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Degradation of naphthalene by bacterial isolates from the Gol Gohar Mine, Iran

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ABSTRACT

Naphthalene is an ubiquitous pollutant of the environment and the biodegradation of this pollutant has been receiving constant scientific consideration. The aim of this study was to isolate and identify bacteria that could degrade naphthalene from three regions of the Gol Gohar Mine at Sirjan, Iran. In this study, the total naphthalene degrading bacteria were quantified with the most probable number (MPN) and the colony forming unit (CFU) methods. The results showed that most of the bacteria communities capable of degrading naphthalene aggregated in the (WG) site. Among 22 isolated bacteria, seven strains were selected for their ability to grow at higher concentrations of naphthalene (300 and 400 mg/l) and biochemical characteristics. Finally, two strains named isolates 72N and 79N were selected for analysis of the 16S rRNA sequences. Strain 72N was identified as *Pseudomonas fluorescens* AHB72N and strain 79N was shown to be related to *Pseudomonas gessardii* AHB79N. The results of biodegradation tests showed that these two strains could degrade 600 mg/l naphthalene in 7 days. The results indicated that strain 79N showed higher potential for removing naphthalene than strain 72N. Practical application of bacterial strains for the degradation of naphthalene from the industrial zones opens interesting prospects. The results of this study provide useful information in evaluating naphthalene degraders isolated from wastewater and industrial sites.

Keywords: Biodegradation; Degrading bacteria; Naphthalene; Gol Gohar Mine

Introduction

The United States Environment Protection Agency (EPA) has designated 16 Polycyclic Aromatic Hydrocarbons (PAHs) as priority pollutants because they are toxic and carcinogenic compounds. The most important sources of environmental pollution worldwide are the discharge of industrial waste and petroleum hydrocarbons into aquatic and terrestrial ecosystems (1, 2). Naphthalene is the simple member of the PAHs group that belongs to 16 PAHs classified as priority pollutants by (EPA) and a common contaminant found in the environment. This di-aromatic substance and its methylated derivatives are considered as some of the majority harmful and toxic substances in the aquatic environment, petroleum and industrial wastes (3). Many procedures such as volatilization, leaching, chemical and photo-oxidation are efficient in decreasing the environmental level of PAHs.

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Microbial activity has been considered to play a considerable function in cause of PAHs elimination from environment (4). Biodegradation using effective microorganisms is commonly the preferred and main pathway of PAHs elimination from polluted environments because of its cost effectiveness and perfect clean up. Bioremediation technology is able to convert these pollutants into microbial biomass, CO_2 and water (5). The main benefits of bioremediation technology are its cost and environmental helpfulness over contractual treatments, such as dredging, volatilization, physical processes and electrochemical remediation (6). Several genera of microorganisms have been prosperously utilized in main hazardous sludge clean-up procedures (7).

Extensive researches have been done on the isolation of bacteria from the natural environment that have the ability of using PAHs compounds as the sole carbon and energy source (8). A great diversity of microorganisms especially bacterial strains have been repetitively isolated mostly from the soil and water that are able to degrade some PAHs like naphthalene, Anthracene and Phenantherne. These isolates are prevalently Gram-negative bacteria, the majority of them belong to the genus *Pseudomonas.* The biodegradation pathways have also been described in bacterial strains belonging to *Aeromonas*, *Bacillus*, *Beijerinckia*, *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Sphingomonas* (9, 10).

The aim of this research was to study the community of naphthalene-degrading bacteria from water and soils of three regions of the Gol Gohar Mine from Kerman province in Iran. Then, we evaluated the biodegradation rate of naphthalene by native isolated bacteria in different concentrations of 1-naphthalene. This research depicts the isolation and identification of two dominant strains of naphthalene-degrading belonging to the genus *Pseudomonas*.

