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Phospholipid Fatty Acid Profiles of Several Oil-Degrading Bacteria

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The analysis of phospholipids fatty acids (PLFAs) provides us a way to distinguish microorganisms at molecular level. Four diesel-degraded bacteria strains isolated from oil-contaminated soils of Shengli oilfield at Yellow River Delta were investigated for PLFAs profiles. All fatty acids, identified by gas chromatography-mass spectrometry (GC-MS), were ranged from 12 to 19 carbon atoms. The PLFA patterns of the bacterial strains were quite different, based on visual observation of the chromatograms. The dominant PLFAs of the four strains were even-numbered saturated acids (C16:0) and monounsaturated acids. Moreover, C18:2ω6c,9c fatty acid was also major component in B3. They were all Gram-negative bacteria according to the analysis of PLFAs, it was consistent with the result of Gram stain. The polyunsaturated PLFA 18:2ω6,9 was used as a fungal biomass indicator, it was verified in this study. The content of *trans*-fatty acids in B1, B2, B4 were all greater than 40%, so we deduced the three strains belonged to aerobic prokaryotes.

Key words: PLFA; GC-MS; Oil-degraded bacteria; Biomarker.

Microbial biomarkers are chemical components of microorganisms, which can be used to describe type of microorganism. The most common biomarkers are fatty acids derived from membrane lipids. Phospholipids are major cell membrane constituents, and their fatty acyl side-chains vary in composition (i.e., length, alkyl-branches and number of double bonds) between different genera[1]. There are a great number of dissimilar fatty acids in bacterial phospholipids and some bacteria contain unique fatty acids[2]. Therefore, phospholipid fatty acids (PLFAs) are used as biomarkers to determine the genera of microorganisms.

Some bacterial groups have specific lipids that are suited as taxonomic biomarkers to detect these bacteria in environmental samples (Boschker and Middelburg, 2002). For other groups it is necessary to compare entire lipid fatty acid profiles with the profiles of reference strains in order to identify them *in situ* (Johnsen *et al*., 2002). The PLFA composition of bacterial membranes is of great value in the understanding of bacterial phylogenic and taxonomic classifications[3]. Lipid analysis of pure cultures was to compare profiles to each other or to establish database to assist in the polyphasic taxonomic description of these organisms. There are now commercial databases available based on whole-cell saponification fatty acid profiles for the taxonomic description of pure cultures of bacteria[4].

Four diesel-degraded bacteria strains isolated from oil-contaminated soils of Shengli oilfield at Yellow River Delta were chosen to investigate the PLFAs profiles. The aim of this study was to characterize the PLFA of four diesel-degraded strains and supply useful data for the taxonomic description of pure cultures of bacteria.

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MATERIALS AND METHODS

Bacterial strains

The bacterial strains were isolated from oil-contaminated soils of Shengli oilfield at Yellow River Delta (Dongying, China). They were identified as *Pseudomonas* (B1), *Alcaligenes* (B2), *Saccharomyces* (B3), *Alcaligenes* (B4) by 16S rDNA sequences.

Liquid cultures were performed in 500 ml flasks containing 300 ml of LB medium (NaCl 10 g, Tryptone 10 g, yeast extract 5 g, distilled water 1 L). All strains were grown at about 30° and harvested in late exponential growth phase. Then, they were washed and frozen-dried.

Phospholipid fatty acid extraction

The extraction procedure was modified based on the method of Frostegard[5]. In brief, a 10 mg sample of frozen-dried strains was extracted with a mixture of $CHCl_3$/ CH_3OH/ phosphate buffer (0.15 M, pH 4) (1:2:0.8, v/v/v). In order to split the phases (organic and aqueous), and then the mixture was left overnight at darkness. The lower $CHCl_3$ layer was collected and dried under N_2 for lipid fractionation. The polar lipids were separated on silica gel columns. Polar lipids were transesterified with methanolic KOH to recover the PLFAs as methyl esters through methanolysis in hexane. The hexane supernatant containing the resultant fatty acid methyl esters (FAMEs) was separated. The samples were then evaporated and dissolved by hexane with a final volume of 1.0 ml.

