Extracellular Glycolipids of Yeasts

Biodiversity, Biochemistry, and Prospects

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INTRODUCTION

Microorganisms are characterized by a great diversity of the so-called secondary metabolites, that is, compounds that are not obligatory participants of metabolism but, nevertheless, provide advantages to producers in their survival under unfavorable environmental conditions and competition for ecological niches. Many of these compounds are biologically active and, hence, have good and promising applications in industry, agriculture, and medicine.

Secondary metabolites include the so-called biosurfactants: lipopeptides, glycolipids, fatty acids, neutral lipids, and phospholipids, as well as some amphiphilic biopolymers. These substances are widespread in microorganisms, from bacteria to fungi. They were found during the studies of microbial growth on hydrophobic substrates, including oils and hydrocarbons, and were supposed to improve the solubility and bioavailability of these substrates. The properties of biosurfactants of different chemical nature and origin, as well as their research and commercial prospects, have been described in a number of reviews (Lang and Wagner, 1987; Rosenberg and Ron, 1999; Kitamoto et al., 2002; Rodrigues et al., 2006; Langer et al., 2006; Arutchelvi et al., 2008; Van Bogaert et al., 2007, 2011). Many reviews are devoted to future potential of biosurfactants in medicine and industry (Banat et al., 2010; Fracchia et al., 2012; Marchant and Banat, 2012; Cortés-Sánchez et al., 2013). Springer Publishers have issued a volume "Biosurfactant" in the series "Advances in Experimental Medicine and Biology" (Sen, 2010) and a volume "Biosurfactants. From Genes to Applications" in the series "Microbiology Monographs" (Soberón-Chávez, 2011).

The following properties of these compounds make them relevant for life science and biotechnology:

- structural diversity;
- multiple biological activities;
- biodegradability;
- nontoxicity;

- the possibility of inexpensive production using simple nutrient media, including those containing industrial and agricultural wastes;
- promising applications as detergents, antibiotics, and amphiphilic compounds.

The extracellular glycolipids of yeast and fungi belong to biosurfactants. These compounds are glycosides of fatty acids containing one or more monosaccharide residues that may contain additional O-substituents at the sugar moiety.

These compounds are mentioned in many reviews on biosurfactants (Lang and Wagner, 1987; Rosenberg and Ron, 1999; Kitamoto et al., 2002; Cameotra and Makkar, 2004; Rodrigues et al., 2006; Langer et al., 2006). However, the reviews devoted specifically to yeast extracellular glycolipids are few (Van Bogaert et al., 2007a,b, 2011; Arutchelvi et al., 2008; Bölker et al., 2008; Kulakovskaya et al., 2008, 2009; Arutchelvi and Doble, 2011; Van Bogaert and Soetaert, 2011

The studies of yeast extracellular glycolipids attract attention due to their numerous activities: from biosurfactant properties providing utilization of hydrophobic substrates to fungicidal properties, as well as a number of other biological activities that make these compounds scientifically and practically promising.

Structural diversity, numerous biological activities, biodegradability, nontoxicity, and possibility of inexpensive production make them attractive for future applications in industry, cosmetology, medicine and agriculture as ecologically pure detergents, fungicides of new generation, and other useful products. Up to date, scientific literature has accumulated quite a lot of data on these compounds, which should be generalized for better understanding of the potential of yeast as a producer of biologically active substances, for development of ecological biotechnologies and research reagents. Although the biological role of extracellular glycolipids in nature is associated primarily with their surfactant properties, the detection of antifungal activity against a broad spectrum of yeast-like fungi in cellobiose lipids (representatives of these compounds) suggests that glycolipid secretion may play a key role in the adaptation to unfavorable environmental conditions. The study of structural peculiarities, the mechanism of action, and distribution of

these natural fungicides may be important for a better understanding of antagonistic relationship between microorganisms, as well as the prospects of their practical application as compounds for plant and crop protection from phytopathogenic fungi and antibiotics and biologically active compounds in medicine.

