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Influence of 2-deoxy-D-glucose on Cell Growth of the Yeast *Saccharomyces cerevisiae*

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Summary

2-deoxy-D-glucose (2-DOG) causes two main effects in the yeast **Saccharomyces cerevisiae***: the first is glucose repression and the second is an inhibitory effect caused by a disturbance of cell metabolism. In this work we have studied both effects of 2-DOG on yeast metabolism and their influence on substrate uptake and growth of biomass. The effect of glucose repression on galactose grown cells was observed at 2-DOG concentration of 20 mg/L, and growth was stopped completely at the concentration of 50 mg/L. The inhibitory mode of action occurred at the concentrations higher than 300 mg/L.*

Keywords: S. cerevisiae, cell growth, 2-deoxy-D-glucose, inhibitory effects, glucose repression

Introduction

Due to high structural similarity with glucose, molecules of 2-deoxy-D-glucose (2-DOG) enter the cell of the yeast *Saccharomyces cerevisiae* by the glucose transport system and are then immediately phosphorylated by any of the three glucose phosphorylating enzymes: hexokinase I and II and glucokinase *(1–4)*. By this reaction 2-deoxy-D-glucose-6-phosphate is formed but it cannot be converted into fructose-6-phosphate by the next glycolytic enzyme, glucose phosphate isomerase *(5)*. As a consequence 2-deoxy-D-glucose-6-phosphate accumulates in a high concentration inside the cell and consecutive energy yielding steps are blocked. The consumption of ATP for the phosphorylation of 2-DOG leads to a starvation of the cell because ATP cannot be regenerated in the following steps of glycolytic pathway which are blocked to a great extent. Furthermore, glucose consumption is decreased *(6)* due to competitive inhibition since both glucose and 2-DOG use the same transport system. Energetic exhaustion of the cell and decreased glucose consumption lead to a decline of the general metabolic efficiency: production of ethanol, polyphosphate, glycerol and trehalose are inhibited *(7)*, as well as the protein synthesis *(1)*.

2-DOG partly undergoes some side reactions via glucose metabolism, basically those which preserve the hexose ring structure *(2)*. *S. cerevisiae* incorporates 2-DOG into guanosine *(8)* and uridine *(9)* nucleotides and glucanes of the cell wall *(10)* which causes an increased sensitivity of the biomass to cell lyses. 2-deoxy-D-glucose-6-phosphate is also converted into 2-deoxy-D-glucose-1-phosphate, 2-deoxy-D-glucose-1,6-diphosphate, 2-deoxy-gluconic-acid *(1)*, deoxy-trehalose and dideoxy--trehalose *(6)*. Interfering with glucose metabolism 2-DOG creates inhibition of cell wall synthesis *(10)*. All these disturbances create a general inhibitory effect of 2-DOG on yeast cells.

Beside this, because of the similarity with glucose, 2-DOG produces the effect of glucose repression. The consequence is that growth on carbon sources other than glucose is strongly repressed in the presence of 2-DOG *(1–4)*. This effect of 2-DOG had made it a substance of choice for selection of non-repressible mutants of *S. cerevisiae* which had a profound significance in studies on glucose repression and a potential in industrial practice as well *(11–13)*.

In this work we have studied both effects (glucose repression and inhibitory effect *per se*) regarding their influence on substrate uptake and cell growth of *S. cerevisiae*.

Materials and Methods

Microorganism and growth media

All studies were made with *S. cerevisiae*, haploid strain ORT-131, from the Collection of Microorganisms of the Faculty of Food Technology and Biotechnology, University of Zagreb.

Basic PY-growth medium *(14)* containing (g/L): peptone 3.5; yeast extract 3.0; KH_2PO_4 2.0; $(NH_4)_2SO_4$ 1.0; $MgSO_4 \cdot 7H_2O$ 1.0, was used for all experiments. Glucose, galactose and 2-DOG were added into basic PY medium as carbon sources in concentrations specified in each experiment. For solid media 20 g/L agar was added. Except agar, peptone and yeast extract all chemicals were of analytical grade.

