene products can be commonly identified by both techniques. Schmid *et al.*[32] investigated a heat shock regulon from *Deinococcus radiodurans*. Also using deletion mutations, transcriptomics and proteomics, they demonstrated the need for the specific regulon in the expression of heat shock genes and showed the importance of several heat shock proteins in heat protection. Morris *et al.*[19] investigated *Dehalococcoides ethenogenes* strain 195 oxidoreductases. They used MALDI-MS/MS and reverse

transcriptase PCR to identify several respiratory enzymes including oxidoreductases, hydrogenases and dehalogenases. These reports demonstrate that transcriptomics and proteomics can provide complementary insights into the metabolism of prokaryotes.

I.IV. General workflow - metaorganisms and development of Burkholderia cepacia Cd44

The experimental design for this project involved an exploratory experiment of a short-term cadmium exposure of a metaorganism, or polybacterial system. As a proof-of-concept of metaproteomics changes in a polybacterial system, we were interested in assessing the ecological effects of a mixed culture of two sequenced organisms. To accomplish this goal, we used two known strains of environmentally-relevant bacteria: *Pseudomonas putida* KT2440 and *Bacillus subtilis*. These two bacteria were cultivated in rich-medium batches, in isolation and in community. Community composition was evaluated by series of Gram stains at different growth stages, i.e., both Gram-negative and Gram-positive cells were observed in comparable amounts, at all stages. Proteome analyses by a quantitative shotgun workflow were performed and are discussed in Chapter 3.

For a more complex system study, all unsequenced bacteria used in this study were obtained from an inoculum from an uncontaminated soil sample from Fort Collins, CO. The source microbial community was inoculated in a continuous-flow simulated wastewater treatment bioreactor that was fed a mixture of organic chemicals. 16S rDNA analyses of this inoculum culture indicated the presence of approximately 50 bacterial strains[46]. For the metaproteomics cadmium resistance study, inocula from the continuous reactor were used for rich-medium cultivations of the culture and short-term cadmium exposures. Proteome analyses using a 2DE workflow were performed and the results are discussed in Chapter 4. This exploratory study led to many questions involving

the detection of proteins in a less complex community and proteins specifically related to cadmium toxicity. Figure 2 shows a schematic of the projects derived from the first metaproteomics study.

The other direction of great interest in this project involved the isolation of a bacterial species with high resistance to cadmium from the unsequenced community. The main goal was to use a variety of proteomics techniques to understand proteome changes under different cadmium environments. For the isolation, a series of inocula were taken from the continuous reactor, and cultivated on media with high cadmium concentrations. These cultures were grown on solid media, so that colonies could be grown independently and undergo several transfers, to improve the isolation of one single species. The fastest growing, most cadmium-resistant organism was identified as a strain of *Burkholderia cepacia* (by 16S rDNA sequencing). In order to clarify the uniqueness of the strain used here and to better describe it, we use the name *B. cepacia* Cd44 in this study, where the strain code represents the highest cadmium concentration tolerated by this strain (0.44 mM Cd^{2+}). The complete details for the isolation procedure are described in Chapter 5, along with the proteome study of adaptation to different cadmium levels.

After understanding the effects of short-term cadmium shocks and cadmium concentration, one evident direction was the evaluation of how other metals affect this bacterium. The reasoning behind the last set of experiments is that after adapting to a highly inhospitable environment, the organism should have the necessary protein machinery to tolerate the presence of other metals. For the next experimental design, exponentially-growing cultures of strain Cd44 were subjected to short-term metal shocks (0.44 mM Cd^{2+}, 0.38 mM Cr^{3+} and 0.62 mM Fe^{3+}) and one was cultured without metals to serve as control. The referred metal concentrations were chosen since they caused visible phenotypic changes in the colonies. After 2 h of metal exposure, cells were

harvested and their proteins extracted and digested prior to a quantitative shotgun proteomics workflow. This experiment is fully presented in Chapter 6.

The last chapter presents the future directions for this research. All these discoveries lead to more intriguing questions and a solid foundation for environmental proteomics has yet to be built. With the advent of systems and synthetic biology, omics-based models can be built so that this work can be applied to bioremediation and other environmental biotechnology fields.

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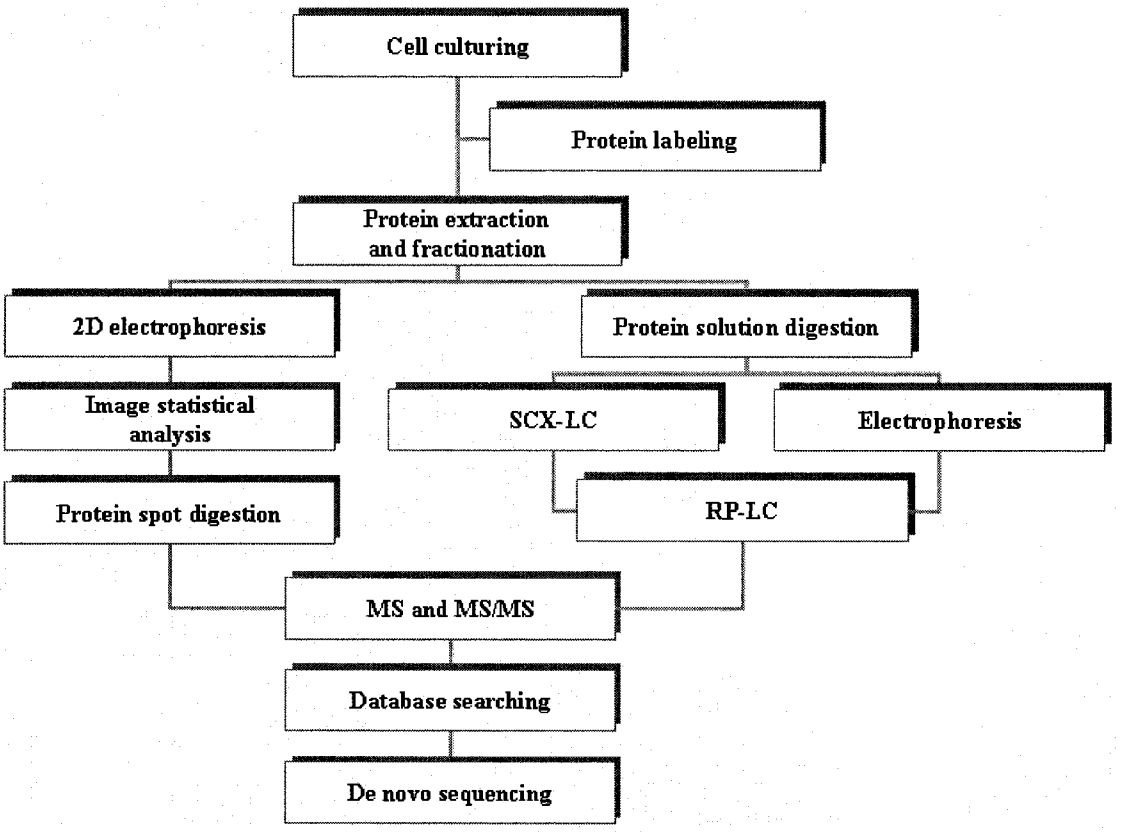

Figure 1: Common proteomics workflows.

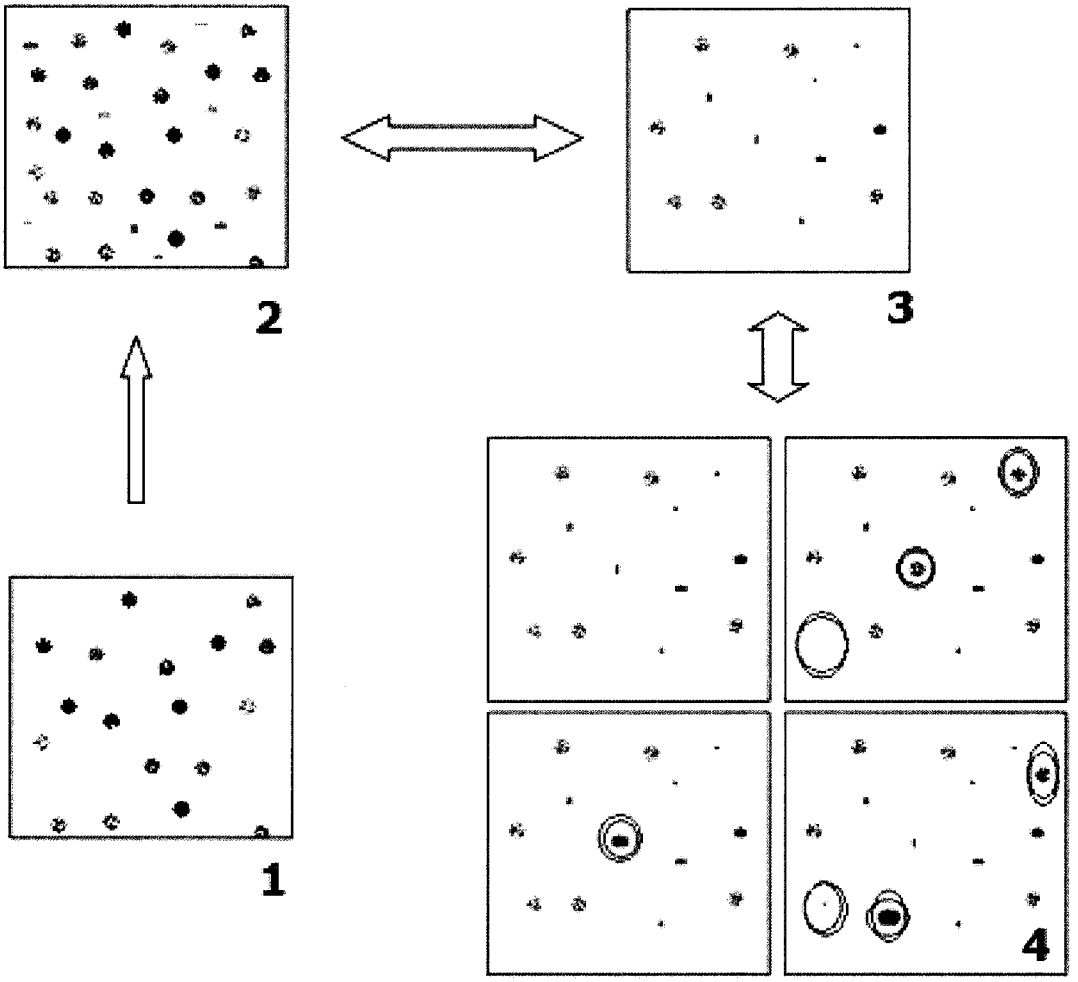

Figure 2: Schematic of project experimental design.1: Metaproteome of a simple culture; 2: Metaproteome of a complex culture; 3: Proteome of one organism under different levels of cadmium stress; 4: Proteome of one organism under different metal stresses after cadmium adaptation.

II. Environmental proteomics: Applications of bacterial proteome profiling in environmental biotechnology

II.I. Introduction

In this review we present the use of environmental proteomics in studies of bacterial physiology, metabolism, and ecology, among other areas. Bacteria and their intricate cell machineries can be widely applied in environmental biotechnology in a range of cleanup processes, including bioremediation. In the bioremediation field, bacterial abilities of potential interest include dehalogenation, methanogenesis, denitrification, sulfate reduction, tolerance of radiation and toxic compounds, versatility regarding carbon and energy sources, as well as use of electron donors and acceptors. Proteomics has a potentially important role in helping uncover the pathways behind these cellular processes. However, environmental samples are often highly complex, which makes proteome studies in this field especially challenging. Some of these challenges are the unavailability of genome sequences for the large majority of environmental bacteria, difficulties in isolating bacteria from certain environments and enriching species.

We compare proteomics with other –omics studies, such as genomics, and phylogenetic classifications, and transcriptomics. Despite all the challenges, proteomics offers a unique and dynamic view into cellular function at specific points in time. We then describe case studies of environmental proteomics of models organisms, such as *Shewanella oneidensis* and Cyanobacteria. And an important aspect of recent environmental proteomics concerns

metaproteomics or microbial community proteomics. This approach has been used by very few research groups and show great potential in the evaluation of function in a community without isolating organisms. This allows for a view of organism interactions, which are impossible to determine using axenic cultures.

The future of proteomics falls into the scope of systems biology, which encompasses the global molecular and network analysis of biological systems. As more research is done, we will be able to make predictions on bacterial activity and apply this directly to bioremediation fields, for example. Proteomics is a unique tool and can offer valuable information into cellular processes. By combining proteomics with other –omics technologies we will be able to more deeply understand previously undescribed cellular mechanisms and to reproduce them.

II.II. Environmental Proteomics

Proteomic studies of environmental samples, such as soil and seawater, are so far limited to some model microorganisms, having the ability to tolerate, degrade or precipitate toxic compounds. In terms of environmental bioprocessing, some other desirable characteristics of those model organisms are versatility regarding carbon and energy sources, as well as electron donors and acceptors. Another aspect is the availability of genome sequences. Some sequenced bacterial species are notorious for their unique abilities: *Shewanella oneidensis* strain MR1 can use more than ten electron acceptors[47], *Deinococcus radiodurans* can tolerate radiation, *Burkholderia* strain LB400 is the most effective tetrachlorobenzene degrader known, among others. These qualities altogether make these organisms extremely attractive for environmental biotechnology applications and proteomics can be used to help

us better understand their functions in specific habitats. One technical advantage of using model organisms regards the availability of sequenced genomes, which almost always improves the quality of protein identifications. Other model organisms do not have much interest in bioremediation, but are of great ecological importance, e.g., understanding mechanisms of survival and adaptation in tundra soils[48].

II.II.I. Physiology of model organisms - *Shewanella oneidensis*

A number of approaches have been used to try to develop environmental proteomics. The direct consequence is that model organisms, such as *Shewanella oneidensis*, are usually targeted. *S. oneidensis* is a Gram-negative bacterium that inhabits oxic-anoxic surfaces in nature. Its bioremediation potential relates to diverse respiratory capabilities, meaning the ability to reduce a wide-range of organic compounds, metal ions and radionuclides. In order to learn more about the bacterium and to improve the performance of different chromatographic and mass spectrometric methods, quantification alternatives, database annotations, etc., there is a collection of physiological studies of *S. oneidensis* to be reviewed here.

At the Pacific Northwest National Laboratory (Richland, WA, USA), several researchers are involved in proteomic analysis of *Shewanella oneidensis*, and their studies are diverse, including a focus on better annotation of hypothetical proteins. Using aerobic, suboxic and anaerobic culture conditions, they apply the AMT tag approach (LC-FTICR-MS) and a combination of filtering approaches for the identification of proteins. The studies focused on annotation of hypothetical proteins[49-51] demonstrate their high-throughput capability. After detecting thousands of tryptic peptides, and using a minimum of two peptides per protein for confident identification, they confirmed the expression of at least 758 conserved hypothetical

proteins[50]. They developed a seven-category annotation scheme based on different functional layers: exact biochemical function, well-defined biochemical function but unknown specificity, general biochemical function based on a superfamily assignment, known biological function derived from genomics data, functional insights derived from genomic data, expressed under certain growth conditions, and organism-specific protein. This method allowed for the new categorization of over 400 proteins, with a defined biological role and confirmed expression in *S. oneidensis*. They also worked on criteria for the confident identification of small proteins (around 100 amino acids in length) using the AMT tag approach[52]. Once again they tried to validate function for the small hypothetical proteins.

There are several other study lines from the Pacific Northwest National Laboratory involving *S. oneidensis* and similar proteomic approaches. To improve their proteomic approaches, they developed a method constituted by smart instrumentation where isotopically-labeled species detected with "interesting" abundance ratios were selected for MS/MS in an automated fashion[53]. For a complex mixture of *S. oneidensis* peptides, they were able to target only species corresponding to proteins differentially expressed under suboxic versus aerobic conditions. Another effort for method improvement was label-free[54] differential proteomics. They used relative peak intensities in conjunction with data normalization (linear regression against the elution time of peptides in the AMT tag database) to quantify protein expression. Another study focused on optimizing a top-down multidimensional chromatographic separation of intact proteins[55] prior to mass spectrometry. They identified a total of 715 intact proteins, with and without PTMs such as loss of N-terminal methionine and methionine oxidation. Furthermore, results combining data from the

two chromatographic separations were illustrated as a 2DE display. Their strategy proved to be high-throughput and efficient in detecting differential protein expression of biologically relevant targets. A more recent study describes the enrichment of active *S. oneidensis* proteins directly involved in metal reduction[56], using liquid chromatography, prior to mass spectrometric identification. A number of proteins were found, including terminal electron-accepting proteins and others not previously associated with metal reduction. This was the first study to identify metal-reducing proteins without the need for purification procedures or loss of protein activity.

Integrative approaches combining proteomics and transcriptomics were also implemented to provide more detailed insight into gene function in *S. oneidensis*. Initial investigation aimed at characterizing a ferric uptake regulator (*fur*)[57, 58], which is an iron-responsive transcriptional repressor. They created a *fur* knockout strain and compared it to the wild type. They found a number of differentially expressed gene products in the fur mutant, including the ones involved in electron transport, energy metabolism, transcriptional regulation, oxidative stress and iron uptake, as well as binding proteins and receptors. They found a strong correlation between transcriptome and proteome data for genes undergoing large changes in expression level. More recent studies compare transcriptome and proteome changes after chronic[59] and acute[60] chromium stress. In the chronic stress study, they exposed *S. oneidensis* cells to 0.3 mM chromate for 24 hours and they observed complete uptake or reduction of the metal. Chromium-exposed cells developed filaments that tended to aggregate and the main molecular response was the up-regulation of genes involved in the prophage formation, DNA metabolism, cell division, biosynthesis, stress protection, and membrane and peptidoglycan response. They also observed down-regulation of genes

involved in chemotaxis, motility and transport. The acute stress study investigated the dynamic response after short-time intervals of exposure to 1 mM chromate. They identified the up-regulation of a number of transporters, receptors, signal transduction systems, sulfur metabolism enzymes, and cellular detoxification and DNA repair proteins. In contrast, energy metabolism proteins were repressed. They also reported comparable induction and repression transcriptomic and proteomic patterns for corresponding proteins. These two studies together show how the presence of chromium can generate a variety of cellular responses, based on the differences of acute and chronic stresses.

Other studies with *S. oneidensis* focusing on cellular states are the comparison of planktonic and biofilm cell growth[61] and characterization of c-type cytochromes underlying their metal reduction strategy[62]. Further method-based studies compare "top-down" and "bottom-up" approaches[63], develop non-denaturing methods for proteome analysis[64], and apply new affinity labeling probes for analysis of the membrane proteome[65].

II.II.II. Physiology of model organisms – Pseudomonads

The bacterial genus *Pseudomonas* has been extensively studied mainly due to species ubiquity. These bacteria are highly versatile with regards to carbon and energy utilization, thus being very active in aerobic decomposition and biodegradation. Because of their very low nutritional requirements, their lifestyles can be autotrophic or lithotrophic, planktonic or sessile, aerobic or anaerobic. They have a great ability to degrade aliphatic and aromatic hydrocarbons, as well as to tolerate non-degradable toxic pollutants.

Pseudomonas putida is generally a model for the investigation of adaptation to harsh environments and toxicity mechanisms in bacteria. Due to its ability to degrade aromatic hydrocarbons, great effort has been put in this direction as an attempt to reduce the amount of

toxic pollutants spread in our environment. Several aromatic compounds are degraded by entering the benzoate pathway, either by transformation to benzoate or catechol, and later directed to the TCA cycle. Proteomic studies of these pathways target the elucidation, at the metabolic level, of the mechanisms of biodegradation and tolerance of toxic aromatic compounds. Kim *et al.*[66] studied *P. putida* cultured in six different monocyclic aromatic compounds, including benzoate, and aimed at determining whether proteins involved in aromatic compound degradation pathways were altered. They were able to identify a total of 190 proteins from 2DE/MS with and without ICAT labeling. Enzymes of the central pathways (benzoate, β-ketoadipate) were confirmed to be induced in the presence of aromatic compounds. Another interesting study looked at *P. putida* grown on phenol, toluene and a mixture of the two[67]. They found that *P. putida* synthesizes different proteins when grown on toluene alone, phenol alone or both. Using 2DE they found seventeen proteins that were exclusively made during phenol growth and ten that were exclusive of toluene. During growth on the mixture, the protein synthesis shifted from toluene-related to phenol-related proteins, which correlated well with the substrate consumption pattern. Those proteins were not identified. A phenol-stress study was conducted by Santos *et al.*[68] focusing on the global mechanism underlying phenol toxicity and tolerance in *P. putida.* They noted the differential expression of proteins involved in the oxidative and general stress response, transcription regulation, transport, cell envelope biosynthesis, cell division, motility, and lipid, amino acid, nucleotide and energy metabolism. Kurbatov *et al.*[69] studied protein expression during growth on carbon sources with different impact on carbon catabolite repression of phenol degradation. In the presence of phenol, they found up-regulation of proteins involved in transport, detoxification, stress response, amino acid, energy, and carbohydrate and

nucleotide metabolism. Toluene-stress proteomic studies of were also accomplished. Segura *et al.*[70] found four categories of toluene-specific proteins: proteins involved in the catabolism of toluene, proteins involved in the channeling of metabolic intermediates to the TCA cycle and activation of purine biosynthesis, proteins involved in sugar transport, and stress-related proteins, suggesting high energy demand for toluene tolerance. Volkers *et al.*[71] also found that the differentially expressed proteins reflected the need for intracellular energy management in the defense against toluene. Other differentially expressed proteins were identified and functionally classified as outer membrane, transport, stress- and translation-related proteins. Other proteome profiling studies of *P. putida* involved growth on acidic amino acids and their amides as sole carbon sources[72], growth on chaotropic solutes causing water stress[73], tolerance of chlorophenoxy herbicides[74], response to tetracycline[75], and methyl tert-butyl ether stress[76].

Other Pseudomonads were also used as a model to study aromatic degradation. A *P. alcaligenes* mutant was built to express gentisate dioxygenase only in the presence of gentisate[77]. Proteomic results showed the up-regulation of a protein with a similar function to gentisate dioxygenase, as well as stress proteins. In another investigation, the same group evaluated the regulatory role of a sigma factor in response to gentisate induction[78]. They observed differential regulation of a number of proteins in response to the inactivation of the sigma factor. Those proteins were enzymes of the gentisate pathway, TCA cycle, pyruvate metabolism and gluconeogenesis, proving the global regulatory role of the sigma factor. *Pseudomonas sp.* K82 also had its aromatic degradation pathways studied. Kim *et al.*[79] looked at the impact of several aromatics (including benzoate) on general metabolic pathways. Two types of catechol oxygenases and related enzymes were identified using

proteomics, suggesting that two cleavage pathways were induced simultaneously for aromatic degradation in *Pseudomonas sp.* K82. Verberkmoes *et al.*[80] compared a "baseline" proteome of a *Rhodopseudomonas palustris* wild-type strain grown under six metabolic conditions, including growth on benzoate. They were able to identify 44 proteins up-regulated during growth on benzoate, including all the enzymes of the benzoate degradation pathway.

Another aspect of importance in the *Pseudomonas* lifestyle is the ability to form biofilms. Proteomics is generally used to describe the physiological differences between planktonic and sessile cells. *P. putida* may contribute to the rhizosphere fitness by forming biofilms and utilizing bacterial communication to express certain phenotypes. The impact of biofilm formation and quorum sensing was investigated by comparative proteomic analyses of *P. putida* and a quorum sensing-deficient mutant[81]. They found that quorum sensing-related proteins overlapped substantially with proteins differentially expressed in sessile cells. Other *Pseudomonas* biofilm studies are from Sauer et al.[82-84]. Their first study investigates phenotypic changes in *P. putida* in response to surface-associated growth. They found a number of changes in gene expression following initial attachment to a surface, including ABC transporters, structural components of flagella and pilli and polysaccharide biosynthesis proteins. The second study compares planktonic cells with mature biofilm, cells, and showed differential expression of more than 800 proteins. The last study here cited deals with dispersal of *P. aeruginosa* cells from biofilms induced by an increase in carbon substrate availability. Glucose was the most efficient carbon source to induce dispersal, leading to 80% reduction in biofilm biomass. Proteins with increased expression in dispersed cells were kinases, flagellar and ribosomal.

Pseudomonas aeruginosa is a facultative human pathogen of high concern in the biomedical field, but also of bioremediation interest. A study by Al-Tahan *et al.*[85] focused on optimizing the effect of biosurfactants on organism cell walls for bioremediation. *P. aeruginosa* was cultured on glucose and hexadecane to investigate the chemical and structural changes that occur in the presence of a rhamnolipid biosurfactant. Results showed that rhamnolipids caused an overall loss in cellular fatty acid content, which was due to release of lipopolysaccharides from the outer membrane. Another study of ecological interest of *P. aeruginosa* concern iron deprivation[86], where the authors compared *P. aeruginosa* and *P. putida.* They found a number of common proteome changes in both species, including ferric uptake regulators, but also a number of differences suggesting important roles of iron-responsive functions in their different lifestyles. Another example is growth under magnesium limitation[87], a condition shown to induce virulence factors. Results showed that magnesium stress response proteins involve bacterial virulence were the most abundant proteins induced. The same group also looked at the *P. aeruginosa* proteome under anaerobic growth[88], where they found more than 100 proteins playing a specific role in anaerobic metabolism.

II.II.III. Physiology of model organisms - Bacilli

The *Bacillus* genus represents models for Gram-positive bacteria. These organisms are also of interest due to their sporulation and biofilm formation ability, as well as virulence of some species. Sporulation and biofilm formation are key mechanisms of survival during environmental stresses. Several proteomic studies have been conducted on *B. subtilis* due to its ease of growth and genetic manipulation. Hecker and collaborators have worked extensively on the proteome of this organism, especially on the allocation of stimulons and

regulons. A review of the application of proteomics to study physiology of *B. subtilis*[89] is available on the literature. Hecker's early studies included starvation for glucose[90-93] or phosphate[90, 91, 94], heat shock[90, 91, 93, 95], salt[90, 91, 93, 96] and ethanol stress[90, 91] as well as oxidative stress[90, 95]. These conditions were tested in order to establish comprehensive two-dimensional proteome maps for *B. subtilis*[97, 98]. The same group also evaluated the *B. subtilis* secretome[99], composed of all the extracellular or secreted proteins. Proteomics showed unique value in this approach since genome-based approaches failed to predict 50% of the actual extracellular proteins. This report of individual secretion machinery components and protein traffic can help us understand mechanisms related to virulence, for example. More recent studies compared gel-free and gel-based approaches[100] and stress-specific versus starvation-specific regulons[101]. Hecker also compared transcriptomics and proteomics of a variety of stresses and demonstrated how proteomics can be used to analyze regulation, structure and function of the sigma dependent general stress regulon[102]. For the comprehensive description of proteins/genes belonging to stimulons or regulons, the proteome approach was complemented with mRNA arrays in order to identify and allocate basic regulation modules. Some combined transcriptome-proteome investigations from the same group involved phosphate starvation[103], stringent response[104], amino acid availability[105] and starvation[106], ammonium starvation[106], catechol and phenol growth[107] and salicylic acid stress[108]. Unique features of interest in Bacilli are virulence and sporulation. Proteome profiles were described for the spores of *B. cereus*[109], *B. subtilis*[110, 111] and *B. anthracis*[112]. Virulence studies mostly concentrate on the secretome – proteome of secreted or extracellular proteins. Virulence factors are generally found between those proteins and were described for a number of Bacilli, namely *B. cereus*[113, 114], *B. thurigiensis*[113], and several *B.*

anthracis[113, 115, 116] strains. Other research groups further investigated Bacilli physiology, including oxidative[117, 118], heat[119, 120] and cold[121-123] stresses, nutrient[124, 125] and phosphate[126] starvation, hexadecane as carbon source[127], biofilm formation[128, 129], as well as full proteome maps[130, 131].

II.II.IV. Physiology of model organisms - Cyanobacteria

Cyanobacteria are photosynthetic bacteria, possessing different lifestyles, from aquatic to terrestrial, unicellular to filamentous, capable of forming symbiotic associations with plants. Cyanobacteria are considered model bacteria, due to availability of genome sequences for some species, and abilities to fix nitrogen and carbon dioxide, as well as produce hydrogen and secondary metabolites; all these characteristics are of great interest in biotechnology. The most commonly studied genera are *Synechocystis*, *Nostoc* and *Anabaena*. An overview of –omics research of cyanobacteria was written by Burja *et al.*[132].

A considerable amount of research has been accomplished for *Synechocystis sp.* PCC 6803. A proteomic map was developed for *Synechocystis* using narrow pH range gels and automated MALDI-TOF[133]. A total of 105 proteins were identified in this study suggesting a baseline proteome for this organism. Choi *et al.*[134] studied the photosynthetic proteome of light- and dark-cultured cells. Using N-terminal Edman sequencing and MALDI-TOF, they identified several proteins involved in photosynthesis and respiration, and other cellular processes. Kashino *et al.*[135] characterized a highly active oxygen-evolving photosystem II complex using proteomic analysis. Using this approach to investigate the structure and function of a photosystem they demonstrated the presence of small polypeptides as of yet unknown. Asadulghani *et al.* studied salt[136] and heat[137] stress in *Synechocystis*. A mutant with an hspA deletion (second most highly expressed gene under salt stress) and the wild-

type strain had their proteomes compared under various salt stresses. The mutant had a growth disadvantage and failed to undergo the ultrastructural changes characteristic of wild-type cells. It also accumulated higher levels of GroES, GroEL1, and GroEL2, suggesting a role for these heat-shock proteins in salt stress. In the heat shock study, they found that light accelerated the heat induction of htpG, groESL1, groEL2, and hspA. They found that light affected the transcription, but not the stability of the mRNA of heat shock genes and also enhanced both the accumulation of GroEL under heat stress and the acquired thermo-tolerance. Slabas *et al.*[138] found major proteome changes in a study of the heat shock response of wild type and a mutant of the histidine kinase 34 gene, which shows increased thermal tolerance. Changes occured not only in the classical heat shock proteins but also in the protein biosynthetic machinery, amino acid biosynthetic enzymes, components of the light and dark acts of photosynthesis and energy metabolism. Heat stress in *Synechocystis* was further studied by Suzuki *et al.*[139] indicating that acclimation to heat shock might be governed by transcriptional and translational regulation. Kurian *et al.* studied heterotrophy[140] and acid stress[141] in *Synechocystis*. In the first case, proteomic alterations and activity levels of selected enzymes indicated a shift in the central carbon metabolism in response to trophic change, down-regulation of the photosynthetic machinery and enhanced levels of proteins involved in glycolysis, oxidative pentose phosphate pathway as well as tricarboxylic acid cycle. In the acid stress study, the periplasmic proteome showed remarkable changes as a function of external pH compared to cytoplasmic proteome. Among the acid- and base-induced proteins, a few were already known for their role in pH homeostasis. A recent study of *Synechocystis* investigated phosphate starvation[142]. They observed a clear physiological response on low phosphate levels, such as a yellowish color, as well as a proteomic response,

where proteins from central carbon metabolism and stress-related were differentially regulated.

The physiology of *Synechocystis* membranes was described by Herranen et al.[143]. By culturing the wild type and several mutant strains under various growth modes (photoautotrophic, mixotrophic, or photoheterotrophic), they were able to identify approximately 20 distinct membrane protein complexes, including the ones involved in photosynthetic electron flow and ATP synthesis, as well as several novel, uncharacterized protein complexes. The Norling laboratory (Stockholm, Sweden) and collaborators have worked extensively on *Synechocystis* plasma and thylakoid membranes. Their first work applies a 2DE separation method for the isolation of pure plasma and thylakoid membranes without any cross-contaminations[144]. The purity of the fractions was verified by immunoblotting, and as a part of their exploratory approach they identified one protein of each fraction. They later focused on further separation and identification of thylakoid[145], plasma[146-148] and outer[149] membrane proteins. Other studies investigated salt stress qualitatively[150], quantitatively using SILAC[151] and specific effects on plasma membranes[152].

Other cyanobacterial species were also studied. Ekman *et al.*[153] Compared the proteomes of free-living *Nostoc sp.* with an isolate from a plant tissue. A number of proteins were found to be down-regulated in the symbiotic stage, including cell envelope, membrane and exopolysaccharide-related, metabolic proteins involved in the pentose phosphate pathway and Calvin cycle, as well as the ones related to dark microaerobic conditions. Stensjö *et al.*[154] studied the quantitative proteome of *Nostoc sp.* PCC 7120 under N_2-fixing and non-N_2-fixing conditions. Upon nitrogen starvation, a range of processes were initiated, and a total of 486 different proteins were accurately identified. Results of metabolic regulation

demonstrated that proteomics represents an important tool for the characterization of heterocysts, specific cells where N_2 fixation takes place. Photoautotrophically and diazotrophically grown *Nostoc sp.* PCC 73102 cells had their proteomes described by Ran *et al.*[155]. Soluble and membrane proteins were studied leading to the identification of 82 proteins that could be divided into 12 functional categories. Many of the identified proteins had general functions, such as housekeeping, but some presented key roles during photoautotrophic and diazotrophic growth or even potential novel functions. A three-dimensional proteomic study of *Nostoc punctiforme* was done by Anderson *et al.*[156]. They evaluated the effect of moderate light and ammonia as single nitrogen source. 1575 proteins were identified, most having metabolic and transport functions. Many of these proteins were oxidative and light stress-related, kinases and response regulators. Barrios-Llerena *et al.* described the proteome of *Anabaena variabilis* using both a shotgun approach[157] and 2DE[158]. Using the shotgun approach, more than 600 proteins were identified whereas 2DE identified 254 proteins only.

II.II.V. Physiology of model organisms – halophiles

Halobacteria and Haloarchaea are of great interest due to their atypical metabolism. They are usually aerobes or facultative anaerobes that utilize photosynthesis to create a proton gradient, which allows them to survive in highly salty environments. The archaeon genus *Halobacterium* is possibly the best studied halophile. These Archaea are adapted to be active and stable in hypersaline environments, making them especially useful for industrial bioprocesses. There are a few literature reviews of *Halobacterium sp.* NRC-1, describing its post-genomic research, namely, their physiological capabilities and role of lateral gene transfer in evolution[159], the acidity of the proteome for function at high salinity[160], and

efficient procedures for discovering novel halophilic enzymes[161]. An interesting structural proteomics study was conducted by Bonneau *et al.*[162] for annotation of unknown *Halobacterium sp.* NRC-1 proteins. They used *de novo* structure prediction to extrapolate putative functions for 1,185 proteins. Proteins were analyzed in the context of a predicted association network composed of several sources of functional associations such as: predicted protein interactions, predicted operons, phylogenetic profile similarity and domain fusion. By combining the association network with transcriptome and genome data, significant improvements were made towards identifying proteins of previously unknown function. Goo *et al.*[163] used ultracentrifugation to separate the soluble and insoluble proteomes from *Halobacterium sp.* NRC-1 cell lysates. They were able to identify 426 proteins, which corresponds to 20% of the theoretical proteome of this organism. Functional classification of the proteins found was accomplished with searches against the KEGG database. A number of metabolic pathways showed more than 50% of their members present in the group of expressed proteins. Shukla[164] developed a proteomics approach for enhanced resolution of the extremely acidic proteome of *Halobacterium sp.* NRC-1. To study the heat shock response, proteomic profiles under normal (42 °C) and heat shock (49 °C) conditions were compared. They identified 30 proteins involved in stress response and protein folding, DNA replication and repair, transcriptional regulation, translation, transport, and housekeeping. Almost one thousand unique *Halobacterium sp.* NRC-1 proteins were identified by Gan *et al.*[165] in a study using biological network analysis with the BMSorter software. This analysis allowed for the study of proteins expressed in different biomodules and interactions between biomodules. Integrated analysis of networks showed evidence of enhanced synthesis of acidic amino acids in an effort to build the highly acidic proteome.

The Kim laboratory has intensely worked on the *Halobacterium salinarum* proteome. They have developed specific protocols for improvement of 2DE[166], strategies for functional characterization of novel halophilic enzymes[167], including the ones responsible for the biodegradation of isopropyl alcohol[168]. Oesterhelt and coworkers also reported their proteomic work on *Halobacterium salinarum.* In multiple studies, they were able to identify 40% of the cytosolic proteome[169] and 20% of the transmembrane proteome[170], a total of 34% of all gene products in *H. salinarum.* In addition, they performed several quantitative study of the membrane proteome to evaluate different labeling techniques[171].

Proteomic studies were also performed in other halophiles. *Haloferax volcanii* was used as a model for protein extraction and purification in two different studies[172, 173], both involving a series of protein washes and improvement of sample quality prior to 2DE. *Halorhodospira halophila* had its proteome analyzed by Samyn *et al.*[174]. They were able to identify more than 30 proteins using subpicomole quantities of protein, some of which are involved in the adaptation to halophilic life conditions. *Halobacillus dabanensis* D-8(T) had its proteome profiled under 1%, 10%, and 20% salinities by Feng de *et al.*[175]. More than one hundred proteins were observed with a changed expression level under different salinity conditions. Twenty seven proteins with a markedly changed expression in hypersaline environments were identified and were functionally related to energy-producing pathways, stress regulators, and proteins involved in survival and adaptation to high salt challenges. Graham *et al.*[176] reported the insoluble proteome of the alkaliphilic and halotolerant deep-sea bacterium *Oceanobacillus iheyensis* HTE831. The 153 proteins identified were functionally classified and physiochemically characterized, which allowed for the identification of proteins believed to be of importance in the alkaliphilic adaptation of *O. iheyensis* HTE831.

Konstantinidis *et al.*[177] used proteomics in order to represent all the genes encoded by the *Natronomonas pharaonis* genome. They identified 929 proteins of which 886 were soluble, representing 41% of the cytosolic proteome. By using shotgun in parallel with 2DE, they identified 700 proteins from each workflow. Overall, they were able to cover about 60% of the cytosolic proteins involved in metabolism and genetic information processing of this organism.

II.II.VI. Metabolism

Other bacterial metabolic abilities include dehalogenation, methanogenesis, denitrification, sulfate reduction, among others. These are metabolic pathways, of bioremediation interest, involving a number of enzymes, and so far not fully understood. Proteomics can provide unique insights into unknown cellular mechanisms, by finding "missing" enzymes[178], which possibly, due to evolutionary pressures, lost their functions or mutated. Some of the currently available studies showing the use of proteomics not only from a biochemical standpoint but also in terms of bacterial physiology and responses to environmental changes are presented.

Some examples of methanotrophic bacterial metabolism follow. Uchiyama *et al.*[179] studied the responses of the bacterium *Methylocystis* sp. M to different water-pollutants, carbon starvation, and temperature shock. Their study demonstrated the existence of stress proteins responding to different stress conditions in this bacterium, which can help in the development of more controlled applications of these organisms in bioremediation. Kao *et al.*[180] investigated the *Methylococcus capsulatus* (Bath) response to different copper concentrations. More than one hundred proteins were differentially regulated, and these included mainly methane and carbohydrate metabolic enzymes, and cellular signaling

proteins. The Vorholt laboratory compared the proteome of *Methylobacterium extorquens* AM1 grown under methylotrophic and nonmethylotrophic conditions[181] (methanol versus succinate as sole carbon source). The majority of the differentially expressed proteins were involved in methanol oxidation to CO_2, assimilation of one carbon units and isoenzymes. A more recent study from the same group evaluated the effects of plant colonization by the same bacterium[182]. They compared colonization of roots, leaves and synthetic medium growth. More than 50 proteins were found to be either leaf- or root-specific, including methanol utilization and stress proteins. They also found a two-domain response-regulator essential for epiphytic growth.

Sulfate-reducers are also of particular interest in bioremediation. Some proteome studies are described here. *Geobacter sulfurreducens* had its proteome described in the presence of several electron donors and acceptors[183]. Around 90% of the total predicted gene products were identified in this study, and most differentially regulated proteins were either hypotheticals or cytochromes. Khare *et al.*[184] used proteomics to understand the metabolic processes involved in metal reduction in the same organism with either fumarate or ferric citrate as electron acceptor. Their results suggested adjustments in membrane transport and specific metabolic pathways in response to different electron acceptors, as well as distinct differences in the oxidative environment within the cell. The metabolism of different carbon sources in *Desulfovibrio vulgaris* was described by Zhang *et al.*[185]. Almost one thousand gene products were identified, including ATP biosynthesis and substrate-level phosphorylation proteins. A large number of hypothetical proteins were also found, leading to a more detailed study[186] that aimed at assigning functions to these proteins according to several non-homology based methods. The Keasling laboratory studied the proteome of *D.*

vulgaris Hildenborough. Redding *et al.*[187] evaluated its proteome under nitrate stress corresponding to 50% growth inhibition. They identified 737 proteins, which represent 22% of the total proteome and span every functional category. The results indicate that this was a mild stress, as proteins involved in central metabolism and the sulfate reduction pathway were unperturbed. Among the up-regulated proteins they observed nitrate reduction proteins, transport systems, and oxidative stress response proteins. Mukhopadhyay *et al.*[188] looked at salt stress, due to its importance in *D. vulgaris* natural habitat. In this study, cells were exposed to high salt concentrations, and analyzed by transcriptomics, proteomics, among other techniques. The showed that ATP synthesis and efflux systems, as well as helicases, chemotaxis genes and osmoprotectants are particularly important in the hyperionic stress.

Escherichia coli has also been studied in the environmental context due to its low complexity and ubiquity. Bebien *et al.*[189] showed different responses of *E. coli* proteins to selenium oxides. One impressive result was the up-regulation of eight enzymes with antioxidant properties, which made the cells more tolerant to oxidative stress. Pferdeort *et al.*[190] investigated the proteome of *E. coli* metabolically engineered for trichloroethylene biodegradation, containing six genes of an evolved toluene ortho-monooxygenase from *Burkholderia cepacia G4*. The cellular physiology showed dramatic alterations due to the insertion of the toluene ortho-monooxygenase gene, with differential regulation of 45 proteins. Another study by Lee *et al.*[191] analyzed the same strain. Using a quantitative proteomics approach, they found that some of the induced proteins were involved in the oxidative defense mechanism, pyruvate metabolism, and glutathione synthesis. Proteins involved in indole synthesis, fatty acid synthesis, gluconeogenesis, and the tricarboxylic acid cycle were repressed.

Rabus and collaborators studied the responses of the denitrifying bacterium strain EbN1 to a variety of environmental stresses. The evaluated anaerobic growth on different carbon sources and focused on the expression of two toluene-related operons, of toluene-adapted cells[192]. They also confirmed the up-regulation of an ethylbenzene pathway in the presence of toluene. In a complementary study they used proteomics and bioinformatics to uncover different mechanisms of regulation of toluene and ethylbenzene pathways[193]. More recently, the same group proposed a genus name for this strain - 'Aromatoleum' sp. strain EbN1, in a study where a total of 556 different proteins were identified[194]. They were able to identify a broad collection of pathway-specific subproteomes, reflecting the metabolic versatility as well as the regulatory potential of this bacterium. The Rabus laboratory also worked on a *Pirellula sp.* strain 1 proteome after growth on glucose and N-acetylglucosamine[195]. A number of proteins were unique to cells grown on N-acetylglucosamine, and those included mostly proteins related to carbohydrate metabolism.

A variety of other species had their proteomes described for specific growth and stress conditions. Callister *et al.* compared aerobic and photosynthetic proteomes[196] of *Rhodobacter sphaeroides* 2.4.1. In a different study membrane, periplasm, outer membrane and chromatophore fractions[197] were compared. A number of proteins were found to be unique of photosynthetic cultures. Protein abundances were compared to provide insights into bioenergetic models for the different models of growth. Novotna *et al.*[198] induced diauxic growth followed by nitrogen starvation on *Streptomyces coelicolor.* The distinct growth states were characterized by distinct protein profiles and identification of stimulons related to heat, cold, salt and bacteriostatic responses. Giuffrida *et al.*[199] evaluated the different proteins expressed in *Acinetobacter radioresistens* when cultivated on aromatic

compounds such as phenol and benzoate. Most of the identified proteins were enzymes involved in aromatic degradation, and some of them had housekeeping functions. *Alcanivorax borkumensis* is a ubiquitous marine petroleum oil-degrading bacterium with an unusual physiology specialized for alkane metabolism. Sabirova *et al.*[200] used proteomic analysis to reveal metabolic features of this organism when cultivated on hexadecane and pyruvate. The proteins identified were related to terminal oxidation of alkanes, oxidation of fatty acids, biosynthesis of fatty acids and phospholipids, biosynthesis of amino acids, respiratory chain and gluconeogenesis, synthesis of cofactors, osmoprotection, pilus formation, transport, information processing and regulation. Morris *et al.*[201] studied *Dehalococcoides* spp reductive dehalogenases - key respiratory enzymes in the anaerobic detoxification of halogenated compounds. They assessed the functional diversity of closely related strains by comparing their reductive dehalogenases. Different growth conditions were tested (dehalogenation of different chlorobenzenes). They identified several reductive dehalogenases homologues, and although the genes are almost identical at the amino acid level, different strains were capable of dehalogenating diverse compounds, depending largely on the suite of reductive dehalogenases expressed. The proteome of *Ralstonia eutropha* was studied by Lee *et al.*[202] to better understand responses to formic acid. Sixty-three differentially expressed proteins in relation to formic acid were found and the greatest change was the induction of ion transporters in relation to maintenance of the acid-base balance. Cells seemed to attempt to overcome the effects of formic acid by increasing ion transporters and proteins that metabolize formic acid. Another study of interest was done by Ho *et al.*[203] and describes the responses of *Stenotrophomonas* sp. OK-5 to stress caused by trinitrotoluene. Two-dimensional protein profiles showed approximately 300 differentially

expressed proteins after exposure to trinitrotoluene. The most important proteins identified by this work were stress-related proteins. Marrero *et al.*[204] investigated the response of *Enterobacter liquefaciens* strain C-1 to cobalt concentration. Twelve proteins were identified to be involved with cellular antioxidant response and resistance to heavy metals.

II.II.VII. Membrane physiology

Membrane physiology studies are usually related to membrane alterations facing specific growth or stress conditions. Methodology for the analysis of membrane proteins has been developed[205], such as purification in aqueous-organic mixtures among other methods compatible with mass spectrometry. Membrane proteome studies are important because the membrane works as a modulable site of communication between intra- and extracellular environments. *Methanococcus jannaschii* had its proteome characterized in response to hydrogen concentration, ammonium availability and growth phase[206]. Significant changes were observed for these conditions and the main response was regulation of motility through modifications of the building blocks of the flagella. Another proteome study of the same organism also focused on flagella as a function of partial hydrogen pressure[207]. Flagella synthesis was induced when hydrogen became limiting. The outer membrane proteome of *Caulobacter crescentus*[208] was characterized for cells grown in rich and minimal media. 41 proteins were identified, and 16 were TonB-dependent receptor proteins that were up-regulated in minimal medium. The same organism was used as a model for different methods of purification of alkaline membrane proteins[209]. 32 proteins were identified and complemented the previous study. The membrane proteome of Acinetobacter radioresistens S13 was analyzed under different growth substrates[210] – acetate, benzoate or phenol. In the presence of phenol, they detected up-regulation of proteins with the following functions:

antiporters, ABC-type sugar transport system, and polysaccharide translocation. In the presence of acetate, up-regulated proteins were an OmpA-like protein, a trimeric porin and glycosyl transferases. Membrane proteome studies were also described for *Paracoccus denitrificans*[211], *Deinococcus radiodurans*[212, 213], and *Streptococcus mutans* biofilms[214].

II.II.VIII. Ecological maps

Genome-wide proteomics is a new concept that envisages creating a proteome map of all possible gene products encoded in a given genome. It represents an advance in the field, but it does not take account for the fact that genomes are not static, and mutations and changes in protein function may be overlooked in these ecological maps. In the metaproteomics approach, proteins are identified from unknown communities, and the construction of ecological maps would be impossible. Some pure culture ecological maps available in the current literature are reviewed.

Christendat *et al.*[215] studied the structural proteome of *Methanobacterium thermoautotrophicum.* They used X-ray crystallography and magnetic resonance spectroscopy to relate protein sequence and solubility. The structures provided clues into biochemical functions that cannot be detected by sequence analysis. Giometti *et al.*[216] worked on a global analysis of the proteome of *Methanococcus jannaschii*, identifying 170 of the most abundant proteins expressed under optimal fermentation conditions. Most of these proteins were involved in energy and intermediary metabolism, cell division and structure, and protein synthesis. More than 60% of the predicted proteome of *Deinococcus radiodurans* was identified in a series of studies[217-219]. An interesting study investigated proteins from *D. radiodurans* recovering from gamma-irradiation[220]. Proteins identified to be relevant to radioresistance had roles in transcription and translation, replication and repair,

general metabolism and signal transduction. Mergeay *et al.*[221] studied heavy metal influence on *Ralstonia metallidurans.* This bacterium originally carries two plasmids bearing several genes for metal resistance. They found plasmid-borne proteins and several gene clusters involved in the response to high concentrations of heavy metals, and a high number of proteins induced or repressed in the presence of copper. Noel-Georis *et al.*[222] built an ecological proteome map of *R. metallidurans* grown in minimal medium. Their map contained 224 unique proteins, and identified undetected open reading frames and proteins not encoded by sequenced genome fragments. *Sulfolobus solfataricus* had its map constructed, containing 47% of the total proteome[223]. In another study, the same group evaluated different concentrations of alcohols and ketones[224]. More than 80% of the proteins showed no discernable changes compared to the control. Another study mapped 324 proteins[225], including proteins from all functional categories.

Several ecological studies focus on cold adaptation of several bacterial species. Nichols *et al.*[226] used proteomics to map the adaptation of *Methanococcoides burtonii* to low temperature. Their results showed that most proteins involved in cold adaptation were involved in lipid biosynthesis and specific changes in membrane lipid unsaturation. Methe *et al.*[227], in a similar study, used *Colwellia psychrerythraea* 34H and found changes to the cell membrane fluidity, uptake and synthesis of cryotolerance compounds and strategies to overcome temperature-dependent barriers to carbon uptake. Proteomic analysis was also used by Qiu *et al.*[48] to investigate the cold adaptation of *Exiguobacterium sibiricum* 255-15. They used an alternative approach involving chromatofocusing and identified 256 proteins, among which 39 were cold acclimation proteins, preferentially or uniquely expressed at 4 °C. The salt and cold adaptation of *Psychrobacter* 273-4 was also evaluated by Zheng *et al.*[228].

Different proteins were identified in cold adaptation in the presence of salt, showing a combination effect of salt and cold on protein expression. The proteome of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 was studied by Danchin (*in silico*[229] and *in vitro*[230]). The first study translated the genome *in silico* and looked at specific features in the amino acid composition that can be related to the cold acclimation. The amino acid distribution in mesophilic and psychrophilic species displayed a few noteworthy differences specifically relevant to growth in the cold. They found that this proteome provided a way to resist to the aging features, making this bacterium a model for the study of cold adaptation. The objective of the second study was to provide information on the interaction between organisms and the ecosystem based on different carbon source usage. Identified proteins were functionally classified, and fell in three main groups: cell envelope and membrane-associated, intermediary metabolism and information transfer.

Proteins of hyperthermophilic organisms are of particular importance since they have an enhanced conformational stability, allowing them to be active at high temperatures. This property can be used to investigate the molecular basis of protein folding and conformational stability. Prosinecki *et al.*[231] studied hyperstable proteins from *Sulfurispharea sp.*, a hyperthermophilic archaeon, able to grow between 70 and 97 °C. They dynamically perturbed the proteome and identified proteins with enhanced stabilities, involved in key cellular processes such as detoxification, nucleic acid processing, and energy metabolism, were identified. These proteins were still biologically active after extensive thermal treatment of the proteome. A *Corynebacterium glutamicum* proteome study revealed a 2DE map presenting more than half of the total proteins encoded by this organism[232]. The proteins identified were the 35 kDa antigen, antigen 84, ATP and cysteine synthase,

elongation factors, enolase and rotamase. In the proteome map of *Corynebaterium efficiens*, more than 300 proteins were identified from intracellular, membrane and extracellular fractions[233]. When compared with the *C. glutamicum* map, species-specific differences were found. This showed environmental adaptations of *C. efficiens*, mostly including molecular chaperones and amino acid biosynthesis enzymes. The aniline degrading bacterium *Acinetobacter lwoffii K24* had its proteome map described for growth with and without aniline[234]. 300 proteins presented differential regulation due to the presence of aniline. Rosen *et al.*[235] created a reference map for the plant pathogen *Agrobacterium tumefaciens* with more than 300 proteins. They showed evidence for post-translational modifications, based on the identification of proteins associated with multiple spots. Among the proteins identified, there were a number of transporters, transcription and translation proteins, and well as metabolic proteins. The dioxin-mineralizing bacterium *Sphingomonas wittichii* strain RW1 had its proteome analyzed in the presence of dibenzofuran and dibenzo-p-dioxin[236]. They were able to identify up to 14 peptide ions belonging to dioxin dioxygenase.

II.II.IX. Wastewater treatment

Applications of proteomics in wastewater treatment mostly characterize the organisms involved in this process and their ecology. They draw attention to the interactions that allow organisms in a community to survive, rather than discussing specific pathways or substrates required for living. MacRae and Smit[237] described 33 different strains of caulobacters present in wastewater. The strains were distinguished based on colony characteristics, DNA restriction fragment length polymorphism analysis, and protein band profiles. They also point out the increasing ability of antibiotic resistance of these strains, indicating environmental adaptation. Jacob *et al.*[238] used 1DE to characterize 24 different strains of

campylobacters present in a wastewater treatment pant. Their work confirms the findings of the different strains, only according to standard protein patterns. A similar study was conducted by Niemi *et al.*[239] with 371 environmental isolates of fecal streptococci samples. Samples were collected from domestic and industrial wastewater, and were characterized and clustered in 7 groups according to their 1DE protein profiles. Samples from each environment had typical species compositions, and their protein profiles varied according to their environments. The protein profiles of whole bacterial cells have been observed to correlate closely with DNA-DNA hybridization results. Maszenan *et al.*[240] did a similar analysis for strains of the species *Acinetobacter*, together with a range of isolates from a biological nutrient-removal activated sludge plant. The proteomic analysis generally supported the taxonomic relationships suggested from earlier DNA-DNA hybridization data. An interesting study was conducted by Wagner-Dobler *et al.*[241] in order to better understand bacterial communities capable of degrading biphenyl, for future bioremediation applications. Different species were identified using 16S rDNA methods, and 1DE of whole-cell proteins was used to provide information on the similarity of strains of the same species. Comparison of normalized protein patterns revealed that all of the representative isolates were very similar to each other, thus coming from the same species. A more recent study involving virus-binding proteins by Sano *et al.*[242] focused on the importance of pathogenic viruses in waterborne diseases. The objective of the study was therefore to discover virus-binding proteins from a bacterial culture derived from activated sludge. 2DE revealed that the isolated virus-binding proteins include a large number of small proteins, with molecular masses smaller than 100 kDa. Amino acid sequences of N termini of five virus-binding

proteins were determined. Finally, homology searches against all protein sequences in the NCBI database showed that the proteins isolated in this study were newly discovered.

II.III. Metaproteomics

Metaproteomics encompasses the proteomic study of metaorganisms. A metaorganism is defined as a collection of organisms evolving as a whole, sharing genes and metabolic capacities. Only a few studies on proteomes of environmental unsequenced mixed cultures have been published in the scientific literature. Valenzuela *et al.*[243] discussed the use of emerging metagenomics and high-throughput proteomic technologies to study biomining communities. The Banfield laboratory has shown pioneer work in this area and presented a review of proteogenomic approaches being used lately for the molecular characterization of bacterial communities[244]. A landmark study was published on a natural acid mine drainage microbial biofilm community[245]. With a shotgun proteomics approach, they identified more than 2000 proteins through the use of a database created from the sequencing of a microbial community sampled from the same mine but at a different location and time. Recently, a strain-resolved community proteomics study[246] was published. They used community genomic data to identify proteins from dominant community members, with strain specificity. Their findings provide evidence for exchange of genes during adaptation to specific ecological niches. Wilmes and Bond investigated a community of microorganisms from a laboratory-scale sequencing batch reactor optimized for enhanced biological phosphorus removal and enriched for polyphosphate-accumulating organisms. In their first study[247], they used a 2DE-MS approach to detect many proteins and identify three. In their subsequent studies[248, 249] they performed comparisons of proteome profiles of activated

sludges with sequentially increasing phosphorus removal performance and profiles of phosphorus removal versus non-phosphorus removal. Kan *et al.*[250] studied Chesapeake Bay microbial communities using a similar workflow and identified eight proteins. Our laboratory also presented a metaproteomics study of an unsequenced bacterial community[251], identifying more than 100 proteins differentially expressed in the presence of cadmium. A different approach consists of extracting proteins directly from soil, with extraction methods compatible with proteomic techniques. Ogunseitan[252] described an extraction method for proteins from soil. Following this approach, Schulze *et al.*[253, 254] studied proteins isolated from dissolved organic matter using mass spectrometry and demonstrated the ability to determine a proteome fingerprint of soil. Among the proteins identified to be abundant in dissolved organic matter, there were cellulases and laccases, which composed a proteomic fingerprint of presence and activity of organisms in an ecosystem. Verberkmoes *et al.*[255] used proteomics to evaluate biological thereat agents in complex environmental matrices. They determined the ability of current mass spectrometric-based methods to detect target species in different matrices at concentrations as low as 6%.

II.IV. Future Directions

Environmental proteomics still have many challenges to overcome, including improving analysis of unsequenced organisms and techniques for extraction of proteins from soil and other matrices. However, advances in the field represent a major step towards a systems view of organisms and metaorganisms. The potential applications are wide, including improvement of wastewater treatments, and bioremediation and monitoring. In a broader view, this represents a major advance in the fields of environmental biotechnology and

microbial ecology. In association with proteomics, transcriptomics and metabolomics[256, 257] are also powerful tools in providing information on gene function and regulatory networks. Only combined studies can correlate metabolic fluxes and physiological changes in organisms.

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III. QUANTITATIVE METAPROTEOMIC ANALYSIS OF A MODEL BACTERIAL COMMUNITY

III.I. Introduction

Differential proteomics is usually applied to study the response of cells and tissues to environmental perturbations (nutrients[258], xenobiotics[259], infection[260]) and genetic alterations (cancer[261, 262], metabolic engineering[263, 264]) by identifying proteins with altered expression levels. In an effort to obtain more than a qualitative description of a proteome there has been a large development of quantitative proteomics methods. Quantitative proteomics can help characterize pathway regulation and complex system networks by providing protein concentration information corresponding to different cellular states. Such information is of utmost importance in the developing field of systems biology[265]. With the increasing rate of acquisition and interpretation of –omics data (including functional proteomics) and high computational power, the new science of systems biology will be able to predict systems behavior when facing different types of perturbations.

Current experimental methods for quantitative proteomics include *in vivo* and *in vitro* methods[266-268]. Stable isotope labeling with amino acids (SILAC)[269, 270] or with small nitrogen- or carbon-containing nutrients[271-273] can be incorporated in the growth medium to directly label proteins while synthesized *in vivo*. *In vitro* quantification methods include labeling of cysteine residues (isotope-coded affinity tags, ICAT)[274, 275], and labeling of N-termini (isobaric tags for relative and absolute quantification, iTRAQ)[276-278]. Other less commonly used quantification

methods were reviewed by Lau *et al*[279]. The iTRAQ technology has a significant advantage over other methods due to its capability of multiplexing up to eight samples in one experimental setup[280]. Another positive aspect includes unbiased peptide labeling, since iTRAQ isobaric tags label lysine side groups and all free amino-terminal groups of the peptides present in a sample. The iTRAQ tags consist of a reporter group, a balance group, and a peptide reactive group that covalently binds to the peptides. The balance group gives all tags the same mass during peptide mass fingerprinting. In the CID stage of a tandem mass spectrometer, there is a neutral loss of the balance group, and the reporter groups are detected in the second MS. The tandem mass spectra include contributions from each sample, and the individual contributions of each sample can be measured by the intensity of the reporter ion peaks.

The objective of this experiment is to evaluate the potential of quantitative metaproteomics, and show that *in vitro* peptide labeling can be successfully used to measure proteome changes in a mixed culture of organisms and in the corresponding pure species. Our goal is to determine the ability of quantitative metaproteomics to reveal unique protein expression responses derived from the interactions of the microorganisms living in co-culture. Comparing microbial proteomes in co-culture with the corresponding pure cultures is key for microbial ecology and understanding mechanisms of bacterial communication and signaling. Due to the challenges associated with metaproteomics of unsequenced organisms (further discussed in Chapter 4), we designed a simple model bacterial mixed culture of two sequenced organisms to use in a quantitative shotgun proteomics experiment. To the best of our knowledge, no such quantitative metaproteomics study is available in the proteomics literature.

The organisms of choice here were bacteria commonly found in soil and considered as models for Gram-positive (*Bacillus atrophaeus*) and Gram-negative (*Pseudomonas putida* KT2440) microorganisms in environmental research. *Pseudomonas putida* KT2440 is a ubiquitous soil organism, non-pathogenic, with a fully sequenced genome[281]. It derives from a toluene-degrading bacterium and it is well-known for its metabolic versatility, including the ability to degrade several xenobiotic compounds. *Bacillus atrophaeus* is the current name for the *Bacillus subtilis* strain DSM675 used in our study[282]. This *Bacillus subtilis* strain was first characterized based on its red pigmentation, but has since been reclassified a number of times. This sequenced species is the best-characterized of all Gram-positive bacteria, and is known for its ability to colonize plants and to produce and secrete industrially important enzymes and metabolites[283]. These organisms were tested for successful growth in community through Gram-staining at every growth stage. After determining community growth in rich medium and at mesophilic temperatures, we compared the community proteome with the proteome of the pure species grown in the same conditions.

III.II. Experimental Procedures

Inocula were added to duplicate 500-mL baffled Erlenmeyer flasks containing 200 mL of 10 g/L tryptic soy broth (BD Diagnostics, Franklin Lakes, NJ, USA) to achieve an initial OD 600 of 0.002 (approximately 0.02 mg/mL of dry cells). A total of eight flasks were incubated (two blanks, two *B. subtilis*, two *P. putida*, two mixed culture of equal numbers of *P. putida* and *B. subtilis*). The culture flasks were then incubated at pH 7, 30 °C, and 200 rpm for approximately 24 h. Protein extraction followed a multi-step process that combined chemical and mechanical

lysis steps to lyse both Gram-positive and Gram-negative cells. Immediately after harvesting, cell cultures were centrifuged at 4 °C and 7500 x g for 10 min. The resulting cell pellets were washed in 30 mL of a pH 7 buffer composed of 3 mM KCl, 1.5 mM KH_2PO_4, 68 mM NaCl, and 14 mM NaH_2PO_4, and centrifuged again. Each cell pellet was resuspended in 2 mL of 10 mM Tris-HCl pH 8, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.5 mM Pefabloc SC (protease inhibitor, Roche Applied Science, Indianapolis, IN, USA), and 0.1% SDS. Cells were then sonicated on ice using a 550 Sonic Dismembrator probe (Fisher Scientific, Hampton, NH, USA) vibrating at 20 kHz for 5 minutes, in cycles of 1 second on and 2 seconds off. After sonication, the suspension was centrifuged at 4 °C and 7500 x g for 15 min and the supernatant isolated. The pellet was resuspended in 2 mL of a second lysis solution, containing 10 mM Tris-HCl pH 8, 1 mM EDTA disodium salt, 0.05 mM dithiothreitol, 0.5 mM Pefabloc SC, 10 % glycerol and 1 mg/mL lysozyme. This was sonicated and centrifuged as before. The two final supernatants were combined, and the protein concentration of the final lysate was determined using the bicinchonic acid (BCA) assay from Pierce Biotechnology Inc. (Rockford, IL USA). Protein samples were divided into aliquots of 100 µg and stored at -80 °C. The two-step extraction procedure assured that proteins from both Gram-positives and Gram-negatives were extracted, and that in the case of pure species, the protein solutions had the same reagents contributing as background in the protein assay. Tryptic digestions of 100 µg of each biological replicate followed the iTRAQ Reagents protocol from Applied Biosystems. Briefly, samples were dissolved in 20 µL of Dissolution Buffer and 1 µL of Denaturant. After full sample dissolution, 2 µL of Reducing Reagent were added, and samples were incubated for 1 h at 60 °C. Cysteines were blocked by the addition of 1 µL of Cysteine Blocking Reagent followed by room

temperature incubation for 10 min. Samples were then digested overnight with trypsin (10 µg) at 37 °C. After complete digestion, samples were were labeled with iTRAQ reagents, also following the iTRAQ Reagents protocol. Samples were labeled according to the following scheme: *B. subtilis*, 115; mixed culture, 116; and *P. putida*, 117.

Labeled peptides were mixed and then fractionated by SCX chromatography using a Polysulfoethyl A column 100 mm x 2.1 mm x 5 µm, 200 Å (PolyLC Inc., Columbia, MD USA). Buffer A was 5 mM potassium phosphate in 25% acetonitrile, pH 3, and Buffer B contained 500 mM potassium chloride added to the buffer A composition. A 35-min linear gradient at 0.2 mL/min was used. Fractions were collected every minute, and purified with C18 PepClean spin columns (Pierce Biotechnology Inc., Rockford, IL USA), according to the manufacturer's protocol. C18-cleaned samples were vacuum-dried and resuspended in 15 µL of 0.1% formic acid. Samples were separated on an Agilent ZORBAX reverse-phase column (C18 wide-pore, 50 mm x 0.075 mm internal diameter) in an Agilent 1200 HPLC system, containing a nanospray directly connected to the mass spectrometer ionization source. The HPLC buffers were 0.1% formic acid in 3% acetonitrile (Buffer A) and 0.1% formic acid in 97% acetonitrile (Buffer B). The method used a 45-min gradient at 0.6 µL/min starting with 5% Buffer B for 5 min, followed by 30 min of ramping to 70% Buffer B. Two other 5-min steps followed at 90% and 5% Buffer B. Mass spectrometry was accomplished by an ESI-QTOF instrument (Agilent 6510, with Chip Cube sample inlet), with the following settings: gas temperature 300 °C; drying gas 5 L/min; and capillary voltage of 1875 V. The auto MS/MS mode was used, with positive ion polarity and centroid data storage. Acquisition ranges were 300 – 2000 m/z for MS (4 spectra/s) and 59 – 1800 m/z for MS/MS (5 spectra/s). Collision energy used a slope of 4 (V/100 Da). Four

precursor ions were collected per cycle, with active exclusion. Internal reference mass standard was used for Chip operation. All datasets were exported to the mzData format. Database searches were conducted on Mascot using the following parameters: all bacterial species in the NCBInr database, decoy search, trypsin digestion allowing for up to two missed cleavages, methionine oxidation and iTRAQ on N-termini and lysines, iTRAQ 4-plex quantification, 0.3 Da tolerance for MS and MS/MS, peptide charges of +1, +2 and +3, and monoisotopic masses.

III.III. Results and discussion

III.III.I. Culture Growth and Protein Extraction

Cells were cultivated in duplicate as described in the previous section. Cell growth in co-culture was monitored by Gram-staining of aliquots collected approximately every 4h. One aliquot was collected from every replicate flask, and spread over three microscope slides. Cells were immobilized on slides by air-drying and Gram stained. The presence of similar counts of Gram-positive and Gram-negative cells was confirmed by using an optical microscope and 40X lens. Averaging of all the observations at different time points showed that *B. subtilis* and *P. putida* were equally abundant in the co-culture. At approximately 20 h of growth, the harvesting OD_{600} for the cultures were: 3.82 for pure *P. putida*, 3.66 for pure *B. subtilis* and 3.94 for the co-culture. During harvesting, *B. subtilis* cell pellets had an orange coloration, *P. putida* cell pellets were tan, and the community cell pellet presented a lighter orange shade. After protein extraction and purification, the discarded cellular material was mostly tan, and the protein solutions retained the colorations described above. Visual inspection indicates that many of the

B. subtilis proteins are pigmented, which could potentially interfere with downstream protein analyses.

III.III.II. Protein Identification and Quantification

Identification and quantification tables for the proteins of interest in this study are present in Appendix I. Those tables contain the minimum information required for a proteome experiment, i.e., protein name and accession numbers, the organism in which the protein was first identified, protein and peptide scores, mass errors, peptide sequence and protein sequence coverage, protein and peptide ratios and standard deviations, peptide weights, and estimation of false discovery rates. The false positive identification rate was calculated for each fraction analyzed by the Mascot decoy search, and the average value for the overall experiment was 2.88%, which can be considered good. Table 1 presents the 63 proteins that were differentially expressed (expression two times larger) in the co-culture. It is interesting to note that MS/MS data were searched against the whole Eubacterial database for protein identifications, but all proteins of interest in this study were highly homologous to pseudomonad proteins.

Figure 3 presents protein quantification for all differentially expressed proteins. The quantification values compare the ratios of *B. subtilis* against mixed culture (blue) and *P. putida* against the culture (red), sorted according to the *B. subtilis* ratios. The leftmost side of the graph shows the proteins that were down-regulated in the *B. subtilis* pure culture, equivalent to up-regulation in the community. In general, proteins that were down-regulated in the *B. subtilis* pure culture were also down-regulated, with less drastic changes, in the *P. putida* pure culture. In other words, these proteins underwent up-regulation in the community environment, most probably due to some community effect, not present in the pure cultures. The right-hand side of

the graph indicates proteins that were up-regulated in the *B. subtilis* pure culture. This side does not show a very good agreement between proteins differentially by the pure cultures, i.e., not all the proteins up-regulated by *B. subtilis* were up-regulated by *P. putida*. This leads to two protein groups that are uniquely differentially expressed in either pure culture. Another interpretation of this event can be that the culture appropriately regulates certain proteins (down in one organism and up in the other) to keep an overall balance of activities.

Among the proteins with the largest expression changes in *B. subtilis*, we find the extreme down-regulation of elongation factor G, acetolactate synthase III large subunit, electron transfer flavoprotein, alpha subunit, and quinone oxidoreductase; and the up-regulation of putative cold-shock DNA-binding domain protein and heat shock protein Hsp20. For *P. putida*, the largest expression changes were the down-regulation of outer membrane lipoprotein OprI and isocitrate lyase and the large up-regulation of DNA-binding protein HU, form N. We further discuss possible roles for these proteins in the biological interpretation paragraphs.

Several issues associated with the quantification aspects of this study merit discussion. First, the newly-developed Mascot quantification algorithm yielded negative quantification values for many peptides (available in Appendix I). This is clearly an error since visual inspection of MS/MS spectra revealed that the iTRAQ reporter ions were present. Due to proprietary issues, we do not understand which parts of the Mascot algorithm are responsible for such discrepancy, nor why only peptide quantification is affected, and not protein quantification. Another quantification issue relates to the clear under-quantification of the 115 isobaric tag (refer to Appendix I for examples), not corresponding to stoichiometric amounts of protein. This could be attributed to experimental errors, but all replications of this experiment presented the same

distortion of the data, and parallel peptide quantification of the samples showed that overall peptide concentrations were equivalent for all three samples (data not shown). Several protein and peptide concentration assays were performed for these samples at various stages of the shotgun protocol, and the quantities were shown to be stoichiometric. To correct for this bias, average values were calculated for the expressed tags and all Mascot ratios were normalized against the mean values, as a correction for the lower average of 115. Since all the quantification values expressed here are ratios, a mean normalization should transform these values into proportional amounts, and reflect the true differential expression ratio of the proteins identified. The mean value normalization technique is used in transcriptomics research among other data correction methods[284].

III.III.III. Protein Functional Classification

Proteins of interest in this study were classified according to the biological processes where they are involved using Gene Ontologies (http://www.godatabase.org/cgi-bin/amigo/go.cgi). Table 1 presents each process where these proteins take part, and Figure 4 shows the more general function category spanning the biological processes. Approximately two-thirds of the proteins identified were involved in metabolism, and the majority of those were related to amino acid and protein metabolism, and well as nucleic acid and DNA metabolism. Some of the proteins in this group also have regulatory functions, but only major functions are used for the functional classification scheme. It is also important to note that approximately 13% of the proteins of interest were involved in cellular defense mechanisms. In addition, the total number of differentially modulated hypothetical proteins is small, reflecting the high homology of the proteins found with proteins present in the NCBI database.

III.III.IV. Biological Interpretation

There are no available metaproteomic studies in the literature that can be used to confirm our results, but here we describe some common trends between protein function and regulation and how that is associated with growth in community. The community of *B. subtilis* and *P. putida* expressed antioxidant and xenobiotic metabolism proteins (organic hydroperoxide resistance protein, antioxidant AhpC/Tsa family) at higher levels than the pure cultures. This might be a direct response to some stress generated by the life in community. Also generally up-regulated in the community are proteins involved in DNA metabolism (DNA-directed RNA polymerase subunits and single-strand binding protein), electron transport (electron-transfer flavoproteins), and energy generation metabolism (catalase/peroxidaase HPI and quinone oxidoreductase). The latter is a group of enzymes, related to increased central carbon metabolism, which can be correlated with better fitness of the organisms living in community in comparison with in pure cultures. Further insights in this direction would be gained by studying the secretome of the community. Among proteins that show unique response in *B. subtilis,* heat- and cold-shock proteins (heat shock protein Hsp20 and cold shock protein CspA) were up-regulated and proteins involved in protein metabolism (chaperonin GroEL and elongation factors) were mostly down–regulated. An alternative interpretation for this regulation can relate to a similar expression of heat- and cold-shock proteins in the community and in *P. putida.* In a similar way, a number of proteins involved in protein metabolism are less abundant in the *B. subtilis* pure culture, meaning that larger concentrations are required for life in community with the other species, which presents no differential expression. Proteins involved in lipid and amino acid metabolism (acyl carrier protein, dihydrolipoamide dehydrogenase, 4-hydroxyphenylpyruvate dioxygenase and

methylmalonate semialdehyde dehydrogenase) are only down–regulated in *P. putida.* This is the opposite case of the one described above, where the community and the pure culture of *B. subtilis* share similarities in specific protein expression groups.

This study is used to further validate the evidence of unique metaproteomic responses observed in the previous chapter. We were able to find major differences in protein expression between the community and the corresponding pure cultures. These responses serve as further foundation for the metaproteomics concept, as they reveal unique evidence for interactions between the two organisms in co-culture. The approach used here is fundamentally different from the one used in the next chapter (2DE versus shotgun) and so is the mixed culture studied. This was the first metaproteomics study to make use of a peptide labeling technology for peptide and protein quantification.

III.IV. Conclusions

Proteomics is the –omics science that most closely relates to cellular physiology. In this quantitative metaproteomics study, we were able to detect and quantify regulation of proteins that are unique to growth in pure cultures or in the community of *B. subtilis* and *P. putida*, two independently-growing soil bacteria. The metaproteomics approach is the only one that provides information on protein regulation due to interactions between organisms. The ecological view of this study is unique in the sense that one organism is the environmental perturbation for the other. This is the first shotgun-based metaproteomics study of a constructed sequenced microbial community employing iTRAQ technology for quantification of proteins.

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Table 1: Summary of all differentially-expressed proteins in the *B. subtilis* and *P. putida* experiment and their expression ratios with respect to mixed culture.

