KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Construction of FUR deletion strains of *Shewanella oneidensis MR-1***

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
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«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Dong Yifei

1. Thesis topic **Construction of FUR deletion strains of *Shewanella oneidensis* MR-1**
Scientific supervisor Olena Okhmat, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Yifei DONG

Scientific supervisor _____ Olena OKHMAT

SUMMARY

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The purpose of this study was to explore the influence of the construction of FUR deletion strains of *Shewanella* on its biological characteristics. The method of gene knockout and mutation is carried out to target FUR, an iron regulatory factor in *Shewanella*, with the aim of analyzing the specific role of FUR in iron transport and absorption through gene knockout technology. The results showed that the growth performance of FUR deficient strains under iron metabolism pathway, microbial fuel cell current generation ability and environmental stress was significantly different from that of wild type strains. Further analysis showed that FUR protein plays an important role in regulating the absorption and utilization of iron ions and bioelectrochemical characteristics in *Shewanella*. The findings of this study provide important references for further understanding the regulatory mechanism of microbial iron metabolism, optimizing the construction of microbial fuel cells, and developing environmentally friendly biotechnology.

Key words: Shewanella, FUR deletion strain, Iron metabolism regulation, Bioelectrochemical properties

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INTRODUCTION

Microbial fuel cell, as a new field of bioelectrochemical system, with its unique biocatalytic way, can convert the chemical energy of organic matter or pollutants into electricity, showing great environmental protection and energy potential. In the process of searching for efficient catalysts, *Shewanella* has become the focus of research because of its excellent electron transport ability and environmental adaptability. In particular, FUR protein in *Shewanella*, as a key regulator of iron uptake mechanisms, plays an important role as a transcriptional regulator. It precisely regulates the expression of genes related to iron metabolism by sensing the concentration of iron ions inside and outside the cell. Therefore, in-depth exploration of the function of FUR protein and its regulatory mechanism is not only crucial for understanding the biological mechanism of iron metabolism and electron transfer in *Shewanella* , but also of great significance for promoting the optimal design and application of microbial fuel cells. The purpose of this study was to construct mutant strains with FUR gene deletion, so as to further analyze the effects of FUR protein deletion on iron metabolism, growth status and electrochemical performance of *Shewanella* . This research is not only expected to provide a new theoretical support for the development of microbial fuel cells, but also to further broaden its application prospects in the field of environmental engineering and energy science. With a better understanding of the function of FUR proteins, we will be able to more effectively harness the important value of microorganisms such as *Shewanella* in sustainable energy development.

The relevance of the topic is *Shewanella* FUR deletion strains and regulation of iron metabolism.

The purpose of the study is the study is to construct FUR deletion strains of *Shewanella* by knockout technology and systematically evaluate changes in iron absorption, utilization and bioelectrochemical properties.

The objectives of the study not only provides an important experimental basis for further understanding of the regulatory mechanism of microbial iron metabolism,

but also provides an important reference for optimizing the design of microbial fuel cells and developing environmentally friendly biotechnology. The in-depth study of the function of FUR proteins will help promote the application and development of microorganisms in the field of environmental engineering and sustainable energy.

The object of the study is *Shewanella* FUR deletion strain.

The subject of the study is *Shewanella* FUR deletion strain.

Research methods is to use in-frame deletion mutation method to knock out the iron regulatory factor FUR of *Shewanella*, construct the FUR gene knockout vector, and obtain *Shewanella* FUR deletion mutant.

The scientific novelty is FUR protein plays an important role in regulating the absorption and utilization of iron ions and bioelectrochemical characteristics in *Shewanella*.

The practical significance of the results obtained is that through systematic construction and evaluation of FUR deletion strains of *Shewanella*, this study aims to reveal the key regulatory role of FUR proteins in iron metabolism and bioelectrochemical properties. The results not only provide an important experimental basis for further understanding of the regulatory mechanism of microbial iron metabolism, but also provide an important reference for optimizing the design of microbial fuel cells and developing environmentally friendly biotechnology. The in-depth study of the function of FUR proteins will help promote the application and development of microorganisms in the field of environmental engineering and sustainable energy.

CHAPTER 1
LITERATURE REVIEW

1.1 Research background and significance

As a cutting-edge bioelectrochemical system, microbial fuel cell is gradually showing its unique advantages. The system uses microorganisms as biocatalysts to convert chemical energy of organic matter or pollutants directly into electrical energy. This innovative technology not only provides a new way for the development of environmentally friendly energy, but also shows great application potential in sewage treatment, ecological restoration and other fields. Among a variety of microbial candidates, *Shewanella* has become the focus of research because of its excellent electron transport efficiency and excellent environmental adaptability. In *Shewanella* and many microbial species, iron ion plays a central role. It is not only the active site of a variety of biological enzymes, but also directly participates in the electron transfer process in the microbial respiratory chain, thus significantly affecting the electrochemical performance of microbial fuel cells.

The FUR protein in *Shewanella*, as a key regulator of iron uptake, demonstrates its importance as a transcriptional regulatory protein. This protein mainly depends on changes in iron ion concentration inside and outside the cell to regulate the expression of genes closely related to iron metabolism. Under the condition of abundant supply of iron ions, FUR binds to iron ions, thereby inhibiting the expression of related genes involved in iron absorption; On the contrary, when iron is in short supply, FUR loses its binding to iron and activates the expression of these genes to regulate the absorption and utilization of iron by cells and ensure the stable state of iron in cells. This unique regulatory mechanism of FUR proteins plays an integral role in the survival and functioning of *Shewanella*.

Any interference or imbalance in this regulatory mechanism may have a significant impact on microbial growth, metabolic activity, and their electrochemical performance in microbial fuel cells.

Therefore, in-depth analysis of the function of FUR protein and its regulatory mechanism is of great significance to elucidate the biological basis of iron ion metabolism and electron transfer of *Shewanella*, and also has significant value for the design and application optimization of microbial fuel cells. The purpose of this study was to further investigate the specific effects of FUR protein deletion on iron metabolism network, growth dynamics and electrochemical characteristics of *Shewanella* by constructing a mutant with FUR gene deletion. This research will not only lay a solid theoretical foundation for the development of microbial fuel cells, but also provide broad prospects for their practical application. By fully understanding the function of FUR proteins, we can more effectively harness the potential of microorganisms such as Shevanella in environmental remediation and energy science, thereby driving innovation and development of sustainable energy technologies.

1.2 Research purpose

The core of this study was to construct FUR protein-deficient strains of *Shewanella* by gene knockout technology, and then systematically analyze the changes in iron uptake, utilization and bioelectrochemical performance of this strain. As a kind of microorganism, *Shewanella* has shown great application potential in the field of microbial fuel cell and environmental remediation. However, in these applications, the role of iron ions cannot be ignored. As the core regulator of iron metabolism in *Shewanella* , FUR (Ferric Uptake Regulator) protein can precisely regulate the expression of genes related to iron metabolism according to changes in iron ion concentration inside and outside the cell, ensuring the maintenance of iron homeostasis in the cell. Therefore, a detailed investigation of the specific role of FUR proteins in the transport and absorption of iron ions is crucial to further understand the regulatory mechanism of microbial iron metabolism and its application value in bioelectrochemical systems.

