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Role of low-concentration monorhamnolipid in cell surface hydrophobicity of *Pseudomonas aeruginosa*: adsorption or lipopolysaccharide content variation

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Abstract A role of rhamnolipid biosurfactant to enhance the biodegradation of hydrocarbons is known to be enhancing bacterial cell surface hydrophobicity (CSH) and adhesion of cells to hydrocarbons. Assumptions regarding the mechanism for rhamnolipid in changing CSH of Gram-negative bacteria are rhamnolipid-induced release of lipopolysaccharide (LPS) from the cell's outer membrane and adsorption/orientation of rhamnolipid on the cell surface. In this study, the relation between cell-wall LPS or rhamnolipid content and CSH of a *Pseudomonas aeruginosa* bacterium subjected to rhamnolipid treatment was investigated to add insights to the mechanism. Results showed that the initial CSH was determined by the type of substrate the cells grow on and the stage of growth. For glucose-grown cells with low initial CSH and high LPS content, rhamnolipid sorption in cell wall had no discernable effect on CSH. For cells grown on glycerol with medium initial CSH and low LPS content, rhamnolipid sorption increased CSH of exponential-phase cells but decreased that of stationary-phase cells. For hexadecane-grown cells with high initial CSH and high LPS content, rhamnolipid sorption decreased CSH of both exponential-phase and stationary-phase cells. The results indicated that CSH has a better correlation to the content of rhamnolipid in the cell wall than to the content of LPS in the presence of rhamnolipid treatment and that rhamnolipid adsorption may be an important mechanism for rhamnolipid to alter CSH of *P. aeruginosa*.

Keywords Cell surface hydrophobicity · Monorhamnolipid · Lipopolysaccharide · Adsorption · *Pseudomonas aeruginosa*

Xiaoling Ma has the same contribution as the first author.

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Introduction

The soil and groundwater contamination owing to petroleum hydrocarbon extraction (Zeng et al. 2013b), pesticide utilization (Zeng et al. 2013a), and heavy metal accumulation (Xu et al. 2012; Tang et al. 2014) has been an important environmental and health issue. With soil and groundwater resources deteriorating fast, bioremediation technology has attracted the attention of the world for its safety, economy, and high ecological compatibility.

Cell surface hydrophobicity (CSH) is an important property of microorganism, which affects the efficiency of various bioprocesses, such as cell-to-cell interaction and adherence of bacteria to hydrophobic organic compounds. Thus, it is recognized as one of the crucial factors in determining microbial adhesion to bioremediation interfaces (Liu et al. 2003, 2004; van Loosdrecht et al. 1987). It was reported that there was a significantly high correlation between CSH and bacteria capability for organic contaminant degradation (Abbasnezhad et al. 2011; Obuekwe et al. 2009; Zhang et al. 2010). As a cell surface property, CSH can be determined by cell-surface-associated compounds (Hazen et al. 1990; Norman et al. 2002; Park and So 2000). In the case of Gram-negative bacteria such as *Pseudomonas aeruginosa*, the cell surface is the outer membrane (OM).

OM consists of an inner leaflet of a complex of phospholipids, an outer leaflet of lipopolysaccharide (LPS), and proteins inserted in the lipid bilayer (Chen and Zhu 2005). LPS has a typical structure consisting of lipid A tail, core oligosaccharide containing 2-keto-3-deoxyoctonic (KDO), and O-antigen from inside to outside (Al-Tahhan et al. 2000; Yokota and Fujii 2007). OM is susceptible to the outer circumstances and can be easily modified by some chemical agents and biosurfactants including rhamnolipid (Chen and Zhu 2005; Gray and Wilkinson 1965; Sotirova et al. 2009).

Rhamnolipid, a kind of biosurfactant with a low critical micelle concentration (CMC), is usually used to stimulate the biodegradation of hydrophobic organic compounds as the substrates (Fu et al. 2007; Kaczorek et al. 2008; Liu et al. 2008; Guangming et al. 2005). In these processes, rhamnolipid often exhibited a role to change bacterial CSH, which was supposed to be one of the reasons for degradation enhancement (Prabhu and Phale 2003; Zeng et al. 2011; Zhang and Miller 1994). For Gram-negative bacteria, loss of LPS in OM in the presence of rhamnolipid is a possible mechanism for rhamnolipid to enhance CSH. Al-Tahhan et al. (2000) found that LPS released to a culture medium in the presence of rhamnolipid resulted in higher cell hydrophobicity and enhanced hexadecane degradation for *P. aeruginosa* ATCC 9027. Chen and Zhu (2005) found a more hydrophobic surface of *P. aeruginosa* cell after rhamnolipid-induced release of LPS from the cell surface and a subsequent enhancement of accumulation of hydrophobic hydrocarbons on the cell surface. In addition, the influence of rhamnolipid on LPS release was different for strains. In the study of Al-Tahhan et al. (2000), *P. aeruginosa* ATCC 27853 released more than 25 % of its LPS in the presence of rhamnolipid, while *P. aeruginosa* ATCC 9027, with a similar improvement of CSH to *P. aeruginosa* ATCC 27853, released only 3.3 to 14 %. Another potential mechanism responsible for the CSH alteration by rhamnolipid is the adsorption or fusion of rhamnolipid on the cell surface. Yuan et al. (2007) found that the adsorption of rhamnolipid on cells of a *P. aeruginosa* strain turned the cell surface to be more hydrophobic, enhancing the adherence of cells onto hydrophobic surfaces. Studies of Zhong et al. (2007, 2008) showed that the adsorption of rhamnolipid changed CSH of *P. aeruginosa* as well as some other microorganisms at different growth phases, in which orientation of rhamnolipid monomers on the cell surface and micelle deposition were supposed to account for the changes at low and high rhamnolipid concentrations, respectively.

In this study, monorhamnolipid biosurfactant was used to treat cells of a *P. aeruginosa* bacterium and the time-course change of CSH, cell-wall LPS content, and cell-wall rhamnolipid content were examined. The bacterium was grown on three different types of substrate to exponential phase or stationary phase to obtain cells with different initial CSHs. Considering that rhamnolipid may penetrate to the inside of cells, for accuracy of the results, the cell-wall content of LPS and rhamnolipid was measured directly after breaking and lysis of cells rather than by measuring the change of LPS or rhamnolipid concentration in culture medium. The objective is to identify the role of rhamnolipid that is prior in modifying CSH for Gram-negative bacteria.

