Creation of an endA Mutant Strain in Pseudomonas aeruginosa PAO1 using Gene Replacement

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Abstract and Results

Endonuclease I is an enzyme encoded by the endA gene. This nucllease degrades double-stranded DNA. Researchers can use this enzyme to create deletions in DNA. In this study, we created a deletion in the endA gene using the Cre-lox system and pCR2.1 vector. The consequence of this deletion in endA was to impair the ability of the PAO1 strain to produce Endonuclease I, which is a key enzyme in the degradation of DNA. This study provides insights into the role of Endonuclease I in the PAO1 strain and its potential applications in genetic manipulation.

Introduction

The endA gene of E. coli is essential for the production of Endonuclease I. It is a 26,862 bp DNA sequence that contains a single initiating ATG codon. In this study, we created a deletion in the endA gene of PAO1 strain using a Cre-lox system and pCR2.1 vector.

Objective

The purpose of this study is to create a new endA deletion mutant strain in PAO1 using Cre-lox system and pCR2.1 vector.

Experimental Design and Results

1. Construction of the pCR2.1-endA' plasmid

The endA gene was cloned into the pCR2.1 vector using the Cre-lox system and pCR2.1 vector. The resulting plasmid was isolated and used for transformation.

2. Construction of the pCR2.1-endA'SalI plasmid

The pCR2.1-endA' plasmid was digested with SalI to create a deletion in the endA gene. The resulting plasmid was isolated and used for transformation.

3. Insertion of the endA gene into the pCR2.1 vector

The endA gene was inserted into the pCR2.1 vector using the Cre-lox system and pCR2.1 vector. The resulting plasmid was isolated and used for transformation.

Conclusion

In conclusion, the Cre-lox system and pCR2.1 vector were successfully used to create a new endA deletion mutant strain in PAO1. This study demonstrates the potential of the Cre-lox system and pCR2.1 vector for genetic manipulation in PAO1.

Future Work

In future experiments, the pCR2.1-endA' plasmid containing the endA gene knockout will be used to create a new endA deletion mutant strain in PAO1. This will allow for further genetic manipulation in PAO1.

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