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Growth and Antioxidant Production of *Bacillus polyfermenticus* SCD in Whey Protein Concentrate (WPC)-based Medium

Gooi Hun Choi, Jang-Hyun Lee, Mi Na Jo, Yo-Chang Yoon, and Hyun-Dong Paik*
Division of Animal Life Science, Konkuk University, Seoul 143-701, Korea

유청단백질농축물을 기본 배지로 한 *Bacillus polyfermenticus* SCD균의 생육과 항산화물질 생산

최귀헌 · 이장현 · 조미나 · 윤여창 · 백현동*
건국대학교 동물생명과학부

ABSTRACT

The cell growth and antioxidant activity of *Bacillus polyfermenticus* SCD were studied in tryptic soy broth (TSB) medium and whey protein concentrate (WPC)-based medium. Overall, higher lactose contents in WPC-35 medium (up to 2.0%), and longer culture times correlated with greater cell viability. In WPC-35 medium with 1.5% and 2.0% lactose, the cell growth of *B. polyfermenticus* SCD was similar to growth in TSB medium. The 1,1-diphenyl-2-picyrylhydrazyl (DPPH) radical scavenging activity of culture supernatant of *B. polyfermenticus* SCD in WPC-35 medium was measured to assess antioxidant activity. The antioxidant activity increased up to 32 hr of culture, reaching a maximum of 75.57% DPPH radical scavenging activity. The antioxidant activity seemed to follow the typical kinetics of primary metabolite synthesis. The antioxidant activity of *B. polyfermenticus* SCD supernatant in WPC-35 medium was more effective and stable than supernatant from TSB medium. These results suggest that WPC-35 medium is effective for the production of antioxidant by *B. polyfermenticus* SCD.

Key words : *Bacillus polyfermenticus* SCD, antioxidant activity, whey protein concentrate

INTRODUCTION

Whey is a nutrient-rich dairy by-product from cheese preparation and contains 4–5% lactose, 0.8–1% proteins, minerals, trace amounts of vitamins and some small organic molecules. However, its proper disposal has been a major environmental problem. The continued growth of the cheese industry, the necessity for the reduction of pollutant in the effluent and the need to maximize returns on raw material have encouraged producers to seek new ways of using cheese whey. For example, the whey proteins are separated and used as food additives and the remains are spray-dried to produce sweet whey powder, which is widely used in the animal feed industry (Cladera-Olivera *et al.*, 2004; Mota *et al.*, 2006; Wu *et al.*, 2006). Glucose is somewhat expensive as a primary carbon source in synthetic media, however whey or UF whey permeate are relatively inexpensive and readily available sources for use as fermentation media. In addition, the use of whey in growth media solves environmental and energy problems related to its disposal (Bury *et al.*, 1998; Rech *et al.*, 2007)

Free radicals and radical-derived reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ion (O_2^-), and hydroxide radical (OH^-) can damage cellular proteins, lipids, and DNA. Antioxidants play a preventive role against diseases such as cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, hepatitis, liver injury, arthritis, immune deficiency diseases and aging by removing the ROS in biological system (Gale *et al.*, 2001; Lee *et al.*, 2000; Schmalhausen *et al.*, 2007; Seifried *et al.*, 2007).

Many synthetic antioxidants and natural antioxidants from

*Corresponding author : Hyun-Dong Paik, Division of Animal Life Science, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-2049-6011, Fax: 82-2-455-1044, E-mail: hdpaik@konkuk.ac.kr

various sources have been studied. However, the possible toxicity of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) has been a subject of concern for many years (Bandyopadhyay *et al.*, 2007; Fujisawa *et al.*, 2004). Therefore, a research has focused on the development and utilization of antioxidants from natural sources, particularly from fruits, seeds, herbs (Boo *et al.*, 2005; Kong *et al.*, 2004; Shon *et al.*, 2004), and many microorganisms (Amanatidou *et al.*, 2001; Kullisaar *et al.*, 2002).

Several studies have suggested that *Bacillus polyfermenticus* SCD is similar to *Bacillus subtilis* strains in terms of morphological and biochemical properties. However, *B. polyfermenticus* SCD is distinct from *B. subtilis* strains in that the former is capable of metabolizing lactose and produces larger amounts of acetic acid and lactic acid from glucose and lactose (Lee *et al.*, 2001).

In our previous studies, supernatant of *B. polyfermenticus* SCD showed potent antioxidant activities based on *in vitro* and *in vivo* models (Paik *et al.*, 2005; Park *et al.*, 2005).

