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ORIGINAL PAPER

Structural characterization of rhamnolipid produced by *Pseudonomas aeruginosa* strain FIN2 isolated from oil reservoir water

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Abstract Biosurfactant-producing microorganisms inhabiting oil reservoirs are of great potential in industrial applications. Yet, till now, the knowledge about the structure and physicochemical property of their metabolites are still limited. The aim of this study was to purify and structurally characterize the biosurfactant from *Pseudomonas aeruginosa* strain FIN2, a newly isolated strain from an oil reservoir. The purification was conducted by silica gel column chromatography followed by pre-RP HPLC and the structural characterization was carried out by GC–MS combined with MS/MS. The results show that the biosurfactant produced by FIN2 is rhamnolipid in nature and its four main fractions were identified to be Rha-C10-C10(46.1 %), Rha–Rha-C10-C10(20.1 %), Rha-C8-C10 (7.5 %) and Rha-C10-C12:1(5.5 %), respectively. Meanwhile, the rarely reported rhamnolipid congeners containing β-hydroxy fatty acids of C6, C9, C10:1 and C11 were also proved to be present in the rhamnolipid mixture produced. The rhamnolipid mixture exhibited a strong surface activity by lowering the surface tension of distilled water to 28.6 mN/m with a CMC value of 195 mg/l.

Keywords *Pseudonomas aeruginosa* · Rhamnolipid · Structure elucidation · ESI–MS · MS/MS · GC–MS

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Introduction

Interest in biosurfactants has been progressively escalating in recent years. To date, researches on their structure and properties are receiving more and more attention. They are amphiphilic molecules with the hydrophilic part composed of sugars, amino acids, or polar functional groups like carboxylic acid groups and the hydrophobic part typically a β-hydroxy fatty acyl group (Lang and Wullbrandt 1999; Lang 2002). The advantage of biosurfactants over their chemical counterparts lies in their low toxicity, higher biodegradability, better environmental compatibility, high selectivity, specific activity at extremes of temperature, pH and salinity, and the ability to be synthesized from renewable feedstocks (Thanomsub et al. 2006). Because of their diverse structure and potential advantages, biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics (Muthusamy et al. 2008; Banat et al. 2010).

The rhamnolipids from *P. aeruginosa*, are one of the most effective biosurfactants and have attracted much attention due to their remarkable tensioactive and emulsifying properties (Maier and Soberon-Chavez 2000; Nitschke et al. 2005a; Ochsner et al. 1996; Soberon-Chavez et al. 2005). Generally, rhamnolipid consists of one (mono-rhamnolipid) or two (di-rhamnolipid) L-rhamnose moieties linked to one up to three β-hydroxy fatty acyl moieties (Jarvis and Johnson 1949; Syldatk et al. 1985). Rhamnolipid, produced by many microorganisms, usually appears as a mixture of different congeners and the yield as well as the composition of these congeners is affected by many parameters, such as the strain, the culture medium and growth conditions (Mata-Sandoval et al. 1999; Hörmann et al. 2010; Déziel et al. 1999; Henkel et al. 2012; Rikalovic et al. 2012; Li et al. 2011; Mehdi et al. 2011; Hoskova et al. 2013).

Rhamnolipids with different structures have different surface activities (Price et al. 2009). Howe et al. (2006) synthesized a variety of compounds based on the basic rhamnolipid structure and they found that the different physicochemical characteristics of rhamnolipids are sensitive to the particular chemical structure. Even slight differences in the composition of the rhamnolipid mixture can also have great consequences for its physicochemical properties (Perfumo et al. 2006; Abdel-Mawgoud et al. 2010). Thus, the study on the rhamnolipid producers and the rhamnolipid mixture produced under different cultivation conditions attracts more and more attention.

Rhamnolipids are believed to be the most promising biosurfactant for actual industrial applications such as microbial enhanced oil recovery (MEOR) and bioremediation. In MEOR, as the indigenous microorganisms can grow well under the physiochemical conditions and microbial ecosystems of an oil reservoir, the in situ MEOR strategy based on the activation of indigenous microflora in oil reservoirs is receiving more and more attention. Although numerous reports on microorganisms and their community structure in oil reservoirs have been presented recently, yet the knowledge about the structure and physicochemical properties of metabolites of microbes from oil reservoirs are still limited.

This paper mainly focuses on the isolation and structural determination of the rhamnolipids produced by a newly isolated bacterial strain from oilfield formation water, *P. aeruginosa* strain FIN2. Four commonly reported rhamnolipid homologues as well as rarely reported rhamnolipid congeners containing hydrophobic moiety of β-hydroxy fatty acids C6, C9, C10:1, C11 have been detected. The results obtained provide new structural information of the rhamnolipids produced by *P. aeruginosa* strain FIN2 from oil reservoir and demonstrate the potential usage of this strain in in situ MEOR.

Materials and methods

Microorganism and cultivation

A biosurfactant-producing bacterium was isolated from formation water samples of the Huabei oilfield in China, and identified as *P. aeruginosa* by means of 16S rRNA gene sequencing. The strain was designated to FIN2 and kept in our Lab. Cultures were performed with 100 ml medium in 250-ml Erlenmeyer flasks incubated at 35 °C with rotary shaking at 145 rev/min for 3 days. The medium contained 2.4 % glucose, 0.6 % urea, 0.1 % yeast extracts, 0.24 % $MgSO_4 \bullet 7H_2O$, 0.004 % $CaCl_2$, 1.5E−4 mol/L $CrCl_3$, 0.1 % phosphate.

Crude sample preparation

The culture broth was centrifuged at 5,000 rev/min for 30 min to remove the cells and the supernatant was adjusted to pH2.0 with 6 M HCl, kept at 4 °C overnight, followed by centrifugation at 5,000 rev/min to collect the precipitate. The precipitate was extracted with ethyl ether and then the ethyl ether was removed by evaporation to obtain the crude sample of glycolipid.

Purification

The crude sample was dissolved in methanol and purified by the following two steps. Firstly, the solution of 0.6 g crude sample in 1.0 ml methanol was subjected to chromatographic separation with the normal pressure silica gel (200 meshes) column ¢ 3 × 15 cm at room temperature. The column was first eluted by 200 ml chloroform–methanol (*v*:*v*, 25:1) at a flow rate of 6.0 ml min^{-1} for removal of pigment, then by 700 ml chloroform–methanol (*v*:*v*, 10:1) to collect glycolipid. The eluate was monitored by a spectrophotometer at a wavelength of 340 nm to identify co-existing pigments and analysed by thin-layer chromatography (TLC) and stained with vitriol-phenol for glycolipid identification. Secondly, the glycolipid obtained was subjected to preparative reverse-phase high-performance liquid chromatography (pre-RP HPLC) for separation of its congeners and homologues. This treatment was carried out on a HPLC system (JASCO Tokyo, Japan) equipped with a Hypersil ODS2 RP-C18 column (5 μm, 21.2 mm × 250 mm). An acetonitrile and 0.05 % TFA solution (in double distilled water) gradient was used starting with 80 % acetonitrile for 3 min, raised to 90 % acetonitrile in 20 min, which was kept for 20 min. The HPLC was operated at flow rate of 15 ml min^{-1} at 35 °C. The eluate was monitored at 214 nm and the peaks representing different components were collected, respectively. Purified component was obtained after evaporating the solvent by rotary evaporator under vacuum.

