Chemical Characterization and Physical and Biological Activities of Rhamnolipids Produced by *Pseudomonas aeruginosa* BN10

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Pseudomonas aeruginosa BN10 isolated from hydrocarbon-polluted soil was found to produce rhamnolipids when cultivated on 2% glycerol, glucose, *n*-hexadecane, and *n*-alkanes. The rhamnolipids were partially purified on silica gel columns and their chemical structures elucidated by combination of one- and two-dimensional ^{1}H and ^{13}C NMR techniques and ESI-MS analysis. Eight structural rhamnolipid homologues were identified: Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-C_{12}, Rha_2-C_{10}-C_8, Rha_2-C_{10}-C_{10}, Rha_2-C_{10}-$C_{12:1}$, and Rha_2-C_{10}-C_{12}. The chemical composition of the rhamnolipid mixtures produced on different carbon sources did not vary with the type of carbon source used. The rhamnolipid mixture produced by *Pseudomonas aeruginosa* BN10 on glycerol reduced the surface tension of pure water from 72 to 29 mN m^{-1} at a critical micellar concentration of 40 mg l^{-1}, and the interfacial tension was 0.9 mN m^{-1}. The new surfactant product formed stable emulsions with hydrocarbons and showed high antimicrobial activity against Gram-positive bacteria. The present study shows that the new strain *Pseudomonas aeruginosa* BN10 demonstrates enhanced production of the di-rhamnolipid Rha_2-C_{10}-C_{10} on all carbon sources used. Due to its excellent surface and good antimicrobial activities the rhamnolipid homologue mixture from *Pseudomonas aeruginosa* BN10 can be exploited for use in bioremediation, petroleum and pharmaceutical industries.

Key words: Pseudomonas, Rhamnolipids, Emulsification, Antimicrobial Activity

Introduction

Surface-active agents produced by microorganisms represent an important and interesting area of research from an environmental standpoint. They possess a number of advantages over their chemical counterparts such as low toxicity, biodegradability, and effectiveness in a wide range of pH values and temperatures. Because of their unique properties to reduce surface and interfacial tension between solids, liquids, and gases, biosurfactants have a potential use in food, pharmaceutical, cosmetic, and petrochemical industries (Desai and Banat, 1997).

Biosurfactants can be categorized in five groups regarding their chemical composition: glycolipids, lipopeptides, phospholipids, fatty acids, and polymeric biosurfactants. Although many microorganisms are able to produce surface-active substances, the rhamnolipids (Rha, rhamnose-containing glycolipids) from *Pseudomonas* species represent one of the most important classes of biosurfactants showing high production yields and a good potential for commercial exploitation (Banat *et al*., 2000). Rhamnolipids are usually produced as a mixture of various homologues (Deziel *et al.*, 1999; Soberon-Chavez *et al.*, 2005), the most common of them being mono-rhamnolipids (Rha_1, one L-rhamnose linked to one or two molecules of β-hydroxyalkanoic acids) and di-rhamnolipids (Rha_2, two units of L-rhamnose similarly linked to β-hydroxycarboxylic acids).

The composition of a rhamnolipid mixture depends on several parameters, the most important being the bacterial strain, medium composition, culture conditions, and culture age (Deziel *et al*., 1999; Mata-Sandoval *et al*., 1999). The composition of the mixture and the ratios between the

homologues determine the properties of the biosurfactant, and even slight differences in the mixture composition can have great consequences for its physicochemical properties (Monteiro *et al.*, 2007).

Di-rhamnolipids are more soluble in water and are more surface-active than mono-rhamnolipids (Peker *et al.*, 2003; Deziel *et al.*, 1999; Mata-Sandoval *et al.*, 1999). It has been shown that di-rhamnolipids can be utilized for bioremediation of phenanthrene-contaminated soils (Zhang and Miller, 1992), pharmacy, and dermatology (Stipcevic *et al.*, 2005, 2006). However, until now, there are few studies reporting an improved production of di-rhamnolipids from mono-rhamnolipids (*e.g.* Deziel *et al.*, 1999; Mata-Sandoval *et al.*, 1999; Wei *et al.*, 2008). More often the quantities of the mono- and di-rhamnolipids in the obtained mixture are either very similar or the mono-rhamnolipid component is predominant (Arino *et al.*, 1996; Sim *et al.*, 1997; Costa *et al.*, 2006; Deziel *et al.*, 2000; Benincasa *et al.*, 2004; Mulligan, 2005).