Bacillus/ MIx	Pseudomonas/Mix	Accession	Protein ID	GO Biological Process
2.02	1.09	gi|129036	2-oxoglutarate dehydrogenase E1 component (Alpha-ketoglutarate dehydrogenase)	tricarboxylic acid cycle
2.34	1.35	gi|148546904	2-oxoglutarate dehydrogenase, E2 subunit, dihydrolipoamide succinyltransferase [Pseudomonas putida F1]	tricarboxylic acid cycle
2.04	0.65	gi|26988503	30S ribosomal protein S1 [Pseudomonas putida KT2440]	cellular protein metabolic process
2.67	1.10	gi|26990146	4-hydroxyphenylpyruvate dioxygenase [Pseudomonas putida KT2440]	amino acid metabolic process
0.86	2.14	gi|26987185	50S ribosomal protein L1 [Pseudomonas putida KT2440]	cellular protein metabolic process
0.38	0.56	gi|26987198	50S ribosomal protein L2 [Pseudomonas putida KT2440]	cellular protein metabolic process
2.13	0.97	gi|26987203	50S ribosomal protein L29 [Pseudomonas putida KT2440]	cellular protein metabolic process
0.31	0.56	gi|26987195	50S ribosomal protein L3 [Pseudomonas putida KT2440]	cellular protein metabolic process
0.28	1.02	gi|26987187	50S ribosomal protein L7/L12 [Pseudomonas putida KT2440]	cellular protein metabolic process
0.26	0.63	gi|29839337	60 kDa chaperonin (Protein Cpn60) (groEL protein)	protein folding
0.12	0.64	gi|15599890	acetolactate synthase III large subunit [Pseudomonas aeruginosa PAO1]	isoleucine / valine biosynthetic process
1.32	0.44	gi|15600516	acetylglutamate kinase [Pseudomonas aeruginosa PAO1]	arginine biosynthetic process
0.48	0.70	gi|26989063	aconitate hydratase [Pseudomonas putida KT2440]	tricarboxylic acid cycle
0.38	0.91	gi|148549005	acyl carrier protein [Pseudomonas putida F1]	fatty acid biosynthetic process
0.55	0.32	gi|26991567	adenylosuccinate synthetase [Pseudomonas putida KT2440]	purine ribonucleotide biosynthetic process
0.75	0.49	gi|26987820	antioxidant, AhpC/Tsa family [Pseudomonas putida KT2440]	oxygen and reactive oxygen species metabolic process
0.63	0.44	gi|26992088	ATP synthase subunit B [Pseudomonas putida KT2440]	plasma membrane ATP synthesis coupled proton transport
0.35	0.75	gi|26987361	ATP-dependent Clp protease, ATP-binding subunit ClpB [Pseudomonas putida KT2440]	proteolysis
0.36	0.58	gi|26988214	betaine aldehyde dehydrogenase, putative [Pseudomonas putida KT2440]	betaine biosynthetic process
0.31	0.61	gi|26990379	catalase/peroxidase HPI [Pseudomonas putida KT2440]	response to oxidative stress
0.43	0.60	gi|13096652	Chain A, Catabolic Ornithine Carbamoyltransferase From	amino acid metabolic

			Pseudomonas aeruginosa	process
0.30	0.68	gi|126359797	chaperonin GroEL [Pseudomonas putida GB-1]	protein folding
0.65	0.46	gi|119856883	chaperonin GroEL [Pseudomonas putida W619]	protein folding
0.46	0.40	gi|32028588	COG0055: F0F1-type ATP synthase, beta subunit [Haemophilus somnus 2336]	ATP synthesis coupled proton transport
2.87	1.40	gi|26989186	cold shock protein CspA [Pseudomonas putida KT2440]	protein folding / response to oxidative stress
0.89	0.32	gi|26991831	D-3-phosphoglycerate dehydrogenase [Pseudomonas putida KT2440]	L-serine biosynthetic process
2.89	0.43	gi|26990879	dihydrolipoamide dehydrogenase [Pseudomonas putida KT2440]	fatty acid catabolic process
3.03	9.69	gi|26987711	DNA-binding protein HU, form N [Pseudomonas putida KT2440]	DNA packaging
3.09	0.81	gi|26989027	DNA-binding protein HU-beta [Pseudomonas putida KT2440]	DNA packaging
0.27	0.44	gi|26987220	DNA-directed RNA polymerase alpha subunit [Pseudomonas putida KT2440]	transcription
0.22	0.96	gi|15599466	DNA-directed RNA polymerase beta subunit [Pseudomonas aeruginosa PAO1]	transcription
2.30	0.81	gi|26987189	DNA-directed RNA polymerase beta' subunit [Pseudomonas putida KT2440]	transcription
0.23	0.93	gi|15598148	electron transfer flavoprotein beta-subunit [Pseudomonas aeruginosa PAO1]	generation of precursor metabolites and energy / electron transport
0.15	0.50	gi|26990893	electron transfer flavoprotein, alpha subunit [Pseudomonas putida KT2440]	generation of precursor metabolites and energy / electron transport
0.11	0.38	gi|26987192	elongation factor G [Pseudomonas putida KT2440]	translational elongation
0.66	0.40	gi|26988324	elongation factor Ts [Pseudomonas putida KT2440]	translational elongation
2.88	1.11	gi|148545498	extracellular solute-binding protein, family 3 [Pseudomonas putida F1]	transport
0.54	0.34	gi|13310130	FadB1x [Pseudomonas putida]	fatty acid catabolic process
0.48	0.45	gi|6855333	glutamine synthetase [Pseudomonas syringae pv. tabaci]	glutamine biosynthetic process
2.07	0.75	gi|148546266	glycine cleavage system T protein [Pseudomonas putida F1]	glycine decarboxylation via glycine cleavage system
4.89	2.21	gi|148547677	heat shock protein Hsp20 [Pseudomonas putida F1]	protein folding / response to oxidative stress
0.40	1.92	gi|535709	HU protein	DNA packaging
1.88	0.50	gi|89893980	hypothetical protein DSY1234 [Desulfitobacterium hafniense Y51]	Unknown
0.12	0.65	gi|26987139	hypothetical protein PP_0397 [Pseudomonas putida KT2440]	Unknown
1.91	2.21	gi|26991516	hypothetical protein PP_4836 [Pseudomonas putida KT2440]	Unknown
0.28	0.59	gi|15598965	inositol-5-monophosphate dehydrogenase [Pseudomonas aeruginosa PAO1]	purine ribonucleotide biosynthetic process
0.37	0.23	gi|26990810	isocitrate lyase [Pseudomonas putida KT2440]	tricarboxylic acid cycle

1.08	0.29	gi|3201828	major outer membrane lipoprotein I [Pseudomonas oleovorans]	lipid modification
2.68	0.65	gi|26991351	methylmalonate semialdehyde dehydrogenase [Pseudomonas putida KT2440]	amino acid metabolic process
0.33	1.06	gi|26991410	molecular chaperone DnaK [Pseudomonas putida KT2440]	response to stress
1.37	0.48	gi|104780737	organic hydroperoxide resistance protein [Pseudomonas entomophila L48]	response to stimulus / response to toxin
0.63	0.22	gi|26989046	outer membrane lipoprotein OprI [Pseudomonas putida KT2440]	lipid modification
0.30	0.31	gi|26987920	outer membrane protein H1 [Pseudomonas putida KT2440]	lipid modification
0.24	0.43	gi|26991177	pterin-4-alpha-carbinolamine dehydratase [Pseudomonas putida KT2440]	tetrahydrobiopterin biosynthetic process
4.64	0.97	gi|148546265	putative cold-shock DNA-binding domain protein [Pseudomonas putida F1]	protein folding / response to oxidative stress
3.66	0.70	gi|17547847	PUTATIVE NAD+ DEPENDENT ACETALDEHYDE DEHYDROGENASE OXIDOREDUCTASE PROTEIN [Ralstonia solanacearum GMI1000]	aromatic compound catabolic process
0.17	0.49	gi|26986817	quinone oxidoreductase [Pseudomonas putida KT2440]	generation of precursor metabolites and energy
2.20	0.52	gi|148546001	ribosomal 5S rRNA E-loop binding protein Ctc/L25/TL5 [Pseudomonas putida F1]	response to stress / translation
0.26	0.60	gi|119858954	ribosomal protein S1 [Pseudomonas putida W619]	translation
3.83	1.11	gi|90020610	Single-strand binding protein [Saccharophagus degradans 2-40]	DNA replication / recombination
0.41	1.79	gi|119860710	Small GTP-binding protein domain [Pseudomonas putida W619]	translation
2.16	1.41	gi|26991396	transcription elongation factor NusA [Pseudomonas putida KT2440]	RNA elongation
2.17	0.56	gi|148548757	UspA domain protein [Pseudomonas putida F1]	response to stress

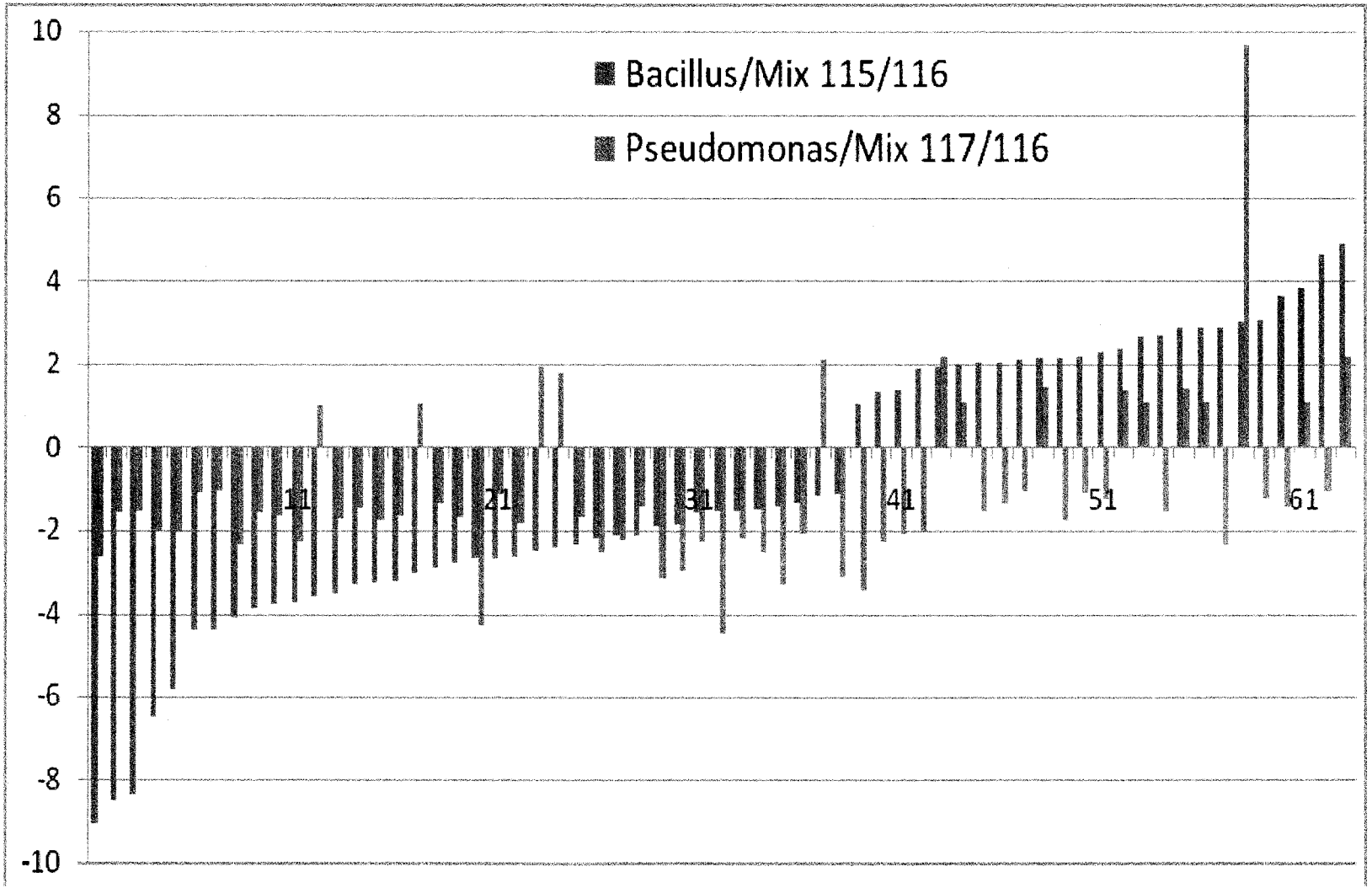

Figure 3: Distribution of all differentially-expressed protein quantification ratios with respect to mixed culture.

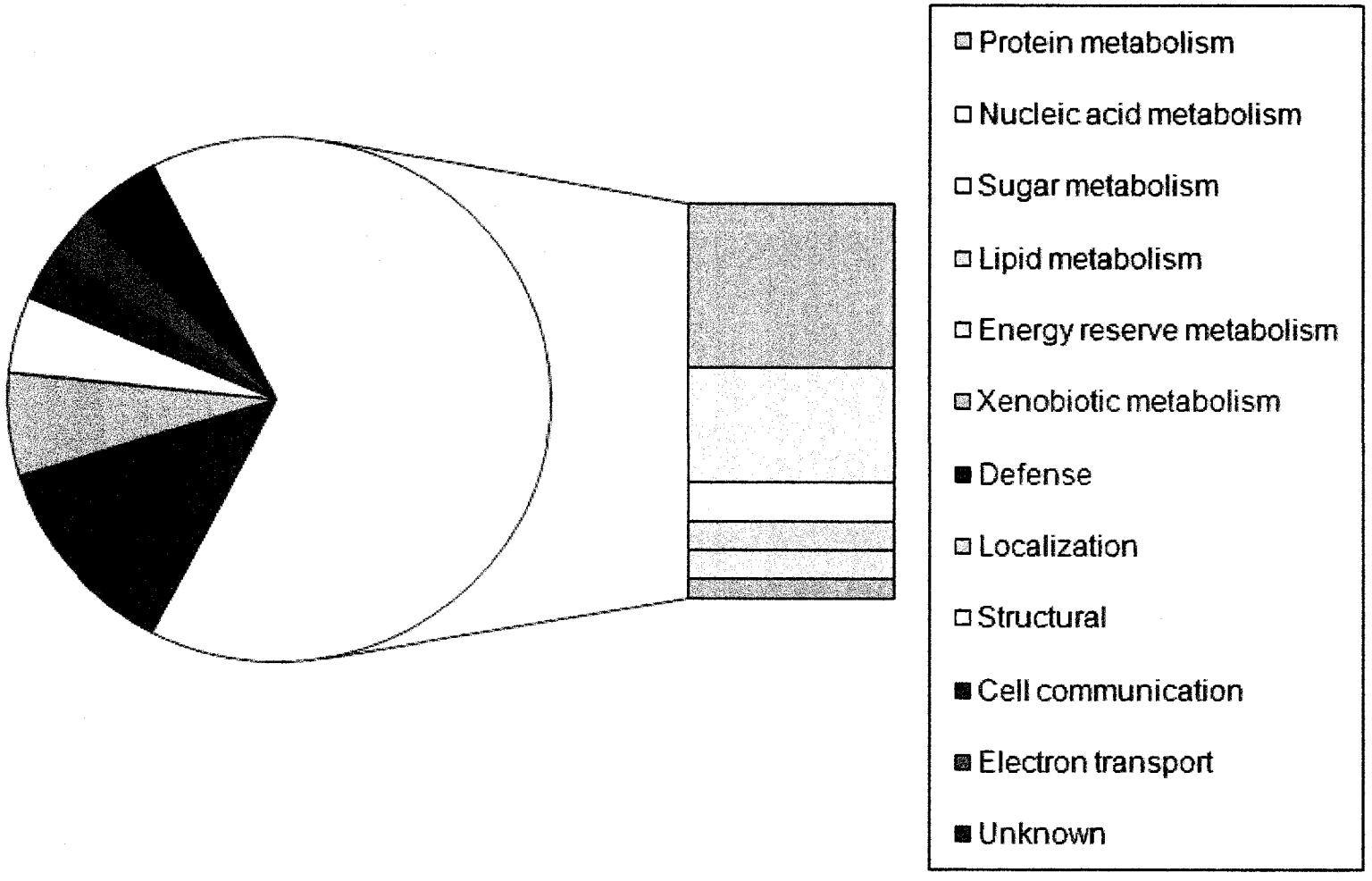

Figure 4: Functional classification of the proteins differentially expressed in the *B. subtilis* and *P. putida* study.

IV. Metaproteomic Analysis of a Bacterial Community Response to Cadmium Exposure

This study was published in the Journal of Proteome Research by Lacerda *et al.*[285].

IV.I.Introduction

Microbial communities impact our lives in many different ways, including our health, environment and industry. A better understanding of microbial ecology would lead to clearer descriptions of processes such as disease, biodegradation, corrosion, and global cycling. However, the complexity of these communities poses significant challenges to their study. A variety of tools have been used in the past to gain insights into microbial communities, including traditional enrichment methods, multispecies modeling, and lipid, DNA- and RNA-based approaches[286-291]. In particular, the development of lipid- and nucleic acid-based profiling techniques has enabled the elucidation of microbial community structures in soils and groundwater[289, 291, 292]. Unfortunately, knowledge of community structure does not necessarily lead to useful information on functions such as metabolic capacity, population dynamics, and physiological responses to variable environmental conditions. In addition to the general inability to extrapolate from phylogenetic data to function, the limitations of standard 16S rDNA / rRNA-based genomic analysis of a mixed culture include the relatively long times required for detectable population changes (several microbial doubling times) and the fact that a variety of organisms can provide the same function. Lately, microbial community genomics, based on DNA extraction from a particular environment, is being hailed as a path forward[293-297]. Although it is an exciting development that has already led to

the discovery of new genes, this approach does not provide direct information on function and is not suited for the study of system dynamics.

Proteomic analysis, in contrast, can reveal rapid physiological responses since proteins can be synthesized and folded within seconds[298]. Direct large-scale measurement of protein expression levels provides an insight into cellular and community activity – information unavailable from any other approach. Most of the work done in the proteomics field so far has focused on single species subjected to different conditions, leaving the potential of microbial community proteomics almost completely unexplored. In this approach, a mixed culture can be viewed as a metaorganism, in which population and meta-proteome shifts are forms of functional responses.

There are several challenges in microbial community proteomics, including representative protein extraction, separation of proteins in highly complex samples (dozens to thousands of bacterial proteomes), and the near complete absence of genomic sequences for the microorganisms in these environmental communities. While the latter leads to an impediment to protein identification, we note that approximately 650 prokaryotic genomes have been sequenced to date (http://www.ncbi.nlm.nih.gov/sutils/ genom_table.cgi) with new sequences completed at an increasing rate. It is thus reasonable to expect some protein sequence homology across species such that an identification could be assigned to a protein from the unsequenced organism.

Although there have been proteomic studies on pure cultures of sequenced organisms of environmental interest[299-301], reports on the proteomic analysis of unsequenced pure cultures are rare[300, 302]. Only six studies on proteomes of unsequenced mixed cultures have been published in the scientific literature. Schulze *et al.*[303] studied proteins isolated from

dissolved organic matter using MS and demonstrated the ability to determine a proteome fingerprint of soil. Wilmes and Bond[304-306] investigated a community of microorganisms from a laboratory-scale sequencing batch reactor optimized for enhanced biological phosphorus removal and enriched for polyphosphate-accumulating organisms. In their first study[306], they used a 2DE-MS approach to detect many proteins and identify three. In their subsequent studies, also 2DE-based, they performed comparisons of proteome profiles of activated sludges with sequentially increasing phosphorus removal performance[305] and profiles of phosphorus removal versus non-phosphorus removal[304]. Neither of the latter studies included information on protein identification. Kan *et al.*[307] studied Chesapeake Bay microbial communities using a similar workflow and identified eight proteins. Recently, a landmark study was published by Ram *et al.*[308] on a natural acid mine drainage microbial biofilm community. With a shotgun proteomics approach, they identified more than 2000 proteins through the use of a database created from the sequencing of a microbial community sampled from the same mine but at a different location and time. These studies either mapped proteomes of certain conditions or focused on single comparisons, rather than investigating a functional response.

Here, we describe the use of proteomics as a tool to obtain functional information about the dynamic response of a microbial community to cadmium stress. Specifically, we demonstrate the abilities to use 2DE gels from a microbial community to detect proteome shifts, to identify protein markers of significant environmental change, and to use mass spectrometry coupled with *de novo* sequencing to identify 158 proteins, 109 of which were unique. The microbial community was obtained from a continuous-flow; stirred-tank bioreactor fed a mixture of organic chemical pollutants. Cadmium was chosen as the

chemical stress because the effects and tolerance mechanisms to this common contaminant are relatively well-studied. This community had no previous exposure to cadmium, and thus the experiment served to assess the response of a naïve community to a chemical stress.

IV.II. Experimental Procedures

The source of the microbial community used in this study was a continuous-flow wastewater treatment bioreactor that was fed a mixture of twelve organic chemicals (acetone, 2-butanone, 2-hexanone, phenol, p-cresol, 2,4-dimethyl phenol, benzene, toluene, m-xylene, chlorobenzene, 1,4-dichlorobenzene, and 1,2,4-trichlorobenzene) at a dilution rate of 0.01 h^{-1}. This culture was maintained at pH 7 ± 0.2 and 20 °C. The continuous culture was originally inoculated from an uncontaminated soil sample from Fort Collins, CO. 16S rDNA analyses of this inoculum culture indicated that it consisted of approximately 50 bacterial strains[309].

The inoculum was added to two replicate 500-mL baffled Erlenmeyer flasks containing 200 mL of 10 g/L tryptic soy broth (BD Diagnostics, Franklin Lakes, NJ, USA) to achieve an initial OD 600 of 0.002 (approximately 0.02 mg/mL of dry cells). The culture flasks were then incubated at pH 7, 20 °C, and 200 rpm for approximately 20 h, when $CdCl_2 \cdot 5/2H_2O$ was added to half of the flasks to achieve a concentration of 0.09 mM (10 mg Cd/L). This concentration of cadmium was chosen to inhibit but not stop culture growth (based on OD 600 measurements). All flasks were incubated further, and both treated and control cultures were harvested 15 min, 1, 2 and 3 h after the time of cadmium addition.

Protein extraction was a multi-step process developed in this project that combined chemical and mechanical lysis steps to lyse both Gram-positive and Gram-negative cells.

Immediately after harvesting, cell cultures were centrifuged at 4 °C and 7500 x g for 10 min. The resulting cell pellets were washed in 30 mL of a pH 7 buffer composed of 3 mM KCl, 1.5 mM KH_2PO_4, 68 mM NaCl, and 14 mM NaH_2PO_4, and centrifuged again. Each cell pellet was resuspended in 2 mL of 10 mM Tris-HCl pH 8, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.5 mM Pefabloc SC (protease inhibitor, Roche Applied Science, Indianapolis, IN, USA), and 0.1% SDS. Cells were then sonicated on ice using a 550 Sonic Dismembrator probe (Fisher Scientific, Hampton, NH, USA) vibrating at 20 kHz for 5 minutes, in cycles of 1 second on and 2 seconds off. After sonication, the suspension was centrifuged at 4 °C and 7500 x g for 15 min and the supernatant isolated. The pellet was resuspended in 2 mL of a second lysis solution, containing 10 mM Tris-HCl pH 8, 1 mM EDTA disodium salt, 0.05 mM dithiothreitol, 0.5 mM Pefabloc SC, 10 % glycerol and 1 mg/mL lysozyme. This was sonicated and centrifuged as before. The two supernatants were combined, and the protein concentration of the final lysate was determined using the Bradford-based protein assay from Bio-Rad (Hercules, CA, USA). Protein samples were divided into aliquots of 200 µL and stored at -80 °C until 2DE analysis.

A volume of sample containing protein sufficient for one 2DE gel (1000 µg) was precipitated to further purify the sample before electrophoresis. Large sample loadings were used in order to increase the amount of protein per spot and facilitate MS analyses. Ten volumes of ethanol were added to one volume of sample and the final solution was incubated at -80°C for 20 min. After incubation, the precipitated sample was centrifuged at 10,000 x g for 3 min and the supernatant removed by pipette. Sample pellets were air-dried until no ethanol residue remained. After this step, samples were ready for rehydration and electrophoresis. The electrophoretic protocols were previously described in detail by

Pferdeort *et al.*[310]. Briefly, isoelectric focusing was carried out using 18 cm, pH 4-7 Immobiline IPG gels (GE Healthcare, Piscataway, NJ, USA) in a Multiphor II (GE Healthcare). SDS-PAGE was carried out in 12% acrylamide gels (18 x 16 x 0.1 cm) in a Protean II xi Multicell (Bio-Rad). After electrophoresis, gels were stained with the PlusOne Silver Staining Kit (GE Healthcare) before imaging. In the gels from which spots were excised, Sypro Ruby (Invitrogen, Carlsbad, CA, USA) and Simply Blue SafeStain (Invitrogen) were also used, following the manufacturers' protocols. Gel images were generated using a UVP Bioimaging System (UVP, Upland, CA, USA). A minimum of four replicate gels for each treatment and control were analyzed using Proteomweaver v. 3.0 analysis software (Definiens AG, Munich, Germany). Spots present in at least three replicates were considered true protein spots. Differential regulation of protein was determined by a change in spot volume of at least two fold.

After analyzing the gel patterns, differentially expressed proteins were chosen for identification and cut out of the gels using 1.5-mm spot pickers (The Gel Company, San Francisco, CA, USA). Digestion, mass spectrometry and database searching were performed according to the methods previously described by Choe *et al.*[311]. In brief, proteins in gel plugs were digested in an Investigator ProGest (Genomic Solutions, Ann Arbor, MI, USA). Digests were de-salted and spotted onto target plates with 5mg/ml alpha-cyano-4-hydroxycinnamic acid. MALDI-MS/MS was performed on a 4700 Proteomics Analyzer running v2.0 software (Applied Biosystems, Framingham, MA, USA). MS was performed in positive ion reflector mode over 850-4000 m/z mass range, with 1000 laser shots per spot and internal calibration. Up to five of the most intense peaks, excluding trypsin autolysis peaks, were selected from each MS spectrum for MS/MS. Tandem MS was performed in positive

ion mode with 5000 laser shots, 1kV collision energy, air at $1E^{-6}$ torr as the collision gas, and default calibration. GPS Explorer (v2.0, Applied Biosystems) was used as an interface between the raw data from the mass spectrometer and a local copy of the Mascot search engine (v1.9, Matrix Science, London, UK). A combined MS and MS/MS search was performed against a local copy of NCBInr (downloaded October 25, 2004). Searches were restricted to the Eubacteria taxonomy with 50 ppm MS and 0.3 Da MS/MS mass tolerances, trypsin specificity allowing for one missed cleavage, and the following three variable modifications: methionine oxidation, cysteine modifications by iodoacetamide and acrylamide. All MS/MS peak lists belonging to the same parent mass were analyzed as one set in order to give the most accurate protein matches.

In addition, *de novo* sequencing was conducted on all datasets using Peaks Studio Version 3.1 (Bioinformatics Solutions, Waterloo, ON, Canada). *De novo* searches were performed for every protein, using their respective MS/MS data in the same set. The parameters used to generate peptide sequences were similar to the ones used in Mascot in order to keep the same level of confidence. Specifically, these parameters were tryptic digestion with one missed cleavage allowed, 0.1 Da MS and MS/MS mass tolerances, carbamidomethylation of cysteine and oxidation of methionine, and allowance for a maximum of three modifications per peptide. *De novo* peptide sequences obtained in this manner were used for a hybrid *de novo* sequence-guided MS/MS search with the Peaks protein ID tool, a process similar to an MS-BLAST search. These searches were conducted against the NCBInr database, with taxonomies restricted to Bacteria, Archaea, "others", and "unrelated", with a monoisotopic mass search type. The "others" and "unrelated" taxons were used in order to cover important datasets such as proteins found in recent metagenomics projects.

A second search was performed for all datasets in order to estimate the rate of false-positive identification. This search used the same *de novo* sequencing parameters, but the searches were conducted against the entire NCBInr database in order to find peptides that would match more significantly to proteins coming from eukaryotic organisms. If a pair of peptides matched a eukaryotic protein with a higher significance, then it was categorized as a potential false positive. The number of potential false positive peptides was divided by the number of total peptides identified to obtain a rate of false positives.

IV.III. Results and Discussion

IV.III.I. Culture Growth

OD measurements (data not shown) showed that cadmium-shocked cultures had slower growth rates than the controls but that the OD still increased. At the time of cadmium addition, the OD 600 was around 3.8. After 3 h of cadmium shock, the OD 600 for the control cultures was around 5.2, while that of the cadmium-treated cultures was 4.0.

IV.III.II. Protein Expression Visualization

Images of 2DE gels from Cd-treated cultures are shown in Figure 5. More than 500 well-resolved spots were detected in each gel. The protein expression analysis was performed in a temporal manner, so that each cadmium treatment was normalized against the control at the same time point. The protein profile in the culture changed significantly within the first 15 min, and continued changes were noted over the entire 3-h post-perturbation period. The early response to cadmium addition was the up-regulation of a large set of proteins – nearly 15% of the total number of proteins detected (Table 2). A substantial number of proteins were present at lower levels in cadmium-exposed cultures, but, overall, more proteins were

up-regulated in response to this environmental change. The observation of up- and down-regulation of proteins due to cadmium exposure shows that cadmium can have both stimulatory and inhibitory effects, as has been noted previously[312, 313]. More than 100 protein expression changes relative to control cultures were observed at each sampling time, consistent with the magnitude of changes (up and down) reported in proteomic studies on cadmium exposure of *Saccharomyces cerevisiae*[314] and *Schizosaccharomyces pombe*[315], and with a transcriptomic investigation of the response of an ectomycorrhizal fungus[316].

An important observation is that the protein levels of at least 100 proteins had changed (by at least two fold) within 15 min of exposure to cadmium. A smaller number of proteins with altered expression levels relative to the control were noted after the other exposure times. Comparison of the proteomes after 15 min and 3 h of Cd exposure revealed major differences, indicating that the cadmium shock led to rapid physiological responses as well as longer term changes. These observations are in contrast to the trend reported for *S. cerevisiae*, for which exposure for 15 min yielded a similar pattern of protein expression as noted for a 60-min exposure[314].

When the temporal expression patterns were tracked for each of the differentially expressed proteins, several trends were evident. However, the three most frequent patterns, accounting for approximately 90% of the differentially expressed proteins, were: (a) differential expression (increase or decrease) after 15 min exposure, and restoration to control levels after 2 h; (b) differential expression at all sampling points; and (c) control levels of expression until around 2 h of exposure, then differential expression (Table 3). The first behavior was also reported in a proteomic study of cadmium shock in *S. pombe*[315].

IV.III.III. Protein Identification and Functional Classification

From the four pairs of gels (Cd-exposed and control, at each time point), approximately 200 unique protein spots were excised, digested, and analyzed by MS/MS. From these, 189 sets of MS/MS data were obtained. A strategy based on sequence homology was explored initially in which repeated identification of proteins from different bacteria within the list of best matches was taken as initial evidence for the identification of the sample protein. A more thorough approach that has been successful for unsequenced species was to use MS/MS data to generate *de novo* peptide sequences followed by BLAST searches of the NCBInr database[302, 317]. A critical tool in this effort was the recently developed commercial software Peaks[318], which accomplished both *de novo* sequencing and MS-BLAST searches. The Mascot-based homology search yielded the identification of 50 proteins, which were then used as pseudo-positive controls for the *de novo* sequencing approach.

De novo sequences were obtained for all 50 of the datasets for which protein identifications had been obtained with Mascot. These sequences were then used in an MS-BLAST search using the parameters described in the Methods section and the same identifications previously produced by Mascot were obtained again in all cases. In some instances, the highest scoring match was not the identical database entry (e.g., different gi), but the two matches were the same protein, with the same cellular function, but in different organisms. The resulting scores were different, even though the parameters used were the same. This happened due to the completely different searching and scoring algorithms and was thus expected. Given that Peaks could confidently identify all of the proteins that were identified by Mascot, the remaining sets of MS/MS data were then analyzed with Peaks. MS-BLAST identifications with confidence levels of at least 90% were obtained for a total of 158

proteins, 109 of which were unique. All protein identifications were made on the basis of two or more peptides. These are listed in Appendix II, along with the assignment to a biological process (obtained from Gene Ontologies (http://www.godatabase.org/cgi-bin/amigo/go.cgi)), trends observed during the whole shock (resistance, tolerance or adaptation), and up- or down-regulation. Appendix II also lists the organism from which the database entry was obtained. It is not the case that our culture contained these organisms; instead, the sequences of the proteins expressed by our culture are highly homologous to those presented. Nearly all proteins identified in this study were bacterial. Although the searches included Archaea and important related databases obtained through metagenomics projects, no significant hits were obtained from these, with the exception of three synthetic constructs (proteins 15, 39 and 90 in Appendix II).

Nineteen proteins were found in two or more 2DE gel spots. In some cases, these proteins were in neighboring spots with similar molecular weight and slightly different isoelectric point values. In pure culture studies, such patterns are attributed to protein isoforms or post-translational or other modifications. However, in other cases (e.g., proteins 25 and 53 in Appendix II), the same protein identification (in different database entries) was obtained from gel spots with significantly different molecular weight and isoelectric point. Since metaproteomics studies inherently include the possibility that different organisms will produce variants of a protein with the same function, proteins from spatially distinct gel spots were classified as unique. Nineteen such proteins were identified here. This classification is a hypothesis that could be investigated further (e.g., by acquiring more complete protein coverage). Proteins in 31 spots for which MS/MS data had been obtained could not be identified through the *de novo* approach due to difficulties in finding an accurate peptide

sequence.

Appendix II also presents the molecular weight and isolelectric point of each protein, gi and accession numbers, protein scores, and mass errors. The peptides with the highest score contributing to the identification are also listed along with the corresponding mass/charge ratio, percent coverage, and potential to be a false positive identification. A false positive rate, calculated based on the total number of peptides identified, was 5.7%. This value is comparable to those found in other studies[308, 319] and can be considered good because of the very large decoy database.

The functional categories and the distribution of the 109 unique differentially expressed proteins are shown in Figure 6 and related to the primary temporal protein expression patterns in Appendix II. It is interesting and important to note that not all proteins in a functional category displayed the same pattern of response to cadmium exposure. For example, defense proteins were found to be differentially expressed for the short term only, at the longest time only, and at both short and long times. Similarly, while some metabolic proteins were found to be differentially expressed only over the first 15 minutes, others were differentially expressed over the entire three-hour monitoring period. The short-term response was also observed in previous cadmium stress studies done on *S. pombe* by Bae *et al.*[315] (proteomics) and on microbial communities by Lorenz *et al.*[293] (soil enzyme activities). Another interesting observation concerns ribosomal proteins that were only differentially regulated during short-term exposure and then restored their normal levels of expression, also observed by Bae *et al.*[315] in the *S. pombe* study.

IV.III.IV. Cadmium Shock Interpretation

Two of the known mechanisms for cadmium detoxification used by bacteria are ATPase

(ABC- or P-type) and chemiosmotic pumps. The latter includes several types of mechanisms: the major facilitator superfamily, the cation diffusion facilitator, and the resistance nodulation division, among others[320, 321]. Among the differentially expressed proteins identified in this study, we observed several of the proteins related to these pumps, including P-type and ABC ATPases (e.g. proteins 22, 25, 37, 59 and 104 in Appendix II). Nies[320] observed that most prokaryotic genomes only encode one mechanism of heavy metal resistance, and that it is rare to find an organism with all of the efflux pumps. The detection here of proteins from several efflux pumps is consistent with the concept of the microbial community as a metaorganism.

While there are no similar reports of the effect of cadmium exposure on mixed cultures, it is of interest to compare the results from this study with those obtained from the limited number of pure culture experiments in the literature. Many proteins identified here were also found previously in other cadmium shock studies[313-316], including dehydrogenases[313, 314, 316] (protein 100), ribosomal proteins[314-316] (proteins 62 and 90), ATPases[314-316] (proteins 22, 25, 37, 59 and 104), oxidoreductases[314, 315] (proteins 47 and 65), catalase[314, 315] (protein 26), superoxide dismutase[314, 315] (protein 64), inorganic pyrophosphatase[314, 316] (protein 43), and transcriptional regulators[314, 316] (protein 97).

IV.III.V. Temporal Expression Patterns

Differentially expressed proteins found in this study were classified according to one of the three behaviors presented in Table 3. Those behaviors may be correlated to the different mechanisms that the members of the culture use to survive the cadmium shock. The "short-term only" type of response suggests a mechanism of resistance to the cadmium in the environment. Proteins presenting that behavior include those involved in nucleic acid and

protein synthesis and xenobiotic metabolism. The "long-term only" response indicates a phase of adaptation and that group contains mostly membrane proteins involved in transport. The last behavior, an immediate response that is sustained, may indicate tolerance of the metal during the shock. Representatives of all protein functionalities were found in this group. Some proteins found here are of special interest in the response to cadmium shock and are discussed in the following paragraphs.

ATPases and other ion transport proteins. ATP synthase (ATPase) is a complex membrane protein involved in ATP synthesis and hydrolysis. It is composed of several subunits functioning as a motor[322] that pumps ions in and out of the cells. Three of those subunits were identified here (proteins 22, 25, 37, 59 and 104) and, interestingly, were found in eight different spots throughout the gels. As noted earlier, this distribution could be attributed to minor sequence differences that might be present in ATPases coming from different species in the community, to post-translational modifications, or to breakdown or truncation products. An important point is the combination of finding ATPases in many spots and their different regulation from all other membrane proteins. While the ATPases were found to follow the pattern of a short-term response, all other membrane proteins involved in secretion, according the Gene Ontologies functional classification, had a longer-term response. As described by Nies[320], ATPases should occur more frequently than other transport proteins since they are the only ones responsible for the detoxification of metal-thiolate complexes, which appear to be the most common form of metals in the intracellular space[323].

Another important group of proteins found here were secretion system proteins (e.g. proteins 14, 53 and 103). These were found to be either up-regulated during the whole post-

shock period or towards the end of the experiment. This behavior may be related to ATPase activity. ATPases can pump ions into the periplasm but not outside of the cell[320]. According to Mitra *et al.*[324], during the early stages of the cadmium shock, 75% of the bioavailable cadmium is in the periplasm, suggesting the need for secretion systems for detoxification. When the ATPase levels returned to pre-shock levels, then up-regulation of the secretion systems was noted.

Macromolecular synthesis and repair proteins. Cadmium is known to enhance the genotoxicity of DNA-damaging agents[325]. A suggested mechanism for this phenomenon is cadmium binding to methyl group acceptor sites in DNA methylases (methyltranferases), inhibiting their activity and causing DNA damage. Consistent with this, an initial increase followed by decrease in the level of DNA methylase (protein 89) was observed. Several DNA repair proteins (proteins 20 and 76) were also rapidly up-regulated. This observation can be associated with the fact that cadmium is known to induce DNA single-strand breakage[325]. Finally, we observed an immediate up-regulation of two ribosomal proteins (proteins 62 and 90). A similar response was noted by Bae and Chen[315] for *S. pombe*; these researchers hypothesized that this response could be attributed to the increased demand for protein synthesis at early stages of the shock. A later reduction of ribosomal protein synthesis is thought to permit cells to save energy and resources for other mechanisms involved in the response to cadmium stress.

Metabolic proteins. Proteins from this group were equally represented in all three trends: resistance, sustained tolerance, and adaptation. It is interesting to point out that proteins involved in sugar metabolism only followed the resistance and tolerance patterns, showing their rapid response to the shock. Cadmium is known to inhibit cell respiration[326], primarily

via mechanisms prior to the entry of electrons into the electron transport system[327]. We observed the down-regulation of several glycolytic enzymes, e.g., glyceraldehyde-3-phosphate dehydrogenase (protein 33) and enolase (protein 1). This could be due to the fact that cadmium can bind cysteine residues[328], leading to a shift in protein molecular weight and making the original spot observed in the control have reduced intensity.

Metallothioneins. Metallothioneins were not detected in this study, although they are a known mechanism for metal resistance via complexation[321]. This lack of detection might be due to their small molecular weight (typically 6 kDa) or it may be the case that metallothioneins were not expressed in this culture; cadmium complexation appears to have a higher energetic cost than cadmium efflux[323]. An interesting observation is that most bacterial metallothioneins are observed in cyanobacteria, and very few metallothioneins have been described among heteretrophic bacteria.

IV.IV. Conclusions

Proteomic analysis revealed significant shifts in the microbial community physiology within 15 min of cadmium exposure, a rapid change not detectable using the phylogenetic profiling tools common to molecular microbial ecology. Furthermore, the proteome of the cells exposed to cadmium for longer times was significantly different from that of the cells exposed for 15 min, suggesting that the community's short-, medium-, and long-term responses to this stress were different. These results demonstrate that 2DE can be used to separate and resolve hundreds of proteins from a microbial community of more than 50 species, and that it is possible to identify proteins from a mixture of bacteria with unsequenced genomes. The protein identified here, and their temporal expression patterns,

not only provided confirmation of the toxicity of cadmium to the organisms in the community but also yielded insights into mechanisms of tolerance and resistance. Evidence for altered metabolism was also obtained. Clearly, the application of proteomics tools can provide important insights into the physiology of microbial communities, thus establishing proteomics as a viable tool in microbial ecology.

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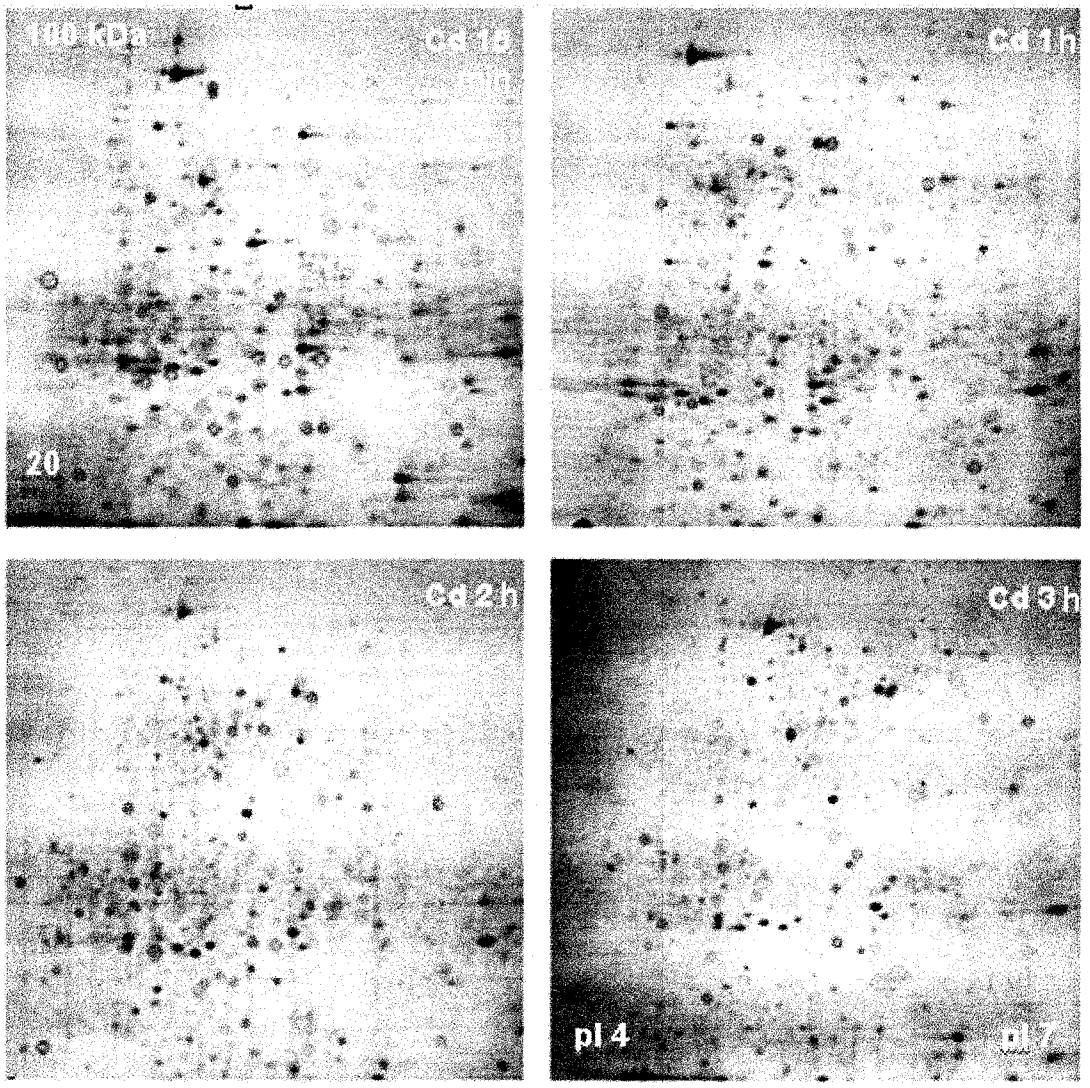


Figure 5: Silver-stained 2DE gel images from the microbial community following the addition of 10 mg/L cadmium. Pink circles represent up-regulated proteins, while blue circles represent down-regulated proteins relative to the controls.

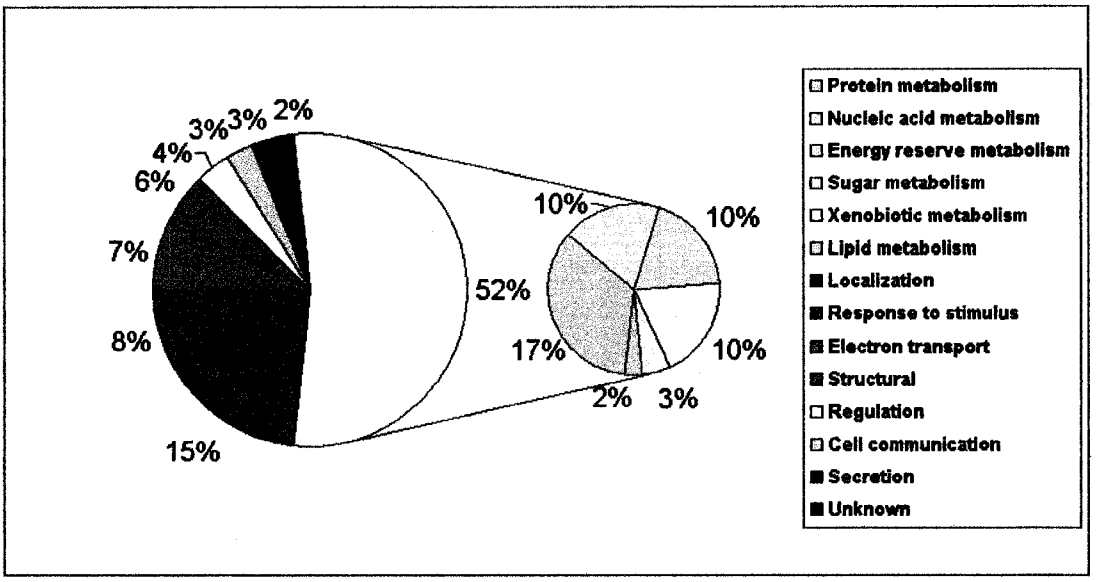
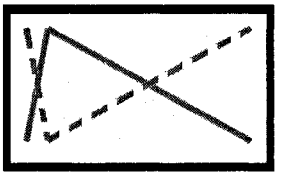
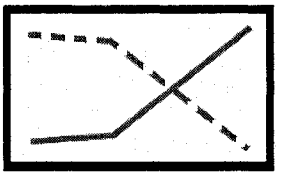
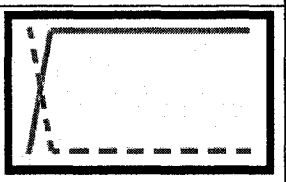


Figure 6: Gene Ontology functional classification of all 109 differentially expressed proteins identified in the cadmium shock experiment.

Table 2: Summary of protein changes in cadmium-exposed vs. control cultures (at least two-fold change from control).

Time point	Down-regulated proteins	Up-regulated proteins	Total proteins detected
15 min	4% (19)	14% (72)	512
1 h	5% (25)	10% (52)	524
2 h	2% (13)	10% (55)	568
3 h	3% (15)	8% (41)	530

Table 3: Summary of the three major protein expression patterns observed following exposure of the microbial community to cadmium. The temporal response patterns represent up- or down-regulation of each cadmium treatment normalized against the control at the same time point.

Temporal response pattern		**Proteins in trend**	**Protein functions**
Short-term only RESISTANCE		30%	• Metabolism • Electron Transport • Cell communication • Structural
Long-term only ADAPTATION		11%	• Secretion • Localization • Metabolism
Short- and long-term TOLERANCE		50%	• All classes

V. QUANTITATIVE PROTEOMIC ANALYSIS OF A SOIL BACTERIUM UNDER DIFFERENT LEVELS OF CADMIUM STRESS

V.I. Introduction

Quantitative proteomics, as described in the previous chapter, can be accomplished by *in vitro* or *in vivo*[329] labeling techniques, at the peptide- or protein-level. Due to the relative ease of growth of bacterial cultures, an important quantification alternative is metabolic labeling, or *in vivo* incorporation of stable isotopes into proteins. In this technique, proteins are labeled as they are synthesized, due to the presence of stable isotopes in the growth medium, i.e., essential components such as amino acids, carbon or nitrogen are supplied as heavy isotopes, leading to synthesis of proteins which only have heavy isotopes in their compositions. Heavy isotopes commonly used for metabolic labeling are ^{15}N (as ammonium sulfate in the medium) and ^{13}C (as $^{13}C_6$ glucose, arginine or lysine). The specific case where heavy amino acids are incorporated directly into the medium is known as SILAC (stable isotope labeling with amino acids in culture)[330, 331]. Small carbon- or nitrogen-containing nutrients (e.g., glucose or ammonium sulfate) are the most common options supplied in defined minimal media.

Proteomic experimental design for metabolic labeling allows for multiplexing of usually two or three samples (e.g., one unlabeled, one labeled with ^{13}C, and one with ^{15}N). Each treatment is cultivated with a different isotope and then combined during cell harvesting or

protein analysis. Sample combination during cell harvesting gives the advantage of reducing experimental error, or at least making it identical for all treatments, for all downstream proteomics steps. However, combination of samples at the protein level allows for generation of single-treatment protein samples that can be used in other complementary analyses. During LC/MS analysis, light and heavy peptides elute at the same retention time, making it easy to compare their mass spectra. The peaks representing the heavy isotopes should be localized in a heavier m/z region in the spectra, based on the number of heavy isotopes found in the peptide being analyzed. This method of targeting heavy isotopes allows for accurate protein quantification at the peptide level and increases the confidence of a protein identification by adding a stoichiometric constraint to the identification. Quantification software packages to use with metabolic labeling are, so far, limited. Here, we propose a manual analysis method, as well as using Mascot, which allows for detection of light and heavy isotopes in the search results.

In this study, we isolated an organism with high cadmium resistance and sought to answer the following research questions. How does cadmium concentration affect proteomes and cadmium resistance mechanisms? Can we successfully quantify the cadmium response of the isolate using metabolic labeling and a combination of proteomic approaches? How does the response of the isolate compare to that of the mixed culture? The central idea is to use proteomics to detect differences between cadmium resistance mechanisms. In our previous metaproteomics study (Chapter 3), we evaluated the short-term effect of cadmium on a community of different types of bacteria. Half of the proteome changes in that study were metabolic proteins, and 15% were transport-related proteins. In this case, we measure the effect of different doses of cadmium on an organism capable of surviving in a wide range of

cadmium concentrations. The isolate is adapted to growing in the presence of cadmium, which leads to possible new tolerance mechanisms other than transport-related. This bacterium was identified by 16S rDNA sequencing as a type of *Burkholderia cepacia.* We named it strain Cd44 in reference to its isolation procedure, further discussed in the experimental section.

V.II. Experimental Procedures

V.II.I. Growth medium

We developed a growth medium optimized to avoid cadmium precipitation, adapted from previous media studies available in the literature[332]. The final medium composition was 18 g/L glucose ($C_6H_{12}O_6$), 4 g/L HEPES buffer ($C_8H_{18}N_2O_4S$), 4 g/L ammonium sulfate ($(NH_4)_2SO_4$ as either ^{14}N or ^{15}N), 2 g/L sodium pyrophosphate ($Na_4P_2O_7$), 0.5 g/L magnesium sulfate heptahydrate ($MgSO_4*7H_2O$), 5 mg/L ferrous sulfate heptahydrate ($FeSO_4*7H_2O$), at pH 6. Glucose and ammonium sulfate were autoclaved separately.

V.II.II. Microorganism

The pure species used in this study was isolated from a mixed culture maintained in a continuous reactor. Cadmium as $Cl_2Cd*2.5H_2O$ was added to media plates in a range of concentrations: 0, 0.02, 0.04, 0.22, 0.44, 0.66 mM. Inocula from the continuous reactor mixed culture were cultivated on these plates, and very few colonies grew in the presence of higher concentrations of cadmium. Colonies from the 0.44 and 0.66 mM, Cd plates were transferred to new plates containing 10 g/L tryptic soy broth, without cadmium. New

colonies from the rich-medium plates were transferred to defined medium with cadmium. The objective of this series of sequential plating was to ensure that the isolated species had the necessary machinery to survive in high cadmium concentrations even after cultivation in a nutrient-rich environment. The isolates were considered to be pure species after eight sequential transfers. No isolates survived at 0.66 mM Cd after all the transfers, and further characterization of the 0.44 mMCd isolates proceeded. A PCR-cloning approach of the 16S rDNA molecule of the isolates was used prior to DNA sequencing. DNA was extracted using UltraClean Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) and 16S gene was amplified using primers 8F and 1492R. Cloning was performed using the TOPO TA Cloning Kit (Invitrogen) and cloned inserts were amplified using M13 PCR. The PCR products were verified on an agarose gel, and had their concentrations determined prior to mixing with T7 sequencing primer. Sequencing was performed at the Macromolecular Resources Facility at CSU. Based on 16S rDNA sequences, we identified the isolate as a strain of *Burkholderia cepacia* on NCBI with a 99.9% confidence. Due to its ability to grow on 0.44 mM Cd^{2+}, this organism was given the strain name of Cd44 and was used in the remainder of this study. Another interesting feature to note is that colonies of this bacterium grown on high cadmium concentrations presented a very distinct dark blue phenotype, shown in Figure 7. This color was clearly visible when the colony reached a large size (2-3 mm diameter), and was only observed on the cadmium-containing plates. *B. cepacia* is a Gram-negative bacterium related to the *Pseudomonas* genus[333]. The *cepacia* designation describes a complex of a number of species, almost always related with lung diseases.

V.II.III. Growth conditions and metabolic labeling

For the metabolic labeling study, duplicate batch cultures of this bacterium were used. In order to compare the effect of different cadmium doses, we used control cultures (without cadmium), low cadmium concentration cultures (0.04 mM), and high cadmium concentration cultures (0.44 mM), all cultivated in the minimal medium described above. Two pair-wise comparisons were performed, with the low cadmium cultivations conducted with $(^{15}NH_4)_2SO_4$. In other words, the low cadmium treatment (^{15}N isotope incorporated) was compared with the high cadmium treatment and with the control, both grown in ^{14}N medium. All cultures originated from a single colony on rich medium agar, and then transferred to 1 mL of defined minimal medium without cadmium in a test tube. Tube cultures were grown for approximately 3 days, and then were transferred to 500 mL shake flasks containing 200 mL of the appropriate medium. Flask cultures were grown approximately 60 h until mid-exponential phase or OD_{600} of 0.5, at 25 °C and 200 rpm.

V.II.IV. Protein extraction

Cell harvesting and protein extraction followed the protocol described by Pferdeort *et al.*[334]. Briefly, cell cultures were centrifuged at 4 °C and 7500 x g for 10 min. The resulting cell pellets were washed in a pH 7 buffer and centrifuged again. Each cell pellet was resuspended in 2 mL of 10 mM Tris-HCl pH 8, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.5 mM Pefabloc SC, and 0.1% SDS. Cells were then sonicated on ice using a 550 Sonic Dismembrator probe vibrating at 20 kHz for 5 min, in cycles of 1 s on and 2 s off. After sonication, the suspension was centrifuged at 4 °C and 7500 x g for 15 min and the supernatant isolated. This procedure was previously described in the first protein extraction

step in Chapter 3. The protein concentration of the final lysates was determined using the bicinchonic acid (BCA) assay. Protein samples were divided into aliquots of 100 μg and stored at -80 °C. ^{14}N and ^{15}N samples were combined in equal amounts prior to shotgun analysis.

V.II.V. Shotgun proteomic workflow

Shotgun proteomics was the method of choice for the analysis of these samples. Complementary approaches were used (1DE and 2DE, followed by LC-MS/MS), but their low throughput and large sample requirements hindered the efficiency of the analysis. The tryptic digestion protocol used for the shotgun work is as follows. A total of 200 μg of sample (100 μg each treatment) were precipitated in three volumes of cold acetone and incubated at -20 °C for at least 1 h. The sample was then resuspended in 70 μL of bicine buffer (100 mM bicine, pH 8.5) and 1 μL of 10% SDS solution. After complete resuspension, the sample was reduced with 5 μL of TCEP solution (200 mM TCEP in bicine buffer) for 10 min at 70 °C. The sample was then alkylated with 4 μL of IAA solution (1 M IAA in bicine buffer) for 1 h at room temperature. An additional 20 μL of TCEP solution was added (to reduce any excess IAA) and incubated for another hour at room temperature. A 400 μL aliquot of bicine buffer was added to the reduced and alkylated sample, making it ready for tryptic digestion. Four microliters of 1 mg/mL trypsin (in 1 mM HCl) was added to the 200 μg sample (in a total of 500 μL solution), and the mixture was incubated for 16 h at 40 °C. After digestion, the sample was vaccum dried to completion and resuspended in SCX buffer A. The chromatographic, mass spectrometric and database searching procedures were the same described in Chapter 4.

Data analysis procedures followed the sequence of steps described here. From the Mascot results, all peptides identified as significant were chosen for ^{15}N constraint identification. All peptide information was gathered to construct the data analysis table: peptide sequence, respective reverse-phase fraction number, m/z and delta mass, expected value, number of missed cleavages. Using the composition calculator (from the proteomics toolkit available through http://db.systemsbiology.net) and the peptide sequences, the number of nitrogen atoms present in each peptide was determined. The expected position of the ^{15}N isotope was calculated by dividing the number of nitrogen atoms by the charge of the peptide, then adding this to the m/z of the ^{14}N peptide. At this exact position there should be a peak representing the ^{15}N labeled peptide with the same sequence as the original peptide. In a constitutively expressed housekeeping protein, with correct labeling, both the ^{14}N and ^{15}N peaks should have the same height. If the ^{14}N and ^{15}N peaks have different heights, this peptide is part of a differentially expressed protein, and thus of interest in this study. If the ^{15}N peak was completely absent, it might have been below the limit of detection of the instrument, the ^{14}N peptide is a false positive, or the protein was completely down-regulated in the low cadmium case. The latter was considered only if all ^{15}N peptides in an identified protein were absent or low. All peptides belonging to proteins of interest were used for *de novo* sequencing of their respective MS/MS info followed by MS-BLAST searching. All *de novo* sequences were compared with the ones given by Mascot and then combined with the results across fractions.

V.III. Results and Discussion

V.III.I. Protein Identification and Quantification

Using a shotgun approach, we identified 72 proteins with expression modulated (expression two times larger) by the presence of cadmium in the culture medium. These proteins and all data pertinent to their identification procedures are presented in Appendix III. Among the proteins whose expression changed to a greater extent in the low cadmium concentration (0.04 mM) relative to the control, 8 were down-regulated and 16 up-regulated. Interestingly, all of these proteins had unchanged expression levels for the high cadmium concentration (relative to low cadmium), or were undetected. For the high cadmium concentration (0.44 mM), 18 proteins were down-regulated and 30 up-regulated relative to the low cadmium case. Among these proteins, 28 also had an expression change in the lower cadmium concentration, but this change was not as large. These proteins are identified in the complete data table. The false positive identification rate was calculated using a decoy database search and reached a total of 3.19%, corresponding to the identification of approximately two proteins for our complete dataset.

Identification of proteins from these samples was especially complicated. The proteins extracted from the cells cultivated in the presence of cadmium were unusually resistant to protein digestion. This was observed by the real-time monitoring of digestion protocols with an assay that measures decreasing protein fluorescence as it is digested (LavaDigest, Fluorotechnics, Sydney, Australia). Several protocol optimization steps were necessary to perform this experiment, and the optimal digest was obtained using trypsin as the protease, but tris-2-carboxyethyl phosphine (TCEP), instead of dithiothreitol (DTT), for the reducing

agent. A possible explanation for this is based on the fact that DTT uses its thiol groups to perform sample reduction, and thus that reduction could potentially be affected by cadmium since interaction of cadmium with polythiols is particularly strong and of biological importance[335], and DTT binds to cadmium as well as with peptides containing sulfhydryl groups[336]. However, the possibility that cadmium salts added to culture medium might be carried over to the cell lysate and there bind nonspecifically to proteins is small[337]. From the observations made in this experiment, it seems more likely that cadmium must be bound to disulfide-containing proteins as they are synthesized. This can also affect protein identification searches. We created variable posttranslational modifications, containing the molecular weight of one or two cadmium ions added to amino acids, to perform database searching. These searches did not identify any protein significantly, although it is possible that the scores were highly penalized by such complex variable modifications.

The quantification procedure used here is functional, but the manual analysis has a very low throughput and is subject to errors, especially regarding quantification of the correct isotopes in the peptide isotopic distribution. In addition, quantification of complete down-regulation of a labeled protein is not accurate since we count on the ^{15}N constraint to consider an identification as true. As a side note, the most current version of Mascot performs ^{15}N metabolic labeling quantification, by searching for the labeled peptides in addition to unlabeled ones, for use as an identification constraint. However, it does not provide the actual peptide amounts or ratios of the peptides found.

V.III.II. Functional Classification and Biological Interpretation

All proteins found in this study were classified according to their functionality, and plotted on graphs according to differential expression based on cadmium concentration (Figure 8). It is interesting to note that for efficient cell growth in a very low cadmium concentration, proteins involved in protein metabolism are the most often induced/repressed. Proteins involved in cellular defense and energy reserve metabolism also play an important role. At high cadmium concentrations, however, we notice that the shares of all protein classes are approximately the same, with the exception of a large amount of proteins with unknown function. It seems that adaptation to high cadmium concentrations require a number of proteins that, as of yet, have not been recognized to have that specific function. Cadmium cellular damage during adaptation is further described below according to protein classes. All the comparisons used here for the biological interpretation are made with adaptation of microorganisms to cadmium-rich environments, and contain both similarities and differences. A previous cadmium adaptation study showed that almost half of the extracellular cadmium is bioaccumulated[338], and cells have to make adjustments to deal with this new condition.

In this study we found the down regulation of ATPase and of a cell wall hydrolase, as well as up-regulation of outer membrane proteins, efflux pumps and ABC transporters. Previous cadmium adaptation studies observed that cadmium adaptation involves a cellular return to its control state by decreasing cadmium-specific transport and compartmentalization of intracellular cadmium[339]. Other modifications towards adaptation involve optimization of ABC transporters[340], and major modifications in membrane morphology and permeability[341].

Changes in cell morphology may be related to the large amount of cadmium localized in the cell envelope, polyphosphate granules (due to precipitation of cadmium-phospahte complexes[340]) and high molecular weight proteins. Cadmium-adapted may release whole membrane fragments, leaving lesions or holes that could account for the increased permeability, ultimately inducing the synthesis of new membrane[339].

We also observed the up-regulation of redoxin proteins and general down-regulation of other electron transporters. This can be explained by a unique intermolecular redox-active disulfide center utilized by redoxins for their protective activity. The richness in disulfide bonds in this catalytic center also makes it a very attractive site for cadmium binding. This might lead to constant induction in synthesis of these proteins to compensate for loss of activity due to cadmium binding. Other explanations for the up-regulation of redoxins are related to the more commonly understood need for redoxins during oxidative stress. Also, higher intracellular content of cadmium in cells probably mediate an increase in H_2O_2-generating compounds[340]. Usually, cross-resistance to oxidative stress in cadmium-challenged cells can be counted for by a parallel increase in glutathione content, which can act both as an antioxidant and as a metal-chelating agent[342]. Glutathione induction was not detected in this study.

Regarding DNA damage by cadmium, we were able to detect the down-regulation of an endoribonuclease and a helicase, as well as up-regulation of DNA polymerase III and a ribosyltransferase, among others. Through the generation of reactive oxygen species, cadmium binds to DNA, causing single-strand DNA damage and disrupts the synthesis of nucleic acids[343]. Cadmium-exposed cells have previously shown to induce DNA repair and

recombination systems and repress genes encoding DNA replication and translation systems[344]. Induced synthesis of proteins appears to occur specifically to protect the single-stranded DNA from further nuclease activity[345].

The proteins involved in protein metabolism found in this study were almost all up-regulated. These included a histone-like bacterial DNA-binding protein, peptide synthetase, ribosomal proteins, proteases, chaperones, elongation factors, and others. Cadmium is known to induce protein synthesis and repair[343]. Cadmium strongly binds to amino acids and peptides and both C- and N-termini, and has larger affinity for those than nucleic acids[336]. The use of chaperones mostly relates to their ability of preventing protein denaturation, especially due to cadmium binding.

Proteins involved in energy reserve were also identified in this study, and we observed down-regulation of glycolysis proteins, and up-regulation of a phasin, among others. Phasin is a storage protein generally induced in adverse environments, with the purpose of conserving energy[346]. This protein was greatly up-regulated in this study, possibly to save energy and prioritize cadmium detoxification. Cadmium is known to repress energy production pathways[336, 340]. A number of other proteins were identified as differentially expressed in this study. However, there is not enough information to put together the last pieces of the puzzle. The current literature does not have any large-scale proteomic study of cadmium adaptation in bacteria that has gone beyond our current findings.

In comparison with the metaproteomics cadmium shock study, we found a number of similarities and differences. The similarities are among transporters (with fast induction during shock), chaperones (preventing protein denaturation) and respiratory enzymes being

repressed (for energy saving). Regarding cadmium adaptation trends, no specific conclusions can be drawn with the current datasets since only a few proteins were observed in all three cadmium concentrations. Based on these proteins only, the cadmium adaptation response is only observable at higher cadmium concentrations, not strongly modulated at 0.04 mM Cd. Metabolic labeling was shown as a good labeling alternative, reducing the amount of experimental work by multiplexing at the cellular level, however, its data analysis is intense and improvements are needed in this direction. For this experiment, it functioned as an important identification constraint but its quantification scheme was subjective and not very robust.

V.IV. Conclusions

Significant shifts in protein regulation were found for the different levels of cadmium, with the final identification of 72 unique proteins involved in cadmium adaptation. The ^{15}N metabolic labeling approach added important constraints for identification and quantification of proteins. Cadmium caused the induction of a complex network of regulatory systems, including DNA and protein synthesis and repair, energy saving, oxidative stress, as well as efflux systems for heavy metals. Many proteins involved in cadmium adaptation are also involved in short-term cadmium resistance. This study proves to be a great contribution to cadmium resistance and adaptation science.

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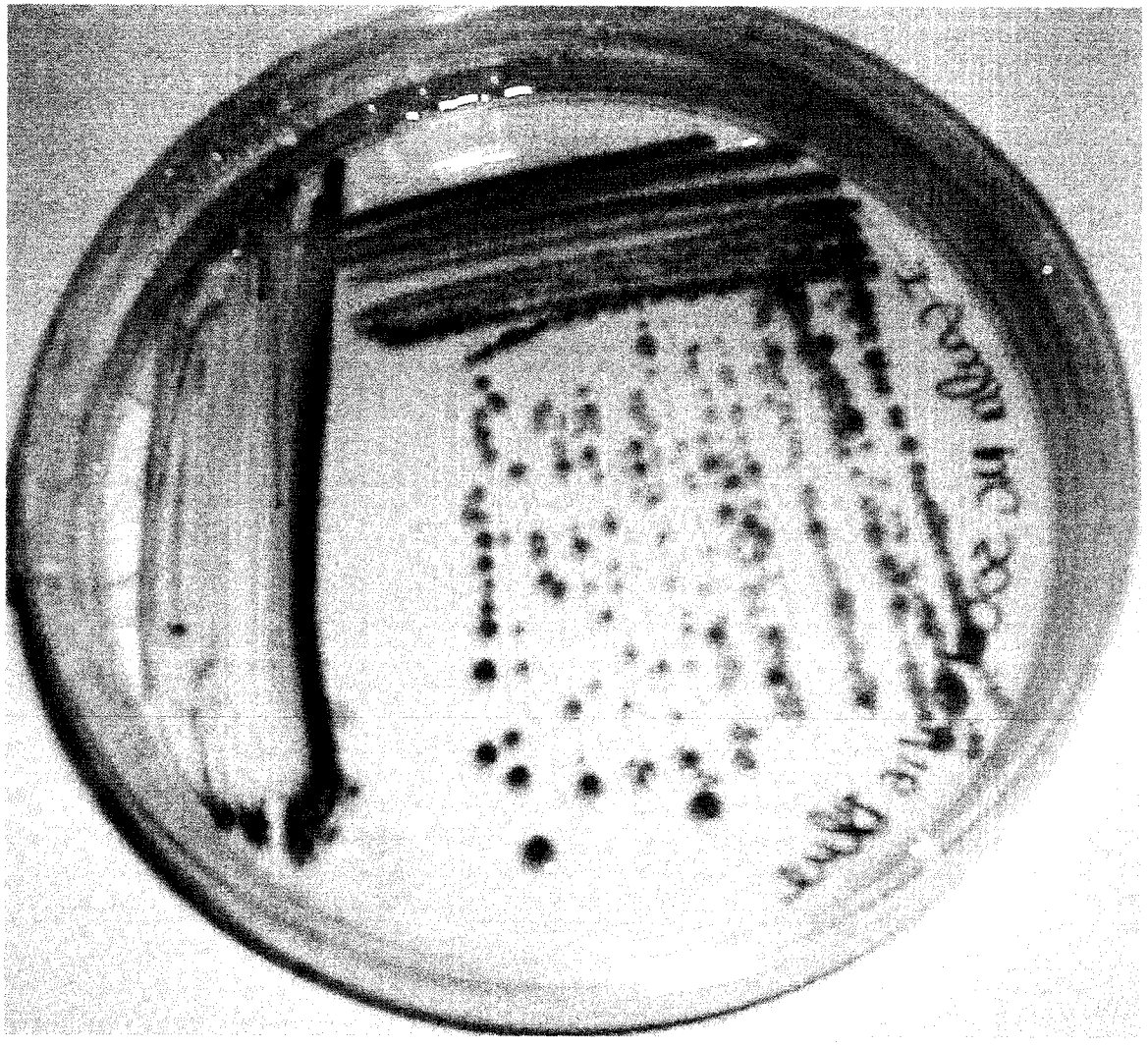

Figure 7: *B. cepacia* Cd44 phenotype during growth on 0.44 mM cadmium.

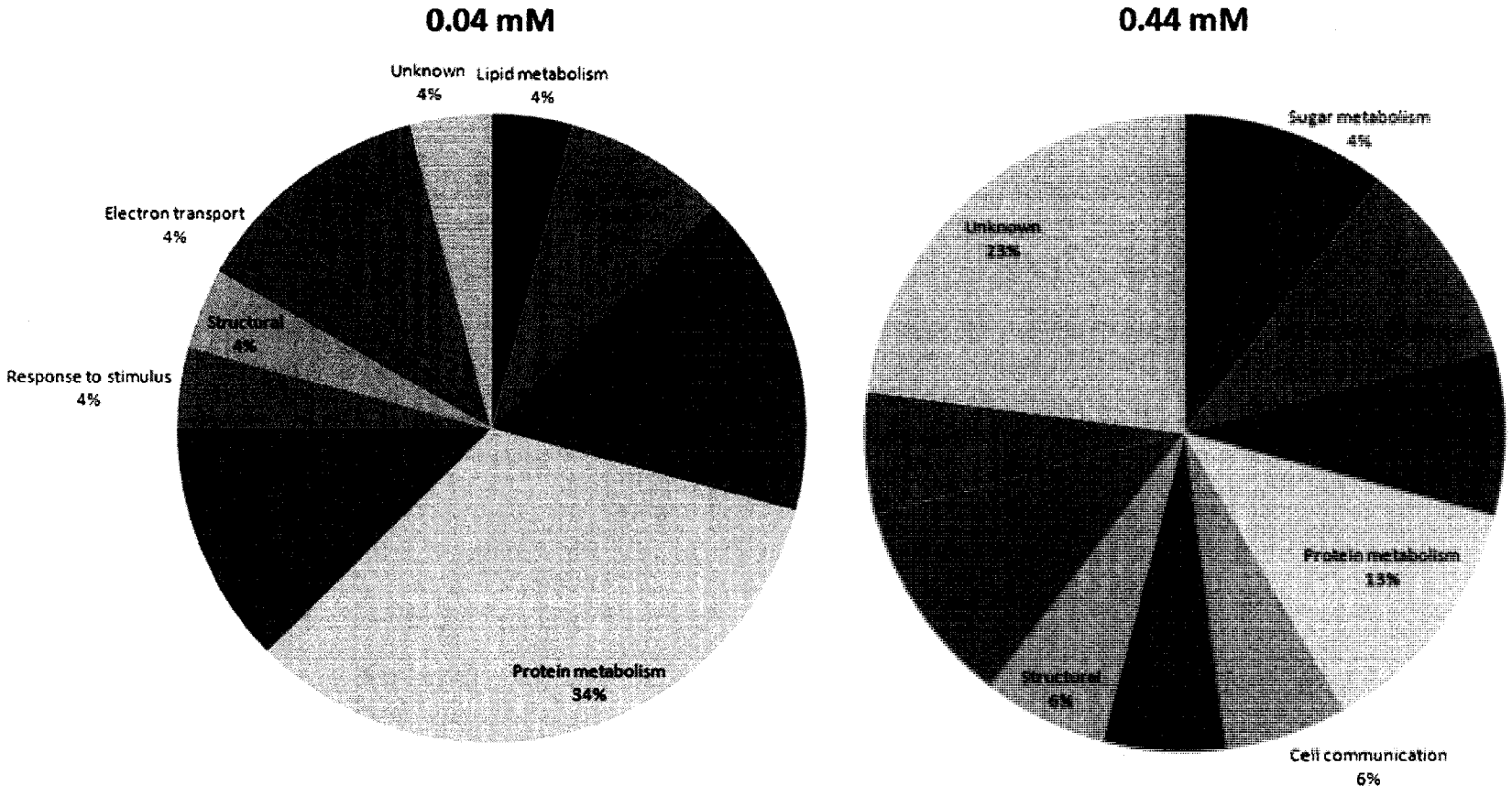

Figure 8: Functional classification of proteins identified in this study, sorted by levels of cadmium.

VI. QUANTITATIVE PROTEOMICS AS A TOOL FOR SYSTEMS BIOLOGY: ASSESSMENT OF METAL STRESSES IN *Burkholderia cepacia*

VI.I. Introduction

Quantitative proteomics methods have been extensively described in the previous chapters. Our final experiment has two major aims: to improve our knowledge of the biology of metal resistance after cadmium adaptation and to compare iTRAQ quantification software packages. After studying *B. cepacia* Cd44 adaptation to different cadmium levels, our next question is how the machinery involved in cadmium adaptation can help this bacterium tolerate other metals. For this study we chose iron(III) and chromium(III) due to some of their observed phenotypic changes on this bacterium and because of some of their known effects, briefly described below. Iron is an essential nutrient for most organisms, but can also be relatively toxic in high concentrations. In the presence of oxygen, iron is both poorly available and potentially toxic, requiring that organisms have well-designed homeostasis mechanisms. The best-known homeostasis mechanisms used by bacteria involve high-affinity iron transport, deposition of intracellular iron stores, redox stress resistance systems, control of iron consumption by down-regulation of the expression of iron-containing proteins, and an iron-responsive regulatory system[347]. All of these mechanisms were expected to be of fundamental importance in the context of this study. Trivalent chromium also has some unique and interesting properties. Cr(III) is considered to be less toxic than the oxidized version because of its tendency to form insoluble hydrated complexes, which cannot cross cell membranes of some microorganisms[348]. However, Cr(III) has been shown to cause DNA damage and inhibit

topoisomerase DNA relaxation activity in bacteria. To cope with chromium toxicity, some bacteria have developed detoxification strategies that include biosorption, diminished intracellular accumulation and extracellular precipitation[349]. The goal here is to detect cadmium-adaptation related proteins that are capable of helping the cells in the resistance to short-term less-toxic metal shocks.

