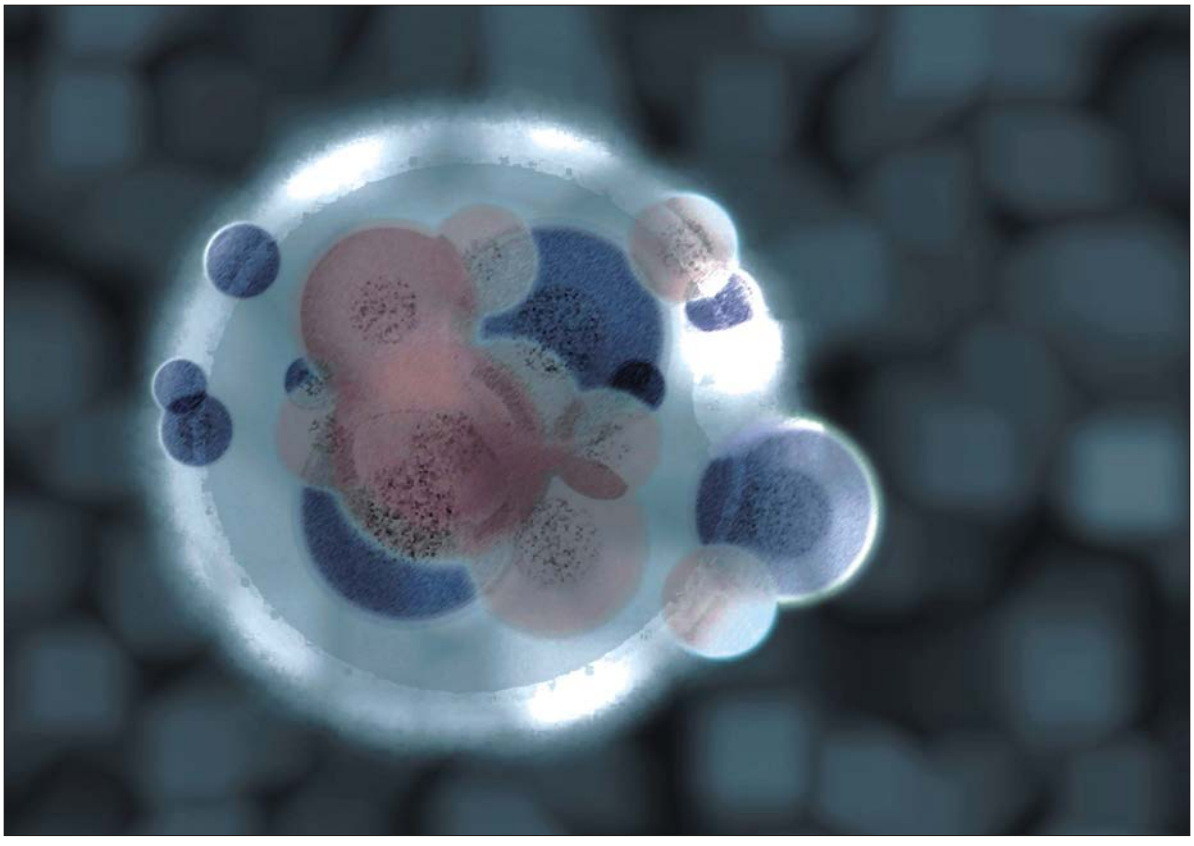

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 5 (3): 253-256, 2005
ISSN 1727-3048

Glycolipids of *Saccharomyces cerevisiae* Cell

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Abstract: Total lipids of *Saccharomyces cerevisiae* were isolated by chloroform and methanol (2:1). Glycolipids were separated from total lipids by silicic acid chromatography. Glycolipid's constituent sugars and fatty acids were analyzed by using Gas Liquid Chromatography. Galactose was the prominent sugar followed by mannose. Relative concentrations of fucose, mannose, galactose and glucose in the glycolipid were 5.3, 35.2, 55.1 and 4.2%. 16:0, 18:0, 18:1, 18:2 and 18:3 were the major fatty acids of the total glycolipids. Oleic acid was the dominating fatty acid followed by linoliec acid. They were separated into different fractions by using DEAE-Sephadex ion exchange chromatography. Glycolipids were fractionated and identified as cerebrosides, ceramide polyhexosides, sulfatides, monoglucosyldiglycerides and diglucosyldiglycerides. Ceramide polyhexosides were present in higher concentration as compared to other fractions.

