

Glycerol promoted the anaerobic production of rhamnolipids by different *Pseudomonas aeruginosa* strains

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Research

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Abstract

Background: Rhamnolipids is the most widely studied and applied biosurfactants. The anaerobic biosynthesis of rhamnolipids has important research and practical significance, such as meeting the in situ production of biosurfactant in anoxic environments and the foamless fermentation of biosurfactants. A few studies have reported the anaerobic biosynthesis of rhamnolipids from rare *Pseudomonas aeruginosa* strains. What did promote the anaerobic biosynthesis of rhamnolipids, the specificity of the rare strains or the effect of specific substrates? Here, anaerobic production of rhamnolipids by different *P. aeruginosa* strains was investigated using diverse substrates. The anaerobic biosynthesis mechanism of rhamnolipids were also discussed from the substrate point of view.

Results: All *P. aeruginosa* strains anaerobically grew well using the tested substrates. But all *P. aeruginosa* strains anaerobically produced rhamnolipids only using substrates containing glycerol and nitrate. Fourier transform infrared (FTIR) spectra analysis confirmed the anaerobic production of rhamnolipids from all *P. aeruginosa* strains. All the anaerobically produced rhamnolipids decreased air-water surface tension from 72.6 mN/m to below 29.0 mN/m and emulsified crude oil with EI_{24} above 65%. Using crude glycerol as low-cost substrate, all *P. aeruginosa* strains can anaerobically grow and produce rhamnolipids to reduce the culture surface tension below 35 mN/m. The glycerol metabolic intermediate, 1, 2-propylene glycol, can also achieve the anaerobic production of rhamnolipids by all *P. aeruginosa* strains.

Conclusions: Not the specificity of the rare *P. aeruginosa* strains but the effect of specific substrates promote the anaerobic biosynthesis of rhamnolipids by *P. aeruginosa*. Glycerol and nitrate are the excellent substrates for anaerobic production of rhamnolipids from all *P. aeruginosa* strains. Results indicated that glycerol metabolism involveed the anaerobic biosynthesis of rhamnolipids in *P. aeruginosa*. Results also showed the feasibility of using crude glycerol as low cost substrate to anaerobically biosynthesize rhamnolipids by *P. aeruginosa*.

Background

Rhamnolipids is the most widely studied and applied biosurfactants [1-3]. Due to its relatively high yield and good activity, rhamnolipids is promising in enhanced oil recovery, environmental bioremediation, agriculture, food, cosmetic, and other fields [3-7].

The anaerobic biosynthesis of rhamnolipids has important research and practical significance, such as meeting the in situ production of biosurfactant in anoxic environments and the foamless fermentation of biosurfactants [8]. Aerobic production of rhamnolipids fails to meet the in situ applications in the anoxic environments, such as deep soil, oil reservoirs, sediments [8-10]. The foam problem in biosurfactant fermentation has been perplexing the production of biosurfactants [11]. Foam is formed by biosurfactant enfolding air. Currently, the foam problem was solved by adding defoamer or collecting the produced foam, which also increased the risk of bacterial contamination [11,12] Biosurfactants fermentation

without aeration can avoid the foam problem. These breakthroughs require to further research the anaerobic synthesis of rhamnolipids, including the involved strain and culture medium.

Although the mainly rhamnolipids producers, *Pseudomonas aeruginosa*, are facultative bacteria [8,13,14], only few studies have reported the anaerobic biosynthesis of rhamnolipids by rare *P. aeruginosa* strains [9,10,15,16]. *P. aeruginosa* can grow and metabolize at both aerobic and anaerobic conditions. But studies on production of rhamnolipids by *P. aeruginosa* were almost performed under aerobic conditions. Few studies reported that rare *P. aeruginosa* strains can anaerobically produce rhamnolipids [8,17].

What did promote the anaerobic biosynthesis of rhamnolipids, the specificity of the rare strains or the effect of nutritional conditions? In previous studies, using facultative anaerobic and denitrifying bacterial strain *P. stutzeri* DQ1, the constructed engineering bacterial strain *P. stutzeri* Rh1 achieved heterologous production of *P. aeruginosa* rhamnolipids under anaerobic conditions [18]. A wild-type *P. aeruginosa* SG that can anaerobically produce rhamnolipids was subsequently screened from production water of Xinjiang oil reservoir [9]. During the process of medium optimization, it was found that strain *P. aeruginosa* SG could anaerobically grew well using glucose and glycerol, but strain SG anaerobically produced rhamnolipids using glycerol not glucose [19]. Whether the anaerobic synthesis of rhamnolipids is caused by the strain specificity of *P. aeruginosa* SG or the promoting effect of glycerol and nitrate? This study aims to analyze this problem by assessing the anaerobic production of rhamnolipids using different *P. aeruginosa* strains fed with diverse substrates. Results will deepen the biosynthesis theory of rhamnolipids and guide the process for anaerobic production of rhamnolipids.

In the present study, different *P. aeruginosa* strains were used. Diverse carbon sources and nitrogen sources were tested for anaerobic production of rhamnolipids by *P. aeruginosa* strains. Anaerobically produced rhamnolipids was confirmed by Fourier transform infrared (FTIR) spectra analysis. The physicochemical properties of rhamnolipids products were also evaluated. The feasibility of using crude glycerol as low cost substrate to anaerobically biosynthesize rhamnolipids by *P. aeruginosa* was studied. Prospects and bottlenecks existing in the anaerobic biosynthesis of rhamnolipids were also discussed. Results will guide to enhance anaerobic production of *P. aeruginosa* rhamnolipids by culture conditions development or strain improvement.

