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RESEARCH ARTICLE

Distributions of phospholipid and glycolipid fatty acids in two strains of different functional *Erythrobacter* sp. isolated from South China Sea

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Abstract The comparison of the fatty acids between aerobic anoxygenic phototrophic bacteria (AAPB) and their phylogenetic relatives has been a fascinating but yet enigmatic topic, enhancing our understanding of physiological variations between these evolutionarily related microorganisms. Two strains of marine bacteria, both phylogenetically falling into *Erythrobacter* sp., were isolated from the South China Sea, and demonstrated, respectively, to be an aerobic anoxygenic phototrophic bacteria (AAPB) (JL475) which is capable of anoxygenic photosynthesis via BChl *a*, and an obligate heterotroph (JL316) with a lack of BChl *a*, on the basis of phylogenetic analysis and pure culture cultivation. Phospholipid fatty acids (PLFA) and glycolipid fatty acids (GLFA) of the two strains were extracted and analyzed by gas chromatography-mass spectrometry. The PLFA in JL475 AAPB are characterized by $C_{18:1}$, $C_{18:2\omega7,13}$ and $C_{18:0}$, with the $C_{18:2\omega7,13}$ being a specific compound for AAPB and in particular for *Erythrobacter longus* and some of its phylogenetically closely related relatives. The JL316 strain is characterized in PLFA by the presence of $C_{18:1}$, $C_{16:1}$ and $C_{16:0}$, and in particular $C_{17:1}$. GLFA do not show any discrimination between the two strains. Four α,ω-dicarboxylic acids, including 1,8-octanedioic acid, 1,9-nonanedioic acid, 1,10-decanedioic acid and 1,11-undecanedioic acid, are present only in JL316 GLFA, presumably derived from metabolic products. C_{14}–C_{16} 2-hydroxy fatty acids were found in the two strains, probably assuming a similar function of their LPS in outer membranes.

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1 Introduction

Aerobic anoxygenic phototrophic bacteria (AAPB), a functional group of marine bacteria, have been receiving great attention due to their important roles in marine carbon cycle and in the evolution of microbial photosynthesis. To date, most AAPB have been mainly isolated from the eutrophic zone and fall into the category of obligately aerobic gram-negative bacteria (Yurkov and Beatty, 1998a). Bacteriochlorophyll *a* (BChl *a*) has been found to be the only bacteriochlorophyll detected in AAPB, and is often used as a tool to estimate the relative abundance of AAPB in phototrophs inhabiting eutrophic zones (Kolber et al., 2001; Schwalbach and Fuhrman, 2005; Cottrel et al., 2006). The diversity of AAPB in typical environments could also be obtained by *puf*M gene analysis (Jiao et al., 2007). By an integrated measurement on the water column distribution of BChl *a*, infrared fluorescent cells and different fluorescence signal at 880 nm wavelength, Kolber et al. (2001) proposed that AAPB might account for more than 11% of the microbial abundance in the Northeastern Pacific Ocean. However, cyanobacterial contribution will affect the estimation of AAPB abundance. Jiao et al. (2007) indicated that AAPB have an extensive distribution in global oceans with the relative abundance being the highest ($3.79 \pm 1.72\%$) in the Indian Ocean, the second ($1.57 \pm 0.68\%$) in the Atlantic Ocean and the lowest in the Pacific Ocean ($1.08 \pm 0.74\%$).

Along with the wide distribution and the high abundance in global oceans, AAPB are often considered as a probable evolutionary link between purple photosynthetic bacteria and aerobic heterotrophs, and may play an irreplaceable

part in the early evolution of photosynthetic bacteria (Shimada, 1995; Kolber et al., 2000). At present, the isolated AAPB have been taxonomically assigned into two marine genera *Erythrobacter and Roseobacter*, and six freshwater genera *Erythromicrobium, Roseococcus, Porphyrobacter, Acidiphilium, Erythromonas* and *Sandaracinobacter* (Yurkov and Beatty, 1998a). However, not all species are capable of photosynthesis via BChl *a*, and an increasing number of non-BChl *a*-producing species in *Erythrobacter* genus have been found during taxonomic surveys on microbial populations in open seas (Ivanova et al., 2005). As these non-BChl *a*-producing bacteria are phylogenetically related but phenotypically distinctive to AAPB, these two groups of bacteria might display an evolutionary direction from BChl *a*-producing aerobic anoxygenic phototrophs to obligately aerobic heterotrophs without BChl *a*. Throughout this possible evolution, there were some adaptations ranging from a permanent loss of photosynthetic abilities to the development of a regulatory mechanism, which controls the expression level of photosynthetic apparatus in response to nutrient change (Kolber et al., 2001).

