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RESEARCHES CONCERNING CHOLESTEROL UPTAKE IN *SACCHAROMYCES CEREVISIAE* CELLS

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Abstract

As sterols requirement in Saccharomyces cerevisiae is not specific for ergosterol, and sterols influences deeply the status of the eukaryotic membranes and further the biotechnological performances, the cholesterol up taking in yeast cells was studied. The most recommendable solution would be to add exogenous cholesterol in anaerobic conditions, in doses like 200-300 mg/l medium.

Key words: *cholesterol, colorimetric method, Saccharomyces cerevisiae, anaerobic conditions.*

Introduction

Sterols play major roles in the building and maintenance of eukaryotic membranes: they mainly regulate membrane fluidity and permeability, ethanol resistance and plasmic H^+-ATPase activity. They also regulate aerobic metabolism, the cell cycle and the uptake of exogenous sterols (Daum et al., 1998). Yeast growth in the absence of both anaerobic growth factors and oxygen leads to the accumulation of a large amount of squalene in membranes and a concomitant extremely low viability of the cells (Jahnke and Klein, 1983; Fornairon-Bonnefond et al., 2002).

Several studies have indicated that when wild-type cells are grown aerobically they seem to be impermeable to exogenous sterols (Molzahn and Woods, 1972; Trocha and Sprinson, 1976). It has been demonstrated that heme competency prohibits uptake under aerobic conditions, but the mechanism of exclusion hasn't been determined (Trocha, Sprinson, 1976).

The sterol requirement in S. cerevisiae is not specific for ergosterol, the most abundant sterol produced by this organism. Sterols differing by single structural changes have been tested for their ability

to support anaerobic growth, and it has been found that many sterols may substitute for ergosterol (Pinto and Nes, 1983).

Experimental

In order to study the cholesterol uptake in anaerobic and semi-anaerobic conditions (very similar to industrial environment) in Saccharomyces cerevisiae a commercial strain (SC Rompak) was used. A gram of compressed yeast was suspended in a liter YPG medium (yeast extract 10g, peptone 10 g, glucose 150 g, distilled water up to 1000ml), previously autoclaved at 121°C for 20minutes and pH adjusted to 5.6. The YPG medium was distributed in 3 fermentation containers (0.33 l each) – one of them, considered blind sample B_f (without cholesterol) and to the other two increasing cholesterol doses were added: sample 1 – 200 mg cholesterol/l, sample 2 – 300 mg cholesterol/l; doses related to 1g fresh yeast (30 % dry matter). Samples were incubated at 24°C for 6 days.

Simultaneously another YPG medium, containing only 20 g glucose, was similarly prepared and distributed also in 3 fermentation containers (0.33 l each) – one of them, considered blind sample B_{sf} (without cholesterol) and to the other two increasing cholesterol doses were added: sample 3 – 200 mg cholesterol/l, sample 4 – 300 mg cholesterol/l; doses related to 1g fresh yeast (30 % dry matter). Samples were also incubated at 24°C for 6 days, but every day samples 3 and 4 were shacked for 10 minutes in an orbital shaker (to increase the amount of O_2 solved in medium).

After 6 days of fermentation, the yeast biomass was separated (by centrifugation) from the spent YPG media and the amount of cholesterol left in the spent media for each sample, and also the biomass growth were determined.

The cholesterol remained was determined using a colorimetric Zlatkis-Zok-Boyle modified method (frequently used for blood serum cholesterol dosage). This method is based on the reaction between ferric chloride and cholesterol, in presence of concentrated CH_3COOH and H_2SO_4, which gives a red color. 10 ml $FeCl_3$ and 0.1 ml spent YPG media were mixed in a test tube, agitated and left for 15 minutes in repose, and centrifuged for 10 minutes at 3000 rotations/minute. In

another test tube 5 ml supernatant and 3 ml H_2SO_4 are mixed together and then left for 20 minutes in repose in a dark environment.

It had also prepared 5 standard cholesterol solutions with 200, 150, 100, 50, 25 mg cholesterol/100 ml conc. CH_3COOH. Each of these standards was used as follows: 0.05 ml of each standard cholesterol solution was mixed with 5 ml 0.05% ferric chloride and 3 ml conc. H_2SO_4. After a 20 minutes repose in a dark environment, absorbance is read at 570 nm for standards and samples, against a blank (5 ml 0.05% ferric chloride and 3 ml conc. H_2SO_4).

An Uvikon S30 spectrophotometer (Kontron Instruments) was used both to choose the wavelength (λ = 570 nm as in fig.1) and to read the absorbance values for standards and samples (fig.3).

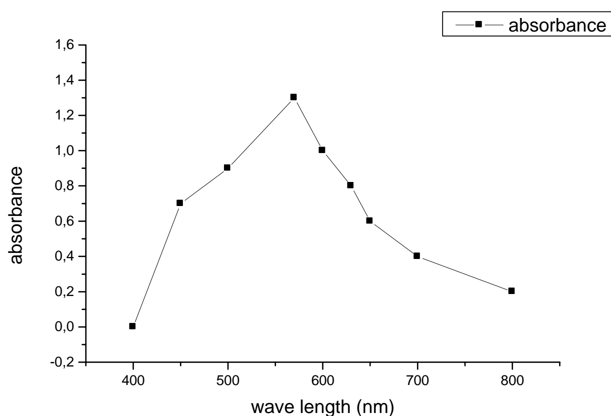

Fig. 1. Selecting the appropriate wave length value for standard and samples absorbance reading

Results and Discussions

In order to evaluate the cholesterol uptake in connection to biomass growth we also read the OD_{600} of yeast suspension in YPD media (D. Batani et al, 2002).