Generalization of the data on the biochemistry, cell biology, and biotechnology of extracellular fungal glycolipids is of concern for microbiologists, biochemists, biotechnologists, and students of the respective specialties.

The book presents modern data on the yeasts producing extracellular glycolipids, their composition, structure and properties, biosynthetic pathways, methods of isolation and identification, antifungal activity, and mechanisms of action. The applied potential of these compounds in medicine, agriculture, and industry is being considered. The emphasis is placed on cellobiose lipids, including their structure, distribution, and antifungal activity.

CONTENTS

CHAPTER 1

Structure and Occurrence of Yeast Extracellular Glycolipids

Secretion of glycolipids, namely fatty acid glycosides, was found in mycoplasms, bacteria (including actinobacteria), mixomycetes, fungi, plants, ascidia, and nematodes. The most-known extracellular glycolipids of yeast fungi are cellobiose lipids, mannosylerythritol lipids (MELs), and sophorolipids.

1.1 THE STRUCTURES OF EXTRACELLULAR GLYCOLIPIDS OF YEAST

1.1.1 Cellobiose Lipids

Cellobiose lipids consist of a residue of cellobiose, the disaccharide composed of two glucose residues linked by a 1,4′-β-glycoside bond, and fatty acid residue as an aglycone.

The simplest compound of this group consists of a cellobiose residue linked through a glycosidic bond to 2,15,16-trihydroxyhexadecanoic acid (Figure 1.1A). The diversity of cellobiose lipids is determined by O-substituents in cellobiose residue and by the number of hydroxyl groups in fatty acid residue. The cellobiose residue may contain acetate groups and/or C_6 or C_8 hydroxy fatty acids as O-substituents (Figure 1.1B, C).

According to the terminology of the review (Kitamoto et al., 2002), the cellobiose lipid without O-substituents in the cellobiose residue is named cellobiose lipid A; those containing C_6 or C_8 hydroxy acids as O-substituents, as well as one or two acetate groups, are named cellobiose lipid B; and the methylated form is named cellobiose lipid C. This terminology has not become prevalent, and the authors of most publications either use the IUPAC nomenclature or call the compounds under study cellobiose lipids, adding the species name of the producer. Authors' trivial names may also be encountered: flocculosin for the cellobiose lipid of *Pseudozyma flocculosa* (Mimee et al., 2005), although such compound is found as a minor in *Ustilago maydis* (Kitamoto et al., 2002; Bolker et al., 2008).