Key words: *Saccharomyces cerevisiae,* glycolipids, sugar, fatty acids

INTRODUCTION

Glycolipids are a diverse class of heterogeneous conjugates composed of sugars and lipids, which are found in species ranging from bacteria to man. They are amphipathic molecule that exits mainly in the plasma membrane with their oligosaccharides portion protruding into the extracellular environment. The content of the glycolipids varies among different cell types. They contain one or more monosaccharides residues bound by a glycosidic linkage to a hydrophobic moiety such as acylglycerol, a sphingoid, a ceramide or prenyl phosphate. They are generally classified as glycoglycerolipids, glycosphingolipids and glycophosphatidyl inositol. The glycolipids of bacteria and plants are generally glycosyl diacylglycerol. The major glycolipids of animal consist of mono or oligosaccharides glycosidically linked to ceramide[1]. In addition to phospholipids and sterols, glycolipids are important structural components of the biomembranes. Due to their capability of undergoing inter hydrogen bonding; they have high transition temperature and impart structural integrity to the membranes. Besides, structural components of, they also play very important role in membrane functions such as in cell-cell communication, as receptors components, as anchors for proteins and as regulators of signal transduction[2,3]. Glycolipids seem to play some role in helping the cells to adjust to unfavorable conditions[4,5]. To the best of our knowledge, these molecules of such an importance i.e. glycolipids from the yeast *Saccharomyces cerevisiae* cells have not been reported so far. In the present investigation, an attempt has been made to study the glycolipids of this industrial important yeast.

MATERIALS AND METHODS

Saccharomyces cerevisiae strain MTCC827, used in the present study, was procured from Institute of Microbial Technology, Chandigarh (Punjab).

Growth of yeast cells: Yeast cells were cultured in 500 mL Erlenmeyer flasks containing 100 mL basal synthetic medium[6] at 27±1°C for 24 h. The composition of the medium per liter was glucose 20 g, peptone 20 g, yeast extract 10 g. The cells were harvested by centrifugation at 5000 x g for 15 min and were washed with 0.01 M phosphate buffer (pH 7.0) to remove adhering metabolites and unused ingredients of the medium.

Lipid extraction: Total lipids were extracted from the yeast cells by the method of Folch *et al.*[7] with some modifications. 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[8]. The column was eluted with the solvents i.e. chloroform, acetone and methanol, respectively. Glycolipids were eluted in the

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acetone fraction. The excess solvent was evaporated on rotary evaporator and the glycolipids were estimated on the basis of their total sugar content. Total sugar content was determined by the method of Dubois[9].

Estimation of sugar's contents in the glycolipids: Purified glycolipids were assayed for hexose, sialic acid and hexosamine content. Hexoses were estimated by using phenol/sulfuric acid method[9], sialic acid by the method of Sevnnerholm[10] and hexosamine by Elson-Morgan method as described by Dische[11].

Analysis of sugars: Different sugars of glycolipids were separated and estimated by GLC. Glycolipids were hydrolyzed in 1N anhydrous methanolic HCl at 100±2°C in sealed vials for 18 h[12]. The resulting monosaccharides were converted to trimethylsilyl derivatives, which are analysed on a Shimadzu GS-17C gas chromatograph, equipped with PTE-5 (5% diphenyl, 95% dimethylsiloxane) column. Detector and injector were maintained at 280°C and 250°C, respectively, the flow rate of carrier gas nitrogen was at 42 mL/min. The oven temperature was programmed from 150 to 250°C at 7°C per min. The analysis was monitored with flame-ionization detector.

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

Fractionation of glycolipids by column chromatography: Glycolipids were fractioned by DEAE cellulose ion exchange column chromatography[8]. The purity of glycolipid subclasses were checked by TLC on silica gel G using chloroform: methanol: water (65:25:4 v/v/v) as solvent system[13]. They were identified by using spray reagents and by comparing their Rf values with those of authenticated standards. Specific spray reagents were used to identify neutral glycolipids, sulfatides, ceramide polyhexosides, cerebrosides and glycosyldiglycerides. Various glycolipids were then quantified on the basis of their hexose content as described by Roe[14].

RESULTS AND DISCUSSION

Glycolipids have been a subject of intensive research because of their structural diversity and physiology. They are also emerging as a class of messenger molecules linked to many different cellular functions[15]. However, glycolipids of yeast cells have not been reported so far. In this communication, glycolipids of *Saccharomyces cerevisiae* have been discussed. Total lipids constitute 4.53% of the dry weight of *Saccharomyces cerevisiae* cells. Similar content of total lipids in yeast cells have also been reported[5,16]. Glycolipids isolated from total lipids by silicic acid chromatography were estimated to be 11.58% of total lipids (Table 1). No storage glycolipids are reported in *Saccharomyces cerevisiae* cells. So, all glycolipids of the yeast cells can be considered to be the membrane constituents. The qualitative and quantitative glycolipid content varies tremendously among various animal cell types[1].

In yeast cell glycolipids, hexoses, sialic acid and hexosamines were found to be main carbohydrate constituents. Quantitatively, hexoses were the major sugars associated with lipids (124.8 μg mg^{-1} glycolipids) whereas hexsoamines and sialic acids were present relatively in small amounts (Table 2). In rat intestinal brush border membranes also, higher amounts of hexose, followed by sialic acid and hexosamine, are reported[17]. However, rat microvillus membrane contained higher proportions of hexosamine, followed by hexose content[4]. The presence of different amounts of hexose, sialic acid and hexosamine in various cell membranes may contribute to the glycosylation patterns of their membrane lipids. Therefore, the glycosylation patterns of the yeast cell membrane lipids may be different from that of other cells. Sugars like sialic acids and hexosamines generally occupy peripheral sites in oligosaccharide chains of glycolipids and glycoproteins. Thus, variations in content of these sugars in various cell membranes usually reflected the differences in their binding behaviour. Rosner[18] has proposed that sialic acids are important for the conformation and functions of glycolipids.

