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Continuous antibiotic production by an immobilized cyanobacterium in a seaweed-type bioreactor

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Abstract

The filamentous cyanobacterium, *Scytonema* sp. TISTR 8208, which produces a cyclic peptide antibiotic, was cultivated for 20 d in a seaweed-type bioreactor containing anchored polyurethan foam strips. Cells immobilized onto the foam strips produced the antibiotic for only several days, and the secreted antibiotic disappeared very rapidly from the medium. Cells accumulated the antibiotic intracellularly in a growth-related manner, and secreted it in the stationary phase. Since the antibiotic has a stable physico-chemical nature, the cells seem to take it up and metabolize it. When continuous cultivation was attempted, stable production of the antibiotic was maintained in the bioreactor for 16 d at a dilution rate of 0.01 h^{-1}. Three times more antibiotic was produced in the continuous culture than in the batch culture by the 16th day.

Introduction

Carbon dioxide is a major greenhouse gas causing global warming. The recent development of technology enables carbon dioxide to be recovered from the emission gases of steam power plants by using alkanolamine. Efforts to recycle the large amounts of carbon dioxide that can be recovered have now acquired great urgency. We have been trying to produce useful materials, such as biodegradable polymer and antibiotics, using carbon dioxide as the sole carbon source. Since cyanobacteria can grow autotrophically using light energy and carbon dioxide, they should be ideal producers of valuable materials. We previously screened nine strains and five genera of cyanobacteria for antibiotic production, and a filamentous cyanobacterium, *Scytonema* sp. TISTR 8208, had the strongest activity against the bacteria tested. The cyanobacterium, which produced a peptide antibiotic with a broad spectrum, was immobilized in polyurethan foam and the optimal medium composition for the production of the antibiotic was determined (Chetsumon *et al.*, 1993). A seaweed-type photobioreactor was then constructed and the optimal physical conditions for batch culture were determined (Chetsumon *et al.*, 1994). Many attempts have been made to cultivate photosynthetic algae continuously for biomass production and wastewater treatment (e.g., Weiss *et al.*, 1985; Javanmardian *et al.*, 1992). Here, we report the continuous culture of *Scytonema* sp. TISTR 8208 in a seaweed-type bioreactor in an attempt to accomplish stable antibiotic production.

Materials and methods

Cultivation of cyanobacterium

Scytonema sp. TISTR 8208, which was obtained from the culture collection of the Thailand Institute of Scientific and Technological Research Center (TISTR), was inoculated into modified BGA medium (MBGA) (Chetsumon *et al.*, 1993) prepared in a 2-litre bottle, cultivated in an incubation room at 30 ± 1 °C, and continuously illuminated by cool-white fluorescent lamps at a light intensity of 150 μmol photon m^{-2} s^{-1}. The

culture was sparged with air containing 5% CO_2 at a flow rate of about 100 ml min^{-1} for agitation and supplying a carbon source. Cells were then inoculated into the bioreactor for batch and continuous cultures.

Batch culture

The seaweed-type bioreactor used is illustrated in Fig. 1. Details of the bioreactor dimensions were described previously (Chetsumon *et al.*, 1995). Cyanobacterial cells of about 1.6 g d. wt were inoculated into 2.3-L bioreactors containing 2.0 L MBGA medium. The bioreactors were incubated in a 30± 1 °C incubation room. The basal conditions of light illumination and flow rate of gas (air containing 5% CO_2) were 150 μmol photon m^{-2} s^{-1} and 500 ml min^{-1}, respectively, unless otherwise stated. A linear bank of fluorescent lamps was used on one side of the bioreactor. Cyanobacterial growth was measured as cell dry weight by the method previously described (Chetsumon *et al.*, 1993). Determination of the antibiotic concentration was done as already described (Chetsumon *et al.*, 1994).

Continuous culture

The cultivation conditions for the continuous culture were the same as in the batch culture. Sterile medium was introduced from the top into the bottom section of the reactor. The medium was fed at various dilution rates, controlled by a peristaltic pump (EYELA microtube pump MP-3, Tokyo Rikakikai Co., Ltd.). The overflow effluent from the reactor was collected in a sterile container. The whole reactor assembly was sterilized prior to fermentation and kept sterile throughout the operation period. A schematic diagram of the continuous system is shown in Fig. 1. Samples were collected at 24-h intervals and analyzed for their antibiotic concentration.