This study deals with the production of rhamnolipid biosurfactants by the strain *Pseudomonas aeruginosa* BN10 isolated from soil polluted with hydrocarbons, found in Western Bulgaria in the vicinity of a metallurgical factory. The obtained rhamnolipids are structurally identified, and the physicochemical and biological properties of the mixture are investigated in view of future potential uses.

Material and Methods

Microorganism and cultivation

The strain *Pseudomonas aeruginosa* BN10 was isolated from soil polluted with hydrocarbons by a standard enrichment technique as described previously (Tuleva *et al.*, 2008). Strain BN10 was maintained in nutrient agar slants (Difco Laboratories Inc, Detroit, MI, USA) at 4 °C. Inocula were prepared by transferring bacterial cells from the storage culture to 250-ml flasks, containing 50 ml of nutrient broth, and incubating at 30 °C and 150 rpm on a rotary shaker. Four ml of this mid-exponential phase culture were inoculated into 1-l flasks containing 200 ml of mineral medium with the following composition (g l^{-1}): $K_2HPO_4 \cdot 3\ H_2O$ (7.0); KH_2PO_4 (3.0); $(NH_4)_2SO_4$ (1.0); $MgSO_4 \cdot 7\ H_2O$ (0.2). The pH value of the medium was adjusted to 7.0. Cultures were incubated while shaking (150 rpm) at 30 °C for 7 d. The carbon sources (2%, v/v) used were glucose, glycerol, *n*-hexadecane, and *n*-alkanes.

Detection of biosurfactant production

Four simple preliminary methods were used for detection of the biosurfactant production: (1) Surface tension (ST) measurement of the supernatant fluid was carried out after centrifugation at 8000 x *g* for 20 min. (2) Emulsifying activity of the culture supernatant was estimated by adding 0.5 ml of sample fluid and 0.5 ml of kerosene to 4.0 ml of distilled water. The tube was vortexed for 10 s, held stationary for 1 min, and then visually examined for turbidity of a stable emulsion. (3) Blue agar plates containing cetyltrimethyl ammonium bromide (CTAB) (0.2 mg ml^{-1}; Sigma Chemical Co., Poole, UK) and methylene blue (5 μg ml^{-1}) were used to detect extracellular rhamnolipid production (Siegmund and Wagner, 1991). Biosurfactants were observed by the formation of dark blue halos around the colonies. (4) Hemolyses of erythrocytes was tested by spotting 10 μl of 100-fold concentrated culture supernatant onto agar plates containing 5% sheep blood (Johnson and Boese-Marrazzo, 1980).

The orcinol assay (Chandrasekaran and Bemiller, 1980) was used for direct assessment of the amount of glycolipids in the sample. The rhamnolipid concentrations were calculated from standard curves prepared with L-rhamnose and expressed as rhamnose equivalents (RE) (mg ml^{-1}).

Isolation and purification of the rhamnolipids

To remove the cells the culture broth was centrifuged at 12500 x *g* for 20 min. Then the supernatant was acidified with 6 M HCl and allowed to stay at 4 °C overnight. The precipitate was collected after centrifugation at 12500 x *g* for 20 min and extracted with an equal volume of ethyl acetate. The organic phase was removed under reduced pressure at 40 °C, and the resulting light brown viscous liquid was considered as the crude extract.

The biosurfactant-containing extract was dissolved in 3 ml of chloroform/methanol/water (65:15:2, v/v/v) and afterwards purified by normal-pressure chromatography on silica gel 60 (particle size, 0.063–0.200 mm; mesh size, 70–230; Merck, Darmstadt, Germany). The surface-active compounds were eluted with dichloromethane/methanol/water (65:15:2, v/v/v), and fractions

(2 ml) were collected. Partially purified biosurfactant was obtained and analysed by thin-layer chromatography (TLC) using as mobile phase the same solvent mixture and spraying with orcinol/sulfuric acid reagent for detection of rhamnolipid compounds.

Characterization of the rhamnolipid mixture

All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance II+ 600 spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany), operating at 600.13 MHz for hydrogen, equipped with a pulse gradient unit.

