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Overproduction of Riboflavin by an *Arthrobacter* sp. Mutant Resistant to 5-Fluorouracil

Effects of pH and Dissolved Oxygen Concentration on Production of Riboflavin

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ABSTRACT

Effects of pH and dissolved oxygen concentration on batchwise riboflavin production by a 5-fluorouracil (5-FU)-resistant mutant of *Arthrobacter* sp. were investigated. The reaction was carried out in a jar fermentor. The optimal pH of culture medium was around 7.3. Dissolved oxygen concentration was almost constant during fermentation at 600 rpm of agitation rate. Production of riboflavin reached a maximum of 160 mg/L after 70 h fermentation under the agitation rate of 600 rpm, aeration rate of 1.0 L/min, and pH 7.0.

Index Entries: Riboflavin; *Arthrobacter* sp.; jar fermentor; pH; dissolved oxygen.

INTRODUCTION

Riboflavin is a water-soluble vitamin, and has been produced by chemical synthesis or some fungous fermentations (*1,2*). We described in the previous paper (*3*) that a 5-FU-resistant mutant of *Arthrobacter* sp. excretes riboflavin to

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the production medium in relatively high yields. In the study, we had found that the specific growth rate and the riboflavin concentration had decreased with increasing culture volume in a shaking flask. This article describes the effects of pH and dissolved oxygen concentration on a maximum specific growth rate and the riboflavin production in a jar fermentor.

MATERIALS AND METHODS

Microorganism and Culture Media

An *Arthrobacter* sp. mutant resistant to 5-FU, strain No. 28-35, isolated in our laboratory was used in the present study. A seed medium containing 0.5% glucose, 1% peptone, 1% yeast extract, 0.3% meat extract, and 0.05% NaCl was employed. Fermentation for the production of riboflavin was carried out in a culture medium containing 1% L-glutamic acid, 1% glucose, 0.5% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.1% yeast extract. Both media were sterilized by autoclaving for 10 min at 121°C before inoculation.

Production of Riboflavin

The mutant strain, No. 28-35, was cultured for 16–24 h at 30°C in a 500-mL shaking flask containing 30 mL of the seed medium. The inoculum was an amount of seed culture medium corresponding to 3% of working volume in fermentation.

Fermentation in the shaking flask was carried out for 96 h at 30°C under the shaking of 140 rpm and 7-cm stroke.

Fermentation in a 2-L jar fermentor (1.5-L working volume) was carried out for 96 h at 30°C under the agitation of 400 or 600 rpm and the aeration of 1.0 L/min. The pH of culture medium during fermentation was automatically controlled by additions of 1*N* HCl and 1*N* NaOH.

To determine cell growth and riboflavin concentration, a fermentation broth was diluted 10-fold with saline. The absorbance of the diluted broth was measured at 660 nm with a Milton Roy spectrophotometer (SPECTRONIC 21D). Cell weight was calculated from a calibration curve. Specific growth rate was calculated from cell concentrations. Riboflavin concentration of the supernatant of the diluted broth was determined by the absorbance at 450 nm. Riboflavin fermentation was evaluated by a maximum specific growth rate (μ_{max}) and a maximum riboflavin concentration (Y_{max}). Dissolved oxygen concentration (DO) in the culture medium was determined with an oxygen electrode in the jar fermentor.

Measurement of Oxygen Absorption Rate

Oxygen absorption rate was measured by the sodium sulfite method (4). The initial concentration of $Na_2SO_3 \cdot 7H_2O$ was 0.3*M*. Copper (II) sulfate pentahydrate was used as a catalyst at the concentration of $10^{-4}M$.