The hexoses were separated and identified by Gas Liquid Chromatography (GLC). Various hexoses present in *S.cerevisiae* glycolipids were identified as fucose, mannose, galactose and glucose (Table 3). Galactose constitutes 55.19% of total sugars, followed by mannose, which was 35.20% of total sugars. Fucose and glucose contributed for the remaining 10%. Glycolipids from yeast and mycelium of *Paracoccidiodes brasiliences* has been reported to contain mannose and galactose[19]. Glucose and galactose have been reported to be the carbohydrate moieties of fungal cerebrosides[20]. Variety of sugars gives rise to wide range of naturally occurring glycolipids and form cell specific patterns at the cell surface[21].

Fatty acids of glycolipids are important as they make them amphipathic molecules. The fatty acid composition of glycolipids from *S. cerevisiae* was determined by GLC. Both saturated and unsaturated fatty acids were present in the glycolipids of *S. cerevisiae*. The most prominent fatty acids present were $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$

Table 1: Lipids and Glycolipid content of *S. cerevisiae*

Lipids	Content (%)
Total lipids	4.53±.32 dry wt.
Glycolipids	11.58±0.14 total lipids

Table 2: Sugar composition of total glycolipids of *S. cerevisiae*

Sugars	Content (μg mg^{-1} glycolipid)
Hexose	124.80±.35
Sialic acid	30.22±.10
Hexosamine	65.53±.23

Table 3: Total glycolipids of *S. cerevisiae*

Sugars	Content (relative%)
Fucose	5.38
Mannose	35.20
Galactose	55.19
Glucose	4.20

Table 4: Fatty acid composition of total glycolipids of *S. cerevisiae*

Fatty Acids	Content (relative%)
Palmitic acid	17.5
Palmitoleic acid	4.8
Stearic acid	16.3
Oleic acid	23.5
Linoleic acid	21.5
Linolenic acid	16.3

Unsaturated index 33.8/66.1 (0.511)

Table 5: Composition of glycolipids of *S. cerevisiae*

Glycolipid fractions	Content (μg mg^{-1} glycolipid)
Cerebrosides	129.69±.06
Ceramide polyhexosides	376.14±.19
Sulfatides	270.33±.47
MGDG	64.00±.01
DGDG	165.34±.11

(Table 4). Quantitatively, major fatty acids in glycolipids were oleic acid and linoleic acid. Saturated fatty acids i.e. palmitic acid and steric acid were present in relatively less amounts. The unsaturation index was 0.511. The major fatty acids present in fungal cerebrosides have been reported to be palmitic acid, stearic acid and oleic acids[20]. Yeast membrane fatty acids have been reported to be highly variable and influenced by both environment and intrinsic factors[22]. Physical properties of membranes are mainly determined by fatty acid composition of its lipids. The presence of a mixture of fatty acids in *Saccharomyces cerevisiae* may enhance the organism's adaptability to different environment conditions.

Glycolipids present in *Saccharomyces cerevisiae* were fractioned by DEAE- cellulose ion exchange chromatography. The purity of these fractions was checked by Thin Layer Chromatography (TLC). They were identified by using specific spray reagents and by comparing their Rf values with those of authenticated standards (data not given). Five different fractions were separated which were identified as cerebrosides, ceramide polyhexosides, sulfatides, monoglucosyldiglycerides (MGDG) and diglucosyldiglycerides (DGDG). The content of various glycolipid fractions were estimated on the basis of their sugar content (Table 5). Ceramide polyhexosides was prominent glycolipid fraction and contributes 376.14 μg mg^{-1} of glycolipids, followed by sulfatides, which was present in 270.33 μg mg^{-1} of glycolipid. These two fractions make 65% of all the glycolipids in *Saccharomyces cerevisiae* cell membrane. Ceramides polyhexosides and sulfatides have been reported to stabilize the plasma membrane[23,24]. Ceramide polyhexosides are also important as the biosynthetic precursor of most of neutral oligoglycosyl ceramides.

Besides ceramide polyhexosides and sulfatides, MGDG and DGDG were also present in glycolipids from *Saccharomyces cerevisiae*. MGDG was present relatively in small amounts as compared to DGDG.

Weislander *et al.*[25] has proposed that the structure of lipid bilayer is maintained by the regulated ratio of the non-bilayer (MGDG) and bilayer forming (DGDG) glycolipids.

Cerebrosides, which constituted 13% of the glycolipid fraction, may also stabilize the yeast cell membrane physically. Cerebrosides are characterized by extensive hydrogen bonding ability and high gel to liquid crystalline phase transition temperature[1]. Wu *et al.*[26] proposed that cerebroside stabilizes the interactions of bulk lipid and protein. In brief, the present study revealed that *Saccharomyces cerevisiae* cells contain a variety of glycolipids. The glycolipid probably helps to stabilize the membrane in various environmental conditions.

ACKNOWLEDGMENT

This study was supported by the grants from the Guru Nanak Dev University, Amritsar, India.

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