In order to achieve the core goal of this study, we first carefully designed and constructed a specific plasmid vector for FUR gene knockout of *Shewanella* by using advanced modern molecular biology techniques, such as PCR (polymerase

chain reaction) and recombinant DNA technology. This process includes the precise design of specific primers, the effective application of PCR technology, and the careful construction and validation of plasmid vectors. Then, we used homologous recombination technology and electric transformation technology to accurately introduce the constructed knockout vector into *Shewanella* to achieve accurate knockout of FUR genes. Finally, through rigorous screening and precise gene sequencing methods, we verified and ensured the successful construction of FUR gene deletion mutants.

After successfully constructing the Schiva strain with FUR gene deletion, we used atomic absorption spectrometry and other means to accurately measure the iron ion content in the missing strain to evaluate the specific effects of FUR gene deletion on iron uptake and metabolism. Further, we compared the differences in iron metabolism pathways between FUR deletion strains and wild-type strains, and further explored the key role of FUR proteins in iron metabolism regulation. In addition, in a microbial fuel cell system, we comprehensively evaluated the bioelectrochemical performance of FUR deficient strains, with particular attention to their differences in current generation efficiency and stability compared with wild-type strains. These detailed experiments not only help us to analyze in detail the mechanism of action of FUR proteins in the microbial electron transport process, but also reveal its potential practical value and broad application prospects in bioelectrochemical systems.

This study aims to explore the core regulatory role of FUR protein in iron metabolism and bioelectrochemical characteristics by carefully constructing and comprehensively evaluating FUR gene deletion strains of *Shewanella* . The results not only provide a valuable experimental basis for further understanding of the regulatory mechanism of microbial iron metabolism, but also provide an important reference for the optimal design of microbial fuel cells and the development of environmentally friendly biotechnology. The in-depth analysis of the function of FUR proteins will help promote the wide application and sustainable development of microorganisms in the field of environmental engineering and sustainable energy.

1.3 Research status at home and abroad

In the in-depth study of *Shewanella*, FUR (Ferric Uptake Regulator) has always occupied the core position, which plays a crucial role in the regulatory mechanism of microbial iron metabolism. FUR proteins have unique sensory abilities to detect subtle fluctuations in iron concentration inside and outside cells, and accordingly precisely regulate the expression of genes closely related to iron metabolism. This regulatory mechanism is indispensable for the survival and reproduction of microorganisms in the natural environment. The discovery and research of FUR proteins provide us with a unique perspective, which helps us to better understand how microorganisms survive and adapt in an environment where iron ions are scarce, and has positive significance for the further development of microbiology and related fields.

1.3.1 Foreign research

In recent years, with the advancement of molecular biology technology, the genetic background and metabolic network of *Shewanella* have been analyzed in more detail, especially in the aspects of iron absorption and regulation mechanism. For example, Xu et al. (2024) analyzed the microbial inheritance and quality changes of golden pomfret under different storage temperatures by high-throughput sequencing technology, and found that storage temperature significantly affected the structure and dynamic changes of the microbial community. Studies have shown that at lower temperatures, the diversity and richness of microbial communities are higher, while at higher temperatures; the proliferation rate of specific pathogenic bacteria is accelerated, resulting in a rapid decline in food quality. This study provides a scientific basis for optimizing the storage conditions of aquatic products and emphasizes the key role of temperature control in extending the shelf life of aquatic products [1]. Han et al. (2024) studied the bioreduction process regulated by the aggregation of graphene oxides by *Shewanella* and found that there were significant environmental risks in the process. These risks are mainly due to the potential toxicity of the intermediate products produced in the biological reduction process,

and the reduction efficiency fluctuates under different environmental conditions. These problems indicate that although *Shewanella* has important application potential in environmental remediation and nanomaterials modification, the stability and safety of the biological reduction process should be considered in practical applications to avoid environmental pollution and ecological risks [2]. In addition, Yang et al. (2024) prepared riboflavin-modified carbon black bioink through 3D printing technology, and applied it to *Shewanella* bioanodes, significantly improving its extracellular electron transfer ability. The research shows that this innovative material has great potential in improving the performance of microbial fuel cells, and may gradually trend to large-scale applications in the future. This research provides a new technological path for the commercialization of microbial fuel cells, while also laying the foundation for the development of highly efficient, biocompatible electron transport materials. [3].

1.3.2 Domestic research

In an iron-rich environment, FUR proteins bind to iron ions to form stable complexes that inhibit the expression of genes associated with iron absorption. However, when iron is scarce, FUR proteins release iron ions, which dissociate and activate these genes to promote iron absorption and ensure that bacteria can survive in iron-restricted environments. Current research on FUR protein regulation has extended to other biological functions besides iron metabolism, such as its role in oxidative stress response and bacterial pathogenicity. Although these studies in *Shewanella* are still in their infancy, FUR proteins have been shown to influence the response of bacteria to environmental stress by regulating specific sRNAs, providing new insights into their complex regulatory networks.

In addition, existing research is also exploring how to genetically engineer FUR proteins to enhance the electrical production capacity and environmental adaptability of *Shewanella*. Wan Yu et al. (2024) conducted an in-depth study on the changes in gene expression of *Shewanella onaidensis* MR-1 during fluoxetine degradation through transcriptomic analysis and functional gene studies. The results showed that

MR-1 activated a series of genes related to metabolism and degradation during the degradation of fluoxetine, revealing the potential application value of this strain in the degradation of environmental pollutants. At the same time, Li Yajun et al. isolated and identified a strain with the function of crude oil degradation and biosurfactant production through experiments, and analyzed its physiological characteristics and degradation mechanism in detail. It was found that the strain had a significant effect on degrading crude oil pollution, indicating that it had broad application prospects in environmental pollution control (Li Yajun et al.) [4]. Zhang Liwei et al. (2024) studied the effects of quorum sensing of Marine *Shewanella* on biofilm formation and attachment metadoration of thick-shell mussels. The results show that quorum sensing regulation can significantly affect biofilm formation and mussel attachment behavior, which provides a new perspective for understanding microbial community behavior and biological attachment mechanism [5].

In summary, the in-depth study of FUR proteins in *Shewanella* not only deepens our understanding of the important role of iron ions in microbial physiology, but also provides new possibilities for developing novel biotechnology applications, such as optimizing microbial fuel cell performance. With the continuous advancement of research, further exploration of FUR proteins in the future is expected to reveal more about the complex mechanism of microbial iron ion regulation, thus further promoting the vigorous development of the field of biotechnology and environmental engineering.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

The purpose of the study is the study is to construct FUR deletion strains of *Shewanella* by knockout technology and systematically evaluate changes in iron absorption, utilization and bioelectrochemical properties. The object of the study is *Shewanella* FUR deletion strain.