Electrospray mass spectrometry (ESI–MS) analysis

Solutions of the dried pure glycolipid in methanol with the final concentration of 1 mg/ml were applied to ESI–MS analysis. The ESI–MS (Micromass Co. LCT KC 317, UK) operated at the condition of ionization source temperature 80 °C, electrolyte voltage 200 V and spray inlet temperature 120 °C. The equipment was run in a positive mode.

GC/MS analysis

The fatty acyl moiety in glycolipid was determined by GC/MS. To do this, about 5.0 mg purified samples together with 1.0 ml 6 M HCl solution were added into a 2 ml ampoule

bottle and sealed before hydrolysing at 120 °C for 1.5 h. The hydrolysate was then extracted with 3.0 ml diethyl ether. The extract was dried and the residue was esterified in 1.0 ml 10 % H_2SO_4 -methanol solution at 55 °C for 6 h. Then 5 ml of ddH_2O was added to the reaction mixture, followed by extraction three times with 3 ml diethyl ether. The methanol-esterified fatty acid was prepared by evaporating diethyl ether at room temperature and then dissolved in 1.0 ml methanol before it was analysed by GC/MS (Hewlett Packard, USA, 6890 chromatograph with a model 5975 mass-selective detector and a HP-5MS capillary column, 30 m × 0.25 mm × 0.25 μm, Helium 40 cm min^{-1}). For GC/MS analysis, the column temperature was initially held at 80 °C for 3 min and then increased to 280 °C at a rate of 8 °C min^{-1} and kept for 10 min. 1.0 μL sample was used with a split ratio of 10:1.

The residues after extraction of the hydrolysate with ethyl ether was dried and reduced by $NaBH_4$, followed by acetylation with anhydrous acetic acid at 40 °C for 40 min before analysis by GC/MS.

ESI Q-Tof MS/MS analysis

To analyse the structure of the glycolipid with means of Q-Tof MS/MS (Quadruple and Time of Flight mass/mass spectrometry), about 1.0 mg of the dried pure glycolipid was dissolved with methanol and analysed by Q-Tof MS/MS (Q-Tof micro YA019) equipped with an electrospray ion source and operated at the following conditions: capillary voltage 2,600 V, sample cone voltage 60 V and collision energy 30 V. The equipment was run in a negative mode.

Surface activity analysis

The rhamnolipid mixture, obtained after purification by normal pressure silica gel chromatography for removal of pigments, was dissolved in water to form solutions with different concentrations. The surface tension of these solutions was determined by surface tensionmeter JK99D at room temperature.

Results

Purification of glycolipid

The crude sample, obtained by ethyl ether extraction of cell-free precipitate of culture broth, was first separated by normal pressure silica gel column chromatography to remove the co-exist pigments and further by pre-RP-HPLC. The elution profile of pre-RP-HPLC is shown in Fig. 1 in which three main components (1, 2 and 4) appeared. Four relatively pure components (1, 2, 4, and 6) were harvested by collection of the corresponding fractions in pre-RP-HPLC.

Molecular weight of glycolipid component

Components 1, 2, 4 and 6 (shown in Fig. 1) were subjected to ESI–MS analysis respectively for molecular weight determination and the MS spectra are shown in Fig. 2. The *m/z* values obtained for components 1, 2, 4 and 6 were 475.3, 649.4, 503.4 and 529.4, respectively. Generally, ESI–MS in negative model shows the *m/z* of $[M–H]^-$ (native molecules or natural molecules is subtracted a hydrogen and thus negatively ionized), hence, In this way, the molecular weights of component 1, 2, 4 and 6 are actually 476.3, 650.4, 504.4 and 530.4, respectively.

Identification of hydrophilic moiety

The sugar, contained in the water-soluble part of the hydrolysate of crude glycolipid by 6 M HCl, was analysed by GC–MS after reduction by $NaBH_4$ and acetylation with anhydrous acetic acid (Crowell and Burnett 1967). The sugar component in the sample was identified as rhamnose and thus, the glycolipid produced by FIN 2 is rhamnolipid in nature.

Identification of hydrophobic moiety

To elucidate the hydrophobic moiety (fatty acids) in rhamnolipid variants, the collected components were hydrolysed, and the fatty acids were harvested by extract of the hydrolysate with ethyl ether, followed by methyl-esterification before analysis by GC–MS. The Total Ion Chromatogram (TIC) of methyl-esterified fatty acids derived from rhamnolipid variants and their corresponding

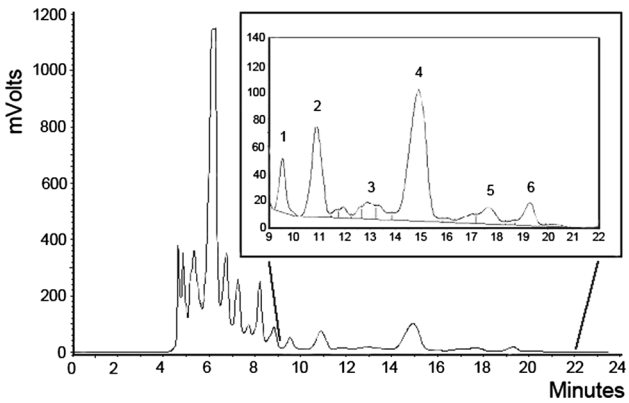

Fig. 1 Preparative HPLC chromatogram of crude glycolipid obtained from culture broth of *P. aeruginosa* strain FIN2

