J Ind Microbiol Biotechnol (2010) 37:701–706
DOI 10.1007/s10295-010-0713-x

ORIGINAL PAPER

Variations of two pools of glycogen and carbohydrate in *Saccharomyces cerevisiae* grown with various ethanol concentrations

M. S. Dake · J. P. Jadhv · N. B. Patil

Received: 11 March 2010 / Accepted: 17 March 2010 / Published online: 7 April 2010

Abstract Glycogen, a major reservoir of energy in *Saccharomyces cerevisiae*, is found to be present as soluble and membrane-bound insoluble pools. Yeast cells can store excess glycogen when grown in media with higher concentration of sugar or when subjected to nutritional stress conditions. *Saccharomyces cerevisiae* NCIM-3300 was grown in media having ethanol concentrations up to 12% (v/v). The effects of externally added ethanol on glycogen and other carbohydrate content of yeast were studied by using alkali digestion process. Fermentative activities of cells grown in the presence of various ethanol concentrations (2–8% v/v) exhibited increase in values of glycogen and other carbohydrate, whereas cells grown with higher concentrations of ethanol (10–12% v/v) exhibited depletion in glycogen and carbohydrate content along with decrease in cell weight. Such inhibitory effect of ethanol was also exhibited in terms of reduction in total cell count of yeast grown in media with 2–16% (v/v) ethanol and 8% (w/v) sugar. These data suggest that, as the plasma membrane is a prime target for ethanol action, membrane-bound insoluble glycogen might play a protective role in combating ethanol stress. Elevated level of cell-surface α-glucans in yeast grown with ethanol, as measured by using amyloglucosidase treatment, confirms the correlation between ethanol and glycogen.

Keywords *Saccharomyces cerevisiae* · Yeast · Glycogen · Ethanol · Osmotolerance

M. S. Dake (✉) · J. P. Jadhv · N. B. Patil
Department of Biochemistry, Shivaji University, Kolhapur, India
e-mail: man1d_2@rediffmail.com

Introduction

The cell wall of yeast accounts for 20% of dry cell weight along with 80% carbohydrate [1]; it has a complex macromolecular organization consisting of glucan, mannan, and chitin. Glucan represents the major structural component, having α- and β-glucan-type polysaccharides made up of glucose units. β-glucan is composed of a major fraction having branched $\beta(1 \rightarrow 3)$-linked polymer with few $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ interchain linkages, plus a minor fraction containing major proportion of $\beta(1 \rightarrow 6)$-linked glucosyl units with about 20% $\beta(1 \rightarrow 3)$ interchain linkages [2, 3]. Mannans present as mannoproteins at the cell surface of yeast determine cell-wall permeability to macromolecules [4]. Chitin is predominantly found in the bud scar region [5]. α-Glucans with $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ linkages constitute glycogen, a water-soluble homopolymer of glucose and a reserve carbohydrate in yeast supplying energy required for cell growth and during budding cell phase. Since the quality of yeast is influenced by the level of reserve carbohydrate, quantitative estimation of glycogen is of great importance [6]. In yeast, presence of two pools of glycogen has been investigated as a cytoplasmic water-soluble glycogen and the remainder referred to as water-insoluble glycogen associated with alkali-insoluble mass [7]. In *Saccharomyces carlsbergenesis*, storage of these glycogen fractions varies depending upon type and concentration of sugar used, conditions of nitrogen limitation, fermentation time, etc. [8]. Previous study regarding yeast indicates that increased level of cell-wall glycogen is related to increase in ethanol yield of yeast cells [9]. Level of glycogen storage and its metabolism are regulated by physiological state of the cell. Glycogen accumulation in yeast is induced by depletion of carbon, nitrogen or sulfur sources [10]. Cells subjected to

