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Fed-batch cultivation for cell growth and spore production by probiotic *B. polyfermenticus* SCD

박규용, 이광호, 김성미, 김원석[1], 백현동
경남대학교 생명과학부, (주)바이넥스 기술연구소[1]
전화 (055) 249-2689, FAX (055) 243-8133

Abstract

The optimal temperature, pH and aeration rate for spore production by *Bacillus polyfermenticus* SCD in 500 ml shake flask and 5-l jar fermenter were found to be 32℃, 7.0 and 1.0 vvm, respectively. When batch culture processes was performed under optimized culture conditions, viable cells were 3.3×10^{10} CFU/ml and spore cells were 3.0×10^{10} CFU/ml. Fed-batch culture processes were also examined with regard to higer maximum viable cell and spore production. The highe viable cells and spores were obtained in 5-l jar fermenter at 72 h cultivation time by strategy in an intermediate feeding mode with 60% glucose solution 150 ml and 5% soybean flour solution 150 ml fed to the fermenter twice, and the productivity of spore cells was significantly increased. Finally, volumetric productivity of spore cells on fed-batch culture indicated 9.9×10^{8} CFU/ml/h, which was approximately 2 times higher than batch culture. Thus, fed-batch culture show a promise as an industrial production method.

Introduction

The emergence of antibiotic-resistant bacteria and natural ways of suppressing the growth of pathogens has contributed to the concept of probiotics. Probiotic bacteria is not only compete and suppress unhealthy fermentation in human intestine, but also produce a number of beneficial health effects of their own. *Bacillus polyfermenticus* SCD which is commonly called as Bisroot■strains, have been appropriately used for the treatment of long-term intestinal disorders, since the live strains in the form of active endospores can successfully reach the target intestine. Fed-batch cultivation is an effective method to enhance productivity compared with batch culture. Thus, fed-batch cultivation has exploited for *B. polyfermenticus* SCD to produce desired active spores at high volumetric productivity. The purpose of this study is to optimize the submerged culture conditions and to get higher spores of probiotic *B. polyfermenticus* SCD in fed-batch cultivation.

Materials and Methods

Producer strain *B. polyfermenticus* SCD was maintained in a vial-bottled containing 20% (v/v) glycerol at -70℃ for further preservation. Seed culture

broth was transferred to tryptic soy broth (TSB; Difco, USA) medium every 12 h for 2 day. Working cultures were propagated in sterile KH5 medium. KH5 medium as a main culture containing (g/l): glucose 20, starch 20, soybean flour 50, $CaCl_2$ $2H_2O$ 1, $(NH_4)_2SO_4$ 2, KH_2PO_4 10, $MgSO_4$ $7H_2O$ 0.3, $MnSO_4 \cdot H_2O$ 0.02, $CuSO_4$ $5H_2O$ 0.01, $ZnSO_4 \cdot 7H_2O$ 0.02, $FeSO_4 \cdot 7H_2O$ 0.02, were used in submerged cultures. The count of viable cells was performed by spread plate counting method on TSB agar plate in petri dish after had incubated at 37℃ for 24 h. The viable spores were also counted by the same method after heating for 10 min at 80℃. At various time intervals, samples were withdrawn and then the glucose concentration was determined by glucose analyzer (YSI Co., Yellow Spring, Ohio, USA). In order to investigate the effects of temperature on cell growth and spore production, *B. polyfermenticus* SCD was cultivated in KH5 media with different temperatures in 500 ml shake flasks with agitation speed 180 rpm for 60 h. In order to find a suitable pH and aeration rate, each fermentation medium was inoculated 2% (v/v) of the seed culture and then cultivated at 32℃ in the 5-l jar fermenter (KoBio Tech Co., Seoul, Korea) with different pHs and aeration rates. In these cases, agitation speed was 500 rpm. Batch culture was performed under optimized fermentation conditions in this study. Namely, batch culture was conducted under the following conditions: temperature, 32℃; aeration rate, 1 vvm; agitation speed, 500 rpm; pH, 7.0; working volume, 3 l. Fed-batch fermentation with an initial volume of 3 liter was also carried out in a 5-l jar fermenter with the same conditions of batch cultivation. When the glucose concentration in the medium reduced to about 2-5 g/l, we supplied the parpared medium solutions to the fermenter by strategy in an intermediate feeding mode. We fed the prepared feeding solutions in the fermenter twice and added soybean flour as a nitrogen source at the last experiment.

