SHORT COMMUNICATIONS

A Continuous Culture Method for Obtaining 100% Triangular Cells of *Trigonopsis variabilis*

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INTRODUCTION

There is considerable variation in the time at which triangular cells develop during growth and in the percentage of triangular cells in populations of *Trigonopsis variabilis*. Methionine induces the highest percentage of triangular cells and this occurs either in early-stationary phase (Sentheshanmuganathan & Nickerson, 1962) or early-exponential phase (Šašek & Becker, 1969; Matthewson & Barnett, 1974). Matthewson & Barnett (1974) also observed that *T. variabilis* responded to different carbon sources by a change in cell shape, which indicates that the dimorphism of *Trigonopsis* cannot be accounted for by a single nutritional requirement. Several reports (Rambelli, 1959; Verona & Zardetto de Toledo, 1958; Picci & Verona, 1963; Packer & Bersten, 1977) describe the temperature dependence of triangular cell formation in diverse growth media in batch culture.

Growth and morphogenesis could therefore be studied more conveniently in chemostat culture which provides a means of altering one environmental condition at a time. The conditions of continuous culture which produce either all triangular cells or all ellipsoidal cells in methionine/glucose medium are described.

METHODS

Growth in continuous culture. *Trigonopsis variabilis* NRRL Y1579 was grown in a 200 ml fermenter (containing 100 ml medium) in which the temperature was maintained by heated water circulating through an outer jacket. The maximum temperature variation was $\pm 0.5^\circ\text{C}$. The sterile air supply was monitored by a flowmeter (Brooks Instrument, Veenendaal, Holland) and was kept constant at 3 l air min^{-1} (1 medium) $^{-1}$ (230 μM -oxygen concentration at 25°C). The vessel was autoclaved and attached aseptically to the medium reservoirs. Fresh medium was added by a variable-speed peristaltic pump (Buchler) and spent medium was removed continuously as a result of the positive pressure of the sealed culture vessel. The pH electrode (combined; Titron Industries, Victoria, Australia) and the O_2 electrode (Clark type; Titron Industries) were sterilized by immersion in 3% (w/v) formaldehyde for 30 min followed by thorough washing with sterile water and exposure to ultraviolet light for 3 h. The initial pH of the medium was 6.0 and the steady state pH was 5.6 ± 0.2 .

Glucose and DL-methionine were added to the basal medium of Sentheshanmuganathan & Nickerson (1962) at 2.0% (w/v) and 1.8 mM, respectively, for nitrogen-limited growth and at 0.2% and 18 mM for carbon-limited growth. With 0.2% glucose in the inflowing medium, no glucose was detected in the effluent medium by glucose oxidase assay. DL-Methionine in the basal salts medium without glucose did not support growth, therefore 2.0% glucose was used for carbon-limited growth. The inoculum was grown for 48 h in the culture vessel in the medium which was to be used for the experiments before fresh medium was metered into the vessel. The culture was assumed to be in steady state when the cell number remained constant for five doubling times. Cell number was measured using a haemocytometer (Spencer bright line) and was plotted against the absorbance ($A_{700}^{1\text{ cm}}$) of the cell suspension. The estimate of triangular cell number was determined on an average total of 250 cells counted on a wet mount slide at a magnification of $\times 1200$.

Table 1. *Growth of Trigonopsis variabilis in chemostat culture with methionine or (NH₄)₂SO₄ as nitrogen source and glucose as carbon source*

Nitrogen concn* (mM)	Glucose concn† (% w/v)	Growth rate (h ⁻¹)	Growth temperature (°C)	Oxygen concn (μM)	Triangular cells (%)
Methionine					
18	0.2	0.075	25	230	100
18	0.2	0.075	30	230	0
18	0.2	0.05	25	230	0
18	0.2	0.05	30	230	0
18	0.2	0.075	25	32	0
1.8	2.0	0.075	25	230	0
1.8	2.0	0.075	30	230	0
(NH ₄) ₂ SO ₄					
20	0.2	0.075	25	230	0

* Methionine was limiting at 1.8 mM and in excess at 18 mM.

† Glucose was limiting at 0.2% and in excess at 2%.

RESULTS AND DISCUSSION

Excess methionine (18 mM) in the medium with 0.2% glucose as the limiting carbon source maintained the cells in a steady state of growth at a constant cell number of 1.3×10^8 to 1.7×10^8 cells ml⁻¹. Dilution rates (growth rates) of 0.05 to 0.08 h⁻¹, equivalent to doubling times of 14 to 8.5 h, could be obtained under these conditions. Only one set of experimental growth conditions, viz. a growth rate of 0.075 h⁻¹ at 25 °C and 230 μM-oxygen concentration, resulted in the production of a 100% triangular cell population in a carbon-limited culture (Table 1). Increasing the temperature to 30 °C at the same growth rate and oxygen concentration caused a drop from 100% to 0% triangular cells in approximately three doubling times.

A decrease in growth rate at either 25 or 30 °C resulted in the formation of 100% ellipsoidal cells. The flow rate of air was adjusted to maintain an oxygen concentration in the medium of 230 μM. Changing the gas mixture from air to 5% (v/v) oxygen in nitrogen reduced the oxygen concentration to 32 μM and only ellipsoidal cells were formed (Table 1).

When the organism was grown in methionine/glucose medium in which the methionine concentration (1.8 mM) was limiting and the concentration of glucose (2%) was in excess, only ellipsoidal cells developed. Ellipsoidal cells also formed at 100% yield when ammonium sulphate replaced methionine in medium containing 0.2% glucose (limiting carbon source) and growth occurred at the same rate, temperature and oxygen tension which produced 100% triangular cells in methionine/glucose medium. Therefore, under the same conditions of culture used to induce triangular cells in methionine medium, ammonium sulphate did not produce them (Table 1). The ability to obtain homogeneous populations of *T. variabilis* reproducibly, either as ellipsoidal or triangular forms in the same medium, eliminates the additional complications resulting from growth on different carbon and nitrogen sources, and will further aid investigations into the dimorphism of *Trigonopsis*.

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