Studies on Production of L-Tryptophan by Fermentative Cultivation

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ABSTRACT

Abstract L-tryptophan is not abundant in the natural world. It mainly exists in the food of animal type such as meat, egg, and milk. For the food of plant type, except potatoes, the content of L-tryptophan in grains like rice and wheat is scare. Four methods to produce L-tryptophan include hydrolysis of protein, chemical synthesis, enzymatic transformation and fermentation. In this study, fermentation is used to produce L-tryptophan. The yield of L-tryptophan may be affected by many factors such as the composition of media, carbon sources, organic and inorganic nitrogen sources, operating conditions such as temperature and pH, and batch or continuous. Corynebacterium glutamicum 21334 (CCRC 12511) was used to begin. Unfortunately, this strain produced no L-tryptophan. Mutant of this strain was then considered to improve the L-tryptophan yield. NTG (N-Methyl-N ’-nitro-N-nitrosoguani -dine) was then used to treat the original strain, and several potential strains were selected. Through a series of screening experiments, the mutant strains still showed no evidence to produce L-tryptophan. In other words, mutant didn ’t succeed in selecting a potential candidate for L-tryptophan production. Therefore, we decided to replace the strain with Brevibacterium flavum ATCC 21427 (CCRC 12509). The strain showed evidence to produce L-tryptophan after preliminary tests, so we used it to study optimal medium composition and culture conditions. The following three major components, carbon sources (glucose, fructose, and sucrose), organic nitrogen sources (yeast extract and peptone), and inorganic nitrogen sources ((NH4)2SO4 and NH4Cl), were considered. The pH value during batch fermentation was also explored. Experimental results showed that carbon and organic nitrogen sources had significant effect to the yield of L-tryptophan, and inorganic nitrogen sources had not. The combination of glucose and yeast extract could have the highest yield of L-tryptophan. Under a medium volume of 30 mL (flask volume 250 mL) and 30 °C, the concentration of L-tryptophan could reach 0.01 g/L after a cultivation of 96 h in a shaker of 150 rpm. In addition, this experiment also studied the effect of pH on the production of L-tryptophan. Three pH ’s (6.0, 7.0 and 8.0) were selected for testing. The results showed that the microbial growth and the L-tryptophan production were the best when the pH was set at 7.0. Furthermore, a fed-batch cultivation of Brevibacterium flavum ATCC 21427 was run with the following conditions ：seed 2％, temperature 30 °C, stirring rate 250 rpm, pH 7.0, working volume 2 L, and mass fraction of glucose around 4％. The concentration of L-tryptophan could reach 0.02 g/L under the above condition after a cultivation of 35 h. The concentration of glucose could almost remain constant if a proper control was imposed. Consequently, the microbial growth could sustain longer because a proper concentration of carbon source could maintain. Therefore, fed-batch cultivation may be a better way than a batch fermentation to produce L-tryptophan. Key words：L-tryptophan, mutant, fermentation, fed-batch cultivation

Keywords : L-tryptophan ; discontinuous fed-batch cultivation ; fermentation ; mutant

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