Studies on efficient homo-L-lactic acid fermentation with lignocellulosic biomass-derived sugars by thermophilic lactic acid bacterium

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（好熱性乳酸菌によるリグノセルロースバイオマス由来の糖を用いた高効率ホモL-乳酸発酵に関する研究）

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Thesis Summary

Optically pure lactic acid (LA) has been widely applied in numerous applications, especially in production of biodegradable and biocompatible poly-LA materials. Lignocellulose has received great attention as substrate for LA fermentation due to its abundance, low-cost, non-competition with food substrates, and high fermentable sugars contents (mainly glucose, xylose, and cellobiose). However, LA from lignocellulose by lactic acid bacteria (LAB) is hampered by non-efficient utilization of xylose, carbon catabolite repression (CCR) of mixed sugars and by-products formation. *Enterococcus faecium* QU 50, a thermophilic LAB isolated and characterized in our laboratory, showed homo-L-LA from xylose at 50°C. This study aimed to establish a cost-effective fermentation system for homo-L-LA production by strain QU 50 from lignocellulose-derived sugars without CCR.

Strain QU 50 fermented 20 g/L and 50 g/L xylose as sole carbon source at 50°C completely to produce LA with high yield of 1.00 g/g. However, Millard reaction (MR) was formed at higher xylose titer. Strain QU 50 couldn't utilize 70 g/L xylose completely and produced LA with yield and productivity of 68.5 g/L with 1.00 g/g and 0.953 g/(L•h), and 58.1 g/L with 0.977 g/g and 0.788 g/(L•h) in batch and fed-batch cultures, respectively. Exclusion of some nitrogen sources from media has alleviated MR but resulted in low LA production. Although the maximum lactate dehydrogenase activity was obtained at 50°C, strain QU 50 exhibited lower stability at 50 °C with more rapid decrease of active cells comparing with those at 37 °C and 43 °C. These results indicated that efficient L-LA culture by strain QU 50 should be performed at 50°C with carbon source <70 g/L with undertaken of cell stabilization.

Using mixed sugars, strain QU 50 simultaneously consumed mixed glucose/xylose with relaxed CCR, while replacing glucose with cellobiose (cellobiose/xylose mixture) led to simultaneous consumption of both sugars without CCR even at any ratio to produce LA. The respective activities of xylose isomerase and xylulose kinase in cellobiose/xylose-grown cells were 3.22 and 1.91 times higher than those of glucose/xylose-grown cells. Strain QU 50 produced 70.8 g/L LA with a yield of 1.04 g/g and a productivity of 2.95 g/(L•h) from simulated energy cane hydrolysate in batch culture. Immobilization of strain QU 50 improved the operational stability of open (non-sterilized) repeated fermentation (three cycles) and led to 61.1–64.3 g/L LA with yields of 1.01–1.02 g/g and productivities of 3.22–3.82 g/(L•h), which indicated that an efficiently cost-effective fermentation system was successfully established for L-LA production from sugar mixtures (Fig. 1).

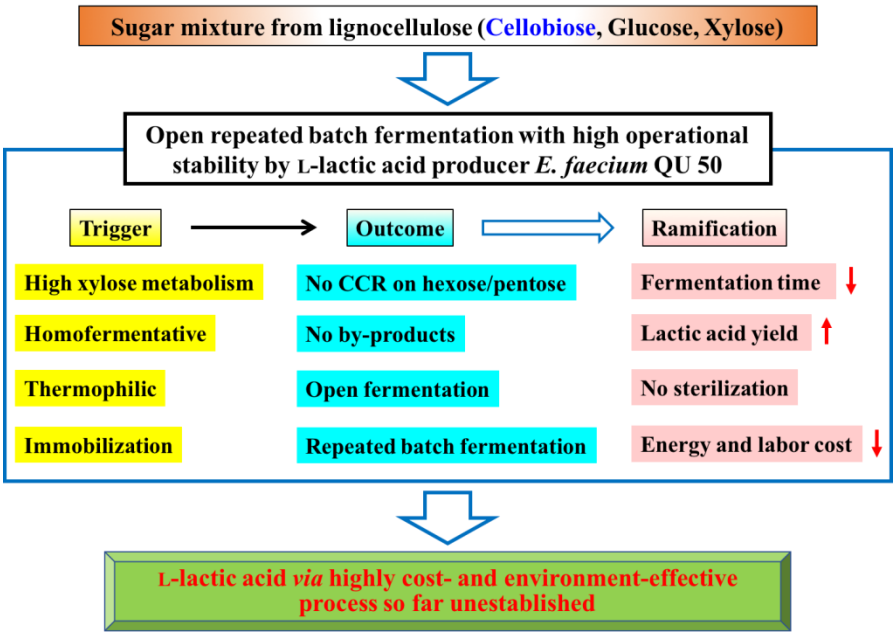

Fig. 1 Thermophilic lactic acid bacterium enabled homo-L-lactic acid fermentation from hexose/pentose without carbon catabolite repression and open repeated production by immobilization.

Finally, rice straw (RS) was utilized as real biomass for LA production by strain QU 50. Acid-pretreated RS (90.9 g-dry mass/L) was hydrolyzed by cellulase (incomplete hydrolysis) and mixed cellulase/cellobiose (complete hydrolysis) that resulted in the respective mixed sugars (glucose/cellobiose/xylose/arabinose) at 5.11/6.29/13.1/1.69 g/L and 22.4/0.0/15.6/2.12 g/L. Open batch and fed-batch cultures of undetoxified rice straw hydrolysate (RSH) were conducted. Homo-LA of 25 g/L and 41 g/L were produced from incomplete RSH without CCR and complete RSH with relaxed CCR, respectively with high yield (LA/sugars) of approx.1.00 g/g. These were equivalent to the yields (LA/RS) of approx. 330 g/kg and 570 g/kg using incomplete and complete RSH, respectively. Incomplete RSH showed several advantages over complete RSH, such as low-cost enzyme consumption of only cellulase, avoiding CCR due to contained cellobiose and low titer of glucose, and potentiality of enhancing sugars titers in repeated hydrolysis by immobilized cellulase on RS. Thus, homo-L-LA production by strain QU 50 from lignocellulose-derived sugars without CCR and by-products was successfully conducted.