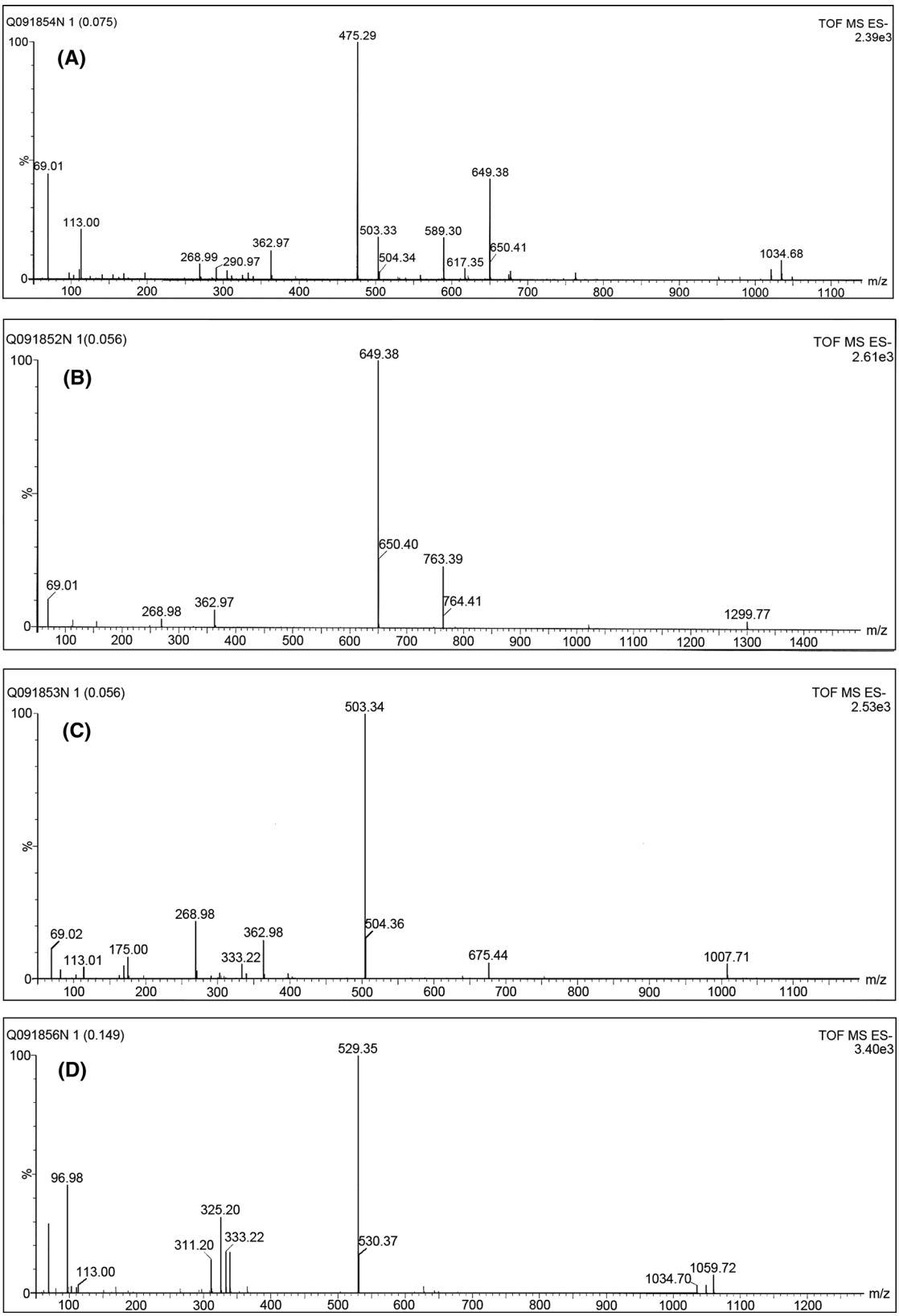

Fig. 2 The ESI–MS spectra in negative model of components 1, 2, 4, 6 (**A**, **B**, **C** and **D** for components 1, 2, 4 and 6, respectively)

mass spectra are shown in Fig. 3 (TIC) and Fig. 4 (MS), respectively.

All the mass spectra show the basic *m/z* 103, which is the typical *m/z* of β-hydroxy fatty acids (Yang et al. 2007). Based on the pseudo-molecular ion and the retention time, the hydroxy fatty acid, represented by their corresponding methanol-esterified products with retention time of 9.4, 12.9 and 15.6 min were identified as β-hydroxyoctanoic acid (C8), β-hydroxydecanoic acid (C10), and β-hydroxydodecanoic acid (C12:1), respectively. As can be seen from Fig. 3, component 1 contains mainly C8 and C10, component 2 and 4 contain mainly the same C10 and component 6 contains C10 and β-hydroxydodecenoic acid (C12:1).

Structural elucidation of components

The purified rhamnolipid components were subjected to ESI–MS/MS for elucidation of their structure. The ESI–MS/MS spectra of component 1, 2, 4 and 6 were shown in Fig. 5 as A, B, C and D, respectively.

Molecules were ionized through electro-spray ionization. The ionized molecules, negative ions in this experiment, were selected by first stage mass analyser, then the aimed ion of $[M–H]^-$ was supplied with extra energy for fragmentation, the *m/z* of fragments were recorded by the second stage mass analyser. The fragmentation usually happened at the polar chemical bands with the charge and/or hydrogen transfer, which are shown in Fig. 6.

In order to confirm the structure, the main ion at *m/z* 475 was fragmented and showed daughter ions at *m/z* 305, corresponding to Rha-C8 and at *m/z* 169, corresponding to the released C10; daughter ions at *m/z* 333, corresponding to Rha-C10, and at *m/z* 141, corresponding to the released C8; daughter ions at *m/z* 311, corresponding to C8-C10, and at *m/z* 163, corresponding to the released rha (Fig. 5A). These daughter ions were consistent with the structure expected for both Rha-C8-C10 and Rha-C10-C8, which is consistent with molecular weight 476.3. However, the Rha-C10-C8 and Rha-C8-C10 could not be separated well through HPLC in this experiment. Their coexistence could be detected by the MS/MS and Rha-C8-C10 was more than the Rha-C10-C8 because the *m/z* 305 formed through first losing the C10 moiety is more intensive than the *m/z* 333 formed by firstly losing C8.

For the component 2 (Fig. 5B), the fragments (*m/z*) 649 → 479 → 169 were formed from losing C10 (170) and RhaRha (310) subsequently. The fragments (*m/z*) 649 → 479 → 309 → 145 were formed from subsequent losing C10 (170), C10 (170) and Rha (164). The fragments (*m/z*) 649 → 339 → 169 were formed from losing Rha–Rha (310) and C10 (170). The fraction 2 should be the molecule Rha–Rha-C10-C10, which is consistent with

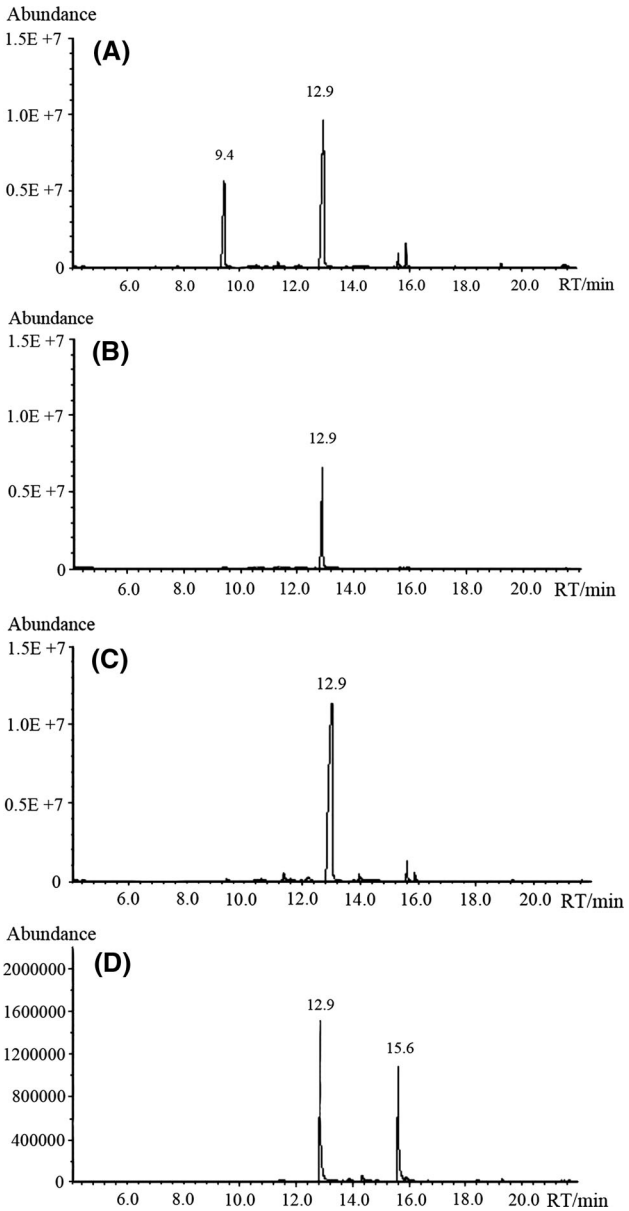

Fig. 3 The TIC spectra of methyl-esterified fatty acids derived from rhamnolipid variants (**A**, **B**, **C** and **D** for component 1, 2, 4 and 6, respectively)

molecular weight of 650.4 and the hydroxyl fatty acids of C10.

