

—Short Communication—

## Production of Glucose-6-Phosphate Dehydrogenase from *Leuconostoc mesenteroides*

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## *Leuconostoc mesenteroides* 의 葡萄糖-6-磷酸 脫水素酵素 生産

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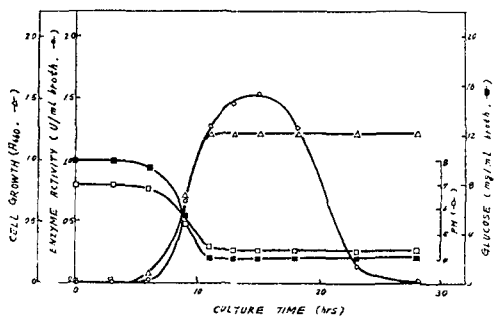
It was previously reported that *Leuconostoc mesenteroides* No. 20-1-10 isolated from Kimchi produced high activity of an intracellular enzyme, glucose-6-phosphate dehydrogenase (1) (EC No. 1.1.1.49). The optimal culture conditions for high production yield of the enzyme were also reported. In particular, it was demonstrated that the production of the enzyme depended greatly on changes of pH of the culture medium. The growth of *L. mesenteroides* accompanied acid production in the medium which caused inhibition of the cell growth and denaturation of the enzyme resulting in decreased enzyme productivity. Thus, it was suggested that pH adjustment of the culture media during cultivation of the microorganism would improve the cell growth and production yield of the enzyme.

The present study demonstrates that glucose-6-phosphate dehydrogenase (G6PDH) decreases drastically as pH of the medium decreases to 4.2, whereas controlling pH of the medium at 7.0 increases greatly the total production yield of the

enzyme.

Figure 1 show the time course of cultivation of *L. mesenteroides* No. 20-2-10 in the AC medium <sup>(1)</sup> (initial pH 7.0) without adjustment of pH during the cultivation. The G6PDH productivity was closely associated with the log phase of the cell growth and the enzyme activity increased continuously to the maximum level even after the cell growth reached a plateau. The total production yield of the enzyme in the harvested cells decreased drastically while the total cell mass remained constant thereafter. No extracellular activity of the enzyme was found in the culture broth suggesting that all of G6PDH activity associated with the cell.

In general, the pH decrease of the culture broth of *L. mesenteroides* if caused by the formation of organic acids, especially lactic acid <sup>(2)</sup>. The residual glucose remained unconsumed at 0.2% level in the medium even after prolonged cultivation indicated an inefficient utilization of the carbon source which was due to inactivation of the



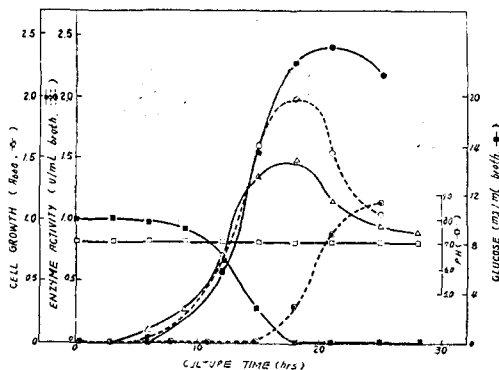
**Figure 1 :** Time Course of Cultivation of *Leuconostoc mesenteroides* No. 20-2-10 in AC Medium without pH Control during the Culture.

The microorganism was cultured in 1 l of the AC media<sup>(1)</sup> at 30°C after inoculation of 10 ml of a seed culture grown for 12 hrs.

- △—△ : Cell growth ( $A_{660}$ )
- : G6PDH activity (U/ml broth)
- : pH of the culture broth
- : Glucose remained in the media (mg/ml broth)

cell growth at acidic pH of the medium. The drastic decrease of the enzyme activity also appears to be caused by inactivation of the enzyme due to acidic pH of the medium. In fact, it was demonstrated that G6PDH deactivated completely at pH 4.2 for 30 min *in vitro* system.

This fact was enable us to adjust pH of the medium during the culture of this microorganism to bring about the higher production yield of the enzyme of interest. pH of the medium was adjusted to 7.0 with an automatic titrator during the course of cultivation. Figure 2 shows time course of the cultivation of *L. mesenteroides* with pH adjustment. As seen in the result, the cell growth was reciprocally associated with the decrease of glucose content of the medium until the nutrient was completely exhausted in the medium. The cell growth showed about 20% increase at the maximum stage with respect to that without pH control. The total G6PDH production reached a maximum stage at 21 hour cultivation while the intracellular G6PDH activity demonstrated 30% increase. The total G6PDH (intracellular and extracellular) increased 56% with respect to that without pH control.



**Figure 2 :** Time Course of Cultivation *Leuconostoc mesenteroides* No. 20-2-10 in AC Medium with pH Adjustment to 7.0 during the Culture.

The culture conditions were the same as Fig 1. except pH adjustment.

- △—△ : Cell growth ( $A_{660}$ )
- : Intracellular G6PDH activity (U/ml broth)
- ⊙—⊙ : Extracellular G6PDH activity (U/ml broth)
- : Total G6PDH activity (U/ml broth)
- : pH of the culture broth
- : Glucose remained in the media (mg/ml broth)

The extracellular enzyme, which was not observed in the previous experiment (Fig. 1), gradually increased with the concomitant decrease of the cell mass. The appearance of the extracellular G6PDH thus seems to be due to autolysis of the microorganism at neutral pH. In this case, the limiting factor for the production of G6PDH is likely to be the glucose content in the medium on which the cell growth is dependent.

In conclusion, controlling pH of the culture medium of *L. mesenteroides* can increase the production yield of G6PDH. It is suggested that the continuous culture of the microorganism with controlled pH as well as nutrients may be more beneficial for the increased production of G6PDH.

### Reference

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