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Improved production of carotenes from synthetic medium by *Blakeslea trispora* in a bubble column reactor

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ABSTRACT

The carotene production from a synthetic medium sterilized at initial pH 11.0 by *Blakeslea trispora* in a bubble column reactor was investigated. Under the above conditions the fungus formed compact aggregates (pellets). As a consequence, a mild oxidative stress in *B. trispora* was occurred indicated by the specific activities of superoxide dismutase (SOD) and catalase (CAT). The oxidative stress resulted in a significant increase in carotene production and a change in the biosynthesis of carotenes. The highest concentration of carotenes (85.0 mg/g dry biomass) was obtained after 8 days of fermentation. In this case, the proportion of β-carotene, γ-carotene, and lycopene was 52.42%, 45.43%, and 2.15%, respectively. Bubble column reactor has a potential for carotene production from synthetic medium sterilized at initial pH 11.0.

1. Introduction

Carotenes are highly unsaturated isoprene derivatives. Naturally occurring carotenes are tetraterpenoids consisting of eight isoprene residues. Carotenes are used as antioxidants to reduce cellular or tissue damage and as coloring agents for food products, such as margarine, butter, cheese, juices, drinks and baked goods [1]. They are produced primarily by plants, fungi, yeasts, and some species of bacteria, algae and lichens. The greatest yield has been obtained from *Blakeslea trispora*, a heterothallic Zygomycota with two mating types (termed "plus" and "minus") [2].

The production of β-carotene from synthetic media by *B. trispora* in shake flask culture has been described [3–6]. Mantzouridou et al. [7–9] studied the production of β-carotene from synthetic medium by *B. trispora* in stirred-tank fermentor using batch and fed-batch culture. Goksungur et al. [10] examined the production of β-carotene from beet molasses by *B. trispora* in stirred-tank and bubble column reactors. The production of lycopene by *B. trispora* in shake flask and stirred-tank fermenter has been studied [11–13]. Recently, in our laboratory, the oxidative stress and the morphological changes in *B. trispora* induced by enhanced aeration during carotene production from synthetic medium in a bubble column reactor were studied [14]. All the studies cited above reported on the production of carotenes from synthetic media with an initial pH of 5.5–7.5. Only one study is available on the production of β-carotene from synthetic medium sterilized under strong alkaline conditions (pH 10–12) [15]. This work reported on the production of β-carotene by *B. trispora* in shake flask culture and the authors did not explain in details the mechanisms by which the above medium affected fungus morphology. In addition, the maximum concentration of β-carotene reported was very low.

The bubble column reactor is one of the most promising devices for gas–liquid mass transfer and is being considered especially for fermentation processes. Bubble column reactor is an elongated non-mechanically stirred reactor with an aspect ratio of height/diameter through which there is a unidirectional flow of gases. Bubble column reactor compared to the conventional stirred tank fermentor has several advantages. There is no mechanical agitation, and the air bubbles forced through the air compressor are responsible for the induced turbulent liquid mixing and the accompanying mass transfer. The fermentation process can be controlled more easily. The required bulk mixing and mass transfer is more cost-effective and requires less energy [16,17]. The production of carotenes from synthetic medium sterilized at initial pH 11.0 by *B. trispora* in a bubble column reactor has not been investigated.

Continuing our work on the production of carotenes, the aim of this investigation was to enhance the production of carotenes from synthetic medium by *B. trispora* in a bubble column reactor. As the initial pH of the medium has an important role in submerged fermentations, the effect of substrate sterilized at pH 11.0 on carotene production, culture morphology and oxidative stress in *B. trispora* was investigated. This attempt drove us to the interest on this

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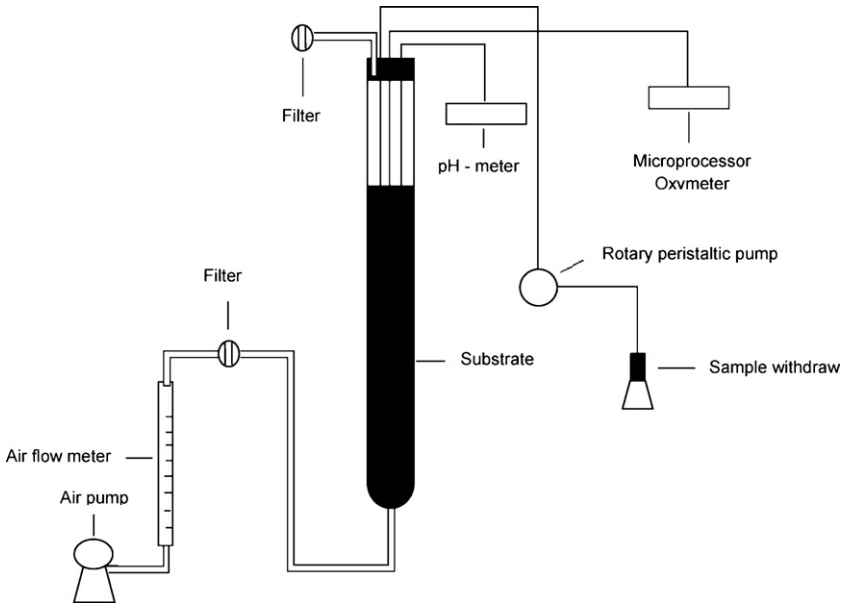