physiological, environmental, and nutritional stress conditions exhibit variations in the level of glycogen, and apart from these parameters, higher concentrations of ethanol are also responsible for creating stress in yeast. Ethanol is toxic to yeast, affecting cell growth, viability [11, 12], intracellular proteins, and glycolytic enzymes [13]. It acts primarily on plasma membrane, resulting in changes in sugar transport and membrane potential [14]. The content of acid-soluble glycogen is correlated with the process of yeast flocculation [15]. Glycogen content is found to be higher in flocculant yeast strains than in powdery varieties [16]. In yeast, presence of glycogen in the form of α-glucans at cell-surface level of yeast and its role in flocculation are confirmed by using amyloglucosidase treatment. However, the effect of ethanol on the two pools of glycogen as well as on cell-surface α-glucans of yeast has not been studied in detail. The aim of our study is to determine the resistance of yeast cell wall (whose major component is glycogen) to ethanol as well as its role in flocculation.

Experimental

Microorganisms and culture conditions

Bottom-flocculating *Saccharomyces cerevisiae* NCIM-3300 was obtained from National Chemical Laboratory, Pune and is routinely maintained on yeast extract peptone dextrose (YEPD) agar at 4°C. Different media compositions were used to study variations in glycogen content resulting from ethanol stress in *Saccharomyces cerevisiae*.

Peptone, yeast extract, and malt extract were from Difco Laboratory, Detroit, MI. Amyloglucosidase was purchased from Sigma Chemical Company, USA, and glucose oxidase kit was from Biolab.

Cell counts were made using a hemocytometer, and viable cells were assessed using methylene blue stain. Glucose was measured using glucose oxidase peroxidase method [17], and total carbohydrate was estimated by phenol sulfuric acid method [18].

Media for set A (increasing sugar concentration, flasks a–e) were 2%, 4%, 6%, 8% or 10% glucose, 0.5% peptone, and 0.3% yeast extract. Media for set B (increasing ethanol concentration and constant sugar concentration, flasks f–k) were 8% glucose, 0.5% peptone, 0.3% yeast extract, and 2%, 4%, 6%, 8%, 10% or 12% (v/v) ethanol. Media for set C (increasing sugar concentration and constant ethanol concentration, flasks l–q) were 2%, 4%, 6%, 8%, 10% or 12% glucose, 0.5% peptone, 0.3% yeast extract, and 10% (v/v) ethanol. Media for set D (for study of inhibitory effect of ethanol on yeast cells, flasks r–y) were 8% glucose, 0.5% peptone, 0.3% yeast extract, and 2%, 4%, 6%, 8%, 10%, 12% or 16% (v/v) ethanol, and a separate dilution medium containing 0.5% peptone and 0.3% yeast extract was prepared as a control to determine the total cell count of yeast. Media for set E (for determination of surface α-glucan content of yeast, flasks A and B) were 8% glucose, 0.5% peptone, and 0.3% yeast extract, with 8% (v/v) ethanol added to only flask B.

All flasks were inoculated, and fermentation was carried out at room temperature (25°C) for 48 h. Then, yeast cells were harvested from each flask under cold conditions and washed twice with distilled water. Cells harvested from all flasks of sets A, B and C were subjected to alkali digestion process to determine the amount of soluble and insoluble glycogen [19]. Yeast cells from set D were used to measure total cell count using a hemocytometer only after taking homogeneous cell suspension.

Amyloglucosidase treatment

About 1 g yeast cells (fixed by using 1.5% glutaraldehyde) suspended in 4 ml sodium acetate buffer (100 mM, pH 4.2) was treated with 0.3 ml amyloglucosidase at 37°C for 1 h. Supernatant fractions obtained after amyloglucosidase treatment were used for analysis of surface α-glucan content using glucose oxidase peroxidase method [17].

Results

Further study regarding changes in glycogen content in yeast cells subjected to ethanol stress is undertaken. The experimental work carried out here will help to investigate whether ethanol externally added to yeast culture medium affects yield of glycogen, surface α-glucan content (related to the flocculation process), cell weight, and total cell count. Similarly it will also help to assess whether insoluble glycogen in yeast plays a protective role against stress conditions created by ethanol. These data can be used to determine optimum storage of glycogen exhibited by yeast in response to ethanol tolerance.

