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THE CONSTRUCTION OF L-LACTIC ACID OVER-PRODUCING GENETIC ENGINEERING STRAIN

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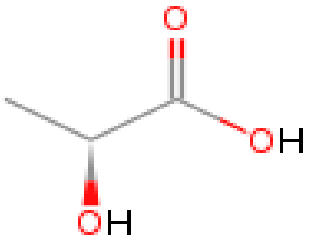
Abstract.
***Object of study**: Lactococus lactis; L-Lactic acid; plasmid PK18mob; plasmid PNZ8150*
***Mission:** Construct the genetic engineering strain- Lactococus lactis to get the high-efficiency, high purity and low-cost production of L-lactate. Knockout gene D-lactate dehydrogenase (ldhA) in genome of Lactococus lactis, with suicide vector PK18mob, with the method of homologous recombination. Then transformation expression vector plasmid PNZ8150-ldh to the Lactococus lactis.*
***Methods :** methods of molecular genetics(PCR, overlab PCR, TA colone; Enzyme restriction, extraction and purification of DNA, ligation,tranfomation, electroporation; sequencing validation), microbiological(cultivaton of microorganisms, single positive colone selection of Kmr .and by reversed selection of sucrose medium.) spectrophotometric.*

1. Introduction

L-lactic acid is an important precursor of many chiral compounds, which have been applied in many fields, such as chemical engineering, agriculture, food industry, pharmacy,especially, polylactic acid has been widely used in environmental protection, such as biodegradation plastic bag. At present, L-lactic acid is many produced high cost and low yield. In this work, L-lactate lactate biosynthesis pathway was reconstructed in Lactococus lactis .The D-lactate dehydrogenase (ldhA) was knocked out successfully. Further, L-lactate dehydrogenase (ldh) overexpression vector was contrasted. The researches above lay a solid foundation for the high-efficiency and low-cost production of L-lactate.

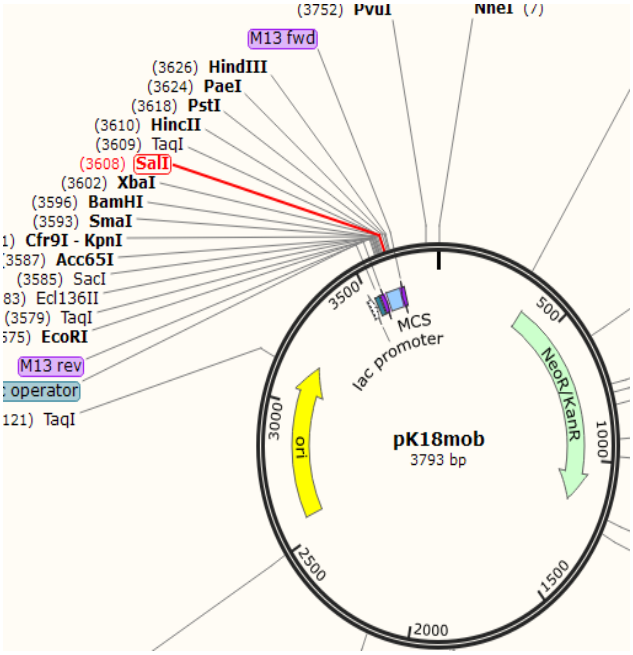


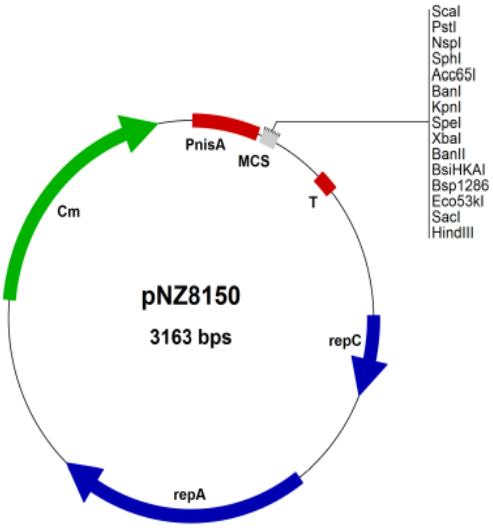
L-Lactic acid

polylactic acid has been used as biodegradation plastic bag

2. Materials and methods

In order to obtain L-lactic of high purity, the D-lactic acid metabolic pathway needs to be blocked in Lactococus lactis. Firstly, the ldhA gene was knockout in Lactococus lactis. The upstream homologous arms of ldhA (ldhA1) and downstream homologous arms of ldhA(ldhA2) were cloned from Lactococus lactis genome and linked by overlap PCR resulted in ldhA1-ldhA2. Then it was inserted in PK18mob; to form the knockout vector. Then transformed to Lactococus lactis. Lactococus lactisΔldhA homologous recombination were screened by positive selection of Kmr and then by reversed selection of sucrose medium.





