INFLUENCE OF GROWTH RATE ON THE ACCUMULATION OF ERGOSTEROL IN YEAST - CELLS

C.ARNEZEDER and W.A.HAMPEL*

Institute of Biochemical Technology and Microbiology,
Technical University of Vienna,
Getreidemarkt 9, A-1060 Vienna (Austria)

SUMMARY

The influence of growth rate on the accumulation of ergosterol in Saccharomyces cerevisiae was studied with glucose, maltose, ethanol and acetic acid as substrates under C- and N-limitations in chemostat experiments. In carbon limited cultures an decrease in ergosterol content with rising dilution rate was observed, whereas in nitrogen limited cells an quite opposite behaviour was attained. A maximum specific rate of ergosterol synthesis of about 2 mg per h per g dry cell mass was calculated for nitrogen limited cultures.

INTRODUCTION

Ergosterol is an interesting raw material for vitamin D₂ production and microbial steroid transformations. Yeasts of the genus Saccharomyces are particularly rich in sterols; ergosterol has been identified as the major sterol and can account for 90% of the total sterol. The level of the sterol components has generally been determined to range from 0.03 to 4.6% of the cell dry weight, accounting for less 1 to 10% of the total cell lipid (Rattray et al.,1975; Elliot,1977; Basarova and Lőblova,1987).

Various factors might influence the synthesis of sterols by yeasts. In particular, the composition of the growth medium and the stage of the growth cycle, and also the level of various enviromental parameters in the course of cell cultivation, have been noted to have an effect on the amount of sterol produced (Rattray et al.,1975; Klein et al.,1954; Klein,1975; El-Refai and El-Kady,1968; Hunter and Rose,1972; Pichova et al.,1985; Novotny et al.,1987).

Since there are only fragmentary data available for correlating growth rate to ergosterol accumulation in yeast cells, systematic experiments were performed in a chemostat.
MATERIALS and METHODS:

Organism:
The yeast used in this study was the strain *Saccharomyces cerevisiae* ATCC 9080 which was maintained on slopes of malt-extract agar.

Cultivation:
*Saccharomyces cerevisiae* was grown at 30°C and at pH 4.5 under aerobic conditions (2 VVM, 650 RPM) in a 2 L fermenter (BTS 03 Applikon B.V., Schiedam, The Netherlands) containing 1 L medium. The modified full synthetic medium (Brändli and Fiechter, 1980) contained per litre: 16 g (NH₄)₂SO₄, 5 g KH₂PO₄, 1 g MgSO₄·7H₂O, 150 mg CaCl₂·6H₂O, 25 mg ZnSO₄·7H₂O, 80 mg FeSO₄·7H₂O, 25 mg MnSO₄·H₂O, 5 mg B₂O₃, 4 mg Na₂MoO₄·2H₂O, 5 mg CoCl₂·6H₂O, 500 mg sodium citrate, 10 μg D-biotin, 20 mg calcium D-pantothenate, 160 mg inositol, 4 mg thiamine, 10 mg pyridoxine and 15 mg nicotinic acid. It was supplemented with the different carbon sources in concentrations specified. For nitrogen limited cultures the amount of (NH₄)₂SO₄ was lowered to 1.1 g/l and the concentration of carbon source increased, so that nitrogen limited growth was ensured. Alkali (2 M KOH) was added automatically to maintain a constant pH and registered by a load cell. The carbon dioxide in exit air was measured by an infrared analyzer (Maihak AG, Hamburg, FRG).

Chemicals:
All chemicals and solvents used in this study were of analytical grade. Solvents used as eluents in HPLC were filtered through a 0.5 um Whatman GF/C glass fibre-filter and degassed ultra-sonically before use.

Analytical procedures:
For dry weight determinations, samples of the yeast cell suspension (10 ml) were centrifuged at 4000 g for 15 min and the pellet was resuspended in water and collected by filtration on a pre-dried and weighed Whatman GF/F glass-fibre filter. The wet filter was pre-dried in a microwave oven for 30 s at 750 W and heated to constant weight (twice at 750 W for 50 s). The results are reproducible to within ±0.2 mg.
Ergosterol was determined as described elsewhere (Arnezeder et al., 1989).

Carbohydrates were estimated by HPLC. The equipment consisted of a model 510 double-piston pump (Waters Division, Millipore, Milford, MA, USA) and a model R 401 refractive index (RI) detector (Waters). Sample injection was carried out with a Rheodyne (Berkeley, CA) 7125 valve equipped with a 20 ul loop. The column temperature was maintained constant within ±0.5°C by a model TCM temperature-control module (Waters). A HP 3393A (Hewlett-Packard, Avondale, PA) programmable recorder-integrator was used for the measurement of retention times and peak areas. Glucose, maltose and acetic acid were separated on a HPX-87N (25cm x 4.6mm i.d.) stainless-steel column packed with 8% crosslinked cation exchange resin in the sodium form (Bio-Rad, Richmond, CA) protected with a suitable cation N (Bio-Rad) guard-column (3cm x 4.6mm i.d.). 0.015 M Na₂SO₄ was used as the mobile phase and pumped through the column at a rate of 0.6 ml/min at a temperature of 85°C.
Ethanol was analyzed using a Perkin-Elmer model 8310 gaschromatograph with flame ionisation detector. It was separated on a Porapak Q (2m x 2.7mm i.d., 100-120 mesh) packed stainless-steel column (Perkin-Elmer Corp., Norwalk, CT, USA). The column was maintained at 240°C with N₂ flow rate of 20 ml/min, and the detector at 260°C. All samples were injected (1ul) on the column after filtration through a 0.45 um HAWP filter (Millipore, Bedford, MA).
Merckoquant test stripes (Merck, Darmstadt, FRG) were used to control nitrogen limitation during cultivations.