Exploring the Hydrocarbon Degradation Potential of Indigenous Bacteria using 16S Sequencing Approach

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Abstract: Total petroleum hydrocarbon (TPH) contamination is a serious problem, as it causes environmental damage and negative effects on human health. In most environments, microbial communities play a critical role in ecosystem functioning. A better understanding of the microbiome profile would increase the ability to manipulate this diversity in order to improve environmental quality through remediation. This paper provides the determination of the correlation between the relative abundance of bacteria with the physicochemical factors and total petroleum hydrocarbon. Metagenomic data were obtained by next-generation sequencing of 16s rRNA V3-V4 region as it is available in NCBI with accession number SR17068254-SRR17068293. Correlation between the relative abundance of bacteria with the physicochemical factors and total petroleum hydrocarbon were obtained by performing the coefficient of rank test on R Studio (v.4.1.3). The results of the correlation test at the phylum level showed that the relative abundance of the Aminicenantes, Chloroflexi, Firmicutes, and Synergistetes groups had a significant strong positive correlation to soil temperature. This is an indication that these group of bacteria can withstand high temperature and extreme environment. Meanwhile, at the class level, Deltaproteobacteria showed a strong positive correlation to the content of TPH. This is an indication that Deltaproteobacteria potentially comprised of many bacteria that has hydrocarbon degradation ability (p value < 0.05).

Keywords: Hydrocarbon, metagenomics, microbiome, pollution, remediation.

1. Introduction

Soil contamination by total petroleum hydrocarbons (TPH) has become a serious environmental problem. Most of the TPH discharged into the ecosystem is done without prior treatment [1], [2]. TPH is a term used to describe the various hydrocarbons found in petroleum [3]. These organic pollutants generally have low decomposition so that they can be trapped in the soil for a long period of time [4], [5]. Furthermore, weathering can increase the absorption of contaminants into soil pores, leading to problems of changing soil physicochemical characters and decreasing bioavailability and biodegradability of biota to pollutants [3], [6].

Finding effective approaches to soil remediation is an urgent need. Although several mechanical and chemical techniques are available, in *situ* bioremediation is a highly efficient and cost-effective approach. Several studies have reported that the use of indigenous microorganisms for in situ contaminant removal is promising, and investigating microorganism attribute in contaminated areas can improve bioremediation management [7].

Meanwhile, direct isolation and culture of microorganisms can only be carried out in limited quantities, so investigation of the overall relative's abundance of all microorganisms present in a given environment is not suitable employing this method. However, the latest metagenomic approach allows researchers to analyze overall microbial diversity. This technique is particularly efficient for studying biodiversity and genome analysis of samples isolated from complex environments where most microorganisms cannot be cultivated under normal laboratory conditions. Metagenomic methods allow researchers to establish correlations between microbial diversity and hydrocarbon levels at contaminated sites [8].

TPH content and physicochemical conditions of different soils will affect microbiome attribute such as high abundance of certain groups of bacteria [9]. TPH can have an indirect effect on the physico-chemical character of the soil [10]–[12]. These changes regularly alter the structure and function of soil microbial communities [13], [14]. TPH can inhibit members of soil communities, or stimulate the growth of microbial populations that can decompose or tolerate hydrocarbons [15]–[19]. Based on this background, potential microbiome on hydrocarbon degradation in TPH polluted areas correlated with soil physicochemical properties is expected to be a reference for remediation.

2. Methods

A. Data Analysis

This research was the further analysis of available data from [20]. The data is available in NCBI with accession number SR17068254-SRR17068293 [20]. Analysis of the correlation between microbiome data with physicochemical data and soil

TPH content was carried out through correlation analysis of coefficients of rank, and correlation network, all statistical tests were performed at a confidence level of 95% or α = 0.05.

3. Results and Discussion

Many environmental factors such as temperature, nutrient availability, electron acceptors and substrates play an important role in bioremediation and influence biodegradation reactions [21]. This is why most researchers have found that many petroleum hydrocarbon degrading bacteria can achieve excellent results during petroleum hydrocarbon degradation under laboratory conditions, but show unsatisfactory results in field-scale tests [22]. In this study, Spearman rank correlation is used to determine the correlation of the relative abundance of the microbiome to various environmental factors including water content, pH, salinity, temperature and TPH content. Spearman correlation testing shows variations in the response of microbiome structure to different environmental factors. One of the main mechanisms by which the distribution of a group within a community can change includes shifts in community composition, with better-adapted species replacing less adaptable species.
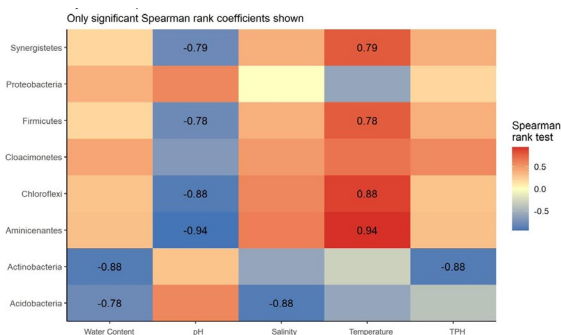