Materials And Methods

Strains and culture conditions

In this study, three *P. aeruginosa* strains (SG, L6-1 and FA1) isolated from different sources were investigated for rhamnolipids production [9]. *P. aeruginosa* SG was used as positive control for anaerobic production of rhamnolipids [9]. LB medium was used to prepare seed culture at 35°C and 180 rpm. For anaerobic production of rhamnolipids, three *P. aeruginosa* strains were cultured in 100 ml serum bottles containing 80 ml anaerobic medium with inoculum amount of 3%, respectively. Briefly, the anaerobic medium was prepared as follows. The medium was boiled for 15 min, and then 99.99% purity of N₂ gas

was injected into the boiling medium for 5 min. Then medium was sub-packaged in serum bottles when it was hot. Press the rubber plug and press the aluminum cap under N₂ gas protection. After sterilization and cooling to 30°C, deoxidizer Na₂S·9H₂O was added into the medium in serum bottles to a final concentration of 0.02% (w/v) [18]. The anaerobic fermentation experiments were performed at 35°C and 50 rpm for 10 days. Three parallel experiments were set for each bacterial strain. The non-inoculated medium was used as the negative control. The medium inoculated with strains *P. aeruginosa* SG was used as positive control.

Analytical methods

Culture samples were taken from serum bottles using sterile syringes. Bacterial strains biomass was represented by OD₆₀₀ values of the culture samples. Culture samples were centrifuged at 10,000g for 10 minutes. The surface tension of supernatant was measured by surface tensiometer (BZY-1, Shanghai Hengping Instrument and Meter Factory, Shanghai, China). The diameter of oil spreading circle formed by culture supernatant was determined as previous reported [20]. The crude oil (7-Middle area, Xinjiang oilfield, China) was used. Emulsification index (EI₂₄) was measured to evaluate the emulsifying activity of produced rhamnolipids products as previous study described [18]. EI₂₄ values were calculated as the height of the emulsified layer (mm) divided by the total height of the liquid column (mm) and multiplied by 100.

***P. aeruginosa* strains fed with different carbon sources**

The common substrates, glucose, sucrose, molasses, soybean oil, palmitic acid, glycerol and starch, were tested as carbon sources for the anaerobic production of rhamnolipids by three *P. aeruginosa* strains. Strains *P. aeruginosa* SG was used as positive control for anaerobic production of rhamnolipids. The concentrations of carbon sources in the medium were all 40 g/l. The medium except carbon source contained 4 g/l of NaNO₃, 4 g/l of K₂HPO₄·3H₂O, 3 g/l of KH₂PO₄, 1.0 g/l of MgSO₄·7H₂O, 0.5 g/l of KCl, 0.5 g/l of NaCl, 0.2 g/l of CaCl₂·2H₂O and 5 ml of trace elements solution. The trace elements solution contained 0.25 g/l of CuSO₄·5H₂O, 0.24 g/l of CaCl₂·4H₂O, 0.29 g/l of ZnSO₄·7H₂O, 0.17 g/l of MnSO₄·H₂O. After the anaerobic fermentation process, the OD₆₀₀, surface tension and oil spreading activity of culture samples were analyzed. Anaerobic production of rhamnolipids by strains was defined as that the surface tension of anaerobic culture was reduced to lower than 40 mN/m and the oil spreading circles diameters formed by anaerobic culture were greater than 10 mm.

***P. aeruginosa* strains fed with possible metabolic intermediates and analogues of glycerol**

The possible metabolic intermediates and analogues of glycerol (hydroxyacetone, 1, 2-propylene glycol and 1, 3-propylene glycol) were also evaluated for the anaerobic production of rhamnolipids by three *P. aeruginosa* strains. After the anaerobic fermentation process, the OD₆₀₀, surface tension and oil spreading activity of culture samples were analyzed.

***P. aeruginosa* strains fed with different nitrogen sources**

The common nitrogen nutrients, NH_4Cl , NaNO_3 , yeast extract and peptone, were tested as nitrogen sources for the anaerobic production of rhamnolipids by three *P. aeruginosa* strains. Strains *P. aeruginosa* SG was used as positive control for anaerobic production of rhamnolipids. The concentrations of nitrogen sources in the medium were all 4 g/L. The medium except nitrogen source contained 40 g/l of glycerol, 4 g/l of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 3 g/l of KH_2PO_4 , 1.0 g/l of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l of KCl, 0.5 g/l of NaCl, 0.2 g/l of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 5 ml of trace elements solution. After the anaerobic fermentation process, the OD_{600} , surface tension and oil spreading activity of culture samples were analyzed. Anaerobic production of rhamnolipids by strains was defined as that the surface tension of anaerobic culture was reduced to lower than 40 mN/m and the oil spreading circles diameters formed by anaerobic culture were greater than 10 mm.

***P. aeruginosa* strains fed with different the complex nitrogen sources**

The complex nitrogen sources of NaNO_3 and yeast extract (3:7, 5:5, 7:3) were also tested for the anaerobic production of rhamnolipids by three *P. aeruginosa* strains. The amounts of complex nitrogen sources of NaNO_3 and yeast extract was 4 g/l. After the anaerobic fermentation process, the OD_{600} , surface tension and oil spreading activity of culture samples were analyzed.

Rhamnolipids extraction from the anaerobic culture

The anaerobically produced rhamnolipids were extracted as previous described [10]. Briefly, the cell free anaerobic culture broth was heated at 80 °C for 30 min to denature the extracellular proteins. Samples were centrifuged at 10,000g for 10 minutes. Using 2 mol/l of HCl-water solution, the pH value was adjusted to 2.0. Chloroform and methanol organic solvent (v/v, 2:1) was used for rhamnolipids extraction. The rhamnolipids products were extracted from the organic phase using a rotary evaporator (50 rpm, 45 °C).

FTIR spectra analysis

Anaerobically produced rhamnolipids was confirmed by FTIR spectra analysis using rhamnolipids produced by strain *P. aeruginosa* SG as positive control. Briefly, 10 mg of rhamnolipids extract and 90 mg of KBr was mixed to make the translucent pellet with pressure of 25 Mpa for 25 s. A NICOLET 380 FTIR spectrometer was used to record the FTIR spectra of the translucent pellet with the wave number from 400 cm^{-1} to 4000 cm^{-1} [10,18].