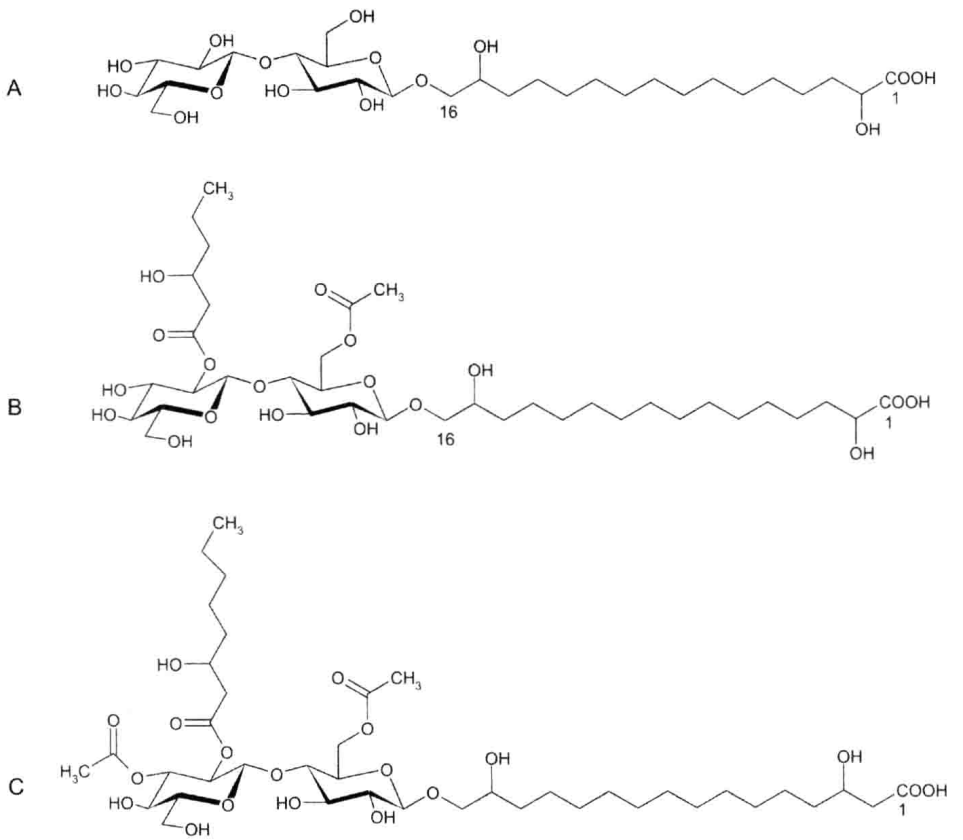

Figure 1.1 Cellobiose lipids of (A) Sympodiomycopsis paphiopedili, (B) Pseudozyma fusiformata, and (C) Pseudozyma flocculosa.

Extracellular cellobiose lipids were isolated for the first time from the culture liquid of smut fungus *U. maydis* (*zeae*) and named ustilagic acids in accordance with the generic name of the producer (Haskins and Thorn, 1951; Lemieux, 1951; Lemieux et al., 1951).

U. maydis was shown to secrete a mixture of non-acylated and acylated derivatives of β-D-cellobiosyl-2,16-dihydroxyl hexadecanoic acid and β-D-cellobiosyl-2,15,16-trihydroxyl hexadecanoic acid, including a relatively rare cellobiose lipid, methylated by the carboxylic group of 2,15,16-trihydroxyhexadecanoic acid (Frautz et al., 1986; Spoeckner et al., 1999; Bolker et al., 2008).

In *Pseudozyma fusiformata* (Kulakovskaya et al., 2005) and *Pseudozyma graminicola* (Golubev et al., 2008b), the major secreted

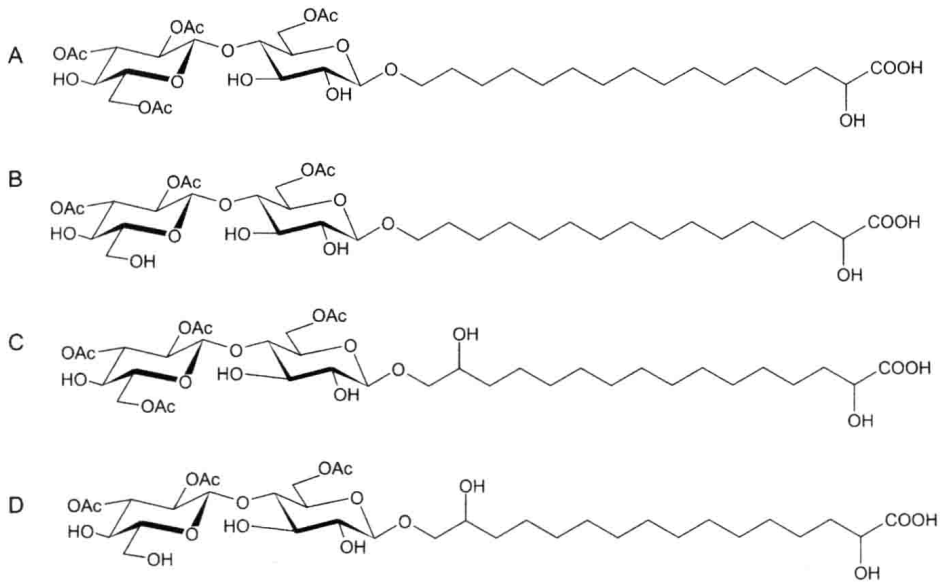

Figure 1.2 Structure of (A) major and (B–D) minor glycolipids of Cryptococcus humicola and Trichosporon porosum.

glycolipid is 2-*O*-3-hydroxyhexanoyl-β-D-glucopyranosyl-(1→4)-6-*O*-acetyl-β-D-glucopyranosyl-(1→16)-2,15,16-trihydroxyhexadecanoic acid (Figure 1.1B); however, some strains of *Ps. fusiformata* also secrete a simpler cellobiose lipid, having no 3-hydroxyhexanoic acid residue as an O-substituent.

