Evaluation and Comparison of the Ability of Indigenous Pseudomonas Bacteria from Musa creek to remove poly Aromatic Compounds

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Introduction
Oil and its compounds are becoming an important problem and the most prevalent type of contamination in the terrestrial and aquatic ecosystems. Among oil hydrocarbons, PAHs are of significant concern because of their properties. PAHs are a large group of organic compounds with two or more fused aromatic rings in linear, angular or cluster arrangements. Many of these compounds have been found to have toxic, mutagenic and carcinogenic properties. Their properties specially change with variations in the number of rings. Thus, it is necessary to clean polluted areas from these compounds immediately. Bioremediation is the most effective option to clean up this class of environmental pollutants. According to its definition, bioremediation techniques are processes applied to microorganisms to breakdown or detoxify pollutants. Bioremediation by suitable bacterial strains is an effective and economical option, based on microorganisms’ ability to degrade contaminations. Isolation of bacteria with high ability of contaminants decomposition is the most important purpose of bioremediation experiments. Therefore the important objectives of present work are isolation and characterization of the best naphthalene degrading bacteria from Musa creek and comparison of their abilities.

Materials & Method

Sampling
In order to isolate Musa Creek indigenous naphthalene degrading bacteria, sediment samples were aseptically collected from 3 polluted stations. All samples were stored in an ice box and brought to the laboratory immediately.

Enrichment, isolation and purification
MSM medium were prepared. Naphthalene was used as a delegate of the PAHs with initial concentration of 60ppm. To increase compatible microbial population with naphthalene, enrichment operations were accomplished pursuant nether instruction. Five grams of sediment samples were added to 10 ml sterile distilled water in the experiment tubes. Serial dilutions were provided and centrifuged at 2000 rpm for 10 minutes. Subsequently, 500μl of adrift substances were mustered from each tubes and added to 100 ml of sterile mineral salt medium (MSM), in beakers. The flasks were incubated in 150 rpm at 30°C for seven days. After this duration, 10 ml of each beaker was transferred to fresh MSM medium and incubated as described above. This function was repeated for 3 times. Thereafter, 1ml of the last enrichment cultures was inoculated to solid MSM medium and spread on the surface of the medium. Distinct colonies appeared after several days. Pure cultures were procured by the implementation of several consecutive subcultures.

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Selecting the best naphthalene degrading bacteria

The best isolates were designated based on bacterial growth rate and their ability to degrade naphthalene. Therefore, 100 ml of MSM medium containing naphthalene was prepared in some 250 ml beakers and pH was adjusted on 7.2. The best isolates were selected on the basis of bacterial growth rate and their ability to degrade naphthalene. Their ability to degrade naphthalene as sole carbon source was determined by diminishing the pH medium due to producing acidic metabolites and the intensification of turbidity due to bacteria growth in the presence of naphthalene.

Identification of bacteria

Two of the best isolates with the lowest pH medium and highest growth were submitted for further studies by performing morphological scrutiny. For accurate characterization, various biochemical tests were carried out such as gram reaction, catalase test, KOH test, mobility, oxide reaction, urease, nitrate reduction, indol production, Oxidative-fermentative test, Lysine decarboxylase, citrate, MethylRed, Voges-Proskauer, TSI, Gelatin liquefaction, Growth at 42°C, growth on MacConkey and Phenylalanine deaminase. Results of these tests were coincided with bergy’s manual of systematic bacteriology, to identify the species levels for these isolates.

Naphthalene degradation by selected bacteria

Degradation quantitative analysis of existent naphthalene in the medium was accomplished by High Performance Liquid Chromatography (HPLC) in specific times. HPLC analysis of naphthalene was performed by a Knauer liquid chromatography (Knauer Instruments, Germany) fitted with C18 column experiments in triplicates.

Three similar cultures with no inoculation were incubated at the same condition in order to evaluate abiotic degradation in sterile mediums such as volatilization, physicochemical or photo-oxidation process. At definite intervals, 5 ml of each culture was picked up and residual naphthalene was extracted with hexane. The residual extracts were dried and concentrated at room temperature.