In the same way, fraction 4 was identified to be the monorhamnolipid Rha-C10-C10 (Fig. 5C) with molecular weight of 504.3 (Fig. 2C), and the fraction 6 (Fig. 5D) is the monorhamnolipid Rha-C10-C12:1 with molecular weight of 530.4 (Fig. 2D).

The loss of hydroxyl fatty acid in rhamnolipids would lead accordingly to the difference of *m/z* in MS/MS spectra. The loss of C8, C10, and C12:1 results in the difference in *m/z* of 142, 170, and 196 respectively. The loss of rhamnose residues would also show the difference of *m/z*, in

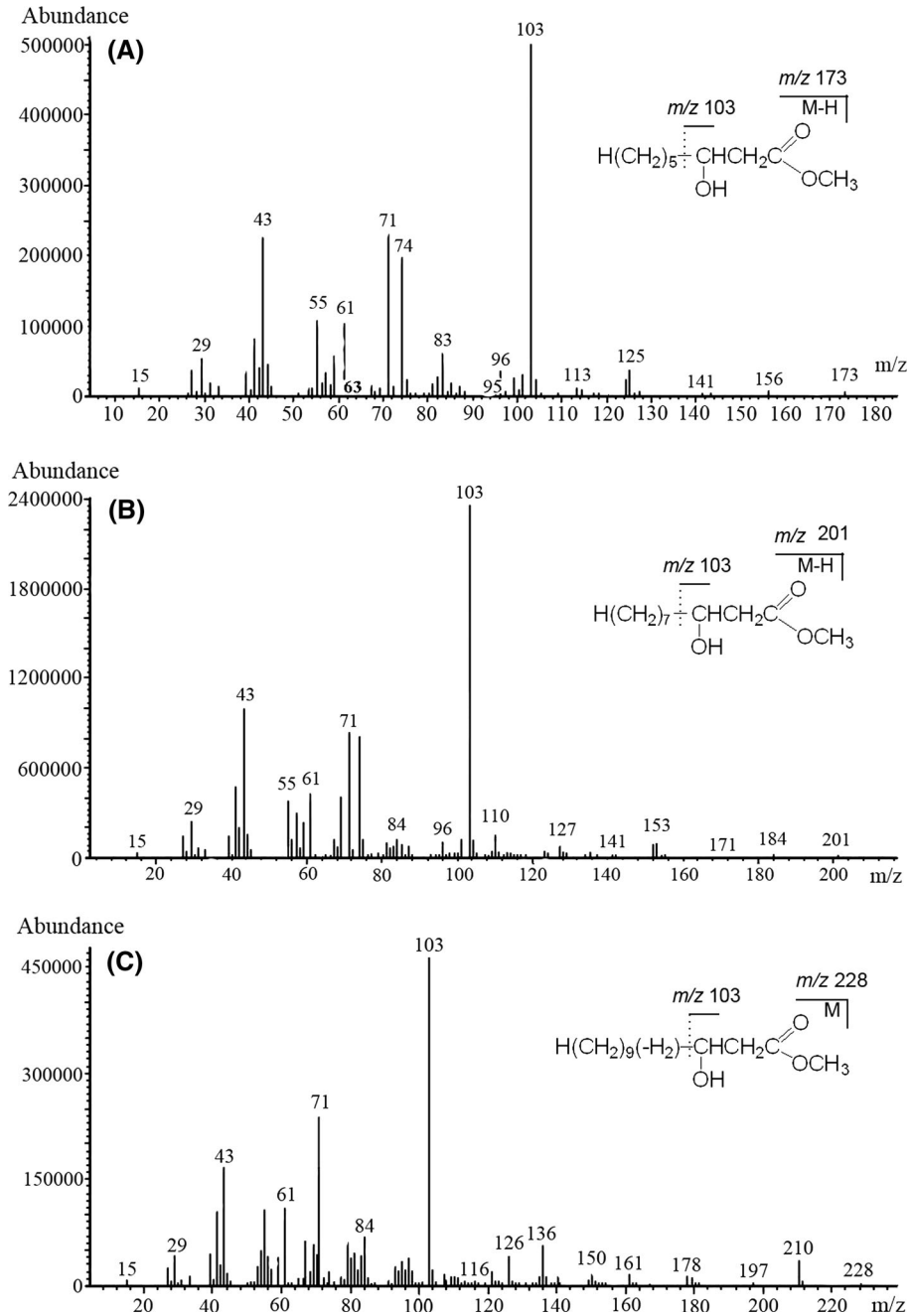

Fig. 4 The MS spectra of methyl-esterified fatty acids at retention time of 9.4 min (**A**), 12.9 min (**B**), and 15.6 min (**C**), respectively

which a single rhamnose in monorhamnolipids showed the difference of 164, and double rhamnose in dirhamnolipids showed the difference of 310. On the other hand, the parts remaining after losing natural fragments can be charged or ionized as well, and show particular ion peaks in the MS spectra. The remaining rhamnose moieties in monorhamnolipids, once charged or ionized show ions at *m/z* 163 and 145. In contrast, the remaining two rhamnose parts in dirhamnolipids show ions not only at *m/z* 145 and 163 but also at *m/z* 291 and 309. The remained C8, C10, C12:1 and C12, once charged or ionized, show ions at *m/z* 141, 169, 195 and 197 respectively. Those were listed in Table 1. All this knowledge might be useful for distinguishing or identifying rhamnolipids.

Relative abundance of main rhamnolipid congenenrs

The relative abundance of rhamnolipid congeners were calculated by their integrated peak area in pre-RP HPLC. All the effluents ranging from retention time of 9–21 min were regarded as rhamnolipid and the relative abundance of the main fraction 1, 2, 4 and 6 were calculated as shown in Table 1. Four monorhamnolipid (Rha-C8-C10, Rha-C10-C8, Rha-C10-C10 and Rha-C10-C12:1) accounts for