Materials and methods

Microorganism and chemicals

P. aeruginosa ATCC 9027 was obtained from the American Type Culture Collection. The culture was maintained at 4 °C on peptone agar slants and transferred monthly. It was activated at 30 °C before use. Highly purified LPS used as the standard for LPS analysis was extracted from *P. aeruginosa* 10 and was purchased from Sigma-Aldrich (St. Louis, MO). Bromoacetophenone (≥99 %) and triethylamine (≥98 %) for rhamnolipid analysis were from Sigma-Aldrich (St. Louis, MO). Other reagents were of analytical grade. The water used throughout the experiments was produced by Labconco WaterPro PS (Kansas, USA) with initial resistivity higher than 18.2 MΩ cm^{-1}.

Rhamnolipid production, purification, and characterization

Rhamnolipid was produced by *P. aeruginosa* ATCC 9027 using the method described by Zhang and Miller (1992). Then, it was separated and purified by the method of Zhong et al. (2007). The component analysis of the purified rhamnolipid was performed by high-performance liquid chromatography-mass spectroscopy (HPLC-MS) (Zhong et al. 2007). The CMC of the purified rhamnolipid in a mineral salt medium (MSM) was measured by the dependence of MSM surface tension on the rhamnolipid concentration, and the data were analyzed using the method of Yuan et al. (2007). MSM was composed of 0.5 % NH_4Cl, 0.5 % Na_2HPO_4, 0.25 % KH_2PO_4, and 0.05 % $MgSO_4 \cdot 7H_2O$, with a pH of 6.8.

Cell growth, surface hydrophobicity, and LPS content

P. aeruginosa ATCC 9027 was enriched in Kay's minimal medium (Warren et al. 1960) with gyratory agitation at 37 °C and 200 rpm. After 24 h, the enriched culture was inoculated at the ratio of 2.5 % (*v*/*v*) into 100 ml MSM containing 2.5 % glucose, glycerol, or hexadecane as the sole carbon source. Then, this inoculated culture medium in a 500-ml Erlenmeyer flask was cultivated on gyratory agitation at 200 rpm and 37 °C. For certain time intervals, the culture medium was sampled to determine cell growth, CSH, and LPS content.

Bacterial optical density at 600 nm (OD_{600}) was used to show cell growth. Culture medium sample was centrifuged at 6,000×*g* for 10 min. The precipitated cells were washed twice with MSM and then resuspended in MSM with a volume equal to the sample. Then, OD_{600} of the cell suspension was determined by the Shimadzu UV-2552 spectrophotometer. After OD_{600} determination, the cell suspension was further used for CSH determination using a modified bacterial adhesion to hydrocarbon (BATH) method as described by Zhong et al. (2007).

Because LPS exists only in the outer membrane of Gram-negative bacteria (Hancock and Nikaido 1978), LPS was extracted using whole-cell lysis described by Inzana and Pichichero (1984), but with some modifications. Firstly, in order to remove all the interfering substances, the pellet was washed twice with phosphate-buffered saline (PBS) which, pH of 7.2, was composed of 0.02 % KCl, 0.02 % KH_2PO_4, 0.8 % NaCl, and 0.216 % $Na_2HPO_4 \cdot 7H_2O$, and each time, cells were separated by centrifugation.Washed cells were then suspended in PBS to give OD_{600} of 1.0. A certain volume of cell suspension was centrifuged at 14,000×*g* for 10 min, and the supernatant was discarded. To extract LPS, 0.3 ml of distilled water and an equal volume of 90 % phenol were added to the cell pellet. Then, the mixture was heated at 68 °C for 15 min with vigorous stirring, quickly chilled to 10 °C, and centrifuged at 6,800×*g* for 15 min. The supernatant was removed carefully with a pipette, and the phenol phase at the bottom was mixed with 0.2 ml of distilled water for a repetition of LPS extraction. The supernatants from the two phenol-water extractions were combined in a centrifuge tube, added with 0.1 ml of 20 % NaCl and 6 ml of 95 % ethanol, and then placed at −20 °C overnight to precipitate LPS. The LPS precipitate and the aqueous phases were separated by centrifugation at 2,000×*g* for 10 min. Then, 0.1 ml of 0.5 M NaCl and 1 ml of 95 % ethanol were added to the solid phase, and LPS precipitation at −20 °C was repeated. Finally, distilled water was added to the precipitate to form 0.1 ml of sample. The LPS concentration in the sample was measured by the thiobarbituric acid assay of KDO (Al-Tahhan et al. 2000) and then converted to cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM.

Treatment on *P. aeruginosa* ATCC 9027 with rhamnolipid

Exponential-phase cells (20 h grown on glucose, 24 h grown on glycerol, or 10 days grown on hexadecane based on growth curves) and stationary-phase cells (36 h grown on glucose, 42 h grown on glycerol, or 30 days grown on hexadecane based on growth curves) of *P. aeruginosa* ATCC 9027 were used for rhamnolipid treatment test. Cells were centrifuged at 6,000×*g* for 10 min, washed with MSM, and resuspended in MSM to obtain OD_{600} of 1.0. Rhamnolipid was supplied to the flask containing 15 ml of cell suspensions to a final concentration 0, 50, or 500 μM. Then, the cells were incubated at 37 °C with gyratory agitation at 200 rpm. After 10, 60, or 240 min, CSH, LPS content, and cell-wall rhamnolipid content were determined.

CSH and LPS were measured using the methods described in "Cell growth, surface hydrophobicity, and LPS content." The quantitative analysis of rhamnolipid content in cell wall was based on 6 ml of cell suspensions (OD_{600}=1.0) in this experiment. First, the cell wall of *P. aeruginosa* ATCC 9027 was separated by the method of ultrasound and centrifugation as described by Weinberg et al. (1983). The cell-wall pellets were collected and resuspended in 2 ml MSM. To adjust the pH to 12.0, 10 % NaOH was added, and then, the temperature of the water bath was kept at 100 °C for 1 min for the lysis of the cell-wall pellets. After that, the suspension was adjusted to a pH of about 2.0 by 2 M HCl. Rhamnolipid was then extracted by 2 ml ethyl acetate; 1.7-ml phase of the ethyl acetate was collected and air-evaporated at 60 °C. The residual rhamnolipid was dissolved in 1 ml of acetonitrile solution containing 8 mM 2-bromoacetophenone and 4 mM triethylamine, and the mixture was then allowed to react at 80 °C for 1 h (Zhong et al. 2007). The content of derivatized rhamnolipid was determined by HPLC using the method of Mata-Sandoval et al. (1999) with some modifications described by Zhong et al. (2007). The measured concentration was converted to adsorbed rhamnolipid content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM.

