Phytane Aerobic Biotransformation by Microbial Consortium and Isolated Bacteria of Petroleum Formation Water from Campos Basin, Brazil.

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ABSTRACT

Samples of petroleum formation water were collected from Campos Basin oil field, Brazil, and submitted to aerobic and anaerobic microbial enrichment assays using appropriate media. The resulting microbial consortia were used in experiments of transformation of biomarkers (phytane and cholestane) under aerobic and anaerobic conditions. The aerobic microbial consortium degraded phytane in 30 days, but the cholestane remained intact. Bacterium A, isolated from the aerobic microbial consortium, was able to degrade phytane in 10 days and is currently under identification. Analysis of the phytane degradation products revealed methyl – phytane as one of its major biotransformation product, which showed a retention time similar to the phytane but with different mass spectra. Additionally, a wide range of polysubstituted unsaturated cyclohexanecarboxylic acids and, cyclopentanecarboxylic acid derivatives were detected. The biodegradation potential of the anaerobic consortium is now being investigated. In this one, the presence of a bacterium belonging to the Petrotoga genus was detected by using cultivation-independent methods.

Keywords: biomarker, biotransformation, petroleum

INTRODUCTION

Bacterial communities present in the reservoir formation water are important agents in the biodegradation of the petroleum constituents. The preferential degradation of long chain n-alkanes in relation to the isoprenoids and steranes, by aerobic microorganisms, has been well demonstrated (Heider et al., 1999), with little details concerning specific bacteria and degradation mechanisms. It is generally accepted that petroleum hydrocarbon degradation by aerobic bacteria leads to an increasing amount of carboxylic acid contents. On the other hand, the anaerobic biodegradation is still a black box. To address this question, aerobic and anaerobic communities living in the same reservoir are under investigation in order to establish the aerobic and anaerobic degradation pathways. Therefore we focused on the Albacora oil field formation water, Campos Basin, one of the largest oil producing basin in Brazil.

METHODS
Sampling: crude oils and formation water were collected from Campos Basin, Brazil. The samples were kept at 4°C before use.

Aerobic Enrichment: Aliquots of 7 formation water samples of (6 mL) were incubated in Zinder medium (Zinder et al., 1984) with organic substrates and vitamins (Vazoller, 1995). The enrichment was incubated under aerobic conditions in rotational shaker (150 rpm), at two different temperatures, 28°C and 55°C.

Anaerobic Enrichment: Zinder medium (Zinder et al., 1984) was prepared as in the previous experiment, but taking care to use a N₂ atmosphere free from oxygen. For the inocula, the N₂ atmosphere was exchanged by N₂/CO₂ (70:30). Samples of formation water collected under anaerobic conditions were inoculated in Zinder medium and a mixture of vitamins, organic and inorganic compounds, according to the methodology reported by Vazoller (1995).

Isolation of Aerobic Microorganisms: aerobic microbial consortium obtained from previous enrichment, was submitted to cultivation in Petri dishes with Nutrient Agar (Difco), and incubated at 28°C during 3 days. Microbial colonies were isolated according to Pelczar (1980).

Biotransformation Assays:

a) 6 mL of the microbial consortium inoculum was added to Zinder medium (28,9 mL) containing sodium bicarbonate solution 10% (0,3 mL), vitamin solution (0,3 mL) and, biomarker solution (0,025 mg/mL of medium: 0,5 mL). The experiment was incubated under aerobic conditions in rotational shaker (150 rpm), at 28°C and 55°C. The biotransformation was monitored during 30 days by GC/MS to determine the conversion rate and the products generated. GC/MS measurements were performed with Hewlett-Packard 6890 gas chromatograph coupled with a mass selective detector Hewlett-Packard 5973. Helium was used as the carrier gas, and GC conditions were the following: oven temperature program was 100 to 290°C at 20°C/min and holding at 290°C for 10 min (capillary column HP5 30 m x 0,25 mm x 0,25 µm); splitless injector, temperature 220°C. The following MS conditions were used: ionization mode, EI⁺; ionization energy 70 eV, mass range, m/z 50 to 700.

b) The isolated strain was cultivated in Nutrient Agar (Difco) and after 24 hours of incubation at 28°C, the cells were suspended in sterile Milli-Q water until 10⁻⁸ cells/mL (Mac Farland scale). 6mL of the suspension were inoculated in basal medium as described above. The biotransformation was monitored by GC/MS.

Petrotoga Identification: An aliquot of the anaerobic enrichment, was sent to CPQBA – Unicamp, for molecular identification using cultivation-independent methods: direct bacterial DNA extraction, PCR amplification of 16S rDNA, cloning and automated sequencing.

RESULTS AND DISCUSSION

Phytane and cholestane were selected for biodegradation experiments. The aerobic and anaerobic microbial communities were enriched in appropriate media, some of which have never been reported before. In the first experiment we have used the aerobic microbial consortium and a 1:1 mixture of phytane and cholestane. Phytane was totally degraded in 30 days, while cholestane did not undergo any visible transformation (Fig. 1).

16 different bacterial strains were isolated from the aerobic consortium. One of these, named Bacterium A (gram-negative rods) degraded phytane in 10 days (Fig. 2). Analysis of the phytane degradation products revealed methyl – phytane as one of its major biotransformation products, which has a retention time similar to the phytane but with different mass spectra (Fig. 3). We have additionally detected a wide range of...
polysubstituted unsaturated cyclohexanecarboxylic acids, cyclopentanecarboxylic acid derivatives. It is generally accepted that the hydrocarbon degradation proceeds via a cyclic pathway involving hydroxyl addition followed by oxidation to ketone and subsequently to carboxylic acid and decarboxylation (Huang et al., 2004; Belhaj et al., 2002; Rodrigues et al, 2000). These were not present in the blank experiment. A suggested biodegradation pathway with the formation of cyclohexanecarboxylic acid derivatives is depicted in Fig. 4.

One should emphasize that the experiments with the isolated strain were performed at 28°C. Experiments at 55°C did not produce any results in 30 days. Molecular techniques will be applied for the identification of Bacterium A.

Anaerobic microbial consortium was submitted to cultivation-independent molecular analyses, which led to the detection of a bacterium belonging to the genus Petrotoga. These organisms have been previously found in deep petroleum reservoirs and have not been described as petroleum tolerant bacteria (Bergey, 2001). Experiments to evaluate the ability of this anaerobic microbial consortium for biotransformation of different petroleum biomarkers (phytane, cholestane, n-alkanes) are under investigation.

CONCLUSION

Results obtained in this study are certainly preliminary, but they clearly indicate that the aerobic bacteria can degrade hydrocarbon and produce a wide variety of compounds by oxidation and cyclization.

REFERENCES


Fig. 1. Total ion chromatogram of phytane and cholestane (30 days).

Fig. 2. Total ion chromatogram of phytane biodegradation by Bacterium A isolated from petroleum formation water (10 days).

Fig. 3. (a) Mass spectra of methyl-phytane; (b) mass spectra of phytane.