Most AAPB are known to proliferate in low temperature and light-saturated environments including the surface water of estuaries (Waidner and Kirchman, 2007), surface seawater, seaweed, beach sand (Shiba et al., 1979) and sediments in the bay (Yorkov and Beatty, 1998a). Nevertheless, a strain isolated from black smoker plume waters in Juan de Fuca Ridge implies that the photosynthesis may have originated at deep-sea hydrothermal vents, and then dispersed to favorable refuges in shallow-water habitats (Yurkov and Beatty, 1998b). In addition, Hiraishi et al. (2000) got four strains of aerobic, mesophilic, acidophilic and BChl *a*-containing bacteria from acidic hot springs and mine drainage. These novel genera supplement our understanding about anoxygenic phototrophs by extending their habitats from eutrophic zones in water columns to extreme environments where most phototrophs can hardly survive.

Pioneering studies were mostly concerned with the distribution, abundance, physiology and bacteriochlorophyll of AAPB in a variety of environments (Shiba et al., 1991; Béjà et al., 2002; Yurkova et al., 2002; Rathgeber et al., 2004; Schwalbach and Fuhrman, 2005; Jiao et al., 2007; Yutin et al., 2007). The distinctive fatty acids were also explored among different species (Koblížek et al., 2003; Yoon et al., 2004; Rontani et al., 2005). However, no comparison in phospholipid fatty acids (PLFA) has been made between BChl *a*-containing AAPB and its phylogenetically related bacteria lacking of BChl *a*. Here, we purely cultured two strains of phylogenetically closely related bacteria isolated from South China Sea to elucidate the difference in PLFA and glycolipid fatty acids (GLFA) between them, and to further link fatty acids to physiological characteristics.

2 Materials and experimental methods

2.1 Bacterial isolation and culture

The two strains of bacteria investigated herein were isolated from the South China Sea, and sequentially chosen for pure culture under the same experimental conditions. They were both inoculated into the seawater medium (RO medium: yeast extract, 1.0 g/L; tryptone, 1.0 g/L; sodium acetate, 1.0 g/L; vitamin B_{12}, 20 μg/L; trace element solution 1 ml/L and artificial sea water added with 30 mg/L streptomycin) which was filtered through 0.2 μm membrane prior to use. After three days of incubation, and being extremely exposed to aerobic conditions with dark-light cycles of 12/12 h at pH = 7.8 and 25°C, cells were harvested in a stationary phase by centrifugation at 8 000 r/min for 15 min, rinsed with nanopure water twice, and dried in an oven at 45°C for one night. The dry samples were lyophilized and stored in a refrigerator until lipid extraction.

2.2 Strain assignment

The two isolated strains, JL475 and JL316 (Table 1), both fall into α-4 subclass of *Proteobacteria* and are subdivided into *Erythrobacter* sp. However, they obviously show different phenotypical characteristics. The JL475 strain can yield BChl *a* which is believed to be indispensable for anoxygenic photosynthesis, whereas the other strain, JL316, could only grow chemoheterotrophically using dissolved organic matter as the main carbon source due to lack of BChl *a*. Their phenotypical difference is also in accordance with the expression of *Puf* gene found only in JL475 but not in JL316. The colony is dark red in JL475 but orange in JL316. For the above reasons, JL475 can be assigned into marine functional group AAPB, and JL316 is a non-AAPB of the same *Erythrobacter* genus.