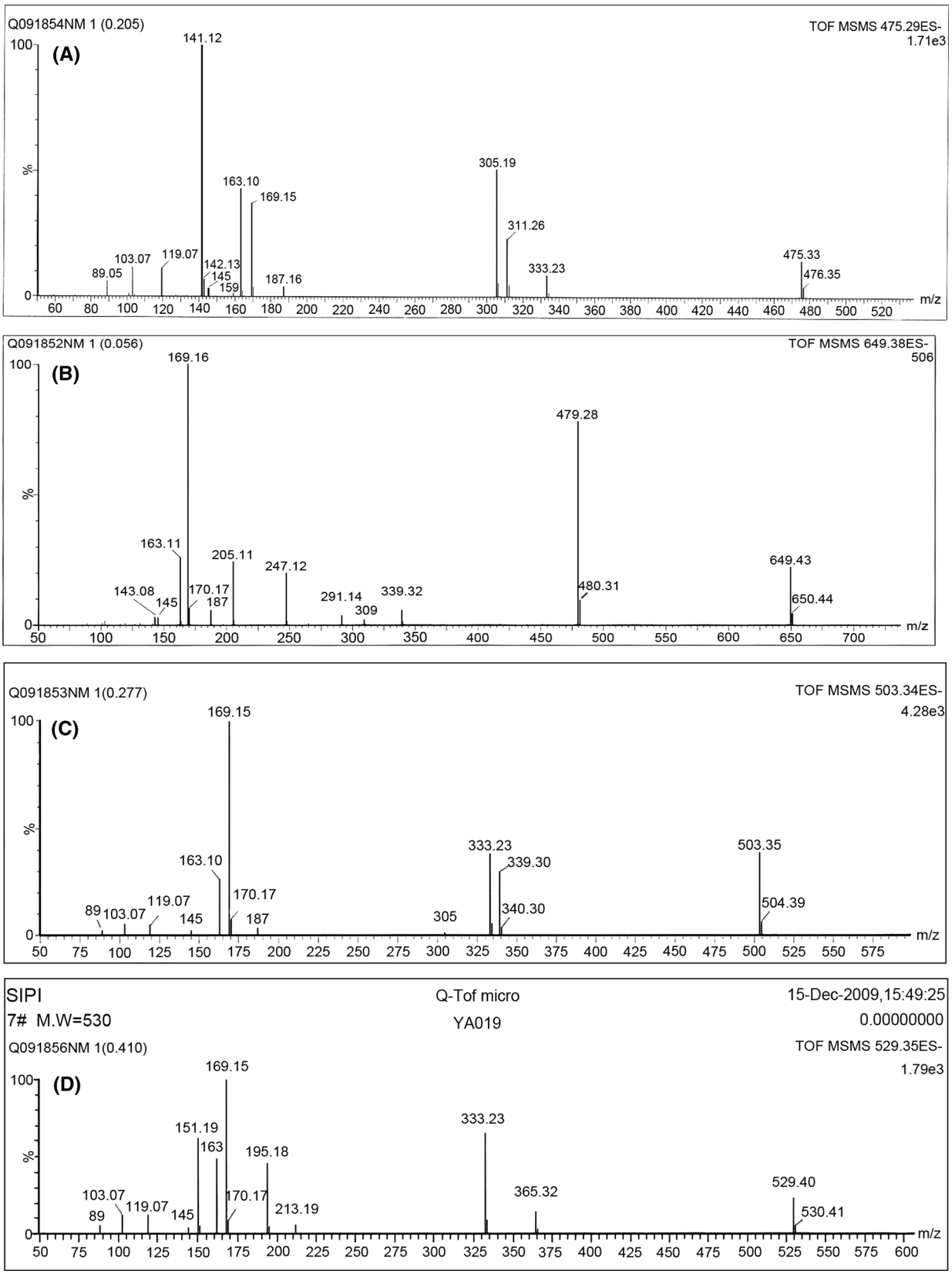

Fig. 5 The MS/MS of rhamnolipid congeners in negative model (**A**, **B**, **C** and **D** for component 1, 2, 4 and 6, respectively)

59.1 % and one dirhamnolipid (Rha–Rha-C10-C10) only for 20.1 %. Other rhamnolipid congeners have a minor content of 20.8 % totally.

Rhamnolipid congeners in trace amounts

The GC/MS with higher resolution and sensitivity is used to screen trace amounts of fatty acids in order to detect the rhamnolipid congeners present in low abundance. The result of chromatography is shown in Fig. 7 and the mass spectra are shown in Fig. 8.

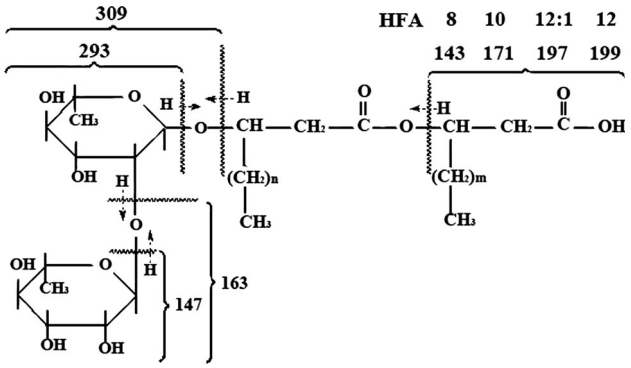

Fig. 6 Molecular structure showing fragmentation pathways

According to the retention time and the appearance of the typical ions at *m/z* 103 of β-hydroxy fatty acids, five β-hydroxyhexanoic acids were identified by the EIC spectra and MS spectra to be β-hydroxyhexanoic acid (C6), β-hydroxynonanoic acid (C9), β-hydroxydecenoic acid (C10:1), β-hydroxyhendecanoic acid (C11) and β-hydroxytetradecanoic acid (C14). Thus, there are rhamnolipids with C6, C9, C10:1, C11 and C14. However, the whole structures of these rhamnolipids are not yet identified due to their trace content.

Surface activity of rhamnolipid mixture

The surface tension of rhamnolipid mixture, obtained after purification by silica gel column chromatography, at different concentrations in neutral water was determined with tensiometer and the results are shown in Fig. 9. As it can be seen from Fig. 9 that surface tension decreased with the increase of the rhamnolipid concentration. However the

Table 1 The characteristics of the rhamnolipids produced by *P. aeruginosa* strain FIN2

Fraction (%)	Hydroxy fatty acids		Molecular weight	Fregmentgs of ramnolipids in MS/MS$^-$	Structures of rhamnolipids	Relative abundance
	Retention time, min	Carbon number				
1	9.4	C8	476.3	475.3, 333.2, 311.3, 305.2, 187.2, *169.2*[a], **163.1**[b], 159, 145, *141.1*, 119.1, 103.1, 89.1	Rha-C8-C10	7.5
	12.9	C10			Rha-C10-C8	
2	12.9	C10	650.4	649.4, 479.3, 339.3, **309**, 291.1, 247.1, 205.1, 187, *169.2*, **163.1**, 145, 143.1	Rha–Rha-C10-C10	20.1
4	12.9	C10	504.4	503.4, 339.3, 333.2, 187, *169.2*, **163.1**, 145, 119.1, 103.1, 89	Rha-C10-C10	46.1
6	12.9	C10	530.4	529.4, 365.3, 333.2, 213.2, *195.2*, *169.2*, **163**, 151.2, 145, 119.1, 103.1, 89	Rha-C10-C12: 1	5.5
	15.6	C12:1				

[a] The value with *italized* means the characteristic *m/z* of hydroxy fatty acids

[b] The value with *bold faced* means the characteristic *m/z* of rhamnose

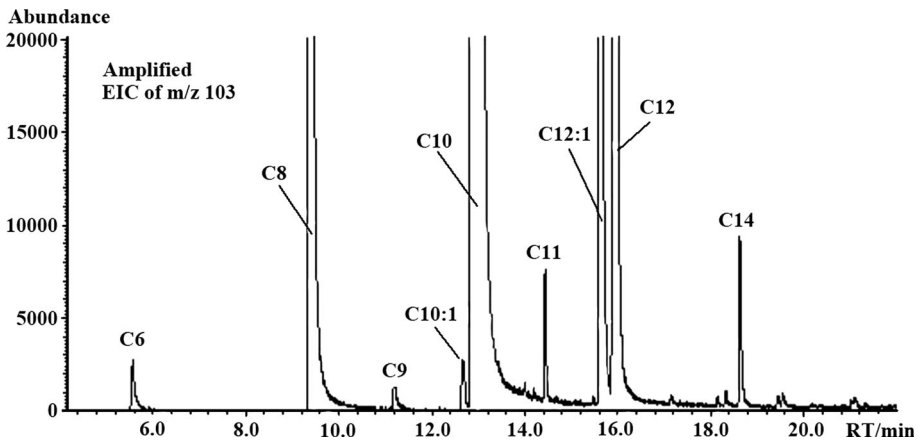

Fig. 7 The chromatogram of fatty acid methyl ester in GC–MS (The amplified EIC spectra at *m/z* of 103 to show specifically methyl esterified hydroxyl fatty acids derived from rhamnolipid mixture with trace amount)