Results

Instability of antibiotic production in batch culture

Scytonema sp. TISTR 8208 was inoculated into the seaweed-type bioreactor and cultivated on anchored polyurethane foam strips under continuous illumination for 20 d. The antibiotic production and consumptions of nitrate and phosphate in the immobilization culture were examined (Fig. 2). Nitrate was consumed

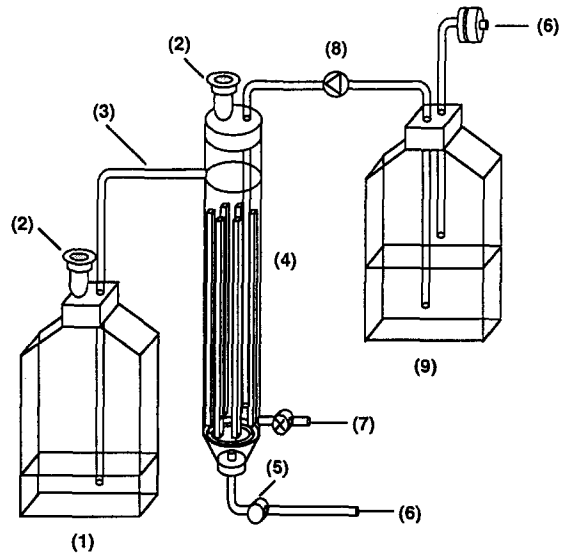

Fig. 1. Diagram of continuous culture apparatus. [(1), storage tank; (2), air outlet; (3), overflow tube; (4), seaweed-type photobioreactor; (5), air filter; (6), air inlet; (7), sampling port; (8), peristaltic pump; (9), feed tank].

completely within 9 days, while the phosphate level did not change after the second day of the culture. The antibiotic started to increase on the 8th day; an abrupt rise occurred on the 13th day, followed by a rapid drop in production on the 15th day. Next, the amounts of intracellular and extracellular antibiotic in a suspension culture were measured to analyse the instability of antibiotic production. A suspension culture was used in this experiment because it is very difficult to harvest cells in an immobilization culture at 24-h intervals. Cells were inoculated into a bioreactor that did not contain polyurethane foam strips and cultivated under the same conditions as those used in the immobilization culture (Fig. 3). Cells kept growing up to the 12th day, when they reached the stationary phase. The antibiotic was produced intracellularly in a growth-associated manner, while the extracellular antibiotic abruptly increased on the 14th day, and then decreased rapidly, in a similar manner to the pattern observed in the immobilization culture. The effect of medium exchange on the stability of antibiotic production was examined in an immobilization culture. Four seaweed-type bioreactors were set up under the optimal conditions for the production of the antibiotic. After the amount of extracellular antibiotic reached a peak in each bioreactor, medium 25, 50, 75, and 100% by volume was withdrawn from the four reactors respectively, and was replaced by the same volume of the