The major extracellular glycolipid of the yeasts *Cryptococcus humicola* (Puchkov et al., 2002) and *Trichosporon porosum* (Kulakovskaya et al., 2010) is 2,3,4-*O*-triacetyl-β-D-glucopyranosyl-(1→4)-6-*O*-acetyl-β-D-glucopyranosyl-(1→16)-2,16-dihydroxyhexadecanoic acid (Figure 1.2A). Minor glycolipids of *Cr. humicola* were revealed containing C_{18} fatty acids with additional hydroxyl groups (Puchkov et al., 2002). Cellobiose lipids differing in the degree of acetylation and in the number of hydroxyl groups in the fatty acid residue were also obtained as minor components from the culture liquid of *Cr. humicola* strains (Puchkov et al., 2002; Kulakovskaya et al., 2006) and *T. porosum* (Kulakovskaya et al., 2010) (Figure 1.2B–D). The differences in cellobiose lipid composition of several strains of *Cr. humicola* were associated with prevalence of compounds with the four or three acetate groups in cellobiose residues (Kulakovskaya et al., 2006).

1.1.2 Mannosylerythritol Lipids

The structural peculiarities of MELs are described in a number of reviews (Kitamoto et al., 2002; Arutchelvi et al., 2008; Morita et al., 2009a; Arutchelvi and Doble, 2011). These glycolipids consist of a mannose residue etherified by erythrite at position 1. One or two fatty acid residues with a number of carbon atoms from 4 to 12 may be present in the mannose residue as O-substituents. The MELs are subdivided into three groups: MEL-A, MEL-B, and MEL-C, different in the quantity and position of acetate groups as O-substituents in the mannose residue (Kitamoto et al., 2002; Arutchelvi et al., 2008; Morita et al., 2009a; Arutchelvi and Doble, 2011) (Figure 1.3). Each of these groups includes a set of glycolipids which differ in the number of fatty acid residues as O-substituents in the mannose residue (mono- and diacylated MELs). Triacylated MELs etherified by the fatty acid residue at the terminal hydroxyl group of erythrite have been found in some strains of *Pseudozyma antarctica* and *Pseudozyma rugulosa* (Fukuoka et al., 2007a). In addition, there may also be numerous MEL stereoisomers.

MELs were found first as minor oily components in culture suspension of *U. maydis* (Haskins et al., 1955; Fluharty and O'Brien, 1969). MEL of *Ustilago* was characterized as a mixture of partially acylated derivatives of 4-*O*-β-D-mannopyranosyl-D-erythritol containing C_2, C_{12}, C_{14}, C_{16}, and C_{18} fatty acids residues (Bhattacharjee et al., 1970). MELs are major extracellular glycolipids of many species belonging to *Pseudozyma* genera (Kitamoto et al., 1990a,b, 1992a,b, 1993, 1995, 1998, 1999, 2001a; Fukuoka et al., 2007a,b, 2008a,b, 2012; Morita et al., 2006a,b, 2007, 2008a–d, 2009a,b, 2010a, 2011c, 2012, 2013). It has been shown that some or other MEL variants may be dominant in certain producers (Table 1.1). Most of the producers secrete not individual compounds but whole sets of MELs with different degrees of acylation and chain lengths of fatty acid residues.