For the second part of this study, we know that data analysis for iTRAQ requires special software packages that perform protein identification as well as quantification of reporter ions. Such quantification procedures vary, and different results can be obtained from different software, depending on how peak intensity is calculated for peptide quantification, and on how peptide abundances are averaged for protein quantification. Currently, iTRAQ data analysis is supported by Mascot (Matrix Science Inc., Boston, MA USA), I-Tracker[350], Libra (http://tools.proteomecenter.org/Libra.php), Pro QUANT (Applied Biosystems, Foster City, CA USA), SpectrumMill (Agilent Inc., Santa Clara , CA USA), and by other software supplied by spectrometer vendors. Here, we present a comparison between the quantification schemes of Mascot and PEAKS (quantification software from Bioinformatics Solutions Inc., slated for commercial release in 2008), and evaluate similarities and differences in the results of these two approaches. Mascot was chosen as a widely used software, not associated with a particular mass spectrometer vendor. PEAKS (Bioinformatics Solutions Inc., Waterloo, ON Canada) is am alternative peptide mass spectrometry data analysis solution, containing database search algorithms for identification of peptides, which use *de novo* sequences both for identification of homologues and in parallel with peptide fragment fingerprinting. PEAKS uses rigorous algorithms for data processing, with improved peak detection, charge recognition, peptide identification and results validation. Well-known for its *de novo*

sequencing algorithms, PEAKS uses tandem mass spectrometry spectra to determine peptide sequences based on the measured fragmentation pattern without reference to sequence database.

Two experiments were designed to accomplish our goals. The first was the quantification of a simple protein mixture (Applied Biosystems six-protein mix provided in the iTRAQ Reagent Methods Development Kit). The second experiment evaluated protein expression in complex samples from a strain of *Burkholderia cepacia* Cd44 exposed to different toxic heavy metals.

VI.II. Experimental Procedures

VI.II.I. Simple sample mixture preparation

This sample was only used for the PEAKS and Mascot quantification comparison. The six-protein mix and solutions used here were provided by the iTRAQ Reagent Methods Development Kit (Applied Biosystems). One vial of protein mix (containing 129 μg or 3.71 nmol) was used according to the iTRAQ Reagents protocol from Applied Biosystems. Briefly, sample was dissolved in 20 μL of Dissolution Buffer and 1 μL of Denaturant. After full sample dissolution, 2 μL of Reducing Reagent were added, and sample was incubated for 1 h at 60 °C. Cysteines were blocked by the addition of 1 μL of Cysteine Blocking Reagent followed by room temperature incubation for 10 min. The sample was then digested overnight with trypsin (10 μg) at 37 °C. After digestion, 34 μL of Dissolution Buffer were added to the resulting peptide mixture, which was then divided into two vials, labeled with 114 and 117 separately. The labeled peptides were mixed in the ratio 114:117=1:3. The peptide mix was purified with cation exchange cartridge - labeled peptides were diluted with

4.4 mL SCX buffer A (described in the upcoming section). The eluate from the cation exchange cartridge was vacuum dried and re-dissolved in 1mL of 0.1% trifluoroacetic acid (TFA). The resulting concentration of this solution was about 1.8 pmol/μL and 1800 fmol were injected into the LC-MS/MS for analysis.

VI.II.II. Complex sample mixture preparation

Burkholderia cepacia strain Cd44, identified by 16S rDNA sequencing, was isolated from a soil bacterial community based on its high cadmium resistance, i.e., ability to grow on 0.44 mM Cd^{2+}. After 24 h of growth in a defined mineral medium, cultures of strain Cd44 were subjected to additions of metals with different toxicities (0.44 mM Cd^{2+}, 0.38 mM Cr^{3+} and 0.62 mM Fe^{3+}) and one was kept without metals to serve as control. After 2 h of metal exposure, cells were harvested and their proteins extracted and immediately digested prior to a shotgun proteomics workflow. Tryptic digestion of 100 μg of each sample followed the iTRAQ Reagents protocol from Applied Biosystems (described in the previous section). iTRAQ labeling also followed the iTRAQ Reagents protocol, with samples labeled according to the following scheme: control (no metal shock), 114; chromium shock, 115; iron shock, 116; and cadmium shock, 117.

VI.II.III. LC-MS/MS

The labeled peptides were mixed and then fractionated by SCX chromatography using a Polysulfoethyl A column 100 mm x 2.1 mm x 5 μm, 200 Å (PolyLC Inc., Columbia, MD USA). Buffer A was 5 mM potassium phosphate in 25% acetonitrile, pH3, and Buffer B contained 500 mM potassium chloride added to the buffer A composition. A 35-min linear gradient at 0.2 mL/min was used. Fractions were collected every minute, and purified with C18 PepClean spin columns (Pierce Biotechnology Inc., Rockford, IL USA), according to the

manufacturer's protocol. C18-cleaned samples were vacuum-dried and resuspended in 15 μL of 0.1% formic acid. Samples were separated on an Agilent ZORBAX reverse-phase column (C18 wide-pore, 50 mm x 0.075 mm internal diameter) in an Agilent 1200 HPLC system, containing a nanospray directly connected to the mass spectrometer ionization source. The HPLC buffers were 0.1% formic acid in 3% acetonitrile (Buffer A) and 0.1% formic acid in 97% acetonitrile (Buffer B). The method used a 45-min gradient at 0.6 μL/min starting with 5% Buffer B for 5 min, followed by 30 min of ramping to 70% Buffer B. Two other 5-min steps followed at 90% and 5% Buffer B. Mass spectrometry was accomplished by an ESI-QTOF instrument (Agilent 6510, with Chip Cube sample inlet), with the following settings: gas temperature 300 °C; drying gas 5 L/min; and capillary voltage of 1875 V. The auto MS/MS mode was used, with positive ion polarity and centroid data storage. Acquisition ranges were 300 – 2000 m/z for MS (4 spectra/s) and 59 – 1800 m/z for MS/MS (5 spectra/s). Collision energy used a slope of 4 (V/100 Da). Four precursor ions were collected per cycle, with active exclusion. Internal reference mass standard was used for Chip operation.

VI.II.IV. Data preparation procedures

All datasets were exported to the mzData format and imported into PEAKS Studio 4.2 sp2. Since a mass spectrometer may take an MS/MS scan of the same peptide several times, replicate spectra were merged (using the PEAKS 'data refine' tool with a parent ion m/z tolerance of 0.1 and a retention time window of 30 seconds) to provide a more complete fragmentation pattern, better signal to noise ratio, and a more representative intensity value for each reporter ion, before submission to both PEAKS and Mascot. The PEAKS 'data refine' tool uses an additive approach to merging. Providing the same merged data set to each quantification algorithm also allowed for easier post-analysis comparison. Data were

acquired in centroid mode, and parent charge states were assigned by the instrument, so MS/MS spectra were not subjected to further preprocessing prior to searching; any deconvolution or deisotoping was done by the search engine.

VI.II.V. Identification and quantification procedures

Database searches were conducted on Mascot and PEAKS using the following parameters: all bacterial species in the NCBInr database, decoy search, trypsin digestion allowing for up to two missed cleavages, methionine oxidation and iTRAQ on N-termini and lysines, iTRAQ 4-plex quantification, 0.3 Da tolerance for MS and MS/MS, peptide charges of +1, +2 and +3, and monoisotopic masses. The data analysis stage of iTRAQ quantification relies on accurate computation of the intensities of reporter ion peaks in MS/MS spectra and rigorous statistical analysis of relative reporter ion intensities from multiple peptides in computation of protein expression ratios. The calculation of peak intensity, though trivial to the human eye, requires complex tasks of a computer algorithm, and appears to be one aspect in which PEAKS and Mascot differ. When starting with profile data, the PEAKS algorithm uses a dynamic baseline subtraction algorithm to recognize how much of the peak's area can be attributed to noise; recognizes the shape of a real peak and what part of the peak can be attributed to an interfering signal; bounds the width of the peak; and determines at what point the peak sinks into the noise. The preprocessing scheme used by Mascot was previously described by Berndt *et al.*[351], and uses a sequence of steps: baseline correction, peak detection, isotope distribution fit, and subtraction of fit. To avoid bias created by any preprocessing algorithm, previously centroided data was used for both PEAKS and Mascot. Calculation of peak intensities (and their ratios), then, was not based on peak area, but on height of a single peak, and could only

be complicated by inadvertent addition of another peak to the reporter ion during deconvolution/deisotoping.

The calculation of a protein expression ratio from the peptide ratios relies on accurate peptide identifications and accurate peptide ratios. Since these are not guaranteed, and are subject to some randomness, differences between the two algorithms evaluated here are noteworthy. The PEAKS quantification algorithm performs a statistical analysis at the protein level. Firstly, PEAKS computes a weighting for each peptide ratio to allow peptide ratios from high quality spectra to be considered more trustworthy than those from spectra where reporter ions are barely visible. Secondly, outliers are removed before protein ratio computation (using Dixon's Test[352]) in PEAKS since some peptide ratios can be erroneous – e.g., those resulting from false positive peptide identifications and/or chemical aberrations. Finally, to facilitate review of results, PEAKS displays the protein expression ratio along with some measures of the success of the statistical analysis: standard deviation of the peptide ratio values as well as the coefficient of variation corresponding to this standard deviation.

A detailed description of MASCOT approach to iTRAQ quantification can be found on the Mascot website (http://www.matrixscience.com/help/quant_reporter_help.html). Briefly, identification and quantification are performed at the peptide level. Ratios for peptide matches are reported depending on peptide modification state, minimum precursor charge, strength of the peptide match, minimum number of fragment ion pairs, among other criteria. The Mascot result report assigns peptide matches to protein hits, and the ratios for individual peptide matches are combined to determine ratios for the protein hits (usually by weighted average). A ratio for a protein hit is only reported if two peptide matches are found. Standard

deviations are only reported if the ratios for the peptide matches are consistent with a normal distribution.

VI.III. Results and Discussion

VI.III.I. Cell growth and observations

This final experiment is the one that most resembles the first metaproteomics experiment described in Chapter 3. It evaluates short-term (2 h) responses of an organism when facing a metal perturbation. The main difference between these experiments is that for this one we have one single organism facing different metal stresses. A metal adaptation experiment (similar to the one in the previous chapter) was conducted to evaluate effects of different metals on the high-cadmium resistance *B. cepacia* strain Cd44. Figure 13 shows some phenotypes observed in the cultivation plates each metal chosen for the experiment. The phenotypes in Figure 13 show a clear difference in coloration, leading to a hypothesis that these metals have an effect even during a short-term exposure, and that this effect could be detected using proteomics technologies.

VI.III.II. Quantification of simple sample mixture

Table 4 lists the identifications and quantifications obtained from both Mascot and PEAKS. High-score identifications were obtained from the Swiss-Prot database for beta-lactoglobulin (human and ovine), beta-galactosidase (*E. coli*), apotransferrin (human), serum albumin (bovine), and lysozyme (chicken). No significant identification was obtained for alpha-lactalbumin, the sixth protein present in the protein mix, with either software. Good protein coverage and number of queries matched were found for all proteins identified in this mixture. Table 4 also summarizes the protein quantification results obtained for this mixture. The

average protein quantification from PEAKS was 14% lower than the expected value of 0.33 for the 114:117 reporter ratio, whereas Mascot provided values that averaged nearly three times the expected value. Protein-level quantification is based on peptide quantification, and also relies on an accurate peptide weighting strategy, which will take into consideration the ionization method used, among other factors. Data analysis suggests that peptide quantification and weighting are the two main causes of the differences in protein quantification found in this study. However, the observed quantification differences might also be attributed to the composition of this particular six-protein mixture. Table 4 further presents the standard deviation from the mean calculated by PEAKS, which is useful to determine the accuracy of the quantification. This information is unavailable from Mascot. An expanded version of Table 4, containing all peptide quantification values, can be found in Appendix IV.

VI.III.III. Quantification of complex sample mixture

The quantification results for the differentially expressed proteins according to Mascot are presented in Appendix IV. The complete tables contain all the information required for a proteomics experiment, namely: protein name and accession numbers, the organism in which the protein was first identified, protein and peptide scores, peptide sequence and protein sequence coverage, protein and peptide ratios and standard deviations, and peptide weights. Database searches were performed as described in the experimental section and similar results (protein identifications) were obtained from both software packages. However, challenges were faced when merging the two datasets, mostly due to the distinct scoring systems in which proteins and peptides are ranked differently. In order to compare the two quantification schemes, it was necessary to construct the results tables manually. Some of these tables are

presented in Appendix IV. The agreement level of Mascot and PEAKS quantification can be described in three levels. Quantification results in good agreement (within 20%) represent 26% of the peptides sampled in this study. Adequate agreement (within 50%) was found for 60% of the peptides and improper agreement (difference greater than 100%) was found in 9% of the peptides analyzed here. Scatter plots with the quantification results of PEAKS and Mascot are presented in Figure 9.

Some extreme cases of disagreement between the two methods are described in further detail below. One example comes from one peptide identified from phasin, with a 115:114 ratio of 17.4 according to Mascot and 3.2 according to PEAKS. This can be attributed to the various peptide quantification factors discussed previously. It is interesting to note that such a large value might be generated by an inefficient removal of outliers during the averaging of the reporter peaks. Further observations of this case can be made by analyzing the spectra of the reporter genes for the specified peptide (Figure 10); visual inspection reveals that the 115:114 peak ratio is closer to 3 than to 17. In contrast to this result for the highest ratio reported by Mascot, the highest ratio reported by PEAKS was 5.8, which corresponded well to the value of 5.98 produced by Mascot. No errors in the PEAKS quantification results were evident on the basis of visual inspection of this sample set. Other cases of differences in peptide quantification can be found in Appendix IV.

The correct protein expression ratios are not known for the complex mixture, so evaluation of the results must be performed by means other than calculation of standard errors. Such approach makes use of the experimental design. Since the peptide mixture was divided into several fractions through SCX chromatography, we can expect to identify a protein in several fractions (though perhaps represented by different peptides), and the same abundance ratios

should be observed for that protein in each fraction. A basis for algorithm comparison is thus the degree of consistency of the protein ratio calculations across the fractions. Examples of such comparisons are shown in Figures 3 and 4. For phasin (gi|78066909|), the 116:114 ratio as calculated by PEAKS is 1.8 with a variance of 0.1 across all fractions, the PEAKS-calculated 115:114 ratio is 2.8 with a variance of 0.5, and the 117:114 ratio for this protein is 4.6 with a variance of 0.9 (Figure 11). The Mascot calculations show considerably higher variations across fractions (115:114 ratio of 10.0 with a variance of 24.4, 116:114 ratio of 4.0 with a variance of 1.5, 117:114 ratio of 5.6 with a variance of 3.3). When this analysis is performed for chaperonin GroEL(gi|107021936|ref|YP_620263.1|), both tools are found to provide consistently similar ratio calculations across the fractions, with PEAKS showing lower variance in two of the three cases (Figure 12). All these calculations are available in Appendix IV.

VI.III.IV. Biological interpretation

Using the procedures described in the experimental section, we were able to identify almost 400 proteins with a false positive identification rate of 1.96%, according to the Mascot decoy search. A total 112 proteins were differentially modulated (expression two times larger/smaller) in response to cadmium, iron or chromium in relation to the control (containing no metals). These findings are extremely interesting since this experiment is based on a short-term metal exposure, and the cadmium treatment shows adaptation characteristics similar to the ones found in Chapter 5. Cadmium is more toxic than the other metals used in this experiment and this can be confirmed by the quantification of the proteomic responses. A Venn diagram of how similar protein expression was among the metals tested is presented in Figure 14. It is important to note that almost half of the proteins

with differential expression were commonly modulated by all three metals, and more than 80% of the total proteins were modulated by cadmium. The complete data tables are presented in Appendix IV, with the raw database searches, and the summary table of the differentially expressed proteins.

Proteins were functionally classified using Gene Ontologies. Figure 15 shows the functional classification pies for each metal. Almost half of the proteins of interest were related to metabolism, and more than half of those were proteins involved in protein and amino acid metabolic pathways. In addition to metabolic changes, proteins of major importance for the metal shocks are proteins involved in localization, structure and defense, also identified by this study. After careful analysis of Figure 15 we can see that the different metals show different functional responses, and these will be explored in detail in the following paragraphs. Since we have extensively exploited cadmium responses in the previous chapters, here we will try to better understand iron and chromium responses.

Iron. Iron acquisition by bacteria can be done by porin proteins (for Fe^{2+}), or by specific iron receptors for Fe^{3+}, usually bound to siderophores[347]. To confirm this first step that requires iron modulation of proteins, we were able to identify and quantify the up-regulation of several porins and a group of outer membrane receptor proteins (COG1629), dedicated to iron transport. For the active transport to happen through the iron receptors, energy needs to be provided by an energy-transducing TonB complex[347]. This step was also observed in our proteome study with the detection of specific TonB receptors (TonB-dependendt siderophore receptor and TonB-dependent haemoglobin/transferrin/lactoferrin receptor). For transport across the periplasmic membrane, ABC transporters are required, and these were also found in our study. Another protein of interested in this study is also related to iron acquisition.

Pathogenic bacteria absolutely require the uptake of haem (or its precursor protoporphyrin IX) for its biosynthetic machinery[347]. The proteome of the organism in this study showed an extreme down-regulation of an iron (III) protoporphyrin IX monomer binding protein. This can be directly connected to the abundance of iron in the shock, making the cell dispose of such mechanism of iron acquisition through haem. Once inside the cell, iron is deposited into proteins specialized in intracellular iron storage. There are three types of iron storage proteins: Dps proteins, ferritins and bacterioferritins, the latter being most commonly found[347]. In this experiment we were able to identify a bacterioferritin with a six-fold expression increase in the iron shock. Intracellular iron (III) can react with reactive oxygen species to produce a hydroxyl radical. This role of iron in redox stress is also observed in this study through the identification on a number on redox proteins involved in cellular defense mechanisms (e.g., redoxins). The ferric-uptake regulator (Fur) protein, which controls iron metabolism, was not identified as a protein of interest in this study. However, it was previously hypothesized that Fur and the bacterial histone-like protein might compete for the same binding site[353], and these were found differentially expressed (up-regulated) in this study. Fur also is responsible for the positive regulation of superoxide dismutase[353], also observed here.

A gene expression study in *P. aeruginosa*[354] recently revealed the iron-dependent expression of iron acquisition systems, protases, ferredoxins, oxidoreductases and dehydrogenases, all identified here. A recent proteome study of iron-starvation of *Bordetella pertussis*[355] revealed the differential expression of a number of proteins also found in this study, namely, superoxide dismutase, acetyltransferase, fumarate hydratase, outer membrane proteins and iron receptors, porins, chaperonins, lipoproteins and secreted proteins.

Chromium. Unlike iron acquisition and intracellular storage mechanisms, chromium tolerance mechanisms are not very well understood. The best described mechanism for chromium (VI) bioremediation involves intracellular reduction to chromium (III) followed by its transport and accumulation on the cell surface. Not much is known about chromium (III), except that it binds tightly to lipopolysaccharides and cell walls of *Bacillus, Salmonella, Escherichia*[349], and *Shewanella*[356]. However, it has been hypothesized that chromium enters the cell as a cation or cationic hydroxyl ($Cr(OH)^{2+}$ or $Cr(OH)_2^{+}$, highly unstable and toxic) after the reduction has taken place extracellularly[357]. In the presence of a high concentration of a complexing agent, such as pyrophosphate of ferrous ions, Cr (III) is complexed, preventing cellular damage. Evidence suggests that this happened in this experiment, and if further confirmed by the amount on DNA damaged usually caused by Cr (III). Cr (III) is known to bind nonspecifically to DNA and other cellular components, cross-link DNA and proteins, interfere with DNA replication, prevent transcription and increase the rate of spontaneous mutations[357]. Our study indicates the induction in the expression of a number of redoxins, proteases and peptidases, and well as DNA single-strand binding proteins, which might be a cellular attempt to prevent DNA and protein damage.

A number of transcriptome and proteome studies of chromate showed similar responses to the ones found in this experiment. This is very interesting since it correlates the intracellular reduction of Cr (VI) to Cr (III) and how the cells prevent damage from newly-formed Cr (III). The proteins found in common with our study were: outer membrane proteins[358, 359], superoxide dismutase[359], thioredoxin[359], cytochrome c protein[359, 360], ABC transporters[360], oxidoreductases[360], TonB-dependent receptors[361], lipoproteins[348] and peptidases[348]. This set of proteins related to chromium response leads us to believe that some sort of chromium

oxidation and reduction is happening inside the cells, despite the fact that they were fed relatively innocuous Cr (III). An alternative -omics study should be used to complement our experiment and provide more information about the response of this strain to Cr (III).

Cadmium. The proteins differentially expressed in this study that were also present in the metaproteomics study of Chapter 3 were TPR repeat (responsible for the assemblage of muyltiprotein complexes), catalase, malate dehydrogenase, elongation factor Tu, two ATP synthase subunits, redoxins, chaperonins, ABC transporters and outer membrane proteins. With the exception of transport-related proteins, responsible for transporting cadmium outside of the cell, these proteins do not provide insights into cadmium tolerance mechanisms. A genetic network would help us understand the regulatory cascade that leads to the differential expression of these proteins during short-term cadmium exposure. Another possibility to consider here is that due to the completely different proteomic approaches, it is difficult to show agreement between the two datasets. We believe that most of the differences are due to the simplicity of the *B. cepacia* proteome when compared to the metaorganism, but the choice of proteomic methods used cannot be disregarded.

VI.IV. Conclusions

This study presented comparable results to previous gene expression studies of exposures to the metals in question. There seemed to be little or no effect of cadmium resistance genes in tolerance to iron on chromium. Valuable knowledge was still gained from this study using the quantification of these metal responses. The cadmium response appeared to be different from the responses obtained for the mixed culture of organisms, leading to the conclusion that this organism was probably not the most active in the short-term cadmium response of the

metaorganism. We also report the fundamental challenges involved in analyzing quantitative proteomic data. The quantification performance of Mascot and PEAKS were compared using iTRAQ datasets from a six-protein mixture and a complex protein sample. The quantification results obtained from PEAKS were found to be more accurate in the case of the simple mixture. The analysis of the complex protein mixture revealed the influence of many variables involved in the quantification, including reporter ion peak intensity calculation and baseline subtraction for peptide quantification, and efficient weighting and removal of outliers for protein quantification. This analysis also demonstrated that the quantification algorithms need further development. As of yet, there is no consensus on how peptide and protein quantification should be accomplished, and direct comparison of results obtained by two software packages can be difficult. Studies of this kind can serve as inputs for future cellular models in the field of systems biology. The study of different components, such as different metals, can be unique in the characterization of microorganisms' behavior at the molecular level.

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Table 4: Six-protein mixture identification and comparison of PEAKS and Mascot protein-level quantification. Two preparations of this protein mixture were combined to provide a ratio of 0.33.

Accession #	Mass (Da)	Score (%)	Coverage (%)	Queries matched	Ratio from Mascot quantification	Ratio from PEAKS quantification	PEAKS coefficient of variance
P02754| LACB_BOVIN	19,883	99	40	25	0.992	0.316	0.115
P67975| LACB_OVIMU	18,151	99	28	26	0.994	0.313	0.110
P00722| BGAL_ECOLI	116,483	99	16	21	1.067	0.237	0.098
P02787| TRFE_HUMAN	77,050	99	13	16	0.928	0.271	0.147
P02769| ALBU_BOVIN	69,294	99	13	13	0.866	0.370	0.252
P00698| LYSC_CHICK	16,239	99	23	3	1.014	0.214	0.152

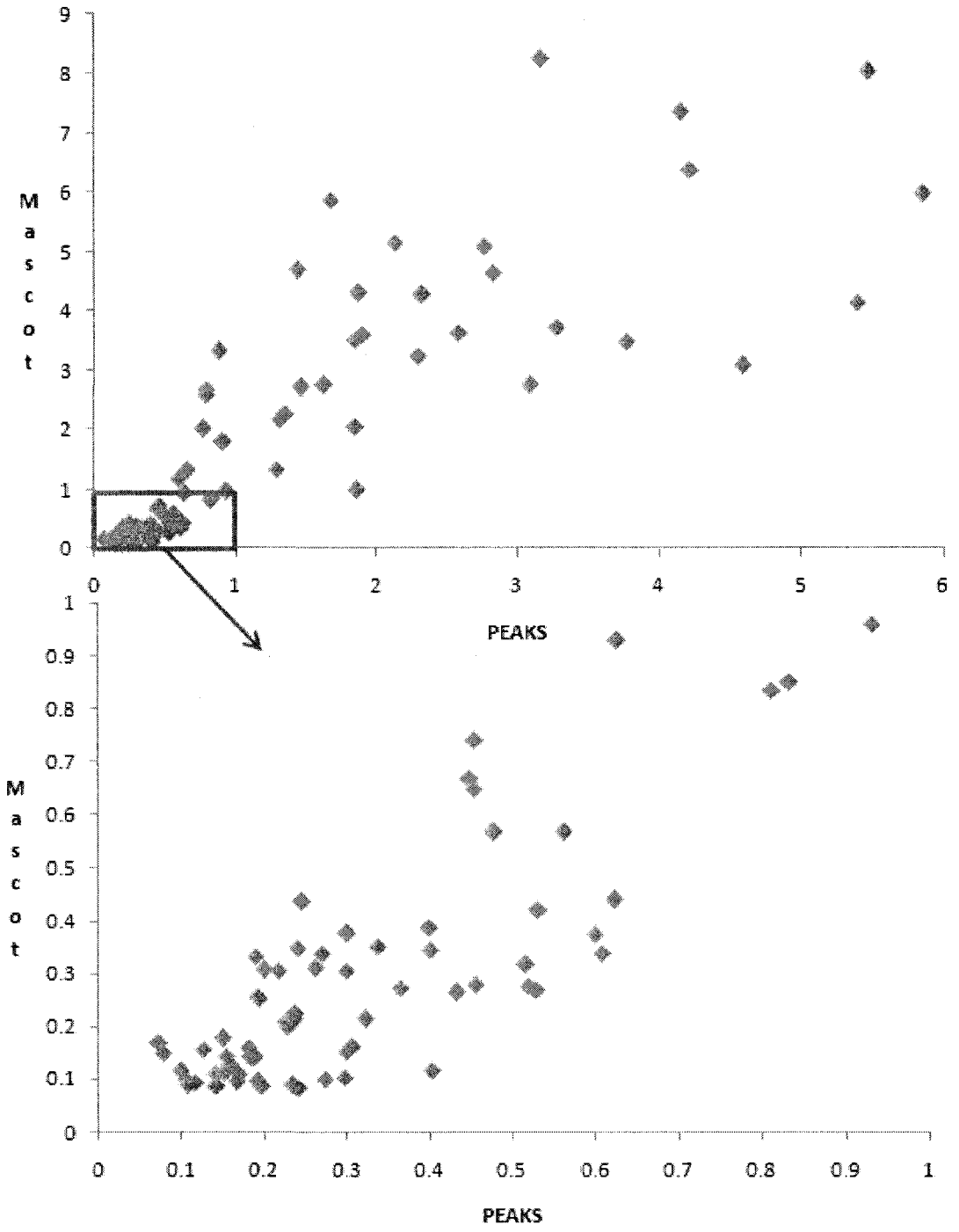


Figure 9: Scatter plots with Mascot and PEAKS quantification results.

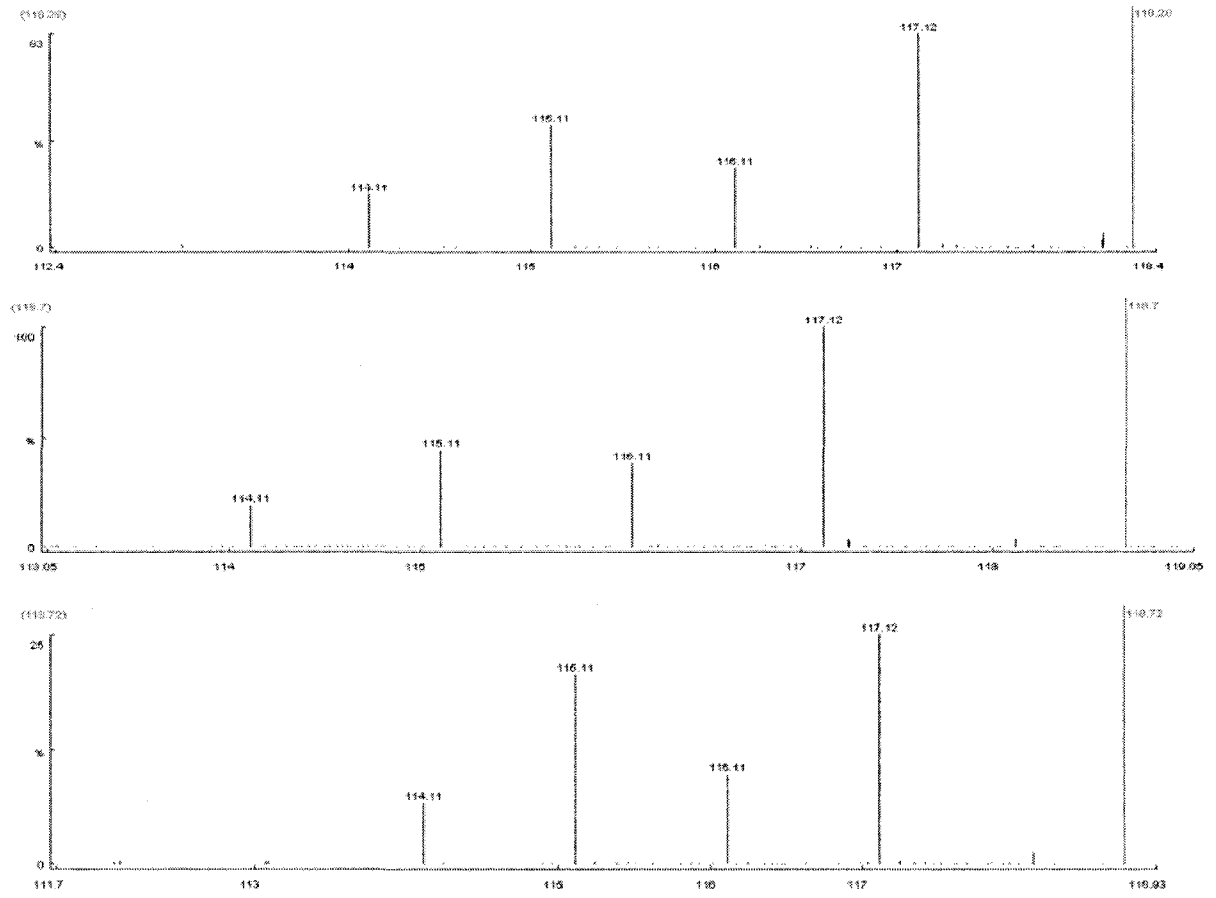


Figure 10: Portions of MS/MS spectra showing reporter ions for three different peptides from phasin (in the complex protein mixture).

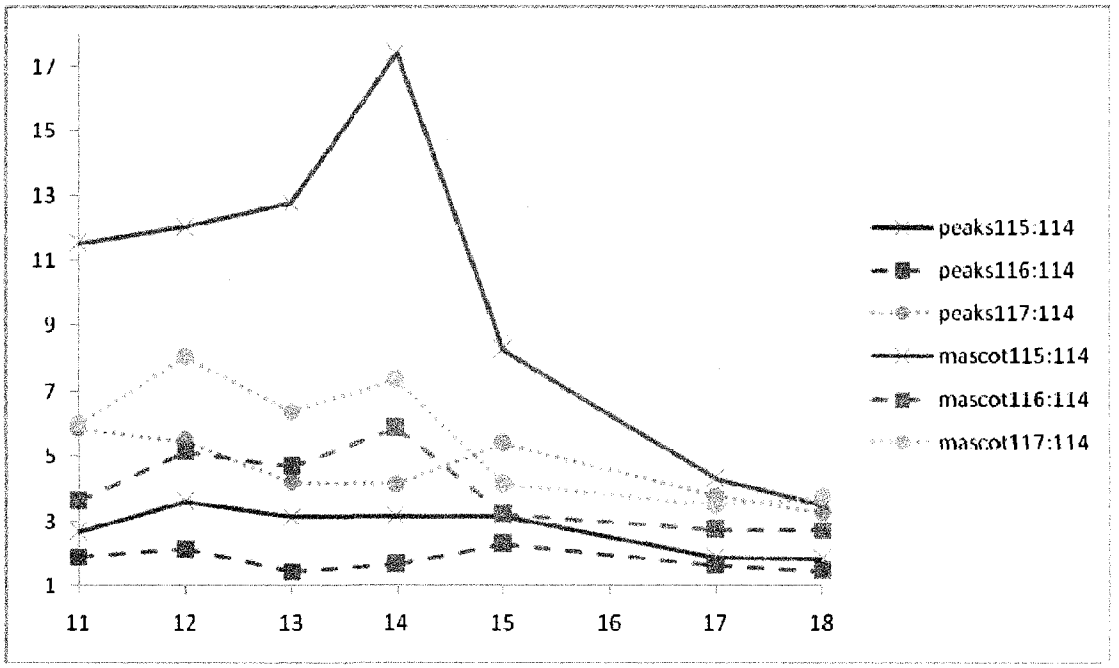


Figure 11: Phasin protein ratios across SCX fractions as calculated by Mascot and PEAKS.

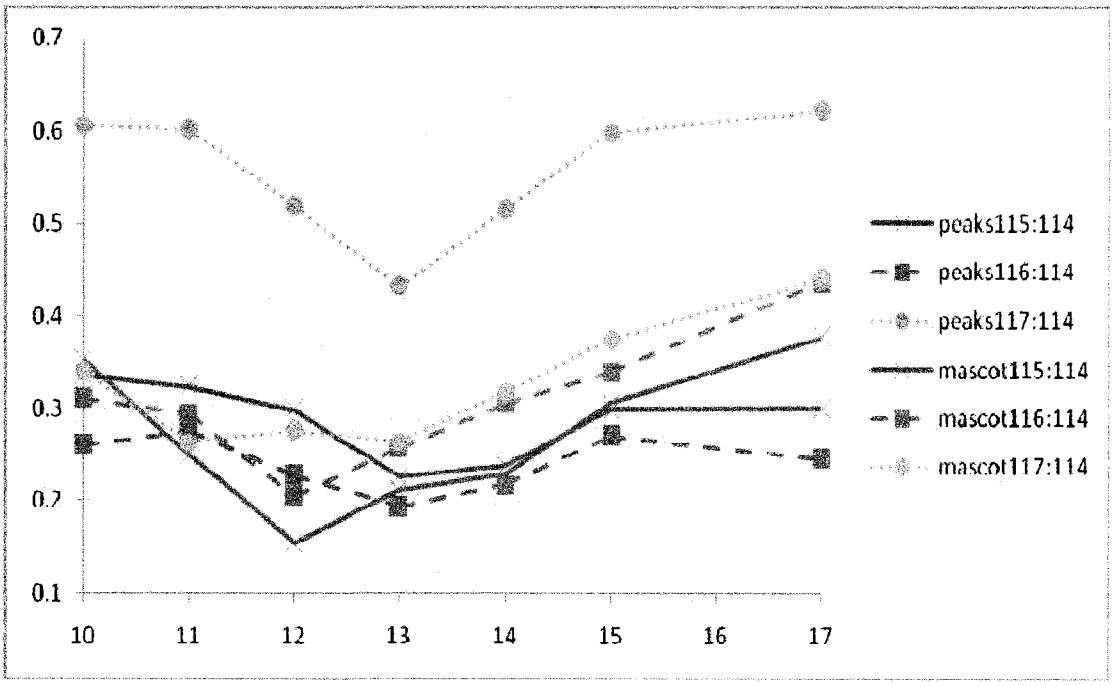


Figure 12: Chaperonin GroEL protein ratios across SCX fractions as calculated by Mascot and PEAKS.

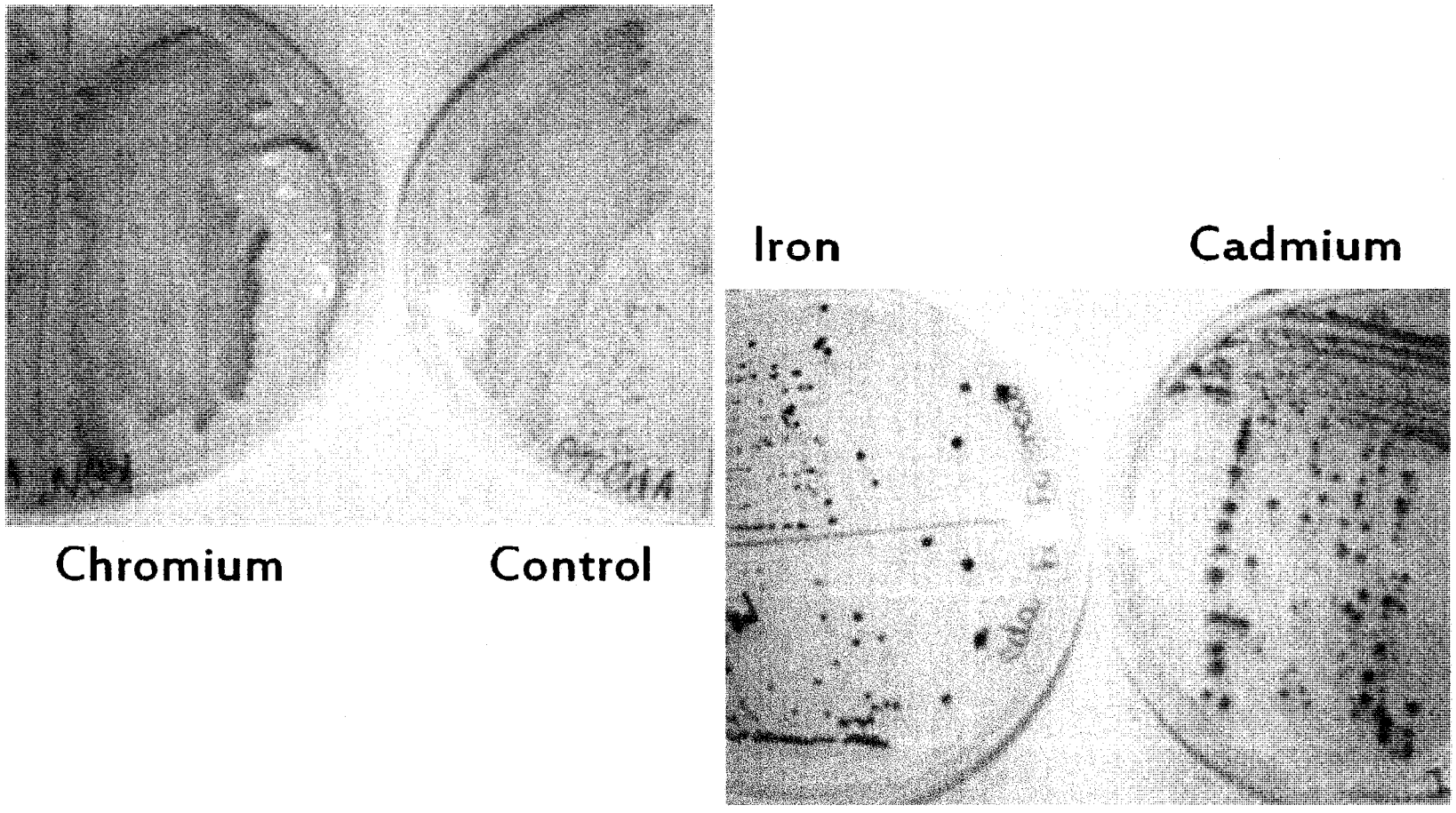


Figure 13: Phenotypic differences of *B. cepacia* Cd44 after cell cultivation on different metals.

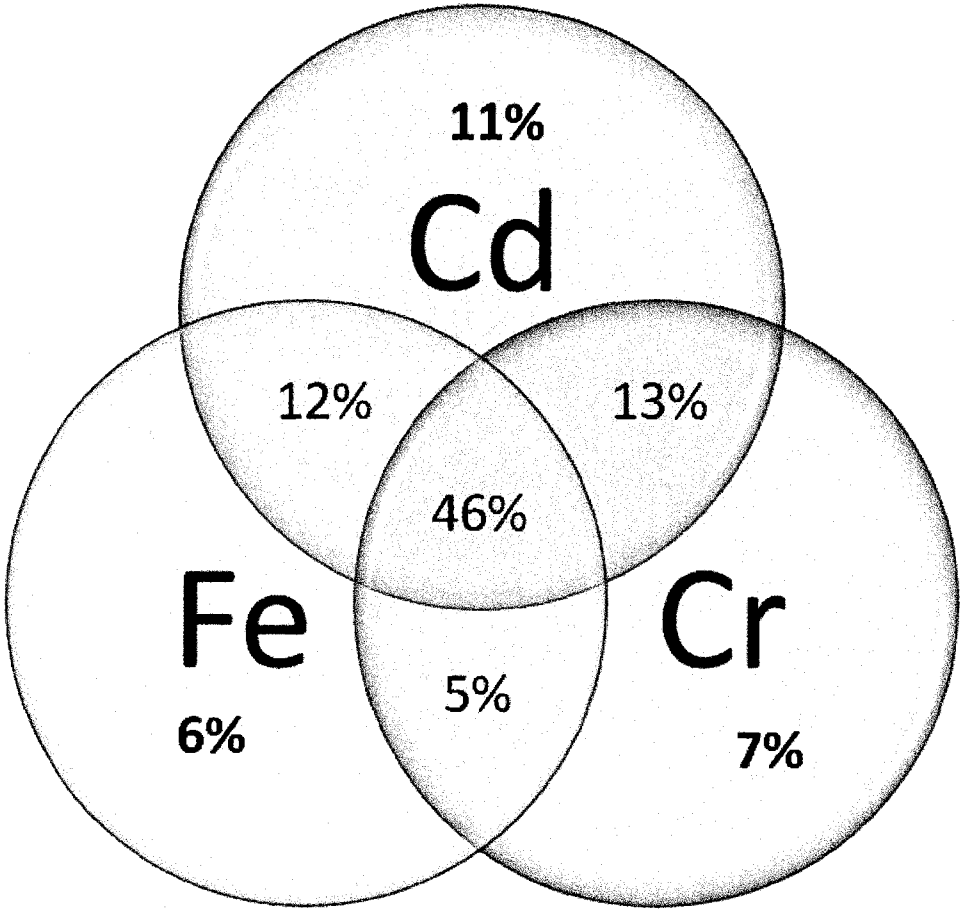
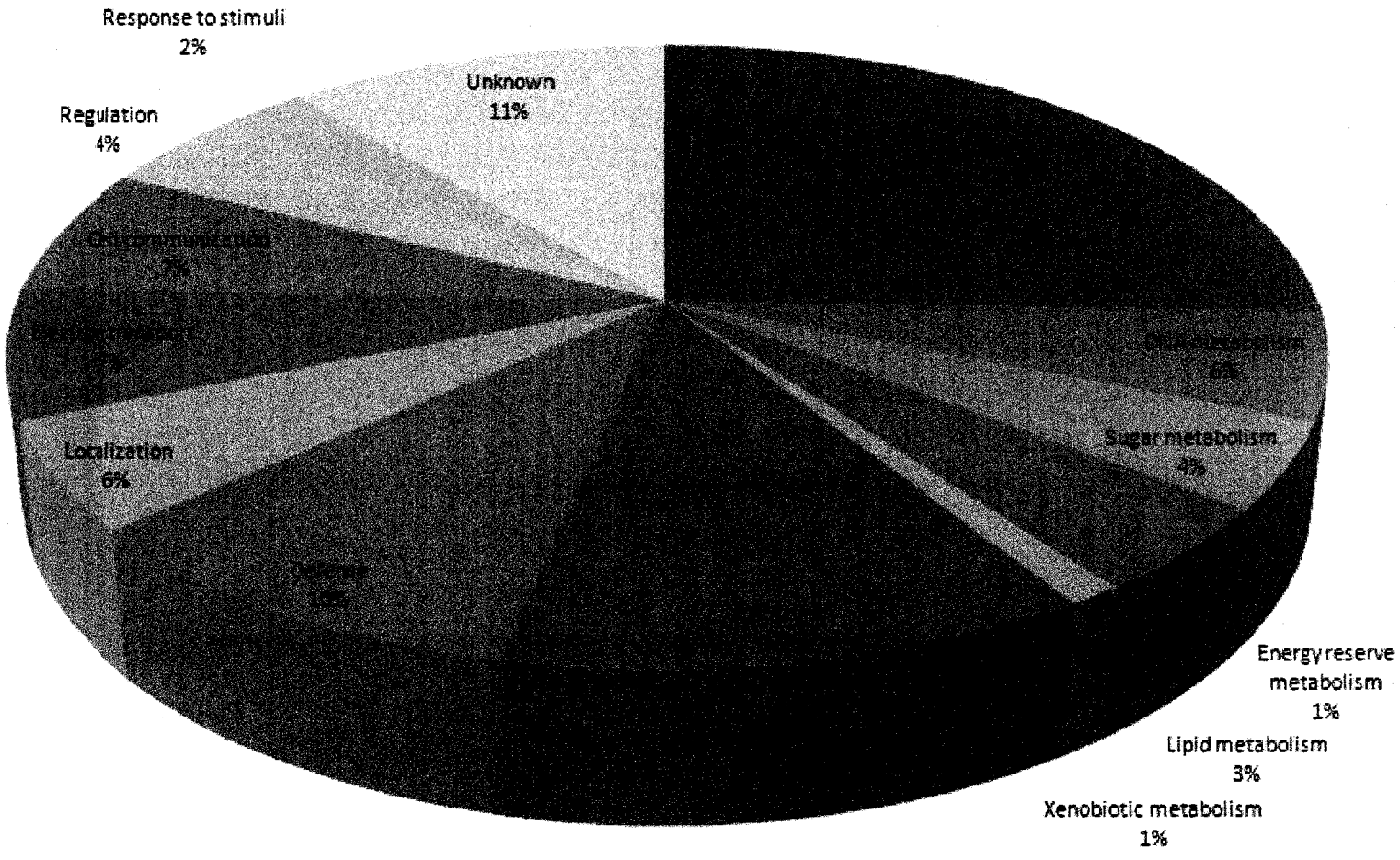
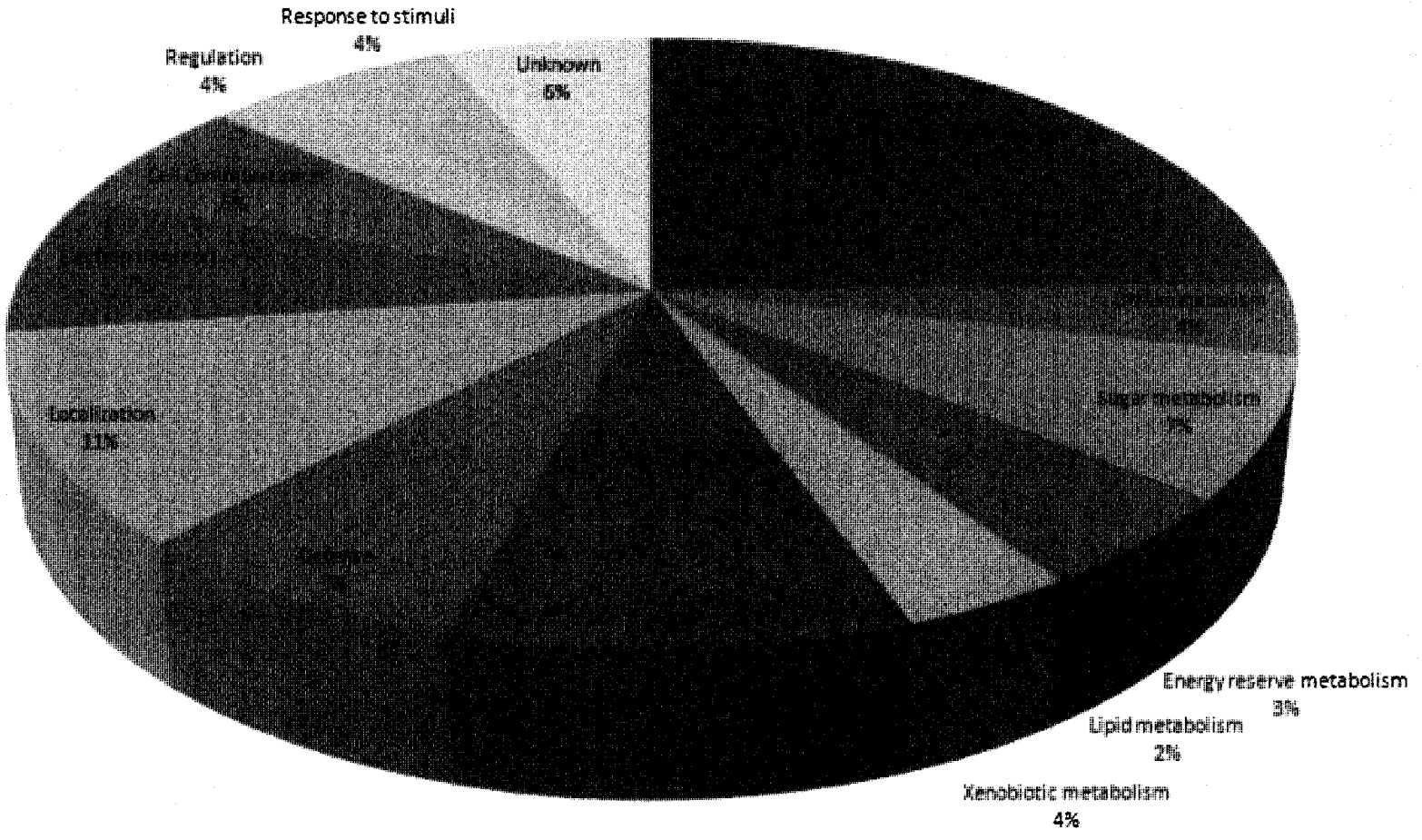


Figure 14: Venn diagram of the similarities in protein expression of the three metal shocks, showing that 46% of the proteins of interest are common to all three shocks.

Cd


Response to stimuli
2%
Unknown
11%
Regulation
4%
Localization
6%
Sugar metabolism
4%
Energy reserve
metabolism
1%
Lipid metabolism
3%
Xenobiotic metabolism
1%

Cr


Response to stimuli
4%
Regulation
4%
Unknown
6%
Localization
11%
Sugar metabolism
7%
Energy reserve metabolism
3%
Lipid metabolism
2%
Xenobiotic metabolism
4%

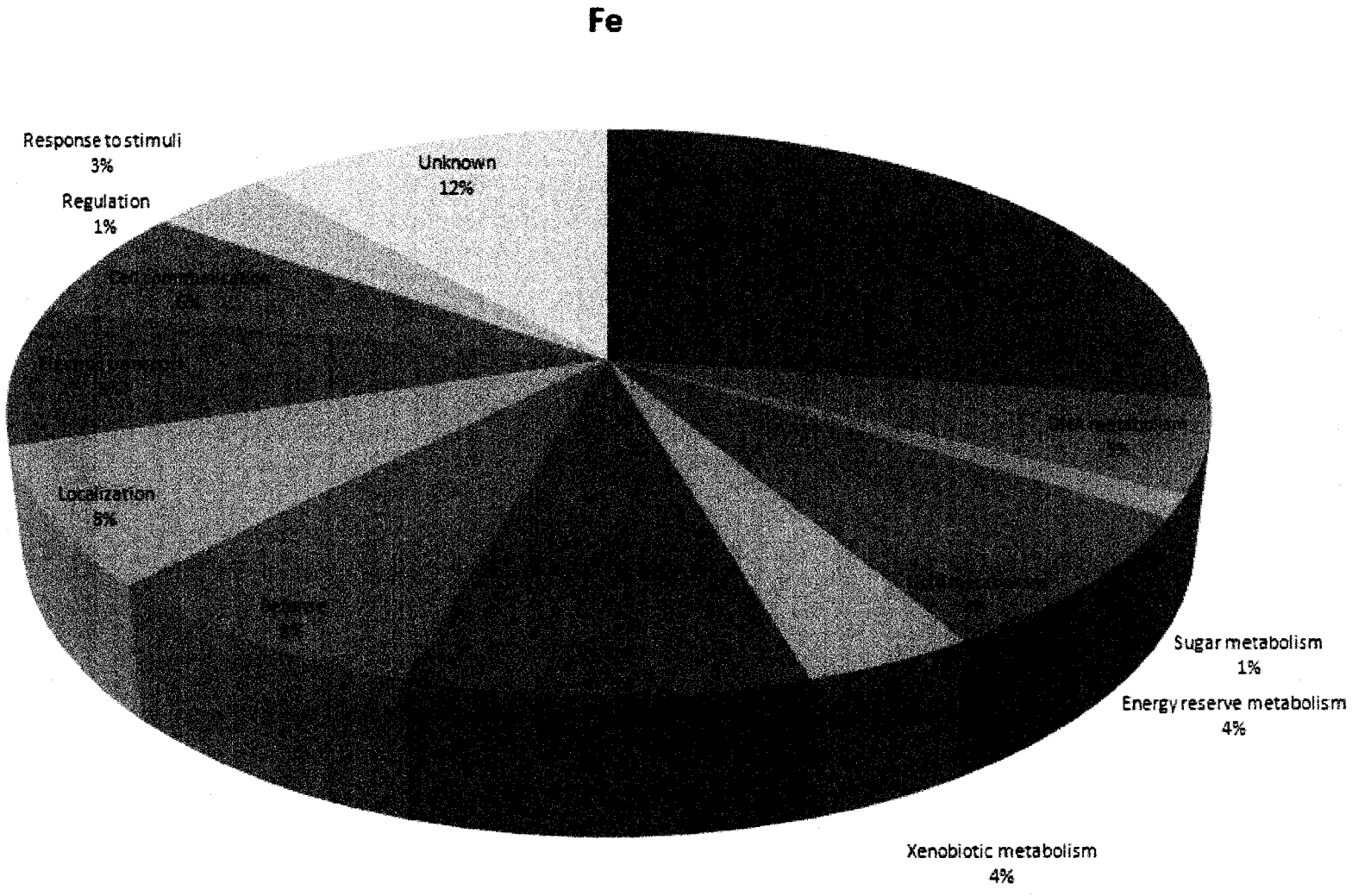


Figure 15: Functional classification of differentially expressed proteins, broken down by metals: cadmium, chromium and iron.

VII. CLOSING REMARKS AND FUTURE DIRECTIONS

Despite all the advances in the proteomics technologies, there is still much to be seen in the bacterial world. In a study of prokaryotic diversity, Torsvik *et al.*[362] showed that the total cell abundance in prokaryotic communities from the most diverse habitats was in the order of 10^9 cells per cubic centimeter and community genome complexity was on average in the order of 10^{10} base pairs. From this information we can deduce that there is a massive number of proteins of potential environmental significance still to be found in proteomic studies. This is just one among all the challenges faced in the application of proteomics in environmental biotechnology.

The other challenge relates to how many proteins can be identified in metaproteomics studies. Assuming an average 3000 open reading frames per bacterial species, and that natural communities usually have hundreds of species, it is doubtful that we could represent a significant portion the whole proteome using the current techniques. In addition to that, the wide dynamic range of proteins, around 10^5 in bacteria[363], makes it impossible to detect lower abundance proteins either by 2DE or shotgun proteomics. Due to all these difficulties in metaproteomics, most environmental proteomics studies so far are based on pure cultures. These studies are relevant but lack the community aspect of protein expression, i.e., they do not consider that proteins are almost certainly expressed differently in a community and thus studying the proteome of a single species leads to the expression of an entirely new set of proteins.

Another challenge discussed here regards the difficulties involved in extracting proteins from mixed cultures, soils and other types of matrices where proteins of interest might lie in the environment. Proteins in nature are almost always combined with other molecules, e.g., lipids or carbohydrates, or even salts or nucleic acids. Due to their diverse physical properties, such as hydrophobicity and charge, these interactions can be weak or strong making it hard to remove those "contaminants" from protein solutions. Extracting proteins from a mixed culture of microorganisms is difficult due to different cell wall complexities. The scenario is more complicated if one considers that soil has several components of "unknown" composition such as humic acids and tannins. These substances have a great influence on electrophoresis and chromatography of proteins, making it necessary to improve immobilization columns and other types of targeted separation methods.

A topic of constant debate in the proteomics field is the identification of proteins from unsequenced organisms. Basically, it would be impossible to find identifications for proteins that are not yet present in translated databases, due to the lack of genome sequencing. To overcome this problem one usually accepts protein identifications coming from highly similar proteins present in sequenced species. This is common in proteomic research, but greatly dependent on the amino acid coverage used for the identification. The first articles to present cross-species identification are from Cordwell *et al.*[364, 365]. Both articles used MALDI-TOF and amino acid sequencing followed by cross-species identification, showing the importance of the development of tandem mass spectrometry. However, the other concern for the future is that as databases increase, most sequences will have one or more potential matches, and for those reasons, other indicators of quality of identification need to be developed.

The technical challenges are many and not only mass spectrometry advances are necessary. Mass spectrometry revolutionized the science of proteomics, but much progress is still needed in other directions such as protein denaturation, purification and quantification techniques, since high sample quality is still imperative for any proteomic study. Furthermore, due to the complexity of these mixtures, and depending on the nature of the perturbations and the organisms, posttranslational modifications are likely to be important. On the other end of analysis, bioinformatics methods coupled with computational power are a major need. Most proteomics experiments can generate incredibly large amounts of data, and file transfer, extraction and/or conversions can be complicated. Software packages that combine all analyses in an interpretable way are not always compatible with the most current technologies.

In this study, we learned that we can combine proteomics approaches to obtain complementary information about a metaorganism. We showed that some types of data can only be obtained by the use of other "-omics" approaches and that all the information can be ultimately combined to demonstrate the acquired knowledge in the field. More specifically, the metaproteomics study of an unsequenced mixed culture led to more complex questions regarding metal resistance and adaptation in bacteria, and how one bacterial species in the culture responded as a member of the metaorganism. Studies of this kind are valuable tools for systems biology, since they can serve as input for future cellular models, and each independent component of this work can be used to model different behaviors and possibly used for training of neural networks. Once further modeling progress is reached, there will be a possibility of applying this information to molecular characterization of other metaorganisms subjected to metal shocks and predict their behavior. Proteomics is used here

as a stepping stone for a much larger wave of molecular work to be produced soon and that will span a variety of sciences combined to reach a unique goal of predicting organismal behavior at the molecular level, both with a static and dynamic view.

Proteomics, however, is a technology that offers a unique view of current status of cells in a certain point in time. Its application to environmental research, much discussed here, still needs progress towards field applications, and the possibility of coupling this technology with metabolic engineering is just one door leading to efficient bioremediation, or any type of contamination remediation process. Another field with large potential for metaproteomics research is the study of acid mine drainage remediation, where very complex communities of organisms are actively participating on reducing the toxic effects of these zones. Other possibilities are industrial metaproteomics, with a slightly different focus, not related to substrate remediation, but instead with the formation of a product. Examples of these are in general fermentations and especially in the biofuel industry. Proteomics has a great potential in these areas, possibly leading to better process control. And, in the fundamental sciences, where metaproteomics has seen its major applications, there are still many areas to be explored, such as microbial ecology of several complex environments, with different colonizing microbes (e.g., tundra soils, polluted seawaters, etc.). The future of the field of biomedical metaproteomics can be especially bright if the necessary advances are made. Currently, in the medical field, the best way to study diseases is by separation of healthy and diseased cells/tissues, to prevent "contamination" of the proteome. But it is known that a number of diseases affect different tissues and cell types, thus metaproteomics can prove a unique tool in demonstrating how much cell isolation is necessary, and also showing unique

intracellular and intercellular (e.g., the case of infections) interactions that are not evident using only "pure" cell studies and other molecular approaches.

A final comment regards the fact that proteomics studies should most definitely be coupled with other technologies. As reviewed in the earlier chapters, the combination of proteomics and transcriptomics usually presents the most complete results in gene expression, and overall is a very powerful tool. We also believe that there are other aspects (inside the metaproteomics field) that can be an essential addition, such as the study of the secretome of communities, or cellular mixtures. The secretome should contain unique proteins that are exported from the cells with very specific functions. The combination of proteomics and metabolomics is another option, which can be coupled to further explain pathway regulation and metabolites that are synthesized in parallel with proteins. Other studies of interest might involve the use of targeted structural proteomics, possibly gene knockouts and other more focused methods that do not use the "survey" procedure, common in –omics technologies. These could be a great complement to more specific case studies, where one cellular activity is known to be present, but not everything related to the cascade of proteomic events is clear. In such case, these more targeted technologies will explain specific proteome features that are not observable using only the functional proteomics technologies. These approaches are the most attractive options in the field of systems and synthetic biology, ultimately leading to organismal models spanning from genomes to metabolic fluxes, incorporating all the necessary information to predict cellular behavior when facing all possible perturbations. This field is in its early infancy, and there is still much to be seen.

List of abbreviations

1DE	One-dimensional electrophoresis
2DE	Two-dimensional electrophoresis
ABC	ATP-binding cassette
AMT	Accurate mass and time
ATP	Adenosine 5'-triphosphate
BCA	Bicinchonic acid
BLAST	Basic local alignment search tool
C18	Octadecyl hydrophobic reverse phase
CID	Collision-induced dissociation
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
FTICR	Fourier transform ion cyclotron resonance
gi	GenInfo identifier
IAA	Iodoacetamide
ICAT	Isotope-coded affinity tag
ID	Identification
IEF	Isoelectric focusing
IPG	Immobilized pH gradient
iTRAQ	Isobaric tags for relative and absolute quantification

KEGG	Kyoto encyclopedia of genes and genomes
LC	Liquid chromatography
MALDI	Matrix-assisted laser desorption and ionization
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NCBInr	National center for biotechnology information non-redundant databse
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PTM	Posttranslational modification
Q/TOF	Quadrupole time-of-flight
rDNA	Ribosomal deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SCX	Strong cation exchange
SDS	Sodium dodecyl sulfate
SILAC	Stable isotope labeling with amino acids in culture
TCA	Tricarboxylic acid
TCEP	Tris-2-carboxyethyl phosphine
TFA	Trifluoroacetic acid
TOF/TOF	Tandem time-of-flight
TPR	Tetratrico peptide repeat

Appendix I:

Peptide summary for all proteins identified in the quantitative metaproteomics of a model culture. (pages 153 to 163)

gi|26990379 Mass: 88830 Score: 113 Queries matched: 8 emPAI: 0.08
catalase/peroxidase HPI [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.419	6	1.160
	116/114	2.704	6	1.275
	117/114	2.212	6	1.132

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1766	923.1251	2766.3534	2766.3470	0.0063	0	77	0.00011	-3.073	-144.755	-138.659	R.VDASQEQTDVESFAVLEPLADGFR.N
1784	941.1563	2820.4469	2820.4456	0.0013	0	36	1.2	-5.086	-97.164	-72.913	R.FIYYVGPVDPVGDEVVGPAGPTTATR.M

gi|15598965 Mass: 51577 Score: 97 Queries matched: 13 emPAI: 0.04
inositol-5-monophosphate dehydrogenase [Pseudomonas aeruginosa PAO1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.157	9	1.783
	116/114	1.104	9	1.309
	117/114	0.862	9	1.478

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1530	748.7670	2243.2793	2243.1953	0.0840	0	85	1.7e-005	0.004	3.090	2.410	R.GIELNIPLVSAAMDTVTEAR.L
770	380.5449	1138.6129	1138.6104	0.0025	0	12	2.8e+002	---	---	---	R.TYTAADVVR.L

gi|26987220 Mass: 39149 Score: 58 Queries matched: 6 emPAI: 0.08
DNA-directed RNA polymerase alpha subunit [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.355	2	1.464
	116/114	2.670	2	1.472
	117/114	1.576	2	1.402

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1115	526.9553	1577.8441	1577.8357	0.0084	0	37	1	0.266	1.986	1.226	-.MQISVNEFLTPR.H
367	554.8127	1107.6109	1107.6046	0.0064	0	21	3.6e+003	---	---	---	K.ALFESIER.D

gi|26986817 Mass: 36791 Score: 70 Queries matched: 7 emPAI: 0.19
quinone oxidoreductase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.181	2	1.060
	116/114	2.111	4	1.328
	117/114	1.388	4	1.442

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
480	515.8158	1029.6170	1029.6192	-0.0022	0	3	2.3e+003	---	---	---	R.TVGSTVLIP.-
1824	991.1924	2970.5555	2970.5420	0.0135	0	67	0.0011	0.172	2.029	1.607	R.SGLYAPPSLPSGLGTEAAGVVEAVGEGVSR.L

gi|15598148 Mass: 30009 Score: 132 Queries matched: 11 emPAI: 0.37
electron transfer flavoprotein beta-subunit [Pseudomonas aeruginosa PAO1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.100	2	2.019
	116/114	0.882	3	1.136
	117/114	1.090	3	2.007

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1629	818.4758	2452.4057	2452.3659	0.0398	0	72	0.00033	0.154	1.105	1.787	R.EIDGGLQTVALNLPAIVTTDLR.L
1327	623.7078	1868.1016	1868.0975	0.0042	0	60	0.0055	0.418	2.904	3.028	K.GVSAGDQLIAGALAPIK.A

gi|3201828 Mass: 9850 Score: 66 Queries matched: 8 emPAI: 0.35
major outer membrane lipoprotein I [Pseudomonas oleovorans]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.306	2	1.638
	116/114	2.429	2	1.221
	117/114	0.940	2	1.069

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
683	581.8137	1161.6128	1161.6111	0.0016	0	55	0.016	2.236	3.105	1.025	R.LTATEDAAAR.A
903	688.8322	1375.6499	1375.6449	0.0049	0	11	3.9e+002	---	---	---	K.AQQTADEANER.A

gi|148546904 Mass: 46013 Score: 66 Queries matched: 13 emPAI: 0.23
2-oxoglutarate dehydrogenase, E2 subunit, dihydrolipoamide succinyltransferase [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.501	2	4.629

	116/114	0.429	2	4.440								
	117/114	0.775	2	3.631								
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide	
97	365.7467	729.4789	729.4758	0.0030	0	12	2e+002	---	---	---	R.LLLDI.-	
1921	1214.6082	4854.4035	4854.3917	0.0118	0	54	0.011	-8.756	-5.200	-26.696	K.GEGDTVLSDELLGSIVEGGAAAAPAAAAAPAAAPAAASADAGEDDPIAAPAAI	

gi|26988214 Mass: 53323 Score: 56 Queries matched: 3 emPAI: 0.06
betaine aldehyde dehydrogenase, putative [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.329	3	1.582							
	116/114	1.840	3	1.399							
	117/114	1.445	3	1.631							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1450	710.7173	2129.1300	2129.1126	0.0174	0	56	0.014	0.771	3.834	4.012	K.APVLVFDDADIDAAIDGIR.T
1672	852.7964	2555.3673	2555.3427	0.0247	0	59	0.0068	-6.929	-52.937	-21.839	R.EVFLIEEPMAAAIGAGLPVEEAR.G

gi|26990146 Mass: 42226 Score: 99 Queries matched: 10 emPAI: 0.25
4-hydroxyphenylpyruvate dioxygenase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.119	2	2.887							
	116/114	0.838	2	1.405							
	117/114	1.232	2	1.474							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1339	717.3416	2149.0028	2148.9982	0.0047	0	37	0.96	2.473	1.206	1.851	R.FMTAPPQTYYEMLEER.L
1380	738.3927	2212.1563	2212.1531	0.0032	0	(42)	0.34	0.304	0.616	0.859	R.ALELGAQPVEIETGPMELR.L

gi|26991351 Mass: 58310 Score: 78 Queries matched: 10 emPAI: 0.06
methylmalonate semialdehyde dehydrogenase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	2.364	3	NN							
	116/114	1.765	3	1.136							
	117/114	1.521	3	1.217							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
887	523.9602	1568.8588	1568.8644	-0.0056	0	18	82	---	---	---	R.DIVNPATQEVLAR.V
1178	630.6826	1889.0258	1889.0241	0.0017	0	60	0.0054	1.445	1.419	1.346	R.TQTINALVGAAFGAAGQR.C

gi|6855333 Mass: 56334 Score: 62 Queries matched: 5 emPAI: 0.06
glutamine synthetase [Pseudomonas syringae pv. tabaci]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.653	3	4.231							
	116/114	2.744	3	2.577							
	117/114	1.653	3	2.317							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
991	558.2795	1671.8168	1671.8236	-0.0068	0	15	1.5e+002	---	---	---	R.DALEDDFFEVGK.M
1158	622.3432	1864.0078	1864.0039	0.0039	0	47	0.1	0.074	1.281	0.955	R.LVPGFEAPVMLAYSAR.N

gi|104780737 Mass: 16435 Score: 99 Queries matched: 9 emPAI: 0.45
organic hydroperoxide resistance protein [Pseudomonas entomophila L48]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.370	2	1.730							
	116/114	2.001	2	1.568							
	117/114	1.284	2	1.795							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
676	449.2704	1344.7893	1344.7856	0.0037	0	39	0.68	-16.014	-14.845	-5.424	K.ALPADASITAK.V
999	560.6424	1678.9054	1678.9012	0.0042	0	60	0.0053	2.359	3.191	2.270	K.VTPLYIAEATSTGGR.D

gi|26987711 Mass: 11253 Score: 63 Queries matched: 4 emPAI: 0.31
DNA-binding protein HU, form N [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	---	1	---							
	116/114	0.331	2	3.280							
	117/114	4.275	2	2.727							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
857	510.9694	1529.8864	1529.8657	0.0208	0	1	4.2e+003	---	---	---	R.NPSTGAAIEIAAK.K

1147	615.0201	1842.0386	1842.0342	0.0044	0	62	0.0032	0.404	1.211	13.889	K.DQLIADIAESIAAPK.A 1145 1146

gi|32028588 Mass: 55436 Score: 121 Queries matched: 16 emPAI: 0.41
COG0055: F0F1-type ATP synthase, beta subunit [Haemophilus somnus 2336]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.506	5	1.642
	116/114	2.200	6	1.174
	117/114	1.165	6	1.147

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
909	532.3033	1593.8880	1593.8848	0.0032	0	54	0.02	-0.034	1.437	0.837	R.YTLAGTEVSALLGR.M 905 906 907 908 910
1498	817.4161	2449.2266	2449.2103	0.0163	0	67	0.00094	-9.390	-54.515	-37.467	R.MPSAVGYQPTLAEEMGVLQER.I 1499 1500

gi|26992092 Mass: 18663 Score: 47 Queries matched: 6
ATP synthase subunit B [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.554	1	---
	116/114	1.737	2	---
	117/114	1.025	2	---

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
801	486.9573	1457.8502	1457.8446	0.0056	0	20	46	---	---	---	R.AQVGALAVGGAEK.I 800
1190	635.0170	1902.0291	1902.0301	-0.0011	0	27	10	---	---	---	K.AQALAEIEQELNSAK.D 1191 1192

gi|126359797 Mass: 62562 Score: 103 Queries matched: 30 emPAI: 0.59
chaperonin GroEL [Pseudomonas putida GB-1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.686	12	1.705
	116/114	4.518	14	1.341
	117/114	4.125	14	1.335

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
945	544.9897	1631.9472	1631.9524	-0.0052	0	62	0.0032	0.169	2.682	1.605	K.MLVGVNVLADAVK.A
1049	575.3334	1722.9783	1722.9750	0.0033	0	41	0.39	0.759	1.367	0.828	R.AAVEEGVVPGGGVALVR.A

gi|26991177 Mass: 14704 Score: 91 Queries matched: 10 emPAI: 0.87
pterin-4-alpha-carbinolamine dehydratase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.27	2	1.191
	116/114	2.217	3	1.673
	117/114	1.269	3	1.655

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
761	707.8765	1413.7384	1413.7374	0.0010	0	39	0.55	-16.022	-14.550	-8.173	R.EIPDWNIEVR.D
781	477.9329	1430.7768	1430.7738	0.0029	0	52	0.031	-0.012	4.875	2.580	K.VTDEELAELIR.E

gi|26988503 Mass: 69800 Score: 100 Queries matched: 20 emPAI: 0.20
30S ribosomal protein S1 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.803	4	1.477
	116/114	0.789	4	1.479
	117/114	0.688	4	1.451

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1620	539.9456	1616.8148	1616.8103	0.0046	0	29	5.8	0.520	0.440	0.382	K.QMGEDPWVAITAR.Y
1826	587.3138	1758.9195	1758.9056	0.0139	0	71	0.0004	-11.742	-23.103	-16.406	K.NAPEAAADTTMAALLR.E

gi|26987198 Mass: 32959 Score: 67 Queries matched: 4 emPAI: 0.10
50S ribosomal protein L2 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.774	2	1.426
	116/114	9.358	2	1.704
	117/114	6.952	2	1.445

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
116	397.2391	792.4637	792.4616	0.0021	0	5	1.2e+003	---	---	---	R.LVDFR.R
1934	623.7078	1868.1015	1868.0975	0.0040	0	62	0.0033	1.135	14.801	9.807	K.GVSAGDQLIAGALAPIK.A

gi|29839337 Mass: 63371 Score: 106 Queries matched: 31 emPAI: 0.29
60 kDa chaperonin (Protein Cpn60) (groEL protein)

Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.794	5	3.787
116/114	6.108	5	NN
117/114	5.151	5	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1421	490.6481	1468.9226	1468.9108	0.0117	0	52	0.032	-12.582	-14.492	-58.129	R.ELLPVLEAVAK.S
1617	539.6608	1615.9605	1615.9575	0.0030	0	54	0.018	0.480	5.251	3.013	K.MLVGVNVLADAVK.A

gi|26990810 Mass: 52436 Score: 86 Queries matched: 16 emPAI: 0.13
isocitrate lyase [Pseudomonas putida KT2440]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.266	4	1.363
116/114	1.427	5	NN
117/114	0.446	5	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1523	518.5980	1552.7722	1552.7977	-0.0254	0	26	11	---	---	---	K.LQEQEFADASK.G
1664	820.4980	1638.9815	1638.9790	0.0025	0	60	0.0053	0.101	1.486	0.426	R.LAADVSGVPTIILAR.T

gi|13310130 Mass: 29810 Score: 54 Queries matched: 8 emPAI: 0.11
FadB1x [Pseudomonas putida]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	2.323	2	8.391
116/114	8.594	2	1.062
117/114	3.917	2	1.118

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1968	427.2444	1278.7114	1278.7283	-0.0169	0	10	4.4e+002	---	---	---	K.SIPVSMMVK.E
3465	576.3464	1726.0175	1726.0120	0.0055	0	44	0.21	4.184	9.052	4.299	R.IVPQAELVEEALK.V

gi|13096652 Mass: 41550 Score: 65 Queries matched: 17 emPAI: 0.17
Chain A, Catabolic Ornithine Carbamoyltransferase From Pseudomonas Aeruginosa
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	4.802	2	2.680
116/114	22.567	2	2.379
117/114	17.996	2	2.513

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1653	602.7945	1203.5744	1203.5716	0.0029	0	22	31	---	---	---	R.MYDAIEYR.G
3190	544.9832	1631.9276	1631.9272	0.0004	0	43	0.23	2.668	14.077	10.664	R.NNMGNSLLLIGAK.L

gi|26991567 Mass: 49916 Score: 75 Queries matched: 5 emPAI: 0.07
adenylosuccinate synthetase [Pseudomonas putida KT2440]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	1.119	3	1.556
116/114	4.212	3	1.291
117/114	1.782	3	1.232