Fig. 1. Correlation analysis on the phylum level

Changes in bacterial community composition correlated with salinity are more likely because of the direct influence of salinity. However, the important thing to consider is that not all observed changes in microbial community composition that correlate with decreased salinity are necessarily due to salinity alone. In salinity factors, it was found that only Acidobacteria showed a significant response, namely negative responses at the phylum level, in addition to the class level (Acidobacteria Gp4, Acidobacteria Gp6 and Betaproteobacteria), family level (Rhodospirillaceae), genus level (Gp6, Gp4, and *Mycobacterium*), and species level (*Aciditerrimonas ferrireducens, Geminicoccus rosesus, Gemmatimonas aurantiaca, Lacibacterium aquatile, Mycobacterium littorale*) showed the same result. This finding is in line with previous research by Canfora (2014), Acidobacteria were also found to be significantly negatively correlated with salinity [23]. Based on correlation analysis between the physicochemical properties of soil, a significant positive correlation was found between TPH content, salinity, and moisture content. At the phylum level (Figure 1), the Acidobacteria and Actinobacteria groups, showed a significant negative response to water content. This is

not directly implying that the relative abundance decreases due to increasing water content, however, it might be due to high TPH and salinity content in locations with high water content as well. Other studies reported that the Actinobacteria group shows higher abundance under environmental conditions with lower salinity [24].

Meanwhile at the level of phylum (Aminicenantes, Chloroflexi, Firmicutes, and Synergistetes), class (Alphaproteobacteria and Synergistetes), order (Methylococcales, Synergistales, and Syntrophobacterales), family (Methylococcaceae, Synergistaceae, and Syntrophaceae), genus *(Smithella and Aminicenantes)* and species (*Smithella propionica*) had a significant negative response to soil acidity. Previous research found that Aminicenantes showed the highest relative abundance in hydrocarbon-affected environments. The detection of Aminicenantes in extreme environments and the patterns of different community structures observed, reflect a high degree of interphylum metabolic diversity and adaptive abilities that allow their survival and growth in a variety of habitats and environmental conditions [25].

Environmental factors such as temperature also showed association with microbiome growth. At the phylum level, the relative abundances of the Aminicenantes, Chloroflexi, Firmicutes, and Synergistetes groups had a significant strong positive correlation with soil temperature. At the class level (Synergistia) shown in Figure 2, the order level (Syntrophobacterales, Synergistales, Methylococales) is shown in Figure 3, the family level (Syntrophaceae, Synergistaceae, and Methylococcaceae) is shown in Figure 4, the genus level (*Smithella*, *Aminicenantes)* is shown in Figure 5, the species level (*Smithella propionica*) showed similar results (Figure 6).
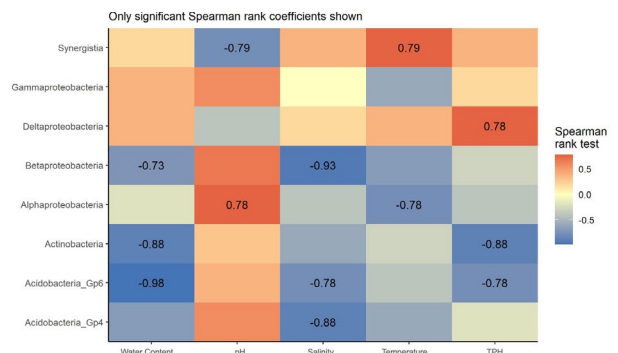

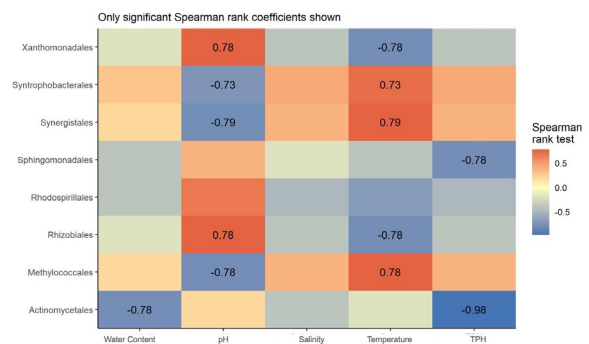Fig. 2. Correlation analysis on the class level

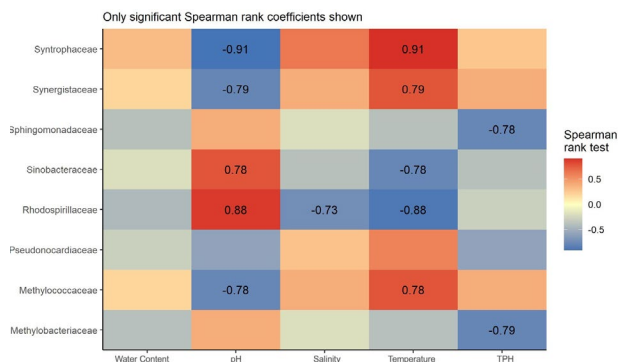Fig. 3. Correlation analysis on the order level

