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. In addition, *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 *Z. mobilis* ZM4 are investigated. D-Lactate dehydrogenase (*d-ldh*) gene from *Leuconostoc mesenteroides* was introduced into free D-(-)-lactic acid producing Z. *mobilis* ZM4 mutant. Afterwards, genes responsible for D-lactate dehydrogenase (ZMO0257) and D-isomer specific 2-hydroxyacid dehydrogenase (ZMO1237) of *Z. mobilis* ZM4 were deleted. The lactic acid productivity was examined with each gene deletion.

Methods

First, D-ldh gene was inserted into ZMO0263-0270(unknown function) of *Z. mobilis* through homologous recombination. Subsequently, genes for ZMO0257 and ZMO1237 were also deleted by homologous recombination. The deletion procedure was confirmed by PCR. Lactic acid fermentation was carried out in a 5L bioreactor containing 2L RM medium (10g/L of yeast extract, 2 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, and 1 g/L $MgSO_4$) with 100 g/L glucose at 30°C. The pH of 5 was maintained by feeding NaOH with an automated system. The concentration of glucose, ethanol, lactic acid, succinic acid, and pyruvic acid 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 D-lactic acid yield reached 33% with a volumetric production rate of 1.37 g $l^{-1}h^{-1}$. There was no significant difference in lactic acid productivity amongst mutant strains. The optical purity of D-(-)- lactic acid observed extremely high at 99.9% in each of the samples.

Table 1. Performances of Lactic acid production mutants

Z. mobilis strains	Glucose (g/l)	Lactic acid		Optical purity	Byproducts	
		D-LA (g/l)	Yield (%)	D-(-)-LA (%)	EtOH (g/l)	Organic acids(g/l)
ZM4	100	-	-	-	> 45	< 1.0
LSI003	100	~ 31	>30.9	>99.9	>32.7	< 0.5
LSI009	100	~ 31	>30.7	>99.9	>33.1	< 0.4
LSI013	100	~ 30	>29.8	>99.9	>33.8	< 0.4
LSI016	100	~ 31	>30.9	>99.9	>32.3	< 0.2

* LSI003: *Z. mobilis* ZM4Δ*ZMO0263-0270::*Ω*spec*^{R} *D-ldh*
* LSI009: LSI003 Δ*ZMO0257*
* LSI013: LSI003 Δ*ZMO1237*
* LSI016: LSI003 Δ*ZMO0257*::Δ*ZMO1237*

Conclusions

We have developed a free D-(-)-lactic acid producing *Zymomonas mobilis* strain by introducing a heterologous D-lactate dehydrogenase (D-*ldh*) gene. However further studies are still required to reduce the fermentation cycle duration, decrease ethanol production, 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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