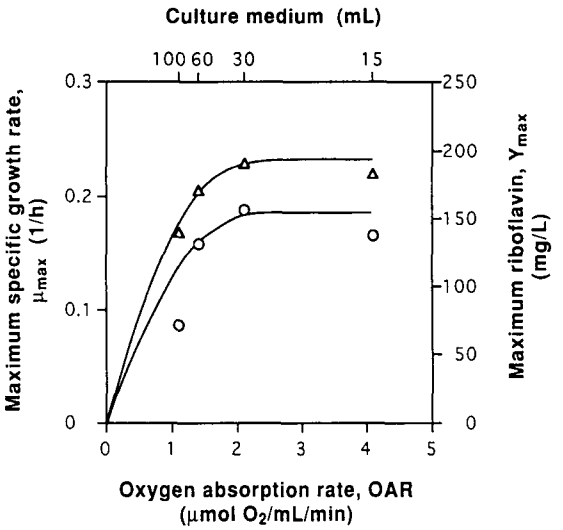

Fig. 1. Effects of oxygen absorption rate on maximum specific growth rate and maximum riboflavin concentration in the shaking flask. Symbols: △, maximum specific growth rate (μ_{max}) and ○, maximum riboflavin concentration (Y_{max}). The OAR values were quoted from ref. (5).

RESULTS AND DISCUSSION

Effects of pH and Culture Volume on Riboflavin Production in a Shaking Flask

The riboflavin production was carried out in the shaking flask, and the pH change was measured (3). The pH was scarcely lowered from the initial value of 7.0 in the early stage of the fermentation because calcium carbonate was contained in the culture medium. However, the pH began to increase after 24 h and reached about 8.0 after 96 h. The excretion of riboflavin continued even after the pH began to increase.

In the flask fermentation, it is well known that the culture volume often affects the cell growth and the production of the target material. It is because the oxygen absorption rate under a given agitation rate depends on the culture volume. The dependency of the oxygen absorption rate (OAR) in μmol O_2/(mL·min) on the culture volume (V_c) in mL had been determined separately as follows (5): $OAR = 24.3V_c^{-0.69}$. Figure 1 presents the dependencies of μ_{max} and Y_{max} on the oxygen absorption rate, that is, on the culture volume. Figure 1 shows that the cell growth and the riboflavin production depend on the oxygen absorption rate, and that at least 2 μmol O_2/(mL·min) of OAR are required to attain 150 mg/L riboflavin production. It is estimated from the OAR-V_c equation described above that the production level 150 mg/L can be attained under the culture volume of smaller than 37 mL.

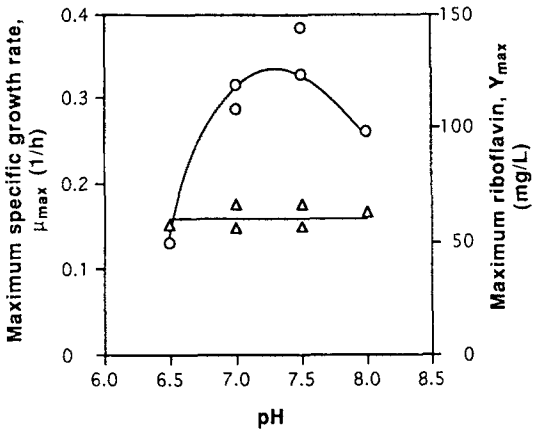

Fig. 2. Effects of pH on maximum specific growth rate and maximum riboflavin concentration in the jar fermentor. Symbols: △, maximum specific growth rate (μ_{max}) and ○, maximum riboflavin concentration (Y_{max}). Culture conditions: volume of culture medium, 1.5 L; agitation rate, 400 rpm; aeration rate, 1.0 L/min; pH, 7.0; and temperature, 30°C.

Effect of pH on Riboflavin Production in a Jar Fermentor

The riboflavin production was carried out in a jar fermentor with agitation of 400 rpm, where the pH of the culture medium was controlled at given values during the fermentation. The pH profiles of the riboflavin production and the specific growth rate are presented in Fig. 2. The value of μ_{max} did not vary between pH 6.5 and 8.0, but Y_{max} had a maximum around pH 7.3. On the basis of this result, riboflavin production was carried out under the pH 7.0.