Materials and methods

Sample collection

Water and mine soil samples were collected from the superficial layer (0–10cm) from three contaminated places in the Gol Gohar Mine in Sirjan (Iran). These sampling sites include: the Magnetit site (WG), the Hematit site (WH) and the Near Hematit site (NH). The differences of these regions are as follows: in the Magnetit site mine wastewater was discharged, at the Hematit site oil contamination was detected, and at the Near Hematit site pollution with heavy metals was observed. The naphthalene contamination in these regions ranged as follows: WG (470 mg/l), WH (420 mg/l) and NH 380 (mg/l). Mine soils samples (200 g) and water samples (300 ml) were taken from 1 to 10 cm beneath the superficial of land using a sterile spoon. The mine soils samples (200 g) and water samples (300 ml) were gathered in the plastic bags and transferred to the microbiology lab, where they were stored at fridge temperature until analysis (11).

Quantification of naphthalene-degrading bacteria in mine samples

Total naphthalene-degrading bacteria were quantified with the most probable number (MPN) procedure using microtiter plates and colony forming unit (CFU) method. For enumeration of naphthalene-degrading bacteria, mine soils or water samples were serially diluted and cultured (100 µl) on Bushnell Hass agar (BH) medium. All plates were incubated at 30±1°C. After seven days, the numbers of grown colonies were counted. The results were described as colony forming units per one gram of soil (CFU gr^{-1}) (12, 13).

Enrichment and isolation naphthalene-degrading bacteria

The Bushnell Hass (BH) medium was used for enrichment and isolation. The composition of BH medium (per liter) was as follows: 0.2 g $MgSO_4$. $7H_2O$, 0.02 g $CaCl_2$. $2H_2O$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3, 0.05 g $FeCl_3$ and trace elements (1ml/l). For preparation of solid culture media, 15 g agar per liter was added to the above media and was spilled into plates. Then 200 mg/l of naphthalene were dissolved antecedently in 0.1 mL acetone and added to the media. After perfect evaporation of acetone, 5 g of mine soils or 5 ml of mine water samples were added to BH medium and the flasks put on the shaker incubator (180 rpm, INFORS AG, Switzerland) at 30°C for 168 hrs. Then 5 ml aliquots were removed to fresh medium. After a series of two another passage process, inoculants from the flask

were streaked out, and phenotypically various colonies purified on BH-agar medium. Phenotypically various colonies prepared from the plates were transferred to fresh media with (200 mg/L) naphthalene to delete agar consuming bacteria. Finally isolates just showing significant growth on naphthalene were stored in stock media with glycerol at -20°C (14, 15).

Assay of growth and naphthalene degradation by isolates

Growth curves of the isolates were commonly estimated indirectly by turbidity estimation as (O.D. at 600 nm). To estimate the remaining naphthalene after the time course standard graphs were prepared using 1 to 10 mg/l of naphthalene, the naphthalene degradeation assay was performed using calibration curve of naphthalene at 276 nm (16).

Biochemical characterization of the isolates

The isolates were characterized with color and morphology. Gram staining and various other biochemical experiments such as oxidation/ fermentation test, production of acid from carbohydrates, nitrate reduction, oxidase test, catalase test and production of gas were tested as described previously and performed based on Bergey's manual for identification of Bacteriology (17).