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2315	488.3156	1461.9248	1461.9153	0.0095	0	26	15	---	---	---	K.TVLHLIPSGILR.E
2682	550.9959	1649.9659	1649.9586	0.0073	0	49	0.057	2.151	6.368	2.589	K.IVDLLTEHAAAVVR.Y

gi|26991410 Mass: 76920 Score: 77 Queries matched: 20 emPAI: 0.04
molecular chaperone DnaK [Pseudomonas putida KT2440]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.910	2	41.943
116/114	5.514	2	1.489
117/114	7.782	2	1.508

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1574	400.8896	1199.6469	1199.6430	0.0039	0	15	1.3e+002	---	---	---	K.EVADFFGK.E
2850	582.0228	1743.0467	1743.0385	0.0081	0	62	0.0031	0.003	4.352	6.082	K.TAIEAAVVALEAAVK.G

gi|26987187 Mass: 14753 Score: 72 Queries matched: 15 emPAI: 0.23
50S ribosomal protein L7/L12 [Pseudomonas putida KT2440]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	---	1	---
116/114	7.154	2	1.242
117/114	9.765	2	1.018

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2243	477.6431	1429.9075	1429.8999	0.0075	0	56	0.013	-3.410	-64.305	-110.039	K.TVLEVVELIK.A
3244	512.0460	2044.1550	2044.1530	0.0020	1	16	1.3e+002	---	---	---	K.EKVDGAPQVVAEGVSK.E

gi|119856883 Mass: 62836 Score: 74 Queries matched: 65 emPAI: 0.43
chaperonin GroEL [Pseudomonas putida W619]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.419	5	NN
	116/114	4.344	6	1.193
	117/114	2.637	6	1.263

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
581	466.3116	930.6086	930.6106	-0.0020	0	37	0.94	0.754	4.511	2.087	R.NVVLAK.S
3746	673.1036	2688.3852	2688.4174	-0.0322	0	37	0.99	1.647	6.480	5.274	K.ANDAAGDGTTTATVLAQAIVNEGLK.A

gi|119858954 Mass: 67694 Score: 98 Queries matched: 25 emPAI: 0.21
ribosomal protein S1 [Pseudomonas putida W619]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.795	6	1.793
	116/114	6.014	5	1.303
	117/114	4.813	6	1.415

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2932	606.6895	1817.0465	1817.0390	0.0076	0	59	0.0065	0.399	6.028	3.545	K.GAIVTLADDIEATLK.A
3600	594.5613	2374.2160	2374.1896	0.0264	0	39	0.68	2.631	9.740	7.570	K.VGDEVHVALDAVEDGFGETK.L

gi|26987192 Mass: 85627 Score: 65 Queries matched: 31 emPAI: 0.08
elongation factor G [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	---	1	---
	116/114	0.181	2	1.400
	117/114	0.091	2	1.098

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
3739	892.8654	2675.5742	2675.5757	-0.0015	0	49	0.059	---	---	---	K.GVPLVLDAVIDFLPAPTEIPAIK.G
3848	613.3661	3061.7941	3061.8157	-0.0216	1	16	1e+002	0.154	0.146	0.095	K.NKGVPLVLDAVIDFLPAPTEIPAIK.G

gi|26988324 Mass: 34540 Score: 103 Queries matched: 36 emPAI: 0.73
elongation factor Ts [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.611	5	1.297
	116/114	13.897	6	1.748
	117/114	7.349	5	1.338

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
3787	705.1417	2816.5378	2816.5285	0.0093	0	39	0.65	5.012	10.494	14.317	K.VGEGIEKPVDDFAAEVAAQVAAAK.Q
3835	737.1172	2944.4399	2944.4358	0.0041	0	64	0.0018	7.169	3.421	5.359	K.NIAMHVAASNPEFLDSSEISAEAIER.E

gi|17547847 Mass: 58670 Score: 80 Queries matched: 3 emPAI: 0.06
PUTATIVE NAD+ DEPENDENT ACETALDEHYDE DEHYDROGENASE OXIDOREDUCTASE PROTEIN [Ralstonia solanacearum GMI1000]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.623	2	1.080
	116/114	0.887	2	1.466
	117/114	0.829	2	1.416

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
935	513.9341	1538.7806	1538.7711	0.0095	0	38	0.86	-7.148	-3.582	-3.687	R.ALDAAHAAADAWGR.T
1492	615.3447	1843.0124	1843.0004	0.0119	0	42	0.31	-6.797	-4.704	-5.039	K.EVLMESIDIHELK.A

gi|148546266 Mass: 42569 Score: 72 Queries matched: 3 emPAI: 0.08
glycine cleavage system T protein [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.305	3	1.324
	116/114	1.263	3	1.239
	117/114	1.260	3	1.241

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1233	573.3097	1716.9072	1716.9069	0.0003	0	31	3.7	0.944	0.897	0.942	R.AASFPGAETIFAHVR.D
2753	821.1274	2460.3603	2460.3589	0.0014	0	41	0.43	0.677	1.270	0.954	K.LKPSNPAELDNLLDAAGYK.A

gi|15599890 Mass: 67727 Score: 99 Queries matched: 8 emPAI: 0.10
acetolactate synthase III large subunit [Pseudomonas aeruginosa PAO1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.124	2	7.529
	116/114	2.106	2	4.970
	117/114	1.802	2	5.021

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1220	855.5078	1709.0010	1708.9967	0.0043	0	61	0.0036	-5.462	-9.150	-7.798	K.IIHIDIDPASISK.T
2897	636.0893	2540.3281	2540.3301	-0.0020	0	38	0.88	-6.822	-4.629	-4.891	K.AIAQAHPDALETLGFAAGSMGPK.V

gi|26991831 Mass: 48242 Score: 51 Queries matched: 4 emPAI: 0.07
D-3-phosphoglycerate dehydrogenase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.353	3	1.557
	116/114	0.792	3	1.530
	117/114	0.340	3	1.377

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
431	419.5801	1255.7185	1255.7159	0.0026	0	9	5.1e+002	---	---	---	K.IADAHFIGIR.S
3396	708.1371	2828.5195	2828.5173	0.0022	1	42	0.33	0.254	0.394	0.209	K.AAGYTNIEYLTGSLPEAELKEK.I

gi|26987820 Mass: 23439 Score: 60 Queries matched: 10 emPAI: 0.71
antioxidant, AhpC/Tsa family [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.150	5	1.250
	116/114	3.075	5	1.225
	117/114	1.996	5	1.183

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1180	563.2910	1686.8510	1686.8457	0.0053	0	22	34	0.844	1.638	1.385	K.AYDVESEGGVAFR.G
1476	610.6339	1828.8799	1828.8765	0.0034	0	38	0.78	1.447	3.922	2.564	K.YTLAADMTHEICK.A

gi|148547677 Mass: 23695 Score: 59 Queries matched: 32 emPAI: 0.49
heat shock protein Hsp20 [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.370	3	1.856
	116/114	1.377	3	1.789
	117/114	4.056	3	1.611

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
444	316.7167	1262.8376	1262.8318	0.0058	0	30	5.5	---	---	---	K.GVLLVHLPK.R
1051	536.6042	1606.7909	1606.7862	0.0048	0	29	5.7	1.549	0.647	2.344	R.HQIDSLFEDFGR.R

gi|148549005 Mass: 9729 Score: 64 Queries matched: 15 emPAI: 2.38
acyl carrier protein [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.561	4	1.476
	116/114	2.991	4	1.468
	117/114	3.622	4	1.339

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1054	537.3179	1608.9320	1608.9330	-0.0011	0	(34)	2.2	0.409	1.595	2.426	K.ITTVQAAIDYVK.A
2479	782.7898	2345.3476	2345.3419	0.0057	1	30	5.3	1.805	9.022	9.317	K.IVAEQLGVKEEEVTIEK.S

gi|26990879 Mass: 55109 Score: 78 Queries matched: 13 emPAI: 0.12
dihydrolipoamide dehydrogenase [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.173	4	1.176
	116/114	0.811	5	1.507
	117/114	0.463	5	1.469

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2126	730.3906	2188.1500	2188.1457	0.0043	0	45	0.15	1.000	0.368	0.281	R.RPVTTDLLASDSGVTIDER.G
3344	692.3730	2765.4631	2765.4592	0.0039	1	33	2.7	1.898	1.974	0.946	K.TEQALKAEGVEVNVGTFPFAASGR.A

gi|148546001 Mass: 23637 Score: 71 Queries matched: 18 emPAI: 0.49
ribosomal 5S rRNA E-loop binding protein Ctc/L25/TL5 [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.765	7	1.396

	116/114	0.697	7	1.353								
	117/114	0.852	7	1.378								
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide	
674	469.9185	1406.7336	1406.7251	0.0086	0	25	14	0.670	0.654	0.811	K.QFIMHADFVR.V	
1101	549.3013	1644.8822	1644.8715	0.0106	0	46	0.13	0.764	0.954	1.454	K.GVEFVALAHGDDK.A	

gi|26987203 Mass: 8308 Score: 55 Queries matched: 14 emPAI: 1.02
50S ribosomal protein L29 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.947	5	2.108							
	116/114	1.829	5	1.827							
	117/114	2.362	5	1.781							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1267	576.3359	1725.9858	1725.9859	-0.0001	0	17	1e+002	---	---	---	K.SAQQLNEQLLGLLR.D
1527	619.0266	1854.0580	1854.0567	0.0013	0	38	0.75	3.928	4.760	6.877	K.ATGQLGQSHLLSQVK.R

gi|129036 Mass: 112324 Score: 110 Queries matched: 3 emPAI: 0.03
2-oxoglutarate dehydrogenase E1 component (Alpha-ketoglutarate dehydrogenase)

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.660	3	1.071							
	116/114	0.652	3	1.242							
	117/114	0.951	3	1.272							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1509	616.3224	1845.9455	1845.9343	0.0112	0	51	0.039	0.677	0.544	0.884	R.TIGAEFTHIVDSEQR.N
1602	629.0428	1884.1065	1884.1036	0.0029	1	59	0.0058	0.292	1.272	0.465	K.GIDKQLVGQVAAEIR.D

gi|26991516 Mass: 18529 Score: 76 Queries matched: 16 emPAI: 0.65
hypothetical protein PP_4836 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.602	3	1.083							
	116/114	1.657	3	1.201							
	117/114	4.874	3	1.121							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2493	588.5720	2350.2590	2350.2543	0.0047	0	21	36	---	---	---	K.DLTFAPGAYHVMLMQPK.D
3528	753.8815	3011.4968	3011.4936	0.0032	0	55	0.014	1.454	1.424	4.566	R.LLSVDSPISDDAQLHEHAMSATGAMK.M

gi|15600516 Mass: 34613 Score: 54 Queries matched: 4 emPAI: 0.10
acetylglutamate kinase [Pseudomonas aeruginosa PAO1]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.923	2	1.074							
	116/114	1.401	2	1.403							
	117/114	0.824	2	1.453							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2834	621.8686	2483.4453	2483.4580	-0.0128	1	26	13	0.975	1.012	0.571	K.AVGINPVVVHGGGPQIGDLLKR.L
1439	550.9955	1649.9646	1649.9586	0.0060	0	28	8.1	5.503	3.800	3.354	K.IVDLLTEHAAAVVR.Y

gi|26989186 Mass: 8714 Score: 50 Queries matched: 11 emPAI: 0.96
cold shock protein CspA [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.647	3	2.700							
	116/114	1.146	3	2.421							
	117/114	2.144	3	NN							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
61	418.2668	834.5191	834.5207	-0.0016	0	22	43	---	---	---	K.VPGFK.A
2748	608.3298	2429.2902	2429.2746	0.0157	0	28	1.1e+002	---	---	---	K.GYGFITPAGGGDDLFVHFK.A

gi|148546265 Mass: 8826 Score: 54 Queries matched: 15 emPAI: 1.71
putative cold-shock DNA-binding domain protein [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.735	2	1.693							
	116/114	0.317	2	1.403							
	117/114	0.408	2	2.115							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
371	366.2157	1095.6252	1095.6168	0.0084	0	26	70	---	---	---	R.AIEGSGFK.S

2301	546.5309	2182.0947	2182.0970	-0.0023	0	28	7.7	-5.651	-13.397	-13.277	K.GYGFITPESGPDLFVHFR.A

gi|148548757 Mass: 33831 Score: 54 Queries matched: 6 emPAI: 0.10
UspA domain protein [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.340	2	2.530
	116/114	1.236	2	1.842
	117/114	0.931	2	1.727

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1292	518.6258	1552.8556	1552.8331	0.0225	0	15	1.4e+002	---	---	---	K.SEEAIAHLAELAR.G
1448	415.4937	1657.9459	1657.9386	0.0073	0	39	0.69	3.627	2.614	1.850	R.SILVVLDPAHAHSR.A

gi|26989027 Mass: 10829 Score: 59 Queries matched: 35 emPAI: 2.01
DNA-binding protein HU-beta [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.487	3	2.097
	116/114	0.962	3	2.084
	117/114	1.042	3	1.501

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1497	559.0039	1673.9899	1673.9807	0.0092	0	26	13	-7.204	-7.216	-8.976	R.ALDAVIESVTGALK.Q
1720	601.3406	1801.0001	1801.0076	-0.0075	0	33	2.5	3.990	1.496	1.827	K.SELIDAIAASADIPK.A

gi|15599466 Mass: 162732 Score: 66 Queries matched: 8 emPAI: 0.04
DNA-directed RNA polymerase beta subunit [Pseudomonas aeruginosa PAO1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	---	1	---
	116/114	0.875	2	1.451
	117/114	1.119	2	1.462

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
777	428.9598	1283.8577	1283.8533	0.0044	1	26	29	1.578	1.178	1.513	R.KLPASVLLR.A
1991	496.5395	1982.1289	1982.1226	0.0062	0	40	0.49	-0.109	0.584	0.740	R.MNVGQILETHLGLAAK.G

gi|26987189 Mass: 168078 Score: 57 Queries matched: 4 emPAI: 0.02
DNA-directed RNA polymerase beta' subunit [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.191	3	1.352
	116/114	3.648	3	1.541
	117/114	3.946	3	1.340

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1229	507.3203	1518.9391	1518.9533	-0.0142	1	15	2.4e+003	---	---	---	-.MKDLLNLLK.N
1701	596.9963	1787.9672	1787.9651	0.0020	0	42	0.33	4.127	3.723	3.706	R.ELLHAIDLEHEIGR.L

gi|26987920 Mass: 23263 Score: 57 Queries matched: 14 emPAI: 0.14
outer membrane protein H1 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.433	2	1.641
	116/114	1.233	2	1.479
	117/114	0.503	2	1.034

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1355	533.2911	1596.8516	1596.8464	0.0052	0	37	1.1	0.683	1.802	0.522	R.TNAATEVGAHGGPK.D
3133	667.6148	2666.4301	2666.4523	-0.0222	1	20	50	---	---	---	K.LRQENLLGSYDLFLPVGDTTK.L

gi|148545498 Mass: 33349 Score: 48 Queries matched: 4 emPAI: 0.10
extracellular solute-binding protein, family 3 [Pseudomonas putida F1]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.933	2	1.320
	116/114	0.647	2	1.041
	117/114	0.955	2	1.360

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
825	439.2594	1314.7563	1314.7499	0.0063	1	20	1.4e+002	---	---	---	K.KDVPQADVR.T
1477	556.6385	1666.8936	1666.8882	0.0054	1	28	8.4	-6.737	-9.331	-6.992	K.AKDTQLAGDAFSR.L

gi|89893980 Mass: 62232 Score: 78 Queries matched: 3 emPAI: 0.05
hypothetical protein DSY1234 [Desulfitobacterium hafniense Y51]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.589	2	1.553
	116/114	1.688	2	1.425
	117/114	1.123	2	1.676

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
3083	528.3070	2636.4987	2636.3876	0.1111	0	38	0.72	1.047	1.225	0.676	K.QLYLASGQHYPDAMVGTVLAAK.N
1991	496.5395	1982.1289	1982.1226	0.0062	0	40	0.49	-0.109	0.584	0.740	R.MNVGQILETHLGLAAK.G

gi|90020610 Mass: 33552 Score: 55 Queries matched: 7 emPAI: 0.10
Single-strand binding protein [Saccharophagus degradans 2-40]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.003	2	2.687
	116/114	1.045	2	1.932
	117/114	1.548	2	2.718

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1233	440.9349	1319.7828	1319.7805	0.0023	0	15	1.7e+002	---	---	---	K.GAPHAPLLEK.K
2170	458.4762	1829.8756	1829.8713	0.0042	0	40	0.54	4.392	1.920	3.410	R.GVAMNPVDHPHGGGEGR.T

gi|26989046 Mass: 9850 Score: 47 Queries matched: 15 emPAI: 0.35
outer membrane lipoprotein Oprl [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.497	2	2.212
	116/114	1.523	2	2.215
	117/114	0.453	2	2.228

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1648	517.9609	1550.8608	1550.8452	0.0156	1	32	3.5	0.140	3.373	1.007	R.KADDAMAAAQK.A
1926	646.3350	1935.9831	1935.9482	0.0348	0	15	1.6e+003	---	---	---	K.GGDLNYDVVIASPDAMR.V

gi|26987185 Mass: 27262 Score: 59 Queries matched: 5 emPAI: 0.12
50S ribosomal protein L1 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	---	1	---
	116/114	2.325	2	1.069
	117/114	6.620	2	1.564

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2047	677.3635	2029.0687	2029.0714	-0.0027	0	54	0.019	-17.977	-67.849	-115.131	R.VAVFTQGPAAEAALAAGADR.V
2192	726.0552	2175.1439	2175.1579	-0.0140	0	5	1.6e+003	---	---	---	R.VTLSTTMGPGLVIDQSSLNV.-

gi|119860710 Mass: 27377 Score: 125 Queries matched: 35 emPAI: 0.77
Small GTP-binding protein domain [Pseudomonas putida W619]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.510	6	1.193
	116/114	2.468	6	1.557
	117/114	5.877	6	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2256	740.3845	2218.1316	2218.1160	0.0155	0	(74)	0.00021	0.259	1.333	6.104	K.ADLVDDAELLELVEMEVR.D
2533	804.0778	2409.2114	2409.2186	-0.0071	0	51	0.041	0.814	6.908	11.701	R.DLLSTYDFPGDDTPIIIGSAR.M

gi|26987139 Mass: 80739 Score: 56 Queries matched: 4 emPAI: 0.04
hypothetical protein PP_0397 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.067	2	1.291
	116/114	1.121	2	1.908
	117/114	0.972	2	1.554

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2105	696.0594	2085.1563	2085.1439	0.0124	0	27	10	0.059	0.744	1.103	R.LLLAIGEPELIDTSTNSR.L
110	398.2076	794.4007	794.3932	0.0075	0	29	4	-20.896	-20.902	-6.938	R.EGGLEF.-

gi|535709 Mass: 10580 Score: 78 Queries matched: 5 emPAI: 0.33
HU protein

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.312	3	1.190
	116/114	6.569	3	1.099
	117/114	16.798	3	1.272

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1493	558.9982	1673.9729	1673.9807	-0.0079	0	31	3.9	---	---	---	R.ALDAVIESVTGALK.A
1645	601.3450	1801.0131	1801.0076	0.0055	0	47	0.11	1.744	7.318	22.128	K.SELIDAIAASADIPK.D

gi|26991396 Mass: 58145 Score: 53 Queries matched: 5 emPAI: 0.06
transcription elongation factor NusA [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.878	2	1.124
	116/114	0.812	2	1.098
	117/114	1.554	2	2.091

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1753	647.6921	1940.0544	1940.0449	0.0095	0	41	0.44	0.752	0.911	3.062	R.DNVIVDLGNNAEALLAR.E
1958	696.3802	2086.1189	2086.1102	0.0088	0	12	3.4e+002	---	---	---	R.IEVPEIAEGLIEVMAASR.D

gi|26990893 Mass: 33667 Score: 60 Queries matched: 4 emPAI: 0.21
electron transfer flavoprotein, alpha subunit [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.185	2	1.025
	116/114	2.404	2	1.203
	117/114	1.602	2	1.121

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2004	731.7143	2192.1211	2192.1140	0.0071	0	21	44	0.182	2.725	1.736	R.AAVDAGFVPNDMQVGQTGK.I
2287	701.4020	2801.5791	2801.5782	0.0009	0	39	0.62	0.190	1.919	1.398	M.TILVVAEYEAGAVAPATLNTVAAAAK.I

gi|26987195 Mass: 26032 Score: 112 Queries matched: 3 emPAI: 0.13
50S ribosomal protein L3 [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.551	2	1.442
	116/114	3.588	2	1.461
	117/114	2.699	2	1.053

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1905	684.7119	2051.1137	2051.1143	-0.0005	0	56	0.014	0.759	4.983	2.846	K.AELFTAGQLVDVTGQSK.G
1309	500.9563	1499.8472	1499.8429	0.0042	0	56	0.013	2.061	4.258	1.608	K.AQALTLTTDLPGR.T

gi|26987361 Mass: 102532 Score: 84 Queries matched: 12 emPAI: 0.10
ATP-dependent Clp protease, ATP-binding subunit ClpB [Pseudomonas putida KT2440]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.501	3	1.910
	116/114	2.869	3	1.378
	117/114	2.857	3	1.327

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1621	593.3199	1776.9378	1776.9202	0.0176	0	24	20	1.002	1.516	1.650	R.MAELQYGVIPDLER.S
1782	653.0272	1956.0597	1956.0537	0.0060	0	60	0.0056	0.122	4.249	4.119	K.VLVEEPSEEDTIAILR.G

gi|26989063 Mass: 101118 Score: 100 Queries matched: 13 emPAI: 0.10
aconitate hydratase [Pseudomonas putida KT2440]

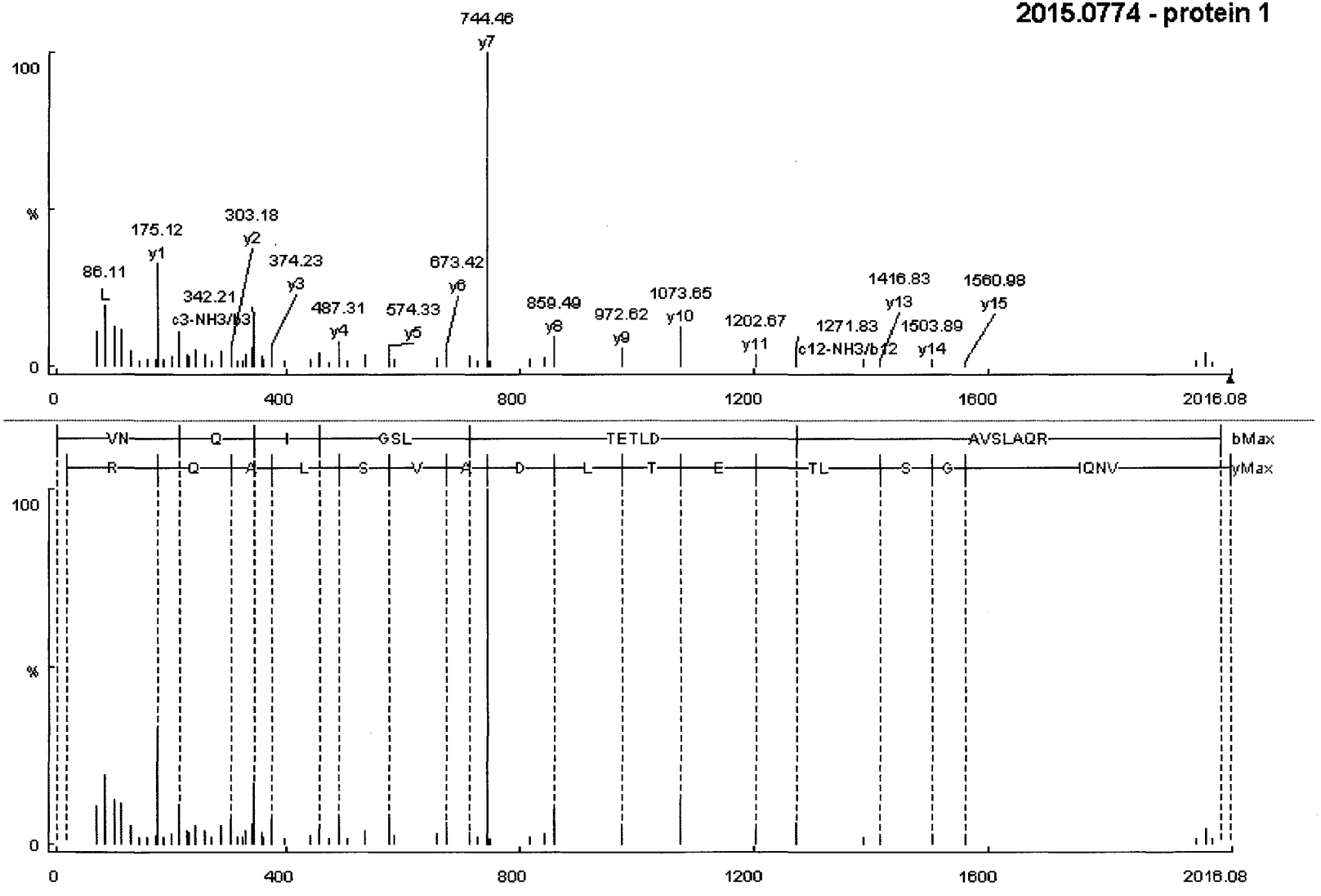
Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.170	3	1.662
	116/114	17.457	3	1.402
	117/114	16.277	3	1.459

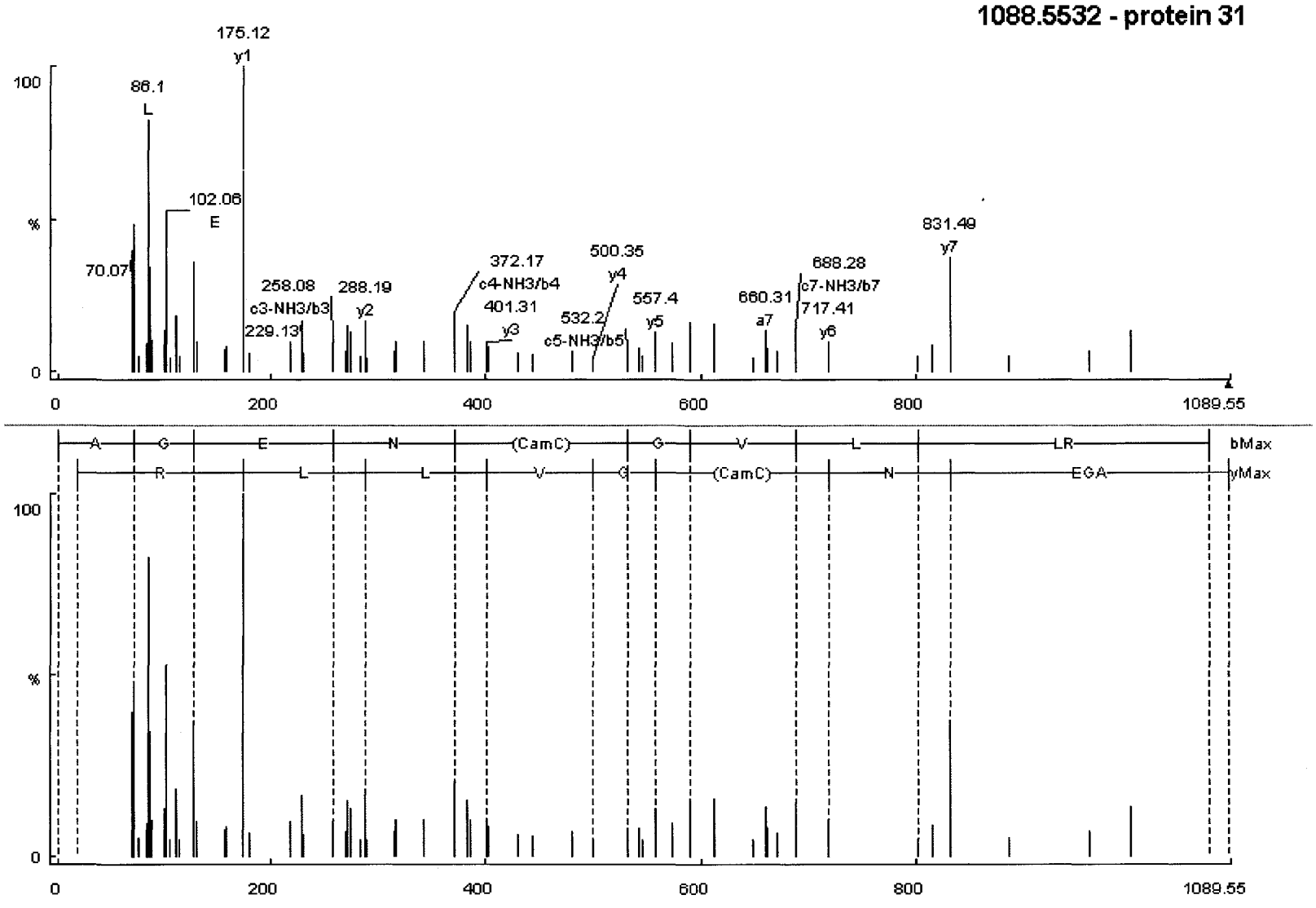
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1650	604.6521	1810.9345	1810.9336	0.0009	0	65	0.0015	11.109	32.831	26.440	K.GFPVAYVGDVVGTGSSR.K
2153	807.7783	2420.3129	2420.3155	-0.0025	0	35	1.6	-9.917	-130.742	-102.099	R.DGIEPQQPGSVGPLAQIEAVK.A

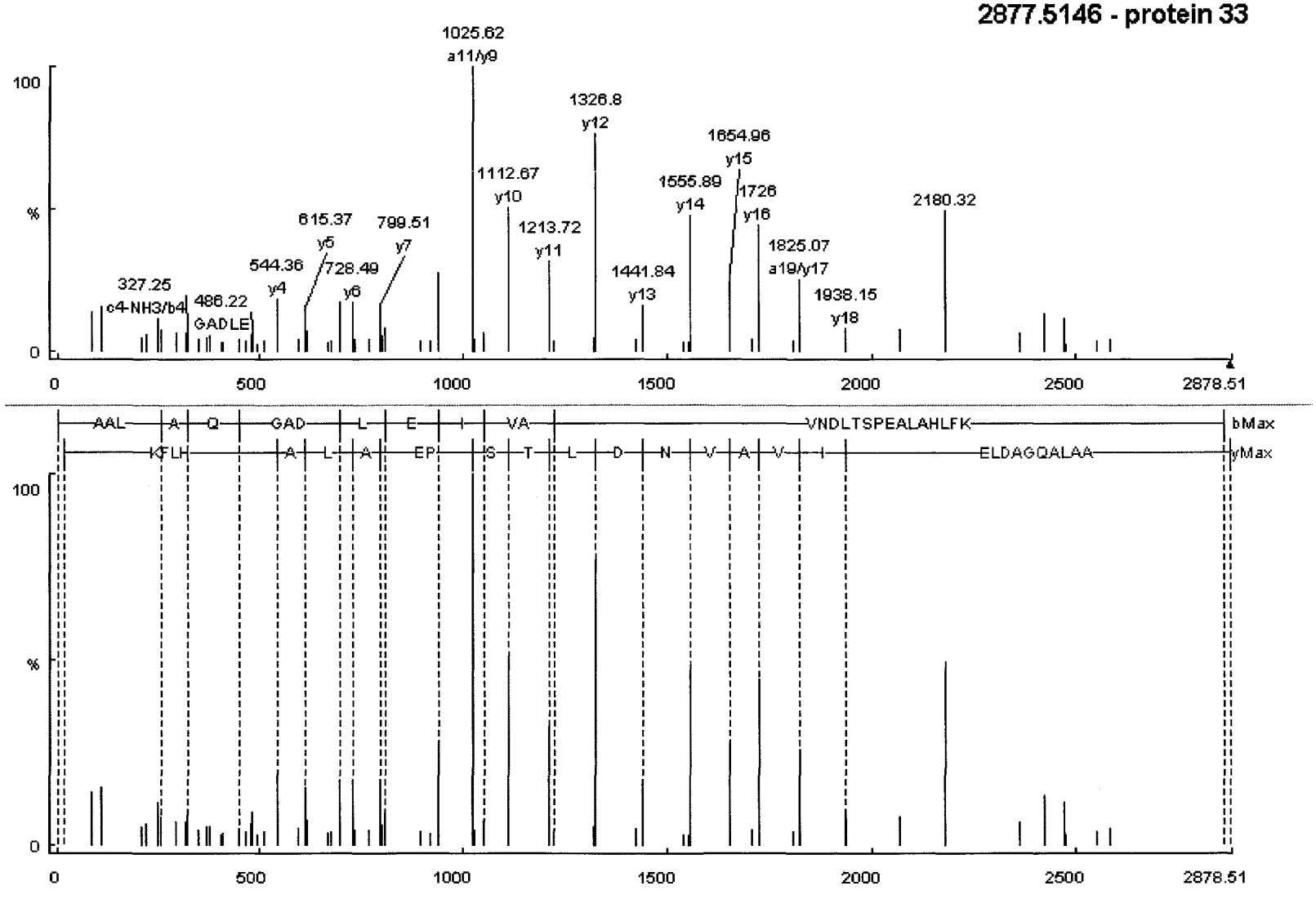
Appendix II:

Part 1: Sample spectra for *de novo* sequencing. Contains spectra with the aligned sequences, y ions (where found) and the corresponding protein information on the top left. (pages 165 to 172)

Part 2: Protein identification table. Contains peptide and protein numbers, protein name, accession and gi numbers, protein and peptide scores, mass errors in Da and ppm, peptide sequence, sequence coverage, protein molecular weight and isoelectric point, organism where protein was first found, trend associated with time course, regulation, theoretical biological process, identification by Mascot or Peaks, and potential for being a false positive. (pages 173 to 199)


2015.0774 - protein 1
744.46
y7
100
%
0
303.18
y2
175.12
y1
374.23
y3
673.42
y6
86.11
L
342.21
c3-NH3/b3
487.31
y4
574.33
y5
859.49
y8
972.62
y9
1073.65
y10
1202.67
y11
1271.83
c12-NH3/b12
1416.83
y13
1503.89
y14
1560.98
y15
0
400
800
1200
1600
2016.08
VN
Q
I
GSL
TETLD
AVSLAQR
bMax
R
Q
A
L
S
V
A
D
L
T
E
TL
S
G
IQNV
yMax
100
%
0
0
400
800
1200
1600
2016.08


1088.5532 - protein 31
175.12
y1
86.1
L
102.06
E
70.07
258.08
c3-NH3/b3
229.13
288.19
y2
372.17
c4-NH3/b4
401.31
y3
500.35
y4
532.2
c5-NH3/b5
557.4
y5
660.31
a7
688.28
c7-NH3/b7
717.41
y6
831.49
y7
100
%
0
0
200
400
600
800
1089.55
A G E N (CamC) G V L LR
R L L V G (CamC) N EGA
bMax
yMax


2877.5146 - protein 33
1025.62
a11/y9
1326.8
y12
1654.96
y15
1112.67
y10
1555.89
y14
1726
y16
615.37
y5
799.51
y7
1213.72
y11
2180.32
544.36
y4
728.49
y6
1441.84
y13
1825.07
a19/y17
327.25
c4-NH3/b4
486.22
GADLE
1938.15
y18
100
%
0
0
500
1000
1500
2000
2500
2878.51
AAL
A
Q
GAD
L
E
I
VA
VNDLTSPEALAHLFK
bMax
KFLH
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L
A
EP
S
T
L
D
N
V
A
V
I
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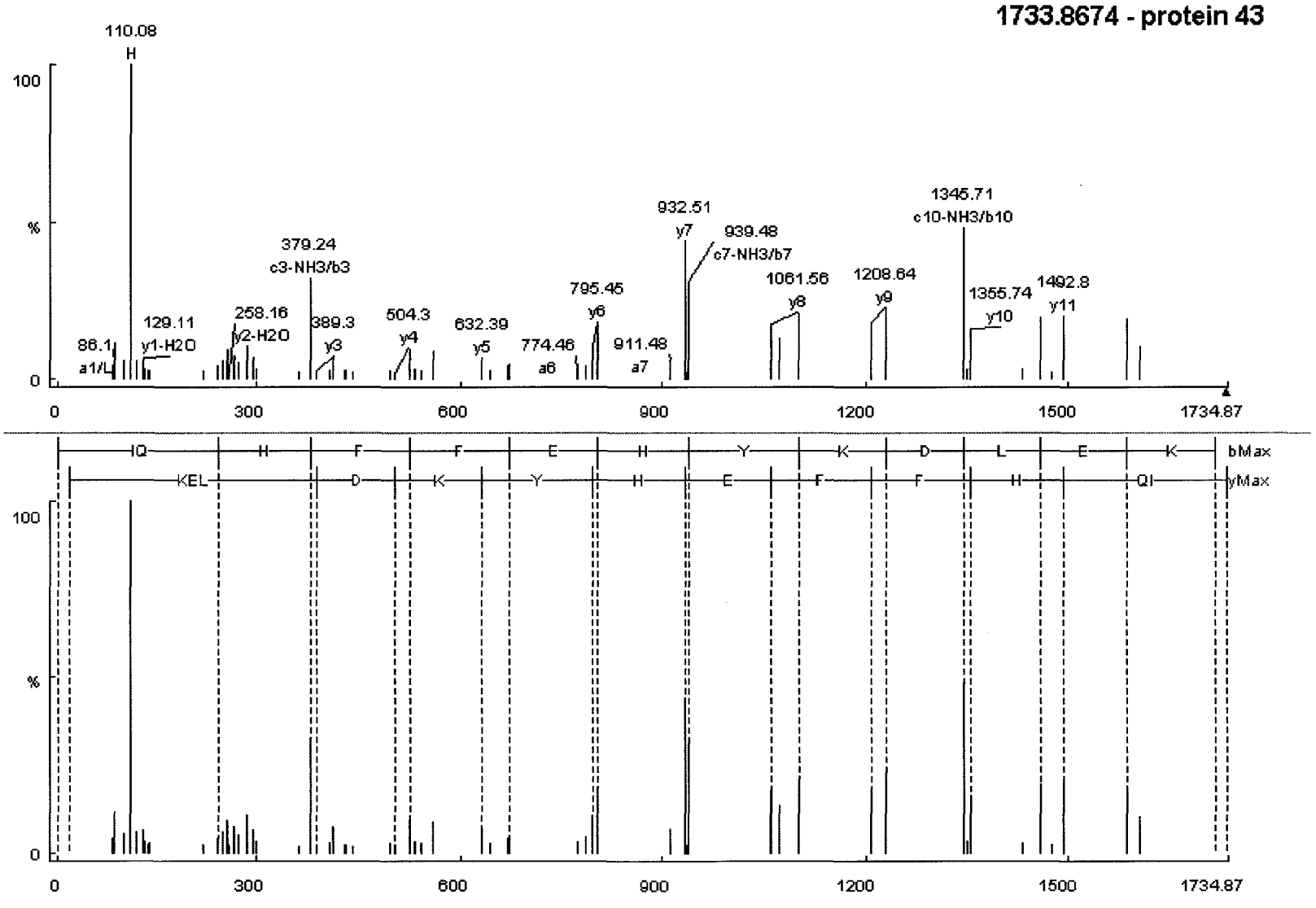
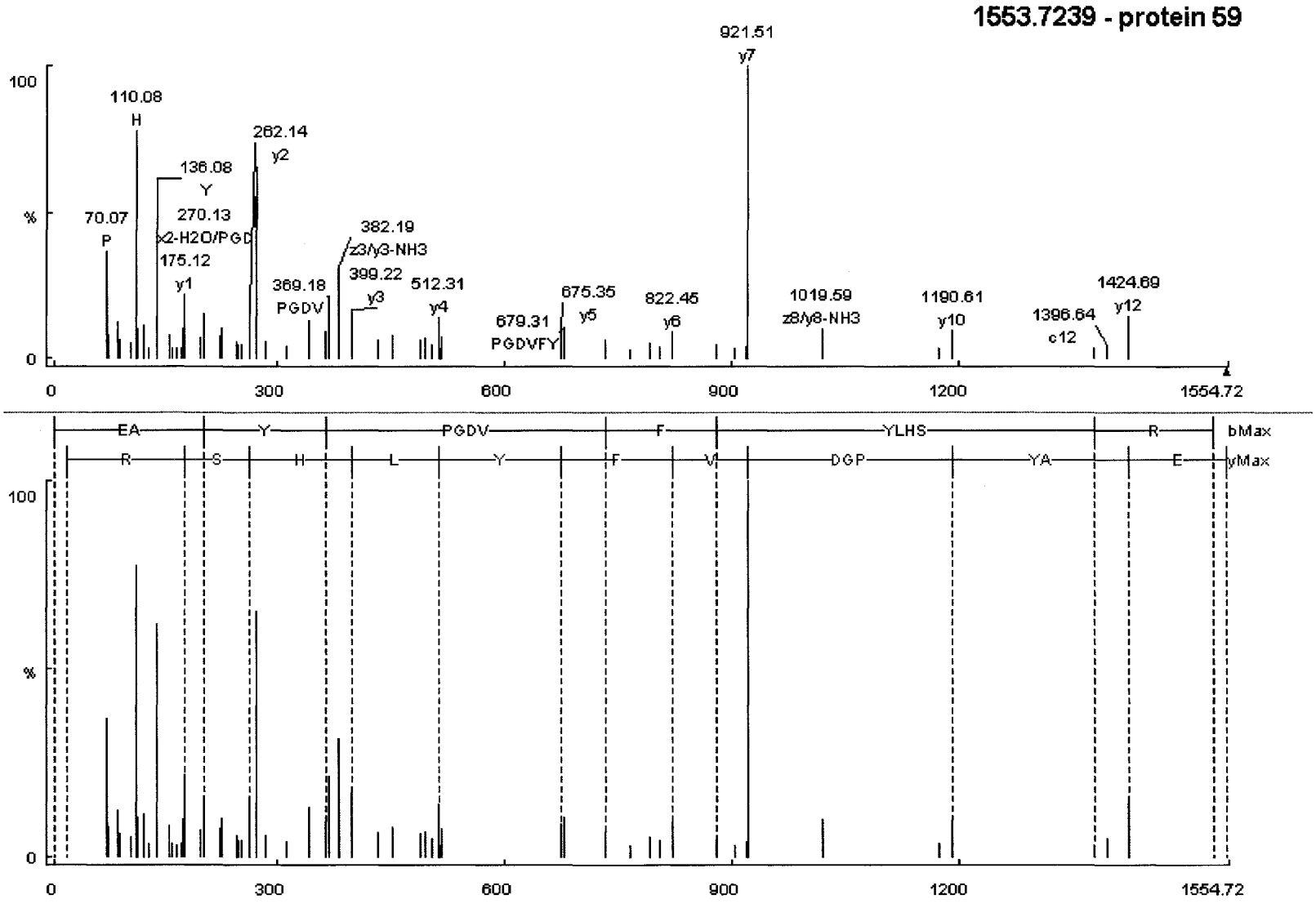
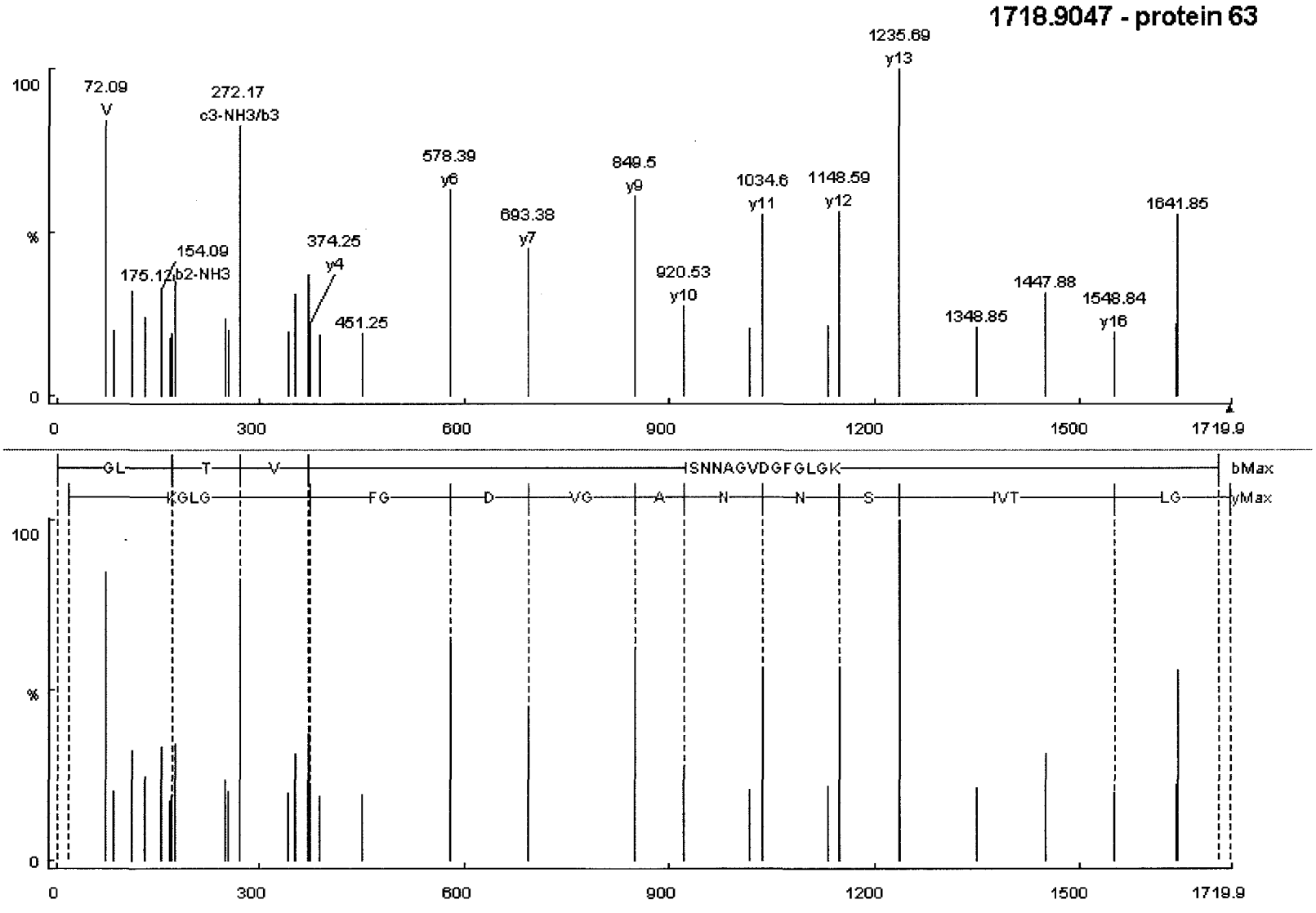
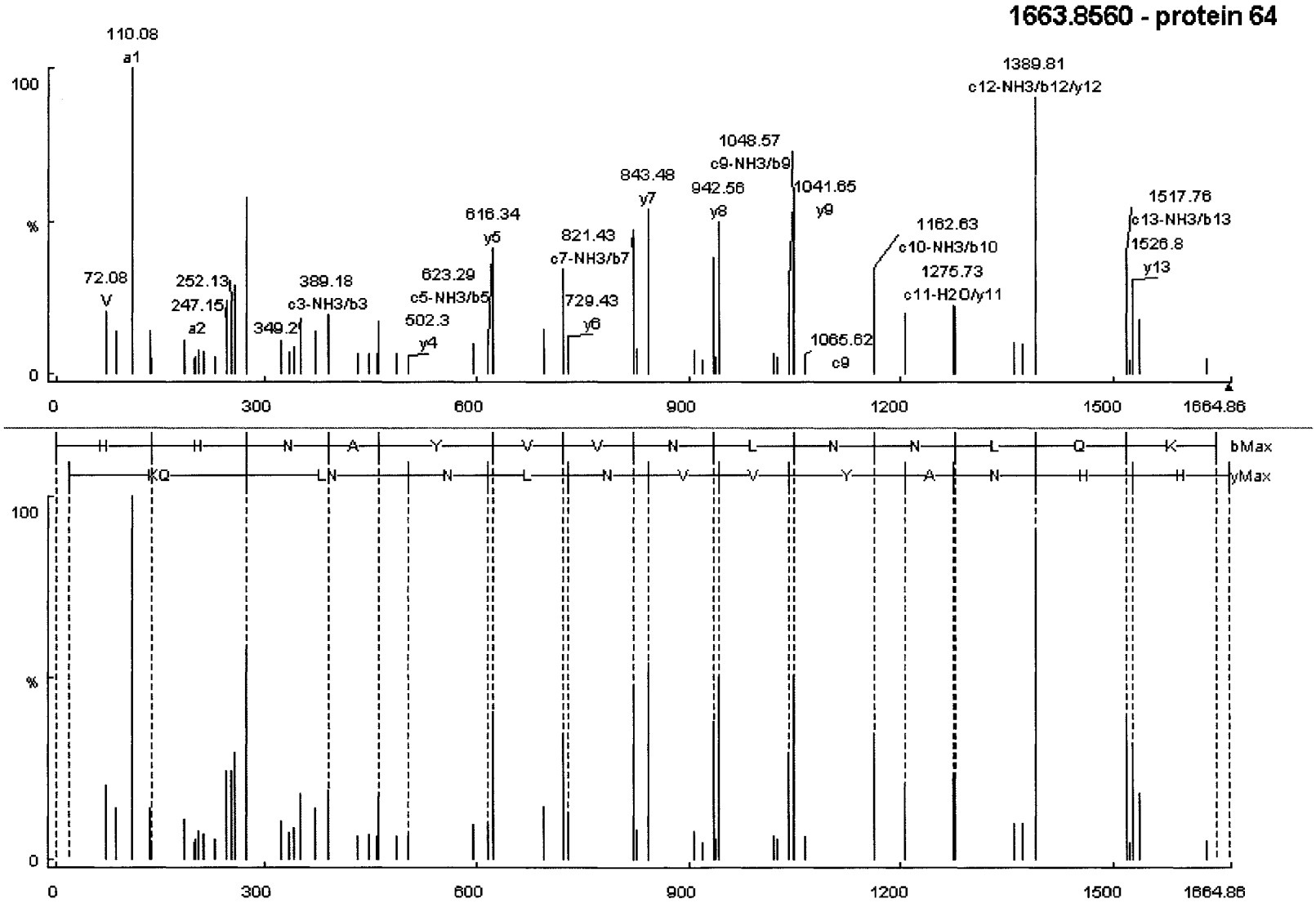
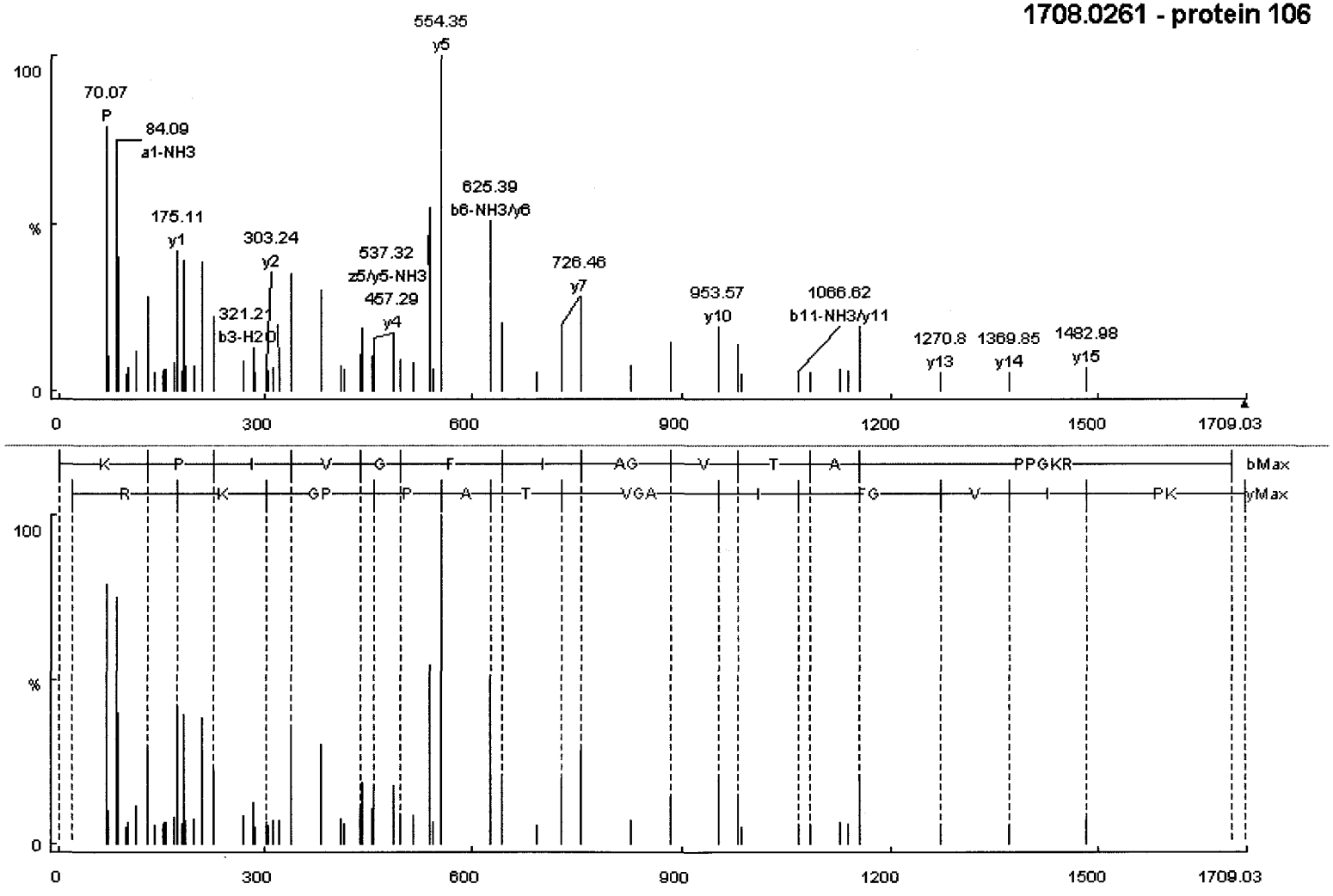
yMax


1733.8674 - protein 43
110.08
H
100
%
0
379.24
c3-NH3/b3
932.51
y7
939.48
c7-NH3/b7
1345.71
c10-NH3/b10
795.45
y6
1061.56
y8
1208.64
y9
1355.74
y10
1492.8
y11
129.11
y1-H2O
258.16
y2-H2O
389.3
y3
504.3
y4
632.39
y5
774.46
a6
911.48
a7
86.1
a1/L
0
300
600
900
1200
1500
1734.87
IQ
H
F
F
E
H
Y
K
D
L
E
K
bMax
KEL
D
K
Y
H
E
F
F
H
QI
yMax
100
%
0
0
300
600
900
1200
1500
1734.87


1553.7239 - protein 59
921.51
y7
110.08
H
262.14
y2
136.08
Y
70.07
P
270.13
x2-H2O/PGD
382.19
z3/y3-NH3
175.12
y1
399.22
y3
369.18
PGDV
512.31
y4
675.35
y5
679.31
PGDVFY
822.45
y6
1019.59
z8/y8-NH3
1190.61
y10
1424.69
y12
1396.64
c12
100
%
0
0
300
600
900
1200
1554.72
EA
Y
PGDV
F
YLHS
R
bMax
R
S
H
L
Y
F
V
DGP
YA
E
yMax
100
%
0
0
300
600
900
1200
1554.72


1718.9047 - protein 63
1235.69
y13
72.09
V
272.17
c3-NH3/b3
578.39
y6
849.5
y9
1034.6
y11
1148.59
y12
693.38
y7
1641.85
154.09
175.12
b2-NH3
374.25
y4
920.53
y10
1348.85
1447.88
1548.84
y16
451.25
100
%
0
0
300
600
900
1200
1500
1719.9
GL
T
V
ISNNAGVDGFGLGK
bMax
IGLG
FG
D
VG
A
N
N
S
IVT
LG
yMax
100
%
0
0
300
600
900
1200
1500
1719.9


1663.8560 - protein 64
110.08
a1
1389.81
c12-NH3/b12/y12
1048.57
c9-NH3/b9
843.48
y7
942.56
y8
1041.65
y9
1517.76
c13-NH3/b13
1526.8
y13
616.34
y5
821.43
c7-NH3/b7
1162.63
c10-NH3/b10
1275.73
c11-H2O/y11
72.08
V
252.13
247.15
a2
389.18
c3-NH3/b3
623.29
c5-NH3/b5
502.3
y4
729.43
y6
349.2
1065.62
c9
100
%
0
0
300
600
900
1200
1500
1664.86
H H N A Y V V N L N N L Q K
bMax
K Q L N N L N V V Y A N H H
yMax


1708.0261 - protein 106
554.35
y5
70.07
P
84.09
a1-NH3
625.39
b6-NH3/y6
175.11
y1
303.24
y2
537.32
z5/y5-NH3
457.29
y4
321.21
b3-H20
726.46
y7
953.57
y10
1066.62
b11-NH3/y11
1270.8
y13
1369.85
y14
1482.98
y15
100
%
0
0
300
600
900
1200
1500
1709.03
K P I V G F I AG V T A PPGKR bMax
R K GP P A T VGA I TG V I PK yMax

peptide #	protein #	Protein Name	Accession #	gi #	Protein Score (%)	Peptide Score (%)
		Unique proteins				
1	1	Enolase	AAZ54466	gi|71914564	98.00	80.00
2	1	Enolase	AAZ54466	gi|71914564	98.00	99.00
3	2	Putative enoyl-CoA hydratase	BAC17791	gi|23492819	93.00	95.00
4	2	Putative enoyl-CoA hydratase	BAC17791	gi|23492819	93.00	85.00
5	3	Phosphopyruvate hydratase	YP_830641	gi|116669708	100.00	99.00
6	3	Phosphopyruvate hydratase	YP_830641	gi|116669708	100.00	99.00
7	4	Chaperone protein HscA	BAC58863	gi|28805586	90.00	81.00
8	4	Chaperone protein HscA	BAC58863	gi|28805586	90.00	98.00
9	5	Glycoside hydrolase, family 3	EAM74380	gi|67986562	95.00	97.00
10	5	Glycoside hydrolase, family 3	EAM74380	gi|67986562	95.00	91.00
11	6	COG3764: Sortase (surface protein transpeptidase)	ZP_00378749	gi|62423589	96.00	92.00
12	6	COG3764: Sortase (surface protein transpeptidase)	ZP_00378749	gi|62423589	96.00	97.00
13	7	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	95.00	99.00
14	7	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	96.00	91.00
15	8	Heterodisulfide reductase, iron-sulfur-binding subunit, putative	AAS95329	gi|46448676	90.00	81.00
16	8	Heterodisulfide reductase, iron-sulfur-binding subunit, putative	AAS95329	gi|46448676	90.00	99.00
17	9	Hydrogenase expression/formation protein, putative	YP_112698	gi|53802535	91.00	90.00
18	9	Hydrogenase expression/formation protein, putative	YP_112698	gi|53802535	91.00	97.00
19	10	Bacterial trigger factor	ZP_00396433	gi|66797675	90.00	95.00
20	10	Bacterial trigger factor	ZP_00396433	gi|66797675	90.00	85.00
21	11	Histidine kinase, HAMP region:Bacterial chemotaxis sensory transducer	ZP_00881851	gi|82496282	95.00	98.00
22	11	Histidine kinase, HAMP region:Bacterial chemotaxis sensory transducer	ZP_00881851	gi|82496282	95.00	91.00
23	12	Peroxiredoxin	AAS77881	gi|45826512	100.00	87.00
24	12	Peroxiredoxin	AAS77881	gi|45826512	100.00	99.00
25	13	Alkyl hydroperoxide reductase subunit C	CAG42129	gi|49243704	99.00	80.00
26	13	Alkyl hydroperoxide reductase subunit C	CAG42129	gi|49243704	99.00	90.00
27	14	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	99.00
28	14	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	95.00
29	15	Enolase 2, (gamma, neuronal)	AAP88878	gi|32880095	90.00	99.00
30	15	Enolase 2, (gamma, neuronal)	AAP88878	gi|32880095	90.00	83.00
31	16	atpD beta synthase chain	CAC88840	gi|18077541	93.00	93.00
32	16	atpD beta synthase chain	CAC88840	gi|18077541	93.00	83.00
33	17	Nucleoside-diphosphate-sugar pyrophosphorylase	NP_599972	gi|19551970	96.00	96.00
34	17	Nucleoside-diphosphate-sugar pyrophosphorylase	NP_599972	gi|19551970	96.00	94.00
35	18	Cell division protein FtsA	ZP_00550701	gi|68177554	90.00	85.00
36	18	Cell division protein FtsA	ZP_00550701	gi|68177554	90.00	93.00

37	19	Nucleoside diphosphate kinase	ZP_00950789	gi|83857261	100.00	99.00
38	19	Nucleoside diphosphate kinase	ZP_00950789	gi|83857261	100.00	99.00
39	20	Chain B, Crystal Structure Of Escherichia Coli Topoisomerase Iv	1S14B	gi|48425514	91.00	96.00
40	20	Chain B, Crystal Structure Of Escherichia Coli Topoisomerase Iv	1S14B	gi|48425514	91.00	91.00
41	21	S-adenosylmethionine synthetase	NP_874743	gi|33239801	95.00	95.00
42	21	S-adenosylmethionine synthetase	NP_874743	gi|33239801	95.00	97.00
43	22	Membrane-bound ATP synthase , F1 sector, beta-subunit	CAG67157	gi|49529445	100.00	99.00
44	22	Membrane-bound ATP synthase , F1 sector, beta-subunit	CAG67157	gi|49529445	100.00	99.00
45	23	Myo-inositol-1-phosphate synthase	EAM74489	gi|67986674	99.00	99.00
46	23	Myo-inositol-1-phosphate synthase	EAM74489	gi|67986674	99.00	99.00
47	24	Hydroxypyruvate isomerase	YP_575224	gi|92115296	91.00	90.00
48	24	Hydroxypyruvate isomerase	YP_575224	gi|92115296	91.00	96.00
49	25	ATP synthase F1, beta subunit	YP_521398	gi|89898927	99.00	99.00
50	25	ATP synthase F1, beta subunit	YP_521398	gi|89898927	99.00	98.00
51	26	Catalase	ZP_01129564	gi|88854898	90.00	100.00
52	26	Catalase	ZP_01129564	gi|88854898	90.00	89.00
53	27	Malate dehydrogenase	CAG69844	gi|49532132	100.00	99.00
54	27	Malate dehydrogenase	CAG69844	gi|49532132	100.00	99.00
55	28	Probable glycine betaine/carnitine/choline ABC transporter	BAB80263	gi|18144216	90.00	83.00
56	28	Probable glycine betaine/carnitine/choline ABC transporter	BAB80263	gi|18144216	90.00	96.00
57	29	Translation elongation factor Tu	YP_832458	gi|116671525	99.00	99.00
58	29	Translation elongation factor Tu	YP_832458	gi|116671525	99.00	92.00
59	30	Chaperonin Cpn60/TCP-1	YP_283653	gi|71906066	90.00	100.00
60	30	Chaperonin Cpn60/TCP-1	YP_283653	gi|71906066	90.00	90.00
61	31	Protein chain elongation factor EF-Tu, possible GTP-binding factor	CAG67260	gi|49529548	99.00	99.00
62	31	Protein chain elongation factor EF-Tu, possible GTP-binding factor	CAG67260	gi|49529548	99.00	95.00
63	32	Chemotaxis sensory transducer	YP_425809	gi|83592057	90.00	90.00
64	32	Chemotaxis sensory transducer	YP_425809	gi|83592057	90.00	86.00
65	33	Glyceraldehyde-3-phosphate dehydrogenase, type I	YP_831567	gi|116670634	100.00	99.00
66	33	Glyceraldehyde-3-phosphate dehydrogenase, type I	YP_831567	gi|116670634	100.00	99.00
67	34	Putative purine nucleoside phosphorylase	BAC71053	gi|29606993	93.00	83.00
68	34	Putative purine nucleoside phosphorylase	BAC71053	gi|29606993	93.00	99.00
69	35	Succinyl-CoA synthetase, beta subunit	ZP_01383504	gi|110595168	99.00	99.00
70	35	Succinyl-CoA synthetase, beta subunit	ZP_01383504	gi|110595168	99.00	99.00
71	36	COG0137: Argininosuccinate synthase	ZP_00135641	gi|46143221	100.00	99.00
72	36	COG0137: Argininosuccinate synthase	ZP_00135641	gi|46143221	100.00	99.00
73	37	Sodium-transporting two-sector ATPase	ZP_01401702	gi|111614628	99.00	99.00
74	37	Sodium-transporting two-sector ATPase	ZP_01401702	gi|111614628	99.00	99.00
75	38	Chaperonin GroEL	YP_465601	gi|86158816	99.00	80.00

76	38	Chaperonin GroEL	YP_465601	gi|86158816	99.00	96.00
77	39	PA3366	AAT51430	gi|49087212	93.00	90.00
78	39	PA3366	AAT51430	gi|49087212	93.00	96.00
79	40	COG0388: Predicted amidohydrolase	ZP_00968336	gi|84319949	98.00	99.00
80	40	COG0388: Predicted amidohydrolase	ZP_00968336	gi|84319949	98.00	95.00
81	41	S-adenosylmethionine synthetase	NP_905981	gi|34541502	96.00	96.00
82	41	S-adenosylmethionine synthetase	NP_905981	gi|34541502	96.00	88.00
83	42	DNA ligase	ZP_01054298	gi|86135717	92.00	95.00
84	42	DNA ligase	ZP_01054298	gi|86135717	92.00	91.00
85	43	Inorganic diphosphatase	ZP_01493388	gi|116269143	92.00	100.00
86	43	Inorganic diphosphatase	ZP_01493388	gi|116269143	92.00	100.00
87	44	Peptidyl-prolyl cis-trans isomerase precursor (PPIase) (Rotamase)	YP_046571	gi|50085061	100.00	98.00
88	44	Peptidyl-prolyl cis-trans isomerase precursor (PPIase) (Rotamase)	YP_046571	gi|50085061	100.00	94.00
89	45	Hydantoin Racemase	BAC50842	gi|27353853	90.00	96.00
90	45	Hydantoin Racemase	BAC50842	gi|27353853	90.00	82.00
91	46	Short-chain dehydrogenase/reductase SDR	YP_611506	gi|99078248	92.00	100.00
92	46	Short-chain dehydrogenase/reductase SDR	YP_611506	gi|99078248	92.00	96.00
93	47	COG1902: NADH:flavin oxidoreductases, Old Yellow Enzyme family	ZP_00108564	gi|23126675	93.00	93.00
94	47	COG1902: NADH:flavin oxidoreductases, Old Yellow Enzyme family	ZP_00108564	gi|23126675	93.00	90.00
95	48	Acyl-CoA dehydrogenase	ZP_01383093	gi|110594751	93.00	99.00
96	48	Acyl-CoA dehydrogenase	ZP_01383093	gi|110594751	93.00	90.00
97	49	Glutamate decarboxylase	YP_557224	gi|91782018	92.00	99.00
98	49	Glutamate decarboxylase	YP_557224	gi|91782018	92.00	86.00
99	50	D-alanine--D-alanine ligase	AAY61886	gi|67004960	91.00	95.00
100	50	D-alanine--D-alanine ligase	AAY61886	gi|67004960	91.00	93.00
101	51	Threonine dehydratase	YP_496379	gi|87199122	93.00	97.00
102	51	Threonine dehydratase	YP_496379	gi|87199122	93.00	91.00
103	52	Alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	ZP_01382731	gi|110594385	95.00	95.00
104	52	Alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	ZP_01382731	gi|110594385	95.00	96.00
105	53	Outer membrane protein A	AAZ06132	gi|70671520	96.00	80.00
106	53	Outer membrane protein A	AAZ06132	gi|70671520	96.00	99.00
107	54	Putative elongation factor tu (EF-TU protein)	ZP_01398858	gi|111611770	98.00	99.00
108	54	Putative elongation factor tu (EF-TU protein)	ZP_01398858	gi|111611770	98.00	90.00
109	55	Riboflavin synthase	CAE09446	gi|34482445	90.00	85.00
110	55	Riboflavin synthase	CAE09446	gi|34482445	90.00	91.00
111	56	Argininosuccinate synthase argG	ZP_00945954	gi|83748945	100.00	99.00
112	56	Argininosuccinate synthase argG	ZP_00945954	gi|83748945	100.00	99.00
113	57	Protease DegS	NP_933383	gi|37678774	90.00	99.00
114	57	Protease DegS	NP_933383	gi|37678774	90.00	81.00

115	58	COG3315: O-Methyltransferase involved in polyketide biosynthesis	ZP_00984343	gi|84359608	95.00	95.00
116	58	COG3315: O-Methyltransferase involved in polyketide biosynthesis	ZP_00984343	gi|84359608	95.00	99.00
117	59	ATP synthase alpha subunit	YP_456092	gi|85060390	99.00	92.00
118	59	ATP synthase alpha subunit	YP_456092	gi|85060390	99.00	99.00
119	60	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	AAD53938	gi|5834372	92.00	99.00
120	60	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	AAD53938	gi|5834372	92.00	92.00
121	61	Dipeptide-Binding Protein	1DPE	gi|1827807	100.00	99.00
122	61	Dipeptide-Binding Protein	1DPE	gi|1827807	100.00	99.00
123	62	50S ribosomal protein L9	CAG69205	gi|49531493	99.00	98.00
124	62	50S ribosomal protein L9	CAG69205	gi|49531493	99.00	97.00
125	63	3-oxoacid CoA-transferase	ZP_01406016	gi|111618999	91.00	98.00
126	63	3-oxoacid CoA-transferase	ZP_01406016	gi|111618999	91.00	81.00
127	64	Superoxide dismutase	ZP_01020473	gi|84712593	100.00	97.00
128	64	Superoxide dismutase	ZP_01020473	gi|84712593	100.00	83.00
129	65	COG1979: Uncharacterized oxidoreductases	ZP_00723379	gi|75239408	100.00	99.00
130	65	COG1979: Uncharacterized oxidoreductases	ZP_00723379	gi|75239408	100.00	99.00
131	66	Alcohol dehydrogenase, NAD(P)-dependent	NP_417484	gi|16130909	100.00	99.00
132	66	Alcohol dehydrogenase, NAD(P)-dependent	NP_417484	gi|16130909	100.00	99.00
133	67	Putative long-chain fatty acid transport protein	CAG67736	gi|49530024	99.00	99.00
134	67	Putative long-chain fatty acid transport protein	CAG67736	gi|49530024	99.00	99.00
135	68	Dihydrolipoamide dehydrogenase (Glycine oxidation system L-factor)	CAG69602	gi|49531890	99.00	99.00
136	68	Dihydrolipoamide dehydrogenase (Glycine oxidation system L-factor)	CAG69602	gi|49531890	99.00	97.00
137	69	Phosphoribosyltransferase	ZP_01405140	gi|111618114	94.00	99.00
138	69	Phosphoribosyltransferase	ZP_01405140	gi|111618114	94.00	94.00
139	70	Cof protein:HAD-superfamily hydrolase subfamily IIB	ZP_00884273	gi|82498818	90.00	90.00
140	70	Cof protein:HAD-superfamily hydrolase subfamily IIB	ZP_00884273	gi|82498819	90.00	90.00
141	71	COG3142: Uncharacterized protein involved in copper resistance	ZP_00752959	gi|75823460	96.00	98.00
142	71	COG3142: Uncharacterized protein involved in copper resistance	ZP_00752959	gi|75823460	96.00	92.00
143	72	CztR	AAG09631	gi|12484568	91.00	88.00
144	72	CztR	AAG09631	gi|12484568	91.00	92.00
145	73	6-phosphogluconate dehydrogenase related protein	ZP_01128349	gi|88813108	92.00	95.00
146	73	6-phosphogluconate dehydrogenase related protein	ZP_01128349	gi|88813108	92.00	92.00
147	74	Ketol-acid reductoisomerase	YP_208304	gi|59801592	93.00	96.00
148	74	Ketol-acid reductoisomerase	YP_208304	gi|59801592	93.00	90.00
149	75	COG0747: ABC-type dipeptide transport system, periplasmic component	ZP_00701576	gi|75188309	100.00	99.00
150	75	COG0747: ABC-type dipeptide transport system, periplasmic component	ZP_00701576	gi|75188309	100.00	99.00
151	76	Single-strand binding protein	ZP_01404310	gi|111617280	99.00	98.00
152	76	Single-strand binding protein	ZP_01404310	gi|111617280	99.00	91.00
153	77	Similar to ABC-type uncharacterized transport system periplasmic component	ZP_00919478	gi|83374708	99.00	80.00

154	77	Similar to ABC-type uncharacterized transport system periplasmic component	ZP_00919478	gi|83374708	99.00	99.00
155	78	ATPase/chaperone ClpC, putative specificity factor for ClpP	YP_396392	gi|81429391	92.00	90.00
156	78	ATPase/chaperone ClpC, putative specificity factor for ClpP	YP_396392	gi|81429391	92.00	94.00
157	79	Prokaryotic Cytochrome C oxidase subunit IV	EAP04289	gi|78362471	91.00	91.00
158	79	Prokaryotic Cytochrome C oxidase subunit IV	EAP04289	gi|78362471	91.00	97.00
159	80	Amidophosphoribosyltransferase	AAK23636	gi|13423064	98.00	98.00
160	80	Amidophosphoribosyltransferase	AAK23636	gi|13423064	98.00	96.00
161	81	Putative phosphodiesterase-nucleotide pyrophosphatase	CAH08056	gi|60493272	94.00	99.00
162	81	Putative phosphodiesterase-nucleotide pyrophosphatase	CAH08056	gi|60493272	94.00	85.00
163	82	Carbonate dehydratase	ZP_01398800	gi|111611711	94.00	92.00
164	82	Carbonate dehydratase	ZP_01398800	gi|111611711	94.00	100.00
165	83	Dihydrodipicolinate synthase subfamily	ABC22665	gi|83576114	90.00	99.00
166	83	Dihydrodipicolinate synthase subfamily	ABC22665	gi|83576114	90.00	80.00
167	84	NeuB, Antifreeze-like protein	ABA05650	gi|74421451	94.00	81.00
168	84	NeuB, Antifreeze-like protein	ABA05650	gi|74421451	94.00	94.00
169	85	Putative alpha-amylase	BAC75106	gi|29611062	90.00	99.00
170	85	Putative alpha-amylase	BAC75106	gi|29611062	90.00	85.00
171	86	Xenobiotic reductase	ZP_01145823	gi|88940385	96.00	96.00
172	86	Xenobiotic reductase	ZP_01145823	gi|88940385	96.00	96.00
173	87	Polyphosphate kinase	YP_427248	gi|83593496	90.00	90.00
174	87	Polyphosphate kinase	YP_427248	gi|83593496	90.00	90.00
175	88	Beta-ketothiolase	BAA33156	gi|3608184	100.00	99.00
176	88	Beta-ketothiolase	BAA33156	gi|3608184	100.00	95.00
177	89	COG0270: Site-specific DNA methylase	ZP_00388127	gi|62526805	91.00	91.00
178	89	COG0270: Site-specific DNA methylase	ZP_00388127	gi|62526805	91.00	91.00
179	90	Ribosomal protein S6 kinase, 70kDa, polypeptide 1	AAQ02612	gi|33304209	94.00	94.00
180	90	Ribosomal protein S6 kinase, 70kDa, polypeptide 1	AAQ02612	gi|33304209	94.00	93.00
181	91	Conserved hypothetical protein	ZP_00810951	gi|77742474	99.00	90.00
182	91	Conserved hypothetical protein	ZP_00810951	gi|77742474	99.00	99.00
183	92	Cytoplasmic trehalase (alpha,alpha-trehalose glucohydrolase)	BAD75963	gi|56380055	92.00	95.00
184	92	Cytoplasmic trehalase (alpha,alpha-trehalose glucohydrolase)	BAD75963	gi|56380055	92.00	87.00
185	93	ATP-binding region, ATPase-like:Histidine kinase, HAMP region	ZP_00808063	gi|77739572	95.00	95.00
186	93	ATP-binding region, ATPase-like:Histidine kinase, HAMP region	ZP_00808063	gi|77739572	95.00	99.00
187	94	Hypothetical protein CwatDRAFT_2986	ZP_00517276	gi|67923814	96.00	96.00
188	94	Hypothetical protein CwatDRAFT_2986	ZP_00517276	gi|67923814	96.00	95.00
189	95	Branched-chain amino acid permease	AAO09050	gi|27360112	97.00	97.00
190	95	Branched-chain amino acid permease	AAO09050	gi|27360112	97.00	99.00
191	96	3-hydroxybutyryl-CoA dehydrogenase	ZP_00859494	gi|78694982	93.00	97.00
192	96	3-hydroxybutyryl-CoA dehydrogenase	ZP_00859494	gi|78694982	93.00	93.00