Fig. 1. Schematic diagram of a bubble column reactor used for carotene production from synthetic medium sterilized at initial pH 11.0 by *B. trispora*.

subject to explain the mechanism by which the above medium affected changes in culture morphology and oxidative stress in *B. trispora.*

2. Materials and methods

2.1. Microorganisms and culture conditions

The microorganisms used in this work were *B. trispora* ATCC 14271, mating type (+) and *B. trispora* ATCC 14272, mating type (−). Both strains were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA). The strains were grown on potato dextrose agar (PDA) (Scharlau, 01-483) Petri dishes at 26 °C for 4 days for sporulation. Ten millilitres of sterile distilled water were added to the Petri dish and the spores were collected by scraping off the medium surface. The spore suspension containing 5.0×10^5 and 1.0×10^6 spores/ml of the strains 14271 and 14272, respectively, was used to inoculate the medium.

2.2. Fermentation conditions

Fermentation was carried out in a 1.4-L glass bioreactor (height 60 cm, diameter 5.5 cm) with a working volume of 0.7-L (Fig. 1). The substrate consisted of (g/l): glucose (Scharlau, GL 0129) 50.0; corn steep liquor (Sigma, C-4648; sterilized at 121 °C for 30 min) 30.0; yeast extract (Scharlau, 07-079) 1.0; casein acid hydrolysate (Scharlau, 07-151) 2.0; L-asparagine (Sigma, A-8381) 2.0; KH_2PO_4 (Merck, 4873) 1.5; $MgSO_4.7H_2O$ (Merck, 5882) 0.5; thiamine·HCl (Sigma, T-4625) 0.005; linoleic acid (Sigma, L-1626) 20.0; and Span 80 (Sigma, S-6760) 10.0. The pH of the medium was adjusted to 11.0 with 10 N NaOH and then sterilized at 121 °C for 20 min. The initial pH of the medium dropped during sterilization from 11.0 to 7.6. After cooling, the medium was inoculated with 7.0 ml of the spore suspension of each strain of *B. trispora* (obtained as described above). The reactor was incubated at 26 °C in a controlled temperature chamber. The air was supplied from the bottom of the column with an air pump at rate of 4 vvm (2.8 l/min)[14]. Water entrainment due to the high specific air flow rate and long-term cultivation was carried out during fermentation. The water entrainment led to the concentration of the product. In order to overcome this problem, sterilized water was added into the medium using a peristaltic pump to maintain the volume of the medium constant during fermentation.

2.3. Analytical techniques

2.3.1. Determination of dry biomass, residual sugars, carotenes, and dissolved oxygen concentration

Every 2 days, fermentation broth was removed from the reactor and analyzed. Dry biomass was determined by filtration of the broth through a Whatman No 3 filter paper under vacuum. The mycelium was washed with distilled water until the filtrate was colorless. One gram of wet biomass was dried at 105 °C overnight. Residual sugars were measured as glucose in the filtrate as described by Dubois et al. [18]. The total carotenes were extracted from the cells with ethanol according to Roukas and Mantzouridou [19]. The carotenes produced were analyzed by high-performance liquid chromatography (HPLC) as described by Nanou et al. [14]. The pH of the fermentation broth was measured using a pH-meter. Dissolved oxygen concentration was determined with a microprocessor oximeter (OXI 96, WTW, Germany). The values were expressed as percentage of the initial level of saturation. The data are the average values ± SD of three independent experiments. Comparison of the means was assessed using the Duncan's multiple range test.

2.3.2. Analysis of the medium composition

The media with an initial pH of 7.5 and 11.0 were sterilized at 121 °C for 20 min. After cooling, the liquid was centrifuged at $5000 \times g$ for 10 min. The supernatant was used for the determination of residual sugars, fatty acids, and the Maillard reaction products (MRPs). The residual sugars were measured as described above. The fatty acids and the MRPs were determined by HPLC using as standard oleic acid (Sigma, O 1630), linoleic acid (Sigma, L 1626), and 5-(hydroxymethyl)-furfural (HMF) (Aldrich, H4, 080-7). HMF was used as standard because this is one of the characteristic substances of MRPs. The HPLC was performed with a P2000 pump, an AS3000 autosampler and a UV6000LP diode array detector. A Thermo HyPurity C18 column (150 × 4.6 mm, 5 μm particle size) thermostated at 30 °C was used. The diode array detector was operated at 285 nm. The mobile phase of 1% acetic acid:acetonitrile 90:10 (v/v) was eluted at a flow rate of 1.0 mL/min.