2.1 Experimental materials

In this study, we selected *Shewanella* as the experimental strain, and the main experimental procedures included genomic DNA extraction, careful construction of gene knockout vector, efficient screening of gene knockout strains, and accurate iron content determination. Materials required for the experiment include standard *Shewanella* strains, specific guide RNA (sgRNA), PCR amplification kits, DNA sequencing analysis reagents, and a range of specialized buffers and enzyme preparations for protein and nucleic acid extraction.

In this study, we chose *Shewanella* as the experimental strain, mainly because of its demonstrated excellent electron transfer ability in the field of microbial fuel cells and its great potential in biotechnology applications. The Shiva strains we used came from the standard collection of China Microbial Culture Preservation Management Committee, and all experimental operations were strictly carried out in the biosafety Class II laboratory to ensure the safety and standardization of the experimental environment. In order to maintain the activity and growth stability of *Shewanella*, we placed it in a specific medium rich in iron ions and cultured it in an oxygen-free environment at 28 ° C and pH 7.0 to simulate its natural growth environment. In addition, in order to ensure the consistency of culture conditions and the accuracy of the experiment, all petri dishes and test tubes were autoclave before use, thus effectively eliminating the potential interference of external microorganisms.

The main instruments used in the experiment included PCR, gel electrophoresis, DNA sequences, atomic absorption spectrometer, and electron microscope.

For sequence search on NCBI, the design sequence required to knock out genes is as follows:

GATTATAGAAACCGAACGTTTACTTTTGAGACCGTTTAACGAGCAGGATGTTGATGCCCTCTATT
TGATGAACAGTAACCCTGCGATGTTGAAATACATTCCAACCGCGCCTTTTACTGATCGCGAGCA
AGCTCGAGAACTGTTCCATAATGTGATCCTACAGGATTATCAGCAACGGGGATTTGGTCGCTGG
GCGGTAGAGCATAAAGCCGATAATAGGGTGATTGGCTTTTGTGGACCCAAATTTATTCCTGAGTT
TAACGAAGTCGAAATTGGCTATCGTTATTTCCCAGAATATTGGGGTAAGGGCATTGGTACTGAA
GCTGCCGAGGCGGCGCTGAGTGTGTTTCCCCAATTTGGGATTAAGCAAACCATTGCTTTGATCTT
AGAGGGGAATTTAGGTTCAGAAACGGTGGCGAAACGCGTGGGCATGTACTGGCGCGAGCACA
GTGAATTTATGGGACATAAGATCAATGTTTACGCCAAGATTTTATGACTAACCCTTTGTTGTAAA
ATAAAAAAACCAGCCGATGGCTGGTTTTTTTATTATTTAAGTTACCGCTAAGGCGCTATTGGAAT
TGGCCGAGCAAGCTGCTAATCTCTTGATTGCAGAACCAATAGGTTCATCCGTGGATAGTGCTTG
CCATTATAGGGGGTCAAATTAAAAACTTAAACCTTTGTTCTGGCTTTATAGGGCTATATGCAGAT
AAATTGTGTTACTGAGCGTGAAATTGCAATAAATTGCGGCAATTCAAGCATAGTTTTTGTAAGGT
TATACCAAGGAATAATGTTCACGATGCGAAAAATTCGTGACAATAAATAGAATAATGACAATAA
AAGGAAACAGTATGTACCAACATATTGTGGTATTAACTGGCGCGGGTATTTCTGCTGAGTCGGG
ATTACGTACCTTTCGCGATCAAGATGGTTTGTGGGAAGAGCATCATATAGAAGATGTGGCAACA
CCAGAAGGCTATGCAAAAGATGCTGAACTCGTCGAACGTTTCTATAACAGTCGCTGGGAGCAAT
TACACTGTGGTACTGTGATGCCTAATGCGGCACATCTTGCATTAGCCAAGTTAGAGGCTGAGTTT
TCTGGGCAGTTATTGGTTGTAACTCAAAATATTGATGATTTACATGAGCGTGCTGGATCTCGTCG
TTTACTGCATATGCACGGCGAACTCT

The primer design is as follows:

FUR MF-1: CTGATGAGTAAAAGCTCGAGGATTATAGAAACCGAACG

FUR MR-1: GCAATCAAGAGATTAGCAGCTTGCTCGGCCAATTCCAA

FUR MF-2: TTGGAATTGGCCGAGCAAGCTGCTAATCTCTTGATTGC

FUR MR-2: GATATGTCGAGCTCGAATTCAGAGTTCGCCGTGCATAT

FUR TF: TGTTTATCAGCCAGAGCGCA

FUR TR: GTACAGCCGTTATTCACTCC

2.2 Experimental method

2.2.1 The target fragment was amplified and purified

In this experiment, we used the cDNA of *Shewanella* as template, FUR MF1/FUR MR1 and FUR MF2/FUR MR2 as specific primer pairs, and performed PCR amplification by PrimerSTAR Max DNA Polymerase (Takara) as DNA polymerase.

The specific PCR amplification procedure is detailed in Tab. 2.1 below. After the successful amplification of two fragments A and B, we used the DNA purification kit (Knogen) and conducted strict purification according to its operating guidelines to ensure the accuracy and reliability of subsequent experiments.

Table 2.1 – **PCR reaction program**

Predegeneration（2 min）	Denaturation（10 s）	Anneal（30 s）	Extend（45 s）	Cycle number	Final elongation（7 min）
98 ℃	98 ℃	55 ℃	72 ℃	35	72 ℃

2.2.2 Fur fusion fragments were obtained by overlapping PCR

Using the previously purified A and B fragments as templates, we performed overlapping PCR amplification with reference to the procedure described in Section 2.2.1. Finally, we succeeded in obtaining a fused AB fragment with a length of 1264 bp. In order to ensure the purity of this segment, PCR product purification kit (Knogen) was used again for purification, and purified fusion fragments were obtained.

2.2.3 Link transformation

The purified target fragment was connected to the PLP12CM carrier by T4 ligase at 16℃ for a long time. The specific configuration of the connection system is shown in Table 2.2. Next, the ligate was introduced into DH5α receptive cells and treated with ice bath for 30 minutes, followed by heat shock at 42℃ for 90 seconds. After heat shock, the cell suspension was transferred to 500 mL antibiotic-free medium and cultured at 37℃ for 1 hour. After the culture, the cell suspension was centrifuged, and the bacteria were uniformly coated in an AGAR dish containing corresponding antibiotics, and then cultured at 37℃ overnight until a single colony was formed.