Ion electrospray (ESI) mass spectra of the same were recorded on a Finnigan ion trap mass spectrometer LCQ DECA (Thermo Quest LC and LC/MS Division, San Jose, CA, USA). The ESI mode was as follows: sheath gas, nitrogen (6 l min^{-1}); positive mode $[M + Na]^+$ at (+) 4 kV or negative mode $[M - H]^-$ at (–) 4 kV of ionization; temperature and voltage of the heated capillary, 300 °C and 25 V, respectively; tube lens offset, 5 V.

Physicochemical characterization of the rhamnolipid mixture

The surface tension measurements of the rhamnolipid solutions were made by the du Noüy ring method using a "Krüss" tensiometer (Hamburg, Germany). Before each measurement the instrument was calibrated against triple distilled water.

The critical micellar concentration (CMC) was determined from the break point of the surface tension vs. logarithm of bulk rhamnolipid concentration curve. Interfacial tension was measured at the aqueous solution/*n*-hexadecane interface. All experiments were performed at 22 °C at natural pH (~5.5).

The emulsification properties of the rhamnolipid mixture were tested on different oil phases: toluene, benzene, *n*-hexadecane, *n*-alkanes ($C_{12}-C_{22}$), kerosene, crude oil, diesel oil, sunflower, and olive oil. The rhamnolipids were dissolved in 5 ml distilled water (0.5%, w/v) and mixed with 5 ml of each hydrophobic substrate, vortexed to homogeneity, and left to stand for 24 h and 168 h. The emulsification index (E_{24} and E_{168}) was calculated by determining the percentage volume occupied by the emulsion after 24 h and 168 h as described by Cooper and Goldenberg (1987). All data were mean values from three independent experiments.

Antimicrobial activity of the rhamnolipid mixture

Antimicrobial activity was determined by the agar diffusion test according to European Pharmacopoeia (1997). Test organisms were suspended in melted nutrient agar and poured into Petri dishes. Holes of 8 mm in diameter were cut in the agar and filled with 100 μl solution of various concentrations of the biosurfactant. The diameter of the clear zone around the point of application of the biosurfactant was measured. Bacterial strains were grown for 24 h at 37 °C in nutrient agar (Serva, Feinbiochemica, Heidelberg, Germany), yeast strains were grown in yeast peptone dextrose agar (YEPD) and fungi in potato dextrose agar (PDA). Yeast and fungal strains were incubated for 72 h at 28 °C. Fifteen bacterial Gram-positive and Gram-negative strains were tested, including: *Bacillus subtilis* ATCC 6633, *Bacillus mycoides* DSMZ 274, *Bacillus cereus* ATCC 11778, *Bacillus megaterium* NRRL 1353895, *Staphylococcus aureus* NRRL B 313, *Streptococcus pneumoniae* ATCC 6308, *Streptococcus piogenes* ATCC 19615, *Micrococcus luteus* ATCC 9631, *Sarcina lutea* ATCC 9341, *Acinetobacter johnsonii* ATCC 17909, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* CECT 17832, *Alcaligenes faecalis* ATCC 8750, *Salmonella thyphimurium* ATCC 16028. The yeast strains were: *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10321, *Rhodotorula rubra* CECT 1158. The fungal strains were: *Penicillium chrysogenum* CECT 2802, *Aspergillus niger* ATCC 14604. The experiments were carried out in triplicate.

Results

Detection of the biosurfactant production

Strain BN10 was submitted to the blue agar test (Siegmund and Wagner, 1991) and to the blood agar test (Johnson and Boese-Marrazzo, 1980) and showed positive results for extracellular rhamnolipid production.

Strain BN10 grew well on all four substrates tested (glycerol, glucose, *n*-hexadecane, and *n*-alkanes) but the reduction of the surface tension was different depending on the carbon source. *n*-Alkanes showed the least reduction (from 70 to 40.3 mN m^{-1}) followed by glucose (33.4 mN m^{-1}). *n*-Hexadecane and glycerol were better substrates for biosurfactant production as the surface ten-

Table I. Chemical composition and relative abundance of the rhamnolipids produced by *P. aeruginosa* BN10 on 2% glycerol.