Results

Characterization of the rhamnolipid

The result of HPLC-MS analysis on the purified rhamnolipid is shown in Fig. 1. *P. aeruginosa* ATCC 9027 produces only monorhamnolipid under the cultivation condition in this experiment. The monorhamnolipid mixture consists of $RhaC_{10}C_{10}$, $RhaC_{10}C_{12}$, $RhaC_8C_{10}$, and $RhaC_{10}C_{12}$-H_2 with the approximate molar ratio of 70:13:10:7 based on the peak area of the species in the chromatograph. The abbreviation $Rha_xC_yC_z(-H_w)$ designates the individual component with x as the number of rhamnose groups, y and z as the carbon atom number of each aliphatic chain in the lipid moieties, and w/2 as the number of unsaturated bonds in lipid moieties. The CMC of the monorhamnolipid in MSM was determined to be 83 μM or 42.1 mg/L based on the dependence of MSM surface tension on rhamnolipid concentration, and the data were analyzed by the method of Yuan et al. (2007).

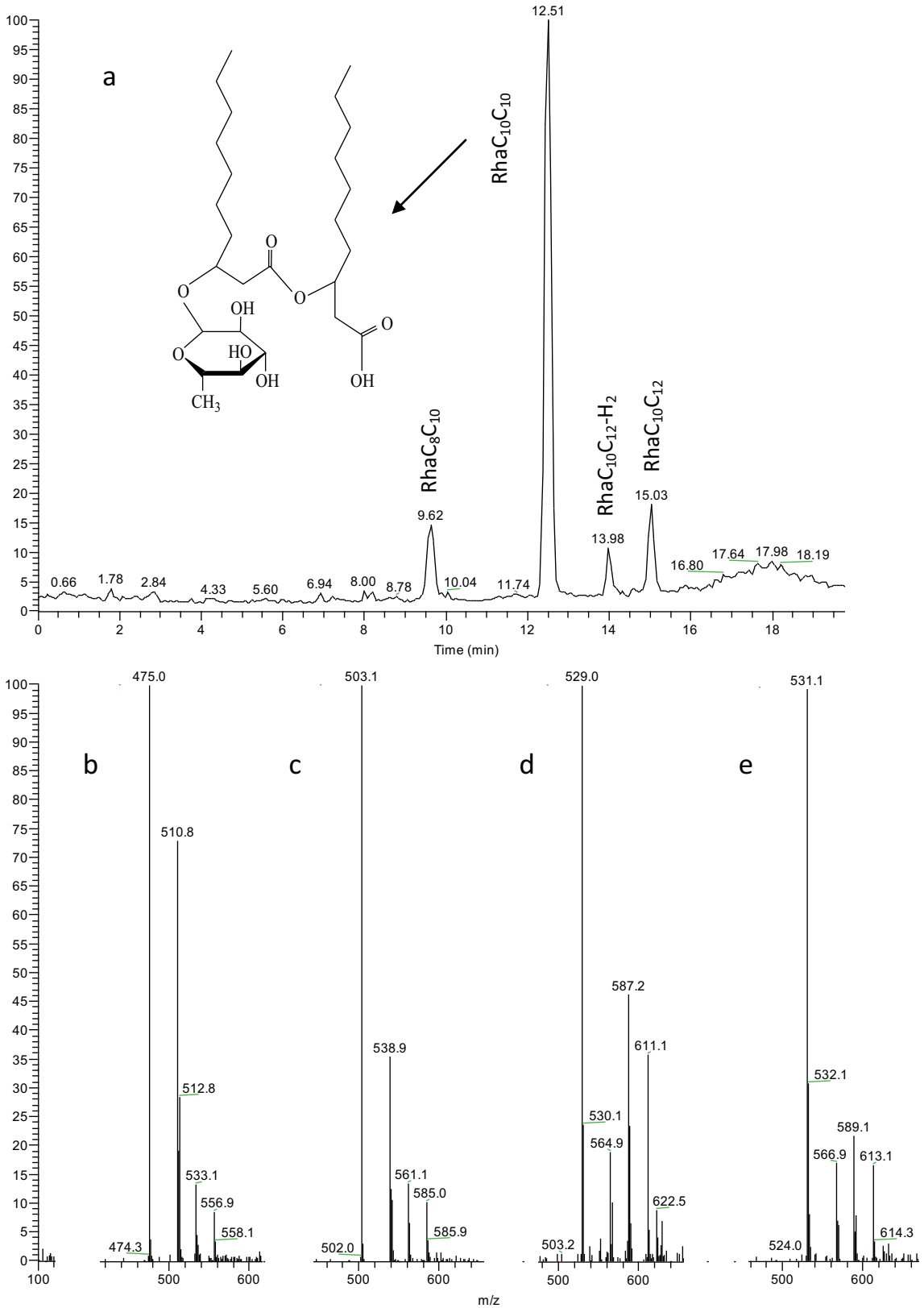

Fig. 1 HPLC-MS chromatogram of purified rhamnolipid from *P. aeruginosa* ATCC 9027. *a*, HPLC chromatogram; *b*, 9.3–9.8 min *m/z* 475.0; *c*, 12.2–12.7 min *m/z* 503.1; *d*, 13.8–14.3 min *m/z* 529.0; *e*, 14.7–15.2 min *m/z* 531.1