The following rarely-occurring extracellular mannose-containing glycolipids have been found in *Pseudozyma parantarctica*: mannosylribitol lipids (with ribitol instead of erythrite), mannosylarabitol lipids (with arabitol instead of erythrite), and mannosylmannitol lipids (with mannitol instead of erythrite) (Morita et al., 2009a, 2012). *Pseudozyma shanxiensis* was found to produce more hydrophilic glycolipids than the previously-reported MELs. These MELs possessed a much shorter

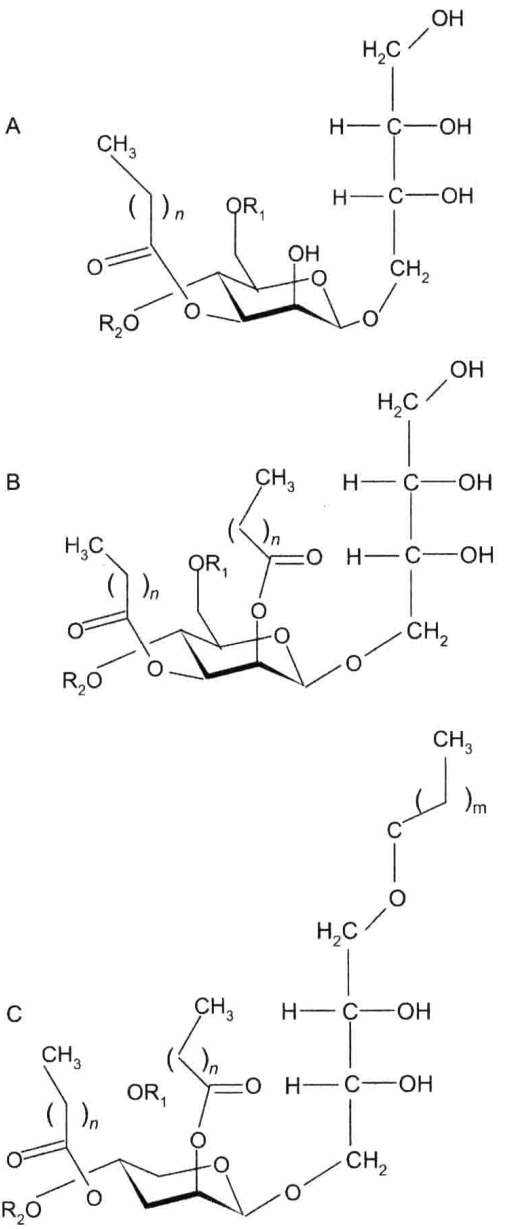

Figure 1.3 Structures of MELs: (A) monoacylated MEL, (B) diacylated MEL, and (C) triacylated MEL; MEL-A: R1 = Ac, R2 = Ac; MEL-B: R1 = Ac, R2 = H; MEL-C: R1 = H, R2 = Ac; n = 4–12; m = 6–16.

chain C-2 or C-4 at the C-2′ position of the mannose moiety compared to the MELs hitherto reported, which mainly possess a medium-chain acid C-10 at the position (Fukuoka et al., 2007b). *Pseudozyma churashimaensis* sp. was now found to produce a mixture of MELs,