Table 2.2 – **Linkage reaction system**

Reagent name	Usage amount
T4 ligase	1 μL
T4 10×Buffer	1 μL
PLP12CM carrier	2 μL
DNA fragments were purified	6 μL
dd H2O	0 μL
Bulk volume	10 μL

2.2.4 Construction and transformation of pLP12Cm-Fur recombinant suicide plasmid

The purified pLP12CM-Fur plasmid was introduced into Escherichia coli β2163 by electrotransformation. The transformed cells were cultured for 5 hours under suitable conditions to ensure a successful transformation and allow the cells to adapt to the new environment. After that, the cell suspension was uniformly coated on an LB plate containing specific antibiotics (CM 20 μg/mL) and additives (DAP 0.3 mM, D-Glucose 0.3%). To verify positive clones, we used primer pairs of FUR-MF1/FUR-MR1 to screen the growing colonies by colony PCR. Once a positive clone is verified, we line it on a new medium for purification culture to ensure the accuracy and reliability of subsequent experiments.

2.2.5 Colony PCR

After looking at the plates, we carefully selected individual colonies and placed them in a pre-labeled centrifuge tube. In order to ensure the growth environment of the colony, 500μL sterile LB liquid medium was pre-added to the centrifuge tube, and the corresponding antibiotics were added. We then did a shake culture for 4 hours until the liquid became cloudy. Next, colony PCR was performed on the cultured liquid using the same PCR amplification system as above. After amplification, we used 1% nucleic acid gel for electrophoretic verification to confirm the quality and size of the amplified products. For positive plasmid colonies, we inoculated them into a medium containing the corresponding antibiotic and continued to culture them for

12 hours. Finally, we extracted plasmids from the cultured colonies for use in subsequent experiments.

2.2.6 Plasmid extraction

The in vitro bacteria solution cultured overnight was removed, centrifuged at 10000g for 1 min to collect the bacteria, and operated according to the instructions of the plasmid small extraction kit. The specific steps are as follows:

In order to carry out the following experimental steps, we first removed 4 ml of fresh bacterial solution and centrifuged it to ensure that the bacterial solution was evenly distributed after centrifugation. Subsequently, 0.25 ml of buffer A was added to each of the four centrifuge tubes, and the bacteria were fully resuspended in the buffer by violent shock operation. After that, an equal amount of 0.25ml buffer B is added to each centrifuge tube and the centrifuge tube is gently reversed 8 times to ensure that the two buffers are evenly mixed. After waiting for two minutes, we can observe that the mixture becomes clear but presents a certain consistency.

We added 0.25 ml buffer N to each of the four mixtures and gently mixed the mixture by turning the centrifugal tube upside down. A white flocculent precipitate was observed to form rapidly. This was followed by centrifugation for 10 minutes. After centrifugation, the supernatant is carefully transferred to special collection tubes inside the kit and these collection tubes are marked in detail. We then centrifuge the tubes for 60 seconds, discard the filtrate and reassemble the tubes. Finally, 0.5 ml of buffer KB was added to each collection tube and centrifuged again for 60 seconds, after which the filtrate was discarded to ensure that the collection tube was reassembled.

0.75 ml buffer PE containing anhydrous ethanol was added to the collection tube and centrifuged for 60 seconds. Subsequently, we discarded the filtrate and reassembled the corresponding collection pipe. To ensure that the impurities are completely removed, we repeat the above steps once. Next, we performed an air separation operation on the collection tube for 2 minutes. We then removed the column from the collection tube into a sterile 1.5 ml centrifuge tube and added 50

microliters of sterile deionized water to the center of the column membrane. After waiting 2 minutes, we performed 1 minute centrifugation to eluate the DNA. After being identified by agarose gel electrophoresis, the DNA was used to construct recombinant suicide plasmids and was properly stored at -20℃.

2.2.7 Insert mutant to construct

In our genetic engineering practice, we first conducted overnight oscillatory culture of E. coli β2163 (PLP12CM-Fur) donor bacteria and Shevanella receptor bacteria in LB-CM-DAP and LB liquid media. We then took equal amounts of cultures of both bacteria (100μL each) and mixed them in a centrifuge tube under sterile conditions. We then performed centrifugation to remove the supernatant and re-suspension of the bacteria using LB liquid medium. To ensure the removal of excess components from the medium, we repeated the centrifugation and re-suspension steps. Finally, we performed a final re-suspension of the cells with a small amount of LB liquid medium (10μL), then added the cells to an LB plate containing 0.3 mM DAP and 0.3% D-Glucose, and cultured them upside down at 37 °C for 6 hours.

In screening mutant strains for successful plasmid insertion, we first re-suspended cultured cells with 1 ml LB liquid medium and performed precise gradient dilution. We then took 100 microliters of diluted bacterial solution and evenly coated it on a LB-selective plate containing 20 μg/mL of chloramphenicol (CM) and 0.3% D-Glucose. In view of DAP deficiency of the donor bacterium Coliform β2163, it could not grow on normal LB plate. Mutant strains without plasmid insertion were also unable to withstand the inhibition of chloramphenicol and could not survive on chloramphenicol tablets. Therefore, only when the plasmid is successfully integrated into the specified region of the *Shewanella* chromosome can the strain successfully grow on the selective plate, thus becoming the target mutant for our screening.

In order to further purify potentially positive clones, several clones were selected from different plates and cultured on LB selective medium containing 20 μg/mL of chloramphenicol (CM) and 0.3% D-Glucose to ensure purified monoclones.

Subsequently, we validated these monoclones using colony PCR to determine whether the homologous arms of the foreign plasmid had successfully integrated into the chromosomes of the target mutant by homologous recombination mechanism. After PCR verification, positive clones were screened for liquid expansion culture, and FUR TF/FUR TR primers were used for PCR amplification. If the PCR product shows a specific band of 1246 bp, it indicates that the foreign plasmid has successfully inserted and may have induced a mutation of the target gene.

2.2.8 Deletion mutant construction

In order to identify and screen clones with specific deletion mutations, we first carefully selected target clones from a set of screened insertion strains and then oscillated them overnight in antibiotic-free LB liquid medium at 37 °C. The cultures were then gradiently diluted and uniformly coated on an antibiotic-free LB plate containing only 0.4% L-arabinose. Finally, the clones carrying the desired deletion mutation were selected by further culture at 37°C and careful observation of the growth of each clone.

After culture, we selected several colonies and simultaneously inoculated them on two LB plates, one containing L-arabinose and the other containing chloramphenicol (Cm). During the inoculation process, we took special care to ensure that the positions of the colonies on the two plates corresponded to each other and were clearly marked for subsequent identification. These plates were then neatly placed in a 37 °C incubator to maintain a constant culture environment and continued to be cultivated to monitor the growth of the colony.

After culture was completed, we screened those clones that could grow on L-arabinose LB plates but could not grow on chloramphenicol containing LB plates. These clones most likely carry the target deletion mutation we need. To further confirm the characteristics of these clones, we performed targeted detection using colony PCR technology combined with FUR TF/FUR TR primers. Wild type strains were also set up as controls. By PCR analysis, we expected that the clone with the correct deletion mutation would display an amplified fragment of 1246 bp, while the

wild-type strain would produce a fragment of 1483 bp, providing us with a clear validation basis.