***P. aeruginosa* strains fed with crude glycerol and nitrate**

To further reduce the medium cost, crude glycerol instead of glycerol was assessed for the anaerobic production of rhamnolipids by three *P. aeruginosa* strains. The concentration of crude glycerol in the medium was 40 g/L. The medium except crude glycerol contained 4 g/l of NaNO_3 , 4 g/l of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$,

3 g/l of KH_2PO_4 , 1.0 g/l of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l of KCl, 0.5 g/l of NaCl, 0.2 g/l of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 5 ml of trace elements solution. After the anaerobic fermentation process, the OD_{600} , surface tension and oil spreading activity were analyzed.

Results And Discussion

Anaerobic production of rhamnolipids by *P. aeruginosa* strains using different carbon sources

Using the tested substrates as carbon sources, the results of biomass (OD_{600}), surface activity and oil spreading activity were shown in Fig. 1. At anaerobic conditions, three *P. aeruginosa* strains obtained the biomass with OD_{600} values between 1.80 to 4.00 using the tested substrates (Fig. 1a). Results indicated that three *P. aeruginosa* strains can grow well under anaerobic conditions using all the tested substrates. Results also confirmed that *P. aeruginosa* are facultative bacteria [8,13,14]. From the point of view of biomass, molasses, glucose and glycerol were more favorable to the anaerobic growth and reproduction of *P. aeruginosa* strains. Molasses, glucose and glycerol as water-soluble substrates may be more conducive to the rapid absorption and anaerobic metabolize by bacterial cells of *P. aeruginosa*.

As shown in Fig. 1b, the surface tension values of anaerobic culture were reduced to lower than 40 mN/m using soybean oil (from 45 mN/m to about 37 mN/m), palmitic acid (from 49 mN/m to about 37 mN/m) and glycerol (from 63 mN/m to about 32 mN/m). Using glycerol, three *P. aeruginosa* strains reduced the surface tension of anaerobic culture with the greatest decrease (49.5%) in the surface tension and the lowest surface tension values. As shown in Fig. 1c, the oil spreading circles diameters formed by anaerobic culture of three strains were greater than 10 mm using soybean oil (about 11 mm), palmitic acid (about 11 mm) and glycerol (about 20 mm). The oil spreading circles diameters formed by bacterial culture can be used to indirectly characterize the biosurfactants yield of bacterial strain [20]. Results demonstrated that three *P. aeruginosa* strains can efficiently produce biosurfactants under anaerobic conditions using glycerol as carbon sources.

Though all the tested substrates can be used as carbon sources for production of rhamnolipids by *P. aeruginosa* under aerobic conditions [21-24]. In this study, only glycerol can be used for anaerobic production of rhamnolipids by *P. aeruginosa* strains among the tested substrates. As previous studies described, strain *P. aeruginosa* SG anaerobically produced rhamnolipids using glycerol [9,19]. In this study, *P. aeruginosa* strains except strain SG also anaerobically produced rhamnolipids using glycerol as carbon source. Here, anaerobic production of rhamnolipids is not induced by the specificity of the rare *P. aeruginosa* (strain SG) but the nutrient factor of glycerol.

Anaerobic production of rhamnolipids by *P. aeruginosa* strains using different nitrogen sources

Using NH_4Cl , NaNO_3 , yeast extract and peptone as nitrogen sources, anaerobic cell growth and rhamnolipids production by *P. aeruginosa* strains were comparatively studied. The results of biomass (OD_{600}), surface activity and oil spreading activity were shown in Fig. 2. Anaerobic culture of three *P.*

aeruginosa strains obtained the biomass with OD₆₀₀ values between 2.50 to 4.50 using the inorganic nitrogen sources, organic nitrogen sources and compound nitrogen sources (Fig. 2a). Three *P. aeruginosa* strains can grow well under anaerobic conditions using different nitrogen sources. Results also confirmed that *P. aeruginosa* are facultative bacteria [13,14]. As shown in Fig. 2a, the organic nitrogen sources were more favorable to the anaerobic growth and reproduction of *P. aeruginosa* strains. The small molecular peptides contained in yeast extract and peptone may provide abundant and available nutrition for cell growth of *P. aeruginosa* [25].

As shown in Fig. 2b, the surface tension values of anaerobic culture were all reduced from 63 mN/m to lower than 40 mN/m using all tested nitrogen sources. Using NaNO₃ as nitrogen sources, three *P. aeruginosa* strains reduced the surface tension of anaerobic culture with the greatest decrease (51.3%) in the surface tension and the lowest surface tension values of about 31 mN/m. As shown in Fig. 2c, the oil spreading circles diameters formed by anaerobic culture of three strains were greater than 10 mm using all tested nitrogen sources. Using NaNO₃ as nitrogen sources, anaerobic culture of three *P. aeruginosa* strains formed oil spreading circles with diameters about 20 mm. Although three *P. aeruginosa* strains also obtained higher biomass using the complex nitrogen sources of NaNO₃ and yeast extract (Fig. 2a), the anaerobic production of rhamnolipids were relatively less (Fig. 2c). Results demonstrated that NaNO₃ was more favorable to the anaerobic production of rhamnolipids by *P. aeruginosa*. Studies also reported that organic nitrogen sources were not conducive to the synthesis of rhamnolipids [26]. Nitrate was considered to be the best nitrogen source for rhamnolipid production under aerobic conditions [27,28]. At anaerobic conditions, nitrate was not only the excellent nitrogen source for rhamnolipids production but also the good electron acceptor for anaerobic metabolism of *P. aeruginosa*. *P. aeruginosa*, as facultative bacteria, can use other electron acceptors except oxygen, such as nitrate [8,15], so *P. aeruginosa* can grow and metabolize at both aerobic and anaerobic conditions [13,14].

