Research & Reviews in Biotechnology & Biosciences

Homepage: www.biotechjournal.in

Volume No: 4, Issue No: 1, Year: 2017 PP: 12-18

Published By: National Press Associates in Association with Lyallpur Khalsa College, Jalandhar, Punjab

ISSN No: 2321-8681

Short Communication

Studies on glycolipids of *S.cerevisiae* in relation to temperature stress

Renuka Malhotra

Department of Biotechnology, DAV College, Jalandhar, Punjab, India

Article History

Received: 01/03/2017
Revised: 15/03/2017
Accepted: 05/04/2017

**Corresponding Author:*
E-Mail: *malhotra7r@gmail.com*

Abstract

The total glycolipid content of S.cerevisiae cells increase in temperature treated yeast cell. Hexoses and sialic acid content decrease whereas hexosamine content increased under temperature stress. The different saccharide moieties of glycolipid of S.cerevisiae were identified. Mannose and galactose content increased as the temperature is raised to 37°C whereas glucose and fucose content decreased. The changes in concentration of these sugars may be due to differential activities of enzymes like β-glucosidase and β-galactosidase. The temperature also affected the glycolipid fractions of yeast S.cerevisiae. The content of cerebrosides increased while sulfatide content decreased during temperature stress suggesting that these molecules may be playing important role in membrane stabilization. The unsaturated fatty acid content of yeast S. cerevisiae glycolipids increased while saturated fatty acid decreased which may be due to conversion of saturated fatty acid to unsaturated fatty acid during temperature stress,helping in modulating the membrane fluidity.

Key words- S.cerevisiae, Temperature, Glycolipid, Stress, Sugar

Introduction

Yeast has evolved numerous mechanism that permit them to grow and thrive in extraordinarily hostile and stressful environment such as alcohol and temperature. The main sites for stress effects in yeast are cellular membranes, the structure and function of which may results increase in membrane fluidity, decrease membrane integrity and altered enzymatic processes [1]. Studies have shown that membrane glycolipids play very important role in adaptation to stress conditions of yeast and rat membrane [2-4] Glycolipids have high transition state due to interlipid hydrogen bonding and therefore impart structural integrity to the membranes of organisms living in harsh environment conditions. Studies have shown that glycoconjugates may be playing some signaling functions during heat stress [5] Temperature is known to effect significantly the content and composition of microbial lipids [6]. T. acidophilum adapts to high temperature by increasing the number of sugar units in their glycolipids [7]. There continues to be a knowledge gap on the molecular mechanisms associated with the ethanol stress response and ethanol tolerance of S. cerevisiae, with research in this area continuing to explore potential genetic engineering strategies for improving microbial ethanol tolerance. Despite the wide spread presence of Glycolipid in the plasma membrane their functions in relation to adaptation to stress conditions in the cell membrane is still under investigation. As fermentation efficiency of all ethanol producing organism's declines under stress conditions and harsh environment so it is imperative that new parameters for developing stress resistance yeast cells should be explored.

Keeping above points in mind we have studied the effect of temperature on yeast membrane glycolipids and their role in adaptation.

Materials and Methods

Yeast strain

S.cerevisiae strain MTCC827 was procured from the Institute of Microbial Technology, Chandigarh (Punjab).

Growth of Yeast Cells

S.cerevisiae cells were grown on yeast peptone dextrose medium in 500ml Erlenmeyer flasks containing 100ml of medium [8] at 27±1°C for 24 h. During growth, cultures were shaken on a rotary shaker at 250 rpm and 27±1°C. The cells were harvested by centrifuging at 5000g for 15 min. and were washed with 0.01 M phosphate buffer (pH 7.0) to remove adhering metabolites and unused ingredients in the medium and given the temperature stress.

Extraction of Lipid

Total lipids were extracted from the yeast cells by the method of Folch et al. [9]. Pure lipids obtained were dissolved in chloroform and stored at -4°C.

Separation of Glycolipids

Glycolipids were isolated from total lipids by silicic acid chromatography [10]. The column was eluted with the solvents chloroform, acetone, and methanol, respectively. Glycolipids were estimated on the basis of their total sugar content. Total sugar content was determined by the method of Dubois et al. [11].

Estimation of Sugar Content in Glycolipids

Purified glycolipids were assayed for hexoses, silaic acid, and hexosamine content [11-13].