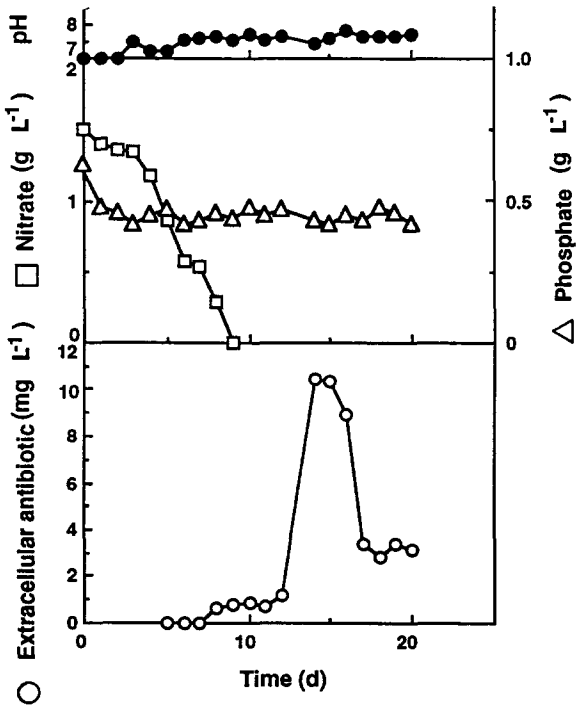

Fig. 2. Secretion of antibiotic by cells immobilized on polyurethane foam strips in batch culture. The amount of antibiotic is expressed as the volumetric production (mg L^{-1}) calculated as the weight of antibiotic per litre of medium. Culture conditions are described in text.

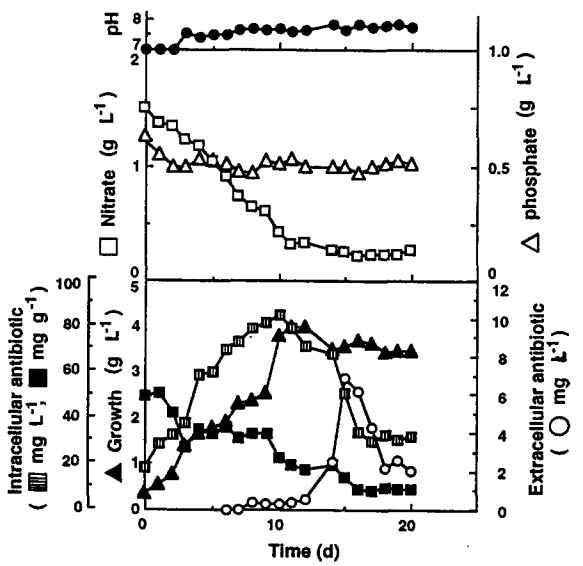

Fig. 3. Changes in intracellular and extracellular concentration of the antibiotic in suspension culture. The volumetric and specific production of antibiotic were calculated as the weight of antibiotic per litre of medium (mg L^{-1}) and per gram dry weight (mg g^{-1}), respectively. The culture conditions were same as those in Fig. 2.

fresh sterilized medium. No improvement in antibiotic production was observed in any of the four bioreactors (data not shown).

Continuous production of antibiotic

First, continuous production of the antibiotic was tried using MBGA medium at a dilution rate of 0.005 h^{-1}. Since a precipitate, probably calcium phosphate and/or magnesium phosphate, formed in the feed tank, the concentration of phosphate was reduced to 0.15 g L^{-1}. As shown in Fig. 2, the antibiotic was secreted after the depletion of nitrate. Therefore, the concentration of nitrate was also reduced to 0.6 g L^{-1}. The remaining chemicals were at half the strength of that used in the MBGA medium. The modified medium (FBGA) was then used for continuous cultures. Figure 4 shows the effect of the dilution rate on antibiotic production and the consumption of nitrate and phosphate. The reactor was operated at each flow rate until the productivity was stable, and maintained for at least 5 days to ensure the stability of the conditions. A dilution rate of 0.015 h^{-1} was too high for all the nitrate to be consumed, and the productivity was the lowest among the dilution tested. Cells consumed all the nitrate and showed the best antibiotic productivity at a dilution rate of 0.01 h^{-1}. Figure 5 shows the long-term stability of antibiotic production in the photobioreactor at a dilution rate of 0.01 h^{-1} in FBGA medium. The productivity was maintained constant for more than two weeks.

Discussion

Elucidation of the structure of the antibiotic produced by *Scytonema* sp. TISTR 8208 is now in progress. From an NMR analysis the antibiotic appears to be a novel cyclic peptide related to scytonemin A (Helms *et al.*, 1988). Cells accumulated a large amount of antibiotic intracellularly in the growth phase, most of which seemed to be metabolized by the cells for energy or as material for the synthesis of protein in the stationary phase, because the amount of the decrease was much more than that secreted into the medium. Since the antibiotic was very stable in the pH range 7.0–9.0 and under heat treatment at 70 °C for 2 h,