The objective of this study was to evaluate the antioxidant activity of *B. polyfermenticus* SCD culture supernatant using whey medium to determine if this industrial residue could be successfully used in bacterial growth medium in order to increase productivity while reducing costs.

MATERIALS AND METHODS

Bacterial strain

B. polyfermenticus SCD was stored at -70°C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) containing 20% (v/v) glycerol.

Culture media and conditions

Inoculum was prepared in 100 mL of TSB medium as a working volume in 500 mL flasks. The temperature was maintained at 37°C and the agitation speed was 150 rpm for 6 hr. A 2% inoculum from the culture was added to 500 mL flasks containing 200 mL of WPC-35 medium (Table 1). The lactose concentrations of each WPC-35 medium tested were 0.5, 1.0, 1.5, and 2.0%. The growth and antioxidant activities were monitored for a 36 hr period at 37°C under shaking condition (150 rpm).

Table 1. Composition of WPC-35 medium

Ingredient	Content (%)
Lactose	51.4
Fat	5.2
Protein	35.7
Ash	7.7

Jar fermentor cultivation

The preculture for jar fermentor cultivation was prepared as follows: 150 mL of TSB medium in a 500 mL baffled flask was prepared and then sealed with a silicon stopper. The preculture was incubated at 35°C and 150 rpm for 9 hr. The main culture consisted of 3 L of WPC-35 medium with a lactose concentration of 1.5% and a 3% inoculums from the preculture incubated in a 5 L Jar fermentor. The agitation speed was 500 rpm and the aeration rate was 1 vvm. Antiform agent (LS-300) was added automatically whenever necessary.

pH experiments were divided into two trials: one maintained at 7.0 ± 0.1 by adding sterile 3 N HCl and 3 N NaOH, and the other set at 7.0 initially without pH-control during the culture.

Scavenging effect on DPPH radicals

The antioxidant activity of *B. polyfermenticus* SCD was measured in terms of radical scavenging activity, using the DPPH method (Lee *et al.*, 2001). Culture broth from the jar fermentor was centrifuged at 20,760×g for 20 min at 4°C and the supernatant was filter-sterilized by passing through 0.45 μm cellulose acetate membrane. One milliliter of 100 μM DPPH ethanol solution was added to 0.2 mL of sample supernatant. The reaction mixture was shaken and incubated for 30 min at room temperature and the absorbance was read at 528 nm using a spectrophotometer (Optizen 2120UV plus, Mecasys Co., Ltd., Korea). The absorbance values were converted into percent antioxidant activity using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{\text{absorbance of sample at 528 nm}}{\text{absorbance of control at 528 nm}}\right) \times 100$$

Viable cells counts

Viable cells were counted using spread plates with TSB agar. The plates were incubated at 37°C for 24 hr.

RESULTS AND DISCUSSION

Effect of lactose concentration in WPC medium

Fig. 1 shows the effect of lactose concentration on the growth of *B. polyfermenticus* SCD in WPC-35 medium. Based on lactose concentrations of 0.5, 1.0, 1.5, and 2.0%, higher lactose contents and longer culture times correlated

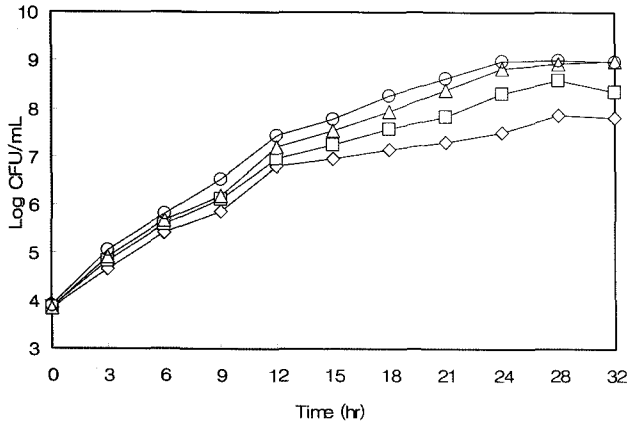

Fig. 1. Effect of various lactose concentrations in WPC-35 medium on cell growth of *B. polyfermenticus* SCD. Symbols: ○, lactose 2.0%; △, lactose 1.5%; □, lactose 1.0%; ◇, lactose 0.5%.