GC-MS analysis

The analysis of the extracts was carried out by gas chromatography coupled to mass spectrometry (GC-MS, Agilent 7890GC-5975MS) equipped with a DB-5 capillary column (30m length, 0.25mm i.d., 0.25μm film thickness). Samples (1 μl) were injected in splitless mode using a 10μl syringe. The temperature program started at 80 °C (kept for 1 min), rising up to 300 °C with a program rate of 12°C min^{-1}, and kept for 5 min. While the injector and interface temperatures were kept at 290°C during the entire analysis.

RESULTS AND DISCUSSION

The PLFAs of the four bacterial strains (B1, B2, B3 and B4) are presented in Fig. 1 to Fig. 4. The chromatogram was obtained in full-scan mode (the mass range m/z 50–500 was scanned). The

Table 1. The compositions and relative abundance of signature PLFAs in B1, B2, B3 and B4 strains

Fatty acids	RRT	Percentage(%)			
		B1	B2	B3	B4
12:0	0.279	0.91±0.03	0.30±0.05	—	—
14:0	0.431	1.59±0.14	1.33±0.08	0.82±0.04	1.22±0.03
a15:0	0.499	1.49±0.09	—	—	—
15:0	0.528	—	0.51±0.06	—	0.21±0.02
16:1ω7c	0.612	5.58±0.21	5.17±0.15	3.71±0.03	3.54±0.03
16:1ω7t	0.618	7.11±0.18	16.98±0.12	4.38±0.08	17.40±0.17
16:0	0.639	24.80±0.40	23.03±0.28	22.88±0.20	23.98±0.19
17:1ω7c	0.728	6.96±0.07	2.93±0.04	—	0.85±0.03
18:2ω6c,9c	0.820	0.70±0.02	0.56±0.02	23.93±0.22	—
18:1ω9c	0.828	1.63±0.11	15.49±0.18	15.02±0.14	18.73±0.16
18:1ω9t	0.839	23.48±0.35	27.37±0.26	7.75±0.09	31.47±0.22
18:1ω7t	0.844	9.87±0.10	—	7.81±0.06	—
18:0	0.864	6.90±0.16	3.83±0.12	10.41±0.14	1.29±0.05
19:1ω9c	0.982	8.56±0.13	1.81±0.10	—	1.04±0.03
19:0	1.000	0.42±0.06	0.23±0.05	3.30±0.06	0.27±0.03
SSFAs		34.62	29.23	37.41	26.97
MUFAs		63.89	69.75	38.67	73.03
trans-FAs		40.46	44.35	19.94	48.87

The relative retention time (RRT) is calculated in relationship to the retention time of 19:0. The relative abundance of signature PLFAs expressed as mean of three replicates ± SD. SSFAs — straight-chain saturated fatty acids; MUFAs — monounsaturated fatty acids.

PLFA patterns of the bacterial strains were quite different, based on visual observation of the chromatograms (Fig. 1 to Fig. 4).

A total of 15 PLFAs were detected in the four strains ranging from 12 to 19 carbon atoms. They are listed in the order of increasing retention time (Table 1). The main PLFAs of the four bacterial strains were even-numbered saturated acids (C16:0) and monounsaturated acids (C18:1ω9c and C18:1ω9t). While B3 strain contained a large number of C18:2ω6c,9c fatty acid, with relatively lower abundance of C18:1ω9t.

As shown in Table 1, we observed for B1 strain a major contribution of C16:0 (24.80±0.40%), C18:1ω9t (23.48±0.35%), C18:1ω7t (9.87±0.10%), C19:1ω9c (8.56±0.13%), C16:1ω7t (7.11±0.18%), C17:1ω7c (6.96±0.07%), C18:0 (6.90±0.16%) and C16:1ω7c (5.58±0.21%). Some fatty acids were also detected as minor compounds such as C12:0, C14:0, Ca15:0, C18:2ω6c,9c, C18:1ω9c and C19:0. The fatty acid C a15:0 was only detected in B1 strain. For B2 strain, the main fatty acids were C18:1ω9t (27.37±0.26%), C16:0 (23.03±0.28%), C16:1ω7t (16.98±0.12%), C18:1ω9c (15.49±0.18%), C16:1ω7c (5.17±0.15%). Some other acids were detected, with abundances of less than 4%, namely, C12:0, C14:0, C15:0, C17:1ω7c, C18:2ω6c,9c, C18:0, C19:1ω9c and C19:0. For B3 strain, the main fatty acids were C18:2ω6c,9c (23.93±0.22%), C16:0 (22.88±0.20%), C18:1ω9c (15.02±0.14%), C18:0 (10.41±0.14%), C18:1ω7t (7.81±0.06%), and C18:1ω9t (7.75±0.09%). Some other acids with abundances of less than 5% were detected. They were C14:0, C16:1ω7c, C16:1ω7t and C19:0. For B4 strain, the main fatty acids were C18:1:9t (31.47±0.22%), C16:0 (23.98±0.19%), C18:1:9c (18.73±0.16%), and C16:1ω7t (17.40±0.17%). The other fatty acids detected in B4 were C14:0, C15:0, C16:1ω7c, C17:1ω7c, C18:0, C19:1ω9c and C19:0.