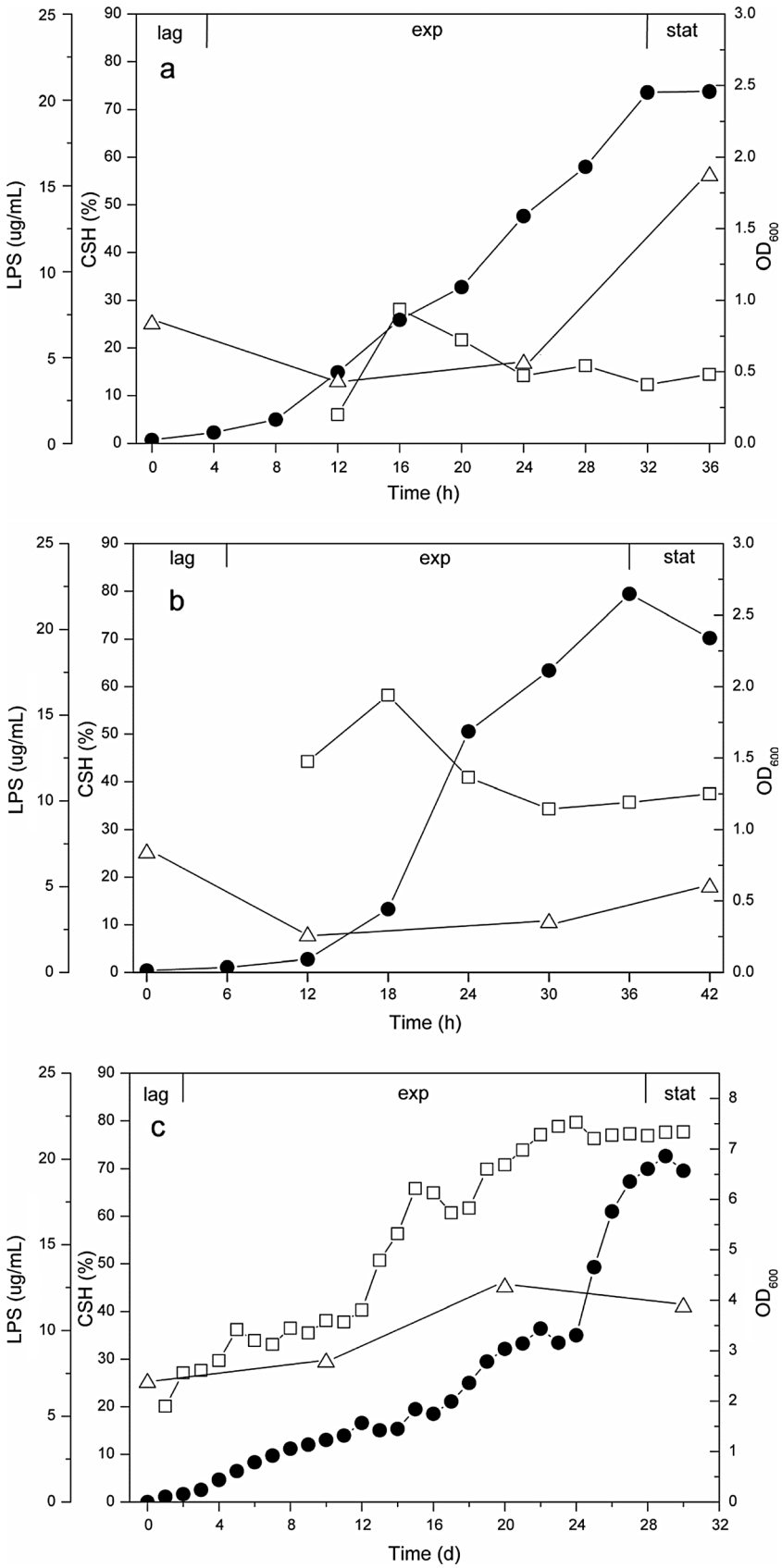

Fig. 2 Growth (*filled circles*), CSH (*open squares*), and LPS (*open triangles*) of *P. aeruginosa* ATCC 9027 when grown on glucose (**a**), glycerol (**b**), and hexadecane (**c**). For LPS, *μg/mL* represented the cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM, and the data of 0 h referred to the sample in Kay's minimal medium at 24 h

Cell growth and cell surface hydrophobicity

Figure 2 shows the cell growth in the culture with different carbon sources and the related CSH and cell surface LPS content. Cells exhibited a fast growth in a period of 32 h with glucose or glycerol as the substrate (Fig. 2a, b). When cells grow from the middle exponential phase to stationary phase on either glucose or glycerol, CSH decreases first and then becomes stable. Totally, CSH is higher for cells grown on glycerol than those on glucose. Cellular LPS content increases from the middle exponential phase to stationary phase, and totally, it is lower for cells grown on glycerol than those on glucose. For these two substrates, change of CSH appears to have a good reverse correlation with that of cellular LPS content.

The features of cell growth and the related change of CSH are different when hexadecane was used as the substrate. Totally, CSH increases along with cell growth during the whole cultivation period, reaching a final high level of BATH rate of 80 % (Fig. 2c). The cell-growing phase can be divided into three stages with incremental growth rate from stage 1 to stage 3 in order of time. At the beginning of each stage, almost a rapid increase of CSH is observed. This result shows the importance of CSH on bacterial growth under water-immiscible organic substrate. The cellular LPS content also increases during the whole growth period, and the LPS content level is comparable to that of cells grown on glucose. Because LPS is the cell's outer membrane structure supposed to cause cell surface hydrophilic (Nikaido 1976; Al-Tahhan et al. 2000), a similar trend in the change of cellular LPS and CSH when cells were grown on hexadecane indicates that cell surface LPS may not be the sole structure determining CSH.

Effect of rhamnolipid treatment and relation between CSH, cellular LPS, and adsorbed rhamnolipid

A method of short-term monorhamnolipid treatment on the *P. aeruginosa* cells in the absence of any carbon source was used to examine the role of rhamnolipid in altering CSH of