DNA extraction and PCR amplification of 16S rRNA genes

The identities of the isolated strains were depicted through sequence analysis of the 16S rDNA gene. The cetyltrimethylammonium bromide (CTAB) method was used to extraction total DNA from the isolates (18). The partial 16S rRNA genes were amplified in a thermal cycler through applying the forward primer, Bac27-F (5′-AGAGTTTGATCCTGGCTCAG-3′) and universal reverse domain particular bacteria primer Uni-1492R (5′-TACGYTACCTTGTTACGACTT-3′). The amplification reaction was carried out in 25 µl volume including, 2 mM $MgCl_2$ (1 µl), 10X PCR reaction buffer (200 mM Tris; 500 mM KCl) (2.5 µl), 2 mM each dNTP (2 µl), 0.15 mM each primer (1 µl), 1U (0.5 µl) Taq DNA polymerase and 2 µl of template DNA (50 pmol). In order to stabilize the reaction, purified water was supplemented. The amplification of 16S rRNA gene for PCR was carried out under following conditions: 95°C for 5 min (1 cycle); 94°C for 1 min, 54°C for 1 min, 72°C for 2 min (35 cycles) and 72°C for 10 min after the final cycle (19). Electrophoretic analysis of DNA was performed using a horizontal agarose gel 2% (w/v) in TBE buffer (Tris 0.089M, Boric acid 0.089 M, Ethylene diamine tetra acetic acid (0.002 M) at pH 8.5). Ethidium bromide (EtBr) was used as nucleic acid staining for agarose gel electrophoresis. PCR products were sequenced by Macrogen (Korea) after the DNA bands detected on a UV light box (254 nm wavelength) through an ultra-bright LED transilluminator. The assembled sequences were subjected to the BLAST server and the DNA data bank of the National Center for Biotechnical Information NCBI, and 16S rRNA genes were accurately aligned with other validly published *Pseudomonas* species and were regained for phylogenetic analysis of the *Pseudomonas* strains. Alignments of sequences were performed through the software Clustal W. Reconstruction of phylogenetic trees was performed using Neighbor-Joining algorithms included in MEGA-5 software package (20, 21).

Analysis of emulsifying activity and bacterial adhesion to hydrocarbon (BATH) of the isolates

The emulsifying activity of the isolates was measured by adding two ml of diesel and two ml of the culture supernatant in an experimental tube and mixing with a vortex for 2 min to procure maximum emulsification and permitted to stand for 24 h. The percentage of the E_{24} emulsifying index was estimated using the following formula:

$\%E_{24}$ = (Total height of the mixture - Height of emulsified diesel)/Total height of the mixture × 100.

The estimation of BATH was assayed to identify modifications in bacterial surface hydrophobicity while growth on minimal salt medium with %0.02 naphthalene (22). In present investigation the method for measurement of BATH was performed in accordance with procedures explained by Pruthi and Cameotra (23).

The effect of different concentrations of naphthalene on growth of selected bacterial strains

The growth rate of selected bacterial strains in various concentrations of naphthalene was determined. The BH medium was supplemented with various concentrations of naphthalene (0.02%, 0.03%, 0.04%, 0.05% and 0.06%). The flasks were incubated for 168 hours at 30±1°C on a rotary shaker incubator, managing at 180 rpm (Shaker INFORS AG, Switzerland). Growth rate was ordinarily estimated indirectly with turbidity measured as optical density (OD600 nm) in a UV-visible spectrophotometer (24, 25).

Gas Chromatography of remaining of naphthalene in BH medium

Gas Chromatography (GC) was used to precisely determine naphthalene degradation. After 168 hrs of incubation, 5 ml of BH medium was utilized to assay remaining naphthalene. For extraction of residual naphthalene in BH medium 1800 µl of ethyl acetate supplemented with 200 µl of methanol were added into BH medium and placed in the vortex for 5 mins. The ethyl acetate was evaporated completely; the extracts were concentrated and added to 1 ml normal hexane. Finally, 1 µl of the extracts was used for assaying the amount of residual naphthalene and injected to Shimadzu GC-14B, prepared with flame ionization detector (FID). In this research the analysis method (GC) for measurement of naphthalene degradation was performed in accordance with procedures explained by Kom et al. (26, 27). The percentage of naphthalene degradation (N%) was estimated with the following formula: $N\% = 100(CI - CF)\ CI^{-1}$, in which CI was the initial concentration of naphthalene, CF was the final concentration of naphthalene after 168 hr of incubation (26, 27).