FTIR spectra analysis of anaerobically produced rhamnolipids using glycerol and nitrate

The anaerobically produced rhamnolipids from three *P. aeruginosa* strains using glycerol and nitrate were confirmed by FT-IR spectra, respectively. Rhamnolipids produced by strain *P. aeruginosa* SG was used as the positive control. As shown in Fig. 3, the FTIR spectra of the anaerobically produced rhamnolipids from *P. aeruginosa* strains L6-1 and FA1 were similar to that of rhamnolipids produced from strain *P. aeruginosa* SG. The FTIR spectra of the anaerobically produced rhamnolipids from three *P. aeruginosa* strains were also similar to the spectra of reported rhamnolipids [29,30]. All the FTIR spectra had the characteristic absorption bands around 2927 cm⁻¹, 2858 cm⁻¹ and 1465 cm⁻¹ causing by the C-H stretching vibrations of aliphatic groups and the absorption bands around 1735 cm⁻¹ causing by the ester groups. All the FTIR spectra also had the absorption area between 1452 cm⁻¹ and 1045 cm⁻¹ causing by the C-H and O-H vibrations that are typical vibrations for carbohydrates. Results showed that three *P. aeruginosa* strains did produce rhamnolipids under anaerobic conditions using glycerol and nitrate.

Activity of anaerobically produced rhamnolipids using glycerol and nitrate

The rhamnolipids-water solutions (200 mg/L) were prepared using the extracted rhamnolipids products. The air-water surface tension and the emulsification index (El_{24}) to crude oil was measured to evaluate their surface activity and emulsifying activity. All the anaerobically produced rhamnolipids using glycerol and nitrate decreased the air-water surface tension from 72.6 mN/m to lower than 29 mN/m. Studies reported the aerobically produced rhamnolipids can decrease the air-water surface tension to lower than 27 mN/m [31]. In this study, the anaerobically produced rhamnolipids from *P. aeruginosa* exhibited excellent surface activity as well. Good surface activity helps to change contact angle, increase wetting activity and even facilitate wetting reversal. The excellent surface activity can also improve the capillary effect and facilitate the flow of groundwater or oil in porous media. These are of great significance for enhanced oil recovery and environmental pollution remediation [32]. Anaerobically produced rhamnolipids all showed better emulsifying activity to crude oil with El_{24} values higher than 65%. Studies reported the aerobically produced rhamnolipids can emulsified crude oil with El_{24} values ranging from 53% to 90% [31,33]. In this study, the anaerobically produced rhamnolipids from *P. aeruginosa* also exhibited good emulsifying activity, which would be significant to enhanced oil recovery and pollution remediation [5,34,35]. The good emulsifying activity of biosurfactants can assist oil dispersion in oil reservoir to reduce oil viscosity and oil flow [36,37]. Besides, the excellent emulsification effect can increase the solubility of hydrophobic pollutants in the environment and increase the bioavailability of hydrophobic pollutants [34,38].

Anaerobic production of rhamnolipids by *P. aeruginosa* strains using crude glycerol

Using crude glycerol as low-cost substrate, all *P. aeruginosa* strains can grow under anaerobic conditions and obtained biomass with OD_{600} values between 2.10 to 2.50. As shown in Fig. 4, three *P. aeruginosa* strains anaerobically produced rhamnolipids to reduce the culture surface tension below 35 mN/m. Compared to using glycerol as carbon source, the diameters of oil spreading circles formed by three *P. aeruginosa* strains anaerobic culture were smaller using crude glycerol. Results showed that using crude glycerol for anaerobic production of rhamnolipids by *P. aeruginosa* is feasible. Previous studies reported aerobic production of rhamnolipids by *P. aeruginosa* using crude glycerol [39,40]. Using glycerol or crude glycerol, the rhamnolipids yields from *P. aeruginosa* were both lower under anaerobic conditions. Enhancing the anaerobic production of rhamnolipids from *P. aeruginosa* is in demand. In future, it is a good choice to develop high-yielding strains of rhamnolipids through biosynthesis pathways regulation and functional genes manipulation. Identifying the biosynthesis pathways and key genes involved in anaerobic production of rhamnolipids are of great importance

Research perspectives to the anaerobic biosynthesis mechanism of rhamnolipids

Glycerol is water soluble, easy to be absorbed and used by microorganisms, and is an excellent metabolic substrate for microorganisms. In this study, only glycerol promoted the anaerobic production of rhamnolipids by *P. aeruginosa*. But the mechanism of anaerobic biosynthesis of rhamnolipids by *P. aeruginosa* using glycerol is still unclear. In this study, from the point of substrate, the possible metabolic intermediates and analogues of glycerol (hydroxyacetone, 1, 2-propylene glycol and 1, 3-propylene glycol)

were also evaluated for the anaerobic production of rhamnolipids by *P. aeruginosa* strains. The results of OD₆₀₀, surface activity and oil spreading activity were shown in Fig. 5. *P. aeruginosa* strains obtained the biomass with OD₆₀₀ values between 2.00 to 3.00 using the possible metabolic intermediates and analogues and analogues of glycerol (Fig. 5a). Three *P. aeruginosa* strains can anaerobically grow well using the possible metabolic intermediates and analogues of glycerol. These substrates are water-soluble and easily enter into the glycolysis and gluconeogenesis pathways, which may be conducive to the rapid absorption and anaerobic metabolize by *P. aeruginosa* [41]. Among the possible metabolic intermediates and analogues of glycerol, only 1, 2-propylene glycol promoted three *P. aeruginosa* strains to produce rhamnolipids under anaerobic conditions, reducing the surface tension of anaerobic culture from 63.2 mN/m to about 31 mN/m (Fig. 5b). Using 1, 2-propylene glycol, the oil spreading circles diameters formed by anaerobic culture of three strains were about 20 mm (Fig. 5c). Results demonstrated that 1, 2-propylene glycol, similar to glycerol, can promote *P. aeruginosa* to anaerobically biosynthesize rhamnolipids.