Water and acetonitril (30:70) were scaled down as HPLC solvent in mobile phase. Naphthalene degradation was balanced at the end of experiments. Factorial ANOVA in SPSS software were used to identify the significance of probability between media containing the selected bacteria and abiotic controls.

Results and Discussion

Bioremediation with suitable bacterial strains is an effective and economical option that is based on microorganisms’ ability to degrade contaminations. Bacterial degradation is a major pathway for the removal of PAHs in the nature, but we can prompt this process with isolating efficient bacteria from contaminated sites and use them for purification of such areas.

In the present investigation, a total of 8 bacteria were separated from oil polluted sediments of Musa creek. They were called from MB10 to MB80. Strains MB30 and MB10 possessed the highest maximal growth. The pH of all cultures declined after 3 days of incubation. MB30 and MB10 had lowest medium pH. In general, these two bacteria with highest medium turbidity and lowest medium pH among the other isolates had the best results in degradation of naphthalene and growth, in the proximity of this contaminant. Therefore, this type of bacteria is suggested for further biodegradation experiments.

Identification results showed that these two bacteria had similar traits of Pseudomonas genes. High derivative ability of pseudomonas sp. is often because of their peculiar genetic to produce the required catabolic enzymes. Accurate identification was accomplished on the basis of Bergy’s manual of systematic bacteriology (1999). Thus, Morphological surveys and biochemical tests on isolates MB10 and MB30 showed similar characteristics with Pseudomonas putida and Pseudomonas aeruginosa, respectively.

Naphthalene depletion analysis results showed that most changes in naphthalene are accrued in incipient hours. This suggests that naphthalene was taken from bacteria or adsorbed to their cell wall.
Showed degradation measures were 96.14±0.662% and 91.48 ±1.1501% by P. aeroginosa and P. putida, respectively, after 120 hours of incubation. Averagely, 14.6% of naphthalene was removed from control cultures, after this period.

One-Way ANOVA statistical analysis was used to identify whether the removal of naphthalene is affected by the presence or absence of bacteria. There were no significant difference in naphthalene degradation between cultures inoculated with P. aeroginosa and P. putida. This analysis showed that there is probability significance in the level of p≤0.05 between two bacterial treatments by abiotic treatment in the removal of naphthalene. In general, there was no considerable mineralization in abiotic controls. This consequence shows that nonbiological agents do not affect the removal of naphthalene from the beakers. Factorial ANOVA showed that there is a considerable decline in naphthalene concentration between the two states of inclusive media degradation with bacteria and the same media condition without bacteria.

In this research we found two kinds of bacteria with high ability to degrade naphthalene belonged to Pseudomonas genus. High derivative ability of pseudomonas sp. is often because of its peculiar genetic to produce the required catabolic enzymes. The ability of P. aeroginosa and p. putida to degrade oil hydrocarbons has been reported by several scholars. 

Kumar et al. (2006) isolated Pseudomonas putida 5a1 and Pseudomonas aeroginosa DHT-GL from oil polluted soils, in Guanaco Asphalt Belt of Venesuela. These species were tested for their ability to degrade naphthalene and some other oil hydrocarbons. The results of oil reduction in MSM medium inoculated with P. putida 5a1 and P. aeroginosa DHT-GL were 44% and 24%, respectively.

Thavasi et al. (2007), isolated 4 bacterial species P. aeroginosa, L.delbrueckii, B.megaterium and C.kustscheri from oil polluted marine environment. Results of the experiments indicated that P. aeroginosa with 85.15% oil degradation had the highest ability to remove oil hydrocarbons among the others. Continuous experiments demonstrated good emulsification of these species at the presence of various hydrocarbon sources such as naphthalene and anthracene.

In general, these experiments proved that there are some bacteria with the ability of degradation and utilization of naphthalene as a sole source of carbon and energy among microbial community in Musa creek. Therefore, we can conclude that bacterial degradation is a pathway to the removal of hydrocarbon contamination in Musa creek and because of their ability to degrade aromatic hydrocarbons we can suggest these bacteria for field experiments and for using in critical oil pollutions.

**Key word**
Musa Creek, Bioremediation, Naphthalene, P. aeroginosa, P. putida