Rhamnolipid	*m/z* $[M - H]^-$	Fragmentation *m/z* (50–800)	Relative abundance (%)
Rha-C_{10}-C_8	476	333, 169, 163	3.7
Rha-C_{10}-C_{10}	503	333, 169, 163	20.6
Rha-C_{10}-$C_{12:1}$	529	333, 169, 163	0.9
Rha-C_{10}-C_{12}	531	333, 169, 163	1.3
Rha_2-C_{10}-C_8	621	479, 311, 169	7.5
Rha_2-C_{10}-C_{10}	649	479, 311, 169	60.1
Rha_2-C_{10}-$C_{12:1}$	675	479, 311, 169	3.3
Rha_2-C_{10}-C_{12}	677	479, 311, 169	2.6

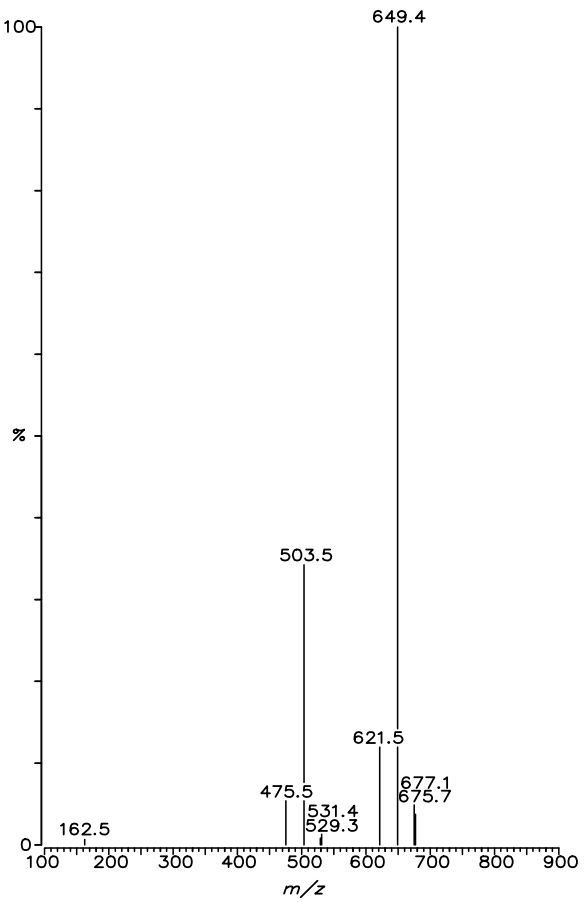

Fig. 1. Negative ion electrospray mass spectrum of the rhamnolipid mixture produced by *P. aeruginosa* BN10.

sion of the cultures was reduced to 28.3 and 27.5 mN m^{-1}, respectively. The supernatant fluids from cultures grown on all tested substrates formed stable emulsions with kerosene, which was also attributed to biosurfactant production.

Surfactants recovery and characterization

When *Pseudomonas aeruginosa* BN10 was cultivated on 2% glycerol, 4.2 g l^{-1} of the biosurfactant were produced after 7 days of incubation. Acid precipitation, extraction with ethyl acetate, and purification by column chromatograhy on silica gel gave the partially purified biosurfactant homologue mixture. To identify the chemical structures of the components in the mixture ESI-MS and NMR spectroscopy were used.

Table I presents data from the mass spectra of the components of the rhamnolipid mixture produced by *Pseudomonas aeruginosa* BN10 on 2% glycerol. Negative ESI-MS gave two main pseudomolecular ions – at *m/z* 503 and at *m/z* 649 (Fig. 1). These pseudomolecular ions represent the molecular weight of the mono-rhamnolipid Rha-C_{10}-C_{10} (rhamnosyl-*β*-hydroxydecanoyl-*β*-hydroxydecanoic acid) and of the di-rhamnolipid Rha_2-C_{10}-C_{10} [*β*-*α*-rhamnosyl (1→2) rhamnosyl-*β*-hydroxydecanoyl-*β*-hydroxydecanoic acid]. Minor ions were also detected – at *m/z* 476, *m/z* 529, and *m/z* 531 (consistent with Rha-C_{10}-C_8, Rha-C_{10}-$C_{12:1}$, and Rha-C_{10}-C_{12}), and at *m/z* 621, *m/z* 675, and *m/z* 677 (consistent with Rha_2-C_{10}-C_8, Rha_2-C_{10}-$C_{12:1}$, and Rha_2-C_{12}-C_{10}).