Table 1 Sample information of the two strains

sample No.	GPS position	sampling water depth/m	seawater salinity/‰	temp./ °C	catalog		
					Puf M gene	BChl *a*	marine function group AAPB
JL475	112.00°E, 20.00°N	75	—	—	√	√	√
JL316	114.50°E, 21.50°N	surface seawater	34.38	21.18	—	—	—

Here '√' means the sample has corresponding characteristic.

2.3 Phylogenetic analysis

The 16S rRNA gene sequencing was performed on the two phylogenetically closely related bacteria. The 16S rRNA gene was amplified and sequenced as described previously (Rainey et al., 1996), and sequences were aligned using the BLAST program (NCBI) to determine the approximate phylogenetic affiliation. Sequence similarities were determined using the maximum-likelihood algorithm, and then phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei, 1987) with MEGA software (Kumar et al., 2004) as shown in Fig. 1. Phylogenetic analysis shows that 16S rRNA gene sequences of the two strains fall within *Erythrobacter* genus, sharing 98.1% similarities. JL475 sequences show 96% similarities with *Erythrobacter vulgaris*, and JL316 were 99.6% similar to its closest phylogenetic relative, *Erythrobacter citreus*. As the two bacteria are phylogenetically related but capable of different anabolism, they are likely to show a linkage in the evolution from anoxygenic phototrophs to obligate heterotrophs. Accordingly, the two phylogenetically related but phenotypically disparate bacteria may provide excellent materials to decipher environmental influence on microbial evolution from anoxygenic phototrophs to heterotrophs.

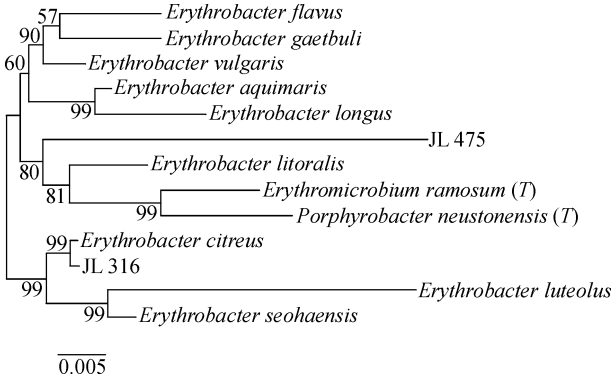

Fig. 1 Phylogenetic positions of JL475 and JL316 based on 16S rRNA analysis. The scale bar represents 0.5 substitution per 100 nucleotide positions

2.4 Lipid extraction and fatty acids identification

Extraction of PLFA and GLFA mainly follows the modified method of Bligh/Dyer (Bligh and Dyer, 1959; White et al., 1979). Five milligrams of lyophilized cells was extracted ultrasonically with chloroform/phosphate buffer/methanol (1∶0.8∶2, v/v/v). Then 5 ml deionized water and 5 ml chloroform were added to the total extracts, and the bottom layer was collected after vortex and kept still overnight. Neutral and polar lipids in the collected layer were separated by sequential eluting with acetone and methanol through a silicic gel column. The dried polar fractions were redissolved in toluene/methanol (1∶1, v/v), subjected to mild alkaline methanolysis to cleave fatty acids from the phospholipid glycerol backbone, and esterified in water bath at a temperature lower than 60°C for at least 30 min. Two ml of hexane was pipetted into the solution which was then neutralized with about 200 μL 1 N acetic acid. Deionized water was added to form two phases, and the upper organic phase was collected and dried under nitrogen gas. Ethylated esterification was performed on neutral lipids, and GLFA ethyl esters were also dried under nitrogen gas flow. A control sample without any bacterial cells was simultaneously conducted to monitor the possible laboratory contamination.

PLFA and GLFA analyses were performed on a HP6890^{plus} gas chromatograph (GC), equipped with an HP-5MS fused silica capillary column (30 m length, 0.25 mm bore, 0.25 μm film thickness), which was interfaced directly with an HP 5973 mass spectrometer (MS). The operating conditions were as follows. Temperature was ramped from 70°C to 280°C at 3°C/min, held at 280°C for 20 min with the injector at 300°C.Helium was used as the carrier gas. The ionization energy of mass spectrometer was fixed at 70 eV with GC and MS interface temperature at 280°C. The compounds were identified based on their mass spectra and literatures, and then quantified by the peak areas.