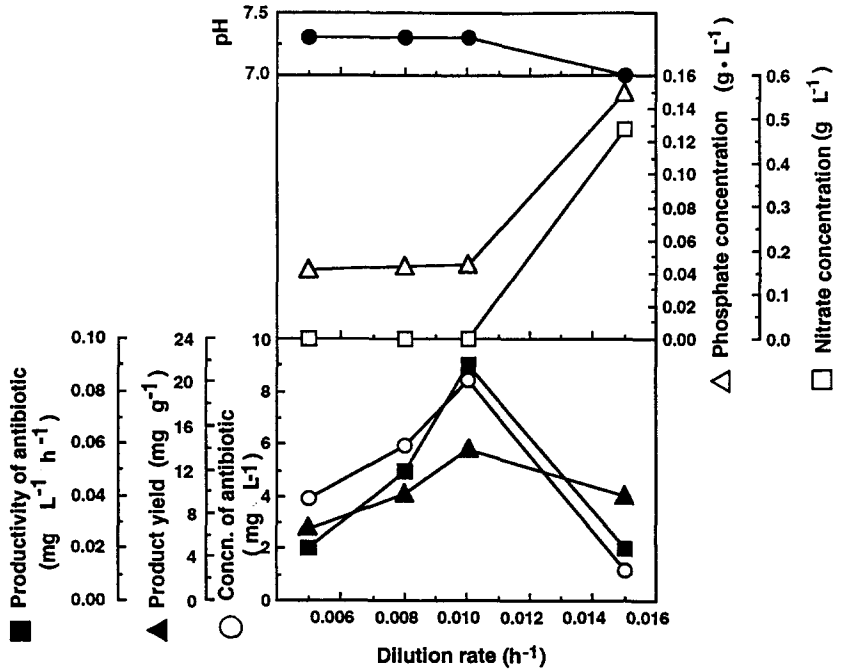

Fig. 4. Effect of dilution rate on antibiotic production in continuous culture. Concentration of antibiotic expressed as mass of antibiotic per litre of medium (mg L^{-1}); product yield expressed as mass per gram of utilized nitrate (mg g^{-1}); productivity is expressed as rate of production (mg L^{-1} h^{-1}). Culture conditions are described in text.

the extracellular antibiotic might also be metabolized by cells or degraded by extracellular protease after its abrupt increase in the medium. Continuous cultivation solved the problem of the instability of the antibiotic in the medium; continuous withdrawal of medium probably makes it difficult for cells to take up the antibiotic again. However, a high dilution rate of 0.015 h^{-1} decreased the productivity, presumably because the nitrate concentration remained too high. In batch culture, immobilized cells secreted the antibiotic after the complete depletion of nitrate in the medium. The nitrate concentration must thus be an important factor in regulating the secretion of the antibiotic into the medium; optimization of the nitrate concentration in the feed medium should increase the productivity of the antibiotic in continuous culture. The addition of an adequate concentration of detergent appears to be effective because a large amount of the antibiotic is accumulated intracellularly in the growth stage.

Continuous and batch cultures produced 66 and 20 mg of antibiotic, respectively, on the 16th day using the same seaweed-type bioreactor. Throughout the continuous culture period no cells became detached from the polyurethane foam strips. The seaweed-type bioreactor thus appears very promising for the recycling of carbon dioxide to produce valuable materials autotrophically using solar energy.

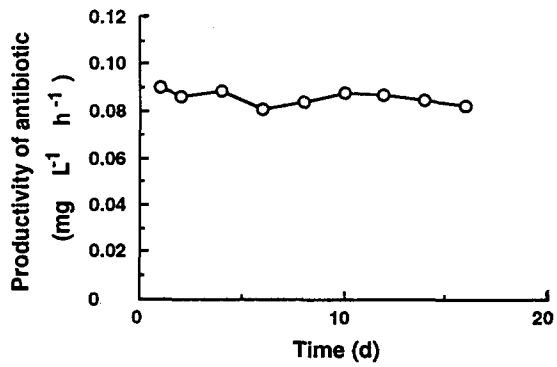

Fig. 5. Stable maintenance of antibiotic production in continuous culture. The dilution rate was 0.01 h^{-1}. Other conditions were the same as those in Fig. 4.

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