BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Genetics and Molecular Microbiology

Evaluation of bacterial diversity recovered from petroleum samples using different physical matrices

Bruna Martins Dellagnezze[a,*], *Suzan Pantaroto de Vasconcellos*[b], *Itamar Soares de Melo*[c], *Eugênio Vaz dos Santos Neto*[d], *Valéria Maia de Oliveira*[a]

[a] *Division of Microbial Resources, Research Center for Chemistry, Biology, Agriculture (CPQBA), University of Campinas – UNICAMP, Campinas, SP, Brazil*
[b] *Federal University of São Paulo (UNIFESP), Department of Biological Sciences, Diadema, SP, Brazil*
[c] *EMBRAPA, National Center for Research on Monitoring and Evaluating Environmental Impact, Jaguariúna, SP, Brazil*
[d] *PETROBRAS R and D Center, Rio de Janeiro, RJ, Brazil*

ARTICLE INFO

Article history:
Received 23 February 2015
Accepted 24 November 2015
Available online 22 April 2016

Associate Editor: Lucy Seldin

Keywords:
Bacterial diversity
Microbial enrichment
Petroleum reservoir
Physical support
16S rRNA

ABSTRACT

Unraveling the microbial diversity and its complexity in petroleum reservoir environments has been a challenge throughout the years. Despite the techniques developed in order to improve methodologies involving DNA extraction from crude oil, microbial enrichments using different culture conditions can be applied as a way to increase the recovery of DNA from environments with low cellular density for further microbiological analyses. This work aimed at the evaluation of different matrices (arenite, shale and polyurethane foam) as support materials for microbial growth and biofilm formation in enrichments using a biodegraded petroleum sample as inoculum in sulfate reducing condition. Subsequent microbial diversity characterization was carried out using Scanning Electronic Microscopy (SEM), Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rRNA gene libraries in order to compare the microbial biomass yield, DNA recovery efficiency and diversity among the enrichments. The DNA from microbial communities in petroleum enrichments was purified according to a protocol established in this work and used for 16S rRNA amplification with bacterial generic primers. The PCR products were cloned, and positive clones were screened by Amplified Ribosomal DNA Restriction Analysis (ARDRA). Sequencing and phylogenetic analyses revealed that the bacterial community was mostly represented by members of the genera *Petrotoga*, *Bacillus*, *Pseudomonas*, *Geobacillus* and *Rahnella*. The use of different support materials in the enrichments yielded an increase in microbial biomass and biofilm formation, indicating that these materials may be employed for efficient biomass recovery from petroleum reservoir samples. Nonetheless, the most diverse microbiota were recovered from the biodegraded petroleum sample using polyurethane foam cubes as support material.

* *Corresponding author.*
E-mail: brunamadell@gmail.com (B.M. Dellagnezze).
http://dx.doi.org/10.1016/j.bjm.2016.04.004

Introduction

Crude oil biodegradation in petroleum reservoirs affects the world production of fuels, making the recovery and refining processes more expensive. For many years, the prevalent occurrence of biodegradation in petroleum wells has been attributed to the aerobic bacterial degradation of hydrocarbons, which can be stimulated by oxygen carried by the infiltration of meteoric waters in the reservoir.[1] However, there is strong evidence for the widespread occurrence of obligate anaerobes in subsurface petroleum systems,[2–4] and the flushing of meteoric water does not indicate that highly reactive oxygen survives transportation to deep reservoirs, since even small concentrations of organic compounds can remove oxygen from an aquifer.