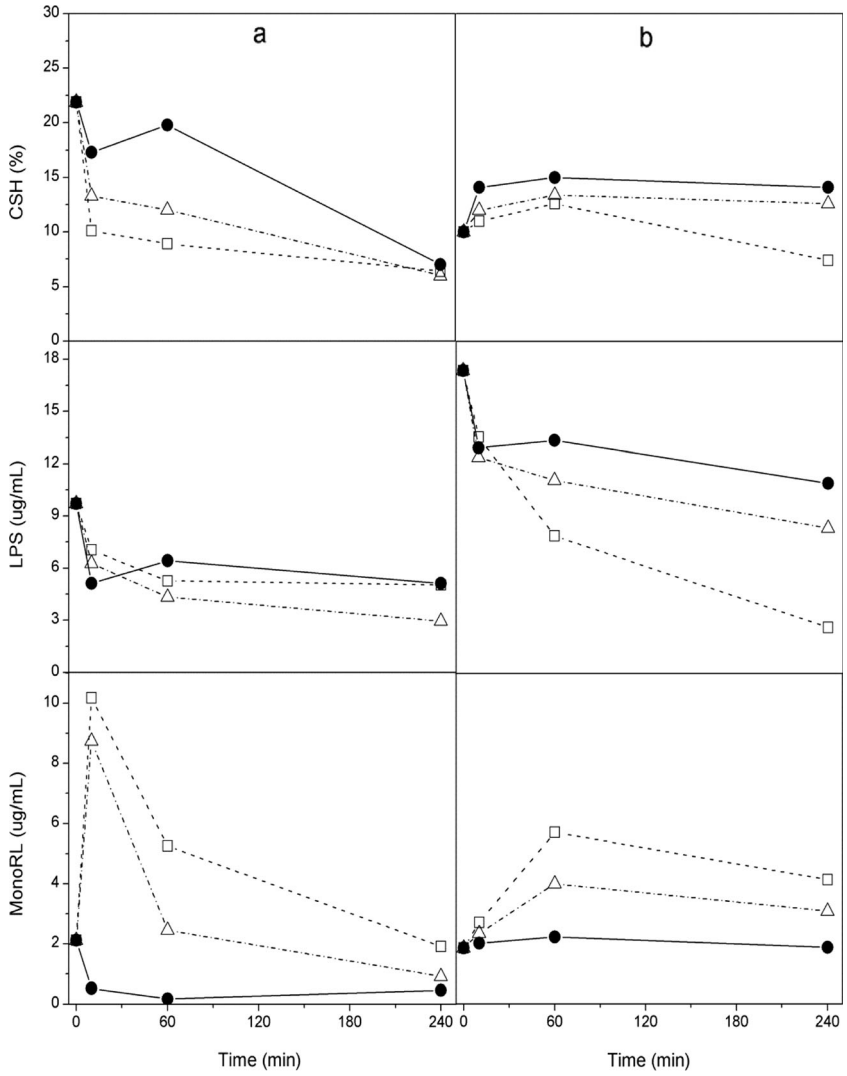

Fig. 3 CSH, LPS, and adsorbed monoRL of *P. aeruginosa* ATCC 9027 exponential-phase (**a**) and stationary-phase (**b**) cells grown on glucose after monoRL addition. For LPS, *μg/mL* represented the cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM. For monoRL adsorption, *μg/mL* represented the amount of monoRL in cell walls of the same amount of bacteria. *Filled circles* indicate no monoRL; *open triangles*, 50 μM monoRL; and *open squares*, 500 μM monoRL

P. aeruginosa ATCC 9027. During this 4-h course of treatment, the growth of cells is minimal due to lack of carbon source (the obtained cells were centrifuged and washed twice with MSM, and *P. aeruginosa* ATCC 9027 does not use the added rhamnolipid as the carbon source for growth), which is demonstrated by the minimal change of OD_{600} of the culture before and after the 4-h period (data not shown). The reason for not examining the effect of rhamnolipid in a cell-growth manner is to avoid any side effects that may arise from reproduction activity of growing cell. Because the LPS regeneration and penetration of rhamnolipid to the inside of cells occur in living cells and also the cells of *P. aeruginosa* ATCC 9027 can produce rhamnolipid, it may not be precise to determine the cellular LPS and adsorbed rhamnolipid content by measuring LPS and rhamnolipid concentration in the culture medium. Thus, the content of cellular LPS and adsorbed rhamnolipid was measured directly by the cell extraction method (see "Materials and methods" for details). The effect of rhamnolipid treatment on CSH, cellular LPS content, and adsorbed rhamnolipid content for exponential-phase and stationary-phase cells grown on the three substrates of glucose, glycerol, and hexadecane is shown in Figs. 3, 4, and 5.

Change of CSH upon the rhamnolipid treatment occurred mainly in the first 10 min of contact and depends on both the carbon source the cells grew on and the stage of growth. For glucose-grown cells with low initial CSH, the change is limited whether the cells are in the exponential or stationary growth stage. For glycerol-grown cells with medium initial CSH, CSH decreases for exponential cells and increases for stationary cells. For hexadecane-grown cells with high initial CSH, CSH decreases for both exponential and stationary cells and the decrease was most significant for stationary cells treated with 500 μM rhamnolipid.

Rhamnolipid can induce loss of LPS from the cell surface of *P. aeruginosa*, which is supposed to be the reason for increased CSH of the cells in the presence of rhamnolipid (Al-Tahhan et al. 2000). In our experiment, the glucose-grown exponential-phase cells, glucose-grown stationary-phase

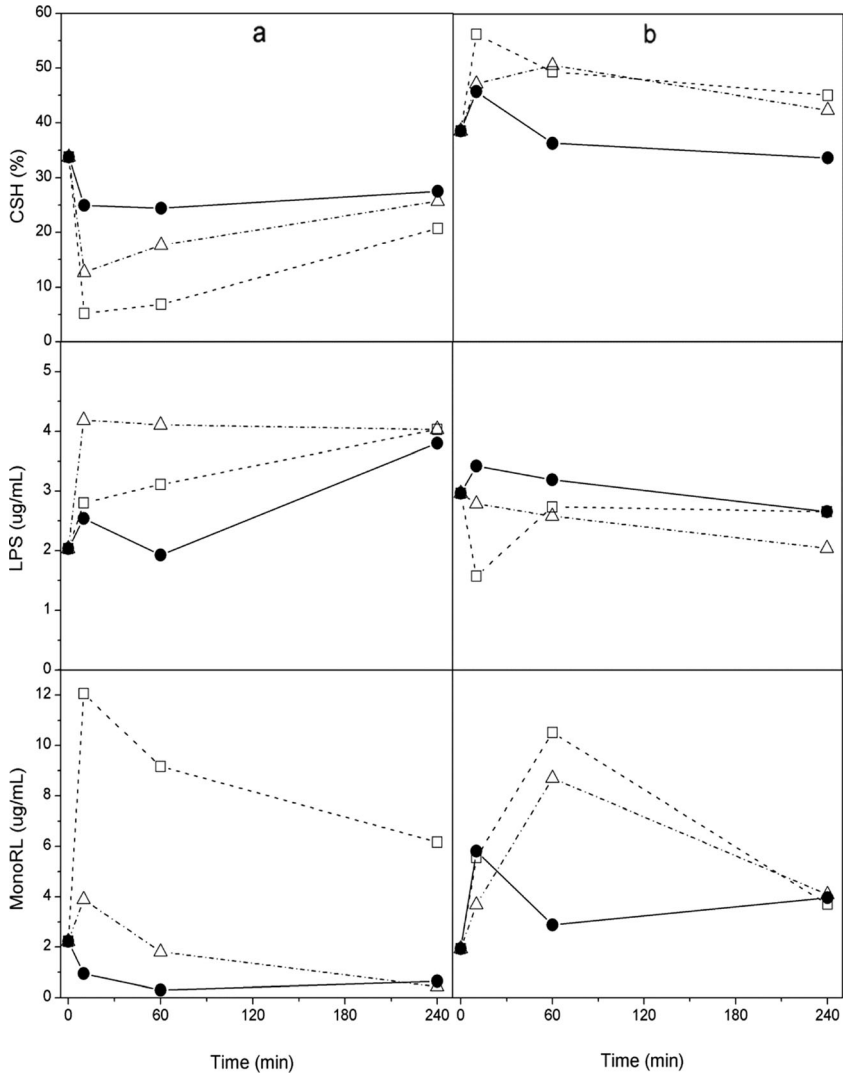

Fig. 4 CSH, LPS, and adsorbed monoRL of *P. aeruginosa* ATCC 9027 exponential-phase (**a**) and stationary-phase (**b**) cells grown on glycerol after monoRL addition. For LPS, *μg/mL* represented the cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM. For monoRL adsorption, *μg/mL* represented the amount of monoRL in cell walls of the same amount of bacteria. Details are described in the legend of Fig. 3