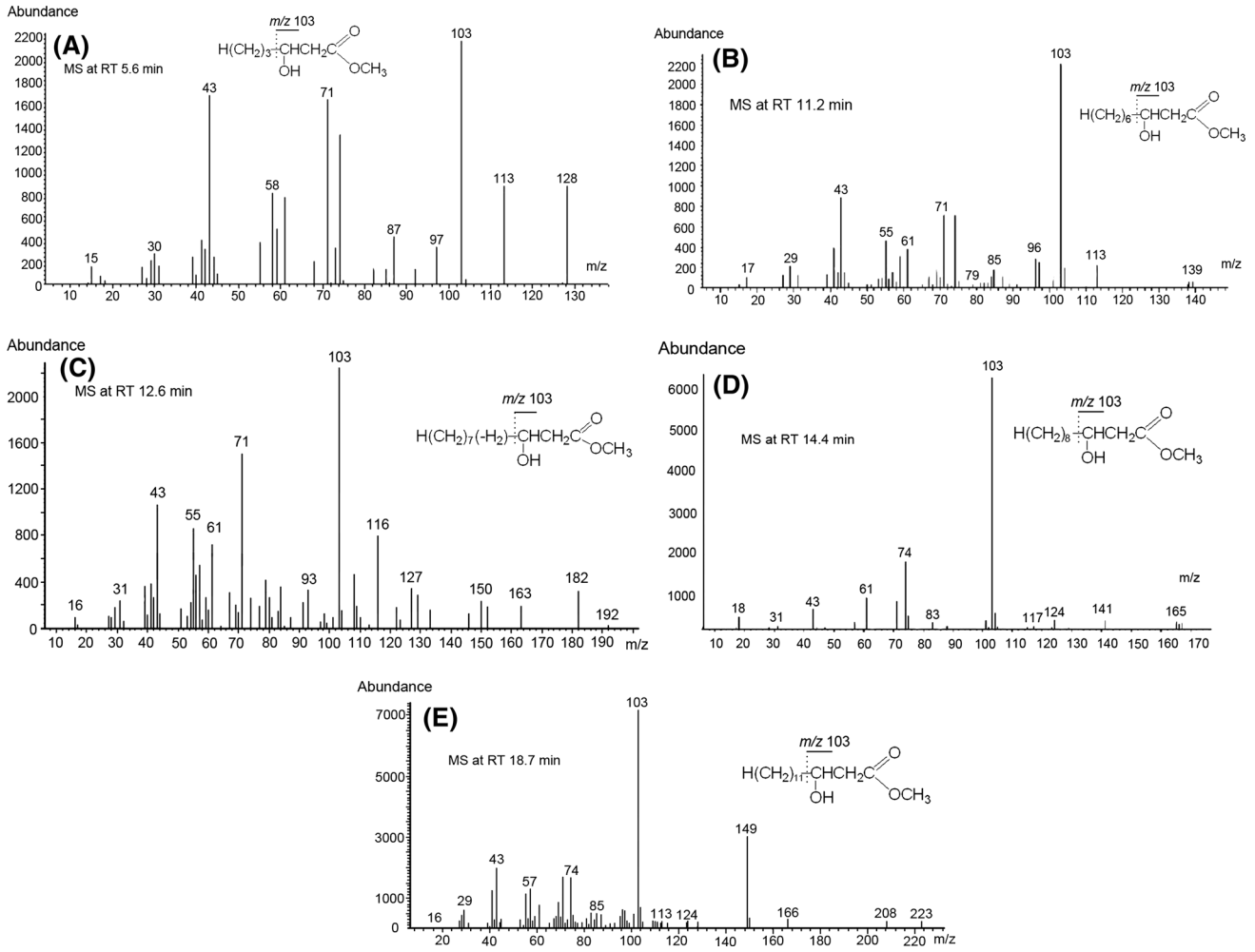

Fig. 8 The mass spectra of methyl-esterified hydroxy fatty acids derived from rhamnolipid mixture at retention time of 5.6 min (**A**), 11.2 min (**B**), 12.6 min (**C**), 14.4 min (**D**) and 18.7 min (**E**), respectively

surface tension reached its lowest value of 28.6 mN/m when the rhamnolipid concentration was above 195 mg/l, which means that the Critical Micelle Concentration (CMC) of the glycolipid was 195 mg/l.

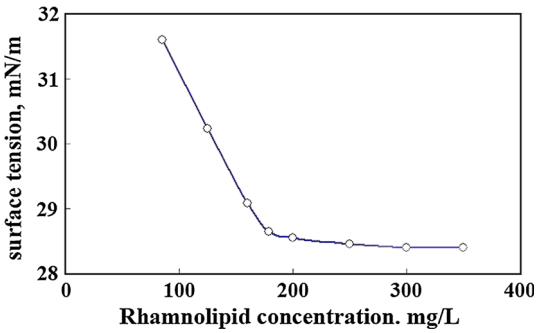

Fig. 9 Effect of rhamnolipid mixture concentration on surface tension activity

Discussion

The bacterium *P. aeruginosa* strain FIN2 produced several rhamnolipid congeners. These rhamnolipids were purified using silica gel column chromatography and pre-RP-HPLC and identified to mainly be Rha-C10-C10, Rha–Rha-C10-C10, Rha-C8-C10 (Rha-C10-C8) and Rha-C10-C12:1. Strain FIN2 produced the monorhamnolipids Rha-C10-C10 and dirhamnolipids Rha–Rha-C10-C10 as the predominant components with the value of Rha-C10-C10/Rha–Rha-C10-C10 to be 2.3. This finding is consistent with previous reports with different strains of *P. aeruginosa* (Sarachat et al. 2010; Nitschke et al. 2010; Rooney et al. 2009). However, other authors have observed that the amount of Rha-C10-C10 is less than that of Rha–Rha-C10-C10 (Deziel et al. 2000). Nitschke et al. (2005b) reported that the predominant rhamnolipid homologue changed with the carbon source. It can be concluded here that different content profiles of rhamnolipid variants may be produced with different strains and/or culture conditions.

Among the rhamnolipid congeners detected in our experiment, the proportion of Rha-C10-C12:1 is in minor quantities (5.5 %). This may be a result of the fact that no unsaturated fatty acid was contained in our culture medium. Benincasa and Accorsini (2008) suggested that the amount of unsaturated fatty acids in the carbon source reflects in the unsaturation of rhamnolipid carbon chains. Our result seems to support this consideration.

Rhamnolipid congeners with C6, C9, C10:1, C11 and C14 were also considered to be present with trace content in the culture broth of strain FIN2 when glucose and urea were used as carbon and nitrogen source respectively. Till now, Rha–Rha-C6-C6:1, Rha–Rha-C9:1 have only been detected in culture broth of *P. aeruginosa* strain NY3 with polycyclic aromatic as carbon substrate (Nie et al. 2010). Rha-C11 was exceptionally extracted from the culture broth of thermophilic bacteria with medium containing yeast extract, tryptone and sunflower seed oil (Rezanka et al. 2011). To our knowledge, rhamnolipid congeners with C9 and/or C10:1 have rarely been reported. Besides the different strains were used in these experiments, the difference in culture medium may play an important role in the composition of the rhamnolipid produced.