In recent work, researchers have suggested that biodegradation processes can occur at the oil–water transition zone, in which microbial life should be possible within water droplets containing active microbial communities.[5] Data gathered from several studies indicate that oil biodegradation in deep subsurface petroleum reservoirs occurs through anaerobic microbial metabolism rather than aerobic mechanisms, resulting in a decrease of light hydrocarbons and an increase of oil density, acidity, viscosity and sulfur content.[6–8] In addition, viable anaerobic hydrocarbon degradation processes have recently been established for both saturated and aromatic hydrocarbons.[9–11] Studies aiming to evaluate intermediate metabolites, characteristic of anaerobic hydrocarbon degradation, have been carried out and allowed the identification of compounds such as reduced 2-naphthoic acids,[12] 2-methylnaphthalene, tetralin, as well as naphthoic acids in petroleum reservoirs.[13]

Sulfate reduction and methanogenesis are the most likely processes responsible for in-reservoir hydrocarbon oxidation.[14] Oil degradation linked to sulfate reduction would explain the consistent hydrocarbon compositional patterns seen in many degraded oils worldwide. Sulfate arises from geological sources, such as evaporitic sediments and limestone, or from the injection of seawater for pressure stabilization, and may lead to significant oil degradation and increased residual-oil sulfur content.[15] Souring in oilfield systems is most commonly due to the action of sulfate-reducing prokaryotes, a diverse group of anaerobic microorganisms that respire sulfate and produce sulfide (the key souring agent) while oxidizing diverse electron donors.[8]

In this sense, efforts have been made by several researchers in order to recover and characterize the anaerobic microbial community inhabiting the deep petroleum biosphere.[2,16,17] The study of genomes of uncultivated microbes have become possible through metagenomics, a cultivation-independent approach that allows to explore the metabolic potential of the unseen biodiversity by cloning large DNA fragments directly isolated from the environment.[18] With the use of the metagenomic approach, bacteria capable of degrading petroleum hydrocarbons, including anaerobes, have been more deeply investigated and their metabolic routes unraveled.[19,20] However, the extremely low amount of DNA obtained from samples derived from petroleum reservoirs using direct nucleic acid extraction procedures is often a restraint when the phylogenetic and/or metabolic diversity of microbial communities are investigated,[21,22] because of the low cellular density and activity found in such hostile environment. Microbial enrichments using different culture conditions, simulating the chemical and physical parameters of natural environments, can be applied to overcome this limitation and increase the recovery of DNA from environments with low cellular density.[23] Although cultivation under laboratory conditions can diminish the biodiversity recovered, this technique allows the selection of microorganisms that have some function of particular interest, such as enzymatic activity or biodegradation ability.[24]

This work aimed to evaluate the efficiency of different matrices, used as physical supports, in recovering anaerobic bacterial diversity from a biodegraded oil sample derived from a petroleum reservoir in Campos Basin (Brazil). The matrices were used in order to evaluate their effect in the increase of biomass, as well as a support for biofilm formation. The relative abundance and diversity of the anaerobic microbiota recovered from the enrichments were compared by using microscopic and molecular analysis (DGGE and 16S rRNA libraries).

Material and methods

Sampling

Petroleum samples were obtained in July 2005 from five production wells at the Pampo Platform, Campos Basin (Macaé, RJ, Brazil), with logistic support from CENPES/Petrobras, as described in details by Vasconcellos et al.[22] Samples were collected in triplicate using 500 mL sterilized Schott bottles, which were completely filled with the samples in order to avoid oxygen influx. Samples were kept on ice during transportation to the laboratory and stored at room temperature for subsequent anaerobic bacterial enrichment assays.

Anaerobic enrichments

The biodegraded petroleum sample (P2) used in this work as inoculum (10% v/v) for the anaerobic enrichments was collected from oil reservoir 2 in Campos Basin, RJ, Brazil.[22] This well was characterized as highly biodegraded, level 5–6, according to Peters and Moldowan,[25] with average temperature 71 °C and approximately 2000 m deep. The petroleum sample was homogenized in water bath at 50 °C. The enrichments were settled in Schott bottles (1 L) containing 500 mL of Zinder medium[26] supplemented with organic substrates (sodium acetate, sodium formate, sodium lactate, yeast extract, methanol) to stimulate the growth of sulfate reducing bacteria, according to methods described by Dubourguier et al.[27] and Silva et al.[28]. The cysteine–HCl solution (2 mM) was added to the enrichments (1%, v/v) as final electron acceptor.

Three different matrices were independently applied as physical supports to allow bacterial biofilm formation and increase biomass recovery under sulfate reducing condition: (1) polyurethane foam cubes (PF) (1 cm^2), (2) slices of shale