The chemical structures of the main components of the mixture as mono- and di-rhamnolipids were confirmed by ^1H and ^{13}C NMR spectral analyses. Resonance signals characteristic for lipids appeared at 13.7 ppm (CH_3) and 22.5–32.7 ppm (CH_2). The ester (C=O) and carboxylic (C=O) signals at 171.5 ppm and at 173.6 ppm were also observed. The rhamnosyl CH_3 protons appeared as two overlapping doublets at 1.28 ppm which correlated with ^{13}C signals at 13.7 ppm and showed strong COSY correlation to C-5 and C-5' protons at 3.65 and 3.71 ppm. The rhamnosyl ring protons were located in the region 3.34–3.79 ppm. Fig. 2 shows the heteronuclear multiple bond correlation (HMBC) spectrum of the obtained di-rhamnolipid surfactant.

Effect of the carbon source on the composition of the rhamnolipid mixtures

When cultivated on water-soluble and -insoluble substrates (glycerol, glucose, *n*-hexadecane, and *n*-paraffines) strain BN10 produced a cul-

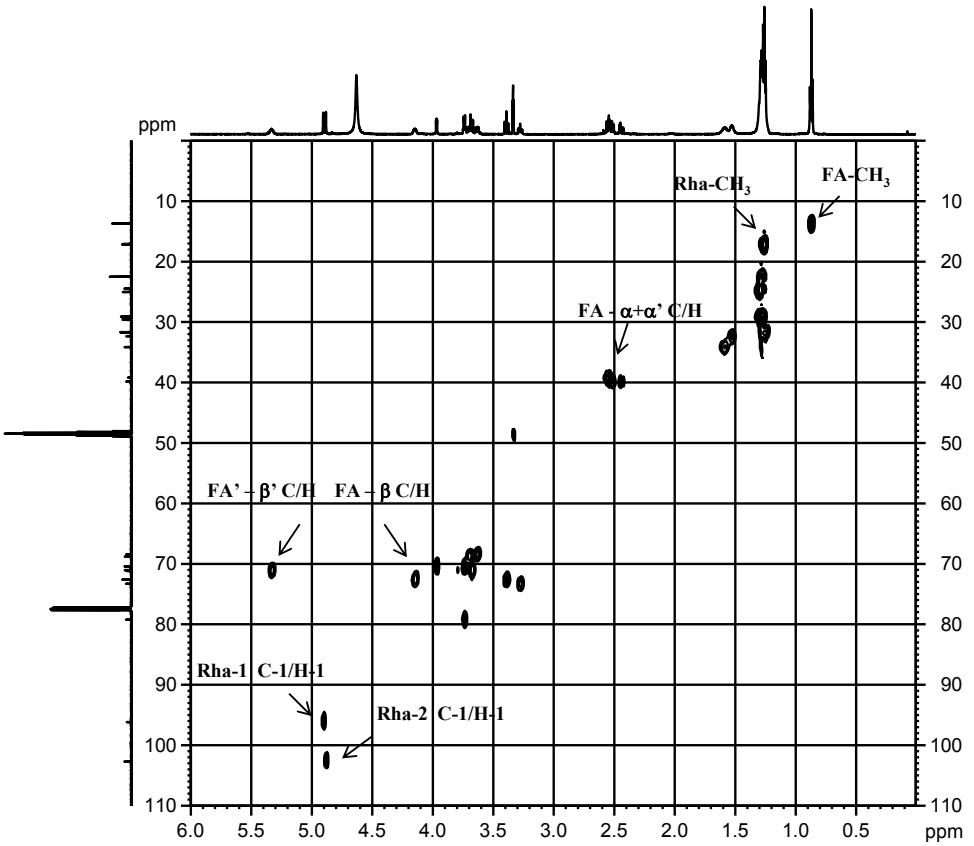

Fig. 2. Heteronuclear multiple bond correlation (HMBC) spectrum of the di-rhamnolipid surfactant produced by *P. aeruginosa* BN10.

ture broth with low surface tension indicating the synthesis of significant amounts of surfactants. Chemical analysis by MS showed that the carbon source did not influence the qualitative composition of the rhamnolipid mixtures. In all cases the main rhamnolipids were at *m/z* 504 and *m/z* 650 corresponding to Rha-C_{10}-C_{10} and Rha_2-C_{10}-C_{10}. In all rhamnolipid mixtures the di-rhamnolipid Rha_2-C_{10}-C_{10} was the dominant component but the proportion of Rha_2-C_{10}-C_{10} to Rha-C_{10}-C_{10} was different (Table II).