2.3 Data analysis method

In this study, data analysis is an important step in evaluating experimental results and verifying research hypotheses. The data obtained during the experiment include gene knockout efficiency, physiological phenotype changes, iron ion content determination results and electrochemical performance of microbial fuel cells. In order to ensure the accuracy and reliability of the data, a variety of statistical analysis methods and software tools are used for data processing and analysis. In general, the selection and application of data analysis methods combined multi-disciplinary technical means such as molecular biology, statistics and electrochemistry to ensure the comprehensiveness and scientificity of experimental data. Through rigorous statistical analysis and data processing, the important role of FUR gene in iron metabolism and bioelectrochemical characteristics of *Shewanella* was verified, providing a solid experimental basis for the research conclusion.

CHAPTER 3
EXPERIMENTAL PART

3.1 Cloning of the FUR gene

3.1.1 Target gene amplification

In the current experiment, in order to accurately construct the missing mutant of the FUR gene, we used PCR (polymerase chain reaction) technology to amplify the upper and downstream homologous arm segments of the target gene. Specifically, we designed two pairs of primers - FUR MF1 and FUR MR1, and FUR MF2 and FUR MR2 - to amplify the homologous arm segments A and B of the upstream and downstream deletion genes for the purpose, respectively. The length of both fragments was set at 632 bp, which was determined based on our in-depth knowledge of the *Shewanella* genome and strict requirements for experimental precision. The process of PCR amplification requires strict temperature and time control to ensure that the primer can bind specifically to the template DNA and carry out chain extension in accordance with the principle of complementary base pairing under the action of DNA polymerase. After many cycles, we obtained a large number of amplified products. In order to verify the effect of PCR amplification, agarose gel electrophoresis, a classical molecular biology technique, was used. In 1% agarose gel, the amplification products were isolated and displayed. As shown in Fig. 3.1, clear and single bands appear on the gel, exactly as expected in size, that is, each fragment is 632 bp in length. This result fully demonstrates the success of PCR amplification, and we have obtained pure, high-quality target fragments. Next, these successfully amplified fragments were gelled and recovered. This is done by cutting the DNA fragments from the agarose gel with a specific tool and purifying them. Through this procedure, we obtained pure DNA fragments that will be used in subsequent gene knockout and recombination experiments.

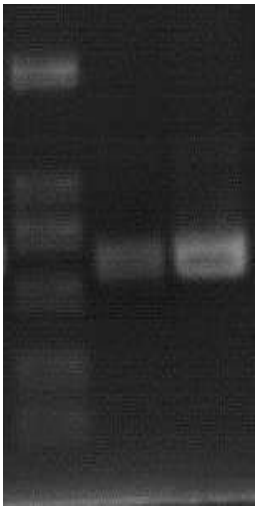

Figure 3.1 – Amplified Fur nucleic acid fragment

Lane 1: DL2000 DNA Marker; Lane 2: Segment A; Lane 3: Segment B

(From bottom to top: 100 bp 250 bp 500 bp 750 bp 1000 bp 2000 bp)

3.1.2 Overlapping PCR fusion

After completing PCR amplification and purification of A and B fragments, we further used these two fragments as templates and fused them through Overlap PCR technology. Overlapping PCR is a method commonly used in genetic engineering that allows us to link two or more pieces of DNA together by a specific overlapping sequence. In the process of overlapping PCR, we first designed the primer so that the downstream part of the A fragment and the upstream part of the B fragment have a certain overlapping sequence. Then, we added A and B fragments and corresponding primers and polymerases for PCR amplification. In the process of amplification, due to the design of primers and the optimization of PCR conditions, the overlapping parts of A and B fragments will combine with each other to form a complete fusion fragment. After the amplification, we verified the amplification results by agarose gel electrophoresis. As shown in Fig. 3.2, a clear and single amplified band appears on the gel between 1000 and 2000 bp, the length of which is consistent with the expected length of the fusion fragment AB (1264 bp). This result demonstrates the success of overlapping PCR, and we successfully obtained the target fusion fragment

AB. Next, we used PCR product recovery kit (Knogen) to cut and recover AB fragments. The purpose of this step is to cut the DNA fragments from the gel and remove impurities through a specific purification process to obtain pure DNA fragments. Through the precise operation of the recovery kit, we successfully recovered AB fragments from the gel and ensured its purity and quality.

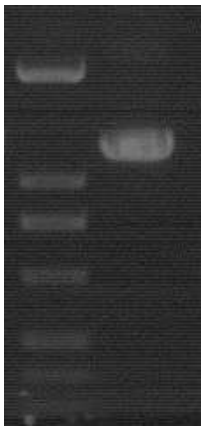

Figure 3.2 – Overlapping amplification amplified fragments
Lane 1: DL2000 DNA Marker; Lane 2: Fusion AB fragment
(From bottom to top: 100 bp 250 bp 500 bp 750 bp 1000 bp 2000 bp)

3.1.3 Transformed into DH5α receptor cells

After the successful purification of the AB fragment, we further advanced the experimental process. The focus of this stage is to link the AB fragment to the suicide vector pLP12Cm and construct a recombinant plasmid for gene knockout through homologous recombination. Homologous recombination is a form of gene recombination that relies on DNA sequence similarity, allowing us to precisely insert foreign DNA fragments into target locations. Before conducting homologous recombination, we first ensured the integrity and activity of the suicide vector pLP12Cm. Suicide vectors are a class of plasmids that cannot replicate autonomously in a particular host bacterium and are commonly used in gene knockout and gene replacement experiments. The pLP12Cm vector carries the chloramphenicol (Cm) resistance gene, which allows us to quickly identify bacteria that successfully introduce plasmids through antibiotic screening. Next, we used molecular cloning technology to link the purified AB fragment to the suicide vector pLP12Cm. In this

step, we use DNA ligase, which, under the right conditions, is able to connect DNA fragments to cuts in the carrier. By precisely controlling the conditions and timing of the connection response, we ensured that the AB fragment could be correctly inserted into the target location of the suicide vector. Once the connection was complete, we transformed the recombinant plasmid into Escherichia coli DH5α receptor cells. Escherichia coli DH5α is a commonly used genetically engineered host bacterium, which has good transformation efficiency and stability, and is suitable for various gene cloning and expression experiments. We introduced recombinant plasmids into receptive cells by thermal breakdown method, and used its high recovery ability to express the plasmids. In order to select the cells successfully introduced into the recombinant plasmid, the transformed cells were coated on LB plates containing Cm resistance for culture. The presence of the Cm resistance gene allowed only cells carrying the recombinant plasmid to grow on a medium containing Cm. After a period of culture, we observed individual colony growth on the LB plate (see Fig. 3.3). These colonies are formed from cells that have successfully introduced recombinant plasmids, which are resistant to Cm and are therefore able to survive and reproduce on a medium containing Cm. To verify the transfer of plasmids in these single colonies, we will conduct subsequent plasmid extraction and validation experiments.