3 Results and discussion

3.1 Phospholipids fatty acids

Microbial cytomembranes usually function as a fundamental structure for cell protection and an important route for the substance exchange between intra- and extracellular environments. However, considerable difference in PLFA profiles of the membranes among specific groups of microorganisms may exist, implying that it's possible to classify microorganisms in terms of the structure diversity of the PLFA related to biological specificity (Zelles, 1999). To date, microbial PLFA have extensively been applied to bacterial taxonomy, the approximate identification of microbial communities and the estimation of biomass in diverse geological settings (Frostegård et al., 1993; Rajendran et al., 1994; Zink et al., 2003), due to the difference in PLFA correlated with the functional distinction of cell membranes. Some indices linked to unsaturated and cyclopropyl fatty acids, e.g. the *trans*/*cis* ratio of two isomeric monoenoic fatty acids with the double bond at the same carbon position, were established to indicate the microbial physiological conditions such as starvation due to the substrate shortage and stress (Guckert et al., 1986; Kieft et al., 1994; Uhlířová et al., 2005).

JL475 and JL316 in our experiments exhibit some difference in PLFA profiles (Table 2). The diversities of PLFA in JL475, the AAPB strain, are greater than those in JL316 lacking BChl *a*. Saturated fatty acids ranging from C_{15} to C_{18} were all identified in JL475 but were below the

detection limit in JL316 (with only a small amount of palmitic acid identified), though the unsaturated fatty acids (UFAs) prevail in both strains. The total amount of UFAs can account for up to 73.37% in JL475 PLFA and even soaring up to 94.2% in JL316 PLFA, collectively indicating that UFAs are the major PLFA in the two strains. Branched fatty acids and cyclopropyl fatty acids in JL475 were found to include $iC_{15:0}$, $iC_{16:1}$, $iC_{17:0}$ and $cyC_{19:0}$, with a relative abundance of 9.32%.

Noticeably, two UFAs, i.e. $C_{18:1}$ and $C_{18:2\omega7,13}$, can comprise approximately 47.84% and 21.59% of the total PLFA in JL475, agreeing well with previously reported PLFA in other species of *Erythrobacter*, in which $C_{18:1}$ is demonstrated to exceed in abundance over $C_{18:2}$ or $C_{16:0}$ (Fuerst et al., 1993; Koblížek et al., 2003; Rontani et al., 2005). Most isolated and identified AAPB were considered to be capable of yielding two kinds of $C_{18:2}$ isomers. Rontani et al. (2005) found that $C_{18:2\omega7,13}$ was in significant concentration among fatty acids from four of five strains of *Erythrobacter longus*, and was associated with the low abundance of $C_{18:2\omega6,9}$ in all samples, the latter of which, however, was proposed to be a possible laboratory contaminant. Nonetheless, apparent $C_{18:2\omega6,9}$ was identified in *Erythrobacter* sp. isolated from surface sea water by Koblížek et al. (2003). The two UFAs, $C_{18:2\omega7,13}$ and $C_{18:2\omega6,9}$, were identified in JL475 but absent in both JL316 and the control in our experiments, suggesting that these two UFAs identified in our samples would not be the laboratory contaminants.

It was proposed that polyunsaturated fatty acids (PUFAs), such as $C_{18:2}$, could only come from oxygenic phototrophs and phototrophic eukaryotes but not from anoxygenic phototrophs (Keyon, 1978). Later on, however, $C_{18:2}$ was found in the pure culture of cyanobacterium *Phormidium luridum* at 24°C (Summons et al., 1996) and in fungi (Stahl and Klug, 1996; Bardgett et al., 1996). Nevertheless, the $C_{18:2}$ identified in cyanobacteria and fungi are quite different in the double bond positions from those found in AAPB, indicating that $C_{18:2\omega7,13}$ might be an indication of AAPB, and could serve as the biomarker of *Erythrobacter* sp. Of all the AAPB species, $C_{18:2}$ is present only in the isolated *Erythrobacter longus* and some relatives which are phylogenetically related to this species, suggesting that $C_{18:2\omega7,13}$ may function as the indicator of *Erythrobacter longus* and its phylogenetically related relatives. This also substantiates that JL475 has a firmly phylogenetic relationship with *Erythrobacter longus* (Denner et al., 2002; Yoon et al., 2003; Rontani et al., 2005).