Diverse rhamnolipid congeners have been reported to be produced by different *P. aeruginosa* strains. Rezanka et al. (2011) revealed 77 rhamnolipid homologues in culture broth of thermophilic bacteria at 65 °C with medium containing yeast extract, tryptone and sunflower seed oil. Also, 28 rhamnolipid components were identified and quantified in *P. aeruginosa* strain 57RP cultures (Déziel et al. 1999). However, in our study, only four main rhamnolipid congeners were detected when strain FIN2 was incubated with simple medium. It is reasonable to believe that the complex substrate, especially the carbon and nitrogen source, may result in complex rhamnolipid components and novel strains may produce novel rhamnolipid congeners.

The ratio and composition of the rhamnolipid congeners can affect the physicochemical properties of the rhamnolipid mixture and sometimes more sensitively. Even slight differences in the composition of the rhamnolipid mixture can cause remarkable changes in their properties (Perfumo et al. 2006; Benincasa et al. 2004). The change in the hydrophilic part (monorhamnolipids vs. dirhamnolipids) and in hydrophobic part (the length of fatty acid chains) can change their properties such as the stability of rhamnolipids in the aqueous phase, capability to solubilize hydrophobic organic compounds (Mata-Sandoval et al. 1999). For example, the CMC values of Rha-C10 or Rha–Rha-C10 (Syldatk and Wagner 1987), Rha-C10-C10 (Dyke et al. 1993) and Rha–Rha-C10-C10 have been studied and reported to be 200, 5–60 and 40–65 mg/l, respectively. The Rha-C10-C10 monorhamnolipid showed higher micellar solubilization capacity than Rha–Rha-C10-C10 with phenanthrene. As the nature and ratio of these two rhamnolipid homologues are strain dependent and/or medium-component dependent as motioned previously and the property of a rhamnolipid mixture depends to a great extent on the individual congeners and their ratios. Thus, it is very important and very helpful to obtain specific rhamnolipid congeners in designing for particular research and industrial applications. It is reasonable to believe that biosynthesis can be directed to form preferentially a desirable rhamnolipid homologue by simply selecting the right substrate. Incubating rhamnolipid producers with simple medium as done in our experiment may be an effective way to obtain rhamnolipids with simple composition and specific rhamnolipid congeners.

It was also observed that among the rhamnolipids containing two different β-hydroxy fatty acid side chains (one being two carbons shorter than the other), those with the shorter chain linked to the rhamnose moiety (Rha–Rha-C8-C10) are more abundant than those with longer chain (Rha–Rha-C10-C8) linked at the same position (Deziel et al. 2000). We arrived at the same conclusions.

Owing to its environmentally friendly nature, biodegradability and effectiveness even at extreme conditions, rahmnolipid is regarded as promising surfactants in industrial applications such as bioremediation and MEOR. As for MEOR, a lot of attentions have been paid to in situ MEOR strategy which is based on the activation of indigenous microflra in oil reservoir. Among the variety of functional bioproducts such as biosurfactant, polysaccharide, carbon dioxide, methane and hydrogen (Almeida et al. 2004), biosurfactant plays the most important role in enhancing oil recovery and has been intensively studied. The rhamnolipid mix of Rha-C10-C10 and Rha2-C10-C10, with the ability to reduce the surface tension of water to 26 mN/m with a critical micellar concentration of 120 mg/l, can recover 27 % of original oil in place after water flooding from a sand pack at a concentration of 120 mg/l (Amani et al. 2013). Golabi's et al. (2012) results shows that biosufactant with a critical micellar concentration of 100 mg/l could reduce the surface tension of distilled water to 26 mN/m and can enhance the oil recovery up to 15 and 7.5 % in the high and low permeable cores, respectively. Our results show that the rhamnolipid mixture produced by *P. aeruginosa* strain FIN2 have the ability to low the surface tension of distilled water to 28.7 mN/m with a CMC of 195 mg/l, which fall in the same range of tensioactive property with the reported ones. Furthermore, a field trial of MEOR demonstrated that indigenous *P. aeruginosa* and *P. pseudoalcaligenes* were stimulated by supplement of nutrients to the test oil reservoir (Zhang et al. 2012). All these results imply that it is possible to activate *P. aeruginosa* strain FIN2 in the oil reservoir and therefore

enhance oil recovery with the production of rhamnolipids with remarkable tension-activity property once the nutrients are available.

Conclusion

The newly isolated strain from oil reservoir, *Pseudomonas aeruginosa* strain FIN2, produced rhamnolipid when it was cultured on glucose as carbon source with urea as nitrogen source. This rhamnolipid was predominated by Rha-C10-C10 and Rha–Rha-C10-C10 homologues with a relative abundance of 46.1 and 20.1 %, respectively. Besides these commonly detected rhamnolipid homologues, rarely reported rhamnolipid congeners with C6, C9, C10:1, C11 have been detected. The rhamnolipid shows the ability to low the surface tension of water to a value of 28.7 mN/m with a CMC of 195 mg/l. These results are significant towards the usage of microorganisms in oil reservoir such as in situ MEOR, the remediation of oil contaminated environments as well as the possibility of obtaining bioproducts with new chemical structure by microorganisms in extreme environments such as oil reservoirs.

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References

Abdel-Mawgoud AM, Lepine F, Deziel E (2010) Rhamnolipids: diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol 86(5):1323–1336. doi:10.1007/s00253-010-2498-2

Almeida PE, Moreira RS, Almeida RCC, Guimaraes AK, Carvalho AS, Quintella C, Esperidia MCA, Taft C (2004) Selection and application of microorganisms to improve oil recovery. Eng Life Sci 4(4):319–325. doi:10.1002/elsc.200420033

Amani H, Muller MM, Syldatk C, Hausmann R (2013) Production of microbial rhamnolipid by *Pseudomonas Aeruginosa* MM1011 for ex situ enhanced oil recovery. Appl Biochem Biotechnol 170(5):1080–1093. doi:10.1007/s12010-013-0249-4

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87(2):427–444. doi:10.1007/s00253-010-2589-0

Benincasa M, Accorsini FR (2008) *Pseudomonas aeruginosa* LBI production as an integrated process using the wastes from sunflower-oil refining as a substrate. Bioresour Technol 99(9):3843–3849. doi:10.1016/j.biortech.2007.06.048

Benincasa M, Abalos A, Oliveira I, Manresa A (2004) Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 85(1):1–8. doi:10.1023/B:Anto.0000020148.45523.41

Crowell EP, Burnett BB (1967) Determination of the carbohydrate composition of wood pulps by gas chromatography of the alditol acetates. Anal Chem 39(1):121–124. doi:10.1021/ac60245a006

Deziel E, Lepine F, Milot S, Villemur R (2000) Mass spectrometry monitoring of rhamnolipids from a growing culture of *Pseudomonas aeruginosa* strain 57RP. Biochim Biophys Acta 1485(2–3):145–152. doi:10.1016/S1388-1981(00)00039-1

Déziel E, Lépine F, Dennie D, Boismenu D, Mamer OA, Villemur R (1999) Liquid chromatography/mass spectrometry analysis of mixtures of rhamnolipids produced by *Pseudomonas aeruginosa* strain 57RP grown on mannitol or naphthalene. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids 1440(2–3):244–252. doi:10.1016/S1388-1981(99)00129-8

Dyke MIV, Couture P, Brauer M, Lee H, Trevors JT (1993) *Pseudomonas aeruginosa* UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil. Can J Microbiol 39(11):1071–1078. doi:10.1139/m93-162

