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Effects of crude oil on phospholipid fatty acid compositions of marine hydrocarbon degraders: estimation of the bacterial membrane fluidity

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Abstract

In this study, we investigated, in vitro, the effects of petroleum hydrocarbons on the phospholipid ester-linked fatty acid composition of *Corynebacterium* sp. Strain 8. The usual ratio of monounsaturated fatty acids E/Z (or *trans*/*cis*) was calculated. This ratio led to unexpected results because we found similar values for growths on either a hydrophobic substrate (crude oil) or a soluble carbon source (rich medium). The use of such an indicator seemed limited for monitoring an environmental stress, so we proposed an index based on the homeoviscous adaptation theory. A membrane viscosity index was defined and applied to *Corynebacterium* sp. Strain 8 (in vitro growth) and to a sedimentary community (in situ experiment). The results allowed us to estimate the membrane fluidity of both an isolated strain and a bacterial community in accordance with the medium hydrophobicity.

Keywords: Phospholipid ester-linked fatty acids; *Corynebacterium* sp.; Petroleum; Homeoviscous adaptation; Marine sediment; Biodegradation

1. Introduction

The subsurface microbial community plays a very important role in the mineralization of organic pollutants. Also, the evaluation of bioremediation performance involves an understanding of functional activities of prokaryotic organisms. However, the study of biomass and community structures in the natural environment is a difficult task (Findlay, 1996) that requires the definition and the use of some biomarkers. Thus, analysis of phospholipid ester-linked fatty acids (PLFAs) in marine sediments appears a useful method to obtain information about the sedimentary microbial community (White, 1988). Generally, PLFAs analysis is mainly oriented to the definition of biomarkers (Lechevalier, 1977; Findlay, 1996; Rajandran et al., 1992) but the PLFAs composition is also of interest to determine the effects of lipophilic substrates and xenobiotics on bacterial membrane properties. In fact, many organic solvents and hydrophobic substances partition into phospholipid bilayers and modify the PLFAs composition. Consequently, the cytoplasmic membrane properties such as fluidity, stability, and permeability are affected (Weber and De Bont, 1996). However, most microorganisms are able to adapt themselves to different organic compounds by modifying their membrane fluidity. The two main mechanisms involve the variation of either the PLFAs saturation degree or the *cis* to *trans* isomerization of the monounsaturated PLFAs. These mechanisms have been related to the homeoviscous adaptation (Shinitzky, 1984) and have been investigated by several authors (Diefenbach et al., 1992; Heipieper and De Bont, 1994; Weber et al., 1994; Heipieper et al.,

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1995). In the present study, our aim was to determine the effects of crude oil on PLFAs composition and, therefore, on bacterial membrane fluidity. For the first time, we studied a culture of a hydrocarbon-degrading bacterium (*Corynebacterium* sp.) on different substrates (rich medium or petroleum) and then carried out an in situ experiment with a sedimentary bacterial community artificially contaminated with crude oil.

2. Materials and methods

2.1. Chemicals and materials

Acetone, dichloromethane, heptane, methanol (chromasolv grade), and diethyl ether (puriss. p.a.) were purchased from Fluka (Germany). TLC Si 60 F254, silica gel 60 (0.063–0.200 mm), and alumina 90 (0.063–0.200 mm) were purchased from Merck (Germany). Filters GF/F (47 mm diameter) and silica gel plus sep-pak cartridge were purchased from Whatman (England) and Waters (Ireland), respectively. Dimethyl disulfur (99%), iodine (99.99%), and pyrrolidin (99%) were purchased from Aldrich. Yeast extract and bactopeptone (Difco) were provided by BD Biosciences (San Jose, CA, USA).

2.2. Culture media

The mineral salt medium (MSM) was composed of 23 g/L of NaCl, 0.75 g/L KCl, 5 g/L of Tris(hydroxymethyl)aminomethane, 1 g/L NH_4Cl, 3.9 g/L $MgSO_4$, 5 g/L $MgCl_2$, 1.5 g/L $CaCl_2$, 0.12 g/L K_2HPO_4, 0.002 g/L $FeSO_4$, $7H_2O$. The rich medium (RM) was obtained by adding 10 g of rich medium (5 g of yeast extract and 5 g of bactopeptone) to 1 liter of MSM. The BAL 250 medium was a 2-g/L MSM solution of Blend Arabian Light petroleum topped at 250 °C (BAL 250).

2.3. Bacterial cultures

The gram-positive hydrocarbon-degrading bacterium *Corynebacterium* sp. Strain 8, previously named *Pseudomonas* sp. Strain P8 (Rontani et al., 1984), was isolated from a subsurface sediment coming from the same site used for the in situ experiment (Hycarfos site, Golfe de Fos, France). *Corynebacterium* sp. Strain 8 was precultured in the dark at 20 °C, under aerobic conditions, in 500-mL inverted T-shaped flasks containing 200 mL of MSM supplemented with 3 g/L of ammonium acetate. Aeration was provided by agitation on a reciprocal shaker (96 rpm).

An experimental protocol has been worked out to estimate the effects of a massive crude oil spill and for monitoring degradation and remediation abilities after a petroleum contamination (Aries et al., 2001).

First, a cell inoculum from the acetate preculture was transferred on the RM and grown for 72 h (late exponential growth phase) under the same conditions described above. Second, cells were centrifuged, washed, and frozen. This sample was called 8RM1. In the same way, another cell inoculum from the acetate preculture was transferred on a hydrophobic medium (BAL 250). The culture was carried out for 21 days (aerobic conditions, 20 °C, late exponential growth phase). Afterward, cells were resuspended in fresh BAL and grown again for 21 days as previously described to prevent any growth of the microorganisms on ammonium acetate traces remaining from the preculture medium. Cells from the second growth were centrifuged, washed, and frozen. This sample was called 8BAL. Finally, a cell inoculum from the 8BAL sample was brought back on RM and grown twice on this medium (72 h, aerobic conditions, 20 °C) to prevent any growth of the microorganisms on petroleum traces. Cells from the second growth were centrifuged, washed, and frozen. This last sample was called 8RM2.

2.4. Sediment sampling and preparation

This experimental protocol is adapted from the methodology used by Gilbert et al. (1996) and Le Dréau et al. (1997b); 200 kg of samples from subsurface sediment of the Hycarfos site (Golfe de Fos, France), was collected by a grab sampler (Smith-Macintyre) and homogenized in a cement mixer. Sampling location is shown in Fig. 1. No sediment size measurement was performed in this study although sediment texture was estimated (muddy sands). The sediment was sifted twice by 2- and 1-mm-pore sieves successively and divided into two equal parts. The first half was noncontaminated and used as a control (NC); the second part was contaminated with a massive amount of petroleum (C). For the contaminated sediment, 18 g of BAL 250/kg sediment was added and homogenized in a cement mixer for 3 h. Mixture sediment was transferred into polyvinyl choride core samples (20 cm in length and 10 cm in diameter). Six core samples, three noncontaminated (T_0NC) and three contaminated (T_0C), were directly analyzed at the initial time. Six other core samples (three contaminated (T_5NC) and three noncontaminated (T_5C)) were introduced into the Hycarfos site sediments (20 m depth, Fig. 1) on October 10, 2001 and collected 5 months later. The 0- to 2-cm layers of every samples were homogenized, and both debris and fragments of mollusks and any macrofauna were manually removed before freezing at −18 °C. Afterward, the PLFAs from the 0- to 2-cm layers were analyzed to consider the aerobic degrading conditions exclusively.