Table 2 PLFA and their relative abundance

sample No.	JL475			JL316		
catalog	components	rentention time/min	relative abundance/%	components	rentention time/min	relative abundance/%
saturated fatty acids	15:0	35.58	0.55	—	—	—
	16:0	38.47	12.78	16:0	39.07	4.72
	17:0	41.71	1.42	—	—	—
	18:0	44.66	0.43	—	—	—
unsaturated fatty acids	16:2	37.32	0.06	—	—	—
	16:1(a)*	37.60	1.00	—	—	—
	16:1(b)	37.84	1.17	16:1	38.10	16.43
	17:1(a)	41.00	0.58	—	—	—
	17:1(b)	41.25	0.73	17:1	41.49	6.60
	18:2ω7,13§	43.34	21.59	—	—	—
	18:2ω6,9	43.70	0.20	—	—	—
	18:1ω7	44.25	47.84	18:1ω7	44.00	71.18
	19:1	45.98	0.20	—	—	—
branched fatty acids	i15:0¶	34.47	0.21	—	—	–
	i16:1	39.93	0.13	—	—	—
	i17:0	40.52	1.66	—	—	—
cyclopropyl fatty acids	cy19:0#	44.86	9.32	—	—	—
unidentifiable compounds			0.13			1.08

*(a) and (b) in the brackets stand for isomeric compounds owing to different double bonds positions or same double bonds positions however with *trans*/*cis* isomerization.

§18 represents the total carbon number of fatty acids backbone, 2 denotes number of double bonds and 7, 13 after *omega* mean the double bonds positions relative to the terminal methyl end rather than to the carbonyl carbon.

¶ i (short for iso) denotes the methyl branch at the next to the most remote carbon center from the carbonyl carbon.

cy represents cyclopropyl fatty acids.

It is generally believed that the closest relatives of AAPB are purple non-sulfur bacteria, which are incapable of producing oxygen in photosynthesis and strictly restricted to the anoxic zones in the water columns (Yorkov and Beatty, 1998a; Kolber et al., 2001). Evidently, the evolutionary direction from purple non-sulfur bacteria to AAPB and then to heterotrophs exactly displays a trend in which photosynthetic genes were gradually lost, and increased reliance on dissolved organic carbon (Jiao et al., 2007). Here the two strains of phylogenetically closely related bacteria, JL475 and JL316, showing an evolutionary tendency from anoxygenic phototrophs to obligate heterotrophs, provide wonderful materials for us to learn more about this evolution. Even though lipids can only exhibit phenotypical characteristics and never replace genes or DNA as proxies of the evolution, their biosynthesis is strongly relevant to the metabolic pathways which require the requisite enzymes. JL475 shows the competence of biosynthesizing $C_{18:2}$ and thus distinguishes itself from JL316, indicating that the two bacteria may use different metabolic pathways in spite of both growing in the same culture media and environments. JL475 shows some similarities in the major PLFA with the purple non-sulfur bacteria cultivated by Kompantseva et al. (2007); they are both dominated by $C_{18:1}$ and $C_{16:0}$, but with a bit of difference being the presence of abundant UFAs $C_{18:2}$ in JL475 AAPB. The PLFA in JL316 mainly consist of $C_{18:1}$, $C_{16:1}$, $C_{17:1}$ and $C_{16:0,}$ characterized by the dominance of unsaturated fatty acids, in particular $C_{17:1}$ accounting for 6.6% of the total PLFA. Such kind of distribution is comparable with those found in some species of *Erythrobacter*, e.g. *E. aquimaris* (Yoon et al., 2004) and *E. citreus* (Denner et al., 2002). These species are believed to be unable to yield BChl *a*. It appears to us that from JL475 AAPB to purple non-sulfur bacteria and then to JL316, PLFA show a loss in $C_{18:2}$ and an appearance of $C_{17:1}$.

