

## Bacterial Degradation of Petroleum and Petroleum Products

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### Abbreviations

MBC: Mixed Bacterial Consortia; PBC: Pure Bacterial Culture; GT: Growth Test; MF Metabolic Fingerprinting; LM: Light Microscopy; TEM: Transmission Electron Microscopy; SEM: Scanning Electron Microscopy; ARDRA: Amplified Ribosomal DNA Restriction Analysis; RAPD: Random Amplified Polymorphic DNA; REP-PCR: Repetitive Extragenic Palindromic Polymerase Chain Reaction; T-RFLP: Terminal Restriction Fragment Length Polymorphism; PFGE: Pulsed Field Gel Electrophoresis; Q-PCR: Quantitative PCR or Real-Time PCR; RT-Q-PCR: Reverse Transcription Q-PCR; SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; HPTLC: High Performance Thin Layer Chromatography; DGGE/TGGE: Denaturing/Temperature Gradient Gel Electrophoresis; LH-PCR: Length Heterogeneity PCR; RISA: Ribosomal Intergenic Spacer Analysis; SSCP: Single Strand Conformation Polymorphism; FGA: Functional Gene Arrays; FISH: Fluorescence In Situ Hybridization; FAME: Fatty Acid Methyl Esters; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid

### Editorial

Petroleum is the major source of energy used in several industries and in our daily life. Subsequently, the release of petroleum hydrocarbons into the environment due to accidental spills or human activities, such as oil exploitation, transportation, and storage are the main causes of water and soil pollution [1,2]. Petroleum is a complex mixture of different hydrocarbons including alkanes, aromatics, asphaltenes, resins, and majority of these compounds are very toxic and carcinogenic for living organisms [1,3-5]. Therefore, the cleaning up of petroleum hydrocarbons in the water and soil environment is a real world problem. Some of the microorganisms, primarily bacteria which naturally exist in water and soil, are capable to degrade toxic petroleum hydrocarbons under different environmental conditions [1,5]. Furthermore, bacteria exposed to such toxic compounds become adapted, exhibiting higher biodegradation rates, as compared with bacteria from unpolluted sites [2,3,6]. Several adaptive mechanisms (e.g., modifications of the membrane or changes in the overall energy status, modifications of cell morphology and cell surface properties, active excretion,

induction of anabolic pathways) were described for bacteria able to survive in the presence of toxic petroleum hydrocarbons [7-9].

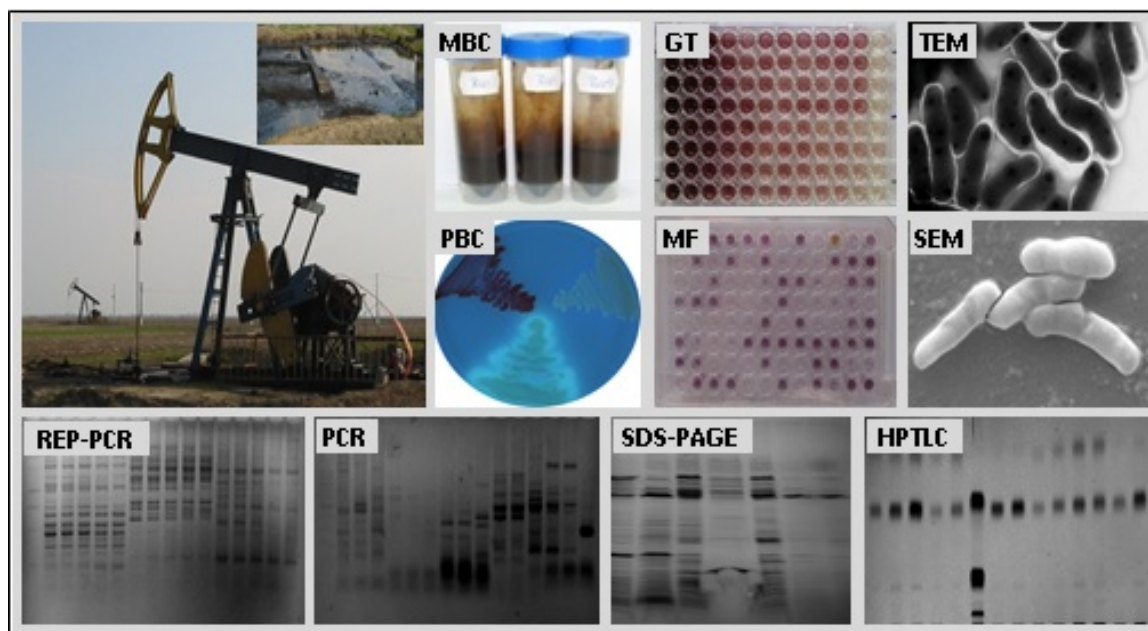
Bioremediation represents the use of bacteria to degrade toxic petroleum hydrocarbons into non-toxic end products, such as carbon dioxide, water and other inorganic compounds, and these processes are an environmentally friendly, efficient, economic and versatile alternative to physicochemical treatment of water and soil polluted with petroleum hydrocarbons [3-5,10-12]. Generally, bioremediation technologies are classified as *in situ* or *ex situ*. *In situ* bioremediation (e.g., natural attenuation, bioaugmentation, biostimulation) involves treating the pollutants at the site, while *ex situ* (e.g., landfarming, composting, biopiles, bioreactors) involves the removal and transporting of the pollutants to another site for treatment [13-15].