193	97	Putative LysR-family transcriptional regulator	YP_289483	gi|72161826	91.00	92.00
194	97	Putative LysR-family transcriptional regulator	YP_289483	gi|72161826	91.00	98.00
195	98	ABC transport system ATP-binding protein	CAE31102	gi|33567188	99.00	99.00
196	98	ABC transport system ATP-binding protein	CAE31102	gi|33567188	99.00	99.00
197	99	Multi-sensor signal transduction histidine kinase	YP_431319	gi|83591310	95.00	92.00
198	99	Multi-sensor signal transduction histidine kinase	YP_431319	gi|83591310	95.00	96.00
199	100	COG1028: Dehydrogenases with different specificities	ZP_00244839	gi|47574804	99.00	99.00
200	100	COG1028: Dehydrogenases with different specificities	ZP_00244839	gi|47574804	99.00	99.00
201	101	Putative secreted amidase	CAA20075	gi|3367750	91.00	88.00
202	101	Putative secreted amidase	CAA20075	gi|3367750	91.00	92.00
203	102	COG0183: Acetyl-CoA acetyltransferase	ZP_00241486	gi|47571433	93.00	92.00
204	102	COG0183: Acetyl-CoA acetyltransferase	ZP_00241486	gi|47571433	93.00	95.00
205	103	Putative membrane protein	ZP_01190318	gi|89337540	94.00	94.00
206	103	Putative membrane protein	ZP_01190318	gi|89337540	94.00	92.00
207	104	ATPas	ZP_00504409	gi|67874829	96.00	94.00
208	104	ATPas	ZP_00504409	gi|67874829	96.00	98.00
209	105	Calcium/proton antiporter	CAH21194	gi|51589564	97.00	97.00
210	105	Calcium/proton antiporter	CAH21194	gi|51589564	97.00	99.00
211	106	Succinyl-CoA synthetase, alpha subunit	ZP_01383505	gi|110595169	100.00	99.00
212	106	Succinyl-CoA synthetase, alpha subunit	ZP_01383505	gi|110595169	100.00	99.00
213	107	Putative universal stress protein	CAE36891	gi|33573230	90.00	82.00
214	107	Putative universal stress protein	CAE36891	gi|33573230	90.00	92.00
215	108	Probable transglycosylase	ZP_00957081	gi|83944715	90.00	80.00
216	108	Probable transglycosylase	ZP_00957081	gi|83944715	90.00	99.00
217	109	TPR repeat	ZP_00839219	gi|78369052	94.00	94.00
218	109	TPR repeat	ZP_00839219	gi|78369052	94.00	94.00
		Proteins found in multiple spots				
219	110	Peptidylprolyl isomerase	YP_569671	gi|91977012	99.00	95.00
220	110	Peptidylprolyl isomerase	YP_569671	gi|91977012	99.00	99.00
221	111	Peptidylprolyl isomerase	YP_569671	gi|91977012	99.00	98.00
222	111	Peptidylprolyl isomerase	YP_569671	gi|91977012	99.00	97.00
223	112	Superoxide dismutase	YP_549757	gi|91788805	99.00	97.00
224	112	Superoxide dismutase	YP_549757	gi|91788805	99.00	92.00
225	113	Superoxide dismutase	YP_549757	gi|91788805	99.00	94.00
226	113	Superoxide dismutase	YP_549757	gi|91788805	99.00	98.00
227	114	Superoxide dismutase	YP_549757	gi|91788805	99.00	98.00

228	114	Superoxide dismutase	YP_549757	gi|91788805	99.00	95.00
229	115	Carbonate dehydratase	YP_550979	gi|91790027	99.00	94.00
230	115	Carbonate dehydratase	YP_550979	gi|91790027	99.00	92.00
231	116	Carbonate dehydratase	YP_550979	gi|91790027	99.00	93.00
232	116	Carbonate dehydratase	YP_550979	gi|91790027	99.00	91.00
233	117	Carbonate dehydratase	YP_550979	gi|91790027	99.00	98.00
234	117	Carbonate dehydratase	YP_550979	gi|91790027	99.00	96.00
235	118	Carbonate dehydratase	YP_550979	gi|91790027	99.00	97.00
236	118	Carbonate dehydratase	YP_550979	gi|91790027	99.00	95.00
237	119	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	94.00
238	119	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	97.00
239	120	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	99.00
240	120	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	95.00
241	121	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	91.00
242	121	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	94.00
243	122	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	98.00
244	122	HMP-like outer membrane protein	AAT42489	gi|48429443	99.00	95.00
245	123	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	99.00	99.00
246	123	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	99.00	99.00
247	124	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	99.00	92.00
248	124	Electron transfer flavoprotein alpha-subunit	CAG69411	gi|49531699	99.00	91.00
249	125	Transporter, EamA family	AAT34148	gi|47505472	99.00	95.00
250	125	Transporter, EamA family	AAT34148	gi|47505472	99.00	90.00
251	126	Transporter, EamA family	AAT34148	gi|47505472	99.00	90.00
252	126	Transporter, EamA family	AAT34148	gi|47505472	99.00	95.00
253	127	Transporter, EamA family	AAT34148	gi|47505472	99.00	91.00
254	127	Transporter, EamA family	AAT34148	gi|47505472	99.00	96.00
255	128	Malate dehydrogenase	CAG69844	gi|49532132	99.00	99.00
256	128	Malate dehydrogenase	CAG69844	gi|49532132	99.00	99.00
257	129	Malate dehydrogenase	CAG69844	gi|49532132	99.00	99.00
258	129	Malate dehydrogenase	CAG69844	gi|49532132	99.00	99.00
259	130	Glyceraldehyde-3-phosphate dehydrogenase, type I	ZP_00413489	gi|66965925	99.00	99.00
260	130	Glyceraldehyde-3-phosphate dehydrogenase, type I	ZP_00413489	gi|66965925	99.00	99.00
261	131	Outer membrane protein A	AAZ06132	gi|70671520	99.00	85.00
262	131	Outer membrane protein A	AAZ06132	gi|70671520	99.00	88.00
263	132	Outer membrane protein A	AAZ06132	gi|70671520	99.00	85.00
264	132	Outer membrane protein A	AAZ06132	gi|70671520	99.00	99.00
265	133	Outer membrane protein A	AAZ06132	gi|70671520	99.00	99.00

266	133	Outer membrane protein A	AAZ06132	gi|70671520	99.00	99.00
267	134	NADH, Flavin oxidoredutase	ZP_01227426	gi|90419516	99.00	93.00
268	134	NADH, Flavin oxidoredutase	ZP_01227426	gi|90419516	99.00	90.00
269	135	Succinyl-CoA synthetase, beta subunit	YP_551463	gi|91790511	99.00	99.00
270	135	Succinyl-CoA synthetase, beta subunit	YP_551463	gi|91790511	99.00	99.00
271	136	Succinyl-CoA synthetase, beta subunit	YP_551463	gi|91790511	99.00	99.00
272	136	Succinyl-CoA synthetase, beta subunit	YP_551463	gi|91790511	99.00	99.00
273	137	Elongation factor EF-Tu	NP_787248	gi|28493087	99.00	95.00
274	137	Elongation factor EF-Tu	NP_787248	gi|28493087	99.00	92.00
275	138	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	97.00	98.00
276	138	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	99.00	99.00
277	139	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	99.00	97.00
278	139	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	99.00	93.00
279	140	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	97.00	93.00
280	140	Translation elongation factor Tu:Small GTP-binding protein domain	ZP_00412370	gi|66964801	99.00	91.00
281	141	Phosphopyruvate hydratase	ZP_01130726	gi|88856065	94.00	99.00
282	141	Phosphopyruvate hydratase	ZP_01130726	gi|88856065	95.00	99.00
283	142	Phosphopyruvate hydratase	ZP_01130726	gi|88856065	94.00	99.00
284	142	Phosphopyruvate hydratase	ZP_01130726	gi|88856065	95.00	99.00
285	143	Phosphopyruvate hydratase	YP_285570	gi|71907983	92.00	99.00
286	143	Phosphopyruvate hydratase	YP_285570	gi|71907983	92.00	99.00
287	144	Argininosuccinate synthase argG	ZP_00945954	gi|83748945	99.00	99.00
288	144	Argininosuccinate synthase argG	ZP_00945954	gi|83748945	99.00	99.00
289	145	Argininosuccinate synthase	CAE43796	gi|33564483	99.00	99.00
290	145	Argininosuccinate synthase	CAE43796	gi|33564483	99.00	99.00
291	146	Argininosuccinate synthase	CAH34287	gi|52208353	99.00	99.00
292	146	Argininosuccinate synthase	CAH34287	gi|52208353	99.00	99.00
293	147	ATP synthase F1, beta subunit	ZP_00670958	gi|71550887	99.00	99.00
294	147	ATP synthase F1, beta subunit	ZP_00670958	gi|71550887	99.00	99.00
295	148	ATP synthase F1, beta subunit	ZP_00670958	gi|71550887	99.00	99.00
296	148	ATP synthase F1, beta subunit	ZP_00670958	gi|71550887	99.00	99.00
297	149	Membrane-bound ATP synthase , F1 sector, beta-subunit	CAG67157	gi|49529445	99.00	99.00
298	149	Membrane-bound ATP synthase , F1 sector, beta-subunit	CAG67157	gi|49529445	99.00	99.00
299	150	ATP synthase F1, beta subunit	YP_521398	gi|89898927	99.00	97.00
300	150	ATP synthase F1, beta subunit	YP_521398	gi|89898927	99.00	99.00
301	151	ATP synthase F1, beta subunit	ZP_01019284	gi|84710982	99.00	99.00
302	151	ATP synthase F1, beta subunit	ZP_01019284	gi|84710982	99.00	98.00
303	152	ATP synthase F1, beta subunit	ZP_01019284	gi|84710982	99.00	97.00

304	152	ATP synthase F1, beta subunit	ZP_01019284	gi|84710982	99.00	99.00
305	153	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	99.00
306	153	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	97.00
307	154	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	99.00
308	154	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	99.00
309	155	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	98.00
310	155	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	97.00
311	156	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	98.00
312	156	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	98.00
313	157	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	99.00
314	157	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	98.00
315	158	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	99.00
316	158	COG0055: F0F1-type ATP synthase, beta subunit	ZP_00244017	gi|47573980	99.00	98.00

Mass Error (Da)	Mass Error (ppm)	M/z (Da)	Peptide Sequence	Sequence Coverage (%)	MW (Da)	pI	protein #
0.0004	0.2002	1998.0966	AGVLVGDDLFVTNAPLLQR	8.96	45269.79	4.46	1
-0.0035	-1.7369	2015.0731	VNQIGSLTETLDAVSLAQR	8.96	45269.79	4.46	1
-0.0399	-16.3379	2442.1787	FPKPVVGMAGGIVMGGGLGLLAACR	13.84	39150.00	4.50	2
-0.0164	-6.3559	2580.2634	GALVT(OxM)PHKVATVELVDELTPTAR	13.84	39150.00	4.50	2
0.0007	0.3474	2015.0774	VNQIGSLTETLDAVSLAQR	10.80	44996.94	4.54	3
-0.0276	-9.4245	2928.5398	TLTDAIGDKVQLVGDDLFVTNPSILQR	10.80	44996.94	4.54	3
-0.0366	-39.2505	932.4728	TLNDEQGR	4.54	66053.91	4.67	4
0.0295	13.3777	2205.1614	GLVEKIIPRNTTIPVARAQEK	4.54	66053.91	4.67	4
0.0483	23.4792	2057.1370	GAQPYSYLHPKLGAASPVSNR	5.27	81468.69	4.72	5
0.0039	1.6384	2380.3460	EVDGIPVAADERLLTDLLRER	5.27	81468.69	4.72	5
0.0074	3.2614	2268.9648	(OxM)AAWLTQIDQSKVVENATTR	13.61	34169.32	4.73	6
0.0005	0.1911	2616.3518	DPDQTETLDAVRGKDLATLVT(CamC)TPR	13.61	34169.32	4.73	6
0.0078	4.6376	1681.9153	VLDPLADKLGAAQGASR	13.18	31578.69	4.79	7
-0.0029	-1.1534	2514.3645	RPIYAGNAIATVQSDEAIIVGTVR	13.18	31578.69	4.79	7
-0.0688	-38.0130	1809.9065	ELLSTMNARQHLQLR	4.35	82149.00	4.79	8
0.0316	16.4079	1925.9044	ADVGQERDAPLTGVEPDR	4.35	82149.00	4.79	8
-0.0138	-6.6246	2083.1356	TLGTAGLSVLSRGYGDCRVTR	15.11	30605.64	4.81	9
-0.0105	-3.2744	3206.6880	CRVTLTRLPNVWWVQYFNSPGQLILNR	15.11	30605.64	4.81	9
0.0262	29.0319	902.4550	EGMQVEIPR	5.79	50268.56	4.84	10
0.0042	2.1649	1939.9998	LAEDLGVQLSDAEFSNTMR	5.79	50268.56	4.84	10
0.0042	1.5818	2655.2842	ARQQGKATLEYPIANPISKVVEDK	9.54	59564.13	4.85	11
0.0004	0.1381	2896.4575	QSSAQIGSILDVIRSIAEQTNLLALNAAR	9.54	59564.13	4.85	11
-0.0239	-15.5824	1533.7839	KNFEVLIEADGLADR	16.67	20688.46	4.85	12
0.0186	9.6175	1933.9660	LGVEIYSVSTDTHFTHK	16.67	20688.46	4.85	12
-0.0052	-3.8411	1353.7797	KITYTMIGDPSQR	15.34	20963.49	4.88	13
-0.0026	-1.2942	2008.9948	AQAFDPKKDQFKEVTQER	15.34	20963.49	4.88	13
-0.0071	-4.9835	1424.7009	SALVSEYNIDASR	23.94	31005.51	4.90	14
-0.0084	-4.3888	1913.9528	QTQINGNFYATSDLITK	23.94	31005.51	4.90	14
0.0145	10.9203	1327.7975	TIAPALISSGLSVVR	7.13	47352.09	4.91	15
-0.0358	-19.0430	1879.9554	GNPTVEVDLYTAKGLFR	7.13	47352.09	4.91	15
0.0007	0.4825	1450.7908	YTLAGTEVSALLGR	12.79	27887.04	4.92	16
-0.0005	-0.2513	1990.0122	DIASLGIYPAVDPLDSTSR	12.79	27887.04	4.92	16
-0.0266	-20.2794	1311.6741	GADLNSILDTHR	11.33	38494.59	4.96	17
0.0006	0.3223	1861.8993	VDASYWRDMGTPSDFVK	11.33	38494.59	4.96	17
-0.0177	-8.6937	2035.9590	IASMTDSGLDIVGIGTSVSR	15.40	43474.90	4.99	18
0.0012	0.4445	2699.4414	QRVIDAAKAIAIPMDREVIHVIPQR	15.40	43474.90	4.99	18

-0.0111	-7.4561	1488.7081	PFFGELVEYMTR	18.63	17740.91	5.00	19
-0.0084	-4.2617	1971.0460	GPIVAAILEKENAVEDFR	18.63	17740.91	5.00	19
-0.0074	-4.0023	1848.9570	SRLTHVLKAKAVLCPGVR	23.71	21688.88	5.05	20
0.0423	13.4470	3145.6826	VIDDGRGMPVDIHPEEGVPAVELILCISVR	23.71	21688.88	5.05	20
0.0195	15.3940	1266.7302	LMPLPISLAHR	5.85	44265.65	5.06	21
0.0077	4.9910	1542.7715	PIQEKTRFLVNPTR	5.85	44265.65	5.06	21
0.0006	0.4390	1366.7372	DVLLFVDNIYR	11.21	50280.01	5.07	22
-0.0032	-1.8659	1714.9705	IIQIIGAVIDVEFER	11.21	50280.01	5.07	22
-0.0092	-4.1364	2224.1472	VAIVGVGN(CamC)AASLVQGVQYYR	11.67	38863.82	5.10	23
-0.0238	-9.6976	2454.2253	DRGIQLERTYQLNVGGNMDFK	11.67	38863.82	5.10	23
-0.0071	-5.5655	1275.7076	VGHIQIAGVPER	12.60	29648.47	5.10	24
0.0059	2.3827	2476.1758	EYKPRAGTRAGLGWFEAWKR	12.60	29648.47	5.10	24
0.0038	2.0766	1829.9105	DEGKDVLFFVDNIYR	7.17	51211.27	5.11	25
-0.0005	-0.2513	1990.0122	DIASLGIYPAVDPLDSTSR	7.17	51211.27	5.11	25
-0.0023	-1.4165	1623.7377	FSSVAGEQGSPDTWR	6.61	55898.84	5.12	26
-0.0348	-16.3690	2125.9697	KWLHSEDSDFGQAGTLYR	6.61	55898.84	5.12	26
-0.0046	-2.6698	1722.9490	VAVTGAAGQIGYSLLFR	14.94	35426.11	5.14	27
-0.0042	-2.0979	2002.0228	VNGEIFIGQGQALNEVASR	14.94	35426.11	5.14	27
0.0180	11.4153	1576.8281	SSPENMCAVKLGNAR	8.20	42391.00	5.14	28
0.0239	13.5794	1760.0133	AEDIMIEDPVSISGKR	8.20	42391.00	5.14	28
-0.0051	-2.7644	1844.8911	QLDEAWAGEN(CamC)GLLLR	16.92	43564.25	5.16	29
-0.0072	-3.6090	1995.0182	DKPFL(OxM)PIEDVFTITGR	16.92	43564.25	5.16	29
-0.0370	-41.7384	886.4735	VTLGPKGR	4.36	57813.19	5.19	30
-0.0067	-3.6436	1838.8522	SLANELDVVEGMQFDR	4.36	57813.19	5.19	30
0.0009	0.8268	1088.5526	AGEN(CamC)GVLLR	6.31	42927.85	5.20	31
-0.0095	-4.8894	1942.9745	HTPFLNGYRPQFYFR	6.31	42927.85	5.20	31
-0.0216	-11.2795	1914.9850	RVGEEQMRLGAVVKDDIR	6.28	69799.58	5.21	32
-0.0298	-12.3890	2405.3616	AALLGEANSHNLTEADRGLLAR	6.28	69799.58	5.21	32
0.0009	0.5294	1699.9232	VPVPTGSATDLTVTVSR	16.07	35807.54	5.24	33
-0.0225	-7.8192	2877.5146	AALAQGADLEIVAVNDLTSPEALAHLFK	16.07	35807.54	5.24	33
0.0217	23.8608	909.4430	THFYEGR	9.12	28416.00	5.26	34
-0.0024	-1.3522	1774.9343	IGADLVG(OxM)STALEAIAAR	9.12	28416.00	5.26	34
-0.0021	-1.7290	1214.6011	FNFDANALFR	10.62	41024.37	5.28	35
-0.0011	-0.5567	1975.9735	LFGGEPANFLDVGGGATPEK	10.62	41024.37	5.28	35
0.0170	13.3159	1276.6676	WFDPQAIMLR	18.34	25813.19	5.29	36
0.0054	3.0203	1787.8977	LVTGIHNEDTIEQYR	18.34	25813.19	5.29	36
-0.0159	-9.3426	1701.8833	IVQ(CamC)IGAVVDVEFPR	6.79	50830.33	5.30	37
-0.0193	-10.5072	1836.8428	MPSAVGYQPTLAEEMGR	6.79	50830.33	5.30	37
0.0508	33.6232	1510.8633	VAGGGVALLR	6.39	57998.52	5.30	38

0.0164	6.4406	2546.3545	SALQNAASIASLLITTEA(OxM)VAETPK	6.39	57998.52	5.30	38
-0.0051	-4.0846	1248.5996	LPYEGLEKEAGR	14.99	38526.73	5.31	39
-0.0083	-5.8461	1419.7544	DCAMKGAELIVR	14.99	38526.73	5.31	39
-0.0110	-7.6616	1435.7278	ANVWGVFSLTGER	8.38	38455.70	5.31	40
-0.0145	-7.3831	1963.9500	ISLII(CamC)DDGNYPEIWR	8.38	38455.70	5.31	40
-0.0278	-16.0332	1733.9014	YVDVQETARRVIER	10.02	47668.87	5.33	41
0.0254	14.4568	1756.9595	AEPVSIYVNTKGRSHVR	10.02	47668.87	5.33	41
0.0159	7.3470	2164.1404	KAEQEATLLNEITYQVGRTR	10.08	74755.61	5.34	42
0.0315	9.6251	3272.6995	ANLDPVQLAGTTVKRASLHNADQIEKLDVR	10.08	74755.61	5.34	42
0.0027	1.5572	1733.8674	IQHFFEHYKDLEK	19.10	20063.33	5.39	43
-0.0627	-16.7700	3738.8257	APSDPLKYEVDKESGAVFVDR	19.10	20063.33	5.39	43
-0.0115	-8.6752	1325.6113	DGFYDGVIFHR	31.95	18536.33	5.40	44
-0.0131	-7.5034	1745.8839	TQAPHSASAQFFINVK	31.95	18536.33	5.40	44
0.0498	25.1512	1980.0278	DVDAIVVACFDDTGVDAVR	21.81	25035.00	5.41	45
-0.0370	-10.3847	3562.9382	ARANEVPVLGLPAAAYVMKVETGNLVFVTGHLVK	21.81	25035.00	5.41	45
-0.0011	-0.7257	1515.8116	IIGVNLSGAFHTMR	16.48	27525.19	5.41	46
0.0020	0.7295	2741.5042	GLAIAEALAGAGARIAVHGLADAAQAQAAIR	16.48	27525.19	5.41	46
0.0072	4.2234	1704.7876	ALETSEIPGIVEQYR	10.54	40194.37	5.43	47
-0.0051	-2.2096	2308.1306	AGFDGVEVHSANGYLLDQFLR	10.54	40194.37	5.43	47
-0.0001	-0.0755	1323.6725	VADMYTVLQAGR	9.34	43104.63	5.43	48
-0.0022	-0.8202	2682.3716	AVLTGGPLGIMQSVMDNVVPYIHDR	9.34	43104.63	5.43	48
-0.0005	-0.5316	940.5946	QRVLIRR	4.34	51251.60	5.46	49
0.0378	23.0635	1638.9509	A(OxM)LLEDIRHAIGHK	4.34	51251.60	5.46	49
0.0105	5.3405	1966.1031	SSALAFDKIHSRSWFLTK	18.69	35891.47	5.47	50
-0.0364	-16.2137	2245.0212	YALEINTHPG(OxM)TPLSIVPEK	18.69	35891.47	5.47	50
-0.0060	-2.2801	2631.4100	MQNRELFAGRNVGVVLTGANIDTR	11.40	44842.84	5.52	51
0.0603	22.6411	2663.3005	TFVHPFDERNVAAGQGTVALE(OxM)LEK	11.40	44842.84	5.52	51
-0.0082	-6.9993	1171.5425	AWHDASDTIR	21.93	20744.52	5.52	52
-0.0044	-1.1803	3727.7683	WAVFFFYPADFTFV(CamC)PTELGDVADHYDELQK	21.93	20744.52	5.52	52
0.0320	21.9353	1458.8387	IKPYVLLGAGHYK	8.31	37951.42	5.52	53
-0.0159	-8.8784	1790.8671	LSTQGFAWDQPIADNK	8.31	37951.42	5.52	53
0.0039	2.7686	1408.6782	YDFPGDDTPIIR	14.90	43156.10	5.53	54
0.0029	1.6211	1788.8906	GITINTAHVEYETANR	14.90	43156.10	5.53	54
-0.0060	-4.7898	1252.6737	FDAVCCVGAVIR	16.03	16769.00	5.54	55
-0.0006	-0.4286	1399.7717	VALEKVLWAAGAR	16.03	16769.00	5.54	55
-0.0186	-9.7690	1903.9806	GIYEAPGMAILHIAYER	13.26	49202.57	5.55	56
0.0004	0.1950	2051.0600	IYKPWLDQTFIDELGGR	13.26	49202.57	5.55	56
0.0070	8.0604	868.4428	LLAVPSLR	11.55	37600.01	5.55	57
0.0002	0.1437	1391.7279	LGLVTALLVLLAVPK	11.55	37600.01	5.55	57

-0.0044	-2.1858	2012.9507 APQAWKRRRLIELGFGVK	22.60	32072.44	5.56	58
-0.0576	-26.0851	2208.1545 ARVALWRALHVRLDAQPHVK	22.60	32072.44	5.56	58
-0.0123	-9.5742	1284.7010 SALAIDAIINQR	4.87	55424.88	5.62	59
0.0011	0.7080	1553.7239 EAYPGDVFYLHSR	4.87	55424.88	5.62	59
-0.0258	-15.8886	1623.8015 KV(OxM)AWDNNPETR	12.64	28944.01	5.62	60
-0.0144	-6.2550	2302.1653 LPLLAADPLPDGGVYVLELSSYK	12.64	28944.01	5.62	60
-0.0023	-1.2617	1822.8947 W(CamC)YKPFEDLIQPAR	12.82	57398.60	5.66	61
0.0151	5.2350	2884.4521 AFDGYWGTKPQIDTLVFSITPDASVR	12.82	57398.60	5.66	61
0.0001	0.0670	1492.7367 AVAATEANTAAFEAR	27.70	15775.42	5.66	62
0.0010	0.3484	2870.5193 HTGEFNIAIQLHHDVVAEVLVTIVSEK	27.70	15775.42	5.66	62
-0.0105	-6.1085	1718.9047 GLTVISNNAGVDGFGLGK	14.07	28432.05	5.71	63
-0.0098	-4.3806	2237.1465 QYLAGELELEFTPQGTLAEK	14.07	28432.05	5.71	63
-0.0026	-1.5626	1663.8560 HHNAYVVNLNNLQK	16.06	21380.52	5.71	64
0.0129	6.0195	2143.0398 ALLTVDVWEHAYYIDYR	16.06	21380.52	5.71	64
0.0079	3.1272	2526.2612 FIAAAANYPENIDPWHILQTGGK	23.51	42084.82	5.72	65
-0.0356	-12.1981	2918.4792 NFFEQLGVPTHLSDYGLDGSSIPALLK	23.51	42084.82	5.72	65
-0.0164	-10.1491	1615.9020 FAEGILLTLIEDGPK	23.51	42070.80	5.72	66
-0.0142	-4.8079	2953.4998 QAFHSAHVQPVFAVLDPVYTYTLPPR	23.51	42070.80	5.72	66
0.0031	3.1016	999.4965 YFWLGDAK	4.51	52130.31	5.73	67
0.0316	18.5574	1702.8204 NGFNLVEYSKDQWSR	4.51	52130.31	5.73	67
-0.0117	-9.8023	1193.5945 TGQFPFAVNGR	6.08	50877.56	5.74	68
-0.0011	-0.6125	1796.0416 LGVIGAGVIGLELGSVWR	6.08	50877.56	5.74	68
-0.0057	-3.9646	1437.7389 AEAGTVQGHLDIAR	16.67	19535.05	5.76	69
-0.0060	-3.4386	1744.8876 IFDKPLAI(OxM)STSSYR	16.67	19535.05	5.76	69
0.0417	22.3599	1864.9485 RDEIMAIGDGDNDISMIR	20.27	34133.74	5.76	70
0.0012	0.3795	3161.6740 RLAKEENIHVHIYIDNVWYVEVFNER	20.27	34133.74	5.76	70
0.0072	5.8084	1239.5746 PSE(OxM)QFIRSER	14.96	27203.97	5.78	71
0.0032	1.9707	1623.7976 D(CamC)DDFIIPVTSRDAR	14.96	27203.97	5.78	71
0.0181	11.0915	1631.8782 QVLMRQTGEVLTR	12.50	25514.33	5.79	72
0.0033	1.6975	1944.0067 QRV(OxM)(OxM)LTARGRLADR	12.50	25514.33	5.79	72
0.0151	9.8006	1540.7274 AETTPLAHPERYR	9.41	36488.27	5.82	73
-0.0024	-1.1781	2037.1692 RSLDPIFKTLAPGRAAAPR	9.41	36488.27	5.82	73
0.0497	26.4650	1877.9530 AEATKEADVV(OxM)LLLPDR	13.95	36394.34	5.84	74
-0.0435	-20.4607	2126.0288 FIQEGNVNYASMTARRR	13.95	36394.34	5.84	74
-0.0018	-1.1790	1526.7103 ELNADDVVFSFDR	12.36	59254.66	5.85	75
0.0022	1.4131	1556.8130 IVTYEWGEYLKR	12.36	59254.66	5.85	75
0.0034	2.9268	1161.6660 LAEIVGQYLR	19.79	19839.00	5.88	76
-0.0010	-0.5283	1892.9341 TFPSGDQVANVTIATTDR	19.79	19839.00	5.88	76
-0.0253	-16.7125	1513.8370 AVLGFQTVLDVAALPR	8.17	70006.63	5.94	77

0.0302	18.7824	1607.8916	VFEHDSARLPAPPR	8.17	70006.63	5.94	77
0.0015	0.4911	3054.4985	KKYLNLERELHKRVIGQDDAVSSISR	9.23	90498.98	5.98	78
0.0283	8.9566	3159.6812	SFSDL)OxM)KPPSVTGELADFTRDLTELAR	9.23	90498.98	5.98	78
0.0311	16.4208	1893.9364	TGIPFWAVMTHSLETSTR	30.56	11855.32	5.99	79
0.0327	17.0129	1922.0757	LAVVQILVHLKYFLHLR	30.56	11855.32	5.99	79
-0.0286	-12.7103	2250.1448	KPVLASET(CamC)ALD(OxM)IGARFVR	12.40	54635.91	6.04	80
-0.0371	-15.4239	2405.3542	LGFLSVDGLYDALEAGQRDPATPR	12.40	54635.91	6.04	80
0.0411	13.7574	2987.4824	SPDMQV(OxM)FRA(CamC)GPDFKAGYESKGFVR	16.92	45807.97	6.08	81
0.0005	0.1486	3363.7483	YRDSVYNALKDVPHIHVWKKEEIPAELNK	16.92	45807.97	6.08	81
0.0446	29.0975	1532.7790	MGTSLEELFVHNR	14.80	24481.31	6.09	82
0.0018	0.9169	1963.0380	VPANQITGLEPGEVFVHR	14.80	24481.31	6.09	82
0.0303	15.5139	1953.0831	EAVGDLPVVLYNVPGR	16.04	31063.04	6.09	83
-0.0060	-1.9433	3087.5757	ADMDMKKKSVLPHAARALDSVDGYHKRTLNVK	16.04	31063.04	6.09	83
-0.0370	-37.2780	992.5419	EVGFSDHSVK	4.58	40007.58	6.10	84
-0.0397	-33.7131	1177.5824	SLREAFGLEVGK	4.58	40007.58	6.10	84
-0.0104	-5.1255	2029.0707	QYLLAPTSLPPSPLGTAFR	5.71	74022.84	6.10	85
0.0228	10.3682	2199.0374	HPLRELLTSSEPLPLLVER	5.71	74022.84	6.10	85
-0.0122	-7.7621	1571.7330	YIANEKFTRETAER	9.01	38012.59	6.10	86
-0.0281	-14.4844	1940.0138	GGSIANRARLLLEVTDAAIR	9.01	38012.59	6.10	86
-0.0668	-35.5346	1879.8563	PERIRDFGGD(CamC)FAAIR	6.76	84155.16	6.11	87
-0.0549	-21.5438	2548.2957	VIT(OxM)D(OxM)PDRFINREVSWLK	6.76	84155.16	6.11	87
-0.0009	-0.6005	1498.8256	FVDEIVPVSIPQR	20.61	40212.76	6.15	88
0.0010	0.4075	2454.2415	VGTDQVGEVI(OxM)GQVLAAGAGQNPAR	20.61	40212.76	6.15	88
-0.0075	-3.6748	2040.9274	AEYGVPQARERVIIIGNR	11.51	44930.13	6.18	89
-0.0496	-24.1138	2056.9163	QVNQHEI(CamC)DYLRYYR	11.51	44930.13	6.18	89
0.0308	24.5309	1255.5593	DTAHTKAERNIR	4.94	59214.90	6.21	90
-0.0576	-35.4729	1623.7753	GKGGYGKVFQVRKVTR	4.94	59214.90	6.21	90
-0.0005	-0.3859	1295.7327	AGLRFVAHPATR	23.97	28473.86	6.22	91
0.0015	0.9370	1600.8457	LADTARIERAWLDAR	23.97	28473.86	6.22	91
-0.0068	-3.8705	1756.8750	VFSSIHQRWEQLVK	7.45	48801.39	6.24	92
0.0586	25.7806	2273.0286	(OxM)DETTHYHPQIGLNKYYR	7.45	48801.39	6.24	92
-0.0403	-25.6409	1571.7075	AADAGRLERGEDR	4.00	48148.15	6.25	93
0.0101	5.2062	1940.0001	LAALAADAGRLRERGEDR	4.00	48148.15	6.25	93
-0.0478	-29.9358	1596.7518	YIPTLTPPDTPLIR	14.59	21201.99	6.27	94
0.0332	20.2962	1635.7756	IEDYDYIEYLQATR	14.59	21201.99	6.27	94
-0.0051	-2.5641	1989.0237	VLPLFEVGMAWLLPTMTAR	14.19	46309.10	6.30	95
0.0459	19.6214	2339.2852	FSILFFAVAMFFSWSQGKLIR	14.19	46309.10	6.30	95
-0.0095	-10.3433	918.4731	(OxM)VAAGQLGR	9.19	29858.53	6.33	96
0.0193	9.6693	1996.0156	ATATDRPDRFIGMHFFNR	9.19	29858.53	6.33	96

0.0410	16.7884	2442.1648	LATKMELDLATDLNDADWI(OxM)R	11.69	33019.19	6.42	97
-0.0426	-30.1770	1411.6730	AIAH(OxM)VVRPVR	11.69	33019.19	6.42	97
-0.0314	-23.3513	1344.6813	QMVAVARALMSAPR	15.90	25311.49	6.44	98
0.0164	5.4803	2992.5364	RENLEMGAYRIDDARRRAELLAT(CamC)LK	15.90	25311.49	6.44	98
-0.0496	-24.3383	2037.9424	LLGAYLYLHQAILKA(OxM)GR	11.45	54222.27	6.46	99
-0.0562	-17.5316	3205.6396	ETLLEGALEDPEVSRRFLGIINHEAQRLR	11.45	54222.27	6.46	99
0.0023	1.5005	1532.7726	AEAGEAVARDIGGAFVR	16.27	25557.41	6.60	100
0.0073	3.0262	2412.2478	GVIISTASVAAYDGQIGQAAYAASK	16.27	25557.41	6.60	100
-0.0007	-0.4502	1554.7598	EVDAL(OxM)TRTAER	10.28	55903.05	6.64	101
-0.0054	-1.9250	2805.2554	LPSLGAEVDAL(OxM)TRTAERLTAAGAEVR	10.28	55903.05	6.64	101
0.0441	29.4627	1496.8090	MIRDAYLCDALR	8.73	41783.40	6.67	102
-0.0498	-20.7827	2396.2214	NVARMAGLLAGLPVEVPGSTVNR	8.73	41783.40	6.67	102
-0.0148	-10.2942	1437.7086	AAIERFRSYTHAR	11.42	33248.01	6.68	103
0.0275	7.4205	3705.9275	YNPVRLLRSLRAFRHNPLFSRAAIERFRSYR	11.42	33248.01	6.68	103
-0.0118	-6.5382	1804.7874	ESPTATRYAELLNILNR	9.22	40165.96	6.92	104
-0.0421	-22.1715	1898.8346	IRERALAFIENIDNPQK	9.22	40165.96	6.92	104
-0.0270	-23.1247	1167.5822	RRLAVQATPR	10.93	39127.33	6.93	105
0.0298	11.3163	2633.3794	V(CamC)DLSEA(OxM)RAALERGTSQIVARLAK	10.93	39127.33	6.93	105
0.0144	8.4308	1708.0261	KPIVGFIAGVTAPPGKR	26.26	31072.11	6.96	106
0.0122	3.6783	3316.7510	SGTLTYEAVAQLTEIGLGQSSAVGIGGDPINGLK	26.26	31072.11	6.96	106
-0.0007	-0.3853	1816.8826	TAEEKGCDLIVMASHGR	25.52	15308.00	6.96	107
0.0239	11.1002	2153.1116	SYQNLLVPVDGSDLSSCDLK	25.52	15308.00	6.96	107
-0.0085	-3.9962	2127.0461	ASVAAERREPSAILTVDWR	13.22	24365.24	6.98	108
0.0239	11.0698	2159.0332	AILTVDWRAAPLFSARTDR	13.22	24365.24	6.98	108
0.0298	18.1947	1637.8440	GLSRAYWATGDERR	8.91	45324.07	7.02	109
0.0493	19.3009	2554.2812	QSL(OxM)LKADFIPALNTLAILYR	8.91	45324.07	7.02	109
0.0055	4.0036	1325.6113	DGFYDGVIFHR	25.32	15938.24	8.76	110
0.0028	2.0382	1373.7677	TLIIPAELGYGAR	21.43	15938.24	8.76	110
0.0049	3.5668	1373.7677	TLIIPAELGYGAR	25.32	15938.24	8.76	111
0.0096	5.4986	1745.8839	TQAPHSASAQFFINVK	21.43	15938.24	8.76	111
0.0034	2.0434	1663.861	HHNAYVVNLNNLQK	23.83	21320.52	6.22	112
0.0372	22.3576	1663.861	HHNAYVVNLNNLQK	23.83	21320.52	6.22	112
0.0179	8.4154	2127.0461	ALLTVDVWEHAYYIDYR	23.83	21320.52	6.22	113
0.0054	2.5198	2143.0398	ALLTVDVWEHAYYIDYR	23.83	21320.52	6.22	113
0.0098	5.8899	1663.8560	HHNAYVVNLNNLQK	23.83	21320.52	6.22	114

0.0176	8.2744	2127.0461	ALLTVDVWEHAYYIDYR	23.83	21320.52	6.22	114
0.0176	11.4824	1532.7790	MGTSLEELFVHNR	7.73	26079.28	6.12	115
0.0215	14.0268	1532.7790	MGTSLEELFVHNR	7.73	26079.28	6.12	115
0.0421	27.4665	1532.7790	MGTSLEELFVHNR	7.73	26079.28	6.12	116
0.0284	18.5284	1532.7790	MGTSLEELFVHNR	7.73	26079.28	6.12	116
0.0086	4.3810	1963.0414	VPANQITGLEPGEVFVHR	7.73	26079.28	6.12	117
0.0017	0.8660	1963.0421	VPANQITGLEPGEVFVHR	7.73	26079.28	6.12	117
0.0063	3.2093	1963.0426	VPANQITGLEPGEVFVHR	7.73	26079.28	6.12	118
0.0077	3.9225	1963.0437	VPANQITGLEPGEVFVHR	7.73	26079.28	6.12	118
-0.0099	-5.1725	1913.9622	QTQINGNFYATSDLITK	15.14	31005.51	4.90	119
0.0081	4.2321	1913.9617	QTQINGNFYATSDLITK	16.55	31005.51	4.90	119
-0.0065	-4.5624	1424.7009	SALVSEYNIDASR	15.49	31005.51	4.90	120
-0.025	-17.1146	1460.7427	VADALNTFPNATAR	21.13	31005.51	4.90	120
0.0178	6.0455	2944.3457	YKLDDMSYHNNEEGTLGNAGVGAFWR	14.08	31005.51	4.90	121
-0.0099	-5.1725	1913.9622	QTQINGNFYATSDLITK	15.14	31005.51	4.90	121
-0.0063	-4.4220	1424.7009	SALVSEYNIDASR	16.55	31005.51	4.90	122
-0.0231	-15.8139	1460.7427	VADALNTFPNATAR	15.49	31005.51	4.90	122
0.0082	4.8754	1681.9153	VLDPLADKLGAAQGASR	19.29	31578.69	4.79	123
0.0091	5.4105	1681.9153	VLDPLADKLGAAQGASR	13.50	31578.69	4.79	123
-0.0045	-1.7897	2514.3635	RPIYAGNAIATVQSDEAIIVGTVR	19.29	31578.69	4.79	124
-0.0038	-1.5113	2514.3677	RPIYAGNAIATVQSDEAIIVGTVR	13.50	31578.69	4.79	124
-0.0194	-8.0654	2405.3474	QEKFSSRKLIGSIIAFGGAALVL	15.61	33294.18	9.60	*125*
-0.0295	-16.9158	1743.9285	YGLLEFTAIEFTALR	7.64	33294.18	9.60	*125*
-0.0346	-19.8403	1743.9285	YGLLEFTAIEFTALR	7.64	33294.18	9.60	*126*
-0.0102	-4.2405	2405.3542	QEKFSSRKLIGSIIAFGGAALVL	12.62	33294.18	9.60	*126*
-0.0398	-22.8220	1743.9285	YGLLEFTAIEFTALR	15.61	33294.18	9.60	*127*
-0.0156	-6.4855	2405.3616	QEKFSSRKLIGSIIAFGGAALVL	7.64	33294.18	9.60	*127*
-0.005	-2.9020	1722.9490	VAVTGAAGQIGYSLLFR	14.94	35426.11	5.14	*128*
-0.0045	-2.6118	1722.9490	VAVTGAAGQIGYSLLFR	14.94	35426.11	5.14	*128*
-0.0048	-2.3976	2002.022	VNGEIFIGQGQALNEVASR	14.94	35426.11	5.14	*129*
-0.0042	-2.0979	2002.0310	VNGEIFIGQGQALNEVASR	14.94	35426.11	5.14	*129*
0.0015	0.8824	1699.9232	VPVPTGSATDLTVTVSR	16.07	35807.54	5.24	*130*
-0.0265	-9.2093	2877.5127	AALAQGADLEIVAVNDLTSPEALAHLFK	16.07	35807.54	5.24	*130*
0.0359	24.6085	1458.8427	IKPYVLLGAGHYK	16.05	37951.42	5.52	*131*
0.0367	25.1570	1458.838	IKPYVLLGAGHYK	12.32	37951.42	5.52	*131*
0.0366	25.0884	1458.8387	IKPYVLLGAGHYK	12.89	37951.42	5.52	*132*
-0.0138	-7.7058	1790.8665	LSTQGFAWDQPIADNK	14.61	37951.42	5.52	132
-0.0142	-7.9291	1790.8671	LSTQGFAWDQPIADNK	16.05	37951.42	5.52	133

-0.0178	-9.9393	1790.8665	LSTQGFAWDQPIADNK	12.32	37951.42	5.52	133
0.0079	4.6340	1704.7876	ALETSEIPGIVEQYR	5.68	40598.47	5.06	134
-0.005	-2.1663	2308.1328	AGFDGVEVHSANGYLLDQFLR	5.68	40598.47	5.06	134
-0.0025	-2.0583	1214.5931	FNFDANALFR	7.77	41181.30	5.21	135
-0.0023	-1.8936	1214.5929	FNFDANALFR	10.36	41181.30	5.21	135
-0.0011	-0.5567	1975.9735	LFGGEPANFLDVGGGATPEK	7.77	41181.30	5.21	136
-0.0015	-0.7591	1975.9735	LFGGEPANFLDVGGGATPEK	10.36	41181.30	5.21	136
-0.0081	-4.3089	1879.8439	HNPFYSNYRPQFYFR	8.31	43493.42	5.36	137
-0.0094	-4.6462	2023.1483	GTLAINSEVEIVGIRPVQK	8.31	43493.42	5.36	137
-0.0067	-3.3584	1995.0182	DKPFL(OxM)PIEDVFTITGR	9.09	43564.25	5.16	138
-0.0049	-2.6560	1844.8911	QLDEAWAGEN(CamC)GLLLR	19.44	43564.25	5.16	138
-0.0072	-3.9027	1844.8911	QLDEAWAGEN(CamC)GLLLR	12.63	43564.25	5.16	139
-0.0095	-4.6661	2035.9583	HNPFYSNYRPQFYFR	14.90	43564.25	5.16	139
-0.0059	-2.9162	2023.1517	GTLAINSEVEIVGIRPVQK	9.09	43564.25	5.16	140
-0.0044	-2.1748	2023.1483	GTLAINSEVEIVGIRPVQK	19.44	43564.25	5.16	140
0.0013	0.6451	2015.0774	VNQIGSLTETLDAVSLAQR	9.86	44825.75	4.50	141
0.001	0.6681	1496.809	VMPVGAANIREAIR	10.56	44825.75	4.50	141
-0.0234	-7.9903	2928.5398	TLTDAIGDKVQLVGDDLFVTNPSILQR	9.86	44825.75	4.50	142
-0.0268	-9.1513	2928.5398	TLTDAIGDKVQLVGDDLFVTNPSILQR	10.56	44825.75	4.50	142
0.0011	0.7349	1496.809	VMPVGAANIREAIR	3.28	45691.08	4.65	143
-0.0208	-7.1025	2928.5398	TLTDAIGDKVQLVGDDLFVTNPSILQR	3.28	45691.08	4.65	143
0.0056	3.1322	1787.8885	LVTGIHNEDTIEQYR	13.26	49202.57	5.55	144
0.0006	0.2925	2051.06	IYKPWLDQTFIDELGGR	13.26	49202.57	5.55	144
-0.0173	-9.0862	1903.9806	GIYEAPGMAILHIAYER	8.76	49292.11	5.21	145
0.0005	0.2438	2051.0554	IYKPWLDQTFIDELGGR	8.76	49292.11	5.21	145
0.0044	2.4610	1787.8885	LVTGIHNEDTIEQYR	7.17	49666.26	5.37	146
-0.0002	-0.0975	2051.0554	IYKPWLDQTFIDELGGR	7.17	49666.26	5.37	146
-0.0001	-0.0588	1701.8934	IVQ(CamC)IGAVVDVEFPR	7.17	50117.59	4.91	147
0.0009	0.4523	1990.0103	DIASLGIYPAVDPLDSTSR	7.17	50117.59	4.91	147
-0.0022	-1.1055	1990.011	DIASLGIYPAVDPLDSTSR	7.17	50117.59	4.91	148
-0.0095	-2.4714	3844.0232	VGSITSIQAVYVPADDLTDPSPATTFAHLDSTVVLSR	7.17	50117.59	4.91	148
0.0005	0.3658	1366.7372	DVLLFVDNIYR	12.93	50280.01	5.07	149
-0.0087	-3.7726	2306.1223	MPSAVGYQPTLAEEMGVLQER	12.93	50280.01	5.07	149
0.0020	1.0929	1829.9105	DEGKDVLFFVDNIYR	14.98	51211.27	5.11	150
0.0007	0.1821	3843.9497	VGSITSIQAVYVPADDLTDPSPATTFAHLDSTVVLSR	14.98	51211.27	5.11	150
0.0077	4.5244	1701.8934	IVQ(CamC)IGAVVDVEFPR	14.77	51181.31	5.17	151
0.0032	1.6080	1990.0021	DIASLGIYPAVDPLDSTSR	10.76	51181.31	5.17	151
0.0072	3.6181	1990.0021	DIASLGIYPAVDPLDSTSR	14.77	51181.31	5.17	152

-0.0011	-0.2862	3844.0251	VGSITSIQAVYVPADDLTDPSPATTFAHLDSTVVLSR	10.76	51181.31	5.17	152
0.0042	2.4678	1701.8934	IVQ(CamC)IGAVVDVEFPR	10.19	50933.25	5.25	153
-0.0018	-1.0576	1836.8376	MPSAVGYQPTLAEEMGR	10.19	50933.25	5.25	153
-0.0060	-3.2665	1701.9004	IVQ(CamC)IGAVVDVEFPR	7.22	50933.25	5.25	154
0.0055	3.2317	1990.0078	DIASLGIYPAVDPLDSTSR	6.16	50933.25	5.25	154
0.0001	0.0503	1836.8376	MPSAVGYQPTLAEEMGR	6.16	50933.25	5.25	155
0.0049	2.6676	1990.0021	DIASLGIYPAVDPLDSTSR	6.79	50933.25	5.25	155
0.0096	4.8241	1829.9105	DEGKDVLFFVDNIYR	6.79	50933.25	5.25	156
0.0004	0.2186	1990.0021	DIASLGIYPAVDPLDSTSR	10.19	50933.25	5.25	156
0.0022	1.1055	1829.9105	DEGKDVLFFVDNIYR	10.19	50933.25	5.25	157
-0.0006	-0.3279	3844.0251	VGSITSIQAVYVPADDLTDPSPATTFAHLDSTVVLSR	7.22	50933.25	5.25	157
0.0028	0.7284	1701.8934	IVQ(CamC)IGAVVDVEFPR	6.16	50933.25	5.25	158
0.0010	0.5876	1829.9105	DEGKDVLFFVDNIYR	6.16	50933.25	5.25	158

Organism	Trend	Regulation	Biological process	Identified by	False positive	protein #
Thermobifida fusca YX	Resistance	Down	Sugar metabolism	Peaks	No	1
Thermobifida fusca YX	Resistance	Down	Sugar metabolism	Peaks	No	1
Corynebacterium efficiens YS-314	Tolerance	Up	Sugar metabolism	Peaks	No	2
Corynebacterium efficiens YS-314	Tolerance	Up	Sugar metabolism	Peaks	No	2
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Peaks	No	3
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Peaks	No	3
Vibrio parahaemolyticus RIMD 2210633	Resistance	Up	Structural	Peaks	No	4
Vibrio parahaemolyticus RIMD 2210633	Resistance	Up	Structural	Peaks	No	4
Kineococcus radiotolerans SRS30216	Tolerance	Down	Sugar metabolism	Peaks	No	5
Kineococcus radiotolerans SRS30216	Tolerance	Down	Sugar metabolism	Peaks	No	5
Brevibacterium linens BL2	Tolerance	Up	Protein metabolism	Peaks	No	6
Brevibacterium linens BL2	Tolerance	Up	Protein metabolism	Peaks	No	6
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	7
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	7
Desulfovibrio vulgaris subsp. vulgaris str. Hildenborough	Resistance	Up	Electron transport	Peaks	Maybe	8
Desulfovibrio vulgaris subsp. vulgaris str. Hildenborough	Resistance	Up	Electron transport	Peaks	Maybe	8
Methylococcus capsulatus str. Bath	Tolerance	Up	Protein metabolism	Peaks	No	9
Methylococcus capsulatus str. Bath	Tolerance	Up	Protein metabolism	Peaks	No	9
Deinococcus geothermalis DSM 11300	Tolerance	Up	Response to stimulus	Peaks	Maybe	10
Deinococcus geothermalis DSM 11300	Tolerance	Up	Response to stimulus	Peaks	Maybe	10
Shewanella sp. MR-4	Resistance	Up	Cell communication	Peaks	No	11
Shewanella sp. MR-4	Resistance	Up	Cell communication	Peaks	No	11
Acinetobacter radioresistens	Tolerance	Up	Response to stimulus	Peaks	No	12
Acinetobacter radioresistens	Tolerance	Up	Response to stimulus	Peaks	No	12
Staphylococcus aureus subsp. aureus MSSA476	Tolerance	Up	Response to stimulus	Both	No	13
Staphylococcus aureus subsp. aureus MSSA476	Tolerance	Up	Response to stimulus	Both	No	13
Acinetobacter junii	Adaptation	Up	Secretion	Both	No	14
Acinetobacter junii	Adaptation	Up	Secretion	Both	No	14
synthetic construct	Resistance	Down	Sugar metabolism	Peaks	No	15
synthetic construct	Resistance	Down	Sugar metabolism	Peaks	No	15
Pseudomonas aurantiaca	Tolerance	Up	Localization	Both	No	16
Pseudomonas aurantiaca	Tolerance	Up	Localization	Both	No	16
Corynebacterium glutamicum ATCC 13032	Resistance	Up	Nucleic acid metabolism	Peaks	No	17
Corynebacterium glutamicum ATCC 13032	Resistance	Up	Nucleic acid metabolism	Peaks	No	17
Desulfuromonas acetoxidans DSM 684	Adaptation	Up	Localization	Peaks	Maybe	18
Desulfuromonas acetoxidans DSM 684	Adaptation	Up	Localization	Peaks	Maybe	18

Croceibacter atlanticus HTCC2559	Tolerance	Up	Nucleic acid metabolism	Peaks	No	19
Croceibacter atlanticus HTCC2560	Tolerance	Up	Nucleic acid metabolism	Peaks	No	19
Escherichia coli	Resistance	Up	Nucleic acid metabolism	Peaks	No	20
Escherichia coli	Resistance	Up	Nucleic acid metabolism	Peaks	No	20
Prochlorococcus marinus subsp. marinus str. CCMP1375	Resistance	Up	Protein metabolism	Peaks	No	21
Prochlorococcus marinus subsp. marinus str. CCMP1375	Resistance	Up	Protein metabolism	Peaks	No	21
Acinetobacter sp. ADP1	Tolerance	Up	Localization	Both	No	22
Acinetobacter sp. ADP1	Tolerance	Up	Localization	Both	No	22
Kineococcus radiotolerans SRS30216	Tolerance	Down	Energy reserve metabolism	Peaks	No	23
Kineococcus radiotolerans SRS30216	Tolerance	Down	Energy reserve metabolism	Peaks	No	23
Chromohalobacter salexigens DSM 3043	Resistance	Up	Energy reserve metabolism	Peaks	No	24
Chromohalobacter salexigens DSM 3043	Resistance	Up	Energy reserve metabolism	Peaks	No	24
Rhodoferax ferrireducens T118	Tolerance	Up	Localization	Both	No	25
Rhodoferax ferrireducens T118	Tolerance	Up	Localization	Both	No	25
Leifsonia xyli subsp. xyli str. CTCB07	Tolerance	Up	Response to stimulus	Both	No	26
Leifsonia xyli subsp. xyli str. CTCB07	Tolerance	Up	Response to stimulus	Both	No	26
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Peaks	No	27
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Peaks	No	27
Clostridium perfringens str. 13	Tolerance	Up	Localization	Peaks	No	28
Clostridium perfringens str. 13	Tolerance	Up	Localization	Peaks	No	28
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	29
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	29
Dechloromonas aromatica RCB	Resistance	Up	Structural	Peaks	No	30
Dechloromonas aromatica RCB	Resistance	Up	Structural	Peaks	No	30
Acinetobacter sp. ADP1	Tolerance	Up	Protein metabolism	Both	No	31
Acinetobacter sp. ADP1	Tolerance	Up	Protein metabolism	Both	No	31
Rhodospirillum rubrum ATCC 11170	Tolerance	Up	Cell communication	Peaks	Maybe	32
Rhodospirillum rubrum ATCC 11170	Tolerance	Up	Cell communication	Peaks	Maybe	32
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Both	No	33
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Both	No	33
Streptomyces avermitilis	Tolerance	Up	Nucleic acid metabolism	Peaks	No	34
Streptomyces avermitilis	Tolerance	Up	Nucleic acid metabolism	Peaks	No	34
Acidovorax sp. JS42	Tolerance	Down	Energy reserve metabolism	Both	No	35
Acidovorax sp. JS42	Tolerance	Down	Energy reserve metabolism	Both	No	35
Actinobacillus pleuropneumoniae serovar 1 str. 4074	Tolerance	Up	Protein metabolism	Both	No	36
Actinobacillus pleuropneumoniae serovar 1 str. 4074	Tolerance	Up	Protein metabolism	Both	No	36
Verminephrobacter eiseniae EF01-2	Tolerance	Up	Localization	Both	No	37
Verminephrobacter eiseniae EF01-2	Tolerance	Up	Localization	Both	No	37
Anaeromyxobacter dehalogenans 2CP-C	Resistance	Up	Structural	Both	No	38

Anaeromyxobacter dehalogenans 2CP-C	Resistance	Up	Structural	Both	No	38
synthetic construct	Adaptation	Up	Localization	Peaks	No	39
synthetic construct	Adaptation	Up	Localization	Peaks	No	39
Pseudomonas aeruginosa C3719	Tolerance	Down	Sugar metabolism	Peaks	No	40
Pseudomonas aeruginosa C3719	Tolerance	Down	Sugar metabolism	Peaks	No	40
Porphyromonas gingivalis W83	Resistance	Up	Nucleic acid metabolism	Peaks	No	41
Porphyromonas gingivalis W83	Resistance	Up	Nucleic acid metabolism	Peaks	No	41
Tenacibaculum sp. MED152	Resistance	Up	Nucleic acid metabolism	Peaks	No	42
Tenacibaculum sp. MED152	Resistance	Up	Nucleic acid metabolism	Peaks	No	42
Polynucleobacter sp. QLW-P1DMWA-1	Adaptation	Up	Energy reserve metabolism	Both	No	43
Polynucleobacter sp. QLW-P1DMWA-1	Adaptation	Up	Energy reserve metabolism	Both	No	43
Acinetobacter sp. ADP1	Tolerance	Up	Protein metabolism	Peaks	No	44
Acinetobacter sp. ADP1	Tolerance	Up	Protein metabolism	Peaks	No	44
Bradyrhizobium japonicum USDA 110	Tolerance	Up	Protein metabolism	Peaks	Maybe	45
Bradyrhizobium japonicum USDA 110	Tolerance	Up	Protein metabolism	Peaks	Maybe	45
Silicibacter sp. TM1040	Tolerance	Up	Regulation	Peaks	No	46
Silicibacter sp. TM1040	Tolerance	Up	Regulation	Peaks	No	46
Nostoc punctiforme PCC 73102	Resistance	Up	Electron transport	Peaks	No	47
Nostoc punctiforme PCC 73102	Resistance	Up	Electron transport	Peaks	No	47
Acidovorax sp. JS42	Tolerance	Up	Electron transport	Both	No	48
Acidovorax sp. JS42	Tolerance	Up	Electron transport	Both	No	48
Burkholderia xenovorans LB400	Tolerance	Up	Protein metabolism	Peaks	No	49
Burkholderia xenovorans LB400	Tolerance	Up	Protein metabolism	Peaks	No	49
Rickettsia felis URRWXCal2	Resistance	Up	Protein metabolism	Peaks	No	50
Rickettsia felis URRWXCal2	Resistance	Up	Protein metabolism	Peaks	No	50
Novosphingobium aromaticivorans DSM 12444	Resistance	Down	Protein metabolism	Peaks	No	51
Novosphingobium aromaticivorans DSM 12444	Resistance	Down	Protein metabolism	Peaks	No	51
Acidovorax sp. JS42	Tolerance	Up	Response to stimulus	Peaks	No	52
Acidovorax sp. JS42	Tolerance	Up	Response to stimulus	Peaks	No	52
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Both	No	53
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Both	No	53
Verminephrobacter eiseniae EF01-2	Tolerance	Up	Protein metabolism	Peaks	No	54
Verminephrobacter eiseniae EF01-2	Tolerance	Up	Protein metabolism	Peaks	No	54
Wolinella succinogenes	Adaptation	Up	Energy reserve metabolism	Peaks	Maybe	55
Wolinella succinogenes	Adaptation	Up	Energy reserve metabolism	Peaks	Maybe	55
Ralstonia solanacearum UW551	Tolerance	Up	Protein metabolism	Both	No	56
Ralstonia solanacearum UW551	Tolerance	Up	Protein metabolism	Both	No	56
Vibrio vulnificus YJ016	Resistance	Up	Protein metabolism	Peaks	Maybe	57
Vibrio vulnificus YJ017	Resistance	Up	Protein metabolism	Peaks	Maybe	57

Burkholderia dolosa AUO158	Resistance	Up	Protein metabolism	Peaks	No	58
Burkholderia dolosa AUO158	Resistance	Up	Protein metabolism	Peaks	No	58
Sodalis glossinidius str. 'morsitans'	Tolerance	Up	Localization	Both	No	59
Sodalis glossinidius str. 'morsitans'	Tolerance	Up	Localization	Both	No	59
Zymomonas mobilis	Tolerance	Up	Protein metabolism	Peaks	No	60
Zymomonas mobilis	Tolerance	Up	Protein metabolism	Peaks	No	60
Escherichia coli	Tolerance	Up	Protein metabolism	Peaks	No	61
Escherichia coli	Tolerance	Up	Protein metabolism	Peaks	No	61
Acinetobacter sp. ADP1	Resistance	Up	Structural	Both	No	62
Acinetobacter sp. ADP1	Resistance	Up	Structural	Both	No	62
Acidovorax avenae subsp. citrulli AAC00-1	Adaptation	Up	Localization	Peaks	No	63
Acidovorax avenae subsp. citrulli AAC00-1	Adaptation	Up	Localization	Peaks	No	63
Polaromonas naphthalenivorans CJ2	Tolerance	Up	Response to stimulus	Both	No	64
Polaromonas naphthalenivorans CJ2	Tolerance	Up	Response to stimulus	Both	No	64
Escherichia coli E110019	Tolerance	Up	Electron transport	Peaks	No	65
Escherichia coli E110019	Tolerance	Up	Electron transport	Peaks	No	65
Escherichia coli K12	Tolerance	Up	Regulation	Both	No	66
Escherichia coli K12	Tolerance	Up	Regulation	Both	No	66
Acinetobacter sp. ADP1	Tolerance	Up	Lipid metabolism	Peaks	No	67
Acinetobacter sp. ADP1	Tolerance	Up	Lipid metabolism	Peaks	No	67
Acinetobacter sp. ADP1	Tolerance	Down	Lipid metabolism	Both	No	68
Acinetobacter sp. ADP1	Tolerance	Down	Lipid metabolism	Both	No	68
Pseudomonas syringae pv. syringae B728a	Resistance	Up	Localization	Peaks	No	69
Pseudomonas syringae pv. syringae B728a	Resistance	Up	Localization	Peaks	No	69
Caldicellulosiruptor saccharolyticus DSM 8903	Adaptation	Down	Protein metabolism	Peaks	Maybe	70
Caldicellulosiruptor saccharolyticus DSM 8903	Adaptation	Down	Protein metabolism	Peaks	Maybe	70
Vibrio cholerae RC385	Tolerance	Up	Xenobiotic metabolism	Peaks	No	71
Vibrio cholerae RC385	Tolerance	Up	Xenobiotic metabolism	Peaks	No	71
Pseudomonas fluorescens	Tolerance	Up	Xenobiotic metabolism	Peaks	No	72
Pseudomonas fluorescens	Tolerance	Up	Xenobiotic metabolism	Peaks	No	72
Nitrococcus mobilis Nb-231	Tolerance	Down	Sugar metabolism	Peaks	No	73
Nitrococcus mobilis Nb-231	Tolerance	Down	Sugar metabolism	Peaks	No	73
Neisseria gonorrhoeae FA 1090	Adaptation	Up	Energy reserve metabolism	Peaks	No	74
Neisseria gonorrhoeae FA 1090	Adaptation	Up	Energy reserve metabolism	Peaks	No	74
Escherichia coli E24377A	Tolerance	Up	Localization	Both	No	75
Escherichia coli E24377A	Tolerance	Up	Localization	Both	No	75
Acidovorax avenae subsp. citrulli AAC00-1	Resistance	Up	Nucleic acid metabolism	Peaks	No	76
Acidovorax avenae subsp. citrulli AAC00-1	Resistance	Up	Nucleic acid metabolism	Peaks	No	76
Rhodobacter sphaeroides ATCC 17029	Tolerance	Up	Localization	Peaks	No	77

Rhodobacter sphaeroides ATCC 17029	Tolerance	Up	Localization	Peaks	No	77
Lactobacillus sakei subsp. sakei 23K	Resistance	Up	Structural	Peaks	No	78
Lactobacillus sakei subsp. sakei 23K	Resistance	Up	Structural	Peaks	No	78
Shewanella sp. PV-4	Adaptation	Up	Electron transport	Peaks	No	79
Shewanella sp. PV-4	Adaptation	Up	Electron transport	Peaks	No	79
Caulobacter crescentus CB15	Tolerance	Up	Sugar metabolism	Peaks	No	80
Caulobacter crescentus CB15	Tolerance	Up	Sugar metabolism	Peaks	No	80
Bacteroides fragilis NCTC 9343	Tolerance	Up	Nucleic acid metabolism	Peaks	No	81
Bacteroides fragilis NCTC 9343	Tolerance	Up	Nucleic acid metabolism	Peaks	No	81
Verminephrobacter eiseniae EF01-2	Tolerance	Down	Electron transport	Both	No	82
Verminephrobacter eiseniae EF01-2	Tolerance	Down	Electron transport	Both	No	82
Rhodospirillum rubrum ATCC 11170	Adaptation	Up	Protein metabolism	Peaks	Maybe	83
Rhodospirillum rubrum ATCC 11170	Adaptation	Up	Protein metabolism	Peaks	Maybe	83
Nitrobacter winogradskyi Nb-255	Adaptation	Down	Response to stimulus	Peaks	No	84
Nitrobacter winogradskyi Nb-255	Adaptation	Down	Response to stimulus	Peaks	No	84
Streptomyces avermitilis MA-4680	Tolerance	Down	Sugar metabolism	Peaks	No	85
Streptomyces avermitilis MA-4680	Tolerance	Down	Sugar metabolism	Peaks	No	85
Acidiphilium cryptum JF-5	Resistance	Up	Xenobiotic metabolism	Peaks	No	86
Acidiphilium cryptum JF-5	Resistance	Up	Xenobiotic metabolism	Peaks	No	86
Rhodospirillum rubrum ATCC 11170	Tolerance	Up	Nucleic acid metabolism	Peaks	No	87
Rhodospirillum rubrum ATCC 11170	Tolerance	Up	Nucleic acid metabolism	Peaks	No	87
Delftia acidovorans	Resistance	Down	Energy reserve metabolism	Both	No	88
Delftia acidovorans	Resistance	Down	Energy reserve metabolism	Both	No	88
Streptococcus thermophilus LMD-9	Resistance	Up	Nucleic acid metabolism	Peaks	No	89
Streptococcus thermophilus LMD-9	Resistance	Up	Nucleic acid metabolism	Peaks	No	89
synthetic construct	Resistance	Up	Structural	Peaks	No	90
synthetic construct	Resistance	Up	Structural	Peaks	No	90
Rhodopseudomonas palustris BisA53	Tolerance	Up	Unknown	Peaks	No	91
Rhodopseudomonas palustris BisA53	Tolerance	Up	Unknown	Peaks	No	91
Geobacillus kaustophilus HTA426	Resistance	Down	Sugar metabolism	Peaks	No	92
Geobacillus kaustophilus HTA426	Resistance	Down	Sugar metabolism	Peaks	No	92
Rhodopseudomonas palustris BisA53	Resistance	Up	Cell communication	Peaks	No	93
Rhodopseudomonas palustris BisA53	Resistance	Up	Cell communication	Peaks	No	93
Crocosphaera watsonii WH 8501	Tolerance	Down	Unknown	Peaks	No	94
Crocosphaera watsonii WH 8501	Tolerance	Down	Unknown	Peaks	No	94
Vibrio vulnificus CMCP6	Tolerance	Up	Localization	Peaks	No	95
Vibrio vulnificus CMCP6	Tolerance	Up	Localization	Peaks	No	95
Bradyrhizobium sp. BTAi1	Tolerance	Up	Protein metabolism	Peaks	No	96
Bradyrhizobium sp. BTAi1	Tolerance	Up	Protein metabolism	Peaks	No	96

Thermobifida fusca YX	Resistance	Up	Regulation	Peaks	No	97
Thermobifida fusca YX	Resistance	Up	Regulation	Peaks	No	97
Bordetella bronchiseptica RB50	Tolerance	Up	Localization	Peaks	No	98
Bordetella bronchiseptica RB50	Tolerance	Up	Localization	Peaks	No	98
Moorella thermoacetica ATCC 39073	Resistance	Up	Response to stimulus	Peaks	No	99
Moorella thermoacetica ATCC 39073	Resistance	Up	Response to stimulus	Peaks	No	99
Rubrivivax gelatinosus PM1	Tolerance	Up	Regulation	Peaks	No	100
Rubrivivax gelatinosus PM1	Tolerance	Up	Regulation	Peaks	No	100
Streptomyces coelicolor A3(2)	Tolerance	Down	Sugar metabolism	Peaks	No	101
Streptomyces coelicolor A3(2)	Tolerance	Down	Sugar metabolism	Peaks	No	101
Rubrivivax gelatinosus PM1	Adaptation	Up	Localization	Peaks	No	102
Rubrivivax gelatinosus PM1	Adaptation	Up	Localization	Peaks	No	102
Mycobacterium flavescens PYR-GCK	Tolerance	Up	Secretion	Peaks	No	103
Mycobacterium flavescens PYR-GCK	Tolerance	Up	Secretion	Peaks	No	103
Clostridium thermocellum ATCC 27405	Tolerance	Up	Localization	Peaks	No	104
Clostridium thermocellum ATCC 27405	Tolerance	Up	Localization	Peaks	No	104
Yersinia pseudotuberculosis IP 32953	Resistance	Up	Electron transport	Peaks	No	105
Yersinia pseudotuberculosis IP 32953	Resistance	Up	Electron transport	Peaks	No	105
Acidovorax sp. JS42	Tolerance	Up	Energy reserve metabolism	Both	No	106
Acidovorax sp. JS42	Tolerance	Up	Energy reserve metabolism	Both	No	106
Bordetella parapertussis	Resistance	Up	Response to stimulus	Peaks	No	107
Bordetella parapertussis	Resistance	Up	Response to stimulus	Peaks	No	107
Oceanicaulis alexandrii HTCC2633	Resistance	Down	Sugar metabolism	Both	No	108
Oceanicaulis alexandrii HTCC2633	Resistance	Down	Sugar metabolism	Both	No	108
Shewanella sp. PV-4	Tolerance	Up	Nucleic acid metabolism	Peaks	No	109
Shewanella sp. PV-4	Tolerance	Up	Nucleic acid metabolism	Peaks	No	109
Rhodopseudomonas palustris BisB5	Tolerance	Up	Protein metabolism	Both	No	110
Rhodopseudomonas palustris BisB5	Tolerance	Up	Protein metabolism	Both	No	110
Rhodopseudomonas palustris BisB5	Tolerance	Up	Protein metabolism	Peaks	No	111
Rhodopseudomonas palustris BisB5	Tolerance	Up	Protein metabolism	Peaks	No	111
Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Both	No	112
Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Both	No	112
Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Peaks	No	113
Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Peaks	No	113
Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Peaks	No	114

Polaromonas sp. JS666	Tolerance	Up	Response to stimulus	Peaks	No	114
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Both	No	115
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Both	No	115
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	116
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	116
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	117
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	117
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	118
Polaromonas sp. JS666	Tolerance	Down	Electron transport	Peaks	No	118
Acinetobacter junii	Adaptation	Up	Secretion	Both	No	119
Acinetobacter junii	Adaptation	Up	Secretion	Both	No	119
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	120
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	120
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	121
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	121
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	122
Acinetobacter junii	Adaptation	Up	Secretion	Peaks	No	122
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	123
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	123
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	124
Acinetobacter sp. ADP1	Resistance	Down	Electron transport	Both	No	124
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Both	No	*125*
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Both	No	*125*
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Peaks	No	*126*
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Peaks	No	*126*
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Peaks	No	*127*
Bacillus anthracis str. 'Ames Ancestor'	Adaptation	Up	Secretion	Peaks	No	*127*
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Both	No	*128*
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Both	No	*128*
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Both	No	*129*
Acinetobacter sp. ADP1	Tolerance	Up	Energy reserve metabolism	Both	No	*129*
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Both	No	*130*
Arthrobacter sp. FB24	Resistance	Down	Energy reserve metabolism	Both	No	*130*
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Both	No	*131*
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Both	No	*131*
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Peaks	No	*132*
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Peaks	No	132
Acinetobacter sp. V-26	Adaptation	Up	Secretion	Peaks	No	133

Acinetobacter sp. V-26	Adaptation	Up	Secretion	Peaks	No	133
Aurantimonas sp. SI85-9A1	Resistance	Up	Electron transport	Both	No	134
Aurantimonas sp. SI85-9A1	Resistance	Up	Electron transport	Both	No	134
Polaromonas sp. JS666	Tolerance	Down	Energy reserve metabolism	Both	No	135
Polaromonas sp. JS666	Tolerance	Down	Energy reserve metabolism	Both	No	135
Polaromonas sp. JS666	Tolerance	Down	Energy reserve metabolism	Both	No	136
Polaromonas sp. JS666	Tolerance	Down	Energy reserve metabolism	Both	No	136
Tropheryma whipplei str. Twist	Tolerance	Up	Protein metabolism	Both	No	137
Tropheryma whipplei str. Twist	Tolerance	Up	Protein metabolism	Both	No	137
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Both	No	138
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Both	No	138
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	139
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	139
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	140
Arthrobacter sp. FB24	Tolerance	Up	Protein metabolism	Peaks	No	140
marine actinobacterium PHSC20C1	Resistance	Down	Energy reserve metabolism	Both	No	141
marine actinobacterium PHSC20C1	Resistance	Down	Energy reserve metabolism	Both	No	141
marine actinobacterium PHSC20C1	Resistance	Down	Energy reserve metabolism	Peaks	No	142
marine actinobacterium PHSC20C1	Resistance	Down	Energy reserve metabolism	Peaks	No	142
Dechloromonas aromatica RCB	Resistance	Down	Energy reserve metabolism	Peaks	No	143
Dechloromonas aromatica RCB	Resistance	Down	Energy reserve metabolism	Peaks	No	143
Ralstonia solanacearum UW551	Tolerance	Up	Protein metabolism	Both	No	144
Ralstonia solanacearum UW551	Tolerance	Up	Protein metabolism	Both	No	144
Bordetella pertussis Tohama I	Tolerance	Up	Protein metabolism	Peaks	No	145
Bordetella pertussis Tohama I	Tolerance	Up	Protein metabolism	Peaks	No	145
Burkholderia pseudomallei K96243	Tolerance	Up	Protein metabolism	Peaks	No	146
Burkholderia pseudomallei K96243	Tolerance	Up	Protein metabolism	Peaks	No	146
Nitrosomonas eutropha C71	Tolerance	Up	Localization	Both	No	147
Nitrosomonas eutropha C71	Tolerance	Up	Localization	Both	No	147
Nitrosomonas eutropha C71	Tolerance	Up	Localization	Both	No	148
Nitrosomonas eutropha C71	Tolerance	Up	Localization	Both	No	148
Acinetobacter sp. ADP1	Tolerance	Up	Localization	Both	No	149
Acinetobacter sp. ADP1	Tolerance	Up	Localization	Both	No	149
Rhodoferax ferrireducens DSM 15236	Tolerance	Up	Localization	Peaks	No	150
Rhodoferax ferrireducens DSM 15236	Tolerance	Up	Localization	Peaks	No	150
Polaromonas naphthalenivorans CJ2	Tolerance	Up	Localization	Both	No	151
Polaromonas naphthalenivorans CJ2	Tolerance	Up	Localization	Both	No	151
Polaromonas naphthalenivorans CJ2	Tolerance	Up	Localization	Peaks	No	152

Polaromonas naphthalenivorans CJ2	Tolerance	Up	Localization	Peaks	No	152
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Both	No	153
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Both	No	153
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	154
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	154
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	155
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	155
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	156
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	156
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	157
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	157
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	158
Rubrivivax gelatinosus PM1	Tolerance	Up	Localization	Peaks	No	158

