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Rhamnolipids—Next generation surfactants?

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ABSTRACT

The demand for bio-based processes and materials in the petrochemical industry has significantly increased during the last decade because of the expected running out of petroleum. This trend can be ascribed to three main causes: (1) the increased use of renewable resources for chemical synthesis of already established product classes, (2) the replacement of chemical synthesis of already established product classes by new biotechnological processes based on renewable resources, and (3) the biotechnological production of new molecules with new features or better performances than already established comparable chemically synthesized products. All three approaches are currently being pursued for surfactant production. Biosurfactants are a very promising and interesting substance class because they are based on renewable resources, sustainable, and biologically degradable. Alkyl polyglycosides are chemically synthesized biosurfactants established on the surfactant market. The first microbiological biosurfactants on the market were sophorolipids. Of all currently known biosurfactants, rhamnolipids have the highest potential for becoming the next generation of biosurfactants introduced on the market. Although the metabolic pathways and genetic regulation of biosynthesis are known qualitatively, the quantitative understanding relevant for bioreactor cultivation is still missing. Additionally, high product titers have been exclusively described with vegetable oil as sole carbon source in combination with *Pseudomonas aeruginosa* strains. Competitive productivity is still out of reach for heterologous hosts or non-pathogenic natural producer strains. Thus, on the one hand there is a need to gain a deeper understanding of the regulation of rhamnolipid production on process and cellular level during bioreactor cultivations. On the other hand, there is a need for metabolizable renewable substrates, which do not compete with food and feed. A sustainable bioeconomy approach should combine a holistic X-omics strategy with metabolic engineering to achieve the next step in rhamnolipid production based on non-food renewable resources. This review discusses different approaches towards optimization of rhamnolipid production and enhancement of product spectra. The optimization of rhamnolipid production with *P. aeruginosa* strains, screening methods for new non-pathogenic natural rhamnolipid producers and recombinant rhamnolipid production are examined. Finally, biocatalysis with rhamnolipids for the synthesis of L-rhamnose, β-hydroxyfatty acids, and tailor-made surfactants is discussed. Biosurfactants are still in the phase of initial commercialization. However, for next generation development of rhamnolipid production processes and next generation biosurfactants there are still considerable obstacles to be surmounted, which are discussed here.

1. Microbial biosurfactants for industrial use

1.1. Introducing biosurfactants

The development of economical and sustainable bioprocesses replacing petrochemical based synthesis of established products has significantly increased since the beginning of the new millennium. One promising substance class currently under investigation for sustainable production, are surfactants based on renewable primary products, generally called biosurfactants (Breucker et al., 1995; Maneerat, 2005). These biosurfactants can be produced either by chemical synthesis like alkyl polyglycosides (APG) or by means of microbial cultivation (Deleu and Paquot, 2004). They are ecologically well acceptable, biodegradable, and many biosurfactants of microbial origin show interesting biological activity (Van Bogaert et al., 2007; Vatsa et al., 2010). Microbial biosurfactants are produced extracellularly by microorganisms when

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Table 1
Structural classification of microbial biosurfactants according to Lang and Trowitzsch-Kienast (2002).

Structural class	Examples
Glycolipids	Mannosylerythritol lipids (MELs)
	Sophorolipids (SL)
	Rhamnolipids (RL)
	Trehalose lipids
Lipopeptides/lipoamino acids	Surfactin
	Lysin lipids
	Ornithine lipids
Polymers	Proteins
	Lipoproteins (e.g., Liposan)
	Polysaccharides
	Lipopolysaccharides (e.g., Emulsan)
Oil/membranes	Glycerolipids
	Phospholipids
	Fatty acids

Table 2
HLB values according to Griffin (1954) and the respective predicted surfactant properties.

HLB value	Predicted property
0–3	Anti-foaming agents
4–6	W/O emulsifiers
7–9	Wetting agents
8–18	O/W emulsifiers
13–15	Typical detergents
10–18	Solubilizers or hydrotopes

growing on water immiscible substrates (Ron and Rosenberg, 2001).

1.2. Characterization of microbial biosurfactants

Traditionally, microbial biosurfactants are classified by their structure and surfactant properties (Lang and Trowitzsch-Kienast, 2002). The most common structural classification as summarized by Lang and Trowitzsch-Kienast (2002) is presented in Table 1.

A fast and simple preliminary method of characterization of biosurfactants is by thin-layer chromatography with sequential staining. Typical solvent systems used for separation and staining solutions for chemical analysis have recently been summarized (Satpute et al., 2010). The actual structure analysis is performed by various spectrometric and classical chemical methods, which have also been summarized by Satpute et al. (2010).

