트랜스케톨래이스가 유실된 바실러스 서브틸리스 SPK1의 유가식 배양을 이용한 자일로스에서 라이보스 생산

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Fed-batch fermentation of transketolase deficient *Bacillus subtilis* SPK1 to produce D-ribose from xylose

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Introduction

D-Ribose is a five-carbon sugar used to synthesize vitamin B2, nucleoside antiviral agents and nucleotide flavor enhancers. End products of chemical conversion with D-ribose and their roles in human body were well summarized in a review (De Wulf and Vandamme, 1997).

Transketolase is a metabolic enzyme involved in non-oxidative pentose phosphate (PP) pathway and creates a reversible link with transaldolase between glycolysis and PP pathway. If transketolase is deactivated, the five carbon sugars, xylose and arabinose which are metabolized by xylose and arabinose operon, respectively may be not used as a sole carbon source but accumulated as an intermediate, ribulose-5-phosphate or ribose-5-phosphate in PP pathway because of blocking of non-oxidative PP pathway. Biological production of D-ribose is carried out by the dephosphorylation of ribose-5-phosphate. It was reported that only *Bacillus* strains deficient in transketolase or D-ribulose 5-phosphate 3-epimerase produced large amount of D-ribose. Several *Bacillus subtilis* and *B. pumilus* strains were selected to accumulate D-ribose and characterized as transketolase mutants (Sasajima and Yoneda, 1971; De Wulf and Vandamme, 1997).

In this study, the fermentation processes in a transketolase deficient *B. subtilis* SPK1 were developed to produce D-ribose from xylose efficiently. Xylose is a component of lignocellulosic biomass reproduced with solar energy.

Materials and Methods

Bacterial strain

The D-ribose producing strain, *Bacillus* sp. was isolated in the highly concentrated sugar solution and donated by Borak Co. (Kyuonggi, Korea) and characterized as a transketolase deficient mutant like other D-ribose producing stains (De Wulf and Vandamme, 1997). Through the NTG and UV mutagenesis, *Bacillus subtilis* SPK1 was selected to consume the carbon sources faster than others.

Medium and Culture conditions

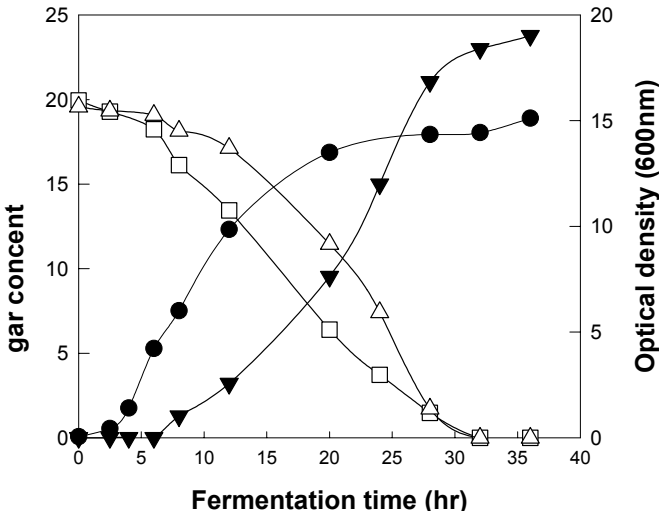
To produce D-ribose, all cultures were performed in a 3.7L jar-fermentor (Type ALF, Bioengineering AG, Sweden) at 37℃, 1vvm of air flow rate and 600rpm, containing 1.5L of a culture medium (10g/L of yeast extract, 5g/L of KH_2PO_4, 5g/L of K_2HPO_4, 1g/L of $MgSO_4 \cdot 7H_2O$ (De Wulf et al., 1996)) with 20g/L of glucose and 20g/L of xylose. After 20~28hrs of batchwise growth of the cells, the feeding solutions containing mixture of glucose and xylose were added into the culture broth continuously to maintain the level of carbon sources.

Assay of cell growth and concentration of carbohydrates

To determine cell growth, optical density of culture broth was determined with a spectrophotometer (Ultraspec 2000, Amersham Parmacia Biotech. AB., Sweden) at 600nm. Culture broth was harvested and the supernatant was taken and diluted appropriately to be analyzed. Concentrations of glucose, xylose and D-ribose in culture broth were determined with high performance liquid chromatography system (M930, Younglin Co., Korea). A 20μL of sample was injected into Aminex HPX-87H ion exclusion column (300mm×7.8mm, Bio-rad Co. U.S.A.) heated at 60℃ and analyzed with reflective index detector (Knauer Co., Germany).