3.2 Glycolipid fatty acids

3.2.1 Non-polar fatty acids

The GLFA profiles usually show almost the same distribution patterns as their PLFA profiles (Zhang et al., 2004). However, the GLFA for both JL475 and JL316 (Table 3 and Fig. 2) are notably greater in diversities than their corresponding PLFA. In particular, $C_{16:0}$ displays an increasing abundance in both GLFA profiles, quite different from its abundance in PLFA profiles. A series of saturated and branched fatty acids with relatively lower

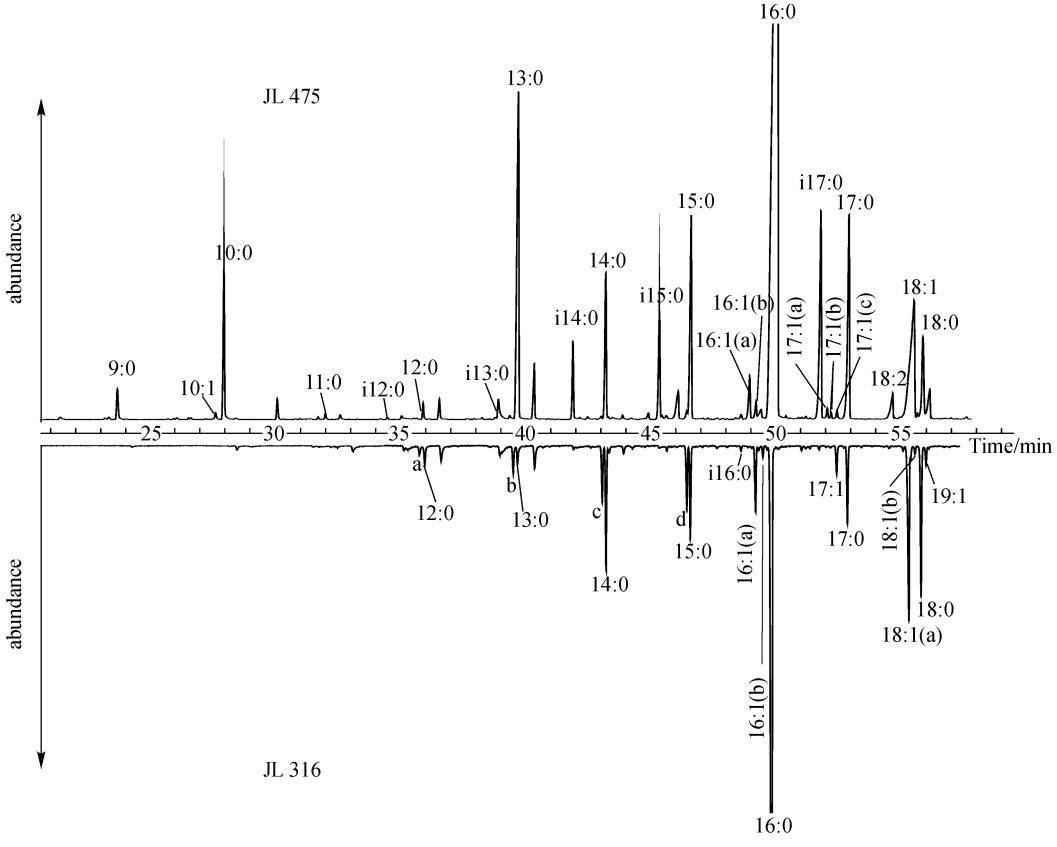

Fig. 2 Comparison of mass chromatographs of GLFA, iethyl esters between two bacteria (m/z 88). a,b,c,d deonte 1,8-octanedioic acid, 1,9-nonanedioic acid, 1,10-decanedioic acid and 1,11-undecanedioic acid, respectively

molecular weight, including $C_{9:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{13:0}$, $C_{14:0}$, $iC_{12:0}$, $iC_{13:0}$ and $iC_{14:0}$ etc., appear in the GLFA of JL475 but are below detection limit in PLFA. GLFA profile in JL316 possesses obviously increasing abundance of saturated fatty acids, and differs from those in JL475 by the absence of $C_{9:0}$, $C_{10:0}$ and $C_{11:0}$. The fatty acids in the GLFA profiles for the two bacterial strains are tremendously dissimilar with previously reported major fatty acids in the phylogenetic relatives of *Erythrobacter* sp., suggesting that GLFA may be unable to serve as the biomarkers for bacterial taxonomy.