Table II. Effect of the carbon source on the production of mono- and di-rhamnolipids by *P. aeruginosa* BN10.

Carbon source	Relative abundance (%)		Rha_2-C_{10}-C_{10}/ Rha-C_{10}-C_{10}
	Rha-C_{10}-C_{10}	Rha_2-C_{10}-C_{10}	
Glycerol	20.6	60.1	2.9
Glucose	8.3	60.3	7.2
n-Hexadecane	21.5	58.6	2.7
n-Alkanes	12.7	47.6	3.7

Physicochemical characterization

The surface tension (σ) measurements as a function of the concentration of the rhamnolipid mixture are shown in Fig. 3. The critical micellar concentration (CMC) was reached at a concentration of 40 mg l^{-1}, and σ at CMC was 29 mN m^{-1}. The reduction of the tension at the interface water/*n*-hexadecane was from 43 to 0.9 mN m^{-1}.

The emulsification index (E) of the rhamnolipid mixture was determined after 24 h and 168 h (Fig. 4). The crude biosurfactant formed stable emulsions with aromatic (toluene, benzene) and aliphatic (*n*-hexadecane, *n*-alkanes) hydrocarbons, and complex hydrocarbon mixtures (crude oil, diesel oil, kerosene). The rhamnolipid biosurfactant gave the highest (80–85%) emulsifica-

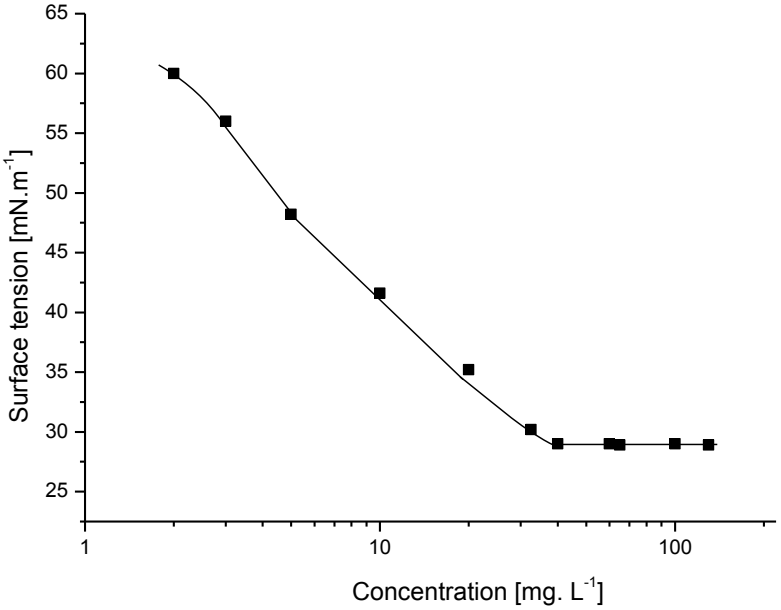

Fig. 3. Surface tension as a function of rhamnolipid concentration.

