Studies on the Overproduction of Indole-Containing Metabolites by a Methanol-Utilizing Yeast, *Hansenula polymorpha*

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Abstract

Production of indole-containing metabolites ("indoles") from methanol has been studied using a mutant of *Hansenula polymorpha* resistant to 5-fluorotryptophan. Whereas the wild-type culture produces only a small amount of indoles, the mutant is partially deregulated and overproduces indoles. Indoles production was studied in batch and continuous culture and in a washed-cell system. When the pH was above 4.0, indoles production was growth-associated, in both minimal and complex media, and batch or continuous culture. When the pH was below or equal to 4.0, a low phosphate concentration was found to improve production. In a phosphate-deficient washed-cell suspension system, the addition of an amino acid such as methionine at 5 mM increased specific productivity by more than 60%. Addition of cycloheximide at 50 mg/L decreased residual growth and increased maximum productivity of indoles by more than 60%. When the antibiotic was added at 1000 mg/L, growth was completely inhibited and indoles production continued for about 35 h.

Index Entries: Methanol utilization, by the yeast, *Hansenula polymorpha*; indoles, overproduction, by a methanol utilizing yeast; fermentation, of a yeast for indoles production; metabolites, yeast produced indole-containing; yeast, production of indoles by; *Hansenula polymorpha*, production of indoles from.

Introduction

The objective of this work was to environmentally examine indoles production by the methanol-utilizing yeast, *Hansenula polymorpha*. Methanol has received at-
tention as a potential carbon-energy source for fermentations because of its relatively low cost and attractive biotechnological features (e.g., miscibility with water, reduced risks of contamination) (3, 9, 11).

An indoles-producing mutant isolated by Denenu and Demain (5) from the methanol-consuming yeast, *Hansenula polymorpha* (8), was used in this work. We used different culture conditions—batch, continuous culture, and a washed-cell system (WCS)—to study the effect of the environment on indoles overproduction.

### Materials and Methods

#### Organisms

The wild-type strain *H. polymorpha* DL-1 (ATCC 26012) (8) and a mutant strain (3-136) resistant to 5-fluorotryptophan (5) were used in this study.

#### Media

The minimal medium used in batch or continuous culture experiments contained the following compounds, in g/L: (NH₄)₂SO₄, 5.0; MgSO₄ · 7H₂O, 1.15; CaCl₂ · 2H₂O, 0.1; NaCl, 0.1; ZnSO₄ · 7H₂O, 0.0014; MnSO₄ · H₂O, 0.00084; FeSO₄ · 7H₂O, 0.00028; CuSO₄ · 5H₂O, 0.00025; Na₂MoO₄ · 2H₂O, 0.00024; CoCl₂ · 6H₂O, 0.00024; thiamine, 0.0004; biotin, 0.000002; and various amounts of KH₂PO₄ and methanol, as described below. Methanol and vitamins were added aseptically to the rest of the medium after the latter was adjusted to pH 5.5 (with NaOH) and autoclaved. Complex medium contained 0.5 g yeast extract/L of the above. The WCS medium (which was devoid of phosphate) contained the following in g/L: (NH₄)₂SO₄, 5.0; MgSO₄ · 7H₂O, 1.15; CaCl₂ · 2H₂O, 0.1; NaCl, 0.1; KCl, 0.1; ZnSO₄ · 7H₂O, 0.0014; MnSO₄ · H₂O, 0.00084; FeSO₄ · 7H₂O, 0.00028; CuSO₄ · 5H₂O, 0.00025; Na₂MoO₄ · 2H₂O, 0.00024; CoCl₂ · 6H₂O, 0.00024; thiamine, 0.0004; biotin, 0.000002; N-2-hydroxyethylpiperazine, N’-3-propanesulfonic acid (HEPPS buffer) 31.5 (125 mM final concentration). The initial pH was adjusted to 7.45. In one experiment, instead of HEPPS buffer, we used 2-(N-morpholino)-ethane-sulfonic acid sodium salt (MES buffer). Buffers and, when needed, methanol, amino acids, and antibiotics, were added aseptically to the rest of the medium.

Vitamins, amino acids, and antibiotics were sterilized by filtration. The remaining components were autoclaved at 120°C for 20 min.

#### Batch and WCS Cultures

Fifty-milliliter aliquots of medium in 250-mL Erlenmeyer flasks were inoculated with an exponential phase culture. The flasks were incubated at 37°C on a rotary shaker (New Brunswick Scientific Co., New Brunswick, NJ) at 220 rpm. For the WCS experiments, log-phase cells produced in batch cultures containing 66 mM phosphate and 10 g/L methanol were centrifuged, washed twice in two-fold con-