Repression experiments

A test tube with 10 mL of PY medium containing 100 g/L glucose was inoculated with a single colony of *S. cerevisiae*. High concentration of glucose was applied in order to create strong glucose repression. After 24 hours of incubation at 27 °C, the whole culture was centrifuged at 2000 rpm for 10 minutes. Biomass sediment was washed twice with cold sterile distilled water to remove the remaining glucose. One microbiological loop of a suspension of washed biomass in distilled water was transferred on Petri plates with solid PY medium containing 20 g/L of galactose, 20 g/L of agar and 20 to 150 mg/L of 2-DOG. Plates were incubated at 27 °C up to six days.

Inhibition experiments

A test tube with 10 mL of PY medium containing 100 g/L of glucose was inoculated with a single colony and cultivated for 24 hours at 27 °C. The whole volume was used to inoculate 100 mL of the PY medium of the same composition. After 24 hours at 27 °C on a rotatory shaker (160 rpm) 10 mL portions of the culture were used as inoculum for 150 mL volumes of PY media in 500 mL Erlenmeyer flasks containing 20 g/L of glucose and varying concentration of 2-DOG (0–900 mg/L). Inoculated cultures were shaken for 3 days at 27 °C. During cultivation samples were taken, cooled on ice and immediately centrifuged at +2 °C (2000 rpm for 10 min). The supernatants were frozen at –20 °C and kept at that temperature until analyses.

Biomass concentration was determined by measuring absorbance at 674 nm. A unit of absorbance corresponded to 0.344 g/L of biomass dry matter.

Glucose concentration was determined enzymatically with Boehringer, Mannheim glucose analytical kits 716.251 (glucose oxydase-UV method). All absorbances were detected by spectrophotometer Cary 1/3, Varian.

Results and Discussion

Repressive effect of 2-DOG

In order to study the repressive effect of 2-DOG, the growth on galactose as a repressible carbon source in the presence of 2-DOG was monitored. Results in Table 1 show that for complete repression concentrations of 2-DOG between 20 and 50 mg/L were sufficient. At the concentration of 20 mg/L some growth was observed after 4 days of cultivation, while at the concentration of 50 mg/L there was no sign of growth even after 6 days of cultivation. Similar results were obtained with several strains of *S. cerevisiae* (data not shown).

Table 1. Cell growth of *S. cerevisiae* on solid galactose media containing different concentrations of 2-DOG

Cultivation time/day	γ(2-DOG)/mg L^{-1}		
	0	20	≥50
1	–	–	–
2	+/-	–	–
3	+	–	–
5	+	+	–
6	+	+	–

(-) no growth observed
(+/-) poor growth
(+) good growth

It has been reported that uptake and metabolism of galactose were repressed *(15,16)*, and the growth stopped due to accumulation of phosphorylated 2-DOG which decreased the intensity of energetic metabolism *(1,10,17)*. A limited growth observed during the cultivation at 20 mg/L of 2-DOG is probably caused by the relatively low concentration of 2-DOG which cannot maintain the full level of repression. Due to an incomplete repression, cells gain enough energy by assimilation of galactose to overcome the inhibitory effect of 2-DOG on energetic metabolism *(18)*.

Inhibitory effect of 2-DOG

The cultivations of *S. cerevisiae* in liquid PY media containing 20 g/L of glucose and various concentrations of 2-DOG were used for studying the inhibitory effect of 2-DOG. Despite the aerobic conditions of cultivation (stirred Erlenmeyer flasks on rotatory shaker), glucose metabolism followed the fermentative pathway because of high initial glucose concentration which caused the Crabtree effect. The concentration of ethanol at the end of the cultivation was between 12 and 14 g/L for all experiments (data not shown).

Compared with cultivation on galactose, a partial inhibition of growth was observed at a much higher concentration of 2-DOG (300 mg/L compared with 20 mg/L for galactose), and even the highest concentration of 2-DOG (900 mg/L) used in these experiments did not stop growth completely.

The inhibition of growth and glucose consumption increased gradually by increasing 2-DOG concentration in the whole employed concentration range. However, significant inhibition was noticeable only at concentrations of 300 mg/L and higher (Fig. 1 and 2). At concentrations higher than 300 mg/L growth was significantly slower (Fig. 1) while the time for complete consumption of glucose was prolonged from 14 hours with 50 mg/L of 2-DOG to 51 hours with 900 mg/L (Fig 2). Furthermore, it was observed that yeast entered the stationary growth phase before complete exhaustion of glucose in the growth medium took place (Fig. 2). 2-DOG affected stoichiometric parameters of cultivation as well as kinetics of the process. It was observed that the coefficient of substrate conversion decreased with the increase of the 2-DOG concentration (Fig. 3).

