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Effects of pure *n*-alkanes and crude oil on bacterial phospholipid classes and molecular species determined by electrospray ionization mass spectrometry

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Abstract

Phospholipids are major components of bacterial membrane. Furthermore, the growth in vitro on xenobiotics such as *n*-alkanes, aromatic compounds or alkanols bring about to a bacterial membrane adaptive response. Concerning this work, we studied the membrane lipid composition of a hydrocarbon-degrading gram-positive bacterium (*Corynebacterium* sp.) on a soluble substrate and we detected four different phospholipid classes: phosphatidylglycerol, phosphatidylinositol, cardiolipin and acyl phosphatidylglycerol. In addition, a study of the lipid composition was performed after an in vitro culture on either pure *n*-alkane or crude oil. The growths on such hydrophobic substrates showed major qualitative and quantitative modifications. In the case of a growth on either heneicosane or crude oil, an increase of odd-numbered fatty acids was observed. Furthermore, the phospholipid polar head group composition was highly influenced by the crude oil addition. These modifications were, respectively, interpreted as the consequence of hydrocarbon assimilation and membrane fluidity adaptation. Finally, *Corynebacterium* sp. was taken back on the initial ammonium acetate substrate in order to determine its restoration abilities after a petroleum contamination.

Keywords: RP-HPLC/ESI/MS; Tandem ESI/MS/MS; Petroleum; Hydrophobic substrates; Gram-positive bacteria; *Corynebacterium* sp

Abbreviations: RP-HPLC/ESI/MS, reversed-phase high performance liquid chromatography/electrospray ionization/mass spectrometry; TLC, thin-layer chromatography; GC/MS, gas chromatography/mass spectrometry; TBABr, tetrabutyl ammonium bromide; PLFAs, phospholipid ester-linked fatty acids; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; FAMEs, fatty acid methyl esters; DMDS, dimethyl disulfide; HDAI, hydrocarbon-degrading activity index = (odd-numbered straight chain SFAs + branched SFAs + odd-numbered straight chain MUFAs + branched MUFAs)/even-numbered MUFAs); PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DPG, diphosphatidyglycerol or cardiolipin; APG, acylphosphatidylglycerol; PI, phosphatidylinositol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-glycerol; IPCs, intact phospholipid classes; IPMS, intact phospholipid molecular species; *n*-C_{20}, eicosane; *n*-C_{21}, heneicosane; BAL 250, Blend Arabian Light petroleum topped at 250 °C

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1. Introduction

Microorganisms characterization are of particular interest in natural environmental samples so as to estimate biomasses and to describe bacterial community structures. There are two different groups of methods in order to obtain such information. The first group involves microbial isolation and microbiological techniques. However, these techniques are time consuming [1], selective and not quantitative [2,3] since most prokaryotes in the environment are viable but not cultivable [4,5]. The second group concerns a variety of biochemical methods that does not need any bacterial cultures or isolations. Among these methods, we can name different approaches such as the molecular biology techniques [6] or the detection of some bio-organic compounds [7]. Concerning this last approach, some compounds, like lipids for instance, were early proposed as biomarkers [8–12]. The most common method is related to phospholipid ester-linked fatty acids (PLFAs) analysis by GC/MS as fatty acid methyl esters derivatives [2,13–16]. Such an analysis provided precious information about cultures of isolated bacteria and especially concerning the effects of xenobiotics such as ethanol and toluene [17–20] or *n*-alkanes [21–24] on PLFA profiles. In the same way, the analysis of PLFAs extracted from in vitro bacterial community grown on crude oil revealed the effects of this complex hydrophobic substrate [21]. Nevertheless, the use of PLFAs possesses some limitations. In one hand, methanolysis lead to a PLFA mixture that corresponds to different phospholipid classes [25] and it results in an important loss of information. In other hand, regarding to in situ samples, the usual lipid extract is a complex matrix that contains various non-phospholipid fatty acid-bearing biomolecules [12]. Thus, these two drawbacks could obscure the sedimentary microbial characterization. More recently, some works were related to the direct intact bacterial phospholipid analysis [26,27,25,28–32]. Afterward, several authors performed intact bacterial phospholipid analysis by HPLC/ESI/MS with a soft ionization technique implying an electrospray interface coupled with a normal phase [30,31] or a reverse phase liquid chromatography column [25,28,29,32].