When the sum of monounsaturated fatty acids (MUFAs) is more than 20%, the bacterium will be considered as Gram-negative bacterium[6].

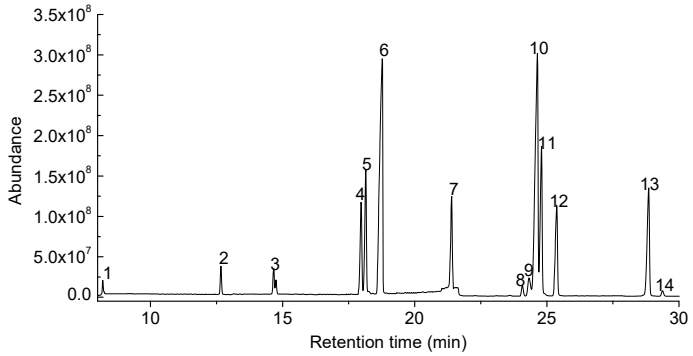

Fig. 1. Chromatogram of B1 obtained in full-scan mode. 1, 12:0; 2, 14:0; 3, a15:0; 4, 16:1ω7c; 5, 16:1ω7t; 6, 16:0; 7, 17:1ω7c; 8, 18 :2ω6c,9c; 9, 18:1ω9c; 10, 18:1ω9c; 11, 18:1ω7t; 12, 18 :0; 13, 19:1ω9c; 14, 19:0

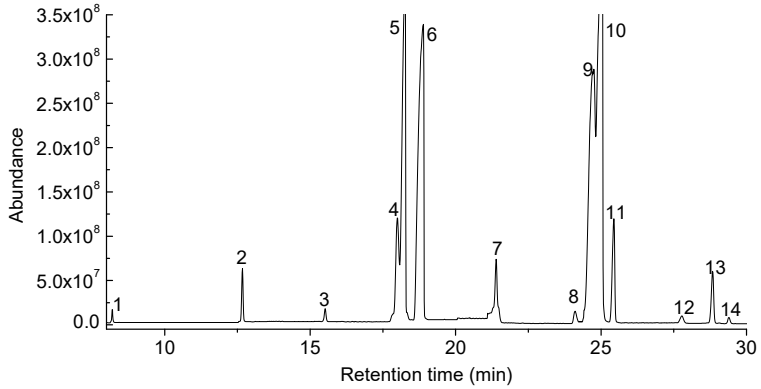

Fig. 2. Chromatogram of B2 obtained in full-scan mode. 1, 12:0; 2, 14:0; 3, 15:0; 4, 16:1ω7c; 5, 16:1ω7t; 6, 16:0; 7, 17:1ω7c; 8, 18 :2ω6c,9c; 9, 18:1ω9c; 10, 18:1ω9c; 11, 18:0; 12, 18:2ω7t,9c; 13, 19:1ω9c; 14, 19:0