Here, results again confirmed that anaerobic production of rhamnolipids is not induced by the specificity of the rare *P. aeruginosa* (strain SG) but the specific substrates. Glycerol and 1, 2-propylene glycol can promote *P. aeruginosa* to anaerobically biosynthesize rhamnolipids. What is the mechanism of anaerobic rhamnolipids biosynthesis by *P. aeruginosa* metabolizing glycerol and 1, 2-propylene glycol? Hauser and Karnovsky cultured *P. aeruginosa* strain using ¹⁴C-labeled glycerol- α -¹⁴C and glycerol- β -¹⁴C under aerobic conditions. And they found that the C₆ unit of rhamnose group in rhamnolipids product was directly condensed from the two molecules glycerol (C₃ unit) without carbon chain rearrangement [43]. The polyol can be oxidized into dihydroxyacetone (DHA), then DHA can be converted into dihydroxyacetone phosphate (DHAP) which enters glycolysis and gluconeogenesis pathways [41,42]. This may be why *P. aeruginosa* can metabolize glycerol and 1, 2-propylene glycol for biosynthesis the precursors of rhamnolipids, rhamnose and fatty acids. But the biosynthesis pathways and key genes involved in anaerobic biosynthesis of rhamnolipids using glycerol is still unclear. Elucidating the mechanism of anaerobic biosynthesis of rhamnolipids is helpful to the development and application of strains high producing rhamnolipids.

The anaerobic biosynthesis of rhamnolipids has important research and practical significance. Anaerobic production of rhamnolipids can meet the in situ production of biosurfactant in anoxic environments, such as deep soil, oil reservoirs, sediments [8-10]. Based on anaerobic production of rhamnolipids, fermentation without aeration can avoid the foam problem in production of rhamnolipids. However, enhancing the anaerobic production yield of rhamnolipids is critical. Future research will be concentrate on elucidating the mechanism of anaerobic biosynthesis of rhamnolipids through revealing the pathways and key genes involved using transcriptome technology.

Conclusions

Tested *P. aeruginosa* strains efficiently produced rhamnolipids under anaerobic conditions. Not the specificity of the rare *P. aeruginosa* strains but the effect of specific substrates promote the anaerobic biosynthesis of rhamnolipids by *P. aeruginosa*. Glycerol and nitrate are the excellent substrates for anaerobic production of rhamnolipids from all *P. aeruginosa* strains. Glycerol metabolism promoted the anaerobic biosynthesis of rhamnolipids in *P. aeruginosa*. Crude glycerol can be used as low-cost substrate for anaerobically producing rhamnolipids by *P. aeruginosa*. Transcriptomic analysis would facilitate to reveal the metabolic pathways and key genes involved in anaerobic production of rhamnolipids in future.

Declarations

Authors' contributions

FZ conceived and designed the study, carried out the experiments and drafted the manuscript. CG participated in analyzing the data. QFC assisted in revising the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Consent for publication

All the co-authors approved the publication of this work in Microbial Cell Factories.

