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Journal of Biotechnology 122 (2006) 285–292
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Effect of nitrogen limitation on the ergosterol production by fed-batch culture of *Saccharomyces cerevisiae*

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Received 7 September 2005; received in revised form 7 November 2005; accepted 25 November 2005

Abstract

The diversity and content of available nitrogen sources in the growth medium both are very important in the accumulation of ergosterol in the yeast cell membrane. Growth on the good nitrogen sources such as ammonia can harvest more yeast cells than on poor ones, but ergosterol content in those yeast cells is relatively lower. Ergosterol content, one of the most variable parameters in ergosterol production by yeast cultivation, is greatly influenced by nitrogen limitation. The aim of our work was to study how the nitrogen sources affected the membrane ergosterol content and increase the total ergosterol yield. On the premise of keeping high ergosterol content in yeast cell, the ergosterol yield was enhanced by increasing the yeast biomass. Direct feed back control of glucose using an on-line ethanol concentration monitor was introduced to achieve high cell density. Ammonia, which acted as nitrogen source, was added to adjust pH during fermentation process, but its addition needed careful control. Cultivation in 5 L bioreactor was carried out under following conditions: culture temperature $30 \pm 1\ ^{\circ}C$, pH 5.5 ± 0.1, agitation speed 600 rpm, controlling ethanol concentration bellow 1% and controlling ammonium ion concentration bellow 0.1 mol/L. Under these conditions the yeast dry weight reached 95.0 ± 2.6 g/L and the ergosterol yield reached 1981 ± 34 mg/L.

Keywords: Ergosterol; *Saccharomyces cerevisiae*; Nitrogen; Fed-batch culture; High cell density

1. Introduction

Sterols are essential structural components of eukaryotic cell membranes. Ergosterol, the main sterol in yeast, is responsible for structural membrane features such as integrality, fluidity, permeability and activity of membrane-bound enzymes (Parks and Casey, 1995). Additionally, it is also an important pharmaceutical intermediate and a precursor of vitamin D_2 (Arnezeder and Hampel, 1990). In yeast, free ergosterol is incorporated in plasma membrane. Ergosterol and some other sterols can be stored as fatty acid esters in lipid particles (Zinser et al., 1993). Ergosterol is commercially produced by yeast cultivation, and opti-

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doi:10.1016/j.jbiotec.2005.11.020

mizing the cultivation process is therefore significant for manufacturing of vitamin D_2 and cortisone.

Nitrogen sources are very important chemical compounds in growth medium. Ammonia is one of the main nitrogen sources in laboratory-scale fermentation. Once inside yeast cells ammonia can react with α-ketoglutarate to produce glutamate and then react with glutamate to produce glutamine (Magasanik and Kaiser, 2002). Glutamate together with glutamine plays an essential role in nitrogen metabolism in microorganism and can be converted into other nitrogen containing compounds (ter Schure et al., 2000). Nitrogen content in medium has great influence on the synthesis of yeast biomass and the accumulation of ergosterol. Growing on the medium of sufficient nitrogen can achieve high yeast biomass but ergsoterol content is relatively low, since the proportion of nitrogenous compounds in yeast cells is about 50 wt.% (Torija et al., 2003). Ergosterol is not a nitrogenous compound and its content in yeast cell is mainly influenced by nitrogen limitation, culture conditions, and growth rate. The ergosterol content increased when the specific growth rate decreased (Arnezeder and Hampel, 1990).

The total yield of ergosterol per volume medium during the cultivation process is mainly determined by the yeast biomass and ergosterol content in yeast cell. In order to improve the ergosterol yield, it is effective to increase the biomass while keeping relatively high ergosterol content. Fed-batch culture is generally applied to achieve more efficient production of proteins or other intracellular products with *Saccharomyces cerevisiae* (Hensing et al., 1995). Gao introduced a DO-control pulse fed-batch method in which pulse feeding was performed according to the DO value, and the total yield of ergosterol reached 1064 mg/L (Gao and Tan, 2003).

In our study, a detail research about the effects of nitrogen sources on ergosterol production by *S. cerevisiae* (Y-E-1) was carried out, and the results showed that high concentration of ammonium ion inhibited the accumulation of ergosterol in yeast cell. Since the high concentration of ethanol during cultivation process was unfavorable for the growth of yeast cells, controlling ethanol concentration by feedback control of glucose feeding rate in a 5 L stirred-tank fermentor during cultivation process was applied to increase the biomass. Controlled by an ammonia probe, ammonia was added to adjust pH, also acting as nitrogen source in the cultivation process. Carried out in such controlled conditions, ergosterol production was efficiently enhanced in yeast cultivation.

2. Materials and methods

2.1. Strain

S. cerevisiae (Y-E-1), preserved in our lab, was obtained by primary screening from 81 wild strains of yeast and random mutagenesis, which was performed to select mutants with an increased production of biomass and ergosterol.

2.2. Medium

Plate medium contained (w/v) (%): yeast extract 1, peptone 2, industrial glucose 2, and agar 2; seed culture medium (w/v) (%): yeast powder 1, peptone 2, and industrial glucose 2; cultivation medium: industrial glucose 60 g/L, corn steep liquor 15 g/L, $NaNO_3$ 7 g/L, KH_2PO_4 6 g/L, $MgSO_4$ 3 g/L, $CuSO_4{\cdot}5H_2O$ 0.2 mg/L, $FeSO_4{\cdot}7H_2O$ 1.0 mg/L, $ZnSO_4{\cdot}7H_2O$ 10.0 mg/L; fed medium: industrial glucose solution 600 g/L.

2.3. Shake flask culture

Firstly, a plate culture was grown at $30 \pm 1\,^{\circ}C$ for 20–24 h. Secondly, the yeast cells were transferred into a 250 mL flask containing 50 mL seed culture medium, and the flask was incubated at $30 \pm 1\,^{\circ}C$ and 180 rpm in a rotary shaker for 24 h. Finally, 5 mL seed culture medium was inoculated into a 250 mL flask containing 25 mL cultivation medium, and the flask was incubated at $30 \pm 1\,^{\circ}C$ and 180 rpm for 48 h.

2.4. Fed-batch culture

First and second step were same as shake flask culture. The bioreactor (Shanghai Baoxing Bioengineering Equipment Ltd., Shanghai, China) containing 2 L cultivation medium was sterilized at $121\,^{\circ}C$ for 20 min. And then the bioreactor was inoculated with 10% (v/v) seed cultures. Cultivation was carried out at $30 \pm 1\,^{\circ}C$, at an agitation speed of 600 rpm and air flow-rate of 10 L/min. A solution of 600 g/L glucose was added into the bioreactor using the feedback