Figure 3.3 – Recombinant plasmid PLP12Cm-Fur transformed Escherichia coli DH5α plate

3.2 Knockout of the FUR gene

3.2.1 Positive colony identification

In the previous phase of experiments, we successfully linked AB fragment to the suicide vector pLP12Cm through homologous recombination and screened out single colonies growing on CM-resistant LB plates. To further verify whether these single colonies successfully carried recombinant plasmids, colony PCR experiments were performed. Colony PCR is a technique that extracts DNA directly from bacterial colonies and performs PCR amplification, which can quickly verify the presence of plasmids without culturing the bacteria. We first randomly selected several single colonies for PCR amplification using specific primers. These primers are designed to cross the junction between the AB fragment and the suicide vector pLP12Cm, ensuring that only colonies carrying the correct recombinant plasmid produce positive results. After PCR amplification, the products were detected by agarose gel electrophoresis. As shown in Fig. 3.4, electrophoresis results show a bright and clear band at approximately 1246 bp, which is exactly the size we would expect after the AB fragment and carrier are connected. This result strongly suggests that we have successfully screened positive clones carrying recombinant plasmoids from a single colony. In order to carry out the follow-up experiment smoothly, we selected one of these positive clones and carried out shake culture.

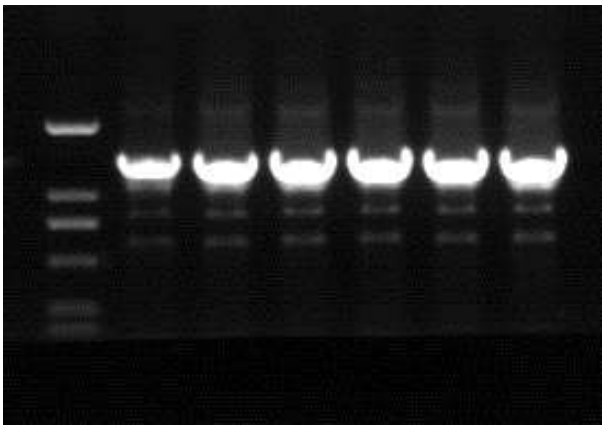

Figure 3.4 – pLP12Cm-Fur colony detection

Lane 1: DL2000 DNA Marker; Lane 2-7: Positive recombinant clone;

(From bottom to top: 100 bp 250 bp 500 bp 750 bp 1000 bp 2000 bp)

3.2.2 Converted to E. coli β2163

After the successful extraction and verification of pLP12Cm-Fur plasmid, we further transferred this plasmid into Escherichia coli β2163 strain by electrical transformation. Electrotransformation is a highly efficient DNA transfer technique, which directly introduces foreign DNA into host cells through electric field action. This technique is particularly suitable for bacteria that are difficult to transform through traditional chemical methods. After mixing pLP12Cm-Fur plasmid with Escherichia coli β2163 strain, we subjected the cells to electric shock treatment using electroconverters. In this process, the action of an electric field creates short pores in the cell membrane, allowing plasmid DNA to pass through these pores and enter the cell interior. Once inside the cell, the plasmid DNA can be replicated and expressed by enzyme systems within the cell, resulting in a stable presence in the E. coli β2163 strain. After electroconversion, the cells were coated on LB plates containing chloramphenicol (Cm) for culture. Since the pLP12Cm-Fur plasmid carries the Cm resistance gene, only cells successfully introduced to the plasmid can grow on the medium containing Cm. As shown in Fig. 3.5, after a period of culture, many single colonies of isolated growth appeared on the plate, indicating that we successfully transferred pLP12Cm-Fur plasmid into Escherichia coli β2163 strain. Escherichia coli β2163 is a conjugative strain, meaning that it is able to pass its own plasmids to other co-cultured microorganisms through conjugation.

Figure 3.5 – Recombinant plasmid pLP12Cm-Fur electrotransβ2163 plate

3.2.3 Identification of positive colonies

After the successful transfer of pLP12Cm-Fur plasmid into Escherichia coli β2163 by electroconversion technology, further validation was performed to ensure the correct insertion and stable presence of the plasmid. This verification process uses colony PCR, a method of extracting DNA directly from bacterial colonies and performing PCR amplification, which enables rapid and direct detection of the presence and insertion of plasmids. When performing colony PCR, we selected FUR MF1 and FUR MR1 as primers. These two primers are designed based on the sequence of FUR gene and its sides in pLP12Cm-Fur plasmid, and they can amplify DNA fragments containing the sequence of FUR gene and some of its vectors. By PCR amplification, we can directly observe the size and number of target DNA fragments, so as to determine whether the plasmid has been successfully inserted into Escherichia coli β2163 strain. After PCR amplification, agarose gel electrophoresis was used to detect the PCR products. Agarose gel electrophoresis is a common DNA separation and analysis technique, which can separate different sized DNA fragments according to size in an electric field and form clear bands on the gel. By comparing the location and size of the bands, we can accurately determine the length and quantity of PCR products. As shown in Fig. 3.6, the detection results of agarose gel electrophoresis were in complete agreement with our expectations. On the gel, we observed two different strands of DNA of different lengths. The shorter band is located at about 1300 bp, which indicates that we have successfully amplified the FUR gene and part of the vector sequence on both sides using FUR MF1 and FUR MR1 primers. The longer bands were located at about 3500 bp, and the electrophoretic results in the images were consistent with expectations.

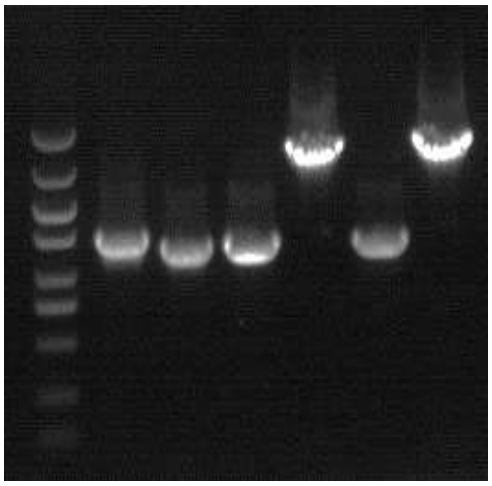

Figure 3.6 – β2163 electric transfer results colony PCR detection
Lane 1: DL100 DNA Marker; Lanes 2-4; 6: positive recombinant clone;
(From the bottom up 100 bp 200 bp 300 bp 450 bp 500 bp 600 bp 700 bp 800 bp 900 bp 1000 bp 1500 bp 2000 bp)