It is well known, that the behavior of petroleum hydrocarbons in the environment is dependent by the nature and concentration of the hydrocarbons present and by the interaction between environmental and biological factors [6]. Hydrocarbons differ in their susceptibility to bacterial attack, and biodegradability of the hydrocarbons generally decreases in the following order: linear alkanes, branched alkanes, monoaromatics, cyclic alkanes, polyaromatic hydrocarbons, and asphaltenes [1,3,5,10]. Environmental factors, such as temperature, the presence of oxygen or other electron acceptors, nutrients, salinity, pH, water, and osmotic stress can limit the biodegradation process by inhibiting the growth of bacteria able to degrade hydrocarbons and/or reducing the bioavailability of hydrocarbons to bacterial attack [3,10,16]. Among biological factors, the bacterial diversity and their metabolic capabilities are major characteristics influencing petroleum hydrocarbons biodegradation. Many studies were carried out with pure culture or mixed bacterial consortia isolated from petroleum hydrocarbons polluted sites [2,5,6,11,17]. Mixed bacterial consortia isolated exhibited higher biodegradation rates, as compared with pure cultures, as a result of the synergistic interactions among different bacteria of the associations, which may lead to the complete degradation of toxic petroleum hydrocarbons into non-toxic end products. Some bacteria of the associations have the ability to secrete specific degradative enzymes and growth factors, while other bacteria have the ability to produce

extracellular polymers, such as biosurfactants and bioemulsifiers which increase the surface area and bioavailability of hydrocarbons for biodegradation [1,2,6,11,16].

In the last decades, advances in microbiology made it possible to combine culture-dependent and culture-independent approaches in order to describe bacterial diversity and bacterial activity in petroleum hydrocarbons polluted sites [10,12,13]. The culture-dependent techniques (e.g., growth test, metabolic fingerprinting; microscopic examination by LM, TEM, SEM; biofilm formation; genetic fingerprinting by ARDRA, RAPD, REP-PCR, T-RFLP, PFGE; quantitative PCR by Q-PCR, RT-Q-PCR; protein analysis by SDS-PAGE; lipid and secondary metabolites analysis by HPTLC) are useful for the isolation and characterization of novel bacterial

strains which possess new degradative pathways. The culture-independent techniques (e.g., clone library method; genetic fingerprinting by ARDRA, DGGE/TGGE, LH-PCR, RAPD, RISA, SSCP, T-RFLP; DNA microarrays by 16S rRNA gene microarrays, FGA; quantitative PCR by Q-PCR, RT-Q-PCR; FISH; lipid analysis by FAME) based on direct nucleic acids (DNA, RNA) and fatty acids analysis are useful for describing community structure, and to distinguish the metabolically active members of the bacterial community [13,18]. Several culture-dependent approaches (**Figure 1**) were also carried out by us for the isolation and characterization of new bacterial strains of the genera *Bacillus*, *Klebsiella*, *Lysinibacillus*, *Pseudomonas*, *Rhodococcus*, *Shewanella*, *Serratia*, and *Vibrio* able to survive in the presence of different toxic petroleum hydrocarbons [19-22].



**Figure 1** Culture-dependent approaches on bacteria isolated from hydrocarbons polluted sites.

To conclude, culture-independent approaches, as well as culture-dependent approaches on pure culture or mixed bacterial consortia isolated from hydrocarbons polluted sites will contribute in the future to the development of new bioremediation strategies for environments contaminated with petroleum and petroleum products.

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