Analysis of Sugars

Different sugars of glycolipid were separated and estimated by gas liquid chromatography (GLC) .TMS derivatives were analyzed on Thermo Trace 1300 GC gas chromatograph equipped with TG 5MS column. Detector and injector temperatures were maintained at 250°C, respectively, and the flow rate of the carrier gas, Helium, was at 1ml/min. The oven temperature was programmed from 60 to 220°C at 10°C/min. Analysis was monitored with a flame ionization detector.

Fatty acid analysis

Fatty acid composition of total glycolipids was determined by GLC after conversion of fatty acids to their methyl ester by treating petroleum ether extracts of methanolysate with 0.5N anhydrous methanolic HCL at 80°C in sealed vials.

Fractionation of Glycolipids by Column Chromatography

Glycolipids were fractionated by DEAE-cellulose ion-exchange column chromatography (10). The purity of glycolipid subclasses was checked by thin-layer chromatography (TLC) on silica gel G plates using chloroform/methanol/water (65/25/4 (v/v/v)) as the solvent system [14]. Various glycolipids were then quantified on the basis of their hexose content estimated by the method of Roe [15].

Results and Discussion

Effect of Temperature on Lipids and glycolipids *of S.cerevisiae*

Changes in membrane lipid composition are one of the first responses to stress conditions. It is believed that these changes are required to adjust the physical characteristics of membrane structures so that they may perform their necessary physiological task when environmental factors changes [16]. Total lipids of *Saccharomyces cerevisiae* cell membrane decreased from 4.01 to 2.34% of their dry weight on an increase in temperature condition whereas the total glycolipid content increased from 20.8% to 30.8% dry weight.(Table 1)

Glycolipids have the capability to undergo interlipid hydrogen bonding via glycerol head group and this provides structural stability to membrane. Therefore the relative higher content of glycolipid under temperature stress may represent an adaptive response during stress condition.

Effect of Temperature on Sugar Composition *of S.cerevisiae*

Temperature treatment of *S.cerevisiae* affected not only the content of glycolipid but the composition of carbohydrate moieties. The hexose content as well as the sialic acid content decreased while hexosamine content first increased at 32°C then decreased at 37°C.(Table 2) Monossaccharides functions as metabolic resources and structural constituent of the cells. Variation in the content of the monosaccharide during stress condition may have protective effect on the membrane [17]. The changes in the content of these monosaccharides may be owing to the alter glycosylation and sialylation of glycolipids or may be due changes in the activity of certain enzymes such as silidases or glucosidases under stress condition.

Effect of Temperature on hexoses composition of Glycolipids of *S.cerevisiae*

Various hexoses present in glycolipid were separated and identified by gas liquid chromatography. Fucose , Mannose, Galactose and glucose were identified as major hexoses in *S.cerevisiae* glycolipid (Table 3). Mannose and galactose content increased while glucose and fucose content decreased as the temperature is increased from 27°C to 37°C. Galactose is main saccharides of glycolipid which constitute 44.33% of monosaccharides moieties in glycolipid of yeast cells. The increase in mannose and galactose monosaccharide may contribute to adaptation of yeast to temperature. The role played by glycol head groups are reported in electrostatic membrane potential (18), leading to membrane packing.

The changes in the concentration of these sugars may be an important target directly or indirectly in biological action of temperature. Various reports suggested that hexose of glycolipids confer special structural properties and specific site recognition on the membrane. The alteration in the distribution of hexose content during temperature stress may help the carbohydrates residue of glycolipids to serve as binding determinant for variety of membrane signals.

Effect of Temperature on Glycolipid Fractions of *S.cerevisiae*

Glycolipids from *S.cerevisiae* were fractioned by DEAE-cellulose ion-exchange chromatography. Different fractions were obtained and identified as cerebrosides, sulfatides, monoglucosylglycerides and diglucosyldiglycerides. The purity of the fractions were checked by thin layer chromatography and different fractions were identified by using different spray reagents and comparing the Rf values. The effects of temperature on the yeast cells were studied on these fractions of glycolipid. (Fig.1)

The cerebrosides content increased as temperature was raised to 37°C while sulfatide content decreased at higher temperature. Increase in cerebroside content and decrease in sulfatide content was also reported under heat stress on canine kidney cells.[19] The increase in cerebroside content may be due to the activation of ceramide galactosyltransferase and cerebroside sulfotransferase [20]. The changes in these glycolipid may be due to the participation of complex sphingolipids in stress inititated ceramide formation [21] therefore an increase in cerebroside content during temperature stress may play important role in stabilizing membrane both physically and structurally.