The MUFAs of four strains were all greater than 20% (63.89% for B1, 69.75% for B2, 38.67% for B3 and 73.03% for B4, respectively). So they were all Gram-negative bacteria. Gram stain of the four strains got the same results. Slater *et al.*[7] have reported that monounsaturates are generally indicative of the *Proteobacteria*. The four strains are rich in MUFAs, however, only B3 strain belongs to *Proteobacteria*. So we can not determine whether a bacterium belongs to *Proteobacteria* according to the present of MUFAs. The straight-chain saturated fatty acids (SSFAs) are ubiquitous and thus not considered to indicate a specific community component[8]. The SSFAs in four strains were 34.62%, 29.23%, 37.41% and 26.97%, respectively, and C16:0 was the dominant saturated fatty acid. Hammel[9] believed that species of *Alcaligenes* and *Pseudomonas* are known to show an abundance of cyclopropyl fatty acids in the phospholipid bilayer. However, cyclopropyl fatty acids were not found in *Alcaligenes* and *Pseudomonas* species in this study. Terminal branched saturated PLFA are considered indicative of the *Firmicutes*[10]. Only a small amount of Ca15:0 (1.49±0.09%) was detected in B1, and did not appear in the other strains, but B1 was not *Firmicutes*. The polyunsaturated PLFA 18:2:6,9 was used as a fungal biomass indicator[11]. A large number of C18:2:6,9 was detected in B3, its content was the highest in all fatty acids. The result of 16s rDNA showed that it was *Saccharomyces*, belonging to fungi. The high percentages of *frans*-fatty acids detected in the composition suggest that the aerobic prokaryotes present[12]. The content of *frans*-fatty acids in B1, B2, B4 were all greater than 40%, so we deduced the three strains belonged to aerobic prokaryotes. Although catabolic gene and 16S rRNA probes have been used successfully to assess the presence of specific microorganisms in environmental

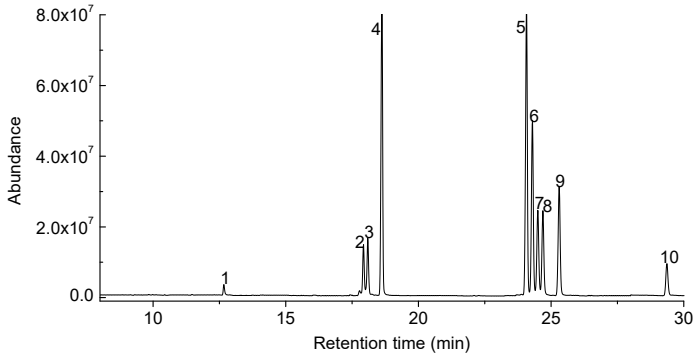

Fig. 3. Chromatogram of B3 obtained in full-scan mode. 1, 14:0; 2, 16:1ω7c; 3, 16:1ω7t; 4, 16:0; 5, 18 :2ω6c,9c; 6, 18:1ω9c; 7, 18:1ω9c; 8, 18:1ω7t; 9, 18 :0; 10, 19 :0

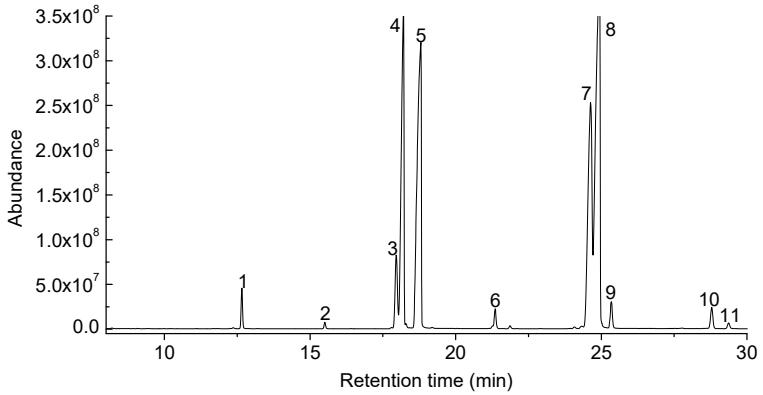

Fig. 4. Chromatogram of B4 obtained in full-scan mode. 1, 14:0; 2, 15 :0; 3, 16:1ω7c; 4, 16:1ω7t; 5, 16:0; 6, 17 :1ω:7c; 7, 18:1ω9c; 8, 18:1ω9c; 9, 18 :0; 10, 19:1ω9c; 11, 19 :0

samples[13]. Such methods, however, are labor intensive and experience limitations when measuring community functionality under stress or competition[14]. PLFAs analysis has provided a fingerprint of community structure, although it does not give precise species composition[15].

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