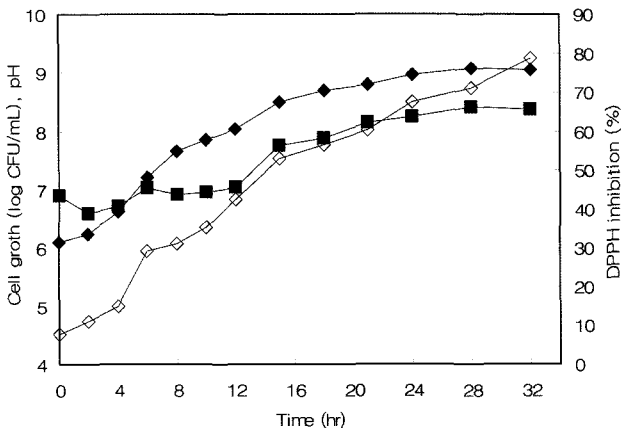

Fig. 2. Cell growth and antioxidant production by *B. polyfermenticus* SCD in a 5 L jar fermenter with WPC-35 medium. Symbols: ◆, viable cells (log CFU/mL); ◇, DPPH inhibition (%); ■, pH.

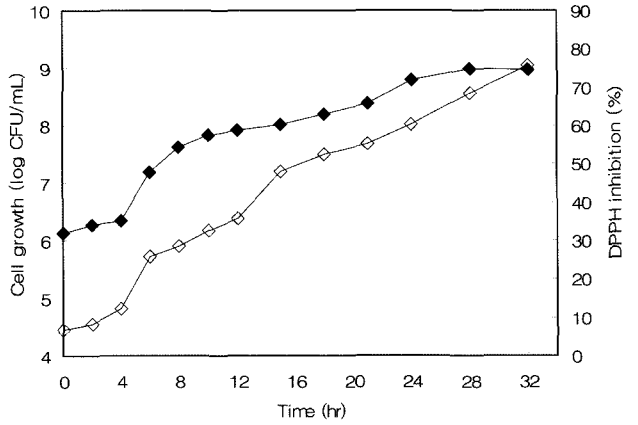

Fig. 3. Cell growth and antioxidant production by *B. polyfermenticus* SCD in a pH 7.0-controlled 5L jar fermenter with WPC-medium. Symbols: ◆, viable cells (log CFU/mL); ◇, DPPH inhibition (%).

with greater cell viability. However, with 1.5% and 2.0% lactose, the viable cell counts were not significantly different. Therefore, WPC-35 medium with 1.5% lactose contents was used to confirm the effect of jar fermentor medium on antioxidant production by *B. polyfermenticus* SCD.

Lee *et al.* (2002) reported that the highest growth of *B. polyfermenticus* SCD was about 8.2 CFU/mL at 12 hr in TSB medium, however based on our experiments, the highest growth of *B. polyfermenticus* SCD was about 9.0 CFU/mL at 28 hr in WPC-35 medium (1.5 and 2.0%).

Antioxidant production in pH uncontrolled fermentation

Free radical elimination is an important antioxidant mechanism. The scavenging of stable DPPH free radicals can be used to measure antioxidant activity in a relatively short time compared to other methods. To demonstrate the antioxidant capacity of culture supernatant of *B. polyfermenticus* SCD in WPC-35 medium, the DPPH assay, which measures proton-radical scavenging activity, was employed.

During pH-uncontrolled fermentation (initial pH of 7.0), the culture supernatant pH in the first three hours decreased to 6.6 in WPC-35 medium, after which it increased to 8.4 (Fig. 2). This results indicate that *B. polyfermenticus* SCD grows well and produces lots of antioxidant substances at weak-alkali conditions. These results are consistent with the results of Lee *et al.* (2002).

Antioxidant production in pH 7-controlled fermentation

Analysis of culture supernatant with the pH fixed at 7.0 ± 0.1 showed that cell viability and antioxidant activity increased with cultivation time (Fig. 3). The antioxidant activity increased for up to 32 hr, reaching a maximum of 75.57% DPPH radical scavenging activity. Antioxidant activity seemed to follow the typical kinetics of primary metabolite synthesis. The rate of growth in TSB medium was higher than in the WPC-35 medium, though the antioxidant activities were similar. However, the antioxidant activity in TSB medium suddenly dropped after reaching its highest levels while WPC-35 medium maintained high levels of antioxidant activity after reaching a maximum.

pH 7-uncontrolled *B. polyfermenticus* SCD fermentation was more effective for antioxidant production compared to growth with pH control.

In conclusion, the present work shows that antioxidant production by *B. polyfermenticus* SCD in WPC-35 medium is more effective and stable than in TSB medium. Further studies are needed to purify and characterize the antioxidant

substances in WPC-35, and to investigate the use of inexpensive whey-based industrial media with other microorganisms.

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