Increasing sugar concentration

Glycogen content in yeast ranges from 1% to 23% total dry cell weight [10]. Study regarding variations in the level of yeast glycogen using media with various sugar concentrations revealed that the amount of soluble and insoluble glycogen increased exponentially with increasing sugar concentration up to 10% (w/v). Similar observations were obtained for soluble and insoluble carbohydrate content. The values of glycogen and carbohydrate are presented in Table 1 (set A). Yeast cells exhibited higher storage level of glycogen as well as carbohydrate after growth in media with 10–12% w/v sugar concentration. Wet weight of yeast

Table 1 Fermentation carried out using media sets A–C

Set	Flask	Glycogen (mg/g cells)		Carbohydrate (mg/g cells)	
		Soluble	Insoluble	Soluble	Insoluble
Set A	a	4.15 ± 0.01	3.13 ± 0.03	16.44 ± 0.04	11.02 ± 0.02
	b	5.25 ± 0.03	5.21 ± 0.01	22.13 ± 0.03	16.23 ± 0.02
	c	5.32 ± 0.02	6.01 ± 0.01	25.41 ± 0.01	21.41 ± 0.01
	d	5.67 ± 0.01	7.42 ± 0.02	25.50	23.21 ± 0.01
	e	4.24 ± 0.01	9.23 ± 0.03	29.02 ± 0.02	27.02 ± 0.02
Set B	f	2.02 ± 0.02	8.03 ± 0.03	26.22 ± 0.02	25.31 ± 0.01
	g	2.62 ± 0.02	10.51 ± 0.01	28.09 ± 0.01	28.03 ± 0.03
	h	3.22 ± 0.02	13.12 ± 0.02	29.03 ± 0.03	31.21 ± 0.01
	i	3.61 ± 0.01	15.02 ± 0.02	30.40	33.40
	j	4.02 ± 0.02	11.41 ± 0.01	21.39 ± 0.01	24.59 ± 0.01
	k	4.21 ± 0.01	6.12 ± 0.02	11.02 ± 0.02	13.61 ± 0.01
Set C	l	0.61 ± 0.01	2.60	19.02 ± 0.02	18.03 ± 0.03
	m	1.79 ± 0.01	4.51 ± 0.01	23.01 ± 0.01	25.22 ± 0.02
	n	2.99 ± 0.01	10.02 ± 0.02	25.01 ± 0.03	27.05
	o	3.41 ± 0.01	15.12 ± 0.02	28.16 ± 0.04	31.03 ± 0.03
	p	4.10	10.37 ± 0.03	19.02 ± 0.02	22.41 ± 0.01
	q	4.30	4.34 ± 0.04	12.52 ± 0.02	10.02 ± 0.02

Set A Variation in glycogen and carbohydrate with increasing sugar concentration (2–10% w/v)

Set B Variation in glycogen and carbohydrate with increasing ethanol content (2–10% v/v); sugar concentration kept constant (8% w/v)

Set C Variation in glycogen and carbohydrate with increase in sugar concentration (2–12% w/v); ethanol concentration kept constant (10% v/v)

Values are expressed as mg/g wet weight of yeast cells; data represent mean ± standard deviation (SD) of three sets of observations

continued to increase with increasing sugar concentration up to 10%, showing further decrease in weight from 5.6 to 4.3 g above 10% sugar concentration, indicating substrate-level inhibition of yeast caused by glucose. Thus 10% sugar concentration is optimum for maximum growth and deposition of glycogen in yeast. Glycogen stored by yeast plays a vital role in providing energy and carbon skeleton required for cell maintenance.