Results

Enumeration of naphthalene-degrading bacteria in collected mine samples

The quantity of naphthalene-degrading bacteria in three mine samples (soil and water) was determined with two enumeration methods (CFU and MPN). The results are presented in Table 1. According to these results the Magnetit site (WG) of Gol Gohar mine had the highest quantity of naphthalene degrader and less naphthalene degrading bacteria exist in the Near Hematite site (NH) of this mine.

Table 1. Quantity of naphthalene degrading bacteria (CFU and MPN) in three mine samples around Gol Gohar mine (South Iran). Abbreviations: Magnetite site (WG), Near Hematite site (NH), and Hematite site (WH).

Sample	MPN Naphthalene degrading bacteria	CFU 10^{-3} Naphthalene degrading bacteria
WG	21×10^{6}	35×10^{4}
NH	93×10^{3}	6×10^{4}
WH	15×10^{4}	20×10^{4}

Isolation and identification of naphthalene-degrading bacteria

Twenty-two naphthalene-degrading bacteria were isolated from enrichment mine soil and water cultures that were established at 30ºC for seven days. The screening results show that all isolated bacteria can degrade (d) naphthalene at a concentration of 200 mg/l. Seven isolates were selected for capability of growth on a higher concentration of naphthalene (300-400 mg/l.). So, these isolates were first classified by biochemical tests. The results of the biochemical examinations are shown in Table 2. The results confirm that all the isolated bacteria were Gram negative and have no motility. From these isolates strains 79N and 72N were able to utilize high concentrations of naphthalene (600 mg/l), therefore as a next step, molecular determination of the isolates was carried out by amplification and sequencing the 16S rRNA gene and comparing the sequences to the database of known 16S rRNA sequences. The

molecular determination shows that these two isolated strains (79N and 72N) belong to *Pseudomonas gessardii* AHB79N and the strain 72N is related to *Pseudomonas fluorescens* AHB72N. The GenBank ID of the bacterial strains in NCBI are LN866625 (Strain AHB79N) and LN866624 (Strain AHB72N). The phylogenetic trees including these two strains are shown in Figure 1.

Table 2. Gram staining and biochemical characterization of strains. Symbols used: +: Positive response, -: Negative response.

Strain name	GRAM STAIN	CATALASE	OXIDASE	O	F	NITRATE	CITRATE	S	I	M	TSI
N71	_	+	_	+	+	+	+	_	_	_	acid/alkali
N72	_	_	+	_	_	+	+	_	_	_	alkali/alkali
N75	_	+	_	+	_	+	+	_	_	_	alkali/alkali
N76	_	+	_	+	+	+	+	_	_	_	acid/ acid
N79	_	+	+	+	_	+	+	_	_	_	alkali/alkali
N80	_	+	+	_	_	+	+	_	_	_	alkali/alkali
N81	_	+	+	+	+	+	+	_	_	_	acid/alkali

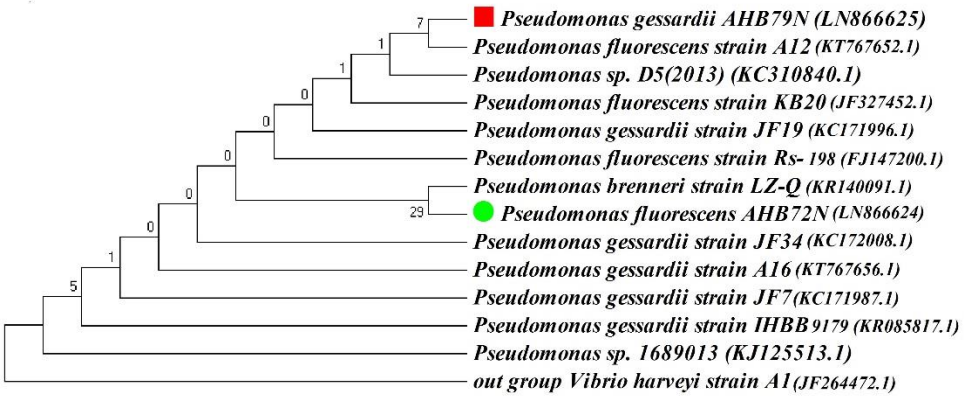

Figure 1. Phylogenetic tree showing the inter-relationships of strains AHB79N and AHB72N with the most closely related type strains of the genus *Pseudomonas* inferred from sequences of 16S rRNA gene. *Vibrio harveyi* strain A1 (JF264472.1) was used as outgroup. The tree was generated using the neighbor-joining method. Bootstrap values expressed as percentages of 1000 replications, are given at the branching point. The accession number of each strain is shown in parentheses.