The so-called hydrophilic–lipophilic-balance value (HLB) is determined by calculating values for the different regions of the molecule, as described by Griffin (1949, 1954) and Davies (1957). The HLB value varies between 0 and 20. According to Griffin the HLB can be calculated as shown in Eq. (1).

$$\mathrm{HLB} = 20 \cdot \left(\frac{\mathrm{MW_{HP}}}{\mathrm{MW_{SA}}} \right) \quad (1)$$

$\mathrm{MW_{HP}}$ indicates the molecular weight of the hydrophilic part and $\mathrm{MW_{SA}}$ indicates the molecular weight of the whole surface-active agent. The HLB value allows prediction of the surfactant properties of a molecule as shown in Table 2.

Surfactant properties are strongly influenced by their net charge. There can be non-ionic, anionic, cationic, or zwitterionic compounds (Lang, 2002; Syldatk and Wagner, 1987). Cationic biosurfactants of microbial origin have not been described. The structure and HLB value may allow estimation of the surfactant properties, but actual characterization is more valuable. Therefore, different analysis methods have been established which could be implemented into screening concepts (Walter et al., 2008) to characterize the surfactant features before actual structure analysis The emulsification index (E24) is a fast and valuable method to determine the emulsifying properties of a surfactant (Cooper and Goldenberg, 1987). Basically, kerosene or another hydrocarbon compound is mixed vigorously with the surfactant and the E24 is determined after 24 h. In addition the surface activity of individual strains can be determined qualitatively with the microplate assay developed and patented by Vaux and Cottingham and modified later by Chen et al. (Chen et al., 2007a; Cottingham et al., 2004; Vaux and Cottingham, 2007). Reduction of surface and interfacial tension, as well as critical micelle concentration (CMC) values, can be determined by tensiometric methods like the Du-Noüy-Ring or Wilhelmi plate approach. Various characterization methodologies have been reviewed in detail by Satpute et al. (2010) and Walter et al. (2008). Further details will be given in Section 3.2.1. Apart from these chemical and physical characterizations, so-called performance indicators are important for the later biosurfactant product itself. These performance indicators may include haptic properties of the surfactant, foaming abilities, odor and color.

1.3. Microbial biosurfactants through the ages

The biotechnological interest in biosurfactants started in the 1980s, then basically focusing on the use of biosurfactants in tertiary oil recovery and bioremediation (Syldatk and Wagner, 1987). Mainly pure hydrocarbons as C-sources were used for their production (Fish et al., 1982; Hisatsuka et al., 1971; Itoh and Suzuki, 1972; Syldatk et al., 1985a). The focus of this basic research was on finding microorganisms able to produce biosurfactants (Laurila, 1985; Wagner et al., 1983), chemical structure elucidation (Edwards and Hayashi, 1965; Itoh et al., 1971; Syldatk et al., 1985b), and analysis of surface and interfacial active properties (Reddy et al., 1983; Syldatk et al., 1985b). The conclusion in the 1980s was that microbial biosurfactants are highly interesting classes of compounds because of their ecological and surfactant properties, but that they were too expensive for industrial use when compared to synthetic surfactants (Syldatk and Wagner, 1987). Limiting factors for economic production were relatively high substrate costs (e.g., pure hydrocarbons) and low product concentrations frequently caused by product inhibition. Additionally, microbial strains were often pathogenic or difficult to handle at a larger scale (Reiling et al., 1986) and producing product mixtures instead of single products results in relatively high costs for downstream processing and purification (Heyd et al., 2008). Although microbial biosurfactants showed surfactant properties and features comparable to those of petrochemically derived surfactants, the economic interest in such biosurfactants in the late eighties, was mainly to produce unusual and valuable sugars like L-rhamnose and fatty acids for nutrition and pharmaceutical application (Linhardt et al., 1989). For example different companies like Suedzucker AG (Mannheim, Germany) and former Hoechst AG (Frankfurt am Main, Germany) applied for various patents for the production and purification of L-rhamnose from rhamnolipids produced by *Pseudomonas aeruginosa* (Giani et al., 1997; Mixich and Rapp, 1990; Mixich et al., 1997). Recent interest in a broader use of biosurfactants as detergents in food (Nitschke and Costa, 2007; Velikonja and Kosaric, 1993), cosmetics (Klekner and Kosaric, 1993), pharmaceutical applications (Leighton, 2010; Rodrigues et al., 2006) and bioremediation (Kosaric, 2001; Pacwa-Plociniczak et al., 2010) can be explained by the increased interest in use of renewable resources or organic waste materials as cheaper substrates (Makkar and Cameotra, 2002) than the relatively expensive pure hydrocarbons. The market potential for biosurfactants has been discussed in detail several times (Banat et al., 2000, 2010; Desai and Banat, 1997; Islas et al., 2010). However, the only