Table 1.1 The Major Extracellular Glycolipids of Yeast Fungi and Their Producers

IUPAC (or Trivial) Names	Species	References
Cellobiose Lipids		
β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→16)-2,15,16-trihydroxyhexodecanoic acid	*Ustilago maydis*	Haskins and Thorn (1951), Lemieux (1951), Lemieux et al. (1951), Bhattacharjee et al. (1970), Frautz et al. (1986), Spoeckner et al. (1999)
	Sympodiomycopsis paphiopedili	Golubev et al. (2004), Kulakovskaya et al. (2004)
2-*o*-3-Hydroxyhexanoil-β-D-glucopyranosyl-(1→4)-6-*o*-acetyl-β-D-glucopyranosyl-(1→16)-2,15,16-trihydroxyhexodecanoic acid	*Ustilago maydis*	Haskins and Thorn (1951), Lemieux (1951), Lemieux et al. (1951), Bhattacharjee et al. (1970), Frautz et al. (1986), Spoeckner et al. (1999)
	Pseudozyma fusiformata	Kulakovskaya et al. (2005, 2007)
	Pseudozyma graminicola	Golubev et al. (2008a,b)
2-*o*-3-Hydroxyoctanoil-3-*o*-acetyl-β-D-glucopyranosyl-(1→4)-6-*o*-acetyl-β-D-glucopyranosyl-(1→16)-3,15,16-trihydroxyhexodecanoic acid	*Ustilago maydis*	Haskins and Thorn (1951), Lemieux (1951), Lemieux et al. (1951), Bhattacharjee et al. (1970), Frautz et al. (1986), Spoeckner et al. (1999)
	Pseudozyma flocculosa	Mimee et al. (2005)
2,3,4-*o*-Triacetyl-β-D-glucopyranosyl-(1→4)-6-*o*-acetyl-β-D-glucopyranosyl-(1→16)-2,16-dihydroxyhexodecanoic acid	*Cryptococcus humicola*	Puchkov et al. (2002), Kulakovskaya et al. (2006, 2007), Morita et al. (2011a), Imura et al. (2012)
	Trichosporon porosum	Kulakovskaya et al. (2010)
Mannosylerythritol Lipids (MELs)		
MEL-A		
4-*O*-[(4′,6′-di-*O*-acetyl-3′-*O*-alkanoil)-β-D-mannopyranosil] *meso*-erythritol	*Ustilago maydis*	Fluharty and O'Brien (1969), Spoeckner et al. (1999), Kurz et al. (2003)
4-*O*-[(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol	*Pseudozyma crassa*	Fukuoka et al. (2008a)
4-*O*-[(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil	*Pseudozyma antarctica*	Kitamoto et al. (1990a,b, 1992a,b, 1999), Morita et al. (2007), Fukuoka et al. (2007a)
	Pseudozyma aphidis	Rau et al. (2005)
	Pseudozyma churashimaensis	Morita et al. (2011c)
	Pseudozyma parantarctica	Morita et al. (2007, 2008c, 2012)
	Pseudozyma rugulosa	Morita et al. (2006a)

(*Continued*)

Table 1.1 (Continued)

IUPAC (or Trivial) Names	Species	References
	Pseudozyma fusiformata	Morita et al. (2007) Konishi et al. (2007)
	Kurtzmanomyces sp.	Kakugawa et al. (2002)
MEL-B		
4-*O*-[(6′-*O*-acetyl-3′-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol	*Ustilago maydis*	Fluharty and O'Brien (1969), Spoeckner et al. (1999), Kurz et al. (2003)
4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol	*Ustilago scitaminea*	Morita et al. (2011b)
	Pseudozyma churashimaensis	Morita et al. (2011c)
4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil	*Pseudozyma crassa*	Fukuoka et al. (2008a)
	Pseudozyma tsukubaensis	Fukuoka et al. (2008b)
	Pseudozyma antarctica	Kitamoto et al. (1990a,b, 1992a,b, 1999), Morita et al. (2007), Fukuoka et al. (2007a)
	Kurtzmanomyces sp.	Kakugawa et al. (2002)
MEL-C		
4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil4-*O*-[(4′-*O*-acetyl-3′-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol	*Ustilago maydis*	Fluharty and O'Brien (1969), Spoeckner et al. (1999), Kurz et al. (2003)
4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil4-*O*-[(4′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol	*Ustilago cynodontis*	Morita et al. (2008a)
4-*O*-[(6′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil4-*O*-[(4′-*O*-acetyl-2′,3′-di-*O*-alkanoil)-β-D-mannopyranosyl] *meso*-erythritol-alkanoil	*Pseudozyma churashimaensis*	Morita et al. (2011c)
	Pseudozyma crassa	Fukuoka et al. (2008a)
	Pseudozyma antarctica	Kitamoto et al. (1990a,b, 1992a,b, 1999), Morita et al. (2007), Fukuoka et al. (2007a)
	Pseudozyma graminicola	Morita et al. (2008d)
	Pseudozyma hubeiensis	Konishi et al. (2007, 2011)
	Pseudozyma shanxiensis	Fukuoka et al. (2007b)
	Pseudozyma siamensis	Morita et al. (2008b)
	Kurtzmanomyces sp.	Kakugawa et al. (2002)

(*Continued*)