Note

Relation between Neutral Lipid Accumulation and the Growth Phase in the Yeast, *Lipomyces starkeyi*, a Fat Producing Yeast

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The yeast, *Lipomyces starkeyi*, is a fat producing yeast and can accumulate a large amount of neutral lipid intracellularly.¹⁾ Physiological studies have led to the prediction that the intracellular lipid content of the fat producing yeast is governed by the progress of the growth phases in a batch culture.^{2~4)} But there have been few detailed studies on the growth in relation to the initiation of and the rate of accumulation of the neutral lipid in the fat producing yeast.

Lipomyces starkeyi IAM 4753 was grown in a glucose (3%)-mineral medium based on the defined medium of Wickerham.⁵⁾ The medium was supplemented with 2 μ g of D-biotin (Daiichi Pure Chemical Co. Ltd., Tokyo, Japan) per liter for its growth requirement.⁶⁾ Five hundred-ml shaking flasks, each containing 100 ml of this medium, were plugged with cotton and autoclaved for 10 min at 115°C. One ml of a suspension in aseptic distilled water of log-phase cells, grown in the same medium, was used to inoculate each flask. Cells were cultured aerobically at 30°C.⁶⁾

At suitable time intervals, samples (usually 5 ml) were taken, and the cell density of the yeast was determined by direct microscopic counting using a counting chamber.⁶⁾ The neutral lipid content of the cells was determined by the method described in the previous paper.⁷⁾ Glucose in the medium was determined enzymatically with a diagnostic glucose assay kit (Glucose C-Test, Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). The dissolved oxygen concentration was measured with an oxygen electrode (Type 7606, Denki Kagaku Keiki Co., Ltd., Tokyo, Japan). All chemicals, except for D-biotin, were of the highest grade available from Wako Pure Chemical Industries Co., Ltd., Osaka, Japan.

The cell density in Fig. 1A is plotted in the usual arithmetic scale. The first 40 hr of the culture, therefore, correspond to the logarithmic phase of growth. After the

logarithmic phase, cell growth gradually decreased (retardation phase).³⁾ In the first stage of the retardation phase, cells grew almost proportionally with culture time (linear growth phase).⁸⁾ Cell growth, then, slowed down in the second stage of the retardation phase and finally reached a stationary phase.

In the logarithmic phase, the cellular neutral lipid content was low and almost constant. The lipid content began to increase in the linear growth phase and increased markedly in the second stage of the retardation phase. As long as glucose remained in the medium, the neutral lipid content continued to increase even in the stationary phase of growth. The neutral lipid accumulation rate was calculated from the data in Fig. 1A. The accumulation rate was accelerated in the linear growth phase, and reached the highest level in the second stage of the retardation phase, and decelerated after that (Fig. 1B).

In general, the linear growth phase appears when dissolved oxygen is inadequate for the growth of microorganisms. This is attributed to the diffusion velocity of oxygen from the gaseous phase to the liquid medium.⁸⁾ In the early stage of the retardation phase, the oxygen level in

FIG. 1. Variation Profiles of Cell Density, Cellular Neutral Lipid Content, Residual Glucose in the Medium, and Neutral Lipid Accumulation Rate in the Course of a Batch Culture.

Lipomyces starkeyi IAM 4753 was cultured aerobically in a simple defined medium at 30°C. Cell density (O) was determined under a microscope and glucose (Δ) was determined enzymatically. Neutral lipid content (□) of the cells was determined by the method reported previously (see ref.⁷⁾).

FIG. 2. Variation Profiles of Oxygen Concentration and Cell Density in the Simple Defined Medium during the Growth of *Lipomyces starkeyi* IAM 4753.

Dissolved oxygen concentration (solid line) was measured with an oxygen electrode. The dotted line indicates cell density. Other experimental conditions were the same as in Fig. 1.

the medium decreased to about a half of that in the logarithmic phase (Fig. 2). These facts support the assumption that a limiting level of dissolved oxygen in the medium or similar related conditions may cause a change in the metabolic direction of *L. starkeyi*, and the metabolic change may result in the initiation of neutral lipid accumulation in the linear growth phase.

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