3.3 Phenotype and validation of mutant strains

3.3.1 pLP12Cm-FUR conjured into *Shewanella*

After successfully transferring pLP12Cm-Fur plasmid into Escherichia coli β2163 by electrotransformation technology, and confirming its positive clone by colony PCR and agar-gel electrophoresis, we proceeded to the next experimental stage: integrating pLP12Cm-Fur plasmid into *Shewanella* by conjugation. E. coli β2163 has the ability to conjugate, that is, it is able to transfer the plasmids it carries to other bacteria through direct contact between cells. In this experiment, we used this property to co-culture a positive clone of Escherichia coli β2163 carrying pLP12Cm-Fur plasmid with *Shewanella*. In the process of co-culture, the Escherichia coli β2163 positive clone was in close contact with *Shewanella*, and the plasmid pLP12Cm-Fur was transferred from Escherichia coli β2163 to *Shewanella* through conjugations. This process requires a certain amount of time and condition control to ensure that the joint action is carried out efficiently. After conjugating, we used *Shewanella* resistance to chloramphenicol (Cm) to screen *Shewanella* cells that successfully received the plasmid. We coated the co-cultured mixture on a plate containing Cm,

and only those *Shewanella* cells that successfully received pLP12Cm-Fur plasmid could grow on the medium containing Cm. After a period of culture, as shown in Fig. 3.7, several well-grown *Shewanella* colonies appeared on the plate, which verified our experimental purpose of successfully integrating pLP12Cm-Fur plasmid into *Shewanella* through conjugations.

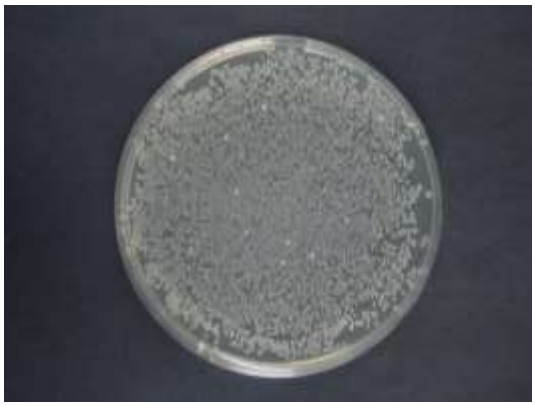

Figure 3.7 – The recombinant plasmid pLP12Cm-Fur is integrated into the *Shewanella* plate

3.3.2 Identification of positive bacteria inserted at specific sites of chromosomes

After successfully delivering pLP12Cm-Fur plasmid to *Shewanella* via conjugation and screening out the *Shewanella* carrier, our goal is to insert specific sequences on the plasmid into specific sites on the *Shewanella* chromosome by homologous recombination. This is a critical step in gene editing and gene knockout experiments because it allows us to precisely change the function or expression of the target gene. To achieve this goal, we designed a plasmid containing homologous sequences on both sides of the FUR gene. When this plasmid has a homologous recombination with the FUR gene on the *Shewanella* chromosome, it can insert a portion of the sequence on the plasmid (including selective markers and/or screening genes) into the FUR gene location on the chromosome, thereby achieving the knockout or replacement of the FUR gene. After co-culture and screening out *Shewanella* with pLP12Cm-Fur plasmid, we further purified and amplified monoclonal colonies by liquid culture. This step is to ensure that we get enough

bacteria for subsequent gene-editing testing. Next, we used FUR TF and FUR TR, a pair of specific primers, for PCR detection of the purified monoclonal colonies. This pair of primers is designed to amplify a specific sequence formed after insertion of a mutation, with an expected size of 1264 bp. Through PCR amplification, we can directly observe whether the insertion mutation occurs successfully and whether the insertion sequence is correct. As shown in Fig. 3.8, the results of agarose gel electrophoresis showed that the PCR products of both monoclonal colonies formed clear and uniform bands, which were located in accordance with the expected 1264 bp size. We can confirm that these two colonies are positive and can be used for subsequent gene function studies and experiments.

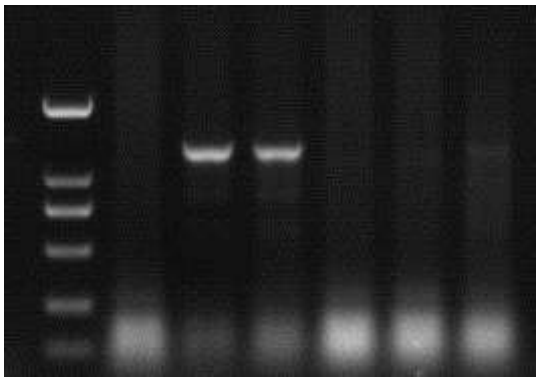

Figure 3.8 –Fur insert mutant detection

Lane 1: DL2000 DNA Marker; Lane 3; 4: positive recombinant clone;

(From bottom to top 100bp 250bp 500bp 750bp 1000bp 2000bp)

3.3.3 The fragment was removed and cultured

In genetic engineering experiments, suicide plasmids (also known as conditional replicating plasmids or induced erasable plasmids) are special plasmids that are capable of self-deletion from host bacterial chromosomes through a process of homologous recombination under specific conditions. This deletion process is usually triggered in the presence of a specific inducer, such as the arabinose mentioned in this case. The deletion mechanism of suicide plasmids facilitates gene editing and gene knockout experiments because it allows researchers to eliminate unnecessary genes

or sequences without the need for constant selection pressure. When *Shewanella* or other bacteria carrying suicide plasmids grow on an LB medium containing arabinose (LB-arabinose plate), specific genes on the suicide plasmids are activated, initiating the process of homologous recombination. This process involves pairing and exchange between homologous sequences on the plasmid and corresponding sequences on the host chromosome, ultimately leading to the deletion of the plasmid from a specific site on the chromosome. After completion of suicide deletion induced by arabinose, the bacteria will no longer carry the plasmid. To verify the success of the deletion process, single colonies grown on LB-arabinose plates are selected and tested to see if they can be grown on a medium that does not contain a selective marker (e.g., chloramphenicol, Cm). This is because suicide plasmids often carry resistance genes (such as Cm resistance genes), which are used to screen for plasmid-carrying bacteria on a medium containing the corresponding antibiotic. If suicide deletion is successful, the bacteria will lose resistance to these antibiotics and therefore cannot grow on a culture medium containing the corresponding antibiotics. As shown in Fig. 3.9, we selected several single colonies on the LB-arabinose plate and inoculated them separately on the LB-Cm plate. By observing the growth of these colonies on the LB-Cm plate, we can screen out those monoclonal colonies that successfully completed suicide deletion and lost Cm resistance. In conclusion, arabinose-induced deletion of suicide plasmid provides an effective means for gene editing and gene knockout experiments. By selecting and verifying monoclonal colonies that can grow on LB-arabinose plates but cannot grow on LB-Cm plates, we can ensure that we have obtained bacterial strains that do not contain suicide plasmids, laying a foundation for subsequent gene function studies.