To our surprise, four α,ω-dicarboxylic fatty acids, i.e. 1,8-octanedioic acid, 1,9-nonanedioic acid, 1,10-decanedioic acid and 1,11-undecanedioic acid, were present in the GLFA profile of JL316 but not in JL475 (Fig.2 and Table 3). These compounds, all eluting prior to the corresponding saturated fatty acids at regular intervals, have never been reported in fatty acid profiles of *Erythrobacter* sp. (Denner et al., 2002; Yoon et al., 2004, 2005; Ivanova et al., 2005). The homologous series of dicarboxylic fatty acids are characterized by significant abundance of $(M-45)^+$ fragment ion due to loss of an ethoxy moiety in their mass spectra (Fig. 3). Possible origins from laboratory contamination could be excluded in consideration of their absence in both JL475 and the control sample. Numerous biological precursors or sources of α,ω-dicarboxylic fatty acids have already been reported. The α,ω-dicarboxylic fatty acids with carbon chain length below 12 were observed in aerosols, and often proposed as the photochemical reaction products of unsaturated fatty acids (Stephanou and Stratigakis, 1993; Kawamura et al., 1996). α,ω-dicarboxylic fatty acids were also found in acid

Table 3 GLFA and their relative abundance

sample No.	JL475			JL316		
catalog	components	retention time/min	relative abundance/%	components	retention time/min	relative abundance/%
saturated fatty acids	9:0	23.66	0.69	9:0	—	—
	10:0	27.93	2.97	10:0	—	—
	11:0	31.99	0.11	11:0	—	—
	12:0	35.89	0.28	12:0	35.89	1.01
	13:0	39.71	9.26	13:0	39.61	1.00
	14:0	43.20	3.07	14:0	43.17	5.45
	15:0	46.62	4.93	15:0	46.55	4.23
	16:0	50.08	49.75	16:0	49.82	51.35
	17:0	52.93	5.34	17:0	52.85	3.89
	18:0	55.88	2.07	18:0	55.81	7.50
unsaturated fatty acids	10:1	27.59	0.13	—	—	—
	16:1(a)*	48.96	0.99	16:1(a)	49.17	3.20
	16:1(b)	49.21	0.29	16:1(b)	49.46	0.52
	17:1(a)	52.06	0.19	—	—	—
	17:1(b)	52.22	0.11	—	—	—
	17:1(c)	52.45	0.14	17:1	52.42	1.45
	18:2	54.69	0.83	—	—	—
	18:1	55.54	7.47	18:1(a)	55.32	11.51
	—	—	—	18:1(b)	55.57	0.35
	19:1	—	—	19:1	56.01	0.79
branched fatty acids	i12:0¶	34.47	0.04	—	—	—
	i13:0	38.91	0.56	—	—	—
	i14:0	41.89	1.42	—	—	—
	i15:0	45.35	2.24	—	—	—
	i16:0	—	—	i16:0	48.59	0.23%
	i17:0	51.81	5.38	—	—	—
other fatty acids			1.74	1,8-octanedioic acid	35.68	0.53
				1,9-nonanedioic acid	39.44	1.34
				1,10-decanedioic acid	43.02	2.67
				1,11-undecanedioic acid	46.41	2.98

*(a),(b) and (c) b in the brackets symbolize isomers

¶ i (short for iso) denotes the methyl branch at the next to the most remote carbon center from the carbonyl carbon