Growth rate and percentage of naphthalene degradation by strains

The results of naphthalene degradation assay and the growth rate of seven isolated bacteria are presented in Table 3. As shown in Table 3, isolated bacteria reached 1.5 OD after 7 days and approximately degraded 50 percent of naphthalene in BH medium, strains 79N and 72N reached 1.8 and 1.35 OD after 7 days and approximately degraded 60 percent of naphthalene in BH medium. Four strains showed high growth rate and naphthalene degradation (79N, 72N, 80N and 81N) at 500 mg/l between 7 isolates. Finally, 2 strains (79N and 72N) that showed high growth rate and naphthalene degradation (55% and 46%) at 600 mg/l were selected for detailed examination. Growth curve (OD. 600 nm) and naphthalene degradation in tube culture of the 79N strain at 30°C in 600 mg/l naphthalene concentration are presented in Figure 2A. The growth of the 79N strain was started after a short delay, and after proceeding for several hours the bacteria entered the log phase. The highest growth occurred 96 hrs after inoculation, and right after it, the stationary phase began.

Table 3. Qualitative degradation, rate of naphthalene removal (%) at 200 mg/l, value of OD 600, (E_{24}%) and (BATH %).

Strain	Qualitative degradation	Rate of naphthalene removal (%)	Value of O.D600	(E24%)	(BATH%)
71N	+ +	45	0.46	46	6
72N	+ + + +	63	1.358	6	38
75N	+ + +	47	0.592	5	21
76N	+ +	52	0.47	8	5
79N	+ + + +	68	1.878	7	8
80N	+ +	57	0.638	5	23
81N	+ + + +	60	1.77	5	5

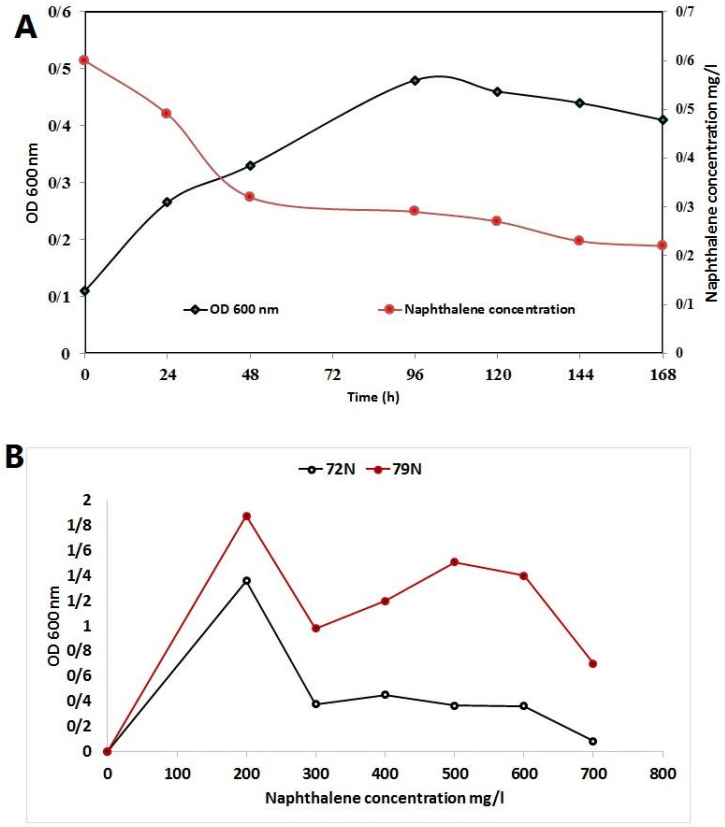

Figure 2. Growth (as O.D. 600 nm) and naphthalene removal in culture of *Pseudomonas gessardii* AHB79N at 30°C in 600 mg/l naphthalene concentration with calibration curve of naphthalene during 168 h incubation (A), and Growth curve of bacteria strains (79N and 72N) in different concentrations of naphthalene (B).