Appendix III:

List of peptides and their respective proteins identified in the metabolic labeling experiment of *B. cepacia* Cd44 cadmium adaptation.(pages 201 to 210)

Fracti	Accession	Mass	Score (%)	Function	Coverage	Description	m/z	z
29		1169.7797	99			COG0459: Chaper	585.8971	2
29		1578.9918	99			COG0459: Chaper	790.5032	2
29		1871.9865	99			COG0459: Chaper	937.0005	2
29	gi|84316904	32,626	99	Protein metabolism	10.54%	COG0459: Chaperonin GroEL (HSP6		
1		814.5794	99			Electron transfer fl	408.29697	2
1		1009.5893	99			Electron transfer fl	505.8019	2
1		1873.9817	99			Electron transfer fl	937.9981	2
1		1086.5884	99			Electron transfer fl	544.30145	2
1	gi|110595124	31,108	99	Electron transport	14.19%	Electron transfer flavoprotein beta-sul		
28		1482.3804	99			formate dehydroge	742.19745	2
28		3149.1553	99			formate dehydroge	788.2961	4
28	gi|107022109	107,094	99	Energy reserve metabolism	4.58%	formate dehydrogenase, alpha subun		
9		1629.8848	99			Histone-like bacter	544.3022	3
9		1538.7776	99			Histone-like bacter	770.39606	2
9		1500.7168	99			Histone-like bacter	751.36566	2
9	gi|67546900	9,605	99	Protein metabolism	60.87%	Histone-like bacterial DNA-binding pr		
4		1055.601	99			Phasin [Burkholder	528.80774	2
4		1167.7267	99			Phasin [Burkholder	584.8706	2
4		1292.6982	99			Phasin [Burkholder	647.3564	2
4		1609.7979	99			Phasin [Burkholder	805.9062	2
4		2010.0638	99			Phasin [Burkholder	1006.0392	2
4		1917.9446	99			Phasin [Burkholder	640.32214	3
4		778.3911	99			Phasin [Burkholder	390.20282	2
4		1302.6128	99			Phasin [Burkholder	652.31366	2
4		1298.6835	99			Phasin [Burkholder	650.349	2
4	gi|78066909	19,801	99	Energy reserve metabolism	57.98%	Phasin [Burkholderia sp. 383]		
38		2024.9752	99			Succinyl-CoA synth	1013.4949	2
38		1559.5756	99			Succinyl-CoA synth	780.79504	2
38		2094.9963	99			Succinyl-CoA synth	1048.5055	2
38	gi|67548608	41,207	99	Energy reserve metabolism	13.14%	Succinyl-CoA synthetase, beta subun		
15		1241.3727	98			hypothetical protei	414.79816	3
15		1971.7881	98			hypothetical protei	986.9013	2
15	gi|77459624	142,944	98	Unknown	2.20%	hypothetical protein Pfl_3402 [Pseud		
28		1184.3883	98			threonine dehydrat	593.2014	2
28		1253.5946	98			threonine dehydrat	627.80457	2
28	gi|106883414	55,844	98	Protein metabolism	3.91%	threonine dehydratase, biosynthetic [I		
5		1474.7782	97			Phosphoribosylantl	738.39636	2
5		1030.5763	97			Phosphoribosylantl	516.2954	2
5	gi|78778922	24,625	97	Nucleic acid metabolism	9.17%	Phosphoribosylanthranilate isomeras		
38		3211.1628	97			probable non-ribos	803.798	4
38		3241.198	97			probable non-ribos	811.30676	4
38		3815.5754	97			probable non-ribos	954.9011	4
38		1933.9723	97			probable non-ribos	967.9934	2
38	gi|53722657	362,971	97	Protein metabolism	3.01%	probable non-ribosomal peptide synth		
34		2885.98	97			type IV pilin biogen	963.0006	3
34		2117.5554	97			type IV pilin biogen	530.3961	4
34	gi|68546839	124,941	97	Structural	2.18%	type IV pilin biogenesis protein, putati		
9		1780.9998	96			2-oxo-acid dehydrc	891.50714	2
9		1781.0065	96			2-oxo-acid dehydrc	594.6761	3
9		1971.0366	96			2-oxo-acid dehydrc	986.5256	2
9		984.51025	96			2-oxo-acid dehydrc	493.2624	2
9	gi|67545539	100,675	96	Electron transport	5.57%	2-oxo-acid dehydrogenase E1 compo		
9		1549.7881	96			COG0450: Peroxir	775.9013	2
9		957.59503	96			COG0450: Peroxir	479.80478	2
9	gi|67738122	18,916	96	Defense	13.02%	COG0450: Peroxiredoxin [Burkholder		
19		535.1919	96			UvrD/REP helicase	536.19916	1
19		1331.577	96			UvrD/REP helicase	666.7958	2
19		1002.83093	96			UvrD/REP helicase	335.28424	3
19		1959.8889	96			UvrD/REP helicase	654.3036	3
19	gi|86747755	127,450	96	Nucleic acid metabolism	3.88%	UvrD/REP helicase [Rhodopseudomc		
4		1414.782	95			Cell division protei	708.39825	2
4		1356.7743	95			Cell division protei	679.3944	2
4	gi|90962024	32,147	95	Localization	8.10%	Cell division protein [Lactobacillus sal		
10		2010.989	95			Homoserine dehyd	1006.5018	2
10		1254.881	95			Homoserine dehyd	419.30093	3
10	gi|77686734	46,980	95	Cell communication	3.94%	Homoserine dehydrogenase [Alkaliph		
22		1581.8818	94			Peptidoglycan-bind	528.3012	3

22		1234.5881	94			Peptidoglycan-bind	618.30133	2
22		2759.5793	94			Peptidoglycan-bind	690.9021	4
22	gi|89211304	61,533	94	Structural	5.86%	Peptidoglycan-binding LysM:Cell wall		
11		823.58685	93			cytochrome c-type	412.8007	2
11		1240.7888	93			cytochrome c-type	621.4017	2
11	gi|89054673	43,761	93	Energy reserve metabolism	4.19%	cytochrome c-type biogenesis protein		
35		1228.5802	93			DNA polymerase II	615.29736	2
35		1656.7767	93			DNA polymerase II	829.3956	2
35	gi|29346774	41,412	93	Nucleic acid metabolism	6.15%	DNA polymerase III, beta chain [Bact		
14		1074.584	93			hypothetical proteir	538.29926	2
14		1032.5853	93			hypothetical proteir	517.2999	2
14		1002.79486	93			hypothetical proteir	502.4047	2
14	gi|20806991	90,746	93	Unknown	3.19%	hypothetical protein TTE0486 [Therm		
21		1706.7909	93			periplasmic ligand	854.4027	2
21		2130.1758	93			periplasmic ligand	1066.0952	2
21		2809.168	93			periplasmic ligand	703.29926	4
21	gi|76811647	70,350	93	Lipid metabolism	6.95%	periplasmic ligand binding lipoprotein		
13		1028.5895	93			Predicted dehydroç	515.302	2
13		2081.9802	93			Predicted dehydroç	1041.9974	2
13	gi|15896641	36,670	93	Electron transport	8.07%	Predicted dehydrogenase [Clostridiun		
6		2129.6636	93			putative isoprenoid	710.89514	3
6		2065.1816	93			putative isoprenoid	689.4012	3
6	gi|91215079	36,722	93	Energy reserve metabolism	11.11%	putative isoprenoid biosynthesis relat		
7		4001.5566	93			sugar ABC transpo	1001.3964	4
7		1660.7897	93			sugar ABC transpo	831.4021	2
7		2537.9644	93			sugar ABC transpo	846.99536	3
7	gi|56962459	46,956	93	Sugar metabolism	12.94%	sugar ABC transporter substrate-bind		
31		1549.7881	92			alkyl hydroperoxide	775.9013	2
31		661.4964	92			alkyl hydroperoxide	662.50366	1
31		2713.1685	92			alkyl hydroperoxide	905.3968	3
31	gi|115422149	20,230	92	Defense	23.63%	alkyl hydroperoxide reductase [Borde		
18		1706.7909	92			carbamoyl-phosphi	854.4027	2
18		1356.7743	92			carbamoyl-phosphi	679.3944	2
18	gi|91775132	116,984	92	Nucleic acid metabolism	2.62%	carbamoyl-phosphate synthase, large		
25		2237.6738	92			COG0046: Phosph	746.8985	3
25		2777.1582	92			COG0046: Phosph	695.2968	4
25		3049.5513	92			COG0046: Phosph	763.3951	4
25	gi|46156887	143,149	92	Nucleic acid metabolism	5.09%	COG0046: Phosphoribosylformylglyci		
8		2738.9753	92			GCN5-related N-ac	913.999	3
8		2266.161	92			GCN5-related N-ac	756.3942	3
8	gi|108803641	19,179	92	Lipid metabolism	24.71%	GCN5-related N-acetyltransferase [R		
7		1856.389	92			hypothetical proteir	929.2018	2
7		2641.1694	92			hypothetical proteir	881.3971	3
7		2611.4783	92			hypothetical proteir	871.5	3
7	gi|68229369	81,736	92	Unknown	7.57%	hypothetical protein Franean1DRAFT		
16		1030.5763	92			interaptin [Legionel	516.2954	2
16		655.49023	92			interaptin [Legionel	656.4975	1
16		1261.5923	92			interaptin [Legionel	631.8034	2
16	gi|52842521	130,787	92	Cell communication	1.89%	interaptin [Legionella pneumophila su		
9		2143.049	92			Linocin_M18 bacte	1072.5319	2
9		1164.6013	92			Linocin_M18 bacte	583.3079	2
9		1502.7557	92			Linocin_M18 bacte	752.38513	2
9		2143.0479	92			Linocin_M18 bacte	715.35657	3
9	gi|78060115	29,035	92	Response to stimulus	26.20%	Linocin_M18 bacteriocin protein [Burk		
1		1995.06	91			5-oxoprolinase (AT	998.5373	2
1		2082.9116	91			5-oxoprolinase (AT	695.3111	3
1		1923.9229	91			5-oxoprolinase (AT	642.3149	3
1	gi|116691651	129,194	91	Protein metabolism	5.28%	5-oxoprolinase (ATP-hydrolyzing) [Bu		
17		3393.273	91			ATPas [Xanthobac	1132.0983	3
17		3126.389	91			ATPas [Xanthobac	782.6045	4
17	gi|89362433	66,161	91	Localization	9.00%	ATPas [Xanthobacter autotrophicus F		
9		1337.7831	91			COG0200: Ribosor	669.8988	2
9		1112.5756	91			COG0200: Ribosor	557.29504	2
9		1426.8578	91			COG0200: Ribosor	476.62653	3
9	gi|84360487	15,096	91	Protein metabolism	21.53%	COG0200: Ribosomal protein L15 [Bu		
9		1545.7366	91			COG2885: Outer m	773.87555	2
9		1291.6083	91			COG2885: Outer m	646.8114	2
9		1082.764	91			COG2885: Outer m	542.3893	2

9	gi|84357159	22,407	91	Localization	15.46%	COG2885: Outer membrane protein ε		
20		2212.4946	91			conserved hypothe	738.5055	3
20		1260.5906	91			conserved hypothe	421.20413	3
20	gi|89199851	74,023	91	Unknown	4.37%	conserved hypothetical protein [Bacill		
38		3312.2603	91			conserved hypothe	1105.0941	3
38		2372.0002	91			conserved hypothe	1187.0074	2
38	gi|71367974	45,671	91	Unknown	11.06%	conserved hypothetical protein [Noca		
17		2806.1765	91			conserved hypothe	936.3994	3
17		1264.5782	91			conserved hypothe	633.2964	2
17		2278.189	91			conserved hypothe	1140.1018	2
17	gi|82738547	116,867	91	Unknown	5.05%	conserved hypothetical protein [Pseu		
14		2207.694	91			hypothetical protei	736.9053	3
14		2525.5635	91			hypothetical protei	632.39813	4
14		749.3962	91			hypothetical protei	750.40344	1
14	gi|94417343	53,263	91	Unknown	10.06%	hypothetical protein PaerP_0100066C		
7		2258.6914	91			hypothetical protei	753.90436	3
7		1526.774	91			hypothetical protei	764.3943	2
7	gi|111220783	36,335	91	Unknown	9.63%	hypothetical protein; putative signal p		
37		1219.1874	91			NADH-ubiquinone	610.60095	2
37		2076.719	91			NADH-ubiquinone	520.187	4
37		1786.7938	91			NADH-ubiquinone	894.4042	2
37	gi|39936010	48,335	91	Structural	9.30%	NADH-ubiquinone dehydrogenase ch		
36		3465.2852	91			Predicted membra	1156.1024	3
36		2951.9639	91			Predicted membra	984.99524	3
36	gi|116617591	98,282	91	Structural	6.33%	Predicted membrane protein [Leucon		
10		2300.687	91			Predicted periplasn	767.90295	3
10		1571.5966	91			Predicted periplasn	786.80554	2
10		1442.7885	91			Predicted periplasn	722.4015	2
10	gi|83622431	27,932	91	Lipid metabolism	17.12%	Predicted periplasmic or secreted lipc		
36		2625.8867	91			putatve zinc-bindin	876.30286	3
36		4260.3516	91			putatve zinc-bindin	1066.0952	4
36	gi|86130443	76,747	91	Defense	7.79%	putatve zinc-binding dehydrogenase [		
12		1776.3839	91			similar to Cation/m	445.10324	4
12		823.58685	91			similar to Cation/m	412.8007	2
12	gi|89359069	71,654	91	Electron transport	2.85%	similar to Cation/multidrug efflux pum		
8		2225.687	90			ABC transporter re	742.9029	3
8		1418.7762	90			ABC transporter re	710.3954	2
8	gi|91975323	26,739	90	Localization	14.17%	ABC transporter related [Rhodopseuc		
16		3312.2603	90			Acetyl-CoA carbox	1105.0941	3
16		1262.5797	90			Acetyl-CoA carbox	632.2971	2
16	gi|104773956	50,786	90	Energy reserve metabolism	8.48%	Acetyl-CoA carboxylase, biotin carbo		
19		1559.376	90			Carboxylesterase,	780.69525	2
19		1082.7805	90			Carboxylesterase,	542.3975	2
19	gi|116625582	56,345	90	Lipid metabolism	4.13%	Carboxylesterase, type B [Solibacter		
38		1605.2655	90			Deoxyxylulose-5-pl	536.09576	3
38		1967.7799	90			Deoxyxylulose-5-pl	984.8972	2
38	gi|83368175	68,735	90	Sugar metabolism	4.94%	Deoxyxylulose-5-phosphate synthase		
9		1240.7406	90			Endoribonuclease	621.37756	2
9		1518.7314	90			Endoribonuclease	760.373	2
9	gi|78067536	13,861	90	Nucleic acid metabolism	17.19%	Endoribonuclease L-PSP [Burkholder		
8		2099.6736	90			hypothetical protei	700.89844	3
8		926.3995	90			hypothetical protei	927.40674	1
8	gi|85707925	117,503	90	Unknown	2.08%	hypothetical protein NAP1_01780 [Er		
24		1833.5819	90			protein of unknown	612.20123	3
24		1583.5883	90			protein of unknown	792.8014	2
24	gi|116625650	88,271	90	Unknown	3.21%	protein of unknown function DUF214		
11		1551.3799	90			protein of unknown	776.6972	2
11		2101.1626	90			protein of unknown	701.3948	3
11	gi|92116781	56,586	90	Unknown	5.51%	protein of unknown function DUF882		
6		2705.9614	90			putative ABC trans	902.9944	3
6		3917.986	90			putative ABC trans	980.5038	4
6	gi|16761557	131,625	90	Localization	4.84%	putative ABC transporter protein [Salr		
26		1301.779	90			serine/threonine pr	651.8968	2
26		1779.7794	90			serine/threonine pr	890.897	2
26	gi|91787132	66,411	90	Protein metabolism	4.14%	serine/threonine protein kinase [Polar		
9		1575.3798	90			serine/threonine pr	788.69714	2
9		722.3903	90			serine/threonine pr	723.3976	1
9	gi|32471645	119,319	90	Protein metabolism	1.68%	serine/threonine protein kinase [Rhod		

30		3085.1213	90			Twin-arginine trans	772.2876	4
30		2044.9741	90			Twin-arginine trans	1023.4943	2
30	gi|109648135	92,825	90	Cell communication	5.08%	Twin-arginine translocation pathway s		
7		1311.76	89			COG0050: GTPas(	656.88727	2
7		1680.9073	89			COG0050: GTPas(	841.46094	2
7	gi|67645167	36,391	89	Protein metabolism	7.78%	COG0050: GTPases - translation elo		
1		2140.115	87			COG0469: Pyruvat	714.3789	3
1		1552.8417	87			COG0469: Pyruvat	777.4281	2
1		1146.542	87			COG0469: Pyruvat	574.27826	2
1	gi|84355614	49,048	87	Energy reserve metabolism	8.35%	COG0469: Pyruvate kinase [Burkholc		
17		2308.7832	87			NLP/P60 [Herpetos	770.6016	3
17		942.5785	87			NLP/P60 [Herpetos	472.2965	2
17	gi|113941631	59,431	87	Unknown	5.04%	NLP/P60 [Herpetosiphon aurantiacus		
37		3287.178	86			nitroreductase fam	822.80176	4
37		1844.7609	86			nitroreductase fam	923.3877	2
37	gi|30264416	24,011	86	Electron transport	20.29%	nitroreductase family protein [Bacillus		
2		3010.462	86			serine hydroxymetl	1004.49457	3
2		4034.1062	86			serine hydroxymetl	1009.5338	4
2		1442.756	86			serine hydroxymetl	722.38525	2
2	gi|78065369	44,842	86	Protein metabolism	15.42%	serine hydroxymethyltransferase [Bur		
3		1311.7244	85			elongation factor T	656.86945	2
3		1332.6385	85			elongation factor T	667.32654	2
3		1327.7184	85			elongation factor T	664.86646	2
3		1200.5438	85			elongation factor T	601.2792	2
3		1680.915	85			elongation factor T	841.4648	2
3	gi|78064909	42,934	85	Protein metabolism	15.00%	elongation factor Tu [Burkholderia sp.		
1		2287.0916	85			serine-type carbox	763.3711	3
1		2313.1216	85			serine-type carbox	1157.5681	2
1		2287.1924	85			serine-type carbox	1144.6035	2
1		2485.1519	85			serine-type carbox	829.3912	3
1	gi|83716854	60,133	85	Protein metabolism	8.12%	serine-type carboxypeptidase family p		
16		2372.7788	83			filamentous haema	594.20197	4
16		2333.1934	83			filamentous haema	1167.604	2
16	gi|115526543	415,701	83	Defense	1.03%	filamentous haemagglutinin family ou		
4		2429.1008	80			Redoxin [Burkholde	810.7075	3
4		1306.6781	80			Redoxin [Burkholde	654.3463	2
4	gi|107021678	17,200	80	Defense	21.56%	Redoxin [Burkholderia cenocepacia A		
1		1943.992	79			Alkyl hydroperoxide	973.00323	2
1		2287.0916	79			Alkyl hydroperoxide	763.3711	3
1	gi|67546142	17,283	79	Defense	21.56%	Alkyl hydroperoxide reductase/ Thiol s		
38		1748.9796	78			malate dehydroger	875.4971	2
38		1759.7727	78			malate dehydroger	880.8936	2
38	gi|33593357	35,664	78	Energy reserve metabolism	8.81%	malate dehydrogenase [Bordetella pe		
35		3444.3884	77			protease Do [Syntr	862.1044	4
35		2115.2646	77			protease Do [Syntr	706.09546	3
35	gi|116749436	49,467	77	Protein metabolism	9.68%	protease Do [Syntrophobacter fumarc		
15		3528.2612	76			Queuine/archaeosi	1177.0944	3
15		1222.5867	76			Queuine/archaeosi	612.3006	2
15		770.69745	76			Queuine/archaeosi	771.7047	1
15	gi|116493017	43,206	76	Nucleic acid metabolism	11.58%	Queuine/archaeosine tRNA-ribosyltra		
1		1516.7074	75			GroEL [Bartonella l	759.36096	2
1		1089.5344	75			GroEL [Bartonella l	545.7745	2
1		1259.662	75			GroEL [Bartonella l	630.83826	2
1		1419.6893	75			GroEL [Bartonella l	474.23703	3
1		1887.021	75			GroEL [Bartonella l	944.51776	2
1	gi|2290621	43,658	75	Protein metabolism	7.35%	GroEL [Bartonella henselae]		
22		2468.6877	75			Heavy metal efflux	823.9032	3
22		1666.7924	75			Heavy metal efflux	834.40344	2
22		1488.7832	75			Heavy metal efflux	745.39886	2
22		770.2884	75			Heavy metal efflux	771.29565	1
22	gi|110599599	117,187	75	Electron transport	4.95%	Heavy metal efflux pump CzcA [Geob		

Peptide	Mr(Calc)	Delta(Mass)	Score(%)	Start	End	As Cd goes up	Change	Accession
AVIAAVEELKK	1169.7019	0.07775879	99.00	123	133	3.67	0-0.04-0.44	
AAVEEGVVPGGGVAL	1578.873	0.11877441	99.00	405	421	3.67	0-0.04-0.44	
ENTTIIDGAGVQADIE/	1871.9224	0.06408691	98.39	94	111	3.67	0-0.04-0.44	
i0 family) [Pseudomonas aeruginosa C3719]						down	0-0.04-0.44	gi|84316904
LGAAIGASR	814.4661	0.11328125	99.00	221	229	1.95	0.04-0.44	
VLHADGASLK	1009.5556	0.03369141	99.00	57	66	1.95	0.04-0.44	
AAVDAGYAPNDLQVG	1873.917	0.06469727	99.00	230	248	1.95	0.04-0.44	
SDRPELTAAK	1086.5669	0.02148438	38.97	186	195	1.95	0.04-0.44	
bunit:Electron transfer flavoprotein, alpha subunit [Acidovorax sp. JS42]						down	0.04-0.44	gi|110595124
GYPASCTTPAEAGMK	1482.6482	-0.26782227	99.00	103	117	2	0-0.04-0.44	
VAAGESESFMASECV	3149.3477	-0.19238281	99.00	225	256	4.33	0-0.04-0.44	
it [Burkholderia cenocepacia AU 1054]						up	0-0.04-0.44	gi|107022109
AQTGETLDTLLEVIK	1629.8826	0.00219727	99.00	19	33	4.28	0-0.04	
GDAVQLIGFGSFGSGI	1538.773	0.00463867	95.98	39	54	3.94	0-0.04	
QELIDAVAAQTGASK	1500.7783	-0.06152344	53.91	4	18	4.56	0-0.04	
otein [Burkholderia vietnamiensis G4]						up	0-0.04	gi|67546900
VQALVDNVAK	1055.5974	0.00354004	99.00	113	122	5.6	0-0.04	
LIELNLQVVK	1167.7227	0.00402832	99.00	34	43	4.89	0-0.04	
ANLESLFGLTTK	1292.6978	0.00048828	99.00	15	26	5.32	0-0.04	
SALNAANTTYETVQK	1609.7947	0.00317383	95.69	137	151	4.02	0-0.04	
DAQELIALQASLSQPV	2010.0632	0.00061035	94.87	61	79	2.69	0-0.04	
QAVEIAETNFNAAAAV	1917.9795	-0.03491211	92.91	155	173	3.55	0-0.04	
AFEGVEK	778.3861	0.00500488	84.13	27	33	4.9	0-0.04	
STLAEGQENAQR	1302.6165	-0.00366211	60.77	44	55	3.85	0-0.04	
NAPAGSETAVAALK	1298.6831	0.00036621	53.05	123	136	6.21	0-0.04	
						up	0-0.04	gi|78066909
VIVEPSTGLLDAQADD	2025.063	-0.08776856	99.00	147	166	5	0-0.04-0.44	
DLDEEDPAEIEASK	1559.6838	-0.10827637	98.90	234	247	3.48	0-0.04-0.44	
IGVPAASIPQARTILQC	2095.2153	-0.21899414	50.12	167	186	3	0-0.04-0.44	
iit [Burkholderia vietnamiensis G4]						down	0-0.04-0.44	gi|67548608
AVELQQSALQR	1241.6729	-0.3001709	89.47	985	995	2.33	0.04-0.44	
APRSPFQVTGTTAALE	1972.0378	-0.24975586	75.33	1252	1270	2.33	0.04-0.44	
omonas fluorescens PfO-1]						down	0.04-0.44	gi|77459624
ESVLAVTIPEK	1184.6653	-0.27697754	94.23	331	341	2.94	0-0.04-0.44	
MTELSAAQYLK	1253.6326	-0.03796387	53.14	1	11	2.94	0-0.04-0.44	
Psychromonas ingrahamii 37]						down	0-0.04-0.44	gi|106883414
DLEKTGALIKFLK	1474.8757	-0.09753418	98.08	204	216	1.56	0-0.04	
MPKTNTLVK	1030.5845	-0.00817871	53.04	1	9	1.56	0-0.04	
e [Prochlorococcus marinus str. MIT 9312]						up	0-0.04	gi|78778922
ESPSRTYWMYRCHHI	3211.457	-0.29418945	99.00	145	171	3	0.04-0.44	
FDSRGYRMDVSRAPL	3241.4712	-0.27319336	78.81	1220	1247	1.5	0.04-0.44	
TYWMYRCHHLVADG(	3815.8088	-0.23339844	61.81	150	180	1.86	0.04-0.44	
EEALAAIWRELLHVEF	1934.0374	-0.06506348	52.50	2110	2125	7	0.04-0.44	
ietase [Burkholderia pseudomallei K96243]						up	0.04-0.44	gi|53722657
APELMVKTRCEDNNE	2886.2039	-0.22387695	99.00	1107	1132	3.33	0-0.04	
TRCEDNNEHCDGCPF	2117.7834	-0.22802734	89.39	1114	1132	2.5	0-0.04	
ive [Shewanella amazonensis SB2B]						down	0-0.04	gi|68546839
ATSLPVPALDAFEPLL	1780.9973	0.00244141	98.35	476	492	3	0-0.04	
ATSLPVPALDAFEPLL	1780.9973	0.00915527	90.37	476	492	3.49	0-0.04	
F(OxM)KYLESRGIAQT	1971.0361	0.00048828	68.59	215	231	3.72	0-0.04	
AEADKKAPR	984.5352	-0.02496338	56.14	728	736	2.88	0-0.04	
nent homodimeric type [Burkholderia vietnamiensis G4]						down	0-0.04	gi|67545539
DAVLLGGSSDNEFVK	1549.7625	0.02563477	99.00	62	76	3.23	0-0.04-0.44	
DKEAGVALR	957.52423	0.07080078	84.08	109	117	3.23	0-0.04-0.44	
ia pseudomallei 668]						up	0-0.04-0.44	gi|67738122
ITFR	535.3118	-0.11993408	88.23	193	196	2.7	0-0.04-0.44	
DALAASDTAQLEK	1331.657	-0.07995606	82.56	279	291	2.7	0-0.04-0.44	
IVDGPHLPR	1002.561	0.2699585	75.14	245	253	2.7	0-0.04-0.44	
AEIASLIASGTMTGPRI	1959.9685	-0.07958984	66.12	569	587	2.7	0-0.04-0.44	
nas palustris HaA2]						down	0-0.04-0.44	gi|86747755
LTSNGRELNVGQK	1414.7527	0.02929688	93.64	171	183	3.07	0.04-0.44	
DVNISLEKNVVK	1356.7612	0.01306152	81.24	139	150	3.07	0.04-0.44	
livarius subsp. salivarius UCC118]						down	0.04-0.44	gi|90962024
PSVSLVFITHRTNEGN	2011.0852	-0.09619141	92.83	391	408	4.25	0.04-0.44	
PSVSLVFITHR	1254.7085	0.17248535	89.85	391	401	4.25	0.04-0.44	
ilus metalliredigenes QYMF]						up	0.04-0.44	gi|77686734
IMSYNDLTYGHLR	1581.761	0.12084961	92.49	395	407	3.22	0.04-0.44	

RFNTTVQELK	1234.667	-0.07885742	88.81	216	225		3.22	0.04-0.44	
IASKFGMEVSKIMSYN	2759.3774	0.2019043	84.15	384	407		3.22	0.04-0.44	
hydrolase, SleB [Halothermothrix orenii H 168]						down		0.04-0.44	gi|89211304
PSLLAAPR	823.4915	0.09533691	84.26	308	315		2.32	0-0.04	
PERAAMVAQLR	1240.6709	0.11791992	70.66	158	168		2.32	0-0.04	
CycH [Jannaschia sp. CCS1]						up		0-0.04	gi|89054673
NAVFMLESYR	1228.5911	-0.01086426	80.68	224	233		1.75	0.04-0.44	
ITMVASDGHKLVRCK	1656.8804	-0.1036377	72.33	169	183		1.75	0.04-0.44	
eroides thetaiotaomicron VPI-5482]						up		0.04-0.44	gi|29346774
MAVSGGTILVK	1074.6106	-0.02661133	83.13	291	301		1.75	0-0.04-0.44	
ITTNPPFED	1032.4763	0.10900879	71.20	280	288		1.75	0-0.04-0.44	
KIGIKLFGK	1002.659	0.13586426	68.97	511	519		1.75	0-0.04-0.44	
oanaerobacter tengcongensis MB4]						up		0-0.04-0.44	gi|20806991
EVVANAGMPPGGISP	1706.8884	-0.09753418	95.82	59	75		2.77	0.04-0.44	
GGDPPREVVANAGMI	2130.064	0.11181641	77.09	53	74		2.77	0.04-0.44	
FPASPLPRFPAFCRN/	2809.4607	-0.2927246	65.25	175	199		2.77	0.04-0.44	
[Burkholderia pseudomallei 1710b]						down		0.04-0.44	gi|76811647
EIVDLANKK	1028.5867	0.00280762	80.64	106	114		1.64	0-0.04-0.44	
DFAKAISEVDNNIYAV/	2082.038	-0.05786133	61.19	17	35		1.64	0-0.04-0.44	
n acetobutylicum ATCC 824]						down		0-0.04-0.44	gi|15896641
TAIEVCEGQQYDVDFI	2129.9211	-0.25756836	88.23	136	153		2.94	0-0.04	
IETAKQIFEDSGAGKA	2065.0693	0.11230469	76.35	268	287		2.94	0-0.04	
ed protein [Psychroflexus torquis ATCC 700755]						up		0-0.04	gi|91215079
ADEFTDDIWGASRKD	4001.8123	-0.25561523	99.00	126	159		1.97	0-0.04-0.44	
VELTFSAWGNPAELK	1660.8462	-0.05651856	90.25	35	49		1.91	0-0.04-0.44	
KDETIYGLPVDCNPYL	2538.181	-0.21655273	84.35	139	159		1.63	0-0.04-0.44	
ling protein [Bacillus clausii KSM-K16]						up		0-0.04-0.44	gi|56962459
DAVLLGGSSDNEFVK	1549.7625	0.02563477	99.00	62	76		3.11	0-0.04-0.44	
TVGGDTL	661.32825	0.16815186	84.09	176	182		3.11	0-0.04-0.44	
PDNTIQHVSVNNLNVC	2713.3892	-0.22070312	77.27	137	160		3.11	0-0.04-0.44	
tella avium 197N]						down		0-0.04-0.44	gi|115422149
EKFKAAMTKIGLGSAF	1706.9502	-0.15930176	90.88	130	145		1.56	0.04-0.44	
SQLGSGTKLPKGGK	1356.7725	0.00183105	83.44	925	938		1.56	0.04-0.44	
subunit [Methylobacillus flagellatus KT]						down		0.04-0.44	gi|91775132
DNPDCADQEFEMKKL	2237.9204	-0.24658203	99.00	999	1017		1.57	0.04-0.44	
NTFEKTPDYVLSAYKL	2777.3215	-0.16333008	81.62	244	268		3.2	0.04-0.44	
EDLDFASVQRDNPEM	3049.398	0.15332031	81.53	463	487		2.5	0.04-0.44	
inamidine (FGAM) synthase, synthetase domain [Haemophilus somnus 2336]						up		0.04-0.44	gi|46156887
LWMQSAAEHASYDPI	2739.2234	-0.24804688	99.00	23	46		2.8	0-0.04	
FLGDLATSGHSFLFVA	2266.138	0.02294922	87.05	51	71		2.8	0-0.04	
ubrobacter xylanophilus DSM 9941]						up		0-0.04	gi|108803641
LVPLAVQLERPRRPGI	1856.1218	0.2672119	99.00	710	725		4	0.04-0.44	
TESPRVVRAETVFVDI	2641.3933	-0.22387695	91.90	686	709		1.8	0.04-0.44	
RPGRLVVFVFDIDAGA	2611.442	0.03637695	88.02	722	746		2.4	0.04-0.44	
_5631 [Frankia sp. EAN1pec]						up		0.04-0.44	gi|68229369
VLDDILKTD	1030.5546	0.02172852	87.41	39	47		3.23	0-0.04-0.44	
PLVEAK	655.39044	0.09979248	62.95	993	998		3.23	0-0.04-0.44	
TDPSDPEAFRK	1261.5938	-0.00146484	60.02	46	56		3.23	0-0.04-0.44	
bsp. pneumophila str. Philadelphia 1]						down		0-0.04-0.44	gi|52842521
ELAPISSSAWEQIEEE	2143.0435	0.00561523	86.68	7	25		2.94	0-0.04	
TFKRSVAGR	1164.6848	-0.08349609	70.31	26	34		2.33	0-0.04	
TIVELTVPFELSR	1502.8342	-0.07849121	64.96	74	86		2.76	0-0.04	
ELAPISSSAWEQIEEE	2143.0435	0.00439453	59.56	7	25		2.65	0-0.04	
cholderia sp. 383]						up		0-0.04	gi|78060115
AVEAVLSDASLPALNA	1995.0637	-0.00366211	99.00	508	527		2.37	0-0.04	
(OxW)QFWIDRGGTFT	2083.0276	-0.1159668	83.17	16	32		1.94	0-0.04	
LDSHVIRAGSGGGGR	1924.0261	-0.10327148	77.08	1114	1130		2.69	0-0.04	
rkholderia cenocepacia HI2424]						down		0-0.04	gi|116691651
TREEAPIDTEVLAPSE	3393.5251	-0.25219727	99.00	592	622		2	0-0.04	
YRPQNFTDLIGQEAM\	3126.578	-0.18896484	78.12	48	74		1.64	0-0.04	
y2]						down		0-0.04	gi|89362433
LPVDEIDLLALK	1337.7805	0.00256348	99.00	85	96		2.38	0-0.04	
KIVVKGLGATK	1112.728	-0.15246582	85.97	119	129		2.19	0-0.04	
KIVVKGLGATKSAR	1426.8982	-0.04040527	82.88	119	132		2	0-0.04	
rkholderia dolosa AUO158]						up		0-0.04	gi|84360487
ITYQADALFDFDK	1545.7351	0.00146484	99.00	78	90		2.09	0-0.04	
DAFWTPATANAK	1291.6196	-0.01135254	73.50	41	52		2.43	0-0.04	
ATLKPLGKQK	1082.6812	0.08288574	51.52	91	100		1.88	0-0.04	

and related peptidoglycan-associated (lipo)proteins [Burkholderia cenocepacia PC184]						up		0-0.04	gi|84357159
FIEQKNSLENAFLKFK	2212.2004	0.29418945	90.11	245	262		1.53	0-0.04-0.44	
NGSVTDREKKK	1260.6787	-0.08813477	82.54	306	316		1.53	0-0.04-0.44	
us cereus subsp. cytotoxis NVH 391-98]						down		0-0.04-0.44	gi|89199851
GDVGVEVITVDQGDW	3312.5466	-0.28637695	99.00	286	315		2.06	0.04-0.44	
RSSLHRLWDDDRSTL	2372.1768	-0.17651367	71.22	227	246		1.85	0.04-0.44	
rdioides sp. JS614]						down		0.04-0.44	gi|71367974
LLTAKTTDQVSYSFTL	2806.4133	-0.2368164	91.60	468	492		4.55	0-0.04	
AYQGGPDTTEAR	1264.5681	0.01013184	69.38	262	273		4.55	0-0.04	
TSQQRLDTFLDSMRV	2278.1487	0.0402832	69.31	686	704		4.55	0-0.04	
domonas putida F1]						down		0-0.04	gi|82738547
AHHPEGQDDGDSRA	2207.9175	-0.22338867	99.00	119	139		2.58	0.04-0.44	
LIGDPEQRYLEDPVR	2525.3535	0.20996094	74.12	189	209		3.63	0.04-0.44	
KEGSGSGE	749.31915	0.07702637	68.63	460	467		2.86	0.04-0.44	
) [Pseudomonas aeruginosa PA7]						down		0.04-0.44	gi|94417343
MADCSDGPSGIGWG	2258.9797	-0.28833008	99.00	1	23		2.67	0.04-0.44	
RLHQGGSHPARRR	1526.8401	-0.06604004	71.76	25	37		1.8	0.04-0.44	
eptide [Frankia alni ACN14a]						up		0.04-0.44	gi|111220783
HESCGQCTPCR	1219.4531	-0.26574707	99.00	343	353		4	0.04-0.44	
EGTGWMWRVLDRM	2076.9985	-0.27954102	75.70	354	370		3.67	0.04-0.44	
STDVVAAIARISYFFK	1786.9617	-0.16784668	59.85	327	342		3.8	0.04-0.44	
ain F [Rhodopseudomonas palustris CGA009]						up		0.04-0.44	gi|39936010
ISFVYTPPLFYYGVIISI	3465.081	0.20410156	99.00	832	861		3	0.04-0.44	
KPLVMTTIPSIPGWQV	2951.6877	0.27612305	82.73	789	815		2.25	0.04-0.44	
ostoc mesenteroides subsp. mesenteroides ATCC 8293]						up		0.04-0.44	gi|116617591
GYGDDGWYQGGGRI	2300.9685	-0.28149414	99.00	22	42		1.75	0-0.04-0.44	
EMLHRQGPKGYTR	1571.7991	-0.20251465	74.27	111	123		8.33	0-0.04-0.44	
DYRDLLHGGGGQR	1442.7014	0.08703613	52.97	98	110		1.54	0-0.04-0.44	
protein [Burkholderia mallei JHU]						up		0-0.04-0.44	gi|83622431
ELTFQVSCSYGPGRY	2626.1394	-0.25268555	99.00	282	303		2.5	0-0.04-0.44	
WTEQRNFETMLNAISI	4260.075	0.2763672	77.44	314	348		3.5	0-0.04-0.44	
Cellulophaga sp. MED134]						up		0-0.04-0.44	gi|86130443
RPRAVQRLLAVWRR	1776.0857	0.29821777	99.00	538	551		2.33	0-0.04-0.44	
DLRPAPR	823.4664	0.1204834	91.24	589	595		2.33	0-0.04-0.44	
p [Xanthobacter autotrophicus Py2]						up		0-0.04-0.44	gi|89359069
RMCEAFDDGAAACTA	2225.9614	-0.27441406	99.00	232	254		3	0-0.04-0.44	
TSGSFGGVGKAARPK	1418.763	0.01330566	78.47	101	115		2	0-0.04-0.44	
lomonas palustris BisB5]						up		0-0.04-0.44	gi|91975323
INAEDSSRDFMPSTG	3312.554	-0.29370117	99.00	339	368		2.25	0.04-0.44	
LPFKQADLGFK	1262.7021	-0.12243652	79.46	321	331		2	0.04-0.44	
xylase subunit [Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842]						down		0.04-0.44	gi|104773956
CMQQALFGDMNFR	1559.6682	-0.29223633	99.00	78	90		4.5	0.04-0.44	
NADPNGSALPK	1082.5356	0.24487305	76.70	485	495		2.43	0.04-0.44	
usitatus Ellin6076]						up		0.04-0.44	gi|116625582
TTGPVLIHVVTRKGK	1604.9727	0.29284668	99.00	276	290		5	0.04-0.44	
GEGVGVEMPERGTVL	1967.9734	-0.19348145	74.03	483	501		4.75	0.04-0.44	
[Rhodobacter sphaeroides ATCC 17025]						up		0.04-0.44	gi|83368175
QTIENVIAILK	1240.739	0.00158691	99.00	53	63		2.65	0-0.04	
VFISYFGEHPPAR	1518.762	-0.03051758	75.14	91	103		2.82	0-0.04	
ia sp. 383]						down		0-0.04	gi|78067536
INFTFEASDNSILTEDE	2099.9648	-0.29125977	99.00	253	270		2.75	0-0.04	
FTVEYIR	926.48615	-0.08666992	73.70	619	625		2	0-0.04-0.44	
ythrobacter sp. NAP1]						up		0-0.04-0.44	gi|85707925
WGVSMMKSGMPPEIE	1833.8577	-0.27575684	99.00	343	358		3.25	0-0.04-0.44	
YHEPFQRAAFYR	1583.7632	-0.17492676	74.04	473	484		2.33	0-0.04-0.44	
[Solibacter usitatus Ellin6076]						up		0-0.04-0.44	gi|116625650
DWRSQEQTTMDR	1551.6738	-0.2939453	99.00	82	93		2.38	0-0.04-0.44	
QPINIVSAYRSPATNAI	2101.1104	0.05224609	78.01	110	128		2.88	0-0.04-0.44	
[Nitrobacter hamburgensis X14]						up		0-0.04-0.44	gi|92116781
KTLSIIVIVASVSTILLA	2705.7031	0.25830078	99.00	25	50		3.5	0-0.04-0.44	
RRVGVVFEDAFLFAG	3917.9917	-0.00561523	78.79	419	453		1.59	0-0.04-0.44	
monella enterica subsp. enterica serovar Typhi str. CT18]						up		0-0.04-0.44	gi|16761557
QAMPLSATQLDK	1301.665	0.11401367	99.28	383	394		1.56	0-0.04-0.44	
GFLNKFPVGIYADLAR	1779.9673	-0.18786621	77.93	343	358		1.56	0-0.04-0.44	
omonas sp. JS666]						up		0-0.04-0.44	gi|91787132
EWGFQFGCDSDASK	1575.6299	-0.25012207	99.00	821	834		3	0.04-0.44	
YKLSGR	722.40753	-0.01721191	74.38	502	507		1.91	0.04-0.44	
lopirellula baltica SH 1]						up		0.04-0.44	gi|32471645

NNLLDQNFLDNCTIGF	3085.4084	-0.28710938	99.00	302	329		2.25	0.04-0.44	
QIVEPQYEAKSDLWIV	2045.0835	-0.109375	77.25	545	561		3.33	0.04-0.44	
;ignal [Desulfitobacterium hafniense DCB-2]						up		0.04-0.44	gi|109648135
LIAPIAMEEGLR	1311.7222	0.0378418	97.51	303	314		2.73	0-0.04	
LLDQGQAGDNVGILLF	1680.916	-0.00866699	55.49	205	220		1.57	0-0.04	
ŋgation factors [Burkholderia mallei NCTC 10247]						up		0-0.04	gi|67645167
AEAIPALQSILDASDGI	2140.1199	-0.00488281	97.99	203	223		3.67	0-0.04	
MIWMARESNK	1552.8098	0.03186035	82.29	242	251		2.86	0-0.04	
MMRAGLDVVR	1146.6001	-0.05810547	59.38	1	10		3.12	0-0.04	
leria cenocepacia PC184]						down		0-0.04	gi|84355614
WGGESPSSGFDCSGI	2309.0422	-0.2590332	98.19	448	468		1.5	0.04-0.44	
GAELTLLAR	942.54974	0.02874756	74.24	355	363		4.45	0.04-0.44	
ATCC 23779]						down		0.04-0.44	gi|113941631
NASLSAMTFMYAAKY	3287.4321	-0.2541504	97.93	133	161		2	0.04-0.44	
MIDECEYEDTIKQTK	1844.8174	-0.05651856	75.68	101	115		2.67	0.04-0.44	
; anthracis str. Ames]						up		0.04-0.44	gi|30264416
GFGPAEAEQVGNLIAI	3010.4517	0.01025391	97.66	371	399		3.24	0-0.04	
YAEGYPGKRYYGG(C	4034.0933	0.01293945	71.47	56	82		3.89	0-0.04	
TESHVMLVDLR	1442.7673	-0.01135254	52.57	312	322		4.13	0-0.04	
'kholderia sp. 383]						up		0-0.04	gi|78065369
LIAPIAMEEGLR	1311.7222	0.00219727	88.19	365	376		4.87	0-0.04-0.44	
TTLTAAITTVLTK	1332.7864	-0.14782715	84.14	26	38		4.55	0-0.04-0.44	
LIAPIA(OxM)EEGLR	1327.717	0.00134277	78.46	365	376		3.68	0-0.04-0.44	
TT(CamC)TGVEMFR	1200.5266	0.01721191	77.93	256	265		4.32	0-0.04-0.44	
LLDQGQAGDNVGILLF	1680.916	-0.00097656	62.00	267	282		4.44	0-0.04-0.44	
. 383]						up		0-0.04-0.44	gi|78064909
LGVAPTPTDLGAFVEE	2287.1846	-0.09301758	87.12	279	300		2.22	0-0.04-0.44	
TALKRDLAQMYESTVI	2313.284	-0.16235352	76.62	522	537		1.98	0-0.04-0.44	
LGVAPTPTDLGAFVEE	2287.1846	0.0078125	76.50	279	300		2.1	0-0.04-0.44	
DLAQM(iTRAQY)ESTV	2485.2537	-0.10180664	65.85	527	546		2.7	0-0.04-0.44	
›rotein [Burkholderia thailandensis E264]						up		0-0.04-0.44	gi|83716854
DAGRDHNDPAAPGGS	2373.0732	-0.2944336	96.94	3764	3786		2.11	0-0.04	
DVANTSAVIPTNAWM	2333.1838	0.00952148	77.96	3168	3188		1.73	0-0.04	
ter membrane protein [Rhodopseudomonas palustris BisA53]						up		0-0.04	gi|115526543
VTLGGNPIDLAGTFPA	2429.2588	-0.15795898	80.25	4	28		4.57	0-0.04	
DLADLSLASFAGK	1306.6768	0.00134277	79.74	33	45		3.94	0-0.04	
,U 1054]						up		0-0.04	gi|107021678
VLNIVPSLDTPT(CamC	1943.9985	-0.0065918	95.48	48	65		2.02	0-0.04	
F(CamC)TTEGLANVV	2287.15	-0.05834961	61.00	93	112		2.24	0-0.04	
specific antioxidant/ Mal allergen [Burkholderia vietnamiensis G4]						up		0-0.04	gi|67546142
DQPVILQLLEIPDEK	1748.956	0.02355957	94.96	34	48		1.53	0-0.04-0.44	
KDLLSVNAQIFTAQGF	1759.958	-0.18530273	66.99	102	117		2.28	0-0.04-0.44	
›rtussis Tohama I]						up		0-0.04-0.44	gi|33593357
IERAEFPDFEGPEQPF	3444.6057	-0.21728516	93.49	66	93		3.5	0-0.04-0.44	
GALVLLVQSSRGTRY'	2115.2527	0.01196289	67.74	456	475		2.25	0-0.04-0.44	
›xidans MPOB]						up		0-0.04-0.44	gi|116749436
AIQIENDLGPDIMMSFI	3528.5444	-0.28320312	93.84	133	162		2.2	0.04-0.44	
WAERGLKAHR	1222.6682	-0.08154297	64.50	171	180		4.75	0.04-0.44	
LVVKNAK	770.5014	0.19604492	64.12	289	295		1.58	0.04-0.44	
nsferase [Pediococcus pentosaceus ATCC 25745]						up		0.04-0.44	gi|116493017
KDRVDDALNATR	1516.8079	-0.10046387	74.59	393	404		4.5	0-0.04	
EKLQER	1089.6387	-0.10424805	66.46	363	368		4	0-0.04	
LENVTLDMLGR	1259.6543	0.00769043	64.08	309	319		3.8	0-0.04	
LENVTLD(OxM)LGR	1419.7512	-0.06188965	63.20	309	319		4.5	0-0.04	
KDRVDDALNATRAAV	1886.9932	0.02783203	61.84	393	408		4.32	0-0.04	
						down		0-0.04	gi|2290621
QKGMVIFLSLLIVALGL	2468.448	0.2397461	86.65	49	70		1.77	0-0.04-0.44	
TGYVGIFKPKDEWK	1666.8718	-0.07946777	66.81	658	671		1.55	0-0.04-0.44	
TGQTGVALDTRGADK	1488.7532	0.0300293	62.37	643	657		1.73	0-0.04-0.44	
LPEEKR	770.42865	-0.14025879	61.23	807	812		1.88	0-0.04-0.44	
›acter sp. FRC-32]						up		0-0.04-0.44	gi|110599599

Appendix IV:

Part 1: Complete data table for simple mixture quantification experiment. (pages 212 to 216)

Part 2: Comparison of Mascot and PEAKS quantification data. (pages 217 to 220)

Part 3: Summary of all proteins identified in the chromium-iron-cadmium shocks experiment. (pages 221 to 225)

Part 4: Complete data table with all peptides identified in the chromium-iron-cadmium shocks experiment. (pages 226 to 248)

Accession	Mass	Score (%)	Coverage(%)	Query matched	UC score	m/z	z	Peptide	RSD	Spectrum Quality
P02754|LACB_BOVIN	19,883	99	40	25	99					
						567.393	2	(iTRAQ4plexK)IIAE(iTRAQ4plexK)	0.25	0.791154816
						431.259	2	(ITRAQI)IAE(ITRAQKK)	0	0.790991988
						602.793	2	(ITRAQI)DALNEN(ITRAQKK)	0	0.791569195
						481.274	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791660048
						499.297	2	(ITRAQA)LP(M16M)HIR	0.6666667	0.791294439
						767.326	2	(ITRAQT)PEVDDEALE(ITRAQKK)	0.30769232	0.791382746
						445.912	3	(ITRAQT)(ITRAQKK)IPAVF(ITRAQKK)	0.3	0.791425808
						542.659	3	(ITRAQV)LVLDTDY(ITRAQKK)(ITRAQKK)	0	0.791690479
						813.502	2	(ITRAQV)LVLDTDY(ITRAQKK)(ITRAQKK)	0	0.79152998
						690.003	3	(ITRAQT)PEVDDEALE(ITRAQKK)FD(ITRAQKK)	0	0.791753068
						481.801	2	(ITRAQL)PAVF(ITRAQKK)	0	0.790676526
						677.409	2	(ITRAQV)LVLDTDY(ITRAQKK)	0.22222222	0.791732759
						481.83603	2	(ITRAQL)PAVF(ITRAQKK)	0	0.79152998
						481.28302	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791569195
						602.863	2	(ITRAQI)DALNEN(ITRAQKK)	0.6	0.791535214
						481.328	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791501938
						1373.36	2	(ITRAQV)YVEEL(ITRAQKK)PTPEGDLEILLQ(ITRA	0.3	0.791694435
						481.319	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791554291
						602.863	2	(ITRAQI)DALNEN(ITRAQKK)	0.8	0.791672967
						481.354	2	(ITRAQG)LDIQ(ITRAQKK)	0.85714287	0.791554291
						602.873	2	(ITRAQI)DALNEN(ITRAQKK)	0.22222222	0.791587057
						481.31	2	(ITRAQG)LDIQ(ITRAQKK)	0.5	0.791434458
						602.853	2	(ITRAQI)DALNEN(ITRAQKK)	0.8888889	0.79161549
						481.328	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791521923
						602.873	2	(ITRAQI)DALNEN(ITRAQKK)	0	0.791576355
P67975|LACB_OVIMU	18,151	99	28	26	99					
						567.393	2	(iTRAQ4plexK)IIAE(iTRAQ4plexK)	0.25	0.791154816
						431.259	2	(ITRAQI)IAE(ITRAQKK)	0	0.790991988
						602.793	2	(ITRAQI)DALNEN(ITRAQKK)	0	0.791569195
						481.274	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791660048
						499.297	2	(ITRAQA)LP(M16M)HIR	0.6666667	0.791294439
						619.365	2	(ITRAQI)IVTQT(M16M)(ITRAQKK)	0	0.791434879
						445.912	3	(ITRAQT)(ITRAQKK)IPAVF(ITRAQKK)	0.3	0.791425808
						611.393	2	(ITRAQI)IVTQTM(ITRAQKK)	0	0.7918039
						619.324	2	(ITRAQI)IVTQT(M16M)(ITRAQKK)	0.25	0.791425808
						542.659	3	(ITRAQV)LVLDTDY(ITRAQKK)(ITRAQKK)	0	0.791690479
						813.502	2	(ITRAQV)LVLDTDY(ITRAQKK)(ITRAQKK)	0	0.79152998
						481.801	2	(ITRAQL)PAVF(ITRAQKK)	0	0.790676526
						611.272	2	(ITRAQI)IVTQTM(ITRAQKK)	1	0.79163889
						677.409	2	(ITRAQV)LVLDTDY(ITRAQKK)	0.22222222	0.791732759
						481.83603	2	(ITRAQL)PAVF(ITRAQKK)	0	0.79152998
						481.28302	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791569195
						602.863	2	(ITRAQI)DALNEN(ITRAQKK)	0.6	0.791535214
						481.328	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791501938
						481.319	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791554291
						602.863	2	(ITRAQI)DALNEN(ITRAQKK)	0.8	0.791672967
						481.354	2	(ITRAQG)LDIQ(ITRAQKK)	0.85714287	0.791554291
						602.873	2	(ITRAQI)DALNEN(ITRAQKK)	0.22222222	0.791587057
						481.31	2	(ITRAQG)LDIQ(ITRAQKK)	0.5	0.791434458
						602.853	2	(ITRAQI)DALNEN(ITRAQKK)	0.8888889	0.79161549
						481.328	2	(ITRAQG)LDIQ(ITRAQKK)	0	0.791521923
						602.873	2	(ITRAQI)DALNEN(ITRAQKK)	0	0.791576355
P00722|BGAL_ECOLI	116,483	99	16	21	97					
						415.557	3	(ITRAQT)DRPSQQLR	0.5	0.791464581
						430.71	2	(ITRAQL)TDDPR	0.5714286	0.79115048
						405.714	2	(ITRAQD)QFTR	0.33333334	0.791460588
						676.87994	2	(ITRAQT)PHPALTEA(ITRAQKK)	0.4	0.79148562
						551.336	2	(ITRAQL)NVENP(ITRAQKK)	0	0.791456516
						522.727	2	(ITRAQF)NDDFSR	0	0.79170785
						801.388	2	(ITRAQA)PLDNDIGVSEATR	0	0.79170785
						440.756	2	(ITRAQT)LFISR	0.33333334	0.79099616
						786.887	2	(ITRAQD)WENPGVTQLNR	0	0.791524627
						492.819	2	(ITRAQQ)LLTPLR	0.2857143	0.791603226
						427.727	2	(ITRAQM)SGIFR	0.33333334	0.791550238
						815.43	2	(ITRAQV)DEDQPFPAVP(ITRAQKK)	0.64285713	0.791559726
						614.313	2	(ITRAQG)DFQFNISR	0	0.791550238
						622.304	2	(ITRAQL)DPNAWVER	0	0.791521491
						575.308	2	(ITRAQW)LPA(M16M)SER	0.875	0.791420779
						567.277	2	(ITRAQW)LPAMSER	0	0.791533332

						507.76797	2 (ITRAQY)WQAFR	0.2857143	0.791621696
						473.32	2 (ITRAQL)AVMVLR	0	0.791452362
						951.55	2 (ITRAQV)NWLGLGPQENYPDR	0.5625	0.791665049
						966.427	2 (ITRAQW)SDGSYLEDQDMWR	0.13333334	0.791501938
						753.416	2 (ITRAQL)WSAEIPNLYR	0.36363637	0.791519151
P02787|TRFE_HUMAN	77,050	99	13	16	99				
						471.755	2 (ITRAQN)TYE(ITRAQKK)	0	0.791679421
						523.872	2 (iTRAQ4plexK)PLE(iTRAQ4plexK)	0.42857143	0.791495103
						437.593	3 (iTRAQ4plexK)PVDEY(iTRAQ4plexK)	0.22222222	0.791439385
						581.834	2 (ITRAQD)SAHGFL(ITRAQKK)	0.8181818	0.791489762
						537.94	3 (iTRAQ4plexK)DSGFQMNQLR	0.23076923	0.791420779
						512.288	2 (ITRAQG)DVAFV(ITRAQKK)	0	0.791101922
						558.282	2 (iTRAQN)PDPWA(ITRAQKK)	0	0.791303371
						714.321	2 (ITRAQE)GYYGYTGAFR	0	0.791823903
						462.243	2 (ITRAQD)LLF(ITRAQKK)	0	0.791732759
						404.232	2 (ITRAQD)LLFR	0	0.791460588
						513.283	3 (ITRAQS)ASDLTWDNL(ITRAQKK)	0.16666667	0.79179501
						769.378	2 (iTRAQS)ASDLTWDNL(ITRAQKK)	0	0.791669943
						609.403	2 (ITRAQV)PPR(M16M)DA(iTRAQKK)	0.75	0.791434879
						589.681	3 (ITRAQM)YLG(ITRAQYY)EYVTAIR	0.35714287	0.791519151
						811.902	2 (ITRAQM)YLGYEYVTAIR	0	0.791495924
						512.279	2 (ITRAQG)DVAFV(ITRAQKK)	0.85714287	0.791603226
P02769|ALBU_BOVIN	69,294	99	13	13	99				
						520.794	2 (ITRAQN)YQEA(ITRAQKK)	0	0.7918039
						474.781	2 (ITRAQT)PVSE(iTRAQKK)	0.5	0.791182875
						403.227	2 (ITRAQA)DLA(ITRAQKK)	0	0.791476107
						553.784	2 (ITRAQA)TEEQL(ITRAQKK)	0	0.791585932
						449.234	2 (ITRAQA)FDE(ITRAQKK)	0	0.791316103
						587.806	2 (ITRAQD)DSPDLP(ITRAQKK)	0	0.791545339
						427.264	3 (ITRAQL)SQ(ITRAQKK)FP(ITRAQKK)	0.5	0.791439385
						531.947	3 (ITRAQH)LVDEPQNLI(ITRAQKK)	0.9230769	0.791660048
						539.319	2 (ITRAQL)VTDLT(ITRAQKK)	0	0.791672967
						536.3	2 (ITRAQY)LYEIAR	0	0.791649707
						525.679	3 (iTRAQ4plexK)QTALVELL(iTRAQ4plexK)	0.5	0.791732759
						651.936	2 (ITRAQQ)TALVELL(ITRAQKK)	0	0.791555033
						726.418	2 (ITRAQL)VNELTEFA(ITRAQKK)	0	0.791771072
P00698|LYSC_CHICK	16,239	99	23	3	99				
						786.83	2 (ITRAQF)ESNFNTQATNR	0	0.791726758
						949.455	2 (ITRAQN)TDGSTDYGILQINSR	0	0.791545339
						595.345	2 (ITRAQG)TDVQAWIR	0.75	0.791237787

Charge Correction	min charge = 1 max charge = 4
Remove Low Quali	quality > 0.65
Deconvolution	true
After Preprocessin	277
Database Options	
Database	swiss
Taxon	All
PEAKS Protein ID	
Title	ITRAQ_QTOF
Database to Searc	swiss
Taxonomy	All
Instrument	Quad-TOF[Quadrupole:ESI(nano-spray):Time of Flight (TOF):CID, CAD, IRMPD (y and b ions)]
Parent Mass Error	0.1Da
Fragment Mass Er	0.1Da
Enzyme	Trypsin[RK:P:C]0
Max Missed Cleava	1
Fixed Modification	ITRAQ(144.10207)@[ARNDCEQGHILKMFSTWYV(N-term)],ITRAQK(144.10207)@[K(Anywhere)]
Variable Modificati	ITRAQY(144.10207)@[Y(Anywhere)],M16(15.9949)@[M(Anywhere)]
Max Variable PTM	2
Precursor Mass Se	Monoisotopic
Mascot	
Title	D:\wyang_work\work\peaks\mascot\F001575.dat
Database	SwissProt
Taxonomy	All entries
Enzyme	Trypsin
Fixed modifications	iTRAQ4plex (K),iTRAQ4plex (N-term)
Variable modificati	iTRAQ4plex (Y),Oxidation (M)
ICAT	
Protein mass	1000
Missed cleavages	1
Peptide tol.	0.1Da

MS/MS tol.	0.1Da
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Peptide charge	1+, 2+ and 3+
Experimental mass	Monoisotopic
Instrument	ESI-QUAD-TOF

Score(%)	Peaks Score(%)	Mascot Score	Peaks Quantification	Mascot Quantification	Peaks SD to Mean Ratio
			0.3158717	0.992063492	0.11528057
28.85657034	0	24.43			
97.27016499	0.45647	26.63			
99.73880597	0.99	50.07			
73.62173552	0.69826	36.5			
98.66464321	0.63523	16.89			
90	0.99	0			
90	0.97929	0			
99.73880597	0.99	49.24			
99.73880597	0.99	61.49			
99.73880597	0.99	109.75			
99.73807832	0.89975	26.9			
99.73880597	0.94417	67.27			
96.16105684	0.37122	16.99			
67.56175538	0.57302	24.47			
97.50945186	0.47992	19.64			
62.72895316	0.47895	30.11			
90	0.97527	0			
69.64382874	0.52897	20.01			
92.84355416	0.23417	7.77			
10.26159969	0.10262	0			
99.32416274	0.77598	31.8			
51.18080274	0.32905	14.92			
97.47647638	0.47655	28.33			
66.78134929	0.53364	21.06			
99.73880597	0.92063	45.72			
			0.31299916	0.994035785	0.11008405
28.85657034	0	24.43			
97.27016499	0.45647	26.63			
99.73880597	0.99	50.07			
73.62173552	0.69826	36.5			
98.66464321	0.63523	16.89			
99.62910093	0.86359	36.09			
90	0.97929	0			
99.73880597	0.99	54.69			
90	0.99	0			
99.73880597	0.99	49.24			
99.73880597	0.99	61.49			
99.73807832	0.89975	26.9			
5.363924786	0.053639	0			
99.73880597	0.94417	67.27			
96.16105684	0.37122	16.99			
67.56175538	0.57302	24.47			
97.50945186	0.47992	19.64			
62.72895316	0.47895	30.11			
69.64382874	0.52897	20.01			
92.84355416	0.23417	7.77			
10.26159969	0.10262	0			
99.32416274	0.77598	31.8			
51.18080274	0.32905	14.92			
97.47647638	0.47655	28.33			
66.78134929	0.53364	21.06			
99.73880597	0.92063	45.72			
			0.23687364	1.067235859	0.097923905
99.05644347	0.71217	25.89			
16.97835769	0.16978	0			
93.15924386	0.24298	10.18			
99.70851186	0.88965	34.14			
99.4259908	0.80325	40.75			
99.73880597	0.99	35.88			
99.73880597	0.99	90.93			
99.66406759	0.91436	22.03			
99.73880597	0.99	47.95			
99.73880597	0.95757	32.34			
99.62996799	0.86387	23.12			
99.73880597	0.95088	75.38			
99.73880597	0.99	53.29			
99.73880597	0.99	49.15			
85.48132678	0.12186	13.16			
99.73880597	0.99	35.48			

99.73880597	0.95841	30.59			
99.73880597	0.96252	32.49			
90	0.97227	0			
99.73880597	0.99	62.1			
99.73361659	0.99	47.3			
			0.27123868	0.928505107	0.14678486
98.7147593	0.64416	29.2			
30	0	5.42			
30	0	24.43			
88.95058443	0.15948	4.08			
29.23332863	0	40.85			
99.73880597	0.97605	47.72			
99.6131595	0.85854	31.27			
99.7360654	0.99	59.63			
55.01202727	0.55012	0			
98.03235923	0.54007	25.92			
99.73880597	0.95399	33.33			
99.73880597	0.99	84.85			
2.480548929	0.024805	0			
98.12365809	0.55208	29.59			
99.73880597	0.99	59.62			
87.70989646	0.14398	15.56			
			0.36982238	0.865800866	0.25170702
99.71138309	0.89062	33.15			
97.72315978	0.50288	15.18			
96.86190495	0.42113	23.4			
99.73880597	0.96945	48.33			
96.72539499	0.41044	31.22			
99.73880597	0.99	50.28			
96.29203769	0.37968	15.25			
99.73612614	0.92651	55.57			
99.73880597	0.98985	48.85			
99.73880597	0.98896	51.16			
19.67462867	0	51.71			
99.65632641	0.91456	41.41			
99.73880597	0.99	44.7			
			0.21394321	1.014198783	0.15237533
99.73880597	0.99	71.6			
99.73614101	0.99	84.79			
91.32374883	0.19877	16.16			

4PF18

	>gi|124893497|gb|EAY67377.1| Extracellular ligand-binding receptor [Burkholderia dolosa			
ProMatch ID:	AUO158];;			
ProMatch Score:	0.999981165			Mascot ratio
Pro ratio (115.1:114.1):	0.2973995	Pro ratio SD 0.06964318	SD to mean:0.23417383	0.104
Pro ratio (116.1:114.1):	0.18772401	Pro ratio SD 0.036108367	SD to mean:0.19234815	0.141
Pro ratio (117.1:114.1):	0.40262154	Pro ratio SD 0.08886342	SD to mean:0.22071204	0.117
ProMatch ID:	>gi|115352222|ref|YP_774061.1| phasin family protein [Burkholderia cepacia AMMD];			
ProMatch Score:	0.979142904			
Pro ratio (115.1:114.1):	1.8413881	Pro ratio SD 0.30168176	SD to mean:0.16383389	3.508
Pro ratio (116.1:114.1):	1.4593192	Pro ratio SD 0.2511421	SD to mean:0.17209539	2.724
Pro ratio (117.1:114.1):	3.2695348	Pro ratio SD 0.52704346	SD to mean:0.1611983	3.726
	>gi|78066693|ref|YP_369462.1| ATP-dependent Clp protease proteolytic subunit			
ProMatch ID:	[Burkholderia sp. 383];			
ProMatch Score:	0.854356706			
Pro ratio (115.1:114.1):	0.32162377	Pro ratio SD -1.0	SD to mean:-3.109223	0.215
Pro ratio (116.1:114.1):	0.36423537	Pro ratio SD -1.0	SD to mean:-2.7454774	0.273
Pro ratio (117.1:114.1):	0.40034539	Pro ratio SD -1.0	SD to mean:-2.4978433	0.346
ProMatch ID:	>gi|78065110|ref|YP_367879.1| outer membrane protein, (porin) [Burkholderia sp. 383];			
ProMatch Score:	0.818098903			
Pro ratio (115.1:114.1):	0.906216	Pro ratio SD 0.1559677	SD to mean:0.17210874	1.801
Pro ratio (116.1:114.1):	0.7920475	Pro ratio SD 0.12078383	SD to mean:0.15249568	2.593
Pro ratio (117.1:114.1):	1.2890229	Pro ratio SD 0.16426615	SD to mean:0.12743463	1.339

4PF17

	>gi|107024372|ref|YP_622699.1| Extracellular ligand-binding receptor [Burkholderia			
ProMatch ID:	cenocepacia AU 1054];			
ProMatch Score:	0.999846399			Mascot ratio
Pro ratio (115.1:114.1):	0.16776972	Pro ratio SD 0.01994337	SD to mean:0.11887348	0.109
Pro ratio (116.1:114.1):	0.11579888	Pro ratio SD 0.0202704	SD to mean:0.17504832	0.094
Pro ratio (117.1:114.1):	0.234114	Pro ratio SD 0.05072277	SD to mean:0.21665841	0.088
	>gi|78066909|ref|YP_369678.1| Phasin [Burkholderia sp. 383];gi|77967654|gb|ABB09034.1|			
ProMatch ID:	Phasin [Burkholderia sp. 383];;			
ProMatch Score:	0.994811714			
Pro ratio (115.1:114.1):	1.8749965	Pro ratio SD 0.19289808	SD to mean:0.10287917	4.31
Pro ratio (116.1:114.1):	1.6261466	Pro ratio SD 0.2452458	SD to mean:0.15081409	2.752
Pro ratio (117.1:114.1):	3.7693918	Pro ratio SD 0.58331734	SD to mean:0.15475105	3.492
	>gi|53724374|ref|YP_104408.1| amino acid ABC transporter, periplasmic amino acid-			
ProMatch ID:	binding protein, putative [Burkholderia mallei ATCC 23344];			
ProMatch Score:	0.98700583			
Pro ratio (115.1:114.1):	0.16633332	Pro ratio SD 0.031469386	SD to mean:0.18919472	0.095
Pro ratio (116.1:114.1):	0.105077095	Pro ratio SD 0.02989218	SD to mean:0.28447855	0.101
Pro ratio (117.1:114.1):	0.24076432	Pro ratio SD 0.06951899	SD to mean:0.2887429	0.084
ProMatch ID:	>gi|115350787|ref|YP_772626.1| chaperonin GroEL [Burkholderia cepacia AMMD];			
ProMatch Score:	0.959949791			
Pro ratio (115.1:114.1):	0.3002123	Pro ratio SD 0.03836527	SD to mean:0.1277938	0.377
Pro ratio (116.1:114.1):	0.2447438	Pro ratio SD 0.0284664	SD to mean:0.11631102	0.437
Pro ratio (117.1:114.1):	0.62185514	Pro ratio SD 0.075165495	SD to mean:0.120873	0.44

>gi|78067415|ref|YP_370184.1| hypothetical protein Bcep18194_A5946 [Burkholderia sp.

