SUSANNE NIESCHER
Freiberg University of Mining and Technology, Freiberg, Germany

MICROBIAL SURFACTANTS AND RHODOCOCCUS – FACTS AND POSSIBILITIES

The article describes possible applications of surfactants of microbial origin (*Rhodococcus biosurfactants*) for decontamination of hydrocarbons.

Описано возможное применение поверхностно-активных веществ микробного происхождения (био-ПАВ семейства *Rhodococcus*) для очистки от загрязнения углеводородами.

Hydrocarbons are released in remarkable amounts as a result of anthropogenic sources, namely the extensive utilisation of petroleum products, often entailing extensive pollution of environmental habitats (Swannell et al., 1996). Their decontamination can be achieved by using the natural potential of microorganisms which have adapted to the hydrophobic nature of various of these hardly bioavailable compounds by developing specific uptake-strategies, a crucial one being the formation of biosurfactants (Hommel, 1990; Makkar & Rockne, 2003).

Biosurfactants are microbial produced, surface-active, amphiphilic compounds which are characterised by a hydrophilic and a hydrophobic molecule part. Due to their physical and chemical properties biosurfactants significantly reduce surface- and interfacial tensions in both aqueous solutions and hydrocarbon mixtures, and hence contribute to enhanced pseudo-solubilisation and emulsification of highly waterinsoluble compounds, such as long-chain n-alkanes, and facilitate the cell-substrate contact (Kitamoto, 2002; Makkar & Rockne, 2003; Philp et al., 2002; Ron & Rosenberg, 2001). Biosurfactants represent a structurally very heterogeneous group. According to their size they can be divided into high- and low-molecular biosurfactants. Glycolipids, e.g. rhamnolipids or trehalose lipids, as one major group of bio-surfactants are by far the most common and best-investigated type. They basically consist of low-molecular, water-insoluble sugars that are acylated with longchain fatty acids or hydroxy fatty acids.

Compared to their synthetic counterparts, surfactants of microbial origin are less toxic to soil micro-organisms, show a better biodegradability (Banat et al., 2000; Kitamoto, 2002) and are more effective under extreme habitat conditions (Cameotra & Makkar, 1998). Biosurfactants show considerable commercial relevance (Banat et al., 2000; Lang, 2002; Makkar & Cameotra, 2002), their emulsifying and emulsion-stabilising properties which are not remarkable changed by shifts in temperature or salinity, and also their low toxicity favour them in a number of environmental applications, namely in bioremediation purposes (Bai et al., 1997; Christofi & Ivshina, 2002; Mulligan, 2005; Ron & Rosenberg, 2002).

However, the realisation of their commercial application requires an economical convenient high-level production which has not yet been established for most of the auspicious biosurfactants so far. Inefficient processing methodology, poor strain productivity and, in many cases, the necessity to use expensive substrates account for the economical hindrance of microbial surfactants (Makkar & Cameotra, 2002), e.g. trehalose-containing glycolipids.

Trehalose lipids are produced by various members of the genus Rhodococcus during growth on liquid, medium- and long-chained n-alkanes (Lang & Philp, 1998). Typical structures evidenced are non-ionic trehalose

monomycolates (Kretschmer, 1983), trehalose di(coryno/nocardio)mycolates (Niescher et al., 2006; Philp et al., 2002), trehalose trimycolates (Tomiyasu et al., 1986), as well as various acylated derivatives of trehalose (Kretschmer, 1983; Philp et al., 2002; Singer et al., 1990). Some strains also produce anionic trehalose lipids, such as trehalose tetraesters, preferably under nitrogen-limited conditions (Rapp & Gabriel-Jurgens, 2003; Ristau & Wagner, 1983).

Compared to the well understood mechanism of rhamnolipid formation by Pseudomonas aeruginosa (Lang & Wullbrandt, 1999; Maier & Soberon-Chavez, 2000), less information is available for the genetic and biochemical background of trehalose lipid biosynthesis in rhodococci. Former studies clearly indicated that the hydrophilic trehalose moiety is synthesised independently from the hydrophobic fatty acid part (Hommel, 1993; Kretschmer, 1983). Hence, analysis of the molecular background of trehalose lipid formation implies asking for the molecular whereabouts of trehalose, too.

Within the OtsAB-pathway, the best characterised and most widely distributed mechanism of trehalose biosynthesis, (Elbein et al., 2003) a trehalose-6-phosphate synthase (OtsA, TPS) catalyses the formation of trehalose-6-phosphate from a nucleoside diphosphate glucose (most common UDP-glucose) and glucose-6-phosphate. In a second step a trehalose-6-phosphate phosphatase (OtsB) catalyses the dephosphorylation to yield trehalose. Recent studies with knock-out mutants revealed that at least in mycobacteria the OtsAB-pathway is most important for trehalose biosynthesis (Murphy et al., 2005). BLAST search of available mycobacteria genomes reveals that these organisms obviously possess only a single gene encoding OtsA. On the contrary, genome data available for Rhodococcus sp. RHAI (Eltis, 2005) and the investigation of two model organisms Rhodococcus opacus 1CP and Rhodococcus erythropolis B7g for the presence of genes encoding putative TPS gave evidence that members of this genus harbour two otsA isogenes.

The presence of a second trehalose-6-phosphate synthase might reflect the unique ability of rhodococci to overproduce trehalose lipids during growth on alkanes, and the involvement of the enzyme in the synthesis of these biosurfactants. The usage of TPS isoenzymes contributing to an enhancement of anabolic versatility is consistent with the existence of multiple homologues of catabolic enzymes known from various rhodococci (reviewed by: Larkin et al., 2005). The catabolic versatility of the genetically well investigated Rhodoccus opacus 1CP becomes consolidated by two chlorocatechol gene clusters, at least three different genes encoding catechol 1,2-dioxygenases, and at least four different hydroxylases for phenol or substituted phenols, respectively (Eulberg et al., 1997; Eulberg et al., 1998; Groning, 2005; Moiseeva et al., 2002). Its linear megaplasmid p1CP carries several genes encoding chlorocatechol catabolic enzymes (Konig et al., 2004). Strain 1CP was originally enriched from soil on 2,4-dichlorophenol (Gorlatov, 1989) and utilises a broad spectrum of (chloro-) aromatic compounds (Gorlatov, 1989; Moiseeva, 1999). The strain 1CP also uses long-chained n-alkanes as sole sources of carbon and energy, and synthesises a novel variant of trehalose dimycolate (Niescher et al., 2006).

The remarkable genetic equipment of various members of the genus Rhodococcus accounts for their extraordinary metabolic potential which together with their robust cellular physiology provides these organisms with an increased ability to adapt to difficult living conditions and harsh environments facilitating their prospective application in biotechnology (de Carvalho & da Fonseca, 2005; Larkin et al., 2005).