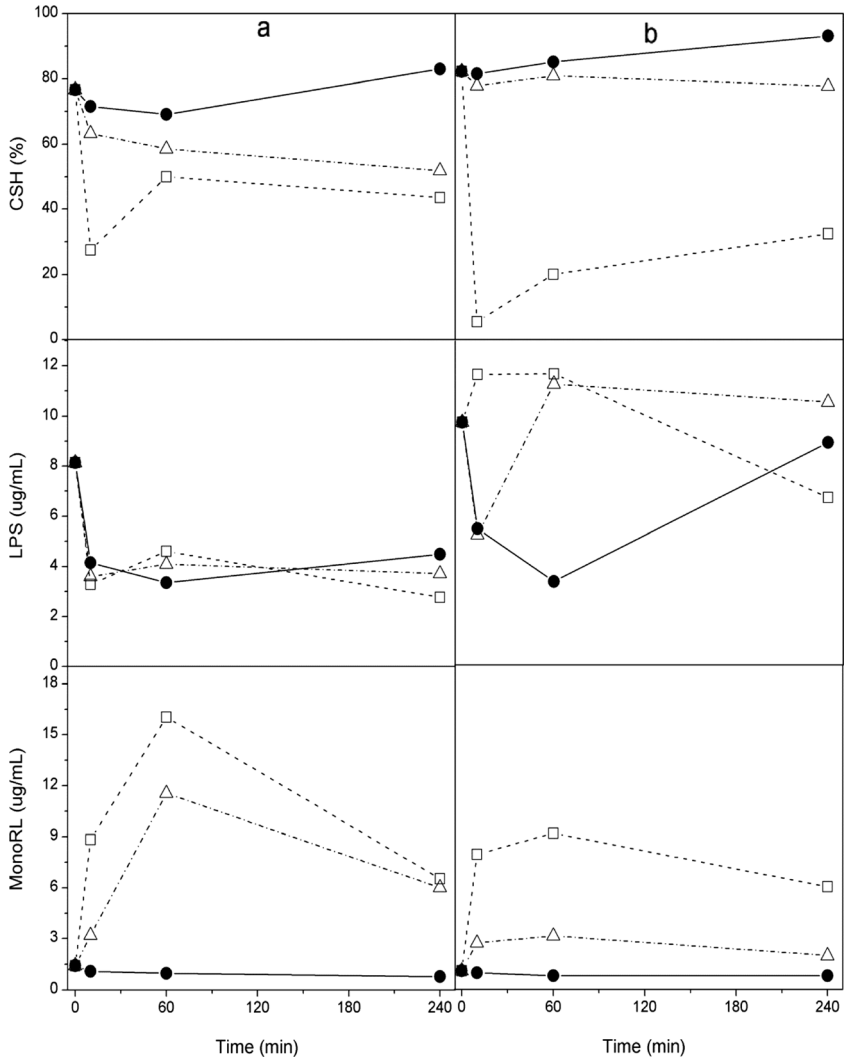

Fig. 5 CSH, LPS, and adsorbed monoRL of *P. aeruginosa* ATCC 9027 exponential-phase (**a**) and stationary-phase (**b**) cells grown on hexadecane after monoRL addition. For LPS, *μg/mL* represented the cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM. For monoRL adsorption, *μg/mL* represented the amount of monoRL in cell walls of the same amount of bacteria. Details are described in the legend of Fig. 3

cells, and hexadecane-grown exponential-phase cells exhibit loss of cellular LPS content during the early stage of rhamnolipid treatment; however, the rhamnolipid treatment does not cause a discernable higher decrease of cellular LPS content at the higher concentration of rhamnolipid (500 μM). No strong relation between the change of CSH and change of cellular LPS content was observed.

For all the six groups of cells, the initial rhamnolipid content in the cell wall is higher than 0 mg/L because *P. aeruginosa* ATCC 9027 produces cell-wall-associated rhamnolipid during growth. The content of cell-wall-associated rhamnolipid is close between log-phase and stationary-phase cells, and basically, it did not increase during the 4-h treatment without addition of extra rhamnolipid. When extra rhamnolipid was added, adsorption of rhamnolipid to the cell wall was observed during the first hour of treatment and the adsorption is faster for exponential-phase cells than for stationary-phase cells. After 10 min or 1 h, the cell-wall rhamnolipid content decreases, which may be a physiological response of the cells to the adsorption, as *P. aeruginosa* bacterium is found to be able to release rhamnolipid actively upon rhamnolipid treatment (Zhong et al. 2007).

Given that the trends of change of CSH and adsorbed rhamnolipid appear related, the data of CSH and cell-wall rhamnolipid content for all the six groups of cells at each time point during rhamnolipid treatment are put together with the expectation of establishing a direct relation between rhamnolipid adsorption and CSH. The data of CSH and cellular LPS content are also plotted for comparison. As shown in Fig. 6a, when all the six groups of cells are considered together, there is a wide distribution of CSH whether the cellular LPS is low or high, and the trend that CSH decreased with increasing cellular LPS content is not obvious. The result indicates that the change of cellular LPS content has a limited impact on CSH in the case of this study and cellular LPS may not be the sole factor determining CSH.

Rhamnolipid adsorption shows an impact on CSH which is dependent on the type of cells (Fig. 6b). For glucose-grown cells with low initial CSH, whether the cells are from the exponential phase or stationary phase, CSH is inert to the

