Identification of New Potential Petroleum Hydrocarbon Degrading Microbial Populations in Petroleum Contaminated Coastal Marine Sediments

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ABSTRACT. In this study, the shoreline of Lebanon, which extends over 225 km along the eastern shore of the Mediterranean Sea, was characterized for its sediment microbial community using 16S rRNA gene pyrosequencing. The shoreline was previously affected with a huge petroleum oil spill and is prone to daily small loads of hydrocarbon contamination. Sediment Petroleum Hydrocarbons (PH) were extracted using an accelerated solvent extractor and measured using GCMS. Physicochemical characteristics of sediment and seawater were also measured. RStudio was used to analyze and correlate the obtained data to identify trends in the microbial community variation in relation to the background sediment PH as well as other sediment and seawater parameters. This allows for assessment of the capacity of the studied marine area to accommodate successful future bioremediation endeavors.

The Shannon diversity index, which ranged from 5.6 to 6.6 in the studied coastal stretch, indicated a very highly diverse and rich microbial ecosystems. Non-metric multidimensional scaling (NMDS) showed no clear grouping among nearby sites; however, insignificant diversion between the wet and dry season microbial communities at each site was observed. Microbial communities showed clear dominance of the phylum *Proteobacteria* (45.7%), which is known for the ability to degrade PH. Phylum *Bacteroidetes* also comprised a large portion of the overall population (18.5%), and is also reported to contain groups of hydrocarbon degraders. Under *Proteobacteria*, the classes *Gammaproteobacteria* and *Alphaproteobacteria*, known for comprising genera of obligate and generalist hydrocarbon degraders in marine settings, dominated the population (24.86% and 11.76%, respectively). A high variation at the genus level was observed, in which several novel genera were identified at high abundances in certain locations, such as marine benthic groups *OTU_4* (5.4%)*, OTU_5* (2.9%) and *OTU_60* (3.2%). When correlating the most abundant microbial groups with the PH, weak negative correlations were observed, especially between PH and *OTU_4*. Given the high temporal and spatial variability in the microbial composition, a high capacity for biodegradation of PH was noticed. Understanding the actual composition of microbial communities in relation to existing petroleum hydrocarbon pollution, as well as in relation to other environmental parameters, would aid in optimizing future bioremediation approaches.

INTRODUCTION

Petroleum hydrocarbon (PH) pollution is a major concern given its huge impacts on entire marine ecosystems. A significant portion of PH is impossible to remove from sediments using common practices. Natural microbial degradation of lingering PH can easily be hindered by several limitations, including the actual composition of the microbial populations that are indigenous to the areas susceptible to contamination. Thus, the variation in the naturally occurring bacteria has a significant impact on the fate of individual hydrocarbons in affected zones. The variety of factors affecting the microbiological ecosystems in coastal marine areas reveal a complex system that is not directed by few simple variables. Hence, studies implementing biological treatment systems for bioremediation of PH contamination in marine sediments often show inconsistent results. This is usually because such attempts employ factors that are external to the composition of the microbial consortia in affected areas, without taking the intrinsic characteristics of the microbiological composition into consideration, in terms of the ability of the sediment to degrade target contaminants.

MATERIALS AND METHODS

Collection of Sediment and Seawater Samples.

Sediment and seawater samples were collected from eleven sampling sites distributed evenly across the shoreline of Lebanon, which extends over 225 km along the eastern side of the Mediterranean Sea. Figure 1 shows the different sampling locations from major coastal cities in Lebanon. These were numerically labeled from 1 to 11 northwards, following the prevailing northerly current along the Lebanese coast. Beach sediments and seawater were collected at each location in triplicate grab samples. Sediments were collected at a seawater depth of around 30-50 cm, while seawater samples were taken from the surface. Fine sandy sediments were collected from all sites except for the rocky beaches of Sarafand and Naqoura, where coarse sediments were sampled. In order to account for the seasonal variations in nutrients and microbial communities, the shoreline sampling was performed during both the dry and wet seasons (July, 2017, and January, 2018, respectively). Access to Naqoura was restricted during the wet sampling event, and thus it was not possible to test for wet season parameters at this location. Sediment samples were analyzed for background nutrients, TPH, and microbial communities, while seawater was analyzed only for background levels of nutrients.

