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New perspectives for microbial glycolipid fractionation and purification processes

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ABSTRACT

Microbial glycolipids produced from renewable sources are of considerable interest in light of their promising biological activities and surfactant characteristics when compared to petroleum derived surfactants. Intense research efforts are currently being made to reduce their production costs and optimize recovery as selected mixtures through downstream processes. Due to the high complexity of natural glycolipid mixtures, efficient purification techniques are also required to examine the biological mechanisms of individual species towards human systems for their application in health-related areas. This review deals with recent advances in the development of glycolipid extraction, fractionation and purification methods, with a particular focus on solid support-free liquid-liquid separation techniques including centrifugal partition chromatography (CPC) and counter-current chromatography (CCC). These techniques offer promising perspectives for the preparative or large-scale separation of glycolipids from complex crude extracts, mainly because of their flexibility in solvent system selection and applicability to a diversity of structures of any polarity.

Mots clés :
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RÉSUMÉ

Les glycolipides microbiens issus de substrats biosourcés sont d'un intérêt considérable aux vues de leurs activités biologiques et de leur caractère surfactant comparé aux surfactants synthétiques. Étant donné la complexité des mélanges naturels de glycolipides, les efforts industriels et scientifiques sont actuellement centrés sur la réduction de leurs coûts de production et l'optimisation des procédés d'extraction, de fractionnement et de purification. Des outils de purification efficaces sont en particulier nécessaires pour isoler les espèces individuelles et étudier leurs mécanismes d'action biologiques. Cette revue présente les dernières avancées des techniques de fractionnement et de purification des principales familles de glycolipides microbiens. Le potentiel des techniques de séparation liquide-liquide sans support solide comme la chromatographie de partage centrifuge et la chromatographie à contre-courant sera en particulier discuté en termes de sélectivité, de possibilités d'applications à l'échelle préparative, et ce à partir d'extraits complexes contenant une grande diversité de structures de polarités différentes.

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1. Introduction

Microbial glycolipids represent a group of structurally diverse low molecular weight compounds generally produced by fermentation processes. Depending on their microbial origin and structural characteristics, they are classified into four main groups including rhamnolipids, sophorolipids, trehalose lipids and mannosylerythritol lipids (Fig. 1). Glycolipid structures are composed of a hydrophilic head linked to a hydrophobic tail. The hydrophilic moiety is typically a mono- or disaccharide unit while the hydrophobic tail is derived from a fatty acid side chain. This amphiphilic structure gives glycolipids interesting surfactant properties, thereby allowing them to reduce surface and interfacial tension between liquids, solids and gases and to form micelles and emulsions in liquid systems [1].

Worldwide surfactant production is about 10 million tons per year [2], and the majority of these compounds are petroleum derived. Microbial glycolipids produced from renewable substrates are an attractive and environmentally friendly alternative to petroleum surfactants because of their biodegradability and low toxicity. Glycolipids are also potentially interesting candidates for different environmental applications such as bioremediation and oil recovery from soil [3,4]. For example, rhamnolipids are able to scavenge organic compounds, which are sorbed to soil particles as well as mobilize contaminants such as heavy metals, oils and other toxic pollutants by pseudo-solubilisation or emulsification during soil washing treatments [5,6]. In addition to their environmental potential, glycolipids have also found a range of applications in the cosmetic, detergent, food, and pharmaceutical industries [7]. Mixtures of rhamnolipids and sophorolipids are able to form microemulsions in different vegetable oils that are applicable to biofuel production, hard surface cleansers and drug delivery [8]. Glycolipids are also becoming interesting alternatives to synthetic drugs due to their antimicrobial and antiviral activities [9–13].

Despite these promising physicochemical and biological properties, the commercialization of glycolipids as microbial biosurfactants has been hampered due to high production costs. Another major difficulty arises from the complexity of the glycolipid mixtures, which are naturally produced by yeast or bacteria. Their structural diversity in fermentation media limits the understanding of individual mechanisms of action and limits their valorization in cosmetic or health-related areas. Efforts are currently being made to improve fermentation and downstream processes to recover glycolipids in higher quantities, with better purity and efficient production costs [14,15]. Thus, the optimization of extraction and purification methods to obtain glycolipids either as selected mixtures or in pure form remains an important challenge.

This paper presents an overview of the different strategies developed in the last several years to obtain highly concentrated glycolipid extracts or to individually characterize these compounds. In particular we will focus on solid support-free liquid-liquid separation techniques such as centrifugal partition chromatography (CPC) or counter-current chromatography (CCC). These separation techniques have shown promising perspectives for the preparative-scale separation and purification of lipids and related compounds [16], but their application to glycolipids and more generally to natural surfactants remains scarce. The main advantages and limitations of CPC and CCC methods regarding glycolipid investigation and valorization will be discussed.

2. The main classes of glycolipids

2.1. Rhamnolipids

Rhamnolipids are mainly produced by *Pseudomonas aeruginosa* and *Burkholderia* sp. [17–19] and are by far the best studied glycolipids due to their fungicide properties and promising applications in the agricultural industry [20,21]. More than 60 different rhamnolipid structures have been identified. They comprise one or two L-rhamnose units linked through a β-glycoside bond to up to three hydroxy fatty acid groups (Fig. 1). The fatty acid chain length varies from 8 to 14 carbon atoms with or without unsaturation. In bacteria cells, rhamnolipids play a crucial role in the uptake and degradation of insoluble substrates and in biofilm development [19]. They possess strong surface active properties interesting for the large-scale production of biosurfactants and are also used to enhance the biodegradation of toxic organic soil contaminants [22]. Rhamnolipids have also demonstrated significant antiviral and antifungal activities against herpes simplex virus types I and II and phytopathogenic fungi, respectively [11–13]. Due to their effect on bacterial cell surface structures, rhamnolipids also display antibacterial activities [23,24].

2.2. Sophorolipids

Sophorolipids are synthesized by a number of non-pathogenic yeasts such as *Candida bombicola*, *Candida apicola* or *Thodotorula bogoriensis* [25]. They are released in the extracellular medium to act as extracellular carbon storage material for the regulation of high osmotic strength produced from high sugar concentrations [26]. Globally, sophorolipids lower the surface tension of water from 73 mN/m to about 30–40 mN/m, with a critical micelle concentration of 40 to 100 mg/L. They show excellent skin compatibility and thus are employed in various cosmetic formulations. Sophorolipids have also demonstrated interesting antimicrobial activity against plant pathogenic fungi [27], a capacity to inhibit the human immunodeficiency virus [10] and more recently a potential for liver cancer treatment [28]. Their structure consists of one sophorose unit (a glucose disaccharide with an unusual β-1,2 bond) which is β-linked to a hydroxy fatty acid containing 16 to 18 carbon atoms and one or more unsaturations [29]. Sophorose can be acetylated at the 6′- and/or 6″ position and the hydroxy fatty acid can be linked to the sophorose unit at the terminal or subterminal position (Fig. 1). The carboxylic end of the fatty acid is either free or esterified at the 4″ position (lactone form). The physicochemical and biological properties of sophorolipids are significantly