Increasing ethanol concentration

It was observed that yeast cells grown in absence of inorganic nitrogen source and other growth factors store abnormally higher amount of insoluble glycogen [9]. This indicates that yeast cells respond to nutritional stress conditions by exhibiting higher level of insoluble glycogen. Insoluble glycogen also serves as a substrate under anaerobic conditions for production of ethanol. Apart from this, another factor producing stress conditions is ethanol, which is toxic to *Saccharomyces cerevisiae* [20] and bacteria such as *Zymomonas mobilis* [21]. Ethanol stress causes enhancement of proton efflux from cells by stimulating activity of plasma membrane H^+–ATPase, resulting in increased membrane permeability of yeast [22]. Ethanol concentration above a threshold value of 4–6% (v/v) is responsible for induction of heat-shock proteins in yeast [23] and also affects membrane lipids by causing fluidization of plasma membrane as well as by increasing proportion of ergosterol [24]. In the present study an attempt was made to determine whether the level of insoluble glycogen varies in response to stress conditions created by ethanol. In view of this, yeast cells were grown in culture media with various ethanol concentrations from 2% to 12% (v/v) along with 8% (w/v) sugar. Variations in the amount of soluble and insoluble glycogen as well as carbohydrate are presented in Table 1 (set B). Values of soluble and insoluble glycogen were observed to increase almost exponentially from 2.02 to 3.6 mg and from 8.03 to 15.02 mg per g wet weight, respectively, with increasing ethanol concentration up to 8% (v/v) added to yeast culture media. Similarly, the amount of soluble and insoluble carbohydrate was also found to increase, from 26.22 to 30.4 mg and from 25.3 to 33.4 mg per g weight of yeast cells, respectively, for set B. However, on increasing the ethanol concentration from 10% to 12% (v/v), values of both glycogen and carbohydrate decreased, from 15.02 to 0.12 mg for insoluble glycogen, from 30.40 to 11.02 mg for soluble carbohydrate, and from 30.40 to 13.01 mg for insoluble carbohydrate. The observed sharp decline in these values is significant. Higher ethanol concentrations (above 8%) also decreased the weight of yeast cells from 4.9 to 4.5 g.

Another remarkable observation from Table 1 is that values of insoluble glycogen and carbohydrate obtained for yeast cells grown in media with 2–8% (v/v) ethanol along with 8% sugar were significantly higher than those observed for cells grown only in presence of 8% sugar. This indicates that yeast cells exhibit a new mechanism of ethanol adaptation, showing increased levels of glycogen and carbohydrate. However, decline in cell weight, glycogen, and carbohydrate content, as observed on addition of 10–12% (v/v) ethanol, indicates reduction in growth rate and viability of cells. It is observed that the ethanol concentration that reduced the specific growth rate by ~50% was 10% (v/v) [25]. Yeast cells grown with ethanol near the limit of tolerance exhibit changes in their fermentative activities as a protective measure of growth. This indirectly proves a positive correlation between ethanol and insoluble glycogen content in yeast. The role of trehalose in inhibiting plasma membrane leakage and thermotropic behavior of lipids was established by previous study [26, 27]. When ethanol increases plasma membrane permeability and fluidity, insoluble glycogen present in the cell wall of yeast is responsible for preventing leakage through cell membrane and thus plays an important role in enhancing ethanol tolerance. Data in Table 1 reveal that yeast cells grown in