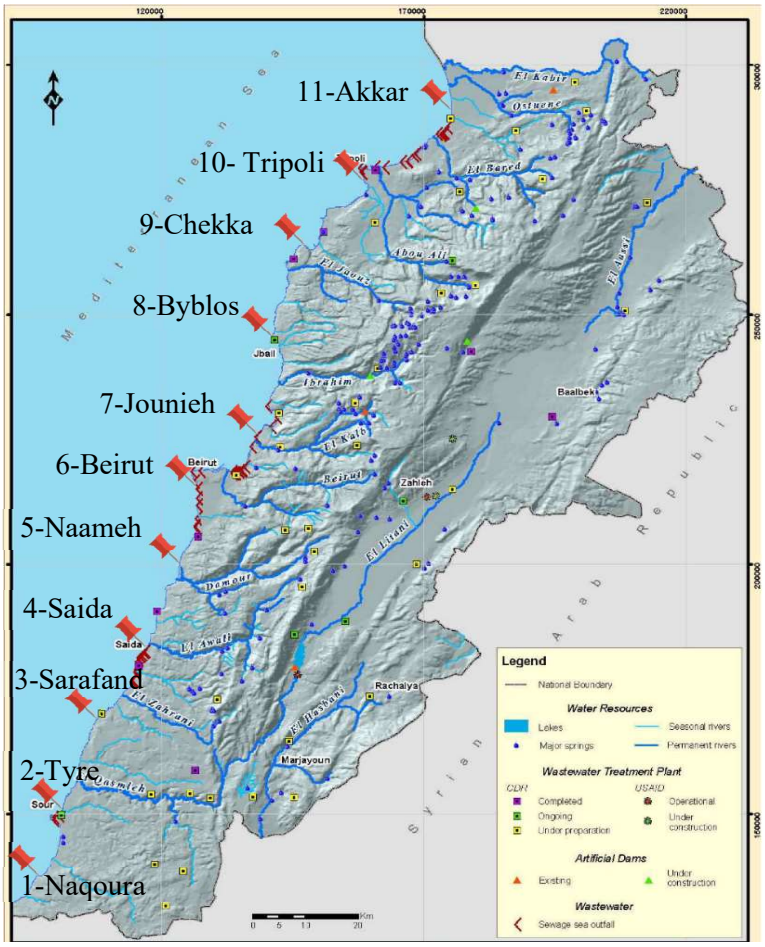

Figure 1. Location of the sediment and seawater sampling sites

NUTRIENTS ANALYSIS. Collected sediment and seawater samples during the dry and wet seasons were analyzed for nitrate, nitrite, ammonia, total Kjeldahl nitrogen, total nitrogen, phosphate, and total phosphorous. Sediment samples were first sieved (2 mm pore size), and aliquots of 30 g of were then extracted with 100 mL of distilled water at 300 rpm for 1 hour (Thermo Scientific MaxQ 6000 Shaker). The extract was then filtered and analyzed. Chemical analysis was performed by spectrophotometry using standard Hach methods.

PETROLEUM HYDROCARBONS EXTRACTION AND ANALYSIS. Sediment samples were extracted using an Accelerated Solvent Extractor (DIONEX ASE 350), following the method described by Richter (2000). Sediment extracts were analyzed for alkanes and polycyclic aromatic hydrocarbons (PAHs) by GC-MS (Agilent 7890A gas chromatography system coupled to an Agilent 5975C mass spectrometer), using an internal standard method described by Campo et al.

(2013). Normal and branched aliphatic alkanes including hydrocarbon chains ranging from nC10 to nC35, along with pristane and phytane, were analyzed.

MICROBIAL CHARACTERIZATION. Microbial communities in the sediments were characterized using 16S rRNA gene sequencing. Sediment samples were thoroughly mixed before DNA extraction. Triplicate DNA extractions were performed for each sediment sample using DNeasy PowerSoil Kit (QIAGEN) according to the manufacturer's instructions, totaling 9 DNA extractions per sampling location. Final DNA extracts from each sampling location were then pooled together in preparation for downstream DNA manipulation. The extracted DNA was then sent for DNASense (Aalborg, Denmark) for sequencing.