Effect of Dissolved Oxygen Concentration on Riboflavin Production in a Jar Fermentor

The riboflavin productions were compared between the fermentations under the agitation of 400 and 600 rpm. The pH was controlled at 7.0. Figures 3 and 4 present the time-courses of DO, cell concentration, and riboflavin production. In the fermentation under 400 rpm as shown in Fig. 3, μ_{max} was 0.26 1/h. The DO decreased quickly in the early stage of the fermentation and hit a bottom at almost 0 ppm. The oxygen absorption rate under the agitation of 400 rpm was 0.4 μmol O_2/(mL·min). The agitation of 400 rpm is insufficient for the riboflavin production. The concentration of riboflavin obtained after 96 h fermentation was 93 mg/L, which was 62% of the maximum obtained in the fermentation by the shaking flask. On the other hand, in the fermentation carried out under 600 rpm, the DO was held almost constant during fermentation as shown in Fig. 4. The maximum specific growth rate was 0.26 L/h, and it was the

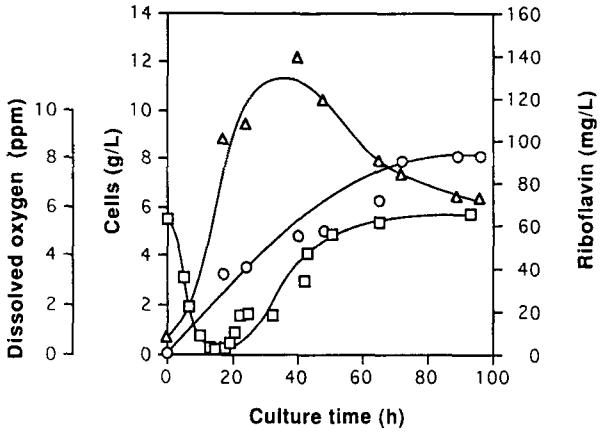

Fig. 3. Time-courses of riboflavin fermentation at 400 rpm of agitation rate. Symbols: □, dissolved oxygen concentration; △, cell concentration; ○, riboflavin concentration. Culture conditions: volume of culture medium, 1.5 L; aeration rate, 1.0 L/min; pH, 7.0; and temperature, 30°C.

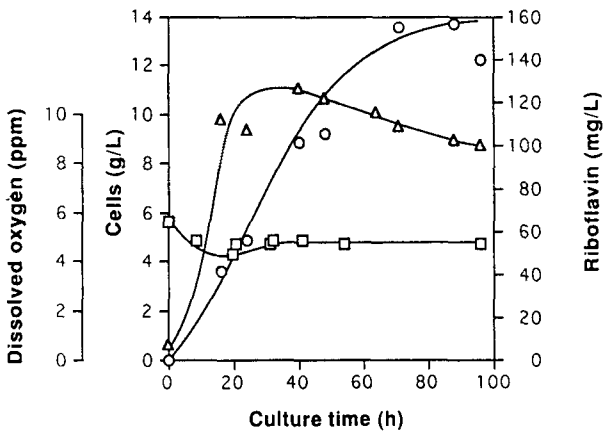

Fig. 4. Time-courses of riboflavin fermentation at 600 rpm of agitation rate. Symbols: □, dissolved oxygen concentration; △, cell concentration; ○, riboflavin concentration. Culture conditions: volume of culture medium, 1.5 L; aeration rate, 1.0 L/min; pH, 7.0; and temperature, 30°C.

same as that at 400 rpm. A high concentration of riboflavin, that is, 160 mg/L, was obtained after 70 h fermentation. It was a little larger than that obtained in the fermentation by the shaking flask. The oxygen absorption rate was 1.8 μmol O_2/(mL·min). The concentration, 160 mg/L, must be the maximum that can be obtained by controlling both pH and DO, since the riboflavin production did not increase under the agitation of above 600 rpm. Since glutamic acid was to be excreted out of cells under aerobic conditions (*6*) and also the accumulation amounts of riboflavin increased by adding detergent, which improved the permeability of cell membrane

(3), the higher agitation rate that is needed to achieve 160 mg/mL appears to improve the permeability barrier through the change of cell membrane.

Since the addition of metal ions or detergent resulted in the stimulation of riboflavin production in the flask experiments (3), we consider that Y_{max} in the jar fermentor would highly increase in the same manner. This concept is currently under investigation with the use of additional effector.

SUMMARY

The riboflavin production by the *Arthrobactor* sp. mutant was carried out by using a jar fermentor, and the optimal conditions in pH and DO for the production were investigated. The maximum production of riboflavin depended on both pH of a culture medium and DO at a logarithmic growth phase. Maximum production of riboflavin reached 160 mg/L under the optimal conditions: agitation rate of 600 rpm (oxygen absorption rate of 1.8 μmol O_2/[ml·min]), aeration rate of 1.0 L/min, and pH controlled at 7.0.

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