A standard graphic linear representation of yeast dry matter concentration (in YPG) versus absorbance (read at 600 nm at Uvikon S30 – Kontron Instruments spectrophotometer, l_{cuve} = 10 mm) was made (Fig.2).

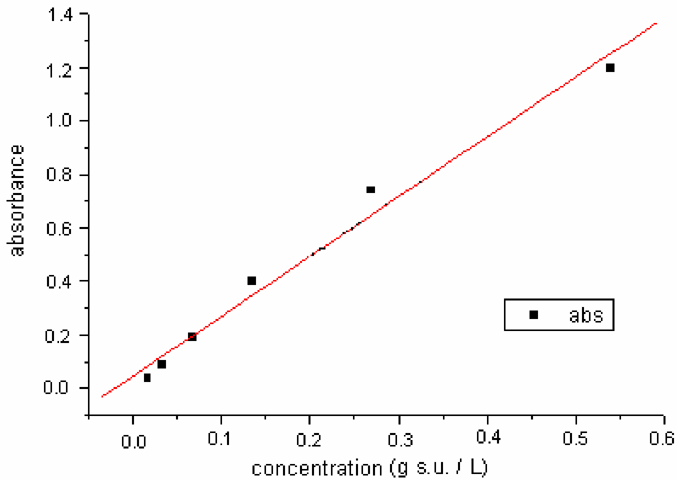

Fig. 2. Standard graphic linear representation of yeast concentration (g d.m./l YPG suspension) versus absorbance (DO_{600})

The assay for cholesterol left in the spent YPG media and biomass growth for each sample leaded to following results:

Table 1. Absorbance readings for both cholesterol assay and biomass growth

Sample	Absorbance read for assaying cholesterol left in spent YPG (OD_{570})	Absorbance read for biomass growth (OD_{600})
B_f	0.0223	0.1924
1	0.0273	0.2981
2	0.0370	0.3064
B_{sf}	0.0223	0.6034
3	0.0252	0.7260
4	0.0280	0.7600

For the beginning it is observable that in semi-anaerobic conditions yeast cells multiplied faster and yielded higher amount of biomass than in anaerobic conditions. Although net amount of cholesterol left for the anaerobic conditions are higher, the cholesterol uptake per cell is almost similar for anaerobic and semi-anaerobic conditions. We have to take into account that the biomass growth for samples B_f, 1 and 2 is lower than in the semi-anaerobic conditions, higher amount of

exogenous cholesterol were uptake comparatively to the anaerobic conditions, by a higher number of yeast cells.

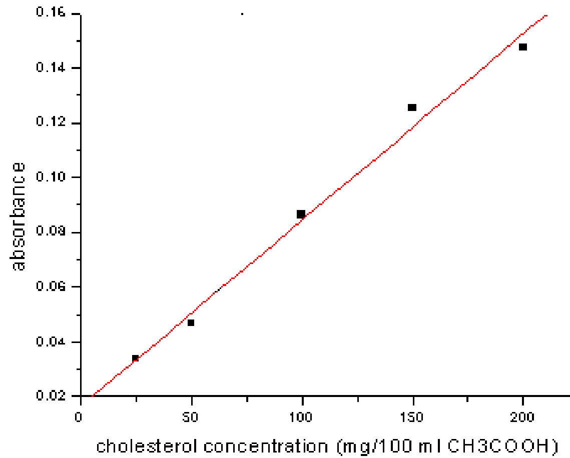

Fig. 3. Standard graphic linear representation of cholesterol concentration (mg/100 ml CH_3COOH) versus absorbance (DO_{570})

Table 2. Cholesterol left in spent YPG and biomass growth

Sample	Cholesterol left (mg/100 ml YPG)	Biomass growth (g d.m./l YPG)	Cholesterol uptake mg/g yeast d.m.
B_f	8.61	0.0652	-
1	15.97	0.1125	112.43
2	30.23	0.1162	72.18
B_{sf}	8.61	0.2627	-
3	12.88	0.3043	51.71
4	17.00	0.3196	67.64

Interpreting table 1 data in the light of figure 2 and figure 3, we obtain a much clear view of the meaning of these results (table 2). The provenience of cholesterol left in the blind samples (no exogenous cholesterol added) is the yeast extract from YPG medium (which is the same for all samples) and is too low to be uptake.

Conclusions

The cholesterol uptake is higher in anaerobical than in semianaerobical conditions as we expected. Under aerobic conditions S. cerevisiae is unable to take sterols from the medium (Andreason and Stier, 1953), an effect termed aerobic sterol exclusion (Lorenz and Parks, 1991).

The most recommendable solution would be to add exogenous cholesterol in anaerobic conditions, in doses like 200-300 mg/l medium, in order to improve the alcoholic fermentation dynamic and biotechnological results.

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