Bacterial surface hydrophobicity (BATH) and emulsifying activity of isolates

The emulsifying Index and BATH tests were examined for all isolated bacteria for selection of prevalent strains. The results for these examinations are presented in Table 3. The highest percentage of BATH was for the 72N and 74N strains (38% and 45%) and the highest emulsification activity (E_{24}) related to strain 71N was 46% (Table 3).

Effect of different concentration of naphthalene on the growth rate of selected isolates

The results showed that all isolated bacteria (22 isolates) had the ability to degrade the initial concentration of naphthalene (200 mg/l). Seven strains showed growth and naphthalene degradation at 400 mg/l concentration of naphthalene. Among these seven isolates four isolated strains (79N, 72N, 80N and 81N) had high growth at 500 mg/l concentration and selected for further experiments. Strains 79N and 72N showed naphthalene degradation at 600 mg/l

concentration after 7 days (Figure 2B). As shown in Figure 2, the maximum concentration of naphthalene that can be degraded by these two strains was 600 mg/l.

Analysis of residual naphthalene with GC by strain 79N

The results of GC analysis of residual naphthalene at BH medium showed that the *Pseudomonas gessardii* AHB79N strain was the best naphthalene degrader and degraded 55% of naphthalene at 600 mg/l concentration after seven days. Results of naphthalene degradation measured by GC are shown in Figs. 3A and 3B. Control A1 is blank naphthalene and A2 for strain AHB79N, respectively.

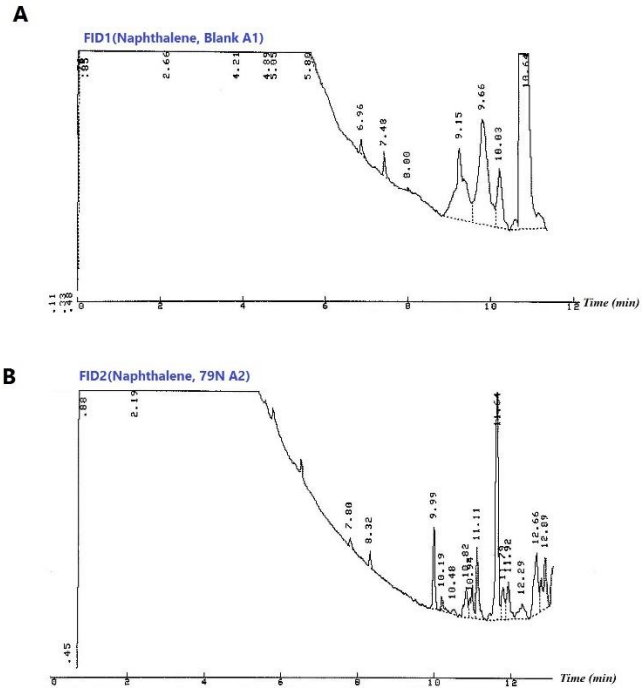

Figure 3. Analysis of naphthalene 600 mg/l by GC after 168 h incubation. A: As blank, and B: By strain 79N.

Discussion

Aromatic compounds are pollutants, often mutagenic and toxic, found in most terrestrial environments that increase in industrial development. Aromatic compounds and are products of incomplete combustion. Main components of petroleum are continuously released into ecosystems, and pose a risk for human health due to their carcinogenic nature and bioavailability in soil and water environments (28). Naphthalene is one of the PAHs widespread in the environment. Many various species of bacteria capable of degrading naphthalene have been obtained, mainly from water and soil environments.