presence of 8% (v/v) ethanol along with various sugar concentrations from 2% to 12% (w/v) exhibit similar effects. Data in Table 1 indicates increase in values from 0.61 to 3.41 mg for soluble glycogen and from 2.6 to 15.12 mg for insoluble glycogen with increasing sugar concentration from 2% to 8% (w/v) along with 10% (v/v) ethanol. Parallel increase in the values of soluble and insoluble carbohydrate was observed, from 19.02 to 28.16 mg and from 18.03 to 31.03 mg, respectively. On the contrary, the corresponding values of glycogen and carbohydrate were seen to decline steeply, from 15.12 to 4.3 mg for insoluble glycogen, from 28.16 to 12.52 mg for soluble carbohydrate, and from 31.03 to 10.02 mg for insoluble carbohydrate, after increase in sugar concentration above 8% (w/v) along with 10% (v/v) ethanol. Wet weight of yeast showed a similar decline from 5.0 to 4.2 g. Thus, higher concentrations of both sugar and ethanol greater than 8% together cause catabolite repression of yeast cells. In view of this, total cell count was measured by growing yeast cells in presence of 8% (w/v) sugar and various ethanol concentrations from 2% to 16% (v/v). Table 2 shows that cell count of yeast grown in media with 2–10% (v/v) ethanol was reduced from 9.14×10^6 to 6.8×10^6 mm^{-3}. However, thereafter, sharp decline in cell count from 2.55×10^6 to 1.02×10^6 mm^{-3} was observed for culture media with ethanol concentration >10% (v/v). This diminution in cell count can be explained by the mechanism of ethanol interacting with plasma membrane to increases its permeability by minimizing hydrophobic interactions, causing free exchange of polar molecules along with ethanol [25]. This ultimately inhibits metabolism in yeast cells by causing denaturation of intracellular proteins and glycolytic enzymes along with reduction in cell growth and viability.

From Tables 1 and 2 it is evident that yeast cells exhibit ethanol tolerance up to 10% (v/v) and display optimum

Table 2 Set D: effect of various ethanol concentrations (2–16% v/v) on total cell count of yeast, with sugar concentration kept constant (8% w/v)

Flask	Ethanol concentration (% v/v)	Total cell count $N/(80 \times 200 \times 400$ mm$^{-3})$
r	2	$(9.41 \pm 0.05) \times 10^6$
s	4	$(9.24 \pm 0.04) \times 10^6$
t	6	$(8.36 \pm 0.01) \times 10^6$
u	8	$(7.54 \pm 0.04) \times 10^6$
v	10	$(6.82 \pm 0.02) \times 10^6$
w	12	$(2.55 \pm 0.05) \times 10^6$
x	14	$(1.22 \pm 0.01) \times 10^6$
y	16	$(1.02 \pm 0.02) \times 10^6$

Total cell count was measured as number of cells per cubic millimeter; values are given as mean ± SD of triplicates

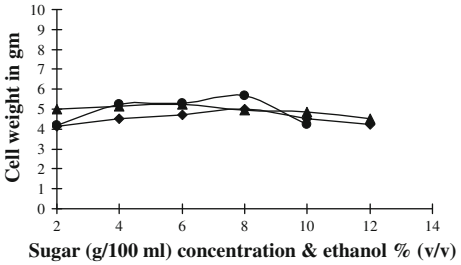

Fig. 1 Variation in wet weight of yeast cells in three different media composition sets: *set A* variation in wet weight of yeast (—●—) with increase in sugar concentration (2–10% w/v); *set B* variation in wet weight of yeast (—▲—) with increase in ethanol concentration (2–12% v/v) along with (8% w/v) sugar; *set C* variation in wet weight of yeast (—◆—) with increase in sugar concentration (2–8% w/v) along with (10% v/v) ethanol. Cell weight is expressed as g/100 ml. Data represent averages of three sets of observations

storage level of glycogen when grown in media containing 8% (w/v) sugar and 8% (v/v) ethanol. By considering all these observations it is essential to investigate whether the same observation is applicable for cell-surface α-glucan of yeast. The relationship between content of insoluble glycogen and the flocculation process in yeast has already been studied [7]. So, it is obvious that glycogen and flocculence must vary concurrently in brewing yeast. However, the actual role of glycogen in the flocculation process was not known. The role of surface α-glucans in yeast flocculation was investigated by using amyloglucosidase [19], an enzyme that specifically cleaves $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ bonds in glycogen-type polysaccharides. On the basis of these experimental data, an attempt was made to determine the role of ethanol in the flocculation process in terms of surface α-glucan content of yeast. In view of this, surface α-glucan content of yeast cells grown in two different media, one with only 8% (w/v) sugar and the other containing both 8% (w/v) sugar along with 8% (v/v) ethanol, was analyzed. We chose these concentrations of sugar and ethanol since they gave maximum yield of glycogen and total carbohydrate, as observed from Table 1 and Fig. 1. Figure 2 exemplifies that those cells grown in presence of