Golabi E, Sogh SRMP, Hosseini SN, Gholamzadeh MA (2012) Biosurfactant production by microorganism for enhanced oil recovery. Int J Sci Eng Res 3(7):1–6

Henkel M, Muller MM, Kugler JH, Lovaglio RB, Contiero J, Syldatk C, Hausmann R (2012) Rhamnolipids as biosurfactants from renewable resources: concepts for next-generation rhamnolipid production. Process Biochem 47(8):1207–1219. doi:10.1016/j.procbio.2012.04.018

Hörmann B, Müller MM, Syldatk C, Hausmann R (2010) Rhamnolipid production by *Burkholderia plantarii* DSM 9509T. Eur J Lipid Sci Technol 112(6):674–680. doi:10.1002/ejlt.201000030

Hoskova M, Schreiberova O, Jezdik R, Chudoba J, Masak J, Sigler K, Rezanka T (2013) Characterization of rhamnolipids produced by non-pathogenic *Acinetobacter* and *Enterobacter* bacteria. Bioresour Technol 130:510–516. doi:10.1016/j.biortech.2012.12.085

Howe J, Bauer J, Andra J, Schromm AB, Ernst M, Rossle M, Zahringer U, Rademann J, Brandenburg K (2006) Biophysical characterization of synthetic rhamnolipids. FEBS J 273(22):5101–5112. doi:10.1111/j.1742-4658.2006.05507.x

Jarvis FG, Johnson MJ (1949) A Glyco-lipide produced by *Pseudomonas Aeruginosa*. J Am Chem Soc 71(12):4124–4126. doi:10.1021/ja01180a073

Lang S (2002) Biological amphiphiles (microbial biosurfactants). Curr Opin Colloid Interf Sci 7 (1–2):12–20. doi:10.1016/S1359-0294(02)00007-9

Lang S, Wullbrandt D (1999) Rhamnose lipids–biosynthesis, microbial production and application potential. Appl Microbiol Biotechnol 51(1):22–32

Li AH, Xu MY, Sun W, Sun GP (2011) Rhamnolipid production by *Pseudomonas aeruginosa* GIM 32 using different substrates including molasses distillery wastewater. Appl Biochem Biotechnol 163(5):600–611. doi:10.1007/s12010-010-9066-1

Maier RM, Soberon-Chavez G (2000) *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. Appl Microbiol Biotechnol 54(5):625–633

Mata-Sandoval JC, Karns J, Torrents A (1999) High-performance liquid chromatography method for the characterization of rhamnolipid mixtures produced by *Pseudomonas aeruginosa* UG2 on corn oil. J Chromatogr A 864(2):211–220

Mehdi S, Dondapati JS, Rahman PKSM (2011) Influence of nitrogen and phosphorus on rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* DS10-129 using glycerol as carbon source. Biotechnology 10:183–189

Muthusamy K, Gopalakrishnan S, Ravi TK, Sivachidambaram P (2008) Biosurfactants: properties, commercial production and application. Curr Sci 94(6):736–747

Nie MQ, Yin XH, Ren CY, Wang Y, Xu F, Shen QR (2010) Novel rhamnolipid biosurfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium *Pseudomonas aeruginosa* strain NY3. Biotechnol Adv 28(5):635–643. doi:10.1016/j.biotechadv.2010.05.013

Nitschke M, Costa SG, Contiero J (2005a) Rhamnolipid surfactants: an update on the general aspects of these remarkable biomolecules. Biotechnol Prog 21(6):1593–1600. doi:10.1021/bp050239p

Nitschke M, Costa SG, Haddad R, Goncalves LA, Eberlin MN, Contiero J (2005b) Oil wastes as unconventional substrates for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* LBI. Biotechnol Prog 21(5):1562–1566. doi:10.1021/bp050198x

Nitschke M, Costa SG, Contiero J (2010) Structure and applications of a rhamnolipid surfactant produced in soybean oil waste. Appl Biochem Biotechnol 160(7):2066–2074. doi:10.1007/s12010-009-8707-8

Ochsner UA, Hembach T, Fiechter A (1996) Production of rhamnolipid biosurfactants. Adv Biochem Eng Biotechnol 53:89–118

Perfumo A, Banat IM, Canganella F, Marchant R (2006) Rhamnolipid production by a novel thermophilic hydrocarbon-degrading *Pseudomonas aeruginosa* AP02-1. Appl Microbiol Biotechnol 72(1):132–138. doi:10.1007/s00253-005-0234-0

Price NP, Ray KJ, Vermillion K, Kuo TM (2009) MALDI-TOF mass spectrometry of naturally occurring mixtures of monorhamnolipids and dirhamnolipids. Carbohydr Res 344(2):204–209. doi:10.1016/j.carres.2008.10.013S0008-6215(08)00508-9

Rezanka T, Siristova L, Sigler K (2011) Rhamnolipid-producing thermophilic bacteria of species *Thermus* and *Meiothermus*. Extremophiles 15(6):697–709. doi:10.1007/s00792-011-0400-5

Rikalovic MG, Gojgic-Cvijovic G, Vrvic MM, Karadzic I (2012) Production and characterization of rhamnolipids from *Pseudomonas aeruginosa* san-ai. J Serb Chem Soc 77(1):27–42. doi:10.2298/Jsc110211156r

Rooney AP, Price NPJ, Ray KJ, Kuo TM (2009) Isolation and characterization of rhamnolipid-producing bacterial strains from a biodiesel facility. FEMS Microbiol Lett 295(1):82–87. doi:10.1111/j.1574-6968.2009.01581.x

Sarachat T, Pornsunthorntawee O, Chavadej S, Rujiravanit R (2010) Purification and concentration of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* SP4 using foam fractionation. Bioresour Technol 101(1):324–330. doi:10.1016/j.biortech.2009.08.012

Soberon-Chavez G, Lepine F, Deziel E (2005) Production of rhamnolipids by *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 68(6):718–725. doi:10.1007/s00253-005-0150-3

Syldatk C, Wagner F (1987) Production of biosurfactant. In: Kosaric N, Cairns WL, Gray NCC (eds) Biosurfactants and biotechnology surfactant science. Marcel Dekker, New York, pp 89–120

Syldatk C, Lang S, Wagner F, Wray V, Witte L (1985) Chemical and physical characterization of four interfacial-active rhamnolipids from *Pseudomonas* spec. DSM 2874 grown on n-alkanes. Z Naturforsch C 40(1–2):51–60

Thanomsub B, Pumeechockchai W, Limtrakul A, Arunrattiyakorn P, Petchleelaha W, Nitoda T, Kanzaki H (2006) Chemical structures and biological activities of rhamnolipids produced by *Pseudomonas aeruginosa* B189 isolated from milk factory waste. Bioresourc Technol 97 (18):2457–2461. doi:10.1016/j.biortech.2005.10.029

Yang SZ, Wei DZ, Mu BZ (2007) Determination of the structure of the fatty acid chain in a cyclic lipopeptide using GC-MS. J Biochem Biophys Methods 70(3):519–523. doi:10.1016/j.jbbm.2007.01.005

Zhang F, She YH, Li HM, Zhang XT, Shu FC, Wang ZL, Yu LJ, Hou DJ (2012) Impact of an indigenous microbial enhanced oil recovery field trial on microbial community structure in a high pour-point oil reservoir. Appl Microbiol Biotechnol 95(3):811–821. doi:10.1007/s00253-011-3717-1