The content of MGDG decreases as the temperature increases from 27°C to 37°C while the content of DGDG decreases by 13% then increases as the temperature increase to 37°C. MGDG and DGDG constitute a major class of lipid membranes. The balance between the amount of DGDG and MGDG is essential for functioning of the biological process [22]. Increase in DGDG content was also reported in cowpea leaves during drought stress [23].The observation can also be used to conclude about the physiological modification of the glycolipid composition in membranes in process of adaptation to stress.

Effect of Temperature on fatty acid composition of Glycolipid of *S.cerevisiae*

The change in fatty acid composition of membrane lipids is one of the important factors which help the organism to adapt the adverse condition. The yeast glycolipid had fatty acid ranging from C_{16} to C_{18}. (Table 4) In glycolipid of temperature treated cell, the content of unsaturated fatty acid increased while that of saturated fatty acid decreased. The changes in membrane fatty acid composition in response to temperature are regulated in such a way that so as to achieve high unsaturation levels. [24]. Similar changes were observed in thermo tolerant diatoms by [25]. The significant changes in both the content and fatty acid composition of glycolipids of yeast suggest that fatty acids might be playing an important role in adaptation to temperature stress by incorporating more unsaturated fatty acid in glycolipids of *S. cerevisiae*.

Conclusion

In brief the presence study revealed that the glycolipid content and the composition of *Saccharomyces cerevisiae* cells was altered under temperature stress.

Glycolipids are known to protect the membrane from chemically unstable conditions. During temperature stress the glycolipids may play important role in adaptation by modulating its sugar residues which may interact with each other via hydrogen bonding helping in modulating the membrane fluidity.

Acknowledgements

This work was supported by University Grant Commission, New Delhi. This paper is a part of UGC Major Research Project. We also thank the director of CIL/SAIF, Punjab university, Chandigarh for providing the necessary facility.

REFERENCES

1. Ingram, L. O. (1986). Microbial tolerance to alcohol: Role of cell membrane. Trends Biotechnol. **4**: 40-44.
2. Curatolo, W. (1987). The physical properties of glycolipids. Biochim. Biophys. Acta **906**: 111-136.
3. Malhotra, R., and Singh, B.(2005),Studies on glycolipids of Sccharomyces cerevisiae in relation to ethanol tress.*Appl. Biochem. And Biotech. Vol*. **126,** 205-213.
4. Singh , B., Gupta, S., Sharma, S., C. (1997), Influence of ethanolon lipid peroxidation in Saccharomyces cerevisiae: Role of superoxide. *Proc. Natl. Acad. Sci. India*. 6861-66.
5. Grewal.R and Akhtar. M.(2010). The effects of ethanol administration on brush border membrane glycolipids in rat intestine. Alcohol.44(6):515-522.
6. Dickson, R.,C. and Lester, R.l. (2002). Review: Sphingolipids functions in Saccharomyces cerevisiae.*Biochem. Biophys. Acta* .**1583**, 13-25.
7. Shimada, H., Nemoto, N., Shida, Y., Oshima, T., and Yamagishi, A. (2008), Effects of pH and temperature on the composition of polar lipids in *Thermo plasma acidophilus*. *American Society for Micro.*,5404-5411.
8. Hayashida,S., Feng, D.D., Ohta,K..(1980). Effect of phosphatidylcholine or ergosterylolleate on physiological properties of Sacchromyces sake.*Agric. Biol. Chem*. **44**, 2561-2567.
9. Folch, J., Lee, M. and Stoche, S. G. H. (1957). A simple method for the isolation and purification of total lipid from animal tissues. J.Biol. Chem. 226: 497-509.
10. Rouser, G., Kritchevsky, G.,and Yamamoto, A.(1976),Column chromatographic and associated procedures for separatons and

determination of phosphatides and glycolipids. *Lipid Chromatography Analysis vol*. 3 (Marinetti, G. V., ed.), Marcel Dekker, New York, 713-776.

11. Dubois , M., Gilles, .KA., Hamilton, J.K. , Rebers , R.A. ,and Smith, F. (1956), Colorometric estimation of carbohydrates by phenol-sulphuric acid methods.*Anal. Chem.* **28**, 350-354..

12. Svennerholm, L.(1957) , Quantitative estimation of sialic acid: a colorimetric resorcinol- Hydrochloride acid method. *Biochim. Biophys. Acta.* 24,604-611.

13. Dische, Z.(1962), Color reactions of hexosamines. *In Method in Carbohydrate Chemistry*, Whistler, R.L. and Wolfram, M.L., eds., Plenum, New York, pp. 575-595.