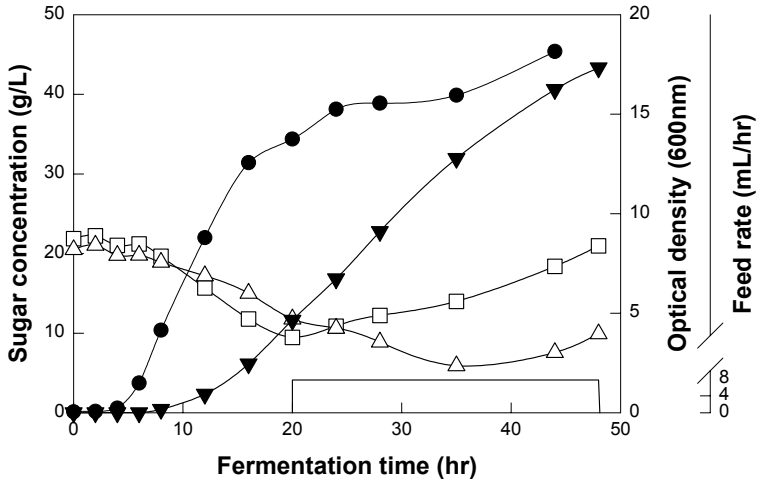
Results and Discussion

A typical profile of batch culture with *Bacillus subtilis* SPK1 using 20g/L of glucose and 20g/L of xylose was shown in the right figure. Cell mass(●) was increased exponentially in 20hrs and maintained to 36hrs. Glucose(□) and xylose(△) were consumed simultaneously and D-ribose(▼) was produced sharply from 7hrs to 36hrs. As a result, 15 of optical density and 24g/L of D-ribose concentration were acquired. Generally xylose is consumed after glucose is exhausted in a type of diauxic growth because of catabolite repression and selectivity of favorable sugar in universal microorganisms. But the transketolase deficient *Bacillus* subtilis SPK1 had an unusual behavior of co-utilization of glucose and xylose. It was coincided with the result of other transketolase mutant *Bacillus subtilis* strain which was probably due to the low intracellular level of fructose-1,6-biophosphate, a catabolite repression element (De Wulf et al., 1996). The same profile was found in the *Escherichia coli* mutant of phosphoenolpyruvate phosphotransferase system (Hernández-Montalvo et al., 2001). In addition, it was reported that *Bacillus subtilis* could not metabolize xylose effectively because of lack of the xylose specific transporter (Schmiedel and Hillen, 1996). As a result, it was supposed that the



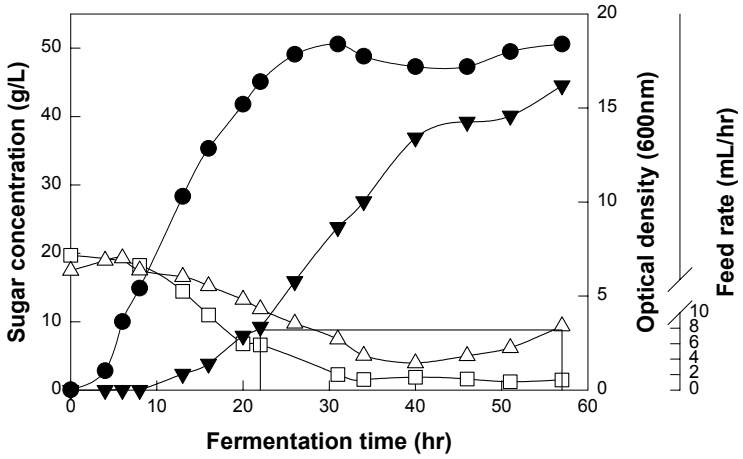
transketolase deficient *Bacillus* sp. lost some elements of catabolite repression in xylose operon and nonspecific xylose transport system was overproduced, for example AraE protein, an arabinose specific transporter regulated by catabolite repression factors, triggered the uptake of xylose in *Bacillus subtilis* (Krispin and Allmansberger, 1998).

To increase the performance of D-ribose production, fed-batch cultivations were carried with a mixture of xylose and glucose, and allowed to determine the point of feeding start. After 20, 24 and 28hrs of batch cultivation with 20g/L of xylose(△) and glucose(□), a sugar solution containing 200g/L of xylose and 200g/L of glucose was fed into culture broth continuously at a rate of 7.7mL/hr to maintain 1g/L-hr of sugar consumption rate(acquired from batch data). Carbon concentrations for the case of 20hr, 24hr and 28hrs were maintained 10g/L, 5g/L and basal level through the fed-batch fermentations, respectively. Upper figure showed the culture profile in which feeding was started after 20hr. Cell mass(●) was increased exponentially from 5hr to 20hr, and maintained or slightly enhanced through the fed-batch mode. D-ribose(▼) was produced from 8hr as a type of mixed-growth in the Luedeking-Piret equation, which production rate was maintained constantly until feeding was stopped. Finally, 18.2 of optical density and 43.5g/L of D-ribose were obtained in a 48hrs of fed-batch cultivation. In sugar consumption, there is an unusual phenomenon that the consumption rates of two sugars were reversed in fed-batch mode. Glucose and xylose were consumed at 0.62g/L-hr and 0.44g/L-hr, respectively in batch culture. After the feeding solution was added, consumption rate of glucose was maintained as same as batch mode, but xylose consumption rate was increased abruptly to 1.31g/L-hr, which was 3 times higher than that of batch mode. The enhancement of xylose consumption rate gave rise to high production rate of D-ribose, 1.2g/L-hr in fed-batch mode and gross productivity of D-ribose was obtained 0.9g/L-hr. Other fed-batch cultures viewed the same profiles. It is uncertain to make clear the reason of changing the xylose consumption rate but probably due to the direction of ATP produced from glucose to xylose utilization. Cell mass was constant in fed-batch mode but glucose consumption rate has the same value as batch mode. It seemed that a surplus of ATP not used for maintenance energy was conversed to xylose metabolism in a reaction of xylulokinase, an enzyme engaged in xylose operon, converses xylulose to xylulose-5-phosphate using ATP.



Upper fed-batch cultivation made a problem to remain high concentration of glucose and xylose, 20g/L and 10g/L, respectively. Another fed-batch fermentation was tried to reduce the residual sugar by changing the composition of feeding solution. After 20hrs of batch culture, a mixture of 200g/L of xylose and 50g/L of glucose was fed into culture broth continuously at a rate of 7.7mL/hr. Profile of

D-ribose production was shown in let figure. Cell mass was increased exponentially to 27hrs and kept constant. Glucose and xylose were consumed simultaneously in batch mode and maintained below 2g/L and 10g/L, respectively. Management of sugar concentration in low level gave the best result of D-ribose concentration of 46.6g/L and productivity of 1.4g/L-hr, which is a 2.3-and 1.5-fold increase compared with the simple batch fermentation, respectively.



Further study will be done to analysis the kinetics of D-ribose production from xylose.

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