IMPROVEMENT OF D-LACTIC ACID PRODUCTIVITY BY GENETIC MANIPULATION OF *ZYMOMONAS MOBILIS*

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Introduction

Zymomonas mobilis is a gram-negative bacterium that has been extensively studied for its ability to produces ethanol. However, *Z. mobilis* can also produce other valuable chemicals such as lactic acid and succinic acid. Lactic acid can be further engineered into Polylactic acid (PLA), which can serve as sustainable replacement for widely used petroleum-based products such as plastic. In this study, the effects of genetic manipulations on *Zymomonas mobilis* ZM4 are investigated. Lactate dehydrogenase (ldh) gene from *Leuconostoc mesenteroides* was introduced into free D-(-)-lactic acid producing mutant *Zymomonas mobilis* strain. Afterwards, genes responsible for pyruvate decarboxylase (*pdc*), phosphoenol pyruvate carboxylase (*ppc)*, and malic enzyme (*sfc)* were deleted. The lactic acid productivity was examined with each gene deletion.

Methods

The gene deletions were performed on ZM4 by FLP-FRT site specific recombination system. The deletion procedure was confirmed by PCR. Lactic acid fermentation was carried out in a 5L bioreactor containing 2L RM medium with 50 g/L glucose at 30°C. The pH of 5 was maintained by feeding NaOH with an automated system. The concentration of glucose, ethanol, lactate, succinic acid, and pyruvate produced were measured from the supernatant of the samples using a high-performance liquid chromatography.

Results

Compared to the wild type strain ZM4, all mutant strains accumulated D(-)-lactic acid as major product. The yield was close to the theoretical yield (almost 1.0g lactic acid/g of glucose) under buffered condition of pH 5.0. Also, multiple gene deletions of *pdc*, *ppc*, and *sfc* helped to improve lactic acid yield and decrease byproduct formation. The optical purity of D-(-)- lactic acid observed extremely high at 99.9% in each of the samples.

Conclusions

Cumulative mutations of *pdc*, *ppc*, *and sfc* shows significant improvement in lactic acid productivity, while reducing byproduct production. However further studies are still required to reduce the fermentation cycle duration and increase lactic acid productivity. These improvements are necessary in order to minimize energy cost and maximize production output on a commercial scale.

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