The obtained results correlate well with the reports on physiological effects of 2-DOG on yeast energetic metabolism *(18)*, transport and metabolism of glucose *(1,6)*,

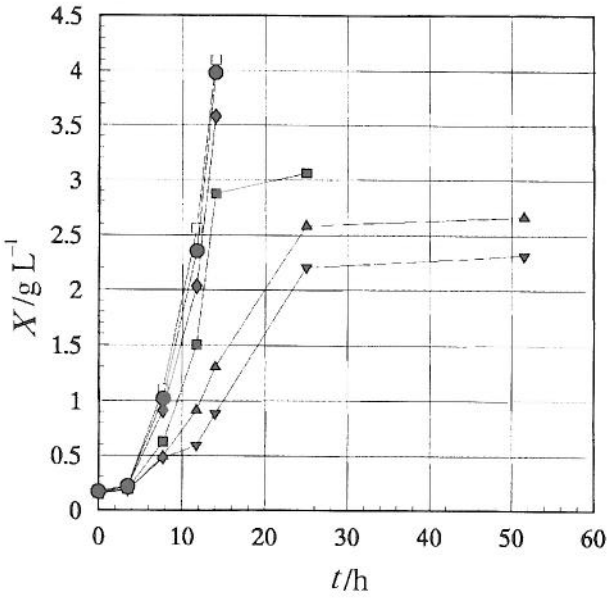

Fig. 1. Comparison of growth curves obtained by cultivation in media containing different concentrations of 2-DOG in the medium (mg/L): 0 (□), 50 (●), 150 (◆), 300 (■), 600 (▲), 900 (▼). X – biomass concentration.

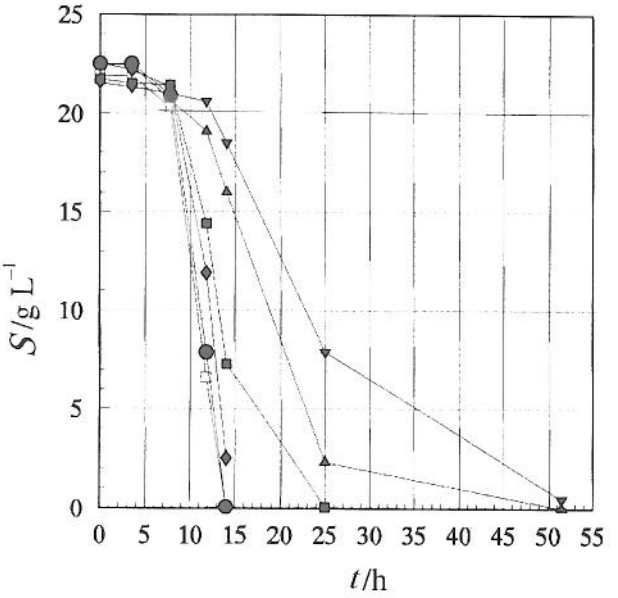

Fig. 2. Comparison of glucose consumption curves obtained by cultivation in media containing different concentrations of 2-DOG in the medium (mg/L): 0 (□), 50 (●), 150 (◆), 300 (■), 600 (▲), 900 (▼). S – glucose concentration.

and cell wall synthesis *(10,17)*. Recent NMR studies *(6,7)* have shown that the effect of 2-DOG is to decrease glucose consumption and the formation of polyphosphates, ethanol, glycerol, trehalose, glutamate, aspartate and succinate, while stimulating the formation of arginine and citrate. It is also stated that hexokinases can phosphorylate 2-DOG as well as glucose. Upon co-addition of 4.5 g/L of glucose and 900 mg/L of 2-DOG into the growing medium it was noticed *(7)* that 2-DOG-6-phosphate quickly reached the limiting value in about 30 minutes of cultivation and 2-DOG consumption became