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Figures

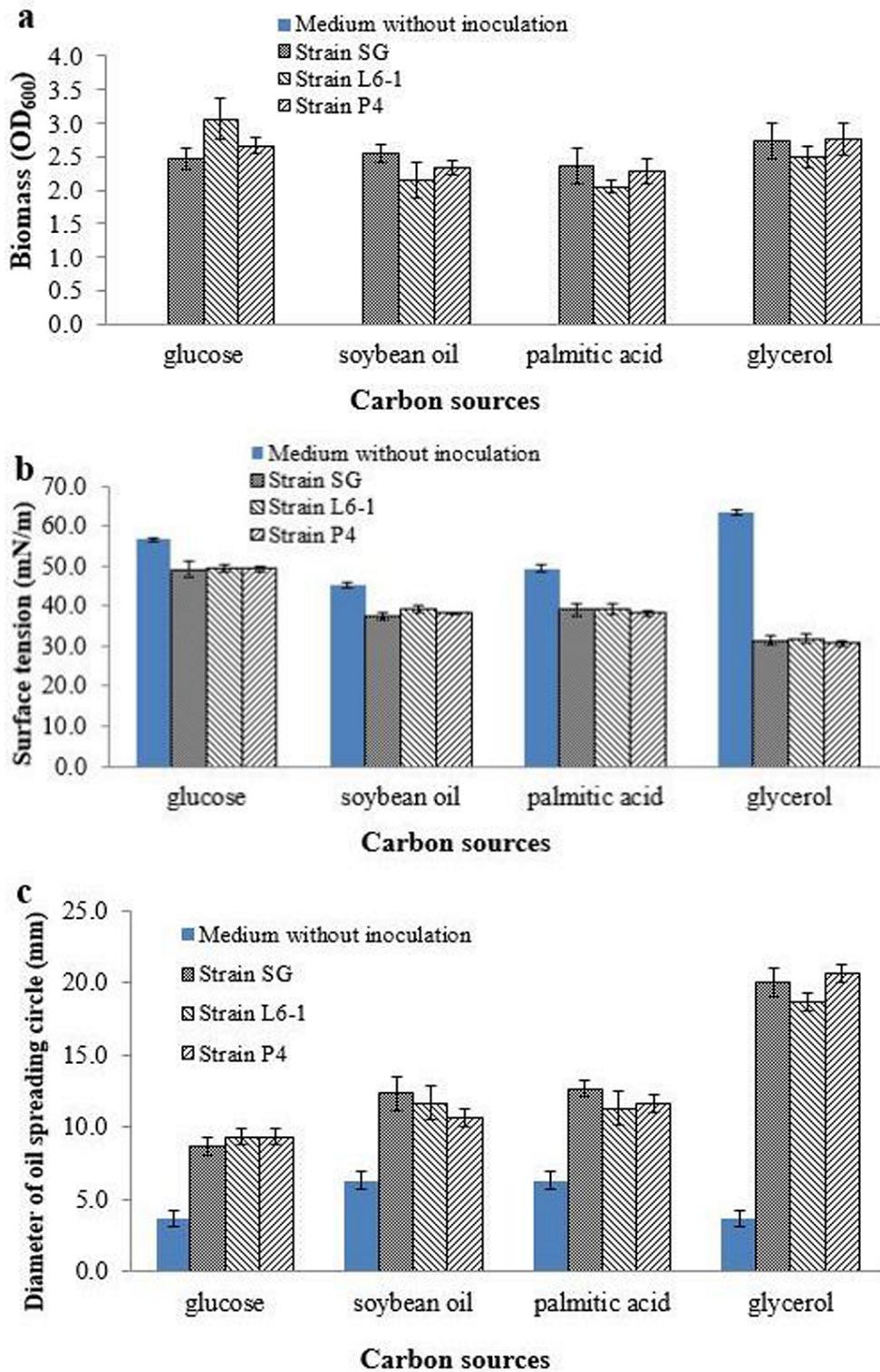


Figure 1

Anaerobic growth and production of rhamnolipids by *P. aeruginosa* strains using different carbon sources: a) biomass (OD₆₀₀), b) surface activity and c) oil spreading activity. Strain *P. aeruginosa* SG was used as positive control.

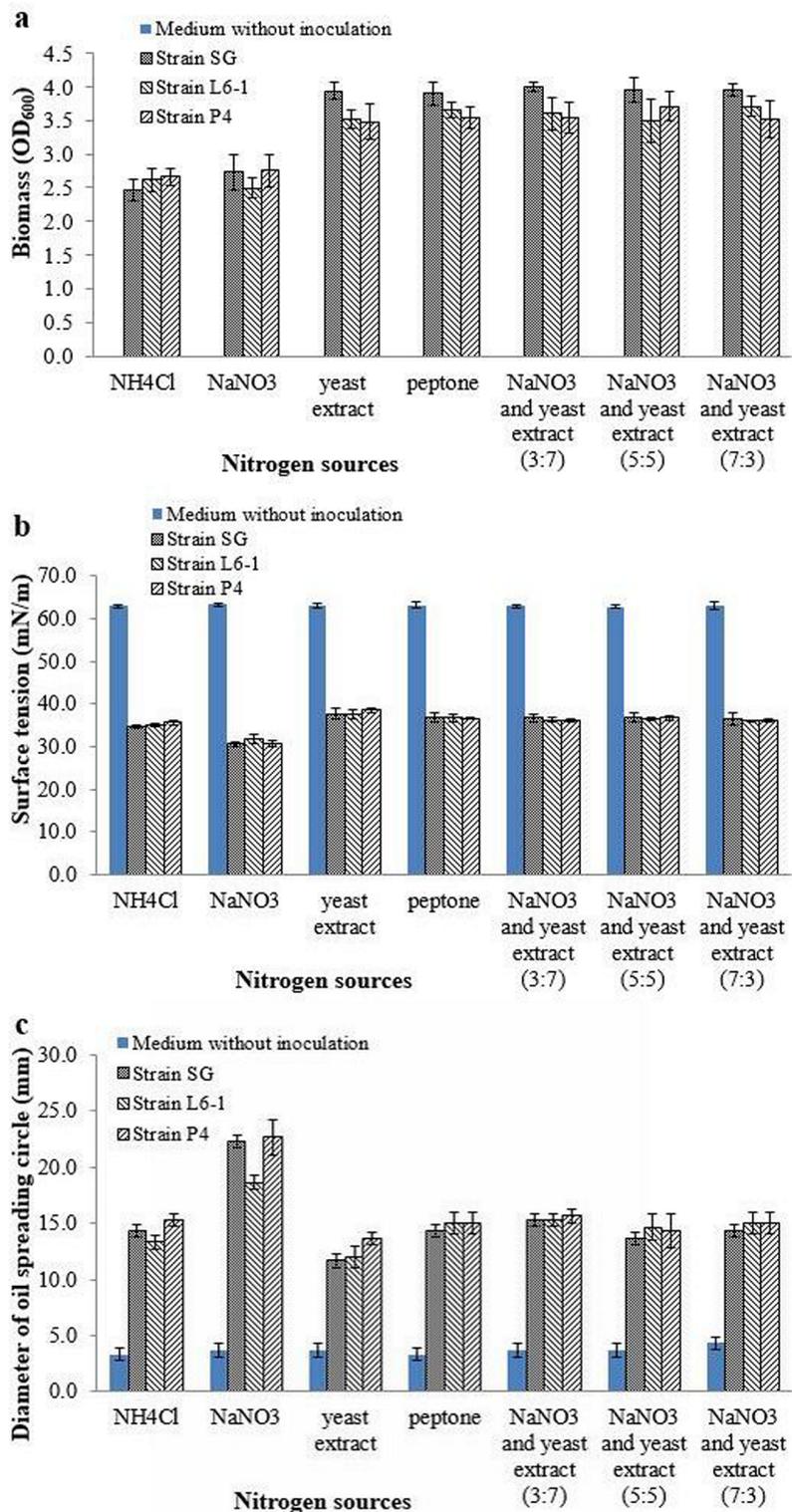


Figure 2

Anaerobic growth and production of rhamnolipids by *P. aeruginosa* strains using different nitrogen sources: a) biomass (OD₆₀₀), b) surface activity and c) oil spreading activity. Strain *P. aeruginosa* SG was used as positive control.