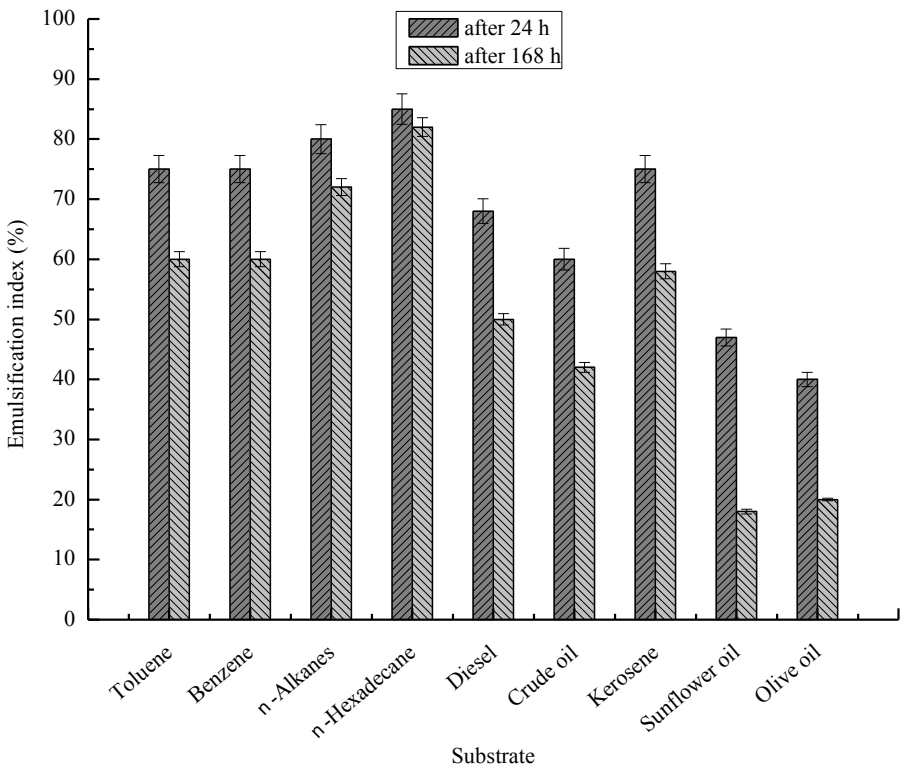

Fig. 4. Emulsification index (*E)* of the rhamnolipid mixture produced by *P. aeruginosa* BN10 with various hydrocarbons after 24 and 168 h. Data shown are means of three experiments.

Table III. Antimicrobial activity of the rhamnolipid mixture produced by *P. aeruginosa* BN10 on 2% glycerol.

Microorganism	Zone of inhibition			
	Crude biosurfactant from *P. aeruginosa* BN10			
	25 μg ml^{-1}	50 μg ml^{-1}	75 μg ml^{-1}	100 μg ml^{-1}
Escherichia coli	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–
Serratia marcescens	–	–	–	–
Alcaligenes faecalis	–	–	–	–
Salmonella thyphimurium	–	–	–	–
Bacillus subtilis	++	+++	+++	+++
Bacillus mycoides	+	++	++	+++
Bacillus cereus	++	+++	+++	+++
Bacillus megaterium	+++	+++	+++	+++
Staphylococcus aureus	+++	+++	+++	+++
Streptococcus pneumoniae	+++	+++	+++	+++
Streptococcus piogenes	++	+++	+++	+++
Micrococcus luteus	+	++	++	+++
Sarcina lutea	+	++	+++	+++
Acinetobacter johnsonii	+	++	+++	+++
Saccharomyces cerevisiae	–	–	–	–
Candida albicans	–	–	–	–
Rhodotorula rubra	–	–	–	–
Penicillium chrysogenum	–	–	–	–
Aspergillus niger	–	–	–	–

Results represent means of three independent experiments; "+" sign indicates inhibition of microbial growth, while "–" sign indicates no growth inhibition. +, Zone of inhibition ca. 10 mm; ++, zone of inhibition ca. 15 mm; +++, zone of inhibition more than 15 mm around the point of application of the biosurfactant.

tion activity on *n*-alkanes and *n*-hexadecane. The emulsion with *n*-hexadecane was most stable, with essentially no decrease in *E*. In contrast, the biosurfactant failed to produce stable emulsions with vegetable oils (sunflower and olive oil).

Antimicrobial activity

In our study the rhamnolipid mixture exhibited strong antibacterial activity against Gram-positive bacterial strains (Table III). Strongest activity at a concentration of 25 μg ml^{-1} was detected for *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Bacillus megaterium*. Fairly good activity (at 50 μg ml^{-1}) was found also for *Streptococcus piogenes*, *Bacillus cereus*, and *Bacillus subtilis*. However, up to a dose of 100 μg ml^{-1} the biosurfactant was not able to inhibit the growth of the tested Gram-negative bacterial, yeast, and fungal strains.

Discussion

When grown on glycerol, *Pseudomonas aeruginosa* BN10 produced a mixture of 8 rhamnolipid congeners including the mono-rhamnolipids Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-C_{10}-$C_{12:1}$, and Rha-C_{10}-C_{12}, and the di-rhamnolipids Rha_2-C_{10}-C_8, Rha_2-C_{10}-C_{10}, Rha_2-C_{10}-$C_{12:1}$, and Rha_2-C_{10}-C_{12}. Generally, the composition of the mixture is determined by many parameters like the bacterial strain, the age of the culture (Mata-Sandoval *et al.*, 2001), the culture medium composition, and specific culture conditions (Costa *et al.*, 2006).

