HYDROCARBON POLLUTION IN TUNISIA COASTS: EVALUATION AND BIOREMEDIATION

Ines Zrafi [1], Amina Bakhrouf [1], Abdelghani Sghir [2] and Dalila Mosbahi Saidane [1]*
[1] Laboratoire d'Analyse, Traitement et Valorisation des Polluants de l'Environnement et des Produits - Dalila.Saidane@fphm.rnu.tn
[2] CEA Genoscope, Evry, France

Abstract
We investigated the composition and spatial distribution of hydrocarbons found in Tunisian's marine environments. Although moderate, these hydrocarbon concentrations should ideally be reduced. Then, we investigated the characterization of bacterial diversity of polluted seawater. Molecular analysis of bacterial composition associated with hydrocarbon composition analysis provided evidence that microbial composition dynamics is related to changes in substrate composition, and there is a close relationship between the proliferation of specialized species and their function in the degradation of the different fractions of oil.
Keywords: Bacteria, Pollution, Coastal Management, Monitoring

Introduction
Sea Coastal were being the receptor of diverse pollutants. Sea water, [1], [2] marine sediment [3] and interstitial water [2] were the subject of this pollution. In the Mediterranean sea, pollution is related to the increase of industrialized and newly industrialized countries. The mediterranean coast in Northern Tunisia is one of such industrialized and populated region [4], [5]. On the other hand, hydrocarbons are one of the most important pollutants that can persist for years [6]. Microbial degradation is the major natural route for cleaning up oil spills [6]. Characterizing microbial diversity and identifying microorganisms which play a key role in the degradation of pollutants could be useful in defining new strategies for bioremediation. The aim of this work is to better understand the present status of environmental pollution by petroleum derives on tunisian coast and to analyze the bacterial diversity during oil degradation in order to contribute to the understanding of the bioremediation technology in a marine environment.

Materials and Methods
Hydrocarbon analysis: Hydrocarbons were extracted using chloroform. The extract (TH) was fractionated into non aromatic hydrocarbon (NAH) and aromatic hydrocarbons (AH) by adsorption liquid chromatography using a column of silica-gel. The TH, NAH and AH were determinate and were analyzed with a gas chromatograph.
Molecular analysis: Direct extraction of total microbial DNA from polluted seawater samples, amplification of 16S rRNA genes, cloning, sequencing, chimera check and phylogenetic rRNA gene sequence analysis were performed. All sequences were imported in the ARB database and compared to the GenBank database using BLAST. They were aligned using the ARB program and then manually checked. A distance matrix was generated and used by the DOTUR computer program to define Operational Taxonomic Units (OTUs). A 97% sequence similarity cut-off was used to define an OTU).
Bioremedation essays : Biodegradation assays were carried out in seawater inoculated directly with fresh culture of an adapted microflora. The efficiency of biodegradation corresponding to each incubation period was calculated for the various fractions of TPH, NAH or AH at various incubation periods.

Results and discussion
1. Evaluation of hydrocarbon pollution
TH concentrations differed from one site to another. NAH and AH levels showed a variation among the collection sites, with an average of 66.22–211.82 µg/g for NAH and 13.84–115.60 µg/g for AH. NAH was predominant. GC traces show unimodal distribution of n-alkanes, equivalent distribution pattern of both odd carbon-numbered alkanes and even-carbon-numbered alkanes. All geochemical markers enable us to make the hypothesis of petroleum source contribution. Our results show that the TPH levels were relatively low compared to those of locations around the world reported to be chronically contaminated by oil.

2. Bacterial diversity of polluted seawater
AM microflora represent the natural bacterial population of seawater from a region adjacent to an oil refinery. A total of 94 clone sequences were obtained for the AM clone library. They show a percentage of sequence identity to the closest related sequences in the public databases ranging between 91% and 100%. The 16S rRNA gene sequences can be grouped into 22 OTUs, of which 16 OTUs are affiliated with cultivated microorganisms while a unique OTU is affiliated with a noncultivated microorganism with $\geqslant$97% sequence identity. A total of 5 OTUs represent novel OTUs sharing less than 97% sequence identity. Detailed analyses of the AM clone library indicated that the abundant phylotypes are distributed within the Alpha-, Beta-, and Gammaproteobacteria subclasses. The minor phylotypes are distributed within the Actinobacteria, the Firmicutes, and the Bacteroidetes groups. The existence of bacteria in petroleum-contaminated sites suggests that many species have evolved specifically in these environments and may be active in the metabolism of hydrocarbon.

3. Bioremediation efficiency of the adapted microflora
The amount of TPH and AH decreased after 3 days. NAH and AH biodegradation reached the highest value (92.6%; 68.7%) at the end of the incubation period. A slight decrease in NAH degradation was detected at day 21. Detailed chromatogram analysis showed visible degradation of n-alkanes ranging from n-C14 to n-C24 during the first week. Branched alkanes were degraded during W2. At the end of W3, the majority of n-alkanes were totally degraded. Community composition changes began during the first week (W1) and remained significant during the whole incubation period. At S3 and S4, we observed a major decrease in bacterial diversity, confirmed by diversity indexes. Bacterial diversity is gradually reduced during the biodegradation process. We found that in W1, W2 and W3. The 16S rRNA sequences are affiliated with the same microorganisms detected in the AM library, with variation in their proportions and the emergence of new species. We concluded that the AM consortium shows a high performance of degradation of both NAH and AH components.

Conclusion
Our findings indicate hydrocarbon contamination in region of study. The use of bacterial diversity analysis could be useful in defining new strategies for bioremediation. Finally, to further elucidate the role of these bacterial groups in hydrocarbon degradation, catabolic gene expression profiling may be necessary.

References
1 - Jaffrennou,C., Stephan L., Giamarchi P., Cabon J.Y., Burel-Deschamps L. and Bautin, F., 2007. Direct fluorescence monitoring of coal organic matter released in seawater. *Journal of Fluorescence*, 17: 564-572.
2 - Pérez-Carrera E., León V.M., Parra, A.G. and González-Mazo E., 2007. Simultaneous determination of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in seawater and interstitial marine water samples, using stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry. *Journal of Chromatography*. A, 2: 1170 (1-2), 82-90.
3 - Wakeham S.G., 1996. Aliphatic and polycyclic aromatic hydrocarbon in Black Sea sediments. *Marine Chemestry* 53: 187-205.
4 - Zrafi-Nouira I., Khedir-Ghenim Z., Zrafi F., Bahri R., Cheraeif I., Rouabhia M. and Saidane-Mosbahi D., 2008. Hydrocarbon Pollution in the Sediment from the Jarzouna-Bizerte Coastal Area of Tunisia (Mediterranean Sea). *Bull. Environ. Contam. Toxicol.* 80(6):566-572.
5 - Burns, K.A., and Teal J.M., 1979. The West Falmouth Oil Spill: Hydrocarbons in the salt marsh ecosystem. *Estuarine and Costal Marine Science*, 8: 349–360.
6 - Kasai Y, Kishira H, Syutsubo K, Harayama S., 2001. Molecular detection of marine bacterial populations on beaches contaminated by the Nakhodka tanker oil-spill accident. *Environ Microbiol.* 3(4):246-55.