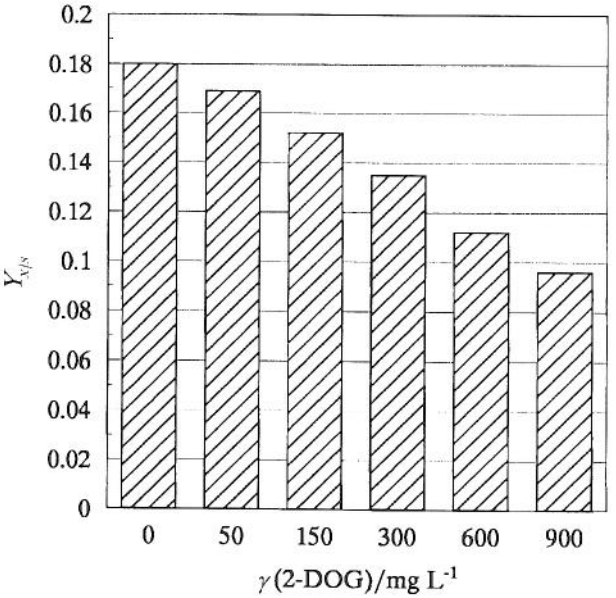

Fig. 3. Influence of 2-DOG concentration on the coefficient of substrate conversion into biomass ($Y_{x/s}$)

negligible. By contrast, the glucose consumption and the production of ethanol and glycerol, although substantially reduced by about 42%, varied linearly with time. Thus, even in the presence of an excess of 2-DOG, glycolysis is only slowed down but not completely inhibited by 2-DOG. However, for elucidation of the inhibitory effect of 2-DOG in yeast cells, more work is left to be done.

References

1. P. K. Maitra, Z. Lobo, *J. Biol. Chem. 246* (1971) 489.
2. J. A. Barnett, *Adv. Carbohydr. Chem. Biochem. 32* (1976) 126.
3. S. Novak, V. Marić, *Prehrambeno-tehnol. biotehnol. rev. 31* (1993) 35.
4. S. Novak, V. Marić, *Kem. Ind. 44* (1995) 341.
5. M. Loureiro-Dias, H. Santos, *FEMS Microbiol. Lett. 57* (1989) 25.
6. M. Herve, J. Wietzerbin, S. Tran-Dinh, *Eur. J. Biochem. 218* (1993) 221.
7. M. Herve, J. Wietzerbin, O. Lebourguais, S. Tran-Dinh, *Biochimie, 74* (1992) 1103.
8. P. Biely, S. Bauer, *Biochim. Biophys. Acta, 121* (1966) 213.
9. P. Biely, S. Bauer, *Biochim. Biophys. Acta, 156* (1968) 432.
10. Z. Kratky, P. Biely, S. Bauer, *Eur. J. Biochem. 54* (1975) 459.
11. S. Novak, T. D'Amore, I. Russel, G. G. Stewart, *J. Ind. Microbiol. 6* (1990) 149.
12. S. Novak, T. D'Amore, I. Russel, G. G. Stewart, *J. Ind. Microbiol. 7* (1991) 35.
13. S. Novak, T. D'Amore, G. G. Stewart, *FEBS Lett. 269* (1990) 202.
14. M. Ogur, R. St. John, *J. Bacteriol. 72* (1956) 500.
15. J. Ramos, V. P. Cirillo, *J. Bacteriol. 171* (1989) 3545.
16. R. Finley, S. Chen, J. Ma, P. Byrne, R. West, *Mol. Cell. Biol. 10* (1990) 5663.
17. J. Zemek, S. Bauer, *Biochim. Biophys. Acta, 264* (1972) 393.
18. S. A. Meredith, A. H. Romano, *Biochim. Biophys. Acta, 497* (1977) 745.

Utjecaj 2-deoksi-D-glukoze na rast kvasca *Saccharomyces cerevisiae*

Sažetak

U stanicama kvasca **Saccharomyces cerevisiae** *2-deoksi-D-glukoza (2-DOG) uzrokuje dva osnovna učinka: prvi je katabolička represija, a drugi inhibicijsko djelovanje zbog poremećaja staničnog metabolizma. U ovom su radu proučavana oba učinka 2-DOG na metabolizam kvasca* **S. cerevisiae** *i njihov utjecaj na potrošnju supstrata i kinetiku rasta biomase. Djelovanje kataboličke represije pri uzgoju na galaktozi primijećeno je već pri koncentracijama 2-DOG od 20 mg/L, a potpuni prestanak rasta pri 50 mg/L. Izravno inhibicijsko djelovanje 2-DOG opaženo je pri koncentracijama većim od 300 mg/L.*