Pseudomonas strains have been reported to produce rhamnolipid biosurfactants on water-soluble substrates such as glucose (Bodour *et al.*, 2003), mannitol (Deziel *et al.*, 1999) or glycerol (Arino *et al.*, 1996; Monteiro *et al.*, 2007) but also on water-insoluble carbon sources (Rahman *et al.*, 2002; Benincasa *et al.*, 2004). The reason why rhamnolipid biosurfactants are produced on both water-soluble and -insoluble substrates is still not well understood. Thus, Perfumo *et al.* (2006) reported that despite the key role of biosurfactants in the hydrocarbon uptake, the water immiscible substrate *n*-hexadecane decreased the variety of rhamnolipids compared with those produced by *Pseudomonas aeruginosa* AP02-1 on water-

soluble glycerol. MS analysis of the rhamnolipid mixtures produced by strain BN10 on different carbon sources clearly demonstrated that their chemical composition did not vary with the type of the carbon source. In our study only the proportion of Rha_2-C_{10}-C_{10} to Rha-C_{10}-C_{10} was different as the highest ratio (7.2) was obtained on glucose. Similar results are reported by Wei *et al.* (2008), only in that study, when obtained on glucose, the Rha_2/Rha_1 ratio equals 2, while the here presented results show a Rha_2/Rha_1 ratio of 7.2. The functional potential of a rhamnolipid mixture is determined by its composition and the ratios of the individual congeners.

The most important properties of rhamnolipids are their surface-active characteristics. The rhamnolipid mixture produced by *Pseudomonas aeruginosa* BN10 reduced the surface and interfacial tension to 29 mN m^{-1} and 0.9 mN m^{-1}, respectively, at a CMC of 40 mg l^{-1}. These results are within the limits of published surface tension and interfacial tension measurements of rhamnolipids, typically showing lowest equilibrium values of σ of 25–30 mN m^{-1} reached at CMC values between 5 and 200 mg l^{-1}, and interfacial tension of below 1 mN m^{-1} (Lang and Wullbrandt, 1999; Helvaci *et al.*, 2004; Guo *et al.*, 2009; Neto *et al.*, 2009; Peker *et al.*, 2003; Pornsunthorntaweea *et al.*, 2008; Abalos *et al.*, 2001). The obtained CMC value is in the lower range of displayed CMC values of rhamnolipid solutions (Lang and Wullbrandt, 1999). So, compared to other measurements (Abalos *et al.*, 2001; Benincasa *et al.*, 2004), the present rhamnolipid mixture shows high efficiency in reducing the surface tension of the solutions to its lowest values.

Rhamnolipids also show the ability to emulsify hydrocarbons and to stabilize emulsions. Haba *et al.* (2003) demonstrated that culture fluid supernatants from different *Pseudomonas* strains were able to form emulsions with kerosene that remained stable for 3 months. Emulsions with *n*-alkanes, aromatics, crude oil, kerosene, coconut and olive oils were stabilized by rhamnolipids, showing a loss of 5–25% of stability after 24 h depending on the carbon source (Patel and Desai, 1997). Similarly, the rhamnolipid surfactant from strain BN10 formed stable emulsions with aromatic, aliphatic, and complex hydrocarbons indicating its possible use in bioremediation and petroleum industries.

Another topic of interest related to the properties of rhamnolipids is their antimicrobial activity. Antibacterial and antifungal effects were reported for several rhamnolipid mixtures (Abalos *et al.*, 2001; Haba *et al.*, 2003; Benincasa *et al.*, 2004). The rhamnolipids from *Pseudomonas aeruginosa* BN10 were highly active against Gram-positive strains. Strongest inhibitory activity was found against *Staphylococcus aureus, Streptococcus pneumonia*, and *Bacillus megaterium.* In recent years Gram-positive microorganisms have developed a broad range of mechanisms to evade antimicrobial agents, so the development of new antibiotics has become increasingly important. Thus, in addition to its emulsifying activity, the application of the rhamnolipid biosurfactant from *Pseudomonas aeruginosa* BN10 as antimicrobial agent is highly promising.

The present study shows that the new strain *Pseudomonas aeruginosa* BN10 demonstrates enhanced production of the di-rhamnolipid Rha_2-C_{10}-C_{10} on all carbon sources used. Due to its good tensio-active and antimicrobial properties, the rhamnolipid homologue mixture produced by this strain can be applied as a ecofriendly material in bioremediation, petroleum and pharmaceutical industries.

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