RESULTS AND DISCUSSION

Microbial Diversity. Principal Component Analysis (PCA) was performed to assess the relatedness of the microbial community composition between the different samples. Samples (dry and wet seasons) from the same site were grouped together and there was no clear grouping between nearby sampling sites (Figure 2). The non-relatedness of microbial communities along the Lebanese coast indicates microbial selectivity due to site specific environmental characteristics, including variation in the sediment and seawater nutrients, and TPH concentrations at each of the studied locations. Considering the relative closeness of the sampling sites (an average of 20 km between nearby sites), the difference in beta diversity among the studied sites indicates a high plasticity in the existing microbial communities, which can easily respond and adapt to variations in the local environment. However, the small divergence in the wet and dry season communities at a given location indicates resistance of the microbial community to significant changes in water and sediment characteristics.

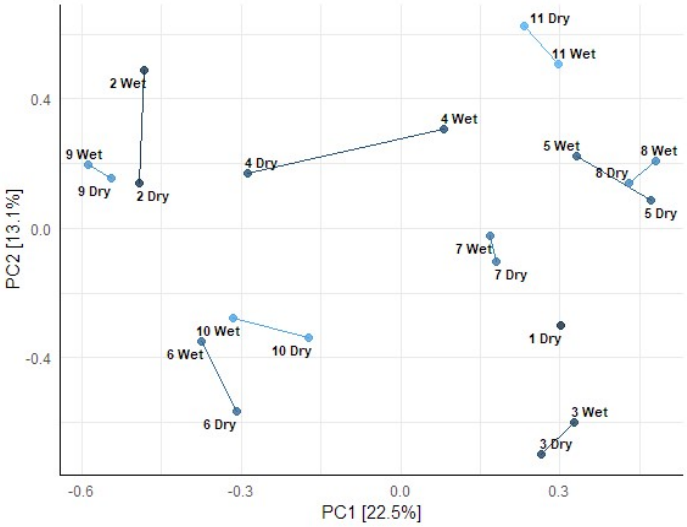

Figure 2. PCA plot showing the relatedness of the sediment microbial communities of the Shore of Lebanon

PH, NUTRIENTS, AND MICROBIAL STRUCTURE CORRELATIONS. The highest negative PH correlation coefficient was observed in the case of sediment TKN (-0.6), indicating that lower PH concentrations were observed in locations with higher concentrations of sediment TKN. This indicates that TKN is a major contributor to the degradation of PH, namely through stimulation of microbial metabolism. The relatively low overall concentration of PH in the sediment samples, ranging from 0.4 to 0.9 mg/kg of wet sediment along the shoreline of Lebanon, combined with no observed strong correlations with the most abundant bacterial taxa, might indicate that there is little impact of the actual PH concentration on the variation of the current microbial population, and that other parameters are rather influencing the observed microbial community structure. However, weak negative correlations were noticed between sediment PH and multiple genera, such as in the case of JTB255 marine benthic group OTU_4 (-0.4), *Rubripirellula* (-0.5), and *Portibacter* (-0.5), suggesting a possible involvement in PH biodegradation. Microbial taxa

showed also correlations with sediment and seawater nutrients. *OTU_4* decreased with increased sediment ammonia levels (-0.6). Same observation was made in the case of *Muriicola* correlated with sediment combined nitrate-nitrite levels (-0.5). Water reactive phosphorous showed weak negative correlations with *Rubripirellula* and *Blastocatella* (-0.5), while sediment reactive phosphorus and total phosphorus showed a week negative correlation with OTU_177. On the other hand, positive correlations between the nutrient levels and multiple bacterial taxa were noted. This was mainly significant in the case of sediment TKN, showing positive correlations with *Zeaxanthinibacter* (0.8), OTU_3 (*Flavobacteria*) (0.6), and *Blastocatella* (0.6). This indicates a major contribution of sediment TKN to the relative abundance of theses microbial taxa. Correlation analysis suggests that local components of the seawater and sediments are highly linked to the diversity of the microbial populations along the coast of Lebanon.

CONCLUSION

The presence of several potential hydrocarbon degrading taxa along the Lebanese shoreline suggests its bioremediation potential of possible oil spills in light of the planned sea excavation and oil extraction activities. The study also provides a baseline knowledge of the microbial communities existing along the Eastern shores of the Mediterranean which are still poorly characterized.

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