Results and Discussion

B. polyfermenticus SCD was cultivated at various temperatures (25-42℃), where the optimum temperature was found to be 32℃. Cell growth and spore production at 32℃ were increased about 118% (8.0×10^9 CFU/ml) and 130% (8.0×10^9 CFU/ml) compared with result of 37℃ incubation, respectively. In comparing the effects of pH on cell growth and spore production, this organism exhibited maximum number of viable cells (3.2×10^{10} CFU/ml) and spore cells (3.0×10^{10} CFU/ml) under pH 7.0-controlled condition. *B. polyfermenticus* SCD was cultivated at various aeration rate from 0.5 to 1.2 vvm in KH5 medium. The maximum number of spore cells (3.0×10^{10} CFU/ml) of this organism was shown at 1.0 vvm. This study showed that aeration rate of 1.0 vvm was sufficient for maximum respiration rate for sporulation of *B. polyfermenticus* SCD in fermentation process. The batch fermentation was performed using KH5

medium under optimized culture condition for 72 h. Figure 1 shows typical time courses of cell growth and spore production under optimal culture conditions in a 5-l jar fermenter. In this case, spores production was reached a maximum level of 3.0×10^{10} CFU/ml after 60 h, while maximum cells concentration indicated 3.3×10^{10} CFU/ml (Table 1). The viable cell growth of microorganism was significantly increased for 8 h, with corresponding depletion of glucose concentration. The residual glucose concentration rapidly decreased during the exponential period of the fermentation process. Two fed-batch fermentations were carried out to evaluate higher spore production than batch fermentation. In comparison to the batch fermentation, when the main fermentation culture was supplied two sources as a carbon and nitrogen, the fed-batch fermentation process gave much better results both for viable cells (8.4×10^{10} CFU/ml) and vegetable spores (7.1×10^{10} CFU/ml) in KH5 medium for 72 h (Table 1 and Fig. 3). Thus, it can be concluded that a fed-batch fermentation process is more effective for spore production, particularly when KH5 medium is used as the culture medium. In contrast, when feeding solution was fed alone as a carbon source (glucose solution) to enhance spore production at fed-batch fermentation, both results were not increased when compared with batch fermentation (Table 1 and Fig. 2).

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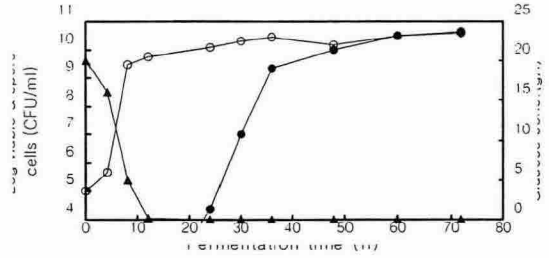

Fig. 1. Profiles of the batch fermentation on cell growth and spore production in the 5-L jar fermenter under optimal culture condition. ○, Log viable cells; ●, Log spore cells; ▲, Glucose concentration

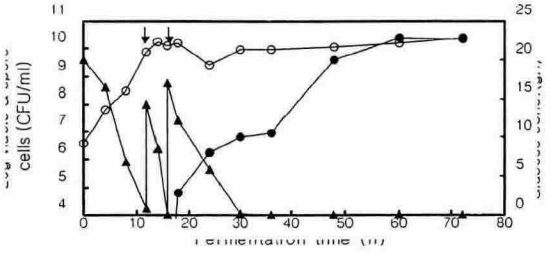

Fig. 2. Profiles of the fed-batch A fermentation on cell growth and spore production in the 5-l jar fermenter. ○, Log viable cells; ●, Log spore cells; ▲, Glucose concentration

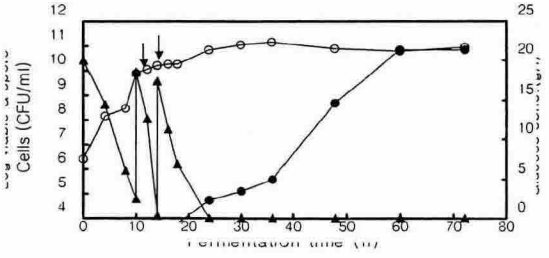

Fig. 3 Profiles of the fed-batch B fermentation on cell growth and spore production in the 5-l jar fermenter. ○, Log viable cells; ●, Log spore cells; ▲, Glucose concentration

Table 1. Comparison of fementation system with batch and two modes of fed-batch cultures for spore production of *B. polyfermenticus* SCD

Fermentation system	Maximum number of viable cells ($\times 10^{10}$ CFU/ml)	Maximum number of spore cells ($\times 10^{10}$ CFU/ml)	Volumetric spore productivity ($\times 10^{8}$ CFU/ml)	Sporulation (%)	Fermentation time (h)
Batch	3.2	3.0	5.0	94	60
Fed-batch A	2.4	2.3	3.2	96	72
Fed-batch B	8.4	7.1	9.9	85	72

A : with glucose solution (60%, 150 ml, twice), B : with A and soybean flour solution (5%, 150 ml, twice)