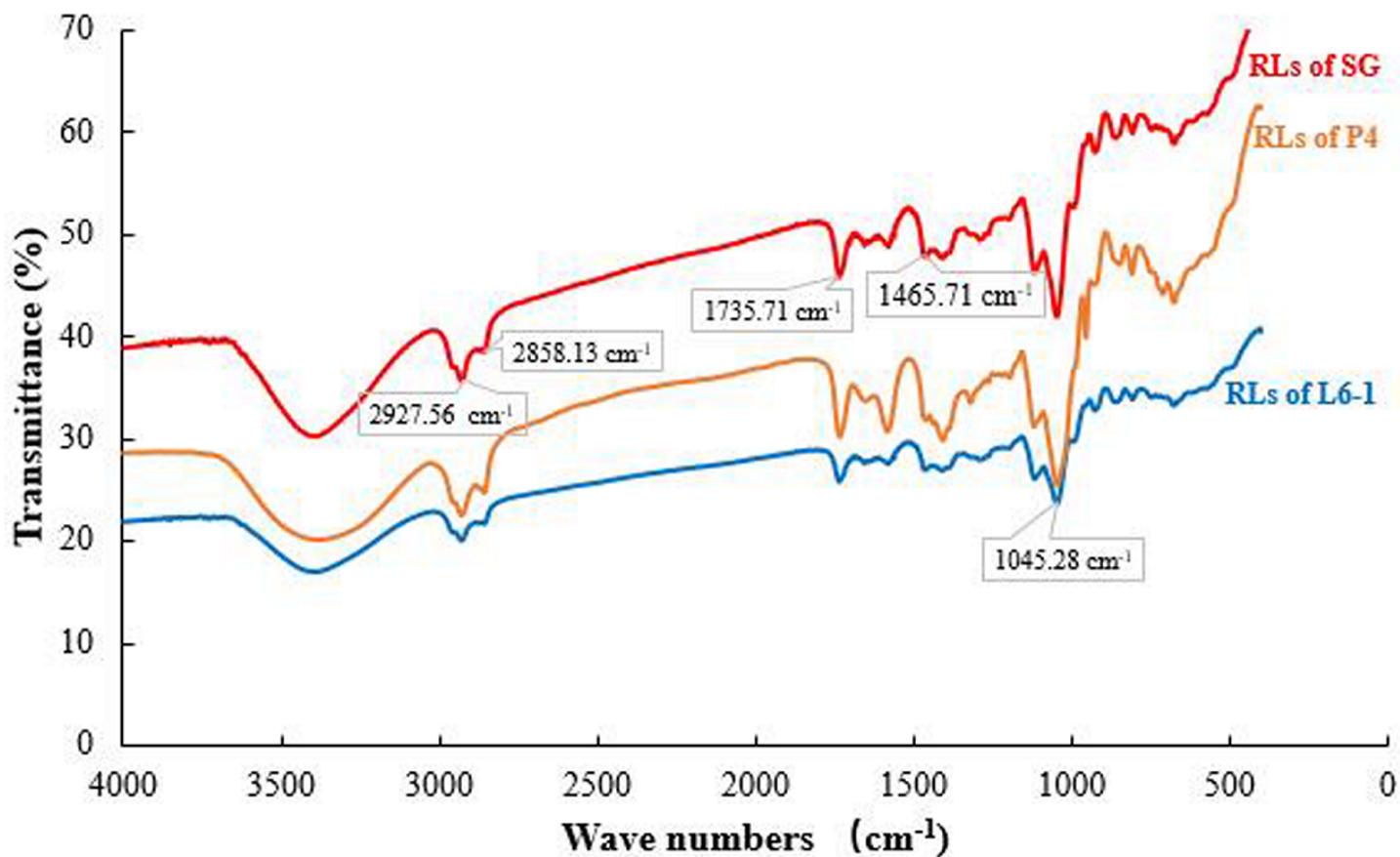


Figure 3

Fourier Transform infrared (FTIR) spectra analysis of anaerobically produced rhamnolipids from *P. aeruginosa* strains using glycerol and nitrate. Anaerobically produced rhamnolipids by strain SG was used as control.