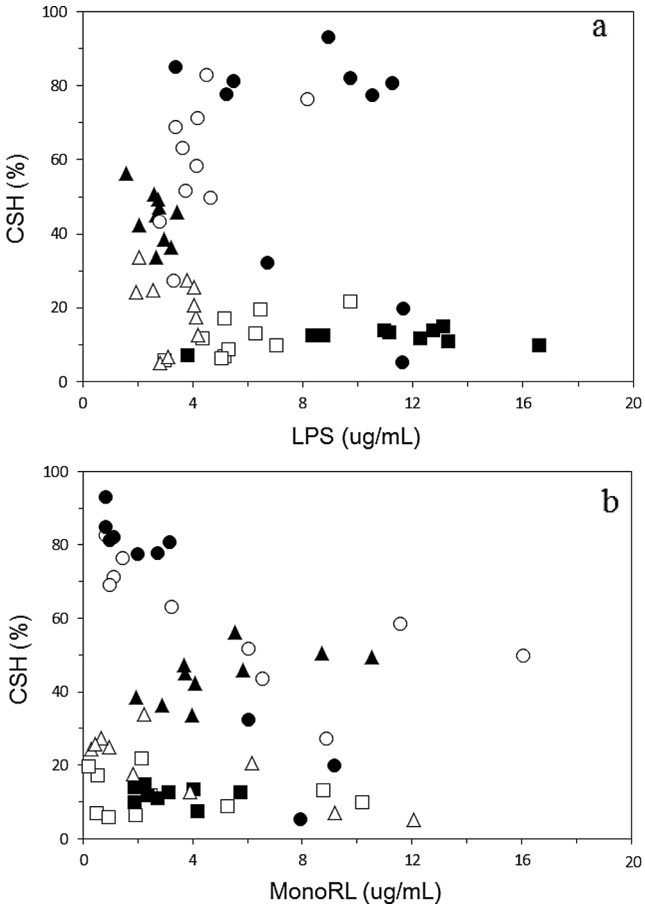

Fig. 6 Relationship between CSH of *P. aeruginosa* ATCC 9027 and LPS content (**a**), and monoRL adsorbed in cell wall (**b**). For LPS, *μg/mL* represented the cellular LPS content in micrograms per milliliter of cell suspension with OD_{600} of 1.0 in MSM. For monoRL adsorption, *μg/mL* represented the amount of monoRL in cell walls of the same amount of bacteria. *Open squares* indicate exponential-phase cells grown on glucose; *filled squares*, stationary-phase cells grown on glucose; *open triangles*, exponential-phase cells grown on glycerol; *filled triangles*, stationary-phase cells grown on glycerol; *open circles*, exponential-phase cells grown on hexadecane; and *filled circles*, stationary-phase cells grown on hexadecane

change of cell-wall rhamnolipid content. For glycerol-grown cells with medium initial CSH, rhamnolipid sorption increases CSH of exponential-phase cells but decreases that of stationary-phase cells. For hexadecane-grown cells with high initial CSH, rhamnolipid adsorption decreases CSH of both exponential-phase and stationary-phase cells. That is, the response of CSH to adsorption of rhamnolipid is specific to both the type of carbon source and growth stage of the cells.

Discussion

Cell surface hydrophobicity is considered to be one of the key factors in affecting the efficiency of various bioprocesses (Feng et al. 2013). In the case of a Gram-negative bacterium such as *P. aeruginosa*, cell surface properties like CSH result from the unique chemical structure of the outer membrane which is primarily composed of LPS. The O-antigen, the part of LPS extending out to the environment, consists of sugars and is hydrophilic. However, the LPS mutants with no O-antigen or LPS loss from the outer membrane is assumed to be able to cause a more hydrophobic cell surface due to the exposure of the hydrophobic fatty acid tails of LPS or phospholipid in the outer membrane. (Nikaido 1976; Al-Tahhan et al. 2000). Other structures, e.g., OM protein, rhamnolipid as a product associating with cell wall, and even substrate adsorbed to the cell surface, may also play a role (Feng et al. 2013; Masuoka and Hazen 1997; Hazen et al. 1986).

In the study of Al-Tahhan et al. (2000), a good relation between the increase of CSH and the amount of LPS released to the culture medium was observed for *P. aeruginosa* ATCC 9027 growing on either glucose or hexadecane in the presence of rhamnolipid. Such a relation between the cellular LPS content and CSH, however, was not established in this study (Fig. 6a). The information on the increase of LPS quantity in the culture medium may not be enough to indicate the decrease of content of the cellular LPS which should be the one directly related to CSH if there is such a relation. There could be the possibility that LPS in cell-wall, during cell growth, is replenished through the synthesis of LPS rather than being constantly lost because of releasing to the medium. It should also be noted that the concentration of rhamnolipid used in their cell growth experiment is 6 mM, which is more than an order higher than that used in our experiment (0.5 mM). This high concentration of rhamnolipid could probably induce much heavier loss of LPS from the cell surface, which may truly pose a strong effect in enhancing cell surface hydrophobicity.

The results of Fig. 6b show that CSH responded to the adsorption of rhamnolipid and the response is specific to both the type of carbon source and growth stage of the cells. This specificity is assumed to result from the difference in the modes of orientation of monoRL molecules adsorbed to the cell surface. Rhamnolipid may adsorb to the cell surface through interaction between rhamnosyl or carboxyl groups and polar structures on the cell surface by electrostatic, dipolar, hydrogen bonding, or short-term forces (e.g., O-antigen of LPS) (Paria and Khilar 2004), turning the bacterial surface more hydrophobic. The adsorption may also be driven by van der Waals and hydrophobic forces between rhamnolipid hydrophobic tail and cell surface nonpolar structures (e.g., lipids and some proteins), and this means that the orientation of rhamnolipid molecule would be inverted with the polar head exposed to the external environment, causing the reduction of CSH (Górna et al. 2011; Zhang and Miller 1994). The ratio between quantities of these polar and nonpolar structures may be a function to the type of carbon source and the growth stage. Thus, the cells exhibit disparity in CSH after growth on different substrates for different time periods, and the pattern

that CSH changes upon rhamnolipid adsorption varies between cell groups.

In conclusion, the study used the cells of a *P. aeruginosa* bacterium treated with rhamnolipid to examine the roles of low-concentration rhamnolipid biosurfactant in the modification of cell surface hydrophobicity. The cellular LPS and cell-wall-associated rhamnolipid were extracted and measured to establish the relations between them and CSH.The results showed that the effect of rhamnolipid adsorption in cell wall on CSH change precedes that of rhamnolipid-induced removal of LPS when rhamnolipid concentration is up to 0.5 mM.The way on how CSH changes, however, depends on the type of substrate the cells grow on and the growth stage, hypothetically as a result of the variation of interaction between the rhamnolipid molecule and cell surface structures and the pattern of orientation of the rhamnolipid molecule on the cell surface. The study added new information to the mechanism on how biosurfactants alter cell surface hydrophobicity of hydrocarbon-degrading microorganism and on the methodology to control cell surface hydrophobicity with biosurfactant, which should be of importance for applications of biosurfactants in the biodegradation of water-immiscible organic contaminants and bioremediation of sites contaminated with organic compounds.