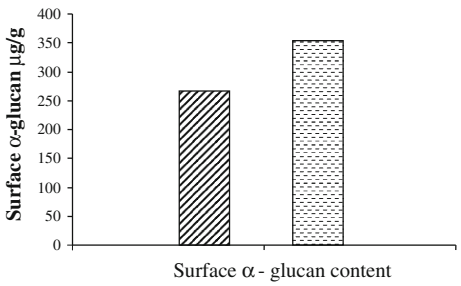

Fig. 2 Variation in surface α-glucan content using two different media compositions: medium A [8% sugar (▨)] and medium B [8% sugar with 8% ethanol (▤)]. Values are presented as µg/g weight of yeast cells. Data represent averages of triplicates

both sugar and ethanol display higher surface α-glucans (353 μg) than do the cells grown only with sugar (267 μg). This indicates that extracellular ethanol not only affects the storage level of cytoplasmic and cell-wall-bound insoluble glycogen but also cell-surface glycogen present in the form of α-glucans. It is obvious that ethanol plays an important role in the flocculation process of yeast by showing higher surface α-glucan content.

Conclusions

Extracellular ethanol affects the level of cytoplasmic and cell-wall-bound insoluble glycogen as well as other carbohydrate in *Saccharomyces cerevisiae*. When cells are grown in media containing 2–8% (v/v) ethanol, increase in the level of both glycogen and carbohydrate was observed, and this effect is considered to be a protective measure shown by yeast towards osmostress. Especially the cell-wall-bound insoluble glycogen plays a dual role by providing energy and carbon skeleton required for cell growth as well as minimizing leakage through plasma membrane. However, ethanol concentration above 10% (v/v) diminishes this protective effect of glycogen by causing inhibition of yeast metabolism, which ultimately results in depletion of cell-wall glycogen, cell growth, and total cell count. The strong evidence regarding ethanol action in entering the hydrophobic interior of yeast cell wall and increasing the polarity of this region [25] proves that ethanol must be causing dissolution of cell-wall-bound insoluble glycogen as well as other carbohydrate and thus lowers yield by causing hydrophobic interaction. Ethanol concentrations above 10% (v/v) is effective in causing hydrophobic interaction and denaturation of glycogen. Similarly, increase in the level of surface α-glucan of yeast grown in media with ethanol indicates the role of ethanol in flocculation and confirms the correlation among glycogen, ethanol, and the flocculation process. Thus, abnormal increase in the level of glycogen can be an indirect measure of ethanol tolerance level of yeast. Such highly ethanol-tolerant yeast strains would be beneficial from the point of view of industrial economics.

Acknowledgments We are grateful to Shivaji University, Kolhapur for a grant from the Departmental Research Fellowship Scheme and for providing the research facilities to carry out this work.