ProMatch ID:	383];				
ProMatch Score:		0.999170482			
Pro ratio (115.1:114.1):		1.3459834	Pro ratio SD 0.3233963	SD to mean:0.24026768	2.252
Pro ratio (116.1:114.1):		0.8730243	Pro ratio SD 0.35047352	SD to mean:0.40144762	3.325
Pro ratio (117.1:114.1):		1.8503891	Pro ratio SD 0.5250313	SD to mean:0.28374103	2.051
	>gi|84356264|ref|ZP_00981118.1| COG0683: ABC-type branched-chain amino acid				
ProMatch ID:	transport systems, periplasmic component [Burkholderia cenocepacia PC184];;				
ProMatch Score:		0.998446584			
Pro ratio (115.1:114.1):		0.19173005	Pro ratio SD 0.030237284	SD to mean:0.15770759	0.095
Pro ratio (116.1:114.1):		0.14085342	Pro ratio SD 0.030174913	SD to mean:0.21422918	0.108
Pro ratio (117.1:114.1):		0.27405673	Pro ratio SD 0.04434729	SD to mean:0.16181792	0.101
ProMatch ID:	>gi|78065429|ref|YP_368198.1| chaperonin GroEL [Burkholderia sp. 383];				
ProMatch Score:		0.99504751			
Pro ratio (115.1:114.1):		0.2991647	Pro ratio SD 0.023924652	SD to mean:0.07997151	0.306
Pro ratio (116.1:114.1):		0.2698212	Pro ratio SD 0.030403193	SD to mean:0.11267904	0.34
Pro ratio (117.1:114.1):		0.59869206	Pro ratio SD 0.0583344	SD to mean:0.0974364	0.375
	>gi|84357159|ref|ZP_00981987.1| COG2885: Outer membrane protein and related				
ProMatch ID:	peptidoglycan-associated (lipo)proteins [Burkholderia cenocepacia PC184];;				
ProMatch Score:		0.990180194			
Pro ratio (115.1:114.1):		0.56090504	Pro ratio SD 0.04207285	SD to mean:0.07500887	0.566
Pro ratio (116.1:114.1):		0.6255123	Pro ratio SD 0.058126472	SD to mean:0.09292618	0.928
Pro ratio (117.1:114.1):		1.8568497	Pro ratio SD 0.2950814	SD to mean:0.15891507	0.992
	>gi|134296380|ref|YP_001120115.1| bacterioferritin [Burkholderia vietnamiensis				
ProMatch ID:	G4];gi|134139537|gb|ABO55280.1| bacterioferritin [Burkholderia vietnamiensis G4];;				
ProMatch Score:		0.999839783			
Pro ratio (115.1:114.1):		2.5836728	Pro ratio SD 0.6421547	SD to mean:0.24854335	3.642
Pro ratio (116.1:114.1):		2.82763	Pro ratio SD 0.8205321	SD to mean:0.29018366	4.641
Pro ratio (117.1:114.1):		3.0882294	Pro ratio SD 0.5352233	SD to mean:0.17331073	2.756
	>gi|84362129|ref|ZP_00986765.1| COG0740: Protease subunit of ATP-dependent Clp				
ProMatch ID:	proteases [Burkholderia dolosa AUO158];;				
ProMatch Score:		0.993755698			
Pro ratio (115.1:114.1):		0.14888456	Pro ratio SD 0.0017171777	SD to mean:0.011533618	0.178
Pro ratio (116.1:114.1):		0.1890229	Pro ratio SD 0.012989008	SD to mean:0.068716586	0.333
Pro ratio (117.1:114.1):		0.3994872	Pro ratio SD 0.018126942	SD to mean:0.045375526	0.388
ProMatch ID:	>gi|78064724|ref|YP_367493.1| Histone-like DNA-binding protein [Burkholderia sp. 383];				
ProMatch Score:		0.991349816			
Pro ratio (115.1:114.1):		0.5904142	Pro ratio SD 0.12292641	SD to mean:0.20820367	1.181
Pro ratio (116.1:114.1):		0.78958035	Pro ratio SD 0.20368893	SD to mean:0.25797114	2.682
Pro ratio (117.1:114.1):		2.7634606	Pro ratio SD 0.84374946	SD to mean:0.30532348	5.089
4PF13					
	>gi|118720065|ref|ZP_01572593.1| Extracellular ligand-binding receptor [Burkholderia				
ProMatch ID:	multivorans ATCC 17616];				
ProMatch Score:		0.999927461			
Pro ratio (115.1:114.1):		0.15860213	Pro ratio SD 0.021488197	SD to mean:0.13548492	0.125
Pro ratio (116.1:114.1):		0.07650985	Pro ratio SD 0.013685544	SD to mean:0.17887297	0.15
Pro ratio (117.1:114.1):		0.18402165	Pro ratio SD 0.0559576	SD to mean:0.30408162	0.141
ProMatch ID:	>gi|107021936|ref|YP_620263.1| chaperonin GroEL [Burkholderia cenocepacia AU 1054];				
ProMatch Score:		0.99957943			

Pro ratio (115.1:114.1):	0.22424653	Pro ratio SD 0.028219327	SD to mean:0.12584063	0.21
Pro ratio (116.1:114.1):	0.19168264	Pro ratio SD 0.03846973	SD to mean:0.20069492	0.257
Pro ratio (117.1:114.1):	0.432092	Pro ratio SD 0.067958616	SD to mean:0.1572781	0.264
	>gi|118700375|ref|ZP_01558444.1| chaperonin GroEL [Burkholderia ambifaria MC40-			
ProMatch ID:	6];gi|118642038|gb|EAV48881.1| chaperonin GroEL [Burkholderia ambifaria MC40-6];;			
ProMatch Score:	0.999365747			
Pro ratio (115.1:114.1):	0.22643688	Pro ratio SD 0.030167837	SD to mean:0.13322845	0.2
Pro ratio (116.1:114.1):	0.19349875	Pro ratio SD 0.041112795	SD to mean:0.2124706	0.253
Pro ratio (117.1:114.1):	0.45510814	Pro ratio SD 0.07224461	SD to mean:0.15874162	0.28
4PF12				
	>gi|6225114|sp|Q9ZFE0|CH60_BURCE 60 kDa chaperonin (Protein Cpn60) (groEL			
ProMatch ID:	protein);			
Pro ratio (115.1:114.1):	0.30640933	Pro ratio SD 0.033816848	SD to mean:0.110364944	0.163
Pro ratio (116.1:114.1):	0.23538978	Pro ratio SD 0.029916424	SD to mean:0.12709314	0.215
Pro ratio (117.1:114.1):	0.5278258	Pro ratio SD 0.05036274	SD to mean:0.09541546	0.269
	>gi|134294995|ref|YP_001118730.1| extracellular solute-binding protein, family 1			
ProMatch ID:	[Burkholderia vietnamiensis G4];			
ProMatch Score:	0.999993742			
Pro ratio (115.1:114.1):	0.47711852	Pro ratio SD 0.0901358	SD to mean:0.188917	0.566
Pro ratio (116.1:114.1):	0.45322913	Pro ratio SD 0.08777553	SD to mean:0.19366701	0.648
Pro ratio (117.1:114.1):	0.832122	Pro ratio SD 0.095454834	SD to mean:0.114712544	0.85
	>gi|78065543|ref|YP_368312.1| ABC sugar transporter, periplasmic ligand binding protein			
ProMatch ID:	[Burkholderia sp. 383];			
ProMatch Score:	0.999979794			
Pro ratio (115.1:114.1):	0.4540604	Pro ratio SD 0.07571702	SD to mean:0.16675538	0.741
Pro ratio (116.1:114.1):	0.44808888	Pro ratio SD 0.0896117	SD to mean:0.19998644	0.667
Pro ratio (117.1:114.1):	0.8111118	Pro ratio SD 0.093182884	SD to mean:0.11488291	0.834
4PF11				
ProMatch ID:	>gi|78066729|ref|YP_369498.1| phage shock protein A, PspA [Burkholderia sp. 383];			
ProMatch Score:	0.999998987			
Pro ratio (115.1:114.1):	0.76779157	Pro ratio SD 0.21046995	SD to mean:0.27412382	1.218
Pro ratio (116.1:114.1):	0.85222304	Pro ratio SD 0.1465992	SD to mean:0.17201976	1.085
Pro ratio (117.1:114.1):	4.731973	Pro ratio SD 1.0359823	SD to mean:0.2189324	7.091
	>gi|115351987|ref|YP_773826.1| phage shock protein A, PspA [Burkholderia cepacia			
ProMatch ID:	AMMD];			
ProMatch Score:	0.999997437			Mascot ratio
Pro ratio (115.1:114.1):	0.3364819	Pro ratio SD 0.060772408	SD to mean:0.18061122	0.352
Pro ratio (116.1:114.1):	0.26019448	Pro ratio SD 0.043521002	SD to mean:0.16726336	0.311
Pro ratio (117.1:114.1):	0.60686123	Pro ratio SD 0.082371235	SD to mean:0.13573323	0.339
	>gi|84355078|ref|ZP_00979968.1| hypothetical protein BcenP_01002743 [Burkholderia			
ProMatch ID:	cenocepacia PC184];			
ProMatch Score:	0.999991179			Mascot ratio
Pro ratio (115.1:114.1):	2.3231199	Pro ratio SD 0.34812346	SD to mean:0.1498517	4.272
Pro ratio (116.1:114.1):	1.3105458	Pro ratio SD 0.21058506	SD to mean:0.160685	2.164
Pro ratio (117.1:114.1):	4.5873666	Pro ratio SD 0.90031004	SD to mean:0.19625857	3.082
	>gi|78062511|ref|YP_372419.1| ABC branched chain amino acid family transporter,			
ProMatch ID:	periplasmic ligand binding protein [Burkholderia sp. 383];			
ProMatch Score:	0.999165177			Mascot ratio
Pro ratio (115.1:114.1):	0.23944779	Pro ratio SD 0.045362826	SD to mean:0.18944767	0.35

Pro ratio (116.1:114.1):	0.1989674	Pro ratio SD 0.035768535	SD to mean:0.17977083	0.307
Pro ratio (117.1:114.1):	0.52994007	Pro ratio SD 0.16874893	SD to mean:0.31843022	0.423
ProMatch ID:	>gi|83718587|ref|YP_443652.1| amino acid ABC transporter, periplasmic amino acid-binding protein, putative [Burkholderia thailandensis E264];			
ProMatch Score:	0.997654438			Mascot ratio
Pro ratio (115.1:114.1):	0.1414465	Pro ratio SD 0.038050976	SD to mean:0.2690132	0.086
Pro ratio (116.1:114.1):	0.106256165	Pro ratio SD 0.027203592	SD to mean:0.25601894	0.088
Pro ratio (117.1:114.1):	0.19608758	Pro ratio SD 0.04832953	SD to mean:0.2464691	0.086

Protein metabolisr	2-C-methyl-D-eryt	2-C-methyl-D-eryt	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [Burkholderia sp. 383]
Sugar metabolism	2-dehydro-3-deoxyphosphoglucona		2-dehydro-3-deoxyphosphogluconate aldolase [Burkholderia sp. 383]
Protein metabolisr	30S ribosomal protein S4 [Ralstonia		30S ribosomal protein S4 [Ralstonia solanacearum GMI1000]
Protein metabolisr	50S ribosomal pr	50S ribosomal pr	50S ribosomal protein L17 [Burkholderia pseudomallei K96243]
Protein metabolism		50S ribosomal pr	50S ribosomal protein L4 [Burkholderia sp. 383]
Protein metabolisr	50S ribosomal pr	50S ribosomal pr	50S ribosomal protein L5 [Burkholderia pseudomallei K96243]
Protein metabolisr	60 kDa chaperoni	60 kDa chaperoni	60 kDa chaperonin [Burkholderia pseudomallei K96243]
Localization	ABC amino acid t	ABC amino acid t	ABC amino acid transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
Localization	ABC branched ch	ABC branched ch	ABC branched chain amino acid family transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
Electron transport	Acetohydroxy aci	Acetohydroxy aci	Acetohydroxy acid isomeroreductase [Burkholderia xenovorans LB400]
Protein metabolisr	acetolactate synth	acetolactate synth	acetolactate synthase III small subunit [Burkholderia pseudomallei K96243]
Sugar metabolism	acetyl-CoA carbo	acetyl-CoA carbo	acetyl-CoA carboxylase [Burkholderia pseudomallei K96243]
Electron transport	Alpha/beta hydrol	Alpha/beta hydrol	Alpha/beta hydrolase [Burkholderia sp. 383]
Localization	amino acid ABC t	amino acid ABC t	amino acid ABC transporter, periplasmic amino acid-binding protein, putative [Burkholderia thailandensis E264]
Electron transport			ATP synthase subunit A [Burkholderia pseudomallei K96243]
Protein metabolisr	ATP-dependent C	ATP-dependent C	ATP-dependent Clp protease proteolytic subunit [Burkholderia sp. 383]
Response to stimu	bacterioferritin [Bi	bacterioferritin [Bi	bacterioferritin [Burkholderia mallei ATCC 23344]
Defense	catalase/peroxida	catalase/peroxida	catalase/peroxidase [Caulobacter crescentus CB15]
Protein metabolisr	Chaperone protei	Chaperone protei	Chaperone protein dnaK (Heat shock protein 70) (Heat shock 70 kDa protein) (HSP70)
Protein metabolisr	Chaperonin Cpn6	Chaperonin Cpn6	Chaperonin Cpn60/TCP-1 [Nitrosospira multiformis ATCC 25196]
Structural	CHRD domain pr	CHRD domain pr	CHRD domain protein [Burkholderia sp. 383]
Protein metabolisr	co-chaperonin Gr	co-chaperonin Gr	co-chaperonin GroES [Burkholderia sp. 383]
Defense	COG0059: Ketol-	COG0059: Ketol-	COG0059: Ketol-acid reductoisomerase [Burkholderia cenocepacia PC184]
Sugar metabolism	COG0245: 2C-methyl-D-erythritol 2,		COG0245: 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [Burkholderia cenocepacia PC184]
Electron transport	COG0596: Predicted hydrolases or a		COG0596: Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily) [Burkholderia cenocepacia PC184]
Protein metabolisr	COG0740: Protea	COG0740: Protea	COG0740: Protease subunit of ATP-dependent Clp proteases [Burkholderia cenocepacia PC184]
Localization	COG0834: ABC-t	COG0834: ABC-t	COG0834: ABC-type amino acid transport/signal transduction systems, periplasmic component/domain [Burkholderia cenocepacia PC184]
Structural		COG0845: Memb	COG0845: Membrane-fusion protein [Burkholderia cenocepacia PC184]
Structural	COG1629: Outer	COG1629: Outer	COG1629: Outer membrane receptor proteins, mostly Fe transport [Burkholderia dolosa AUO158]
Regulation			COG1842: Phage shock protein A (IM30), suppresses sigma54-dependent transcription [Burkholderia dolosa AUO158]
Defense			COG2077: Peroxiredoxin [Burkholderia cenocepacia PC184]
Structural	COG2885: Outer	COG2885: Outer	COG2885: Outer membrane protein and related peptidoglycan-associated (lipo)proteins [Burkholderia cenocepacia PC184]
Structural	COG3203: Outer	COG3203: Outer	COG3203: Outer membrane protein (porin) [Burkholderia cenocepacia PC184]
Protein metabolisr	Cpn60 [Achromot	Cpn60 [Achromot	Cpn60 [Achromobacter piechaudii]
DNA metabolism		DNA-binding prot	DNA-binding protein HU-alpha [Burkholderia xenovorans LB400]
Defense	DSBA oxidoreduc	DSBA oxidoreduc	DSBA oxidoreductase [Burkholderia sp. 383]
Defense	Dyp-type peroxidase [Burkholderia s		Dyp-type peroxidase [Burkholderia sp. 383]
DNA metabolism	elongation factor Ts [Burkholderia sp		elongation factor Ts [Burkholderia sp. 383]
Cell communicatic	Extracellular ligar	Extracellular ligar	Extracellular ligand-binding receptor [Burkholderia multivorans ATCC 17616]
Cell communicatic	extracellular solut	extracellular solut	extracellular solute-binding protein, family 3 [Burkholderia vietnamiensis G4]
Protein metabolisr	glutamate/asparta	glutamate/asparta	glutamate/aspartate periplasmic binding protein precursor [Burkholderia pseudomallei K96243]
Protein metabolism			groEL [Francisella tularensis]
Defense	haem catalase/pe	haem catalase/pe	haem catalase/peroxidase [Burkholderia sp. 383]
Cell communicatic	Haemagluttinin/Autotransporter adhe		Haemagluttinin/Autotransporter adhesin [Burkholderia sp. 383]
DNA metabolism		Histone-like DNA-	Histone-like DNA-binding protein [Burkholderia sp. 383]
DNA metabolism			Histone-like nucleoid-structuring protein H-NS [Burkholderia sp. 383]
Unknown		hypothetical prote	hypothetical protein Bamb_5534 [Burkholderia cepacia AMMD]
Unknown	hypothetical prote	hypothetical prote	hypothetical protein Bcep18194_A3887 [Burkholderia sp. 383]
Unknown		hypothetical prote	hypothetical protein Bcep18194_A4154 [Burkholderia sp. 383]
Unknown	hypothetical prote	hypothetical prote	hypothetical protein Bcep18194_A5946 [Burkholderia sp. 383]
Unknown		hypothetical prote	hypothetical protein Bcep18194_A6405 [Burkholderia sp. 383]
Unknown			hypothetical protein Bcep18194_B2288 [Burkholderia sp. 383]
Unknown		hypothetical prote	hypothetical protein BMAA2076 [Burkholderia mallei ATCC 23344]
Unknown	hypothetical prote	hypothetical prote	hypothetical protein BPSL0466 [Burkholderia pseudomallei K96243]
Unknown		hypothetical prote	hypothetical protein CV0454 [Chromobacterium violaceum ATCC 12472]
Protein metabolisr	immunogenic prot	immunogenic prot	immunogenic protein ChaPs [Piscirickettsia salmonis]
Response to stimu	iron (III) protopor	iron (III) protopor	iron (III) protoporphyrin IX monomer binding protein [Burkholderia cenocepacia]
Electron transport	ketol-acid reductc	ketol-acid reductc	ketol-acid reductoisomerase [Burkholderia sp. 383]
Response to stimuli			Linocin_M18 bacteriocin protein [Burkholderia sp. 383]
Lipid metabolism	lipoamide dehydr	lipoamide dehydr	lipoamide dehydrogenase [Vibrio parahaemolyticus]
Localization	Lysine-arginine-o	Lysine-arginine-o	Lysine-arginine-ornithine ABC transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
Structural			major outer membrane protein homolog, OmpA2 [Haemophilus ducreyi 35000HP]
Sugar metabolism	maltose-binding protein [Burkholderi		maltose-binding protein [Burkholderia pseudomallei]
Structural	OmpA/MotB [Burkholderia phymatun		OmpA/MotB [Burkholderia phymatum STM815]
Lipid metabolism	outer membrane l	outer membrane l	outer membrane lipoprotein [Burkholderia sp. 383]
Structural	outer membrane p	outer membrane p	outer membrane protein, (porin) [Burkholderia sp. 383]
Structural	outer membrane p	outer membrane p	outer membrane protein, OmpA/MotB family [Burkholderia sp. 383]
Protein metabolisr	Peptidase S1C, D	Peptidase S1C, D	Peptidase S1C, Do [Burkholderia sp. 383]
Structural			Peptidoglycan-binding, LysM family protein [Burkholderia sp. 383]

Regulation	phage shock protein A, PspA [Burkh	phage shock protein A, PspA [Burkholderia vietnamiensis G4]
Energy metabolisr	Phasin [Burkholde	Phasin [Burkholde	Phasin [Burkholderia sp. 383]
Structural	porin, Gram-negative type [Burkhold		porin, Gram-negative type [Burkholderia cenocepacia AU 1054]
Electron transport		potassium-transp	potassium-transporting ATPase c chain [Burkholderia pseudomallei K96243]
Defense		PROBABLE THIC	PROBABLE THIOREDOXIN 1 (REDOX FACTOR) PROTEIN [Ralstonia solanacearum GMI1000]
Protein metabolisr	protease Do [Burl	protease Do [Burl	protease Do [Burkholderia vietnamiensis G4]
Unknown	protein of unknow	protein of unknow	protein of unknown function DUF541 [Burkholderia sp. 383]
Protein metabolisr	protein of unknow	protein of unknow	protein of unknown function DUF883, ElaB [Burkholderia sp. 383]
Protein metabolism		putative periplasn	putative periplasmic cytochrome c protein [Burkholderia pseudomallei K96243]
Cell communicatic	putative secreted	putative secreted	putative secreted substrate binding protein [Burkholderia pseudomallei K96243]
Protein metabolism		ribosome recyclin	ribosome recycling factor [Burkholderia thailandensis E264]
Regulation	RND efflux syster	RND efflux syster	RND efflux system, outer membrane lipoprotein, NodT family [Burkholderia sp. 383]
DNA metabolism	single-strand binc	single-strand binc	single-strand binding protein [Burkholderia ambifaria MC40-6]
Defense	Superoxide dismu	Superoxide dismu	Superoxide dismutase [Burkholderia sp. 383]
Xenobiotic metabc	thiamine biosynth	thiamine biosynth	thiamine biosynthesis protein ThiC [Burkholderia sp. 383]
Defense			thiol peroxidase [Burkholderia multivorans]
Cell communication		TonB-dependent	TonB-dependent haemoglobin/transferrin/lactoferrin receptor [Burkholderia sp. 383]
Cell communicatio	TonB-dependent siderophore recept		TonB-dependent siderophore receptor [Burkholderia cepacia AMMD]
Regulation	TPR repeat protein [Burkholderia sp		TPR repeat protein [Burkholderia sp. 383]
Protein metabolisr	tryptophan synthe	tryptophan synthe	tryptophan synthase subunit alpha [Burkholderia sp. 383]
Lipid metabolism			VacJ-like lipoprotein [Burkholderia sp. 383]
Protein metabolism			YtfP [Bacillus subtilis]

Function	Cr	Fe	Cd
Cell communication		COG3471: Predicted periplasmic/secreted protein [Burkholderia cenocepacia PC184]	
DNA metabolism	elongation factor	elongation factor Tu [Burkholderia sp. 383]	
Electron transport	5-oxopent-3-ene-	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase [Burkholderia sp. 383]	
Electron transport	COG1622: Heme/copper-type cytochrome/quinol oxidases, subunit 2 [Burkholderia cenocepacia PC184]		
Energy metabolisr	inorganic pyrophc	inorganic pyrophosphatase [Burkholderia sp. 383]	
Energy metabolism		PhaB [Burkholderia sp. DSMZ 9242]	
Lipid metabolism		acyl carrier protein [Burkholderia pseudomallei K96243]	
Localization	ABC sugar transporter, periplasmic ligand binding protein [Burkholderia sp. 383]		
Localization	COG1653: ABC-type sugar transport system, periplasmic component [Burkholderia dolosa AUO158]		
Localization	COG2854: ABC-type transport system involved in resistance to organic solvents, auxiliary component [Burkholderia dolosa AUO158]		
Localization	phosphate ABC transporter, periplasmic phosphate-binding protein [Burkholderia vietnamiensis G4]		
Localization		transport-associated protein [Burkholderia sp. 383]	
Protein metabolisr	Chaperonin Cpn6	Chaperonin Cpn60/GroEL [Burkholderia sp. 383]	
Protein metabolisr	Peptidase S10, serine carboxypeptidase [Burkholderia sp. 383]		
Response to stimu	Molybdenum-pter	Molybdenum-pterin binding protein [Burkholderia sp. 383]	
Sugar metabolism	fumarate hydratase [Burkholderia sp. 383]		
Sugar metabolism	malate dehydrogenase [Burkholderia sp. 383]		
Unknown	hypothetical protein Bcep18194_A5185 [Burkholderia sp. 383]		
Xenobiotic metabc	COG4154: Fucose dissimilation pathway protein FucU [Burkholderia cenocepacia PC184]		
Xenobiotic metabolism		dihydrodipicolinate synthase [Burkholderia sp. 383]	
Xenobiotic metabc	Toluene tolerance	Toluene tolerance [Burkholderia sp. 383]	

7	2.663	0.992	1.317	gi|78061223	Peptidase S10, serine carboxypeptidase [Burkholderia sp. 383]
7	3.345	2.037	3.127	gi|78062970	tryptophan synthase subunit alpha [Burkholderia sp. 383]
8	0.23	0.187	0.702	gi|78064993	Toluene tolerance [Burkholderia sp. 383]
8	0.569	0.288	0.846	gi|6683781	PhaB [Burkholderia sp. DSMZ 9242]
8	0.299	0.23	1.145	gi|78060990	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase [Burkholderia sp. 383]
8	1.764	0.627	1.345	gi|17546788	30S ribosomal protein S15 [Ralstonia solanacearum GMI1000]
8	3.337	2.228	1.713	gi|78060839	Molybdenum-pterin binding protein [Burkholderia sp. 383]
8	1.295	3.831	2.148	gi|53718809	potassium-transporting ATPase c chain [Burkholderia pseudomallei K96243]
8	2.66	2.038	2.355	gi|1169373	Chaperone protein dnaK (Heat shock protein 70) (Heat shock 70 kDa protein) (HSP70)
8	0.418	0.672	2.795	gi|78065034	TPR repeat protein [Burkholderia sp. 383]
8	2.05	1.49	6.412	gi|134295978	phage shock protein A, PspA [Burkholderia vietnamiensis G4]
8	1.584	3.297	7.566	gi|83721123	ribosome recycling factor [Burkholderia thailandensis E264]
8	3.832	1.861	17.568	gi|115351594	TonB-dependent siderophore receptor [Burkholderia cepacia AMMD]
9	0.36	0.195	0.227	gi|53720532	glutamate/aspartate periplasmic binding protein precursor [Burkholderia pseudomallei K96243]
9	0.94	0.984	0.577	gi|78065089	protein-disulfide isomerase-like [Burkholderia sp. 383]
9	1.128	0.847	2.017	gi|2293312	YtfP [Bacillus subtilis]
9	3.282	2.44	3.443	gi|78067416	Peptidase S1C, Do [Burkholderia sp. 383]
9	9.415	5.869	4.293	gi|118699862	single-strand binding protein [Burkholderia ambifaria MC40-6]
9	2.805	1.898	4.313	gi|84354942	COG0245: 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [Burkholderia cenocepacia PC184]
10	0.136	0.073	0.217	gi|78065802	thiamine biosynthesis protein ThiC [Burkholderia sp. 383]
10	1.311	0.477	0.298	gi|34495909	hypothetical protein CV0454 [Chromobacterium violaceum ATCC 12472]
10	0.285	0.655	0.391	gi|84351996	COG0596: Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily) [Burkholderia cenocepacia PC184]
10	2.892	1.224	0.399	gi|78060284	Haemagluttinin/Autotransporter adhesin [Burkholderia sp. 383]
10	0.806	0.633	0.462	gi|1770287	groEL [Francisella tularensis]

10	0.282	0.563	0.47	gi|9964068	maltose-binding protein [Burkholderia pseudomallei]
10	0.579	2.078	2.143	gi|78061073	TonB-dependent haemoglobin/transferrin/lactoferrin receptor [Burkholderia sp. 383]
10	2.149	3.253	2.237	gi|78065158	outer membrane lipoprotein [Burkholderia sp. 383]
10	2.542	2.057	3.405	gi|78065624	outer membrane protein, OmpA/MotB family [Burkholderia sp. 383]
10	2.579	1.112	3.987	gi|107026730	porin, Gram-negative type [Burkholderia cenocepacia AU 1054]
10	1.318	1.756	4.871	gi|78066650	Peptidoglycan-binding, LysM family protein [Burkholderia sp. 383]
10	0.784	5.497	5.194	gi|78064724	Histone-like DNA-binding protein [Burkholderia sp. 383]
10	4.315	1.538	6.704	gi|118029223	OmpA/MotB [Burkholderia phymatum STM815]
10	4.66	16.731	10.213	gi|134296822	protease Do [Burkholderia vietnamiensis G4]
10	3.082	5.976	17.023	gi|78067259	protein of unknown function DUF541 [Burkholderia sp. 383]
11	0.18	0.196	0.166	gi|78065290	haem catalase/peroxidase [Burkholderia sp. 383]
11	0.117	0.112	0.172	gi|78065231	ABC amino acid transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
11	0.279	0.172	0.199	gi|78065428	co-chaperonin GroES [Burkholderia sp. 383]
11	0.132	0.263	0.213	gi|78067546	CHRD domain protein [Burkholderia sp. 383]
11	0.255	0.229	0.371	gi|78060035	Alpha/beta hydrolase [Burkholderia sp. 383]
11	1.764	0.379	0.465	gi|78067874	hypothetical protein Bcep18194_A6405 [Burkholderia sp. 383]
11	0.933	0.849	0.484	gi|53721005	ATP synthase subunit A [Burkholderia pseudomallei K96243]
11	0.464	0.606	0.839	gi|78065543	ABC sugar transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
11	0.743	1.071	0.987	gi|2306819	acetoacetyl-CoA reductase [Alcaligenes sp. SH-69]
11	0.992	0.913	1.035	gi|78064927	50S ribosomal protein L2 [Burkholderia sp. 383]
11	1.365	2.502	1.638	gi|53720045	acyl carrier protein [Burkholderia pseudomallei K96243]
11	1.006	2.267	3.345	gi|53718340	putative periplasmic cytochrome c protein [Burkholderia pseudomallei K96243]
11	2.358	4.135	3.531	gi|78065361	hypothetical protein Bcep18194_A3887 [Burkholderia sp. 383]
12	0.109	0.102	0.097	gi|118720065	Extracellular ligand-binding receptor [Burkholderia multivorans ATCC 17616]
12	0.322	0.344	0.189	gi|134294561	extracellular solute-binding protein, family 3 [Burkholderia vietnamiensis G4]
12	0.271	0.269	0.216	gi|78062511	ABC branched chain amino acid family transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
12	0.163	0.212	0.241	gi|78061080	Lysine-arginine-ornithine ABC transporter, periplasmic ligand binding protein [Burkholderia sp. 383]
12	0.185	0.189	0.323	gi|30525581	immunogenic protein ChaPs [Piscirickettsia salmonis]
12	0.292	0.278	0.464	gi|53718106	hypothetical protein BPSL0466 [Burkholderia pseudomallei K96243]
12	0.689	0.868	0.496	gi|78060115	Linocin_M18 bacteriocin protein [Burkholderia sp. 383]
12	1.046	1.114	0.668	gi|53720821	50S ribosomal protein L3 [Burkholderia pseudomallei K96243]
12	0.381	0.703	0.805	gi|84361010	COG1653: ABC-type sugar transport system, periplasmic component [Burkholderia dolosa AUO158]
12	0.533	0.762	0.818	gi|134294995	extracellular solute-binding protein, family 1 [Burkholderia vietnamiensis G4]
12	1.858	2.685	1.43	gi|84355507	COG3471: Predicted periplasmic/secreted protein [Burkholderia cenocepacia PC184]
12	2.26	2.131	1.601	gi|78064909	elongation factor Tu [Burkholderia sp. 383]
12	1.386	2.071	1.729	gi|78066870	dihydrodipicolinate synthase [Burkholderia sp. 383]
12	3.436	1.458	1.739	gi|134295363	phosphate ABC transporter, periplasmic phosphate-binding protein [Burkholderia vietnamiensis G4]
12	0.612	0.641	1.786	gi|78067292	bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase protein [Burkholderia sp. 383]
12	1.591	1.958	2.06	gi|49524076	thiol peroxidase [Burkholderia multivorans]
12	0.331	2.349	2.256	gi|84355531	COG2885: Outer membrane protein and related peptidoglycan-associated (lipo)proteins [Burkholderia cenocepacia PC184]
12	3.09	5.534	3.523	gi|78067407	RND efflux system, outer membrane lipoprotein, NodT family [Burkholderia sp. 383]
12	1.276	3.109	4.279	gi|53715901	hypothetical protein BMAA2076 [Burkholderia mallei ATCC 23344]
12	1.111	2.067	5.194	gi|78065626	hypothetical protein Bcep18194_A4154 [Burkholderia sp. 383]
12	3.567	2.375	5.57	gi|84354134	COG3203: Outer membrane protein (porin) [Burkholderia cenocepacia PC184]
12	2.051	4.998	7.088	gi|84359100	COG1629: Outer membrane receptor proteins, mostly Fe transport [Burkholderia dolosa AUO158]
13	0.154	0.294	0.212	gi|82703461	Chaperonin Cpn60/TCP-1 [Nitrosospira multiformis ATCC 25196]
13	0.199	0.539	0.419	gi|17547713	30S ribosomal protein S4 [Ralstonia solanacearum GMI1000]
13	0.998	0.914	0.553	gi|78064765	ATP synthase subunit B [Burkholderia sp. 383]
13	1.012	0.7	0.567	gi|78064766	ATP synthase subunit epsilon [Burkholderia sp. 383]
13	1.309	1.271	1.039	gi|78062997	succinate dehydrogenase [Burkholderia sp. 383]
13	0.496	1.268	1.592	gi|78066654	hypothetical protein Bcep18194_A5185 [Burkholderia sp. 383]
13	1.021	1.892	2.232	gi|78063135	hypothetical protein Bcep18194_B2288 [Burkholderia sp. 383]
13	1.376	1.438	3.755	gi|78060562	*Histone-like nucleoid-structuring protein H-NS [Burkholderia sp. 383]*
13	2.312	2.035	4.487	gi|53720809	50S ribosomal protein L5 [Burkholderia pseudomallei K96243]
13	1.652	4.463	5.284	gi|17545907	PROBABLE THIOREDOXIN 1 (REDOX FACTOR) PROTEIN [Ralstonia solanacearum GMI1000]
14	0.08	0.149	0.065	gi|69880176	iron (III) protoporphyrin IX monomer binding protein [Burkholderia cenocepacia]
14	0.118	0.104	0.14	gi|84356699	COG0834: ABC-type amino acid transport/signal transduction systems, periplasmic component/domain [Burkholderia cenocepacia PC184]
14	0.163	0.161	0.141	gi|83718587	amino acid ABC transporter, periplasmic amino acid-binding protein, putative [Burkholderia thailandensis E264]
14	0.148	0.17	0.157	gi|84355249	COG0059: Ketol-acid reductoisomerase [Burkholderia cenocepacia PC184]
14	0.175	0.166	0.163	gi|53720894	putative secreted substrate binding protein [Burkholderia pseudomallei K96243]
14	0.21	0.246	0.247	gi|22121836	Cpn60 [Achromobacter piechaudii]
14	0.215	0.232	0.273	gi|53721514	60 kDa chaperonin [Burkholderia pseudomallei K96243]
14	0.262	0.446	0.319	gi|78066693	ATP-dependent Clp protease proteolytic subunit [Burkholderia sp. 383]
14	0.438	0.56	0.54	gi|84360616	COG2854: ABC-type transport system involved in resistance to organic solvents, auxiliary component [Burkholderia dolosa AUO158]
14	0.941	0.991	0.554	gi|78065036	protein of unknown function DUF877 [Burkholderia sp. 383]
14	0.408	0.991	1.35	gi|84355822	COG1622: Heme/copper-type cytochrome/quinol oxidases, subunit 2 [Burkholderia cenocepacia PC184]
14	1.179	1.481	1.408	gi|78066914	two component transcriptional regulator, LuxR family [Burkholderia sp. 383]
14	2.237	3.375	2.318	gi|53720591	acetyl-CoA carboxylase [Burkholderia pseudomallei K96243]
14	1.705	1.226	2.547	gi|84356766	COG2077: Peroxiredoxin [Burkholderia cenocepacia PC184]

14	4.345	5.902	2.947	gi|53716294	bacterioferritin [Burkholderia mallei ATCC 23344]
14	2.304	2.707	4.459	gi|78065378	protein of unknown function DUF883, ElaB [Burkholderia sp. 383]
14	18.374	5.701	8.112	gi|78066909	Phasin [Burkholderia sp. 383]
14	1.609	3.896	8.265	gi|91785774	DNA-binding protein HU-alpha [Burkholderia xenovorans LB400]
15	0.214	0.21	0.197	gi|16127273	catalase/peroxidase [Caulobacter crescentus CB15]
15	0.414	0.676	0.345	gi|78060116	Dyp-type peroxidase [Burkholderia sp. 383]
15	0.19	0.291	0.357	gi|84354934	COG0740: Protease subunit of ATP-dependent Clp proteases [Burkholderia cenocepacia PC184]
15	0.359	0.447	0.371	gi|53718833	acetolactate synthase III small subunit [Burkholderia pseudomallei K96243]
15	0.133	0.206	0.457	gi|78067733	DSBA oxidoreductase [Burkholderia sp. 383]
15	0.248	0.381	0.527	gi|78067213	inorganic pyrophosphatase [Burkholderia sp. 383]
15	0.584	0.785	0.61	gi|78066912	2-oxo-acid dehydrogenase E1 component homodimeric type [Burkholderia sp. 383]
15	0.802	0.999	0.8	gi|17549025	elongation factor EF-2 [Ralstonia solanacearum GMI1000]
15	0.593	0.645	1.085	gi|107026783	aconitate hydratase 1 [Burkholderia cenocepacia AU 1054]
15	1.241	1.379	1.188	gi|78064933	30S ribosomal protein S17 [Burkholderia sp. 383]
15	0.573	0.457	1.429	gi|78065405	transport-associated protein [Burkholderia sp. 383]
15	1.196	1.309	1.93	gi|33594012	putative dnaK suppressor protein [Bordetella pertussis Tohama I]
15	1.055	1.869	2.041	gi|78064992	VacJ-like lipoprotein [Burkholderia sp. 383]
15	1.209	2.228	3.303	gi|115360277	hypothetical protein Bamb_5534 [Burkholderia cepacia AMMD]
15	0.798	2.917	4.809	gi|84354646	COG0845: Membrane-fusion protein [Burkholderia cenocepacia PC184]
15	2.103	1.953	5.401	gi|78066797	elongation factor Ts [Burkholderia sp. 383]
16	0.146	0.178	0.162	gi|2689039	lipoamide dehydrogenase [Vibrio parahaemolyticus]
16	0.249	0.15	0.313	gi|53720794	50S ribosomal protein L17 [Burkholderia pseudomallei K96243]
16	0.486	0.446	0.392	gi|78067343	Superoxide dismutase [Burkholderia sp. 383]
16	0.411	0.333	0.581	gi|78063463	Chaperonin Cpn60/GroEL [Burkholderia sp. 383]
16	0.966	1.23	0.661	gi|78066856	Peptidyl-prolyl cis-trans isomerase, cyclophilin type [Burkholderia sp. 383]
16	2.463	1.172	1.048	gi|78063001	malate dehydrogenase [Burkholderia sp. 383]
16	0.411	1.485	2.01	gi|78065223	2-dehydro-3-deoxyphosphogluconate aldolase [Burkholderia sp. 383]
16	1.149	1.572	2.143	gi|33151319	major outer membrane protein homolog, OmpA2 [Haemophilus ducreyi 35000HP]
16	2.513	3.556	2.182	gi|78065110	outer membrane protein, (porin) [Burkholderia sp. 383]
16	1.066	1.055	3.017	gi|84362104	COG1842: Phage shock protein A (IM30), suppresses sigma54-dependent transcription [Burkholderia dolosa AUO158]
17	0.123	0.268	0.154	gi|91782599	Acetohydroxy acid isomeroreductase [Burkholderia xenovorans LB400]
17	0.187	0.27	0.17	gi|78067059	ketol-acid reductoisomerase [Burkholderia sp. 383]
17	0.45	0.5	0.573	gi|84352918	COG4154: Fucose dissimilation pathway protein FucU [Burkholderia cenocepacia PC184]
17	2.035	1.605	1.751	gi|78065675	fumarate hydratase [Burkholderia sp. 383]
17	5.69	3.523	2.233	gi|78067415	hypothetical protein Bcep18194_A5946 [Burkholderia sp. 383]
18	0.559	0.469	0.445	gi|78064925	50S ribosomal protein L4 [Burkholderia sp. 383]

gi|78061223 Mass: 63061 Score: 72 Queries matched: 4 emPAI: 0.12
Peptidase S10, serine carboxypeptidase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.663	4	1.492
	116/114	0.992	4	1.177
	117/114	1.317	4	1.372

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1592	600.9508	1799.8306	1799.8482	-0.0176	0	55	0.014	3.075	1.212	2.156	R.DLAAMYDATVSDTGAR.M
1605	606.2893	1815.8461	1815.8431	0.0030	0	(17)	98	0.733	0.607	0.637	R.DLAAMYDATVSDTGAR.M

gi|78062970 Mass: 30057 Score: 48 Queries matched: 2 emPAI: 0.13
tryptophan synthase subunit alpha [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.345	2	1.129
	116/114	2.037	2	1.750
	117/114	3.127	2	3.045

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1718	701.3792	2101.1158	2101.1177	-0.0019	0	24	21	3.039	1.170	4.735	R.AAQIDPIFLLAPTSTDER.I
1821	827.0983	2478.2730	2478.2625	0.0105	0	24	19	0.710	-0.065	0.973	K.GVNVVPYQNQDQVYADLISGR.L

gi|78064993 Mass: 25823 Score: 91 Queries matched: 6 emPAI: 0.27
Toluene tolerance [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.230	4	1.185
	116/114	0.187	4	2.135
	117/114	0.702	3	1.078

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
904	709.3952	1416.7759	1416.7704	0.0054	0	47	0.1	0.226	0.337	0.471	R.ADPADTDVVVK.T
1811	812.7720	2435.2941	2435.2635	0.0306	0	44	0.2	---	---	---	K.ADGSLDIVSTSNAATPLTTADK.A

gi|6683781 Mass: 28408 Score: 142 Queries matched: 3 emPAI: 0.12
PhaB [Burkholderia sp. DSMZ 9242]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.569	2	1.668
	116/114	0.288	2	1.070
	117/114	0.846	2	1.410

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1250	638.6844	1913.0315	1913.0340	-0.0025	0	56	0.013	0.265	0.315	1.266	K.AEVGEIDVLVNNAGITR.D
1382	799.4005	2395.1797	2395.1737	0.0060	0	86	1.4e-005	0.288	0.229	0.393	R.AATGQAIEAAQSGFDAVSETATR.G

gi|78060990 Mass: 32382 Score: 103 Queries matched: 3 emPAI: 0.10
5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.299	2	2.307
	116/114	0.230	3	1.882
	117/114	1.145	3	1.367

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1140	560.6534	1678.9383	1678.9376	0.0008	0	53	0.025	0.586	0.103	1.568	R.AIDPATLPLVSGEPR.I
1035	502.9356	1505.7849	1505.7807	0.0042	0	50	0.05	0.528	0.711	1.102	R.QAIDSSVDATLSR.M

gi|17546788 Mass: 11803 Score: 90 Queries matched: 3 emPAI: 0.29
30S ribosomal protein S15 [Ralstonia solanacearum GMI1000]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.764	3	NN
	116/114	0.627	3	1.633
	117/114	1.345	3	1.374

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1264	658.3552	1972.0437	1972.0347	0.0089	0	55	0.016	2.407	0.631	2.579	R.GTNDTGSPEVQVALLTAR.I
1083	529.3009	1584.8809	1584.8229	0.0580	0	35	1.7	2.887	0.866	6.933	R.IPEGVASDAQDAIR.A

gi|78060839 Mass: 15116 Score: 74 Queries matched: 3 emPAI: 0.23
Molybdenum-pterin binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.337	3	1.514

	116/114	2.228	3	2.462								
	117/114	1.713	3	1.316								
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide	
1245	634.6766	1901.0079	1900.9976	0.0103	0	37	0.93	6.446	2.463	1.707	K.AAGGAEIVAIVTNDSVDR.L	
467	524.7745	1047.5345	1047.5682	-0.0337	0	37	1.1	---	---	---	K.DALDTLTR.Q	

gi|53718809 Mass: 21245 Score: 78 Queries matched: 4 emPAI: 0.16
potassium-transporting ATPase c chain [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.295	2	1.387							
	116/114	3.831	2	1.026							
	117/114	2.148	2	1.163							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
844	676.3726	1350.7306	1350.7377	-0.0072	0	45	0.16	1.791	3.945	1.787	K.LNLALDAAQAAH.-
1216	617.6863	1850.0370	1850.0383	-0.0013	0	33	2.8	0.324	0.018	0.062	K.IAAILNAATPPADALER.I

gi|1169373 Mass: 78292 Score: 61 Queries matched: 3 emPAI: 0.04
Chaperone protein dnaK (Heat shock protein 70) (Heat shock 70 kDa protein) (HSP70)

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	2.660	3	1.576							
	116/114	2.038	3	1.142							
	117/114	2.355	3	1.315							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1169	577.6528	1729.9367	1729.9332	0.0034	0	38	0.77	3.808	2.641	1.619	K.VSDIDDVILVGGQTR.M
1326	695.3574	2083.0504	2083.1153	-0.0649	0	23	24	2.484	1.606	3.318	R.ISHIDTEQVQAELGAGK.V

gi|78065034 Mass: 24288 Score: 68 Queries matched: 3 emPAI: 0.14
TPR repeat protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.418	3	1.569							
	116/114	0.672	3	1.407							
	117/114	2.795	3	1.181							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1222	626.9948	1877.9624	1877.9493	0.0132	0	42	0.3	0.416	0.480	3.442	R.DTLGESALFPPETTTR.A
1403	623.0678	2488.2421	2488.2213	0.0208	0	26	14	-0.055	0.035	0.035	K.SVAVVDDSTAYGQGLADEFEK.K

gi|134295978 Mass: 26929 Score: 101 Queries matched: 9 emPAI: 0.26
phage shock protein A, PspA [Burkholderia vietnamiensis G4]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	2.050	6	1.388							
	116/114	1.490	6	1.214							
	117/114	6.412	6	1.263							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
745	629.8527	1257.6909	1257.6799	0.0110	0	26	11	---	---	---	R.ALQAGDEALAR.E
1151	567.9699	1700.8879	1700.8451	0.0428	0	74	0.00019	7.180	0.682	11.795	K.GLLNDAADSVQDPSR.D

gi|83721123 Mass: 23679 Score: 71 Queries matched: 6 emPAI: 0.30
ribosome recycling factor [Burkholderia thailandensis E264]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.584	4	NN							
	116/114	3.297	3	1.168							
	117/114	7.566	4	1.159							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1185	885.4798	1768.9450	1768.9441	0.0010	0	45	0.16	0.306	0.489	5.011	R.EADLGLNPATAGDLIR.V
98	366.7227	731.4309	731.4412	-0.0102	0	26	17	0.396	-0.021	0.049	K.VVSQR.D

gi|115351594 Mass: 84070 Score: 70 Queries matched: 7 emPAI: 0.04
TonB-dependent siderophore receptor [Burkholderia cepacia AMMD]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	3.832	3	1.240							
	116/114	1.861	3	1.480							
	117/114	17.568	3	1.360							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1136	555.9705	1664.8897	1664.8929	-0.0032	0	26	12	---	---	---	R.VDPYMIDSITVLR.G

1376	783.0960	2346.2662	2346.2675	-0.0013	0	44	0.22	3.015	1.047	10.171	R.GPTSVLYGAGDPGAIIDVQTK.T

gi|53720532 Mass: 37735 Score: 73 Queries matched: 8 emPAI: 0.18
glutamate/aspartate periplasmic binding protein precursor [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.360	2	1.282
	116/114	0.195	2	1.583
	117/114	0.227	2	1.247

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
845	456.2509	1365.7308	1365.7222	0.0086	0	44	0.18	0.297	0.130	0.191	K.TVVTTAGTTSER.L
1257	590.6400	1768.8982	1768.8610	0.0373	0	30	5.5	0.481	0.319	0.294	R.AVAFMMDDALLAGER.A

gi|78065089 Mass: 28848 Score: 66 Queries matched: 3 emPAI: 0.12
protein-disulfide isomerase-like [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.940	2	1.369
	116/114	0.984	2	1.105
	117/114	0.577	2	1.648

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1280	607.3219	1818.9439	1818.9420	0.0018	0	34	2.2	0.616	0.869	0.938	R.GMNVTGTPTIFLPDGR.R
1250	581.9817	1742.9232	1742.9172	0.0060	0	32	3	0.549	1.145	0.051	R.GTTVDEPALLDALER.N

gi|2293312 Mass: 50131 Score: 61 Queries matched: 3 emPAI: 0.07
YtfP [Bacillus subtilis]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.128	2	1.058
	116/114	0.847	2	1.300
	117/114	2.017	2	1.163

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
791	665.3900	1328.7655	1328.7534	0.0121	0	31	3.4	1.077	0.664	1.768	K.AQSVVDALLNR.L
1394	695.3719	2083.0940	2083.0820	0.0120	0	30	5.1	1.297	2.938	2.476	R.QAFVGLSQDQYGSITLGR.Q

gi|78067416 Mass: 54678 Score: 77 Queries matched: 5 emPAI: 0.06
Peptidase S1C, Do [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.282	2	1.156
	116/114	2.440	2	1.741
	117/114	3.443	2	1.012

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
935	481.9164	1442.7274	1442.7236	0.0039	0	38	0.76	2.807	1.212	3.400	K.LASNDGGPVEQGR.L
955	482.6062	1444.7969	1444.7796	0.0173	0	39	0.69	---	---	---	R.IAYGDPASVPVGR.Y

gi|118699862 Score: 54 Queries matched: 7
single-strand binding protein [Burkholderia ambifaria MC40-6]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	9.415	4	1.519
	116/114	5.869	4	1.216
	117/114	4.293	4	1.402

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
974	724.8578	1447.7011	1447.6708	0.0303	1	11	4.3e+002	---	---	---	R.GGEQMERGGGGGGR.A
1129	798.4637	1594.9129	1594.9164	-0.0035	0	43	0.27	18.650	10.819	9.913	K.VILVGNLGADPEVR.Y

gi|78066722 Mass: 17982 Score: 89 Queries matched: 6 emPAI: 0.19
2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.104	2	1.109
	116/114	0.444	2	1.326
	117/114	5.146	2	1.234

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1016	494.9509	1481.8310	1481.8324	-0.0014	0	48	0.073	1.942	0.527	4.323	R.ANIAADLGLPLDR.V
1403	730.4100	2188.2081	2188.2095	-0.0015	0	41	0.41	2.607	2.311	2.477	K.AAGFAIQNVDSTVIAQAPK.L

gi|78065802 Mass: 76007 Score: 66 Queries matched: 7 emPAI: 0.04
thiamine biosynthesis protein ThiC [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.136	2	1.494
	116/114	0.073	2	2.261
	117/114	0.217	2	1.398

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2066	613.6573	1837.9502	1837.9302	0.0201	0	43	0.27	0.092	0.107	0.158	R.EITQADTPTGFGGEK.N
2671	705.4086	2113.2041	2113.2027	0.0014	0	23	23	---	---	---	R.NSPVPIGTVPIYQALEK.V

gi|34495909 Mass: 19265 Score: 64 Queries matched: 2 emPAI: 0.17
hypothetical protein CV0454 [Chromobacterium violaceum ATCC 12472]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.311	2	1.278
	116/114	0.477	2	1.077
	117/114	0.298	2	1.180

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2117	626.6497	1876.9271	1876.9401	-0.0129	0	32	3.5	0.992	0.442	0.248	R.SGGVELGTPAAYDNNIR.A
1278	496.9450	1487.8132	1487.8106	0.0026	0	32	3.3	0.314	0.877	0.565	K.VISPIEDTPAFR.A

gi|84351996 Mass: 32756 Score: 71 Queries matched: 7 emPAI: 0.21
COG0596: Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily) [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.285	3	1.567
	116/114	0.655	3	1.795
	117/114	0.391	4	1.356

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1611	542.6816	1625.0229	1625.0193	0.0036	0	47	0.1	0.701	0.175	0.389	K.AVLVSAVPPLMLK.T
1751	557.9497	1670.8273	1670.8243	0.0030	0	28	8.7	0.193	-0.004	0.303	K.AFSETDQTEDLK.S

gi|78060284 Mass: 149500 Score: 119 Queries matched: 5 emPAI: 0.02
Haemagluttinin/Autotransporter adhesin [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.892	4	1.131
	116/114	1.224	4	1.105
	117/114	0.399	4	1.311

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2580	691.7047	2072.0921	2072.1106	-0.0184	0	63	0.0026	2.129	1.561	0.665	K.NVAAGAADTDAVNVQQLK.N
1151	731.3892	1460.7638	1460.7705	-0.0067	0	56	0.012	2.429	0.799	1.050	R.ASETNQVQATIR.E

gi|1770287 Mass: 63685 Score: 57 Queries matched: 46 emPAI: 0.11
groEL [Francisella tularensis]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.806	7	1.296
	116/114	0.633	8	1.627
	117/114	0.462	8	1.341

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1841	576.0034	1724.9884	1724.9907	-0.0022	0	55	0.016	1.428	1.050	0.337	R.AAVEEGIVAGGGVALIR.A
2360	994.9690	1987.9234	1987.7762	0.1472	0	2	2.9e+003	---	---	---	K.EAAPAMPMGGGMGGMPGMM.-

gi|9964068 Mass: 50258 Score: 69 Queries matched: 9 emPAI: 0.14
maltose-binding protein [Burkholderia pseudomallei]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.282	3	1.903
	116/114	0.563	3	NN
	117/114	0.470	4	1.225

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1424	515.6359	1543.8858	1543.8813	0.0044	0	33	2.4	0.325	0.667	0.559	K.VISGDAPSAAQIK.G
2003	603.9959	1808.9659	1808.9698	-0.0039	0	36	1.1	0.280	0.638	0.343	K.DFAVAGGAGAAAMTALK.T

gi|78061073 Mass: 85767 Score: 67 Queries matched: 12 emPAI: 0.08
TonB-dependent haemoglobin/transferrin/lactoferrin receptor [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.579	2	1.173
	116/114	2.078	2	1.750
	117/114	2.143	2	1.177

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
934	459.2477	1374.7214	1374.7225	-0.0011	0	39	0.65	0.482	1.001	1.778	K.AVSTSANELSPR.V
2430	676.3548	2026.0426	2026.0462	-0.0037	0	28	8.3	0.655	2.928	2.430	R.TTSNPQDVYSESLLGK.L

gi|78065158 Mass: 15985 Score: 76 Queries matched: 10 emPAI: 0.78
outer membrane lipoprotein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.149	7	1.244
	116/114	3.253	7	1.300
	117/114	2.237	7	1.413

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
948	691.3621	1380.7096	1380.7119	-0.0023	0	44	0.2	2.769	3.367	4.255	R.SITQAASGEAFR.A
3136	915.4928	2743.4566	2743.4708	-0.0143	0	32	3.5	2.163	1.205	0.491	R.IQSDGGGSAIGTIGGGALGAVAGSAIGGGK.G

gi|78065624 Mass: 26875 Score: 73 Queries matched: 32 emPAI: 0.42
outer membrane protein, OmpA/MotB family [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.542	10	1.366
	116/114	2.057	10	1.322
	117/114	3.405	10	1.478

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1492	527.6125	1579.8158	1579.8238	-0.0080	0	46	0.12	6.377	2.093	9.789	R.DAFWTPATANAK.C
2414	672.3338	2013.9796	2013.9799	-0.0003	0	(27)	11	4.748	5.793	5.300	K.IEGMNTEVVVATGYTDR.I

gi|107026730 Mass: 41742 Score: 83 Queries matched: 13 emPAI: 0.26
porin, Gram-negative type [Burkholderia cenocepacia AU 1054]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.579	6	1.573
	116/114	1.112	6	1.142
	117/114	3.987	6	1.558

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
840	658.8867	1315.7588	1315.7581	0.0006	0	59	0.0063	2.440	1.547	2.620	K.SSQEIVALGLR.H
2838	746.7178	2237.1315	2237.1311	0.0004	0	24	22	0.931	1.011	1.444	K.AIFQLENGFNSASGALGQGGR.M

gi|78066650 Mass: 18091 Score: 111 Queries matched: 3 emPAI: 0.19
Peptidoglycan-binding, LysM family protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.318	3	1.077
	116/114	1.756	3	1.540
	117/114	4.871	3	1.149

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1769	565.6516	1693.9330	1693.9342	-0.0012	0	54	0.019	1.483	2.172	5.799	R.TVTLSGSVADLDTK.A
1021	473.9505	1418.8298	1418.8337	-0.0039	0	57	0.0095	1.193	1.163	1.385	K.TAVVTGAASGIGK.E

gi|78064724 Mass: 11617 Score: 64 Queries matched: 10 emPAI: 0.68
Histone-like DNA-binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.784	2	4.912
	116/114	5.497	2	1.073
	117/114	5.194	2	1.350

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1976	597.3315	1788.9728	1788.9825	-0.0097	0	28	7.7	0.089	5.225	4.055	K.QELIDAVAAQTGASK.A
2223	640.3720	1918.0942	1918.0866	0.0076	0	36	1.4	---	---	---	K.AQTGETLDTLLEVIK.K

gi|118029223 Mass: 25938 Score: 81 Queries matched: 8 emPAI: 0.13
OmpA/MotB [Burkholderia phymatum STM815]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.315	2	1.494
	116/114	1.538	2	1.353
	117/114	6.704	2	1.472

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
774	430.5645	1288.6717	1288.6753	-0.0036	0	35	1.7	---	---	---	K.QLIACLAPDR.R
1492	527.6125	1579.8158	1579.8238	-0.0080	0	46	0.12	6.377	2.093	9.789	R.DAFWTPATANAK.C

gi|134296822 Mass: 54549 Score: 78 Queries matched: 11 emPAI: 0.12
protease Do [Burkholderia vietnamiensis G4]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.660	4	1.123
	116/114	16.731	5	1.243
	117/114	10.213	5	1.315

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
773	430.2617	1287.7634	1287.7642	-0.0008	0	28	7.2	---	---	---	K.IDASGLPTVK.I
1070	481.9155	1442.7246	1442.7236	0.0010	0	50	0.042	4.991	17.285	11.437	K.LASNDGGPVEQGR.L

gi|78067259 Mass: 27465 Score: 64 Queries matched: 13 emPAI: 0.26
protein of unknown function *DUF541* [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.082	2	1.517
	116/114	5.976	2	1.716
	117/114	17.023	2	1.808

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
546	388.2283	1161.6630	1161.6597	0.0033	0	28	9.2	3.320	4.864	13.453	R.ADAALSQAK.G
1078	722.3898	1442.7650	1442.7599	0.0051	0	36	1.1	1.538	13.179	40.138	K.DPGSLTAQLNQR.A

gi|78065290 Mass: 85322 Score: 96 Queries matched: 12 emPAI: 0.12
haem catalase/peroxidase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.180	5	1.321
	116/114	0.196	5	1.268
	117/114	0.166	5	1.085

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1350	750.4284	1498.8423	1498.8378	0.0044	0	68	0.00069	0.343	0.275	0.167	K.NAGVAVTVPFAPGR.A
2540	664.3588	1990.0547	1990.0404	0.0143	0	28	9.3	0.342	0.162	0.160	R.FAPLNSWPDNVSLDK.A

gi|78065231 Mass: 37192 Score: 99 Queries matched: 15 emPAI: 0.41
ABC amino acid transporter, periplasmic ligand binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.117	8	1.763
	116/114	0.112	8	NN
	117/114	0.172	8	1.475

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1326	745.9235	1489.8325	1489.8232	0.0093	0	65	0.0016	0.042	0.057	0.142	K.VVDDAISQVEK.S
2054	585.3039	1752.8899	1752.8660	0.0238	0	34	1.8	0.326	0.751	0.315	R.AVAFMMDDALLAGER.A

gi|78065428 Mass: 11897 Score: 86 Queries matched: 11 emPAI: 1.76
co-chaperonin GroES [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.279	7	NN
	116/114	0.172	7	1.208
	117/114	0.199	7	1.151

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
769	645.3521	1288.6896	1288.6931	-0.0036	0	47	0.097	0.219	0.127	0.232	K.VDGNELLVMR.E
1370	502.9402	1505.7986	1505.8003	-0.0017	0	39	0.59	0.215	0.066	0.196	R.EEDIMAVVNAK.-

gi|78067546 Mass: 16277 Score: 91 Queries matched: 4 emPAI: 0.46
CHRD domain protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.132	4	1.180
	116/114	0.263	4	1.241
	117/114	0.213	4	1.163

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2589	676.7156	2027.1249	2027.1142	0.0107	0	42	0.34	0.174	0.178	0.219	R.LSANLQPSSEVPPTATK.G
1145	722.3894	1442.7643	1442.7599	0.0043	0	49	0.063	1.336	3.105	9.227	K.DPGSLTAQLNQR.A

gi|78060035 Mass: 32743 Score: 91 Queries matched: 16 emPAI: 0.47
Alpha/beta hydrolase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.255	8	1.180

	116/114	0.229	9	NN								
	117/114	0.371	10	1.094								
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide	
1691	407.2653	1625.0319	1625.0193	0.0126	0	57	0.0099	-0.012	0.001	0.295	K.AVLVSAVPPLMLK.T	
1816	557.9516	1670.8330	1670.8243	0.0087	0	34	1.8	0.250	0.270	0.339	K.AFSETDQTEDLK.S	

gi|78067874 Mass: 19078 Score: 94 Queries matched: 34 emPAI: 1.65
hypothetical protein Bcep18194_A6405 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.764	12	1.175							
	116/114	0.379	12	1.266							
	117/114	0.465	12	1.226							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
806	651.8533	1301.6921	1301.6890	0.0031	0	40	0.5	2.170	0.456	0.300	R.NLPFSYYVR.A
1208	731.3928	1460.7710	1460.7705	0.0005	0	54	0.021	2.936	0.831	0.918	R.ASETNQVQATIR.E

gi|53721005 Mass: 60482 Score: 103 Queries matched: 9 emPAI: 0.17
ATP synthase subunit A [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.933	6	1.163							
	116/114	0.849	6	1.146							
	117/114	0.484	6	1.096							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1409	507.9781	1520.9125	1520.9048	0.0077	0	43	0.26	0.513	0.841	0.626	R.ILEVPVGPELVGR.V
1598	801.9521	1601.8896	1601.8859	0.0037	0	60	0.0049	0.824	1.080	0.416	R.NQGTVISVTDGIVR.I

gi|78065543 Mass: 50068 Score: 97 Queries matched: 23 emPAI: 0.38
ABC sugar transporter, periplasmic ligand binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.464	9	2.068							
	116/114	0.606	8	1.424							
	117/114	0.839	9	1.651							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
900	443.6113	1327.8120	1327.8067	0.0053	0	48	0.076	0.563	0.573	0.920	K.AATPGQLALAK.T
2279	632.6823	1895.0249	1895.0073	0.0176	0	49	0.069	0.231	0.463	0.292	K.VPTTWPEFFAVADK.L

gi|2306819 Mass: 28794 Score: 97 Queries matched: 5 emPAI: 0.25
acetoacetyl-CoA reductase [Alcaligenes sp. SH-69]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.743	4	1.159							
	116/114	1.071	3	1.031							
	117/114	0.987	4	NN							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1214	487.9592	1460.8559	1460.8442	0.0117	0	23	27	0.662	0.772	0.716	R.IINISSVNGEK.G
2806	709.3876	2125.1411	2125.1333	0.0078	0	74	0.0002	0.746	1.033	1.670	K.GVTVNTVSPGYIGTDMVK.A

gi|78064927 Mass: 32624 Score: 104 Queries matched: 3 emPAI: 0.10
50S ribosomal protein L2 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.992	3	1.103							
	116/114	0.913	3	1.099							
	117/114	1.035	3	1.130							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2264	630.0090	1887.0053	1887.0006	0.0047	0	52	0.029	1.165	1.084	0.900	K.GLTVGQQLMSGSEAPIR.A
1282	741.9254	1481.8362	1481.8324	0.0038	0	52	0.027	0.309	0.829	1.683	R.ANIAADLGLPLDR.V

gi|53720045 Mass: 9571 Score: 105 Queries matched: 8 emPAI: 1.54
acyl carrier protein [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.365	7	1.210							
	116/114	2.502	7	1.219							
	117/114	1.638	7	1.136							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1418	761.9172	1521.8199	1521.8273	-0.0074	0	65	0.0016	1.925	2.890	1.812	K.ITTVQQAIDYAR.A

2060	586.6818	1757.0235	1757.0178	0.0057	0	40	0.47	0.769	0.982	2.089	K.IVAEQLGVAEAEIK.N

gi|53718340 Mass: 29139 Score: 71 Queries matched: 11 emPAI: 0.38
putative periplasmic cytochrome c protein [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.006	5	2.271
	116/114	2.267	6	1.701
	117/114	3.345	6	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
579	596.7794	1191.5443	1191.5386	0.0057	0	31	3.9	1.090	2.625	3.666	K.TPLACGSEPQ.-
3435	991.5228	2971.5465	2971.5525	-0.0060	0	40	0.5	-0.180	1.347	2.156	K.LSDEDVTAVTAWLAAQPAPANPVPAPAR.S

gi|78065361 Mass: 29231 Score: 87 Queries matched: 10 emPAI: 0.54
hypothetical protein Bcep18194_A3887 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.358	5	1.558
	116/114	4.135	5	1.506
	117/114	3.531	5	1.600

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1448	771.9412	1541.8678	1541.8647	0.0030	0	48	0.069	1.341	6.816	2.342	R.LDQLNQQVATLR.G
2793	705.7278	2114.1617	2114.1575	0.0042	0	39	0.66	5.508	2.303	5.900	R.AADALVAIGTNQLEQGQK.A

gi|118720065 Mass: 48746 Score: 130 Queries matched: 27 emPAI: 0.69
Extracellular ligand-binding receptor [Burkholderia multivorans ATCC 17616]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.109	18	1.304
	116/114	0.102	18	1.270
	117/114	0.097	18	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2608	830.0888	2487.2446	2487.2373	0.0073	0	59	0.0059	0.237	0.145	0.091	K.SVAVVDDSTAYGQGLANEFEK.K
3110	982.1840	2943.5302	2943.5222	0.0081	0	71	0.00043	0.146	0.073	0.144	K.IYSDAGIVQISPSATNPAYTQQGFK.T

gi|134294561 Mass: 32173 Score: 87 Queries matched: 5 emPAI: 0.10
extracellular solute-binding protein, family 3 [Burkholderia vietnamiensis G4]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.322	2	1.041
	116/114	0.344	2	1.612
	117/114	0.189	2	1.386

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
282	616.3441	1230.6736	1230.6690	0.0046	0	20	44	---	---	---	K.AINDALDSLR.K
2436	782.7299	2345.1679	2345.1661	0.0018	0	67	0.001	0.310	0.194	0.247	K.EALDFSEPYTYSAAQLIQR.K

gi|78064858 Mass: 45014 Score: 141 Queries matched: 28 emPAI: 0.64
ABC branched chain amino acid family transporter, periplasmic ligand binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.114	16	1.342
	116/114	0.109	16	1.267
	117/114	0.102	16	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1572	906.4692	1810.9239	1810.9183	0.0056	0	55	0.017	0.112	0.024	0.034	K.ITLQLDPQDDAADPR.Q
2709	861.7584	2582.2534	2582.2447	0.0087	0	86	1.3e-005	0.047	0.082	0.137	K.LADLAGDATDNVVCSEAGASLEK.M

gi|78061080 Mass: 32042 Score: 97 Queries matched: 13 emPAI: 0.48
Lysine-arginine-ornithine ABC transporter, periplasmic ligand binding protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.163	9	1.274
	116/114	0.212	9	1.260
	117/114	0.241	9	1.084

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
791	488.2812	1461.8216	1461.8105	0.0112	0	35	1.6	0.068	0.143	0.199	K.GAGLMPTAESLK.G
2596	827.0951	2478.2635	2478.2625	0.0010	0	62	0.003	0.490	0.653	0.357	K.GVNVVPYQNQDQVYADLISGR.L

gi|30525581 Mass: 63022 Score: 70 Queries matched: 9 emPAI: 0.11
immunogenic protein ChaPs [Piscirickettsia salmonis]

Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.185	4	1.063
116/114	0.189	4	1.256
117/114	0.323	4	1.129

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1221	823.4824	1644.9502	1644.9476	0.0025	0	65	0.0016	0.173	0.161	0.242	K.MLDGVNLLANAVK.V
3090	730.9104	2919.6125	2919.5606	0.0519	0	5	1.4e+003	---	---	---	K.AMLEDIAILTGGTVISEEVGLDLEK.A

gi|53718106 Mass: 33376 Score: 107 Queries matched: 3 emPAI: 0.10
hypothetical protein BPSL0466 [Burkholderia pseudomallei K96243]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.292	3	1.159
116/114	0.278	2	1.704
117/114	0.464	3	1.177

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2143	721.0750	2160.2032	2160.1912	0.0120	0	58	0.0087	0.385	0.457	0.647	K.DAIALESLTSPYANLIAVR.A
883	500.3107	1497.9102	1497.9122	-0.0021	0	49	0.068	0.576	0.605	1.642	K.GIGGGAPLGALLSK.A

gi|78060115 Mass: 29928 Score: 141 Queries matched: 13 emPAI: 0.69
Linocin_M18 bacteriocin protein [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.689	8	1.247
116/114	0.868	9	1.171
117/114	0.496	9	1.171

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2346	763.3937	2287.1592	2287.1453	0.0139	0	76	0.00014	0.764	0.683	0.381	R.ELAPISSSAWEQIEEEVAR.T
2671	852.4423	2554.3050	2554.3037	0.0013	0	65	0.0014	0.205	0.909	0.644	R.LAGVDGPYSVVLGSDAYTALSEAR.D

gi|53720821 Mass: 25547 Score: 131 Queries matched: 3 emPAI: 0.13
50S ribosomal protein L3 [Burkholderia pseudomallei K96243]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	1.046	3	1.308
116/114	1.114	3	1.347
117/114	0.668	3	1.121

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2358	764.7337	2291.1793	2291.1767	0.0026	0	57	0.0092	0.591	0.659	0.530	R.IFTAEGDSIPVTVLDVSDNR.V
948	761.9202	1521.8258	1521.8273	-0.0015	0	74	0.00018	1.682	4.141	2.226	K.ITTVQQAIDYAR.A

gi|84361010 Mass: 49245 Score: 76 Queries matched: 7 emPAI: 0.21
COG1653: ABC-type sugar transport system, periplasmic component [Burkholderia dolosa AUO158]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.381	4	1.920
116/114	0.703	4	1.749
117/114	0.805	4	1.083

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
974	515.6361	1543.8865	1543.8813	0.0052	0	53	0.027	0.397	0.768	0.768	K.VISGDAPSAAQIK.G
1696	632.6799	1895.0180	1895.0073	0.0107	0	23	23	0.511	0.450	0.647	K.VPTTWPEFFAVADK.L

gi|134294995 Mass: 50079 Score: 137 Queries matched: 21 emPAI: 0.47
extracellular solute-binding protein, family 1 [Burkholderia vietnamiensis G4]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.533	18	1.376
116/114	0.762	17	NN
117/114	0.818	18	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
974	515.6361	1543.8865	1543.8813	0.0052	0	53	0.027	0.397	0.768	0.768	K.VISGDAPSAAQIK.G
2787	888.8040	2663.3902	2663.3962	-0.0060	0	84	2e-005	0.987	0.595	0.717	K.ALVDLDQATLTSPQMLSVFDTVR.K

gi|84355507 Mass: 27495 Score: 108 Queries matched: 10 emPAI: 0.41
COG3471: Predicted periplasmic/secreted protein [Burkholderia cenocepacia PC184]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	1.858	7	1.177
116/114	2.685	7	1.111
117/114	1.430	7	1.125

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
603	461.9648	1382.8727	1382.8741	-0.0014	0	27	10	---	---	---	R.LAEVPGIIGVK.E
2971	921.4779	2761.4119	2761.4190	-0.0072	0	81	3.7e-005	1.874	2.870	1.006	K.LAGQLSNLMQVANVEFSLSPEAQR.T

gi|78064909 Mass: 46694 Score: 110 Queries matched: 29 emPAI: 0.61
elongation factor Tu [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.260	14	1.270
	116/114	2.131	14	1.286
	117/114	1.601	14	1.267

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1596	609.3443	1825.0111	1825.0179	-0.0069	0	55	0.016	3.385	3.135	1.906	K.LLDQGQAGDNVGILLR.G
2118	718.7034	2153.0883	2153.0949	-0.0066	0	55	0.015	1.061	0.607	0.890	R.AVDGAFLMPVEDVFSISGR.G

gi|78066870 Mass: 33958 Score: 101 Queries matched: 13 emPAI: 0.20
dihydrodipicolinate synthase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.386	8	1.267
	116/114	2.071	8	1.235
	117/114	1.729	8	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2043	695.3914	2083.1522	2083.1799	-0.0277	0	52	0.032	0.788	1.847	1.656	K.TIAEAVDLPVILYNVPGR.T
2537	815.1064	2442.2973	2442.2950	0.0023	0	49	0.057	1.568	4.626	4.145	R.GSIPAIVTPMLEDGSLDLPAFR.K

gi|134295363 Mass: 40763 Score: 88 Queries matched: 6 emPAI: 0.17
phosphate ABC transporter, periplasmic phosphate-binding protein [Burkholderia vietnamiensis G4]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.436	4	1.152
	116/114	1.458	4	1.191
	117/114	1.739	4	1.156

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1122	533.3018	1596.8836	1596.8867	-0.0031	0	30	4.4	---	---	---	K.WNDPAIAALNPK.V
2168	725.3830	2173.1272	2173.1388	-0.0116	0	58	0.0091	2.225	1.152	1.988	K.AADSLDYISLPPAVEAEIR.K

gi|78067292 Mass: 46283 Score: 107 Queries matched: 3 emPAI: 0.07
bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.612	3	1.057
	116/114	0.641	3	1.112
	117/114	1.786	3	1.066

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
883	500.3107	1497.9102	1497.9122	-0.0021	0	49	0.068	0.576	0.605	1.642	K.GIGGGAPLGALLSK.A
2143	721.0750	2160.2032	2160.1912	0.0120	0	58	0.0087	0.385	0.457	0.647	K.DAIALESLTSPYANLIAVR.A

gi|49524076 Mass: 18897 Score: 74 Queries matched: 6 emPAI: 0.39
thiol peroxidase [Burkholderia multivorans]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.591	5	1.206
	116/114	1.958	5	1.361
	117/114	2.060	5	1.194

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1997	689.0008	2063.9806	2063.9778	0.0027	0	48	0.082	0.950	0.820	1.459	R.FCTTEGLENVVTASTFR.T
2016	693.3692	2077.0858	2077.0670	0.0188	0	26	12	2.071	1.971	2.949	K.VLNIVPSLDTPTCATSTR.K

gi|84355531 Mass: 22554 Score: 120 Queries matched: 9 emPAI: 0.52
COG2885: Outer membrane protein and related peptidoglycan-associated (lipo)proteins [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.331	8	1.224
	116/114	2.349	8	NN
	117/114	2.256	8	1.143

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
481	664.8964	1327.7783	1327.7694	0.0089	0	55	0.016	0.199	2.530	2.299	R.AQSVVNALVQR.G
2240	739.6962	2216.0667	2216.0613	0.0053	0	65	0.0015	0.370	0.749	1.118	R.LSAQGMGPSNPIADNATEAGR.A

gi|78067407 Mass: 56586 Score: 100 Queries matched: 7 emPAI: 0.18
RND efflux system, outer membrane lipoprotein, NodT family [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.090	7	1.194
	116/114	5.534	7	1.250
	117/114	3.523	7	1.074

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
265	608.3632	1214.7119	1214.7104	0.0014	0	47	0.086	2.961	6.580	3.617	R.VSVLNIEAAR.A
2077	704.7352	2111.1839	2111.1871	-0.0032	0	53	0.028	5.348	2.765	3.024	K.ISLTGAFGTGSPTLGGLFK.A

gi|53715901 Mass: 23104 Score: 112 Queries matched: 2 emPAI: 0.15
hypothetical protein BMAA2076 [Burkholderia mallei ATCC 23344]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.276	2	1.089
	116/114	3.109	2	1.209
	117/114	4.279	2	1.064

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1577	604.6642	1810.9707	1810.9668	0.0039	0	60	0.0046	1.174	2.559	4.030	R.TYAASLAQLGSQDAK.F
1495	885.4750	1768.9354	1768.9441	-0.0087	0	52	0.033	0.353	0.445	1.695	R.EADLGLNPATAGDLIR.V

gi|84354134 Mass: 41800 Score: 95 Queries matched: 10 emPAI: 0.16
COG3203: Outer membrane protein (porin) [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	3.567	5	1.207
	116/114	2.375	4	1.188
	117/114	5.570	5	1.328

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
270	407.5632	1219.6678	1219.6652	0.0026	0	36	1.4	2.577	-0.314	11.496	K.GSEDLGGGLK.A
443	658.8999	1315.7853	1315.7581	0.0271	0	59	0.0058	4.705	2.794	7.416	K.SSQEIVALGLR.H

gi|78065626 Mass: 24471 Score: 89 Queries matched: 9 emPAI: 0.47
hypothetical protein Bcep18194_A4154 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.111	9	1.153
	116/114	2.067	9	1.155
	117/114	5.194	9	1.219

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1380	574.9831	1721.9275	1721.9225	0.0049	0	50	0.048	1.303	2.966	8.098	K.LVSAIGNSAEMQAK.Q
1684	629.3618	1885.0636	1885.0764	-0.0128	0	39	0.57	0.664	1.513	8.620	K.QLVPAILSDALSENK.T

gi|84359100 Mass: 85076 Score: 79 Queries matched: 7 emPAI: 0.08
COG1629: Outer membrane receptor proteins, mostly Fe transport [Burkholderia dolosa AUO158]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.051	4	2.458
	116/114	4.998	4	1.257
	117/114	7.088	4	1.631

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
668	473.9160	1418.7262	1418.7236	0.0027	0	45	0.14	1.987	3.765	3.037	R.SIGATVSAAGGNDR.V
1962	676.3523	2026.0351	2026.0462	-0.0112	0	34	2.1	---	---	---	R.TTSNPQDVYSESLLGK.L

gi|82703461 Mass: 64924 Score: 96 Queries matched: 19 emPAI: 0.22
Chaperonin Cpn60/TCP-1 [Nitrosospira multiformis ATCC 25196]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.154	8	1.147
	116/114	0.294	8	1.090
	117/114	0.212	8	1.086

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
605	441.5811	1321.7216	1321.7155	0.0061	0	40	0.52	0.188	0.185	0.155	K.VGAATEVEMK.E
991	756.9684	1511.9223	1511.9167	0.0057	0	56	0.012	0.202	0.360	0.192	R.DLLPVLEQVAK.A

gi|17547713 Mass: 25912 Score: 106 Queries matched: 5 emPAI: 0.27
30S ribosomal protein S4 [Ralstonia solanacearum GMI1000]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.199	5	1.158

	116/114	0.539	5	1.296								
	117/114	0.419	5	1.097								
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide	
1302	563.3125	1686.9157	1686.9022	0.0135	0	54	0.019	0.129	0.526	0.483	K.GNTGENLLQLLESR.L	
783	473.9147	1418.7222	1418.7236	-0.0014	0	52	0.035	6.608	2.206	15.713	R.SIGATVSAAGGNDR.I	

gi|78064765 Mass: 54457 Score: 98 Queries matched: 18 emPAI: 0.26
ATP synthase subunit B [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.998	9	1.414							
	116/114	0.914	9	1.223							
	117/114	0.553	9	1.240							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1092	526.9714	1577.8923	1577.8899	0.0024	0	50	0.05	2.048	1.360	0.900	R.FTLAGTEVSALLGR.M
1891	683.0353	2046.0840	2046.0798	0.0042	0	48	0.085	0.231	0.459	0.244	R.DIIAILGMDELSPEDK.L

gi|78064766 Mass: 16273 Score: 127 Queries matched: 4 emPAI: 0.21
ATP synthase subunit epsilon [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.012	3	1.229							
	116/114	0.700	3	1.154							
	117/114	0.567	3	1.012							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2707	844.8124	2531.4155	2531.4234	-0.0079	0	51	0.036	0.805	0.554	0.567	K.FVALPGETGELGILPGHTPLITR.I
1494	885.4723	1768.9300	1768.9441	-0.0141	0	76	0.00013	1.753	2.357	5.338	R.EADLGLNPATAGDLIR.V

gi|78062997 Mass: 69144 Score: 108 Queries matched: 3 emPAI: 0.05
succinate dehydrogenase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.309	3	1.165							
	116/114	1.271	3	1.112							
	117/114	1.039	3	NN							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1474	581.3365	1740.9878	1740.9743	0.0135	0	62	0.0034	0.963	1.036	0.692	R.VEALELENLIEVAR.A
2069	706.0366	2115.0879	2115.0831	0.0048	0	46	0.13	0.684	1.524	2.162	R.GGLTAGGALSTNDGPNFPAVR.Q

gi|78066654 Mass: 21518 Score: 148 Queries matched: 6 emPAI: 0.34
hypothetical protein Bcep18194_A5185 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.496	4	1.124							
	116/114	1.268	4	1.096							
	117/114	1.592	3	1.011							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
983	753.8993	1505.7840	1505.7807	0.0033	0	78	8.6e-005	0.553	1.269	1.574	R.QAIDSSVDATLSR.M
1024	761.9185	1521.8224	1521.8273	-0.0049	0	70	0.00044	1.251	3.667	1.845	K.ITTVQQAIDYAR.A

gi|78063135 Mass: 21175 Score: 130 Queries matched: 5 emPAI: 0.34
hypothetical protein Bcep18194_B2288 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.021	5	1.193							
	116/114	1.892	5	1.271							
	117/114	2.232	5	1.319							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
980	502.6064	1504.7972	1504.7967	0.0005	0	62	0.0035	0.772	1.484	1.922	R.QSIDASVNATLSR.L 977 978 979 981
2830	865.1285	2592.3638	2592.3625	0.0013	0	68	0.00089	1.555	2.548	2.752	K.TLIDLVSESGISELEVTEGEGK.V 2828 2829 2831 2832 2833

gi|78060562 Mass: 12629 Score: 142 Queries matched: 7 emPAI: 0.61
Histone-like nucleoid-structuring protein H-NS [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.376	4	1.055							
	116/114	1.438	4	NN							
	117/114	3.755	4	1.175							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
956	746.4108	1490.8071	1490.7851	0.0220	0	83	2.7e-005	1.362	1.280	4.045	R.AAEFQAIVDDIR.T 951 952 953 954 955 957