In this paper, we reported the use of an ion-pairing RP-HPLC/ESI/MS technique so as to determine the intact phospholipid classes (IPCs) and molecular species (IPMS) of *Corynebacterium* sp., which possess hydrocarbon-degrading abilities [33,34]. The hydrocarbon-degrading abilities of many prokaryotes are well known [35], but there is very few studies about Corynebacterium genus [33,34]. Besides, a large part of the works, which are related to the effects of xenobiotics on bacterial membrane, focused on gram-negative bacteria studies [17–19,36]. Consequently, we investigated the lipid membrane composition of *Corynebacterium* sp. since few information are available as concerns the gram-positive bacteria. *Corynebacterium* sp. was cultured either on a soluble substrate such as ammonium acetate or on hydrophobic substrates such as pure *n*-alkanes (either *n*-C_{20} or *n*-C_{21}) or crude oil. The effects of the three different hydrophobic media on PLFA, IPC and IPMS composition as well as on fatty acyl chain parity were monitored in order to differentiate the nature of the xenobiotics. Afterward, *Corynebacterium* sp. was taken back on the initial ammonium acetate substrate so as to determine its restoration abilities. Furthermore, a study of the phospholipid fatty acyl chain stereochemistry by tandem ESI/MS/MS provided useful information concerning the membrane properties of *Corynebacterium* sp.

2. Experimental

2.1. Chemicals and materials

Acetone, dichloromethane, methanol, heptane, water (chromasolv grade), diethyl ether (puriss. p.a.) and tetrabutyl ammonium bromide (ion-pair reagent, purity ≥99%) were purchased from Fluka (Germany). TLC Si 60 F254, Silica gel 60 (0.063–0.200 mm) were purchased from Merck (Germany). GF/F filters (47 mm Ø) and 1 g Silica gel plus Sep-Pak™ cartridges were purchased from Whatman (England) and Waters (Ireland), respectively. Boron trifluoride (10% in methanol, m/v), dimethyl disulfure (99%), iodine (99.99%) and pyrrolidin (99%) were purchased from Aldrich (USA). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol P-6956 (POPG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine P-5203 (POPE), 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-glycerol P-6412 (DMPG), cardiolipin or diphosphatidylglycerol sodium salt from bovine heart C-0563 (DPG) and L-α-phosphatidylinositol sodium salt from bovine liver P-5766 (PI) were purchased from Sigma (USA).

2.2. Culture media

The mineral salt medium (MSM) was composed of 23 g l^{-1} of NaCl, 0.75 g l^{-1} KCl, 5 g l^{-1} of tris(hydroxymethyl)aminomethane, 1 g l^{-1} NH_4Cl, 3.9 g l^{-1} $MgSO_4$, 5 g l^{-1} $MgCl_2$, 1.5 g l^{-1} $CaCl_2$, 0.12 g l^{-1} K_2HPO_4, 0.002 g l^{-1} $FeSO_4{\cdot}7H_2O$. The ammonium acetate medium was obtained by an addition of 3 g of ammonium acetate to 1 l of MSM. The eicosane and heneicosane media were 1 g l^{-1} MSM solutions of *n*-C_{20} and *n*-C_{21}, respectively. The BAL 250 medium was a 2 g l^{-1} 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., previously named *Pseudomonas* sp. Strain P8 [37], was isolated from a sub-surface sediment coming from the Hycarfos site (Golf de Fos, France). *Corynebacterium* sp. was pre-cultured in the darkness at 20 °C, under aerobic conditions and in 500 ml inverted T-shaped flasks containing 200 ml of MSM supplemented with 3 g l^{-1} of