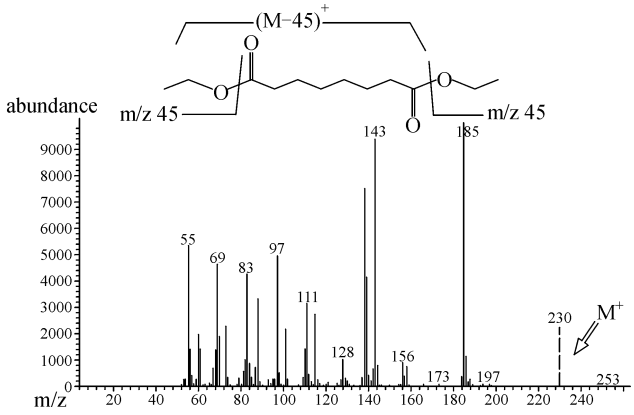

Fig. 3 Mass spectra of 1,8-octanedioic acid, diethyl ester and its fragmentation pattern

digestion products of reticular red soils (unpublished data) and metabolic intermediate products (Erb et al., 2007). Oxidation of ω-hydroxy acids by yeast and bacteria was reported to produce α,ω-diacids. However, α,ω-diacids with a long chain length could occur as polymer ester constituents of some higher plants (Lehtonen and Ketola, 1993) whereas their short-chain homologs can also be biosynthesized by seagrasses (Volkman et al., 1980). In consideration of the appearance only in JL316 in our experiments, the α,ω-dicarboxylic fatty acids might not be the oxidized products. Instead, we propose that these α,ω-dicarboxylic fatty acids might be biosynthesized as metabolic products in JL316.

3.2.2 Hydroxy fatty acids

Hydroxy fatty acids are often diagnostic for the occurrence of gram-negative bacteria in microbial community (Volkman et al., 1998). Generally, these acids serve as the constitute parts of lipopolysaccharide (LPS) of the outer membrane of gram-negative bacteria with the carbon number ranging from 10 to 20 and usually dominated by C_{14}. Higher plants may also generate C_{16} to C_{22} hydroxy fatty acids which were proposed to stem from cutin and suberin (Cardoso and Eglinton, 1983). The two strains of cultured bacteria, JL475 and JL316, both pertaining to gram-negative bacteria, also yield evident abundance of hydroxy fatty acids, including 2-hydroxy acids of C_{14}–C_{16} with C_{14} being the dominant homologue. Trace amount of $C_{16:1}$ 2-hydroxy acid was found in JL316. The distribution of hydroxy fatty acids in our samples is consistent with the previous work on five strains of AAPB in *Erythrobacter* sp. conducted by Rontani et al. (2005): they both show the main 2-hydroxy acids of C_{14}–C_{16}. Exceptionally, a small amount of C_{13} 2-hydroxy acid was detected in *Erythrobacter vulgaris* sp. nov. isolated from the marine invertebrates in South China Sea (Ivanova et al., 2005). The comparable distribution of 2-hydroxy acids in JL475 and JL316 might be related to the similar function of their LPS in the outer membrane, and it thus appears difficult to separate different strains on the basis of hydroxy acids.

4 Conclusions

Two strains (JL475 and JL316) of phylogenetically closely related bacteria, both falling within *Erythrobacter* genus, were isolated from South China Sea. The JL475 strain is demonstrated to be capable of anoxygenic photosynthesis via BChl *a* and belongs to the marine functional group AAPB, whereas the JL316 strain metabolizes in a different pathway due to lacking of BChl *a* and is an obligate heterotroph. The PLFA in JL475 AAPB are characterized by $C_{18:1}$, $C_{18:2\omega7,13}$ and $C_{18:0}$, with the $C_{18:2\omega7,13}$ being a specific compound for AAPB and in particular for *Erythrobacter longus* and some of its phylogenetically closely related relatives. The JL316 strain is characterized in PLFA by the presence of $C_{18:1}$,$C_{16:1}$ and $C_{16:0}$, and in particular $C_{17:1}$. GLFA do not show any discrimination between the two strains. Four α,ω-dicarboxylic acids, including 1,8-octanedioic acid, 1,9-nonanedioic acid, 1,10-decanedioic acid and 1,11-undecanedioic acid, are present only in JL316 GLFA, presumably derived from metabolic products. C_{14}–C_{16} 2-hydroxy fatty acids were found in the two strains, inferring the similar function of their LPS in the outer membrane.

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