References

1. Fleet GH (1981) Cell walls. In: Rose AH, Harrison JS (eds) The yeast, 2nd edn, 14, yeast organelles. Academic, London, pp 199–277
2. Manners DJ, Masson AJ, Patterson JC (1973) The structure of a β(1–3)-D-gluan from yeast cell walls. Biochem J 135:19–30
3. Fleet GH, Manners DJ (1976) Isolation and composition of an alkali soluble glucan from the cell walls of *Saccharomyces cerevisiae*. J Gen Microbial 94:180–192
4. Zlotnik H, Fernandez MP, Bowers B, Cabib E (1984) *Saccharomyces cerevisiae* mannoproteins form an external cell wall layer that determines wall porosity. J Bacteriol 159:1018–1026
5. Cabib E, Bowers B (1971) Chitin and yeast budding. Localization of chitin in yeast bud scars. J Biol Chem 246:152–159
6. Cabib E, Rothman-Denes LB, Huang KP (1973) The regulation of glycogen synthesis in yeast. Ann N Y Acad Sci 210:192–206
7. Smith ZG, Patil NB, Smith EE (1977) Two pools of glycogen in *Saccharomyces*. J Bacteriol 130(2):818–825
8. Jadhav JP, Dake MS, Patil NB (2006) Variations of two pools of glycogen and ethanol in *Saccharomyces carlsbergenesis*. Asian J Chem 18(2):1443–1450
9. Arvindekar AU (1995) Biochemistry of cell wall α-glucans in *Saccharomyces*. Ph.D thesis, Shivaji University, Kolhapur
10. Lillie SH, Pringle JR (1980) Reserve carbohydrate metabolism in *Saccharomyces cerevisiae* : response to nutrient limitation. J Bacteriol 143(3):1384–1394
11. Thomas DS, Rose AH (1979) Inhibitory effect of ethanol on growth and solute accumulation by *Saccharomyces cerevisiae* as affected by plasma membrane lipid composition. Arch Microbiol 122:19–55
12. Thomas DS, Hossack AJ, Rose AH (1978) Plasma membrane lipid composition and ethanol tolerance. Arch Microbiol 117:239–245
13. Nagodawithana TW, Whitt JT, Cutaia AJ (1977) Study of the feed back effect of ethanol on selected enzymes of the glycolytic pathway. J Am Soc Brew Chem 35:179–183
14. Leao C, Van Uden N (1984) Effects of ethanol and other alkanols on passive proton influx in the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 774:43–48
15. Patel GB, Ingledew WM (1975) The relationship of acid soluble glycogen to yeast flocculation. Can J Microbiol 21:1608–1613
16. Masschelein CA, Ramos CJ, Castau C, Devreux A (1963) Mechanism of phenotypic variations in the flocculence character of yeast. J Inst Brew 69:332–338
17. Lloyd JB, Whelan WJ (1969) An improved method for the enzymatic determination of glucose in the presence of maltose. Anal Biochem 30:467–470
18. Dubois M, Gilles KA, Hamilton JK, Robers PA, Smith F (1956) Colorimetric method for determination of sugar and related substances. Anal Chem 28:350–356
19. Arvindekar AU, Patil NB (2002) Glycogen—a covalently linked component of the cell wall in *Saccharomyces cerevisiae*. Yeast 19:131–135
20. Agudo LD (1992) Lipid content of *Saccharomyces cerevisiae* strain with different degrees of ethanol tolerance. Appl Microbiol Biotechnol 37:647–665
21. Barbosa MF, Yomano LP, Ingram LO (1994) Cloning, sequencing and expression of stress genes from the ethanol producing *Zymomonas mobilis*: the groESL operon. Gene 148:51–57
22. Piper PW (1995) The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional ovelap. FEMS Microbiol Lett 134:121–127
23. Gille C, Sigler K, Hofer M (1993) Response of catalase activity and membrane fluidity of aerobically grown *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* to aeration and presence of substrates. J Gen Microbiol 139:1627–1634
24. Alexandre H, Berlot JP, Charpentier C (1994) Effect of ethanol on membrane fluidity of protoplasts from *Saccharomyces cerevisiae* and *Kloeckera apiculata* grown with or without ethanol, measured by fluorescence anisotropy. Biotechnol Tech 8:295–300

25. Alexandre H, Rousseaux I, Claudine C (1993) Ethanol adaptation mechanism in *Saccharomyces cerevisiae*. Biotechnol Appl Biochem 20:173–183
26. Mansure JJ, Panek AD, Crowe LM, Crowe JH (1994) Trehalose inhibits ethanol effects on intact yeast cells and liposomes. Biochim Biophys Acta 1191:309–316
27. Leslie SB, Israeli E, Lighthart B, Crowe JH, Crowe LM (1995) Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Appl Environ Microbiol 61:3592–3597