# Studies on L-lactic acid fermentation with mixed sugars derived from lignocellulose by Enterococcus mundtii QU 25

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論文題名　：Studies on L-lactic acid fermentation with mixed sugars derived from lignocellulose by *Enterococcus mundtii* QU 25
（*Enterococcus mundtii* QU 25 によるリグノセルロース由来混合糖を用いた L-乳酸発酵に関する研究）

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## 論　文　内　容　の　要　旨

Lactic acid (LA; 2-hydroxypropionic acid), is the most widely occurring carboxylic acid, having a prime position due to its versatile applications in food, pharmaceutical, textile, leather, and other chemical industries. Recently, LA production has gained a significant amount of interest due to the application as a feedstock for the production of poly-LA, a polymer present in medical applications and environmentally friendly biodegradable plastics. With the development of industrial bioconversion, microbial fermentation from non-food feedstock such as lignocellulosic biomass has become the dominant for LA production. Production of optically pure LA from lignocellulose-derived mixed sugars is attractive but challenging because most of microbes exhibit carbon catabolite repression (CCR) in the presence of preferable sugar such as glucose. Utilization of xylose is generally inhibited by glucose, which becomes a major obstacle to economical utilization of mixed sugars derived from lignocellulosic biomass. *Enterococcus mundtii* QU 25, an optically pure L-LA-producer, which was isolated and characterized in our laboratory, presented a high activity of β-glucosidase grown in cellobiose as a sole carbon source. Furthermore, strain QU 25 was proved to homoferment xylose to L-LA via pentose phosphate/glycolytic pathway rather than phosphoketolase pathway. Therefore, this study aimed at homo-L-LA production from mixed sugars without CCR by *E. mundtii* QU 25.

Firstly, *E. mundtii* QU 25 exhibited apparent CCR of xylose in glucose/xylose mixture in batch culture; however, replacement of glucose by cellobiose (cellobiose/xylose mixture) led to simultaneous consumption of both sugars without CCR. The production of LA and activity of enzymes related to xylose metabolism were also investigated. Xylose isomerase and xylulokinase activities in cellobiose/xylose-grown cells were three times higher than those in glucose/xylose-grown cells. The addition of yeast extract and

ammonium hydroxide effectively improved sugar utilisation and cell growth. Under the optimal conditions with simulated lignocellulosic hydrolysates, a high L-LA concentration (up to 163 $g \cdot L^{-1}$) was obtained with a yield of 0.870 $g \cdot g^{-1}$ and maximum productivity of 7.21 $g \cdot L^{-1} \cdot h^{-1}$ without CCR in the fed-batch fermentation. Thus, we could establish rapid and simultaneous consumption of hexose and pentose sugars by using a LA bacterium strain, which significantly increased production of highly purity L-LA.

Secondly, the effects of citrate supplements on LA production from mixture of cellobiose and xylose with *E. mundtii* QU 25 were studied. LA production and sugar consumption increased with the concentration of citrate. In addition, under a harsh condition with initial 150 $g \cdot L^{-1}$ LA, sugars utilization and cell growth were improved by citrate supplement. It is proposed that increasing citrate would be useful strategy to improve further the ability of this strain to cope with strongly acidic conditions. Moreover, in the presence of citrate, high LA yield around 1 $g \cdot g^{-1}$ with 128 $g \cdot L^{-1}$ LA was obtained from simulated energycane hydrolysate. Interestingly, combined citrate with yeast extract stimulated cell growth to 6.49 $g \cdot L^{-1}$ and shortened the co-fermentation period into 70 h. We suppose that citrate metabolism endowed the cells extra ability to counteract lactate toxicity, so the cell decline was delayed by extra citrate.

Finally, in order to improve L-lactic acid productivity, continuous co-fermentations were investigated by *E. mundtii* QU 25 using mixed sugars derived from lignocellulose. *E. mundtii* QU25 exhibited apparent CCR in glucose/xylose mixture with continuous fermentation mode, which resulted in a high ratio (10.4:1.0) of consumed hexose to pentose. Replacement of glucose by cellobiose (cellobisoe/xylose mixture) overcame the obstacle of CCR and improved ratio of consumed hexose to pentose to 1.86:1.0 effectively. The effects of dilution rates and sugar concentrations on L-LA production were further investigated in conventional continuous culture with cellobisoe/xylose mixtures. As a result, 22.9 $g \cdot L^{-1}$ lactic acid with a yield of 0.871 $g \cdot g^{-1}$ and productivity of 4.57 $g \cdot L^{-1} \cdot h^{-1}$ were observed at a dilution rate of 0.2 $h^{-1}$ from cellobiose 50 $g \cdot L^{-1}$ and xylose 30 $g \cdot L^{-1}$. This is the first demonstration of co-fermentation of cellobiose and xylose by *Enterococcus* under continuous culture conditions.