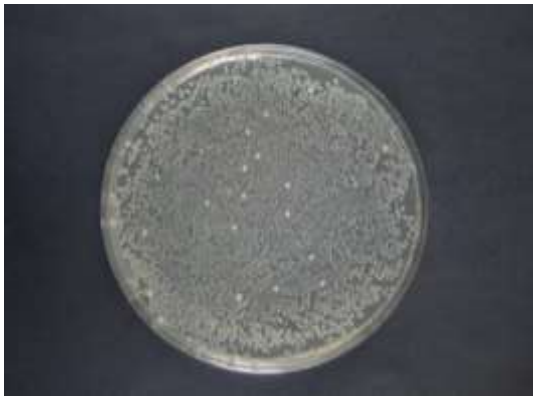

Figure 3.9 – *Shewanella* Fur deletion mutant plate

3.3.4 Identification of knockout fragment colonies

In gene editing experiments, verifying the accuracy of missing mutants is a key step to ensure the reliability of subsequent studies. In the experiment you provided, using primer FUR TF/FUR TR to perform PCR detection on single colonies treated with suicidal plasmid-mediated deletion mutation, and compare the amplification results with those of wild-type strains, which is a common verification method. First, primers FUR TF/FUR TR are carefully engineered to ensure that they can specifically amplify the DNA segment containing the target gene (FUR gene) and its surrounding sequence. In wild-type strains, due to the integrity of the FUR gene and its surrounding sequence, PCR amplification using this pair of primers would result in a DNA fragment 1731 bp long. This fragment contains all or most of the sequence of the FUR gene, as well as other DNA sequences between primer binding sites. However, in deletion mutant clones, the results of PCR amplification will differ from those of wild-type strains because the suicide plasmid introduces precise deletions in the FUR gene or its surrounding sequence through homologous recombination. Specifically, if the deletion occurs somewhere inside or on either side of the FUR gene, then the PCR product will be a shorter piece of DNA. In this experiment, the amplification of the correct deletion mutant clone produced a fragment of 1445 bp, which was significantly smaller than the 1731 bp fragment of the wild-type strain. This result proves that in the deletion mutant clone, the FUR gene or part of its

surrounding sequence has been successfully deleted. To visualize the results of PCR amplification, experimentalists usually perform gel electrophoresis analysis. In Fig. 3.10, the PCR products of the wild-type strains and the deletion mutant clones are loaded into the holes of the agar-gel and separated by electrophoresis. Because of differences in the size and charge of the DNA fragments, they migrate to different locations in the gel. Larger DNA fragments (such as the 1731 bp fragment of the wild type) will migrate more slowly and sit higher in the gel; Smaller DNA fragments, such as the 1445 bp fragment from the deletion mutant clone, will migrate faster and be lower in the gel. In the gel electrophoresis, we can see two distinct bands: one in the higher position, representing the amplification product of the wild type strain; The other, located lower, represents the amplification product of the deletion mutant clone. This apparent difference not only proves that the deletion mutation occurred, but also indicates that the size of the deletion is as expected.

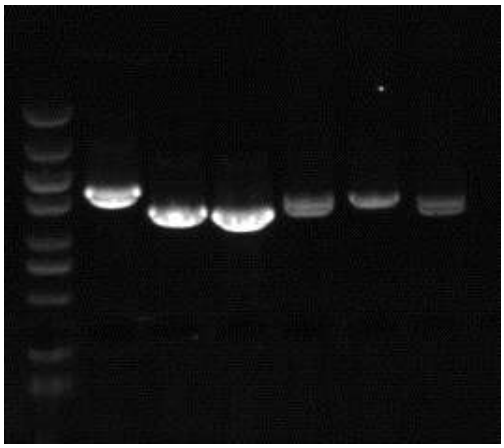

Figure 3.10 – Detection of Fur deletion mutations in *Shewanella*

Lane 1: DL5000 DNA Marker; Lane 3-7: missing mutant;

Lane 2: Wild type strain.

(From bottom to top 100 bp 250 bp 500 bp 750 bp 1000 bp 1500 bp 2000 bp 3000 bp 5000 bp)

CONCLUSIONS

1. In this study, FUR deletion strains of *Shewanella* were successfully constructed, and their performance under iron ion regulation, bioelectrochemical characteristics and environmental stress was preliminarily investigated. Through the analysis and discussion of the experimental results, we can draw the following conclusions and look forward to the future research direction.

2. In order to further study the regulatory mechanism of FUR gene on Fe2+ in *Shewanella*, this thesis first amplified the target knockout region by PCR technology, and synthesized the fusion fragment by overlapping PCR strategy. Subsequently, by homologous recombination technology, we successfully linked the fusion fragment to pLP12Cm vector and transformed it into DH5α strain for plasmid amplification and culture. Next, we extracted the knockout plasmid and introduced it into Escherichia coli β2163 by electroconversion technology. Subsequently, pLP12Cm-FUR plasmids were transferred to *Shewanella* by conjugation. In *Shewanella*, the suicidal plasmid pLP12Cm-FUR undergoes two homologous recombination processes, resulting in precise knockout of the FUR gene. Finally, we verified the gene deletion strains by PCR. The FUR deletion strains successfully constructed in this experiment will provide valuable materials for further research on iron transport mechanism.

3. This study verified the importance of FUR proteins for iron metabolism in *Shewanella* . By knocking out FUR genes, we observed significant changes in iron content, metabolic pathways and growth performance of mutant strains. This suggests that FUR proteins play a key regulatory role in *Shewanella* and are essential for maintaining iron homeostasis within the bacteria. Secondly, this study revealed the effect of FUR loss on the bioelectrochemical characteristics of *Shewanella* . We found that the current generation ability of FUR deficient strains in microbial fuel cells was significantly inhibited, indicating that FUR proteins not only affect iron metabolic pathways, but also may be involved in the regulation of microbial electron transport. In addition, we also conducted a preliminary study on the adaptability of FUR deletion strains under environmental stress. The results showed that the growth

performance of FUR deletion strains under oxidative stress, high salt and low pH was different from that of wild type strains. This suggests that FUR proteins may play an important role in the environmental adaptation of *Shewanella* , but its specific regulatory mechanisms need to be further studied.

4. In summary, this study has made some progress in revealing the function and regulatory mechanism of FUR protein, exploring the bioelectrochemical characteristics and environmental adaptability of *Shewanella* . However, there are still some issues and challenges that need to be further addressed. For example, the regulatory network of FUR proteins has not been fully elucidated, and its interaction with other regulators and signaling mechanisms still need to be further studied. In addition, in practical applications, how to use FUR missing strains to build efficient microbial fuel cells and cope with the challenges of complex environmental conditions is also one of the key directions of our future research.

5. Therefore, future research will focus on the following aspects: further elucidating the functional mechanism of FUR protein and exploring its role in iron metabolism regulation and environmental adaptation; To further study the bioelectrochemical characteristics of FUR deficient strains and optimize the construction and application of microbial fuel cells; To strengthen the study on the adaptability of FUR deficient strains under complex environmental conditions, and explore its potential application value in ecological restoration and environmental governance. These efforts will provide important theoretical and practical support for further understanding of the regulatory mechanism of microbial iron metabolism, promoting the development of microbial fuel cell technology and solving environmental pollution problems.

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