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References

Abbasnezhad H, Gray M, Foght JM (2011) Influence of adhesion on aerobic biodegradation and bioremediation of liquid hydrocarbons. Appl Microbiol Biotechnol 92(4):653–675

Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66(8):3262–3268

Chen G, Zhu H (2005) *lux*-marked *Pseudomonas aeruginosa* lipopolysaccharide production in the presence of rhamnolipid. Colloid Surf B 41(1):43–48

Feng W, Swift S, Singhal N (2013) Effects of surfactants on cell surface tension parameters and hydrophobicity of *Pseudomonas putida* 852 and *Rhodococcus erythropolis* 3586. Colloid Surf B 105:43–50

Fu HY, Zeng GM, Zhong H, Yuan XZ, Wang W, Huang GH, Li JB (2007) Effects of rhamnolipid on degradation of granular organic substrate from kitchen waste by a *Pseudomonas aeruginosa* strain. Colloid Surf B 58(2):91–97

Górna H, Ławniczak Ł, Zgoła-Grześkowiak A, Kaczorek E (2011) Differences and dynamic changes in the cell surface properties of three *Pseudomonas aeruginosa* strains isolated from petroleum-polluted soil as a response to various carbon sources and the external addition of rhamnolipids. Bioresour Technol 102(3):3028–3033

Gray G, Wilkinson S (1965) The effect of ethylenediaminetetra-acetic acid on the cell walls of some gram-negative bacteria. J Gen Microbiol 39(3):385–399

Zeng GM, Zhong H, Huang GH, Fu HY (2005) Physicochemical and microbiological effects of biosurfactant on the remediation of HOC-contaminated soil. Prog Nat Sci 15(7):577–585

Hancock R, Nikaido H (1978) Outer membranes of gram-negative bacteria. XIX. Isolation from *Pseudomonas aeruginosa* PAO1 and use in reconstitution and definition of the permeability barrier. J Bacteriol 136(1):381–390

Hazen K, Plotkin B, Klimas D (1986) Influence of growth conditions on cell surface hydrophobicity of *Candida albicans and Candida glabrata*. Infect Immun 54(1):269–271

Inzana T, Pichichero M (1984) Lipopolysaccharide subtypes of *Haemophilus influenzae* type b from an outbreak of invasive disease. J Clin Microbiol 20(2):145–150

Kaczorek E, Chrzanowski Ł, Pijanowska A, Olszanowski A (2008) Yeast and bacteria cell hydrophobicity and hydrocarbon biodegradation in the presence of natural surfactants: rhamnolipides and saponins. Bioresour Technol 99(10):4285–4291

Liu XL, Zeng GM, Tang L, Zhong H, Wang RY, Fu HY, Liu ZF, Huang HL, Zhang JC (2008) Effects of dirhamnolipid and SDS on enzyme production from *Phanerochaete chrysosporium* in submerged fermentation. Process Biochem 43(11):1300–1303

Liu Y, Yang SF, Li Y, Xu H, Qin L, Tay JH (2004) The influence of cell and substratum surface hydrophobicities on microbial attachment. J Biotechnol 110(3):251–256

Liu Y, Yang SF, Liu QS, Tay JH (2003) The role of cell hydrophobicity in the formation of aerobic granules. Curr Microbiol 46(4):0270–0274

Masuoka J, Hazen KC (1997) Cell wall protein mannosylation determines *Candida albicans* cell surface hydrophobibity. Microbiology 143(9):3015–3021

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

Nikaido H (1976) Outer membrane of *Salmonella typhimurium*: transmembrane diffusion of some hydrophobic substances. Biochim Biophys Acta 433(1):118–132

Norman RS, Frontera-Suau R, Morris PJ (2002) Variability in *Pseudomonas aeruginosa* lipopolysaccharide expression during crude oil degradation. Appl Environ Microbiol 68(10): 5096–5103

Obuekwe CO, Al-Jadi ZK, Al-Saleh ES (2009) Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment. Int Biodeterior Biodegrad 63(3):273–279

Paria S, Khilar KC (2004) A review on experimental studies of surfactant adsorption at the hydrophilic solid–water interface. Adv Colloids Interf Sci 110(3):75–95

Park KM, So JS (2000) Altered cell surface hydrophobicity of lipopolysaccharide-deficient mutant of *Bradyrhizobium japonicum*. J Microbiol Methods 41(3):219–226

Prabhu Y, Phale P (2003) Biodegradation of phenanthrene by *Pseudomonas sp*. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation. Appl Microbiol Biotechnol 61(4):342–351

Sotirova A, Spasova D, Vasileva-Tonkova E, Galabova D (2009) Effects of rhamnolipid-biosurfactant on cell surface of *Pseudomonas aeruginosa*. Microbiol Res 164(3):297–303

Tang WW, Zeng GM, Gong JL, Liang J, Xu P, Zhang C, Huang BB (2014) Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: a review. Sci Total Environ 468:1014–1027

van Loosdrecht M, Lyklema J, Norde W, Schraa G, Zehnder A (1987) The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol 53(8):1893–1897

Warren RAJ, Ells AF, Campbell J (1960) Endogenous respiration of *Pseudomonas aeruginosa*. J Bacteriol 79(6):875

Weinberg J, Ribi E, Wheat R (1983) Enhancement of macrophage-mediated tumor cell killing by bacterial outer membrane proteins (porins). Infect Immun 42(1):219–223

Xu P, Zeng GM, Huang DL, Feng CL, Hu S, Zhao MH, Lai C, Wei Z, Huang C, Xie GX (2012) Use of iron oxide nanomaterials in wastewater treatment: a review. Sci Total Environ 424:1–10

Yokota S-i, Fujii N (2007) Contributions of the lipopolysaccharide outer core oligosaccharide region on the cell surface properties of *Pseudomonas aeruginosa*. Comp Immunol Microbiol 30(2):97–109

Yuan X, Ren F, Zeng G, Zhong H, Fu H, Liu J, Xu X (2007) Adsorption of surfactants on a *Pseudomonas aeruginosa* strain and the effect on cell surface lypohydrophilic property. Appl Microbiol Biotechnol 76(5):1189–1198

Zeng G, Chen M, Zeng Z (2013a) Risks of neonicotinoid pesticides. Science 340(6139):1403–1403

Zeng G, Chen M, Zeng Z (2013b) Shale gas: surface water also at risk. Nature 499(7457):154–154

Zeng G, Liu Z, Zhong H, Li J, Yuan X, Fu H, Ding Y, Wang J, Zhou M (2011) Effect of monorhamnolipid on the degradation of *n*-hexadecane by *Candida tropicalis* and the association with cell surface properties. Appl Microbiol Biotechnol 90(3):1155–1161

Zhang C, Jia L, Wang S, Qu J, Li K, Xu L, Shi Y, Yan Y (2010) Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. Bioresour Technol 101(10): 3423–3429

Zhang Y, Miller RM (1992) Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant). Appl Environ Microbiol 58(10):3276–3282

Zhang Y, Miller RM (1994) Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. Appl Environ Microbiol 60(6):2101–2106

Zhong H, Ming Zeng G, Yuan XZ, Yan Fu H, Huang GH, Ren FY (2007) Adsorption of dirhamnolipid on four microorganisms and the effect on cell surface hydrophobicity. Appl Microbiol Biotechnol 77(2): 447–455

Zhong H, Zeng GM, Liu JX, Xu XM, Yuan XZ, Fu HY, Huang GH, Liu ZF, Ding Y (2008) Adsorption of monorhamnolipid and dirhamnolipid on two *Pseudomonas aeruginosa* strains and the effect on cell surface hydrophobicity. Appl Microbiol Biotechnol 79(4):671–677