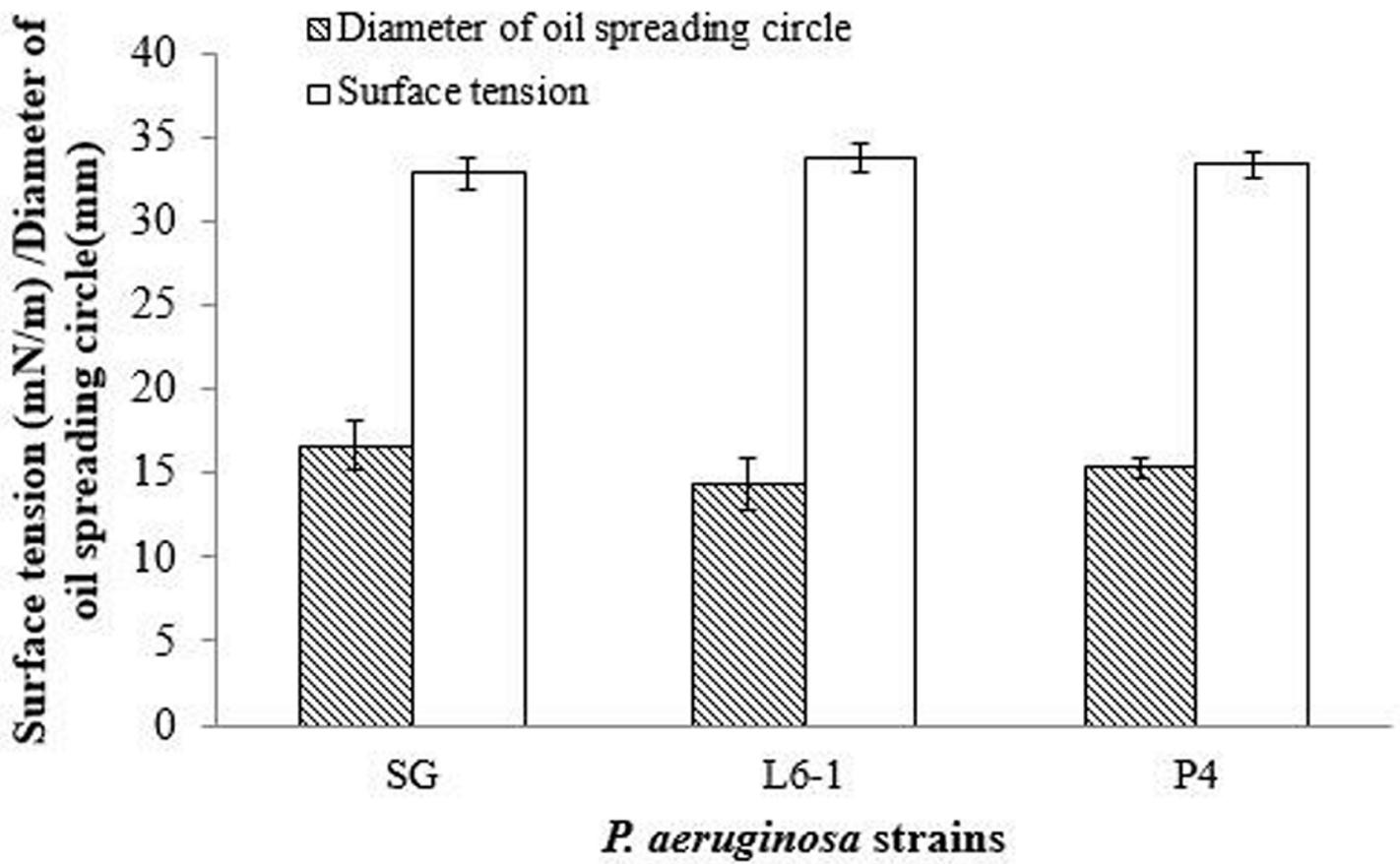


Figure 4

Anaerobic production of rhamnolipids by *P. aeruginosa* strains using crude glycerol.

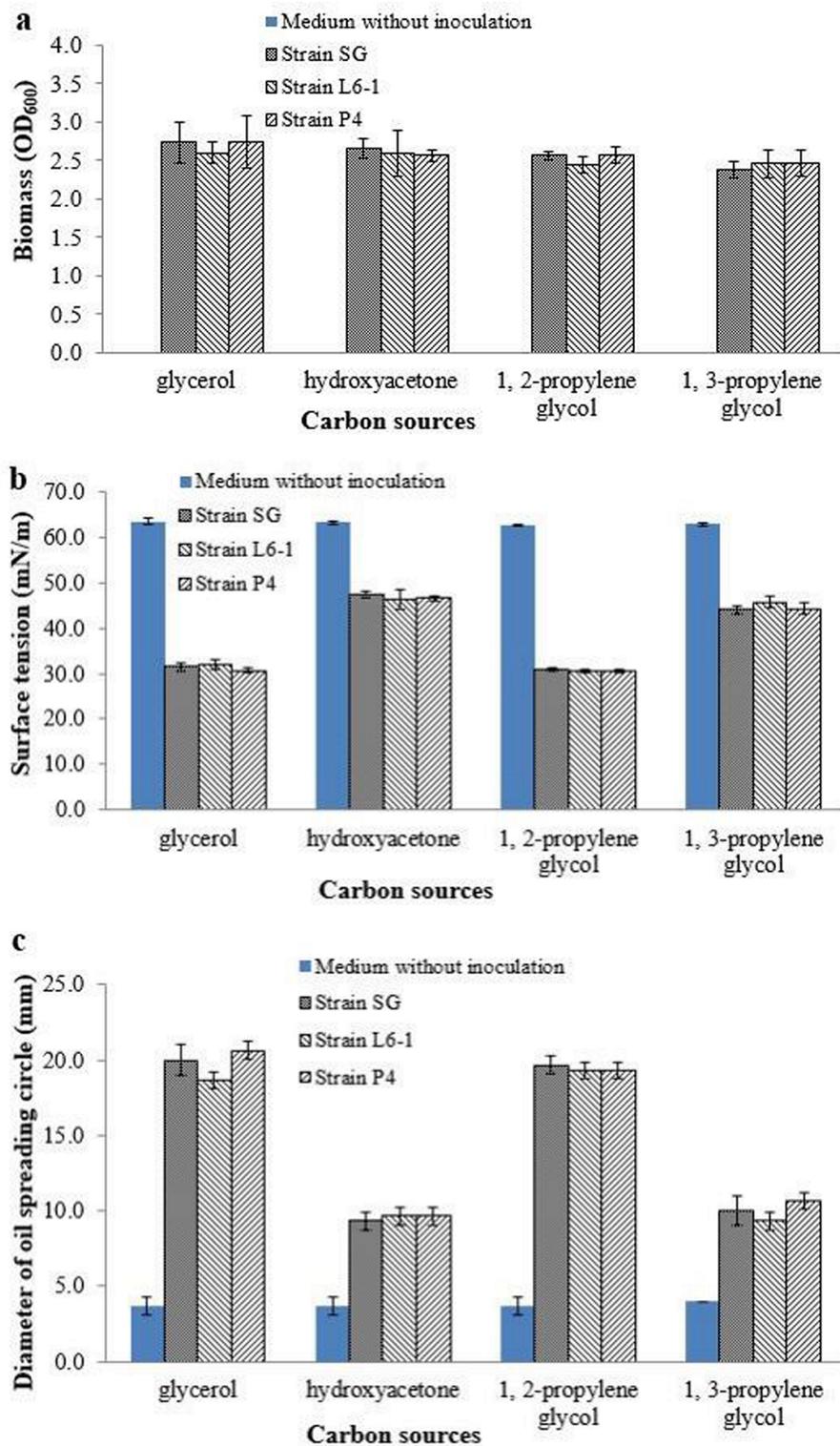


Figure 5

Anaerobic growth and production of rhamnolipids by three *P. aeruginosa* strains using the possible metabolic intermediates and analogues of glycerol as carbon sources: a) biomass (OD₆₀₀), b) surface activity and c) oil spreading activity. Glycerol was used as positive control.