14. Stahl, E.(1965), Thin Layer Chromatography: *A Laboratory Hanadbook*, Springer- Verlag, New York,p.7.

15. Roe, J.H.,(1955). The determination of sugar in blood and spinal fluid with anthrone reagent. *J.Biol. Chem*. **212**, 335-343.

16. Quinn, P.J., (1988). Effects of temperature on cell membranes *Symp Soc Exp Biol*. **42,**237-58.

17. Ianutsevich, A. E, Danilve, A.O., Groza, V.N., Kotlova, R. E. and Tereshina, M.V. (2015). Membrane lipids and cytosol carbohydrates in *Aspergillus niger* under osmotic, oxidative and cold impact. *Microbiology. Vol.***85 (3)**, 302-310.

18. Rog, T., Vattulainen, I., Bunker, A. and Karttunen, M. (2007) Glycolipid membranes through atomistic simulations: Effect of glucose and galactose head groups on lipid bilayer properties. *J.Phys. Chem.B*. **111 (34),**10146-10154.

19. Niimura, Y., Moue, T., Takahashi N, and Nagai K.(2010) Modification of sphingolipids and sulfolipids in kidney cell lines under heat stress: Activation of monohexosylceramide synthesis as a ceramide scavenger. Glycobiology. **20(6),**710-720 .

20. Niimura., Y. and Nagai, K. (2008) ,) Metabolic responses of sulfa tides and related Glycolipid in Madin-Darby canine kidney (MDCK) cells under osmotic stresses. *Comp. Biochem. Physiol. Biochem.Mol. Biol.* **149(1),**161-7.

21. Balogh, G., Peter, M., Glatz, A., Gombos, I., Torok, Z., Horvath, I., Harwood, J.L., Vigh, L(2013). Key role of lipids in heat stress management. *FEBS Lett* **587(13)**,*FEBS Letters*. **587(13)** ,1970-1980.

22. Pali, T., Garab, G., Horvath, L.I., Kota, Z.(2003) Functional significance of the lipid-protein interface in photosynthetic membranes. *Cell Mol. Life. Sci* .**60**,1591–1606.

23. Franklin, M.,L. T., Gigon, A., Fernandes, D, M., Fodil,Z. Y. and Thi-Pham T. A.(2007)) Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. *Physiologia Plantarum.Vol*. **131(2)** ,201-210.

24. Falcone, L. D., Ogas, P.J. and Somerville, R. C.(2004). Regulation of membrane fatty acid composition by temperature in mutants of Arabidopsis with alterations in membrane lipid composition. *BMC Plant Biology*.

25. Rousch, M., J., Bingham, E., S. and Sommerfeld, R.,M.(2003*)* Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress.*Journal of Experimental Marine Biology and Ecology. Vol.* **295 (2),** 145-156.

Table 1

Effect of Temperature on Lipids and Glycolipids of *S.cerevisiae*

Growth Temperature (°C)	Total lipids (% dry weight)	Glycolipid 1 (%o f total lipid)
27	4.53±0.32	20.58±0.14
32	3.70±0.09	23.02±0.85
37	2.39±0.06	30.85±0.98

Table 2

Effect of Temperature on Sugar Composition of Glycolipids of *S.cerevisiae*. Data represents 3 replcates± SE

Growth Temperature (°C)	Hexose (µg/mg glycolipid)	Sialic acids (µg/mg glycolipid)	Hexosamine (µg/mg glycolipid)
27	82.03±0.33	30.22±0.10	65.30±0.23
32	52.03±0.16	24.03±0.16	70.20±0.23
37	44.04±0.13	28.04±0.13	30.30±0.42

Table 3

Effect of Temperature on hexoses composition of glycolipid

	Hexoses (Relative %)			
Temperature (°C)	Fucose	Mannose	Galactose	Glucose
27	21.80	18.52	44.33	14.78
32	17.90	20.71	50.70	10.48
37	7.62	25.04	59.94	6.78

Table 4

Effect of temperature on fatty acid of Saccharomyces cerevisiae

Growth Temperature (°C)	Fatty acids (Relative %) 16:0	16:1	18:0	18:1	18:2	18:3	Unsaturation index
27	17.50	4.80	16.28	23.50	21.50	16.3	66.11
32	15.90	5.13	13.08	39.86	10.94	18.69	71.62
37	8.46	5.84	10.11	46.20	10.94	21.01	80.10