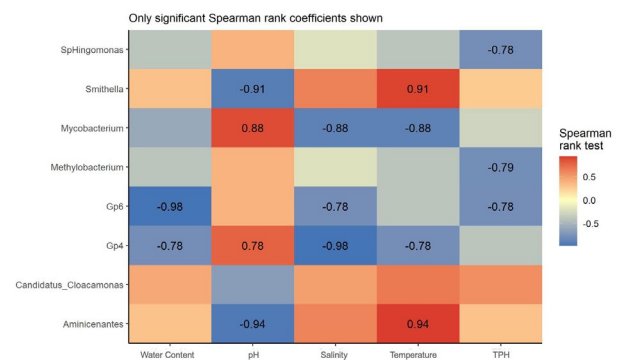Fig. 4. Correlation analysis on the family level

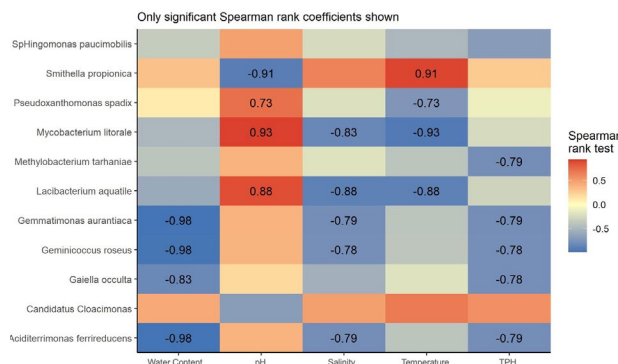

Fig. 5. Correlation analysis on the genus level

Fig. 6. Correlation analysis on the species level

Analysis of environmental factors in the form of TPH levels, at the phylum level Actinobacteria was the only group that has a significant negative response. At the class level (Acidobacteria Gp6 and Actinobacteria), order level (Actinomycetales and Sphingomonadales), family level (Sphingomonadaceae, Methylococcaceae), genus level (*Sphingomonas, Methylobacterium, Gp6*), species level (*Methylobacterium tarhaniae, Gemmatimonas aurantiaca, Geminicoccus roseus, Gaiella occulta, Aciditerrimonas ferrireducens*) also has a negative correlation with TPH level. However, at the class level, Deltaproteobacteria showed a strong positive correlation to TPH content implying their potential as degradation agents, this is in line with the previously mentioned that Deltaproteobacteria comprise many groups of hydrocarbon degraders [20].

It is known that bacterial growth requires a sufficient amount of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and various elements. However, the main components of petroleum hydrocarbons are only carbon and hydrogen, therefore the environment must have enough other nutrients to ensure the growth of degrading bacteria. The addition of naturally available nitrogen and phosphorus-containing nutrients has been successfully applied to stimulate petroleum biodegradation on a number of different coasts [22], [26]. For aerobic degradation processes, using oxygen as an electron acceptor is important, but usually inadequate in petroleum-contaminated environments due to limited air permeability.

Gogoi *et al.* (2003) reported that up to 75% of hydrocarbon contaminants were degraded within 1 year in field tests by controlling and regulating aeration [27]. However, providing an adequate supply of oxygen to stimulate bioremediation of petroleum pollutants in the environment is expensive and inefficient. Therefore, the application of bulking agents such as sawdust into the soil to increase permeability or other electron acceptors (NO_3, Fe^{3+}, or Mn^{2+}) into anoxic environments to stimulate anaerobic microorganisms is often more economical than oxygen supplementation [28], [29].

4. Conclusions

The locations with the highest contamination are inhabited by microbiome groups that have resistance to high temperatures and are resistant to chemical stress. The relative abundance of some microbiome groups has a significant positive correlation to soil physicochemical properties, while there are some groups that have a significant negative correlation to soil physiochemistry. At the phylum level, the relative abundances of the Aminicenantes, Chloroflexi, Firmicutes, and Synergistetes groups have a significant strong positive correlation with soil temperature. At the class level, Deltaproteobacteria.

The correlation analysis has shown the pattern of certain group of bacteria has the positive correlation with temperature in TPH content. However, it is still necessary to analyze the correlation with a wider range of temperature and TPH content so that the more comprehensive pattern could be evaluated. On the other hand, it is necessary to explore and develop cultivation methods for special groups that have not been isolated until now so that later the assessment process of the ability and efficiency of degradation of hydrocarbons under varying conditions can be carried out.

This research suggests that a possible remediation strategy is bio-stimulation. On the other hand, applicative research in the form of analysis of the ability of potential local isolates also needs to be carried out so that validation of bioremediation strategies can be applied directly in polluted locations.

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