592	658.8913	1315.7680	1315.7581	0.0099	0	59	0.006	3.564	2.426	5.505	K.SSQEIVALGLR.H 589 590 591 593 594

gi|53720809 Mass: 22609 Score: 109 Queries matched: 10 emPAI: 0.15
50S ribosomal protein L5 [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.312	2	1.396
	116/114	2.035	2	1.015
	117/114	4.487	2	1.141

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
222	523.8273	1045.6401	1045.6406	-0.0005	0	37	1.2	---	---	---	R.FVTVALPR.V 223
761	703.9276	1405.8407	1405.8384	0.0023	0	72	0.00035	1.627	2.008	3.958	R.GLNISITTTAK.T 760 762

gi|17545907 Mass: 13526 Score: 92 Queries matched: 7 emPAI: 0.96
PROBABLE THIOREDOXIN 1 (REDOX FACTOR) PROTEIN [Ralstonia solanacearum GMI1000]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.652	5	1.118
	116/114	4.463	5	1.187
	117/114	5.284	5	1.177

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
502	645.4238	1288.8330	1288.8362	-0.0032	0	45	0.17	2.457	3.706	8.118	R.GIPTLILFK.N
700	688.3709	1374.7271	1374.7265	0.0006	0	47	0.11	1.676	4.731	5.592	K.SQLTAFLDSHL.-

gi|69880176 Mass: 85197 Score: 76 Queries matched: 21 emPAI: 0.04
iron (III) protoporphyrin IX monomer binding protein [Burkholderia cenocepacia]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.080	3	1.294
	116/114	0.149	3	1.207
	117/114	0.065	3	1.013

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2057	614.8732	2455.4638	2455.4739	-0.0101	1	21	37	---	---	---	K.KVSLADLIVLAGAAGVEQAAK.N
2733	737.6253	2946.4721	2946.4715	0.0006	0	55	0.016	0.091	0.101	0.066	K.THGAGPASNVGPEPEAAGLEEQGLGWK.S

gi|84356699 Mass: 37181 Score: 84 Queries matched: 16 emPAI: 0.29
COG0834: ABC-type amino acid transport/signal transduction systems, periplasmic component/domain [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.118	6	1.562
	116/114	0.104	6	1.327
	117/114	0.140	6	1.139

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
588	497.6183	1489.8329	1489.8232	0.0098	0	34	1.9	---	---	---	K.VVDDAISQVEK.S
947	862.4700	1722.9254	1722.9218	0.0036	0	50	0.046	0.115	0.106	0.116	K.GLNLNFPLSDSMK.K

gi|83718587 Mass: 45088 Score: 94 Queries matched: 15 emPAI: 0.53
amino acid ABC transporter, periplasmic amino acid-binding protein, putative [Burkholderia thailandensis E264]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.163	9	1.212
	116/114	0.161	9	1.115
	117/114	0.141	9	1.153

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1357	675.3927	2023.1563	2023.1670	-0.0107	0	46	0.12	0.072	0.101	0.133	K.VVAVVGHLNSGTSIPASK.I
2144	830.4265	2488.2575	2488.2213	0.0362	0	48	0.08	0.066	0.110	0.094	K.SVAVVDDSTAYGQGLADEFEK.K

gi|84355249 Mass: 39885 Score: 87 Queries matched: 16 emPAI: 0.49
COG0059: Ketol-acid reductoisomerase [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.148	8	1.207
	116/114	0.170	8	1.208
	117/114	0.157	8	1.163

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1634	547.0552	2184.1918	2184.1895	0.0023	0	49	0.058	0.101	0.135	0.131	R.GTYSQGGGVPHLIAVAQNK.S
2098	822.7657	2465.2754	2465.2789	-0.0035	0	38	0.75	0.155	0.212	0.244	K.GADVVMMLLPDEQIADVYAK.E

gi|53720894 Mass: 45015 Score: 82 Queries matched: 13 emPAI: 0.53
putative secreted substrate binding protein [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.175	7	1.281
	116/114	0.166	7	1.424
	117/114	0.163	7	1.279

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1357	675.3927	2023.1563	2023.1670	-0.0107	0	46	0.12	0.072	0.101	0.133	K.VVAVVGHLNSGTSIPASK.I
2732	736.8887	2943.5256	2943.5222	0.0034	0	36	1.1	0.210	0.150	0.174	K.IYSDAGIVQISPSATNPAYTQQGFK.T

gi|22121836 Mass: 22060 Score: 73 Queries matched: 11 emPAI: 0.33
Cpn60 [Achromobacter piechaudii]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.210	4	1.269
	116/114	0.246	4	1.119
	117/114	0.247	4	1.049

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
644	756.9666	1511.9187	1511.9167	0.0020	0	53	0.023	0.124	0.211	0.220	R.DLLPVLEQVAK.S
1445	695.0898	2082.2475	2082.2656	-0.0181	1	20	48	---	---	---	K.ISTIRDLLPVLEQVAK.S

gi|53721514 Mass: 62704 Score: 106 Queries matched: 12 emPAI: 0.17
60 kDa chaperonin [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.215	6	1.177
	116/114	0.232	6	1.092
	117/114	0.273	6	1.061

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
277	425.5916	1273.7530	1273.7485	0.0045	0	53	0.025	0.201	0.254	0.324	K.AVAAAVDELK.K
644	756.9666	1511.9187	1511.9167	0.0020	0	53	0.023	0.124	0.211	0.220	R.DLLPVLEQVAK.A

gi|78066693 Mass: 25092 Score: 102 Queries matched: 11 emPAI: 0.46
ATP-dependent Clp protease proteolytic subunit [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.262	8	1.213
	116/114	0.446	8	1.176
	117/114	0.319	6	1.044

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
661	760.9674	1519.9203	1519.9217	-0.0015	0	59	0.0058	0.230	0.272	0.353	K.AYGLIDQVLLK.R
1115	622.6730	1864.9971	1864.9877	0.0094	0	43	0.27	0.603	0.806	0.344	R.LNQLLAQHTGQDVER.I

gi|84360616 Mass: 64898 Score: 53 Queries matched: 6 emPAI: 0.05
COG2854: ABC-type transport system involved in resistance to organic solvents, auxiliary component [Burkholderia dolosa AUO158]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.438	3	1.104
	116/114	0.560	3	1.054
	117/114	0.540	3	1.052

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1519	529.2997	2113.1699	2113.1785	-0.0086	0	11	3.9e+002	---	---	---	K.FNDTVDTYALKPVAK.G
2404	678.1194	2708.4484	2708.4530	-0.0045	0	42	0.31	0.362	0.572	0.597	R.TYSGALAQLKPDQQIQYPPFR.A

gi|78065036 Mass: 59300 Score: 91 Queries matched: 3 emPAI: 0.06
protein of unknown function DUF877 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.941	3	1.445
	116/114	0.991	3	1.096
	117/114	0.554	3	1.198

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1414	691.3753	2071.1041	2071.1006	0.0035	0	40	0.46	0.474	0.893	0.417	R.LISTQLSAVMHAPEFQR.L
341	653.8544	1305.6942	1305.6911	0.0031	0	51	0.036	2.274	1.788	1.603	K.GANAFVAGSVNR.E

gi|84355822 Mass: 59522 Score: 65 Queries matched: 6 emPAI: 0.06
COG1622: Heme/copper-type cytochrome/quinol oxidases, subunit 2 [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.408	2	1.129
	116/114	0.991	2	1.011
	117/114	1.350	2	1.261

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
354	441.9217	1322.7432	1322.7437	-0.0005	0	46	0.13	0.375	0.984	1.133	K.AAIAAAADYAK.A
485	479.2577	1434.7512	1434.7558	-0.0045	0	19	63	---	---	---	K.TGSADANAELAK.H

gi|78066914 Mass: 25555 Score: 78 Queries matched: 3 emPAI: 0.13
two component transcriptional regulator, LuxR family [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.179	3	1.102
	116/114	1.481	3	1.122
	117/114	1.408	3	1.069

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2888	768.1680	3068.6428	3068.6646	-0.0219	0	45	0.14	1.147	1.248	1.374	R.LIADNAALPIIFVTGHGDVPMAVSTMK.K
2495	916.1453	2745.4140	2745.4241	-0.0102	0	33	2.3	1.093	3.649	1.662	K.LAGQLSNLMQVANVEFSLSPEAQR.A

gi|53720591 Mass: 17385 Score: 98 Queries matched: 5 emPAI: 0.19
acetyl-CoA carboxylase [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.237	4	1.377
	116/114	3.375	4	1.285
	117/114	2.318	4	1.289

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2245	865.1252	2592.3539	2592.3625	-0.0086	0	46	0.11	2.128	4.146	3.220	K.TLIDLVSESGISELEVTEGEGK.V
410	452.9407	1355.8004	1355.8007	-0.0003	0	52	0.029	0.389	1.038	0.827	K.GAGNSLALLIQR.D

gi|84356766 Mass: 18750 Score: 74 Queries matched: 10 emPAI: 0.39
COG2077: Peroxiredoxin [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.705	6	1.075
	116/114	1.226	6	1.086
	117/114	2.547	6	1.051

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
398	449.2668	1344.7787	1344.7857	-0.0069	0	24	20	---	---	---	R.AVVVLDAQDK.V
2915	616.3409	3076.6683	3076.6923	-0.0239	1	50	0.044	1.551	1.023	2.482	M.SKVTLGGNPIDLAGTFPAVGAQAADFK.L

gi|53716294 Mass: 20650 Score: 77 Queries matched: 23 emPAI: 1.13
bacterioferritin [Burkholderia mallei ATCC 23344]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	4.345	14	1.236
	116/114	5.902	14	1.210
	117/114	2.947	14	1.168

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1056	612.0035	1832.9888	1832.9655	0.0233	0	35	1.7	2.491	3.982	2.275	K.NELTAINQYFLHAR.M
1366	507.7845	2027.1091	2027.1118	-0.0027	0	42	0.33	14.981	17.766	5.706	R.VFMLDGLPNLQDLHK.L

gi|78065378 Mass: 12696 Score: 151 Queries matched: 19 emPAI: 1.61
protein of unknown function DUF883, ElaB [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.304	12	1.121
	116/114	2.707	12	1.139
	117/114	4.459	12	1.084

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
500	722.9361	1443.8576	1443.8541	0.0036	0	82	3.2e-005	1.499	1.909	3.637	K.AADVQVVVVEK.G
606	746.4378	1490.8611	1490.8436	0.0175	0	69	0.00059	2.919	4.018	5.061	K.TVLSDAEDLLK.Q

gi|78066909 Mass: 22095 Score: 146 Queries matched: 109 emPAI: 21.40
Phasin [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	18.374	69	NN
	116/114	5.701	69	NN
	117/114	8.112	69	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
853	829.4875	1656.9605	1656.9654	-0.0048	0	67	0.00096	31.510	9.821	13.539	M.SLLTPEQIAAAQK.A
1776	1150.1378	2298.2611	2298.2674	-0.0063	0	79	7e-005	5.103	2.898	2.235	K.DAQELIALQASLSQPVAEK.V

gi|91785774 Mass: 11619 Score: 130 Queries matched: 26 emPAI: 6.92
DNA-binding protein HU-alpha [Burkholderia xenovorans LB400]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.609	18	1.158
	116/114	3.896	19	1.256
	117/114	8.265	19	1.364

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1039	609.9998	1826.9774	1826.9771	0.0004	0	54	0.019	0.814	1.259	2.397	K.GDAVQLIGFGSFGSGK.R
1188	960.0457	1918.0768	1918.0866	-0.0099	0	76	0.00013	0.908	2.700	3.894	K.AQTGETLDTLLEVIK.K

gi|16127273 Mass: 85441 Score: 82 Queries matched: 2 emPAI: 0.04
catalase/peroxidase [Caulobacter crescentus CB15]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.214	2	1.444
	116/114	0.210	2	1.012
	117/114	0.197	2	1.025

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
842	500.9546	1499.8419	1499.8218	0.0201	0	39	0.57	0.295	0.212	0.192	R.VDLIFGSHAELR.A
693	471.9076	1412.7009	1412.7018	-0.0009	0	43	0.23	2.547	2.808	4.234	R.DLHADVTDDLR.G

gi|78060116 Mass: 39097 Score: 80 Queries matched: 3 emPAI: 0.08
Dyp-type peroxidase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.414	3	1.079
	116/114	0.676	3	1.108
	117/114	0.345	3	1.038

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1017	540.3278	1617.9616	1617.9688	-0.0072	0	35	1.6	0.356	0.758	0.340	R.VAVATPGDILLHIR.A
1185	585.6480	1753.9222	1753.9193	0.0029	0	45	0.15	0.713	1.070	0.968	R.GLSSAASAANAAIDHVR.D

gi|84354934 Mass: 9188 Score: 67 Queries matched: 9 emPAI: 0.90
COG0740: Protease subunit of ATP-dependent Clp proteases [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.190	4	1.136
	116/114	0.291	4	1.184
	117/114	0.357	4	1.042

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
872	760.9695	1519.9245	1519.9217	0.0028	0	49	0.062	0.204	0.337	0.319	K.AYGLIDQVLLK.R
1070	555.6827	1664.0262	1664.0238	0.0024	0	(18)	74	---	---	---	K.AYGLIDQVLLK.R

gi|53718833 Mass: 19300 Score: 61 Queries matched: 5 emPAI: 0.17
acetolactate synthase III small subunit [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.359	3	1.127
	116/114	0.447	3	1.246
	117/114	0.371	3	1.084

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
782	490.2681	1467.7824	1467.7804	0.0020	0	24	20	---	---	---	K.VVDLTDGAHIER.E
1410	631.3590	1891.0552	1891.0649	-0.0097	0	37	0.93	0.390	0.280	0.337	R.HIISVLLENEPGALSR.V

gi|78067733 Mass: 26448 Score: 52 Queries matched: 8 emPAI: 0.27
DSBA oxidoreductase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.133	4	1.409
	116/114	0.206	4	1.291
	117/114	0.457	4	1.150

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2137	783.1349	2346.3830	2346.3888	-0.0058	1	49	0.068	0.283	0.353	0.416	K.LLKDYAIDGVPTVVVQGK.Y
2297	817.4568	2449.3487	2449.3461	0.0027	0	3	2.7e+003	---	---	---	K.TGPAYANSIPGTAQVLDFLVK.Q

gi|78067213 Mass: 21685 Score: 55 Queries matched: 5 emPAI: 0.16
inorganic pyrophosphatase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.248	3	NN

	116/114	0.381	3	1.068							
	117/114	0.527	3	NN							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
905	519.2889	1554.8448	1554.8398	0.0050	0	42	0.28	0.239	0.344	0.489	K.VEGWAGIEAAHK.E
1763	699.0591	2094.1554	2094.1574	-0.0020	1	13	2.6e+002	---	---	---	K.SIDDVPEYLKDQIK.H

gi|78066912 Mass: 107396 Score: 41 Queries matched: 4 emPAI: 0.03
2-oxo-acid dehydrogenase E1 component homodimeric type [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.584	3	1.104							
	116/114	0.785	3	1.192							
	117/114	0.610	3	1.061							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
176	475.8335	949.6524	949.5249	0.1275	1	2	3.5e+003	---	---	---	K.SGALMRR.M
1952	737.3935	2209.1587	2209.1613	-0.0026	0	39	0.6	0.479	0.718	0.679	R.HWVTVAALNALADEGTIER.K

gi|17549025 Mass: 83374 Score: 49 Queries matched: 5 emPAI: 0.04
elongation factor EF-2 [Ralstonia solanacearum GMI1000]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.802	2	1.042							
	116/114	0.999	2	1.076							
	117/114	0.800	2	1.110							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
811	495.2857	1482.8352	1482.8398	-0.0046	0	35	1.6	0.835	1.072	0.885	R.NIGISAHIDAGK.T
1675	510.5344	2038.1085	2038.1091	-0.0006	0	14	2e+002	---	---	---	R.EFGVEATVGKPQVAYR.E

gi|107026783 Mass: 108336 Score: 66 Queries matched: 9 emPAI: 0.03
aconitate hydratase 1 [Burkholderia cenocepacia AU 1054]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.593	3	1.253							
	116/114	0.645	4	1.190							
	117/114	1.085	4	1.196							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1050	415.7559	1658.9945	1658.9932	0.0012	1	16	1.3e+002	---	---	---	K.KAVEAGLTVDPK.I
2048	768.1028	2301.2865	2301.2967	-0.0101	0	50	0.048	-0.121	0.677	0.935	R.ANFLASPPLVVAYAIAGNITR.D

gi|78064933 Mass: 11900 Score: 75 Queries matched: 3 emPAI: 0.29
30S ribosomal protein S17 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.241	3	1.120							
	116/114	1.379	3	1.149							
	117/114	1.188	3	1.158							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
457	404.5803	1210.7190	1210.7156	0.0034	0	49	0.057	1.183	1.171	1.506	K.TVTVLIEHR.V
3309	636.3580	3176.7535	3176.7198	0.0337	2	26	13	0.708	0.636	1.784	R.SKGGAITLEGTMPAQDQIDKAEAAAK.G

gi|78065405 Mass: 14198 Score: 70 Queries matched: 4 emPAI: 0.54
transport-associated protein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	0.573	4	1.294							
	116/114	0.457	4	1.145							
	117/114	1.429	4	1.219							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2845	705.3810	2817.4948	2817.4908	0.0041	1	44	0.2	0.244	0.426	0.806	K.GGAITLEGTMPAQDQIDKAEAAAK.G
3309	636.3580	3176.7535	3176.7198	0.0337	2	26	13	0.708	0.636	1.784	R.SKGGAITLEGTMPAQDQIDKAEAAAK.G

gi|33594012 Mass: 19194 Score: 59 Queries matched: 5 emPAI: 0.18
putative dnaK suppressor protein [Bordetella pertussis Tohama I]

Quantitation:	Ratio	Weighted	N	SD(geo)							
	115/114	1.196	2	1.257							
	116/114	1.309	2	1.021							
	117/114	1.930	2	1.297							
Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
901	518.9468	1553.8187	1553.8171	0.0016	0	45	0.15	1.471	1.281	2.435	R.ATIEEEHALELR.T

1452	638.3647	1912.0722	1912.0863	-0.0141	0	14	2.1e+002	---	---	---	R.LLARPTATLSLEAQER.R

gi|78064992 Mass: 34254 Score: 57 Queries matched: 7 emPAI: 0.20
VacJ-like lipoprotein [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.055	5	1.208
	116/114	1.869	5	1.332
	117/114	2.041	5	1.193

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
629	450.5637	1348.6692	1348.6680	0.0013	0	28	8.1	0.770	2.173	1.421	K.HTADFGITMGR.Y
2432	646.6036	2582.3854	2582.3948	-0.0093	1	29	6.3	0.606	1.294	1.763	R.SNLLGAGDVLDAAALDKYSFVR.N

gi|115360277 Mass: 21030 Score: 73 Queries matched: 7 emPAI: 0.35
hypothetical protein Bamb_5534 [Burkholderia cepacia AMMD]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.209	5	1.139
	116/114	2.228	5	1.173
	117/114	3.303	5	1.370

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1061	554.6407	1660.9004	1660.8978	0.0026	1	38	0.73	1.414	3.044	1.382	K.RQSIDASVNATLSR.L
1760	524.2945	2093.1489	2093.1482	0.0006	1	35	1.4	1.502	2.582	4.472	R.KSEGWSAGADASVAVVK.V

gi|84354646 Mass: 44093 Score: 88 Queries matched: 5 emPAI: 0.16
COG0845: Membrane-fusion protein [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.798	3	1.197
	116/114	2.917	3	1.122
	117/114	4.809	3	1.024

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1172	581.9996	1742.9771	1742.9883	-0.0112	1	44	0.2	0.992	3.405	5.025	R.QAGLQVAVVGKDDR.A
2845	705.3810	2817.4948	2817.4908	0.0041	1	44	0.2	0.244	0.426	0.806	K.GGAITLEGTMPAQDQIDKAEAAAK.G

gi|78066797 Mass: 34685 Score: 91 Queries matched: 4 emPAI: 0.10
elongation factor Ts [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.103	2	1.086
	116/114	1.953	2	1.031
	117/114	5.401	2	1.031

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1787	701.7184	2102.1333	2102.1462	-0.0129	1	43	0.27	2.286	2.016	5.227	K.ALTEADGDLAKAEELLR.V
3006	737.3748	2945.4699	2945.4875	-0.0176	0	48	0.082	0.143	0.116	0.091	K.THGAGPASNVGPEPEAAGIEQQGLGWK.S

gi|2689039 Mass: 57372 Score: 38 Queries matched: 7 emPAI: 0.12
lipoamide dehydrogenase [Vibrio parahaemolyticus]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.146	2	1.497
	116/114	0.178	2	1.018
	117/114	0.162	2	1.319

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1999	510.0887	2545.4069	2545.4083	-0.0014	0	23	24	0.099	0.181	0.127	R.TNVPHIFAIGDIVGQPMLAHK.G
2032	513.2870	2561.3986	2561.4032	-0.0046	0	(15)	1.5e+002	---	---	---	R.TNVPHIFAIGDIVGQPMLAHK.G

gi|53720794 Mass: 16509 Score: 63 Queries matched: 7 emPAI: 0.20
50S ribosomal protein L17 [Burkholderia pseudomallei K96243]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.249	2	1.005
	116/114	0.150	2	1.251
	117/114	0.313	2	1.310

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
722	543.6943	1628.0612	1628.0602	0.0010	1	21	38	---	---	---	R.KVVEPLITLGK.K 721 723 724 725 726
981	601.3564	1801.0475	1800.9647	0.0828	0	42	2.9	---	---	---	R.NMSNSLIEHEVIK.T

gi|78067343 Mass: 22819 Score: 69 Queries matched: 25 emPAI: 0.51
Superoxide dismutase [Burkholderia sp. 383]

Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.486	3	1.581
116/114	0.446	2	1.040
117/114	0.392	3	1.138

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2159	677.8722	2707.4597	2707.4605	-0.0009	1	45	0.16	---	---	---	K.KADGSLDIVSTSNAATPLTTADK.A 2160 2161
2743	878.4676	3509.8413	3509.8398	0.0015	0	24	18	0.178	-0.004	0.299	K.HHQAYVTNLNNLIPGTEFENLSLEEIVK.K 2738 2739 2740 2741 2742

gi|78063463 Mass: 62862 Score: 65 Queries matched: 17 emPAI: 0.23
Chaperonin Cpn60/GroEL [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.411	4	1.154
116/114	0.333	5	NN
117/114	0.581	5	NN

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
524	504.9797	1511.9173	1511.9167	0.0006	0	26	11	0.411	0.280	0.580	R.DLLPVLEQVAK.S 525 526
564	516.3204	1545.9395	1545.9455	-0.0061	1	39	0.64	0.473	0.322	0.464	K.AVAAAVDELKK.I 565 566 567 568

gi|78066856 Mass: 19752 Score: 48 Queries matched: 6 emPAI: 0.37
Peptidyl-prolyl cis-trans isomerase, cyclophilin type [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.966	2	1.184
116/114	1.230	2	1.184
117/114	0.661	2	1.236

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
907	587.6825	1760.0257	1760.0409	-0.0153	1	24	22	1.203	1.531	0.867	K.VVEGQDVVDKIK.G
1830	599.5633	2394.2241	2394.2998	-0.0758	1	24	19	1.404	1.301	1.837	K.AYVDSGVAEGATLVVDGRTVK.V

gi|78063001 Mass: 38372 Score: 61 Queries matched: 3 emPAI: 0.09
malate dehydrogenase [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	2.463	2	1.047
116/114	1.172	2	1.018
117/114	1.048	2	1.091

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
578	517.2841	1548.8305	1548.8382	-0.0076	1	30	4.9	2.567	1.153	0.963	K.RIEGLEIDAFSR.E
700	538.6639	1612.9698	1612.9756	-0.0058	1	31	4.1	2.990	1.307	1.337	K.VKLPDTDIAVVR.R

gi|78065223 Mass: 23697 Score: 64 Queries matched: 5 emPAI: 0.14
2-dehydro-3-deoxyphosphogluconate aldolase [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	0.411	3	1.147
116/114	1.485	3	1.136
117/114	2.010	3	1.073

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
2624	772.6593	3086.6081	3086.6106	-0.0025	0	30	4.3	0.314	1.292	1.926	R.ASQLADDIVVGVGTITKPEHCEQAK.R
767	554.9678	1661.8817	1661.8818	-0.0002	1	34	2	0.975	1.142	1.771	K.RQAIDSSVDATLSR.M

gi|33151319 Mass: 50242 Score: 47 Queries matched: 9 emPAI: 0.07
major outer membrane protein homolog, OmpA2 [Haemophilus ducreyi 35000HP]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	1.149	2	1.058
116/114	1.572	2	1.196
117/114	2.143	2	1.067

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
589	521.2954	1560.8644	1560.8723	-0.0079	1	38	0.84	1.095	1.798	2.257	R.KQLIACLAPDR.R
2257	692.3630	2765.4228	2765.3318	0.0910	0	9	5.6e+002	---	---	---	R.FGQGAQMMMDEPTPVAEPEVVSK.N

gi|78065110 Mass: 41957 Score: 56 Queries matched: 6 emPAI: 0.16
outer membrane protein, (porin) [Burkholderia sp. 383]
Quantitation:

Ratio	Weighted	N	SD(geo)
115/114	2.513	4	NN
116/114	3.556	3	1.085
117/114	2.182	4	1.150

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
770	556.6083	1666.8030	1666.8041	-0.0011	0	53	0.026	2.815	3.304	1.812	K.HLYSMASGVMQGSR.F
2063	652.1107	2604.4138	2604.4165	-0.0027	0	3	2.8e+003	---	---	---	K.YQLTPALILGAAYDYTQGSK.I

gi|84362104 Mass: 26722 Score: 57 Queries matched: 17 emPAI: 0.12
COG1842: Phage shock protein A (IM30), suppresses sigma54-dependent transcription [Burkholderia dolosa AUO158]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	1.066	3	1.208
	116/114	1.055	3	1.251
	117/114	3.017	3	1.132

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
368	468.6077	1402.8014	1402.8024	-0.0010	0	44	0.2	0.994	1.108	3.205	K.DVAASALGGIGGK.N
1156	475.2608	1897.0140	1897.0158	-0.0018	1	13	2.4e+002	---	---	---	K.NLSEDFQKLEDK.V

gi|91782599 Mass: 39964 Score: 52 Queries matched: 4 emPAI: 0.08
Acetohydroxy acid isomeroreductase [Burkholderia xenovorans LB400]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.123	3	1.166
	116/114	0.268	3	NN
	117/114	0.154	3	1.197

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1536	547.3077	2185.2016	2185.1735	0.0281	0	6	1.2e+003	---	---	---	R.GTYSQGGGVPHLIAVAQDK.S
1719	574.5777	2294.2817	2294.2739	0.0078	0	46	0.12	0.102	0.217	0.113	K.QVTIIGYGSQGHAHALNLK.E

gi|78067059 Mass: 39858 Score: 70 Queries matched: 11 emPAI: 0.17
ketol-acid reductoisomerase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.187	5	1.290
	116/114	0.270	5	1.063
	117/114	0.170	5	1.136

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1530	547.0574	2184.2006	2184.1895	0.0111	0	24	21	0.337	0.276	0.215	R.GTYSQGGGVPHLIAVAQNK.S
1719	574.5777	2294.2817	2294.2739	0.0078	0	46	0.12	0.102	0.217	0.113	K.QVTIIGYGSQGHAHALNLK.D

gi|84352918 Mass: 16988 Score: 56 Queries matched: 3 emPAI: 0.20
COG4154: Fucose dissimilation pathway protein FucU [Burkholderia cenocepacia PC184]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.450	2	1.231
	116/114	0.500	2	1.002
	117/114	0.573	2	1.036

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
990	472.0257	1884.0738	1884.0703	0.0035	0	29	5.9	0.538	0.501	0.592	K.NLDPLLHADILHTLR.A
1590	554.0294	2212.0886	2212.0865	0.0021	0	27	9.9	0.592	1.312	2.340	K.EKPQATGHDEASWAQNR.R

gi|78065675 Mass: 52624 Score: 62 Queries matched: 3 emPAI: 0.06
fumarate hydratase [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	2.035	3	1.214
	116/114	1.605	3	1.234
	117/114	1.751	3	1.109

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
1156	495.2872	1977.1196	1977.1251	-0.0055	1	25	17	1.577	1.017	1.936	R.AIIDAADEIIAGKHPR.E
1363	524.0411	2092.1352	2092.1309	0.0043	0	37	0.99	0.269	0.658	0.451	K.AALDAYQVHPVHQEVK.K

gi|78067415 Mass: 16158 Score: 58 Queries matched: 11 emPAI: 0.46
hypothetical protein Bcep18194_A5946 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	5.690	2	2.911
	116/114	3.523	2	6.691
	117/114	2.233	2	3.367

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
852	595.6166	1783.8279	1783.8247	0.0032	0	32	3	1.299	0.244	0.415	R.YTVHASYQGNEESR.A
1073	481.0189	1920.0465	1920.0461	0.0003	1	26	13	6.119	3.844	2.411	R.FTGKGGEFLADVHVR.I

gi|78064925 Mass: 25490 Score: 47 Queries matched: 5 emPAI: 0.28
50S ribosomal protein L4 [Burkholderia sp. 383]

Quantitation:	Ratio	Weighted	N	SD(geo)
	115/114	0.559	2	1.867
	116/114	0.469	2	1.859
	117/114	0.445	2	2.039

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	115/114	116/114	117/114	Peptide
385	463.6107	1387.8103	1387.8058	0.0046	0	20	48	0.479	0.403	0.369	R.NLPHVAIVEPR.Y
820	572.0159	1713.0258	1713.0168	0.0090	0	27	11	1.348	1.126	1.202	R.LSVVEDIILEAPK.T

Appendix V:

Two-dimensional electrophoresis protocols.

2-D Electrophoresis Stock Solutions

Washing Solution
(stored at room temperature on Glover 122 benchtop)

Component	Final Concentration	For 1.0 L solution
KCl	3.0 mM	0.2237 g
KH_2PO_4	1.5 mM	0.2041 g
NaCl	68 mM	3.9739 g
NaH_2PO_4	9.0 mM	1.0798 g
Nanopure H_2O		To final volume of 1.0 L

(Filter using large vacuum filter apparatus and 0.22 um Millipore filters stored under Glover 122 benchtop)

Lysis Solution (Gram -)
(stored in 10 mL aliquots at -20°C in Glover 106 freezer)

Component	Final Concentration	For 0.2 L solution
Tris-HCl (pH 8.0)	10 mM	40 mL of 50 mM Tris-HCl (pH 8.0) stock solution
$MgCl_2$ (or $MgCl_2 \cdot H_2O$)	1.5 mM	0.0286 g (or 0.0610 g)
KCl	10 mM	0.1492 g
Sodium dodecyl sulfate (SDS)	0.1% (w/v)	0.2 g
Dithiothreitol (DTT)	0.5 mM	0.0154 g
Pefabloc SC	0.5 mM	0.0240 g
Nanopure H_2O		To final volume of 200 mL

(50 mM Tris-HCl (pH 8.0) stock, DTT, and Pefabloc stored at 4°C in Glover 106 refrigerator; filter using large vacuum filter apparatus and 0.22 um Millipore filters stored under Glover 122 benchtop)

Rehydration Stock
(stored in 0.5 mL aliquots at -20°C in Glover 106 freezer)

Component	Final Concentration	For 60 mL solution
Urea	8.0 M	28.83 g
CHAPS	2.0% (w/v)	1.2 g
Dithiothreitol (DTT)	0.3% (w/v)	0.18 g
Bromophenol blue	Trace	few grains
Nanopure H_2O		To final volume of 60 mL

(DTT stored at 4°C in Glover 106 refrigerator)

SDS Electrophoresis Buffer
(stored in 4 L flask on Glover 106 benchtop)

Component	Final Concentration	For 4.0 L solution
Tris base	25 mM	12.11 g
Glycine	192 mM	57.68 g
SDS	0.1% (w/v)	4.0 g
Nanopure H_2O		To final volume of 4.0 L

For Lower Tank Buffer, add 1.6 g Sodium Azide per 8 L buffer.

Equilibration Stock
(stored in 12.5 mL aliquots at -20°C in Glover 106 freezer)

Component	Final Concentration	For 0.5 L solution
Urea	6.0 M	180.18 g
Glycerol	30.0% (v/v)	300 mL of 50% (v/v) glycerol stock
SDS	2.0% (w/v)	10.0 g
Tris-HCl, pH 6.8	24 mM	24 mL of 0.5 M Tris-HCl, pH 6.8
Nanopure H_2O	~40 mL	To final volume of 500 mL

Reducing Solution: add 0.24 g DTT in 12.5 mL. Acetylation Solution: add 0.30 g IAA + few grains of bromophenol blue, in 12.5 mL.

Electrode solutions
(stored at room temperature above Glover 106 benchtop)

Component	Final Concentration	For 0.1 L solution
$NaOH / H_3PO_4$	1 mM / 6 mM	4 mg / 40 uL of 85% (v/v) H_3PO_4
Nanopure H_2O		To final volume of 100 mL

(Filter using small vacuum filter apparatus and 0.22 um Millipore filters stored in drawer of Glover 106 benchtop)

Tris-HCl Stocks (1.5 M (pH 8.8) and 50 mM(pH 8.0))
(stored at 4 °C in Glover 106 refrigerator)

Component	Final Concentration	For 0.5 L solution
Tris base	1.5 M (50 mM)	90.855 g (3.0285 g)
Nanopure H_2O		400 mL
HCl		adjust to pH 8.8 (adjust to pH 8.0)
Nanopure H_2O		To final volume of 500 mL

(Filter using small vacuum filter apparatus and 0.22 um Millipore filters stored in drawer of Glover 106 benchtop)

Protein Extraction for E. coli

1. Upon reaching the appropriate growth point, harvest the cell culture by centrifuging in a reusable 500 mL centrifuge bottle in the large rotor at 13000xg and 4°C (solid state and centrifuge chamber) for 15 minutes.
(Reusable 500 mL centrifuge bottles and 35 mL centrifuge tubes are stored on the bottom shelf of the supply cabinet beneath the Glover 122 benchtop)

2. Decant supernatant (pour the supernatant off semi-quickly with cell pellet on the upper side of the bottle to minimize disturbance to the cell pellet and cell mass loss), resuspend pellet in ~25-30 mL of Washing Solution by vortexing, and transfer to a smaller (35 mL) reusable centrifuge tube, rinsing any missed cell mass into the smaller tube with additional Washing Solution:

(Washing Solution is stored at room temperature on Glover 122 benchtop)

Component	Final Concentration	For 1.0 L solution
KCl	3.0 mM	0.2237 g
KH_2PO_4	1.5 mM	0.2041 g
NaCl	68 mM	3.9739 g
NaH_2PO_4	9.0 mM	1.0798 g
Nanopure H_2O		To final volume of 1.0 L

(Filter using 0.22 um Millipore filters and vacuum filter apparatus located in cupboard of Glover 122.)

3. Centrifuge suspended cells in small rotor at 30000xg and 4 °C for 10 minutes.

4. Decant supernatant, add fresh Washing Solution to the centrifuge tube, and resuspend the pellet by vortexing. Centrifuge once more in the small rotor at 30000xg and 4 °C for 10 minutes.

5. Decant the supernatant; resuspend this final pellet in 5 mL of Lysis Solution (Gram -) by vortexing.

(Lysis Solution Gram - is stored in 10 mL aliquots at –20°C in Glover 106 freezer; DTT and Pefabloc stored at 4°C in Glover 106 refrigerator)

Component	Final Concentration	For 200 mL solution
Tris-HCl (pH 8.0)	10 mM	40 mL of 50 mM Tris-HCl (pH 8.0) stock solution
$MgCl_2$ (or $MgCl_2 \cdot 6H_2O$)	1.5 mM	0.0286 (or 0.0610) g
KCl	10 mM	0.1492 g
Sodium dodecyl sulfate (SDS)	0.1% (w/v)	0.2 g
Dithiothreitol (DTT)	0.5 mM	0.0154 g
Pefabloc SC	0.5 mM	0.0240 g
Nanopure H_2O		To final volume of 200 mL

(Filter using 0.22 um Millipore filters and vacuum filter apparatus located in bottom drawer of Glover 106 benchtop.)

6. Sonicate cell suspension at maximum level (level 5 for micro tip) on ice for 5 minutes (1 sec on: 2 sec off).

7. Centrifuge the lysate in the small rotor at 30000xg and 4 °C for 20 minutes to remove cell debris. Decant the supernatant into aliquots without disturbing the pellet, and store the aliquots at -80 °C until use.

Protein Extraction for Mixed culture

1. Follow steps 1-7 from the protein extraction from the *E. coli* protocol.

2. Resuspend the final pellet in 3 mL of Lysis Solution (Gram +) by vortexing.

(Lysis Solution Gram + is stored in 10 mL aliquots at –20°C in Glover 106 freezer; DTT stored at 4°C in Glover 106 refrigerator)

Component	Final Concentration	For 200 mL solution
Tris-HCl (pH 8.0)	10 mM	40 mL of 50 mM Tris-HCl (pH 8.0) stock solution
Lysozyme	0.1% (g/v)	0.2 g
EDTA sodium salt	1 mM	0.074 g
DTT	10 mM	0.308 g
Pefabloc	0.5 mM	4 g
Glycerol	10% (v/v)	20 mL
Nanopure H_2O		To final volume of 200 mL

(Filter using 0.22 um Millipore filters and vacuum filter apparatus located in bottom drawer of Glover 106 benchtop.)

3. Sonicate cell suspension at maximum level (level 5 for micro tip) on ice for 5 minutes (1 sec on: 2 sec off).

4. Centrifuge the lysate in the small rotor at 9000 rpm for 20 minutes to remove cell debris. Add the supernatant to the protein solution extracted previously.

5. Proceed to protein concentration determination. Otherwise, save the protein solution in 1 mL aliquots in the -80 oC freezer.

Protein Concentration Determination - Biorad Protein Assay (based upon Bradford method)

1. Turn on visible lamp on spectrophotometer (click on "VIS OFF" at bottom of screen, which should turn red and change to "VIS ON") to allow 30 min warming up, and click on "PROTEIN" under the "Applications" menu. If not already open, open method "A:/BSACARLA" (click on the path listed to the right of "Method name:" and then click on "A:/BSACARLA" from the list of methods that you will be presented with).

2. Prepare dye reagent by diluting Dye Reagent Concentrate 1:5 with Nanopure water.
(Both diluted reagent and concentrate are stored at 4 °C on 2-D PAGE reagent shelf in Glover 106 refrigerator)

3. Pipette 5.0 mL of diluted dye reagent into each clean glass test tube to be used (need six tubes for standard curve and two tubes (duplicates) for each sample to be tested).
(Test tubes are stored in one of the proteomics cupboards on Glover 106)

4. Prepare six dilutions of Bovine Serum Albumin (BSA) standard for standard curve by mixing the following amounts of standard with the dye reagent in standard test tubes, making sure to rinse all of the standard into the dye reagent by aspirating and dispensing the dye reagent through the pipette tip five times:
(Standard is stored at 4 °C on 2-D PAGE reagent shelf in Glover 106 refrigerator)

Volume of Standard added (uL)	0	12.5	25.0	37.5	50.0	62.5
Final Mass of Standard present (ug)	**0**	**25.0**	**50.0**	**75.0**	**100.0**	**125.0**

Based on BSA standard concentration of 2 ug/uL

5. Prepare sample for testing by micro centrifuging a 1 mL aliquot of lysate from protein extraction at 13000 rpm for 15 minutes, then transferring the supernatant to a new microcentrifuge tube. This must be done prior to the assay to remove cell debris or aggregates will form in the dye reagent – 1mL samples separated after sonication in the previous protocol.

6. Prepare sample dilutions by mixing specific volumes of sample supernatant from step 4 with the dye reagent in sample test tubes, again making sure to rinse all of the sample into the dye reagent by aspirating and dispensing the dye reagent through the pipette tip five or six times. The volumes added are dependent upon the concentration of the protein in the sample, since the mass of protein added to the dye reagent must fall within the range covered by the standards, thus this requires some trial and error. To avoid "overshooting" the standard range, start by adding 10 uL of sample to the dye, shaking the test tube to enable mixing throughout the dye reagent, and comparing the shade of the blue color formed to those observed in the six standard tubes. Add more in this way until the shade of the sample tube falls within the range observed in the standard tubes. Record the total volume of sample added, and prepare a duplicate by adding this same volume of sample to a new test tube of dye reagent.

7. Vortex each tube (standards and samples) for 15 seconds each, and incubate for 15-30 minutes at room temperature. Absorbance will increase over time.

8. Transfer 0.5 to 1.0 mL of the contents in tube to a clean plastic cuvette and read the absorbance at 595 nm.
(Cuvettes are stored in cupboard on Glover 122)

a. Load the standard cuvettes into the spectrophotometer with the "0 ug BSA" cuvette in the farthest cell (this is the "blank") and the other standard cuvettes in mass-ascending order towards the closest cell. Replace the cell cover and close the chamber lid.
b. Click on "Auto sampler" and ensure that the number of samples is set to 6. If it is not, click on the number listed and reset using the window that opens.
c. Click on "BLANK" in the bottom left hand corner and allow the spectrophotometer to read the blank, repeat until the blank reading (in the bottom center of the screen, to the left of "Abs") settles to zero (0.0000).
d. Once the blank has settled to zero, click on "Read 1" to the left of the top standard listed. The auto sampler will automatically read all of the standards and record them beside the appropriate theoretical mass listed.
e. Once the standards have been read, click on "Dispstndcurve" at the top of the screen. A window will open displaying the mass versus optical density curve for these standards. Ensure that the r^2 value for the linear fit is >0.99, then click "Exit" in the top right hand corner of this sub window to close it.

f. Now that the standard curve is constructed, remove all of the standard cuvettes EXCEPT for the blank (the farthest cuvette) and replace them with the sample cuvettes (you will be able to measure up to five at a time). Replace the cell cover and close the chamber lid.

g. Click on "Samples" in the top left hand corner of the screen, a new screen will open.

h. Click on "Auto sampler" on the right side of the screen, and set the "number of cells" to the number of sample cuvettes that you will be reading (including the blank).

i. Again, click on "BLANK" and allow settle to zero, repeating if necessary.

j. Click on "Read Samples" in the top left hand corner of the screen. The auto sampler will automatically read all of the samples (including the blank) and record the absorbance readings and the calculated "CONC" (mass) of protein present (from the standard curve) of each sample, including the blank.

k. Repeat steps (i) and (j) twice, for a total of three readings of each sample. If the readings are not stable (i.e., the absorbance read for a particular sample varies by more than 0.0050 between the readings), then click on "Save Clear" at the top of the screen and click "OK" on the pop-up screen (without choosing the box to save the results). This will clear all previous readings from the screen and allow you to retake the three readings on a clear screen, making it easier to print only the results that you want.

l. Once stable readings are obtained, click on "Print" at the top of the screen to print out your results, and label the readings on the printout with the appropriate sample IDs and sample volumes added to the dye reagent in step (6).

m. Remove these sample cuvettes and replace them with the next set of sample cuvettes to be read, if any. Repeat steps (h) – (m) as needed.

9. From the three readings taken for each sample cuvette, calculate the average mass (listed under "Conc" in units of "UG") in each sample duplicate. Determine the concentration in each sample duplicate by dividing the average mass by the volume of sample added to the dye reagent in step (6). Determine the average concentration of each sample by averaging the concentrations calculated for each duplicate of that sample.

10. Dispose of the used dye reagent as hazardous waste (0.01% Coomassie brilliant blue G250 in 50% phosphoric acid and 25% methanol). Clean out used cuvettes and test tubes with 70% ethanol followed by soap and water, and rinse well with Nanopure water. Dry test tubes upside down on a test tube rack.

Immobilized pH Gradient (IPG) Strip Rehydration

1. Prepare Rehydration Stock (if needed):

(Rehydration Solution is stored in 1.5 mL aliquots at –20°C in Glover 106 freezer)

Component	Final Concentration	For 60 mL solution
Urea	8.0 M	28.83 g
CHAPS	2.0% (w/v)	1.2 g
Dithiothreitol (DTT)	0.3% (w/v)	0.18 g
Bromophenol blue	Trace	Few grains
Nanopure H_2O		To final volume of 60 mL

(DTT stored at 4 °C in Glover 106 refrigerator)

2. Remove the necessary number of Rehydration Stock aliquots from the freezer and thaw in lukewarm water. Calculate the volume of sample required from the appropriate protein mass to be loaded:

Stain used	Protein mass loaded	Volume of sample needed
Coomassie	600 ug	600 ug / [conc(ug/uL)]
Silver/Sypro	200 ug	200 ug / [conc(ug/uL)]

3. Determine the volume of Rehydration Stock required for each sample from the appropriate total sample volume and the volume of sample to be added:

IPG strip length	Final volume of sample solution	Volume of Rehydration Stock needed
13 cm	250 uL	250 uL – (volume of sample)
18 cm	400 uL	400 uL – (volume of sample)

**If the amount of sample needed is greater than about 25% of the final sample solution (with Rehydration Stock), the sample must be concentrated in a centrifugal concentrator until the this volume limit is met. **

4. Pipette the appropriate amount of Rehydration Stock (calculated in step #3) into a clean, labeled microcentrifuge tube for each sample. Prepare the sample mixture by adding the appropriate amount of sample (calculated in step #2) to the Rehydration Stock in each test tube, making sure to rinse all of the sample into the Rehydration Stock by aspirating and dispensing the mixture through the pipette tip five or six times. Don't refreeze leftovers of sample or Rehydration Stock.

5. Determine how much IPG Buffer (ampholyte) is to be added to the sample mixture (use the IPG Buffer that corresponds to the same pH gradient and range as the IPG strips to be used):

IPG strip pH range	Concentration of IPG Buffer	Volume of IPG Buffer to be added
4-7, 3-10L or NL	2.0% (v/v)	2 uL / 100 uL
6-11	0.5% (v/v)	0.5 uL / 100 uL

(IPG Buffer is stored at 4 °C in small Nalgene box in Glover 106 refrigerator)

6. Prepare the final sample mixture by adding the appropriate amount of IPG Buffer to the sample mixture prepared in Step #5, making sure to rinse all of the IPG Buffer from the pipette tip by aspirating a couple of times. Vortex each sample mixture for 20 seconds on the highest setting to ensure complete mixing.

7. For the following steps, clean forceps with 70% ethanol between samples to prevent cross-contamination. IF DOING MORE THAN ONE IPG, THEN DO THESE STEPS FOR ONE SAMPLE AT A TIME.

8. Transfer all of the sample mixture to the far end (closest to the leveling bubble) of a trough on the Rehydration Tray.

9. Quickly:

a. USE GLOVES AND FORCEPS to remove one IPG strip from the package and remove the thinner plastic backing.

(IPG strips are stored at –20°C in the Glover 106 refrigerator/freezer)

b. Holding the blunt end of the thick, permanent backing with forceps, place the pointed (+) end of the strip GEL SIDE DOWN into the sample, ensuring that any bubbles present in the sample liquid are on top of the IPG strip. Lower the blunt end of the strip, occasionally raising and lowering as needed, such that the mixture evenly coats the strip.

10. Top the IPG strips with mineral oil in order to prevent evaporation. Add the mineral oil slowly from both sides so that the sample liquid is not driven to either end by the movement of the oil. Level the Rehydration Tray using the built-in bubble level and the adjustable feet on the front of the tray. Let sit, undisturbed, for 12-24 hours, preferably overnight, at room temperature.

Isoelectric Focusing (IEF)

1. Turn on pump and refrigerator (set point = 20°C) in order to cool the Multiphor cooling block. Make sure that the clamps on the tubing *[connecting the pump to the Pharmacia Multiphor (IEF apparatus)]* are arranged such that the water will flow.

2. Apply ~15 mL of (used) mineral oil evenly over the surface of the Multiphor cooling block and replace the Immobiline Strip Tray. Apply mineral oil on the Strip Tray, and place the alignment card. Make sure that the mineral oil is evenly distributed between these surfaces to ascertain that sufficient heat transfer is achieved. Connect the red and black electrode plugs on the Strip Tray to their corresponding jacks on the Multiphor unit.

3. Remove a rehydrated IPG strip from the rehydration tray and remove excess mineral oil by gently wiping across a KimWipe (gel side AWAY from the KimWipe!!).

4. Carefully, place the IPG (GEL SIDE UP) into a trough of the alignment card with the positive ends (marked + or pointed) of all IPGs towards the anode (red, + electrode) of the Multiphor. IF RUNNING MORE THAN ONE IPG, MAKE SURE THAT THE GEL EDGES OF ALL STRIPS ARE ALIGNED AND THAT THE GEL SIDES ARE FACING UP!

5. Cut two paper wicks to a length sufficient to fit across the ends of all IPGs. Soak each strip in the appropriate pH solution (the anodal strip (red, + end) should be soaked 6 mM H_3PO_4, and the cathodal strip (black, - end) should be soaked in 0.5 mM NaOH). Remove excess water by firmly blotting (DO NOT RUB!) the strips with a KimWipe.
(paper wicks are stored in a zip-lock bag in the scissors drawer in Glover 106)

6. Using forceps, place paper strips across the appropriate ends of the IPGs such that they overlap the end ¼" of gel on the IPGs (which is why all strips must be aligned in step #2).

7. Attach electrodes by aligning the electrodes over the appropriate paper strip (i.e., the red (+) anode should be aligned over the paper strip covering the pointed gel edge). This should be done such that the colored plastic piece of each electrode is on the side of the Strip Tray that has the metal strip on the outside. Press the electrodes gently, but firmly, into the paper wicks and gels so that good electrical contact is made.

8. Cover the gels and the entire inner surface of the Strip Tray with fresh mineral oil, taking care not to pour mineral oil directly on the gels as this may disturb their alignment.
(fresh mineral oil is stored on the shelf of 2-D PAGE supplies above the Glover 106 benchtop)

9. Replace the cover, taking care to slip the hooks in the back under the lip of the unit base and to line up the electrode plugs with the jacks on the underside of the cover. Level the apparatus using the bubble level and the four adjustable feet of the unit. Connect to power supply by plugging the red and black plugs into the corresponding receptors on the power supply (EPS 3500 XL)
(bubble level is stored in drawer in Glover 106)

10. Choose program 1 on the power supply. Set for GRADIENT, maximum 30 mA and 20 W).
18 cm IPG (pH 3-10/4-7): **linearly increase** voltage from 0 V to 500 V over 1.0 min;
linearly increase voltage from 500 V to 3500 V over 5.0 h;
hold voltage at 3500 V for 17.5/19.0 h. (total 71250/76500 Vh)

11. With program number blinking, hit RUN. Check if there is current (initially 0.1 mA), and after about 5 min, check the migration of the blue dye.

12. When the program has finished, hit EXIT/STOP then unplug the Multiphor from the power source. Remove the cover from the Multiphor, move the electrodes, and discard the paper strips.

13. If necessary, let the gels run for some more hours (up to 4 h), using program 9 on the power supply. Set for STEP, maximum 30 mA and 20 W).
18 cm IPG (pH 3-10/4-7): **hold** voltage at 3500 V for 4 h

14. Use ethanol-cleaned tweezers to pick up each IPG and remove excess mineral oil from each by drawing across a clean KimWipe, plastic backing side down, and holding upside down over the KimWipe to allow mineral oil to drip off. Place each strip into an individual trough of the clean rehydration tray and proceed immediately to SDS equilibration and SDS PAGE (preferred). If 2nd dimension can not be completed immediately after IEF, use an IPG packaging to wrap the gels and store at -80 ^{o}C until processed (do NOT carry out SDS Equilibration prior to storage at -80 ^{o}C!).

15. Every two weeks, remove the Strip Tray from the Multiphor unit, discard excess mineral oil on Strip Tray surface, and remove the alignment card from the Strip Tray. Wash both the alignment card and the Strip Tray with soap and water, and rinse both the Strip Tray and alignment card with Nanopure water. Wipe up any excess mineral oil from the cooling block and replace the cover on the Multiphor.

Polyacrylamide Gel Preparation (10 gels)

1. Wipe down multi gel-casting chamber with 70% (v/v) ethanol.

2. Clean inner side of larger glass plate and two spacers with 70% ethanol, place the glass plate (clean side out) against back wall of chamber (or against separation sheet of previous gel sandwich if repeating this step for additional slab gels), and position a clean spacer on each side of the clean glass plate.

3. Clean both sides of alignment card with 70% ethanol and place between spacers, bottom flush with plate. Clean inner side of smaller glass plate with 70% ethanol and place against spacers and alignment card. Place a separation sheet against smaller glass plate.

4. Repeat previous steps for each additional slab gel to be made.

5. Once all gel sandwiches are made, fill any additional space in the chamber with available acrylic blocks. Use separation sheets to fill any remaining space to the edge of the chamber. Make sure that all space to the edge of the chamber is filled so that pressure will be exerted on the gel sandwiches by the front wall when attached, ensuring that the gel sandwiches will not shift during casting and that gel will only be introduced to the space between the spacers of each sandwich.

6. Attach the front wall of the chamber such that the port is at the bottom. Tighten the screws in a random order until all are completely tightened (hand-tight). Clamp tubing on inlet port, and ensure that chamber is on a flat, level surface.

7. Check that gel sandwiches are tightly packed within the chamber by testing that the spacers are tightly in place. If so, remove all alignment cards from the gel sandwiches.

8. Prepare gel solution in a 500 mL vacuum flask, with stir bar stirring as each component is added.

Solution for 12% acrylamide gels (V=500 mL)	
H_2O	165 mL
30% acrylamide mix	200 mL
1.5 M Tris (pH 8.8)	125 mL
10% SDS	5 mL
10% (w/v) ammonium persulfate	5 mL
TEMED	0.2 mL

(Acrylamide and Tris stored at 4 °C in Glover 106 refrigerator; ammonium persulfate, SDS and TEMED stored at room temperature in Glover 106 proteomics shelves.)

9. Add all components except ammonium persulfate and TEMED (initiator), and then vacuum for 5-10 minutes to remove all gas from solution. IMMEDIATELY after adding TEMED, transfer gel solution to space between front sandwich using a funnel (gel solution will enter other gel sandwiches from the bottom).

10. Fill chamber with gel solution to within 1 cm of top of shorter plate (top edge of chamber), then QUICKLY top off each gel with 1 mL water-saturated butanol (50% isobutanol or n-butanol) to allow an even surface to form. Only the butanol (upper) phase is pipetted down the larger plate of each block so that no convection is introduced (could cause deformations as the gels polymerize).
(NOTE: Nanopure water may be used instead if applied VERY slowly, but water-saturated 1-butanol is preferred since it will not mix with the gel solution as easily)

11. Allow polymerization to occur for at least one hour, confirming polymerization by the state of any excess gel solution in the vacuum flask. If not used immediately in second dimension, cover top edge of gel with Nanopure water, wrap sandwiches and store at 4°C for up to one week.

Polyacrylamide Gel Preparation (1 gel)

12. Wipe down gel-casting stand and base sealers with 70% (v/v) ethanol. Level the gel-casting stand using the bubble level.

13. Clean inner side of larger glass plate and two spacers with 70% ethanol, place the glass plate (clean side up) on KimWipes, and position a clean spacer on either side. Clean both sides of alignment card with 70% ethanol and place between spacers, bottom flush with plate. Clean inner side of smaller glass plate with 70% ethanol and place on top of spacers and alignment card.

14. Place plate sandwich within clamping blocks and tighten **slowly**!

a. Use holding area on gel-casting stand. Larger plate is closest to tightening knobs. Make sure spacers and plates are flush at the bottom. Remove alignment card when tightened.

b. Measure 18 cm from the bottom of the small plate, and mark this level on the small plate with a fine point permanent marker (this will be the final height of your slab gel).

c. A small, labeled piece of filter paper can be put in the bottom left corner of each plate block to serve as a label for each gel and to help orient all gels.

15. With casting stands situated such that the holding area is away from you, clamp one plate block into each stand in the back base sealer position (the front base sealer position should be open), with small plates facing towards front (away from holder on stand).

16. Prepare gel solution in a vacuum flask that is ~2X the total volume of gel solution to be made, mixing with a stir bar as each component is added. Make ~40 mL of gel solution (PER GEL) so that excess is available to monitor extent of polymerization.

Solution for 12% acrylamide gels (V=100 mL)	
H_2O	33 mL
30% acrylamide mix	40 mL
1.5 M Tris (pH 8.8)	25 mL
10% SDS	1 mL
10% (w/v) ammonium persulfate	1 mL
TEMED	0.04 mL

(Acrylamide and Tris stored at 4 °C in Glover 106 refrigerator; ammonium persulfate, SDS and TEMED stored at room temperature in Glover 106 proteomics shelves.)

17. Add all components except ammonium persulfate and TEMED (initiator), then vacuum for 5-10 minutes to remove all gas from solution. IMMEDIATELY after adding TEMED, pour gel solution between the glass to the 18 cm line marked on the smaller plate of each plate block on each casting stand. Then, quickly clamp the remaining plate blocks into the front base sealer position on each casting stand and pour gel solution between these glass plates to the 18 cm line. Tap the casting stands as needed to dislodge any bubbles that may have become attached to the filter paper labels at the bottom of each gel.

18. QUICKLY top off each gel with 1 mL water-saturated butanol (50% isobutanol or n-butanol) to allow an even surface to form. Only the butanol (upper) phase is pipetted down the larger plate of each block so that no convection is introduced (could cause deformations as the gels polymerize).

(NOTE: Nanopure water may be used instead if applied VERY slowly, but water-saturated 1-butanol is preferred since it will not mix with the gel solution as easily)

19. Allow polymerization to occur for at least one hour, confirming polymerization by the state of any excess gel solution in the vacuum flask. Monitor the butanol level to prevent the top surfaces of the gels from becoming exposed to the air and drying out. If gels are to be kept overnight before use, top each with Nanopure water, wrap and store upright at 4 °C for up to one week.

SDS PAGE

11. Prepare SDS Electrophoresis Buffer (if needed):

(SDS Electrophoresis Buffer is stored in 4 L flask on Glover 106 benchtop)

Component	Final Concentration	For 4.0 L solution
Tris base	25 mM	12.08 g
Glycine	192 mM	57.68 g
SDS	0.1% (w/v)	4.0 g
Nanopure H_2O		To final volume of 4.0 L

(For lower tank buffer, check the levels indicated and add 1.6 g sodium azide per 8 L (0.02%) to control microbial growth; wash the lower tank with soap and rinse well with Nanopure water every 6 months)

12. Make sure that the clamps on the tubing *[connecting the pump to the BioRad MultiCell (PAGE apparatus)]* are arranged such that the water will flow through the cooling cores. Turn on pump and refrigerator (set point = 8 °C) in order to cool MultiCell cooling cores.

13. Prepare Equilibration Stock (if needed):

(Equilibration Stock is stored in 12.5 mL aliquots at –20°C in Glover 106 freezer)

Component	Final Concentration	For 0.5 L solution
Urea	6.0 M	180.18 g
Glycerol	30.0% (v/v)	300 mL of 50% (v/v) glycerol stock
SDS	2.0% (w/v)	10.0 g
Tris-HCl, pH 6.8	24 mM	24 mL of 0.5 M Tris-HCl, pH 6.8
Nanopure H_2O	~ 40 mL	To final volume of 500 mL

14. Once isoelectric focusing is finished, remove each IPG from the alignment tray and remove excess mineral oil from each strip as described in Step #14 of Isoelectric Focusing protocol. Place each IPG into a separate trough of a clean Rehydration Tray (gel side UP). If frozen, let IPGs thaw for ~15 min before reduction.

15. Prepare Reducing Solution by adding dithiothreitol (DTT) to a final concentration of 2.0% (w/v) in an appropriate volume of Equilibration Stock (3 mL needed per IPG strip). This requires 0.24 g DTT per 12.5 mL Equilibration Stock. Cover each IPG with ~3 mL of Reducing Solution, and incubate at room temperature for 15 minutes. *(DTT stored at 4 °C on 2-D PAGE reagent shelf in Glover 106 refrigerator)*

16. Prepare Acetylation Solution by adding iodoacetamide (IAA) to a final concentration of 2.5% (w/v) and a few grains of bromophenol blue to an appropriate volume of Equilibration Stock (3 mL needed per IPG strip). This requires 0.30 g IAA per 12.5 mL Equilibration Stock; add enough bromophenol blue to obtain a deep blue color. *(IAA stored at 4 °C on 2-D PAGE reagent shelf in Glover 106 refrigerator)*

17. After 15 minutes of reduction, transfer each IPG to a new clean trough (gel side UP), cover each with ~3 mL of Acetylation Solution, and incubate at room temperature for 5 minutes.

18. After 5 minutes of acetylation, transfer each IPG to the appropriate slab gel:

a. Using ethanol-cleaned tweezers, remove the IPG from the trough, and place onto the inner side of the large glass plate (gel side UP) of the appropriate slab gel, with the pointed (acidic) end to the left side of the slab gel.

b. Trim both ends of the IPG (trim larger piece on basic (right) end) `using ethanol-cleaned scissors. IT IS IMPORTANT THAT THE IPG BE ORIENTED WITH THE ACIDIC END AT THE LEFT SIDE OF THE GEL PRIOR TO TRIMMING SO THAT PROPER ORIENTATION OF 2-D GELS CAN BE EASILY MAINTAINED.

c. Using ethanol-cleaned metal spatula, gently slide the IPG down the glass plate to the top of the slab gel surface, and press to remove any bubbles lodged between the IPG and the slab gel so that there is uniform contact.

(Tweezers, scissors, and metal spatula are stored in a holder above the Glover 106 benchtop)

19. Snap each slab gel sandwich onto a cooling core, by aligning the pins on the sides of the cooling core with the notches on the outside of each clamping block and pressing the sandwich into the black latch mechanism on the cooling core. Make sure that the gasket on the cooling core is uniformly flush against the smaller plate just below the top of the plate, or else leaks will occur during the run. DO THIS WITHOUT DISCONNECTING THE COOLING CORES FROM THE REFRIGERATION TUBING.

20. If there are an odd number of slab gels to be run, an upper chamber for the lone gel can still be formed by constructing a mock sandwich and snapping it onto the cooling core.

21. Fill each upper chamber with SDS Electrophoresis Buffer to the top edge of the larger glass plate. This will be more than sufficient to ensure that the electrode (located under the black crossbar at the top of the upper chamber) will be submerged.

22. Replace the chamber lid on the Multicell, making sure that the red electrodes on all cooling cores are aligned on the same side as the red electrode of the lid. (All red electrodes should be on the left side of the unit of the electrical cables are coming out of the lid and pointing directly at you.) Connect to power supply by plugging the red and black plugs into the corresponding receptors on the power supply (EPS 3500 XL).

23. Choose program 1 on the power supply, reprogram if necessary so that total current divided by the number of gels being run is equal to 40 mA per gel (V = 3000 V, P = 400 W, time = 3 h).

24. Monitor the gels to ascertain that the buffer level in each of the upper chambers has not drastically decreased, that the line of bromophenol from the IPGs is migrating down through the slab gel, and that the set current is reading on the power supply.

25. At the end of the run, note the distance that the line of bromophenol has traveled through the slab gel. If the line has not migrated to within 3-4 mm of the bottom of the slab gel, then continue the electrophoresis at the same settings for an additional time until this distance has been reached and note the total time required.

26. When complete, pick up each cooling core and pour the upper chamber buffer into a plastic tub set across the top of the Multiphor unit (this buffer can be dumped down the sink with lots of running water). Unsnap the gels from the cooling cores by pulling the clamping blocks away from the cooling core while simultaneously pushing down on the black latch mechanism.

27. Remove the plate sandwiches from the clamping blocks, and remove one of the spacers by gently but firmly pushing the spacer to the side, away from the slab gel. Once out, use the spacer as a wedge to gently pry the two glass plates apart. Do this is such a way that you are supporting the spacer with your fingers and not just using it as a crowbar...the spacer cannot handle that much strain and will break if it is bent too much!! Once the plates are apart, the slab gel should stick to one of the plates. Carry the gel on this plate to a trash can and use the spacer to gently separate the IPG from the slab gel and push the IPG into the trash.

28. Holding the glass plate at the top edge (the top is the edge without gel so that you can grip it better), dip the bottom edge of the slab gel into the fixation solution for the adequate staining procedure, invert the plate so that the gel is on the underside, and hold the glass plate near the surface of the fixation solution. Let the gel use its weight to separate from the glass plate.

Coomassie Blue Staining

Carry out all staining steps in the plastic Nalgene® staining boxes. **These boxes are not to be used for silver staining methods!**

Note: The sensitivity of this method should be around 30 ng/spot.

Step	Components	For 1 gel	For 6 gels	Time
Fixation/Staining[1]	Ethanol	112.5 mL	675 mL	1 h
	Acetic acid (glacial)	25 mL	150 mL	(minimum 30 min)
	Coomassie R250 dye	0.25 g	1.5 g	
	Nanopure water	112.5 mL	675 mL	
Destain[2]	Ethanol	25 mL	150 mL	minimum 30 min x 4 washes
	Acetic acid (glacial)	25 mL	150 mL	
	Nanopure water	200 mL	1200 mL	

[1]Filter this solution prior to use with vacuum filter apparatus and 0.2 um Millipore filters.

[2]Destain carried out to lower background staining. Gel may be stored at 4 °C in this solution for up to 1 week prior to tryptic digest of spots.

Dispose of all solutions as hazardous waste labeled with final concentration (after dilution from all solutions) of each component.

Simply Blue Staining

1. **Rinse** the gel 3 times for 5 minutes with 100 mL deionized water to remove SDS and buffer salts, which interfere with binding of the dye to the protein. Discard each rinse.

2. **Stain** the gel with enough SimplyBlue™ (~100 mL) to cover the gel. Stain for 1 hour at room temperature with gentle shaking. After incubation, discard the stain (down the sink with lots of water).

Note: If you need to leave the gel overnight in the stain, add 10 mL of 20% NaCl (w/v) in water for every 100 mL of stain. The procedure using NaCl allows for better sensitivity (binding of the dye).

3. **Wash** the gel twice with 250 mL of water for 1-3 hours. Gels can be left in 20% NaCl (w/v) in water at 4 °C for up to a week without loss of sensitivity. There is a small amount of dye in the water that is in equilibrium with the dye bound to the protein, so proteins will remain blue.

Silver Staining Method

Based on method of PlusOne Silver Staining Kit, GE Healthcare, modified by Yan et al., Electr. 21: 3666, 2000

Carry out all staining steps in the stainless steel staining trays. **The plastic Nalgene® staining boxes labeled SILV ER are to be used for storage only!!**

Note: The sensitivity of this method should be around 1 ng/spot.

Step	Components	For 1 gel	For 6 gels	Time
Fixation	Ethanol Glacial acetic acid Nanopure water	100 mL 25 mL 131.7 mL	600 mL 150 mL 790 mL	30 min
Sensitizing	Ethanol Sodium thiosulphate (5% w/v) Sodium acetate Nanopure water	75 mL 10 mL 17 g 162.5 mL	450 mL 60 mL 102 g 975 mL	30 min
Washing	Nanopure water	250 mL	1500 mL	3 x 5 min
Silver reaction	Silver nitrate solution (2.5% w/v) Nanopure water	25 mL 225 mL	150 mL 1350 mL	20 min
Washing	Nanopure water	250 mL	1500 mL	2 x 1 min
Developing	Sodium carbonate Formaldehyde (37% w/v) Nanopure water	6.25 g 0.05 mL 254 mL	37.5 g 0.3 mL 1525 mL	~ 5 min
Stopping	EDTA-Na2•2H2O Nanopure water	3.65 g 250 mL	21.9 g 1500 mL	10 min – up to 1 week at 4 °C

Dispose of all solutions as hazardous waste labeled with final concentration (after dilution from all solutions) of each component.

Sypro® Ruby Staining

Carry out all staining steps in the plastic Nalgene® staining boxes (polymethyl pentene box, polypropylene cover, low-density polyethylene plug). **These boxes are not to be used for silver staining methods!**

Note: Volumes listed are for 1 large gel or 2 mini gels; for more gels, multiply volumes by the number of gels. The sensitivity of this method should be on the order of 10ng/spot.

Step	Components	For 1 gel	For 6 gels	Time
Fixation	Methanol Acetic acid (glacial) Nanopure water	25 mL 17.5 mL 207.5 mL	150 mL 105 mL 1245 mL	30 min
Staining	Sypro® Ruby Stain solution	250 mL	1500 mL	24 h (minimum 30 min)
Destain[1]	Methanol Acetic acid (glacial) Nanopure water	25 mL 17.5 mL 207.5 mL	150 mL 105 mL 1245 mL	2-3 changes over 24 h (minimum 30 min)

[1]Destain carried out to lower background fluorescence. Gel may be stored in this solution for up to 1 week prior to tryptic digest of spots.

Sypro® Ruby Protein Gel Stain solution must be disposed of as hazardous waste labeled as "Ruthenium in organic solvents". Dispose of all other solutions as hazardous waste labeled with final concentration (after dilution from all solutions) of each component.

Gel Imaging

29. Clean all the trays in the imager before using it. If imaging silver or Coomassie stained gels, put the white filter on top of the UV light, underneath the gel tray. If imaging Sypro, put the gel tray directly on top of the UV source.

30. Make sure all the lamps and the camera are on. The UV light should be set at 302 nm (UV-B). Set the UVP parameters according to the stain used:

Stain	*Overhead Light*	*Filter*	*Control*
Coomassie Blue	White	Coomassie	Safety switch
Silver Nitrate	White	Coomassie	Safety switch
Sypro Ruby	365 nm	Ethidium bromide	Safety switch

(The Filters can be used differently, and in most cases the "Clear" option works well for all kinds of stain).

31. Put the gel on the imaging tray, and remove all the bubbles between the gel and the tray. Put the tray in the UVP chamber, close the door, and start previewing the image.

32. Use the software "Labworks", and start "Video / Digital Capture". Use the camera settings to optimize the amount of light on the image, and to focus the whole gel.

33. If the image is good, stop previewing by hitting "Stop / Capture". If imaging Sypro, go to "Options, Acquire" and increase the exposition time to 1-2 seconds, until the acquired image is visible.

34. After capturing, go to "Edit / Convert to" and choose "Gray Scale 16", and save your image. Further image improvements can be made by "Edit / Display range", by narrowing the range of black and white.

35. Finally, hit the AOI button, and select the area of the gel that is of interest for image analysis (cut out useless portions of the gel image). Save the new AOI image. Open it, and "Edit / Resize" it to 1000 x 1000 pixels, allowing distortion. This is the final image that should be transferred to the Proteomweaver computer.

36. The files should be saved in the folder "C:/Gels" of the "Proteomweaver" computer, and contain information on gel number, sample type, and stain used, and date. All gels should have the same size (1955 kb).

Image Analysis

1. Start "Proteomweaver", and create a new experiment with sample type reference, and stain used.

2. In step 1, load all the necessary gel images, putting different treatments in different groups. Rename the groups.

3. Follow all the steps in Proteomweaver, making sure to:
- Edit the parameters of detection for all the gels using the "Parameter Wizard" in Step 2.
- Edit spots that are not correct in Step 3.
- Edit bad matching in Step 5.

4. Go to the end of the analysis and identify differentially expressed spots. Select these spots for MS analysis. Annotate this info on Proteomweaver.

In-gel Tryptic Digestion

Solutions needed:

30 mM potassium ferricyanide stock solution (1.266 g in 100 mL of water)
100 mM sodium thiosulfate stock solution (1.58 g – 2.46 g if pentahydrate - in 100 mL of water)
0.1% TFA in 60% acetonitrile (ACN)
200 mM ammonium bicarbonate (ABC) stock solution (1.58 g in 100 mL of water)
100 % acetonitrile (ACN)
20µg/mL trypsin solution (1 vial of trypsin + 900µL 40mM ABC + 100µL 0.1% HCl)
5% formic acid (FA)

First day:

Destain:

1. Rinse gel with water. Carefully excise protein spots of interest using Spot Picker, and place each excised gel spot into a separate, autoclaved deplasticized tube. Take care to excise only the spot of interest, without including gel from other nearby spots. Use a fresh tip to excise each spot.

2. If the gel is silver stained, proceed to the following step. Otherwise skip to step 6.

3. Prepare a Working Solution by mixing a 1:1 ratio of the potassium ferricyanide and sodium thiosulfate stock solutions. (NOTE: This solution is unstable and should be prepared fresh for each gel spot destained.)

4. Cover excised gel piece with 40 uL Working Solution, vortexing while monitoring the stain intensity. After the brown colour has completely disappeared, remove the supernatant. Replace Working Solution 2 times.

5. Cover the gel piece with 100 uL of 200 mM ABC in 40% ACN and incubate at 37 °C for 30 minutes. Remove and discard the solution from the tube. Repeat once.

6. Store all excised gel pieces at -80°C until used, or proceed directly to the next steps.

7. Add 40 uL ACN and incubate for 5 min until the gel pieces shrink. Remove ACN and dry the gel piece in a Speed Vac for 5 minutes.

- Speed Vac settings for all runs: Pre heat to T = 50 °C, RC OFF, Auto run to chosen time. Vacuum should reach around 5 mTorr.

Tryptic digestion

8. Prepare trypsin solution right before use. Add 20 uL (0.4 ug of trypsin) of the trypsin solution prepared for in-gel digests to the gel sample. Add 20 uL of 40 mM ABC to the gel sample. Incubate for 4 hours to overnight at 37 °C.

Second day:

Peptide extraction

9. After overnight incubation, spin down the water droplets condensed inside the lid of the microcentrifuge tube. Remove the liquid from the gel piece and transfer the liquid to a new labeled tube.

10. Extract peptides from the gel matrix by adding 20 uL of 40 mM ABC. Vortex at room temperature for 15 min, and recover the supernatant after a brief spin.

11. Add 40 uL of 5% FA and extract at 37°C for 15 min with agitation. Spin down the gel particles and collect supernatant.

12. Add 40 uL ACN and incubate at 37°C for 15 min with agitation. Spin down the gel particles and recover the supernatant.

13. Pool all extracts and dry down to 5 uL in a vacuum centrifuge. Dry digests can be stored at –20°C for weeks.

Cleanup of Peptide Fragments – DEPLASTICIZED TUBES ONLY

Solutions	C18 Pipette Tips protocol
Wetting solution	100% acetonitrile (ACN)
Sample preparation	0.5% TFA in B&J water
Equilibration solution	0.1% TFA in B&J water
Wash solution	0.1% TFA in B&J water
Elution solution*	0.1% TFA/60% ACN

* For electrospray, elute with 1% formic acid/50% methanol.

1. Prepare enough of these solutions for all digested samples. Since the samples will be bound to the tips, aliquot 40 µL of washing and elution solution to different tubes, according to the number of samples.

2. Using the maximum volume setting of 10 µL, aspirate **wetting solution** into the tip. Dispense to waste. Repeat 10 times.

3. Aspirate **equilibration solution.** Dispense to waste. Repeat 10 times.

4. If samples are dry, add 10 µL of **sample preparation** solution to the tubes, and sonicate for 15 min.

5. Bind peptides and proteins to ZipTip pipette tip by fully depressing the pipette plunger to a dead stop. Aspirate and dispense the sample 10 cycles for maximum binding of complex mixtures.

6. Aspirate **wash solution** into tip and dispense to waste. Repeat once.

7. Dispense 5 µL of **elution solution** into a new tube. A second 5 µL elution can be done using the 0.1% TFA/70% ACN solution.