The PNZ8150 plasmid is a shuttle expressed plasmid between Lactococus lactis and E.coli. In order to confirm its genetic stability,100 generations were subcultured. Then the expression plasmid PNZ8150-ldh containing heterogeneous ldh from Entrococous Feacalis was constructed. Then double digestion plasmid validation. Transformation PNZ8150-ldh to Lactococus lactis.

3. Result and discussion

The cultures in plats showed that Lactococus lactisΔldhA recombination could not grow in basal medium with the sole carbon source of D-lactic acid, indicating that the D-lactic acid metabolism of the micro organism was interrupted owing to the delection D-ldh.

The resulte showed that stability rates reached to 98% and 94% with and with out the antibiotics pressure respectively, which indicated good genetic stability of plasmid PNZ8150 in Lactococus lactis.

plasmid PNZ8150-ldh was successfully constructed.

gene inactivation in D -lactise (plan 1.1 homologous recombination)
(plan1,2 crispr/cas9)
1,1 homologous recombination

1,1,1),D-ldh PCR amplificationa
1,1,2),over lab ldh1, ldh2 ldh1-ldh2
1,1,3),TA colone
Ldh1-ldh2 fragment +A
Ligation with T vector –PMD19T or EZ
1.1.4)Transformation to E,coli DH5
1.1.5)selection and validation
I.Medium: X-g-Gal(blue-white)
Amp
II.double digestion:EcoRI,HIdIII
III. perform PCR to identify the positive clones
IV.sequencing validation
1.1.6), After double digestion EcoRI,HIdIII, Gel extraction and purification,get target fragment
1.1.7), Digestion suicide vector PK18mobSacB with EcoRI,HIdIII,too
1.1.8)Ligation

Digested suicide vector PK18mobSacB	100ng
Digested Target fragment	20 ng
10x T4 ligase buffer	1 ul
T4 ligase	1 ul
H_2O	Up to 10 ul
Total volume	**10 ul**

Ligate overnight at 16 ℃.

1.1.9),transformation to E.coli Dh5

1.1.10)selection and validation

I.Medium: X-g-Gal(blue-white)

Kar

II.double digestion:EcoRI,HIdIII

III. perform PCR to identify the positive clones(primer:down Ldh1 and up ldh2)

УДК 338

DELAY ANALYSIS OF A MODIFIED CUMULATIVE ARQ

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Abstract. *For the wireless channel characteristics, this paper modifies the cumulative ARQ in order to improve the performance of the traditional cumulative ARQ. We put in sequence confirmed feedback to satisfy the demands of the new generation cellular mobile communication. An analysis model is developed for parameter manipulation in the proposed framework. Finally, the simulation results show that the performance of modified cumulative ARQ is better than the traditional mechanism.*

1. Introduction

With the continuous growing of communication, the broadband wireless access technology which is based on a series of the IEEE802.16 standard becomes a hot spot of broadband metropolitan area network wireless access technology gradually. As one of the most mainstream mobile communication standards, the IEEE802.16m has more superior performance. At present, a frequently used error control technology is ARQ, which play an important role in broadband wireless communication such as 4G, WiFi and UWB. It has been catching many researchers' attention over the years.

Reviewing the previous researches, we find that there is still plenty of room for improving to the transmission mechanism of cumulative ARQ, especially the feedback mechanism. Overall, in this paper, we modify the transmission mechanism of cumulative ARQ in IEEE 802.16m networks and study its performance. An absorbing Markov chain and a three-queue model are developed for providing a simple and efficient approach to investigate the important performance metrics, such as goodput, PDU delivery delay, and SDU delivery delay.

2. Modified cumulative ARQ

In the process of the traditional cumulative ARQ mechanism transmission, when the first lost PDU appears, the successful received PDU before the first lost PDU will be fed back. This PDU and the PDUs after it will wait to the next transmission opportunity to retransmit. In this way, the transmission causes large delivery delay and channel waste. So on the basis of the discussion, we propose a modified scheme which adds to the sequence ACK in the traditional cumulative ARQ.