Most of the naphthalene-degrading bacterial strains that were earlier discovered belong to the genus *Pseudomonas*. The naphthalene-derivative gene clusters in this genus were highly analogous to the naphthalene (NAH) gene cluster from the NAH7 plasmid in *Pseudomonas putida* strain G7 (29). Different bacterial strains such as: *Pseudomonas*, *Rodococous*, *Marinobacter*, etc., have earlier been demonstrated to be able to degrade aromatic compounds, but most of the study exhibited that *Pseudomonas* has a higher capability to degrade the naphthalene (30, 31). In addition, naphthalene biodegradation was studied using the bacterial strain *Pseudomonas putida* S2 (32). Bioremediation method is the application of microorganisms especially bacterial to eliminate environmental contamination such as aromatic compounds. This method is applied for hazardous toxic substances and also for aromatic contamination cleanup. Bacteria convert this compound into less injurious compounds and use them as

the only source of carbon and energy (33).

Researchers have applied various methods for assay naphthalene biodegradation by naphthalene-degrading microorganisms. For example naphthalene removal assay was carried out using the standard calibration curve of naphthalene (34-37). Kom et al. (27) applied GC to estimate the removal of naphthalene values. In the present investigation, we isolated 22 naphthalene-degrading bacteria from the Gol Gohar mine in Iran; seven of these isolates were checked for growth on the BH medium containing 400 mg/l of naphthalene. Two bacterial isolates 79N and 72N were selected on the basis of their best growth on naphthalene (600 mg/l). The residual concentration of naphthalene, determined by GC analysis and the AHB79N isolate, showed a degradation of 55% during the 168 hr incubation. Phylogenetic analysis based on the 16S ribosomal RNA sequences of two strains showed that the isolated bacteria (strains 79N and 72N) belong to *Pseudomonas gessardii* AHB79N and *Pseudomonas fluorescens* AHB72N, respectively. The results corroborate the opinion that the mine environment is a suitable source of bacterial strains for aims of aromatic compound biodegradation.

Conclusion

The naphthalene-degrading bacterial strains were isolated from three contaminated regions in the Gol Gohar mine. This finding supports the fact that naphthalene-degrading microorganisms are widely distributed in the various environments of the mine. Our experiments demonstrated that two species of bacteria *Pseudomonas gessardii* AHB79N and *Pseudomonas fluorescens* AHB72N could effectively degrade naphthalene in a BH medium. In addition, *Pseudomonas gessardii* AHB79N demonstrated a biodegradation ability of around 55% against the naphthalene tested. In conclusion, this strain should be further exploited in the aromatic polluted regions after careful examinations.

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REFERENCES

1. Karimi, B., Habibi, M., and Esvand, M. (2015) Biodegradation of naphthalene using *Pseudomonas aeruginosa* by up flow anoxic-aerobic continuous flow combined bioreactor. *J. Environ. Health Sci. Eng.*, **13**, 1-10.
2. Keith, L.H., and Telliard, W.A. (1979) Priority pollutants I-A perspective view. *Environ. Sci. Technol*., **13**, 416-423.
3. Heitkamp, M.A., Freeman, J.P., and Cerniglia, C.E. (1987) naphthalene biodegradation in environmental microcosms: Estimates of degradation rates and characterization of metabolites. *Appl. Envrion. Microbiol*., **53**, 129-136.
4. Yuan, S.Y., Wei, S.H., and Chang, B.V. (2000) Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. *Chemosphere*, **41**, 1463-1468.
5. Ahn, Y., Sanseverino, J., and Sayler, G.S. (1999) Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils. *Biodegradation*, **10**, 149-157.
6. Robles-Gonzalez, I.V., Fava, F., and Poggi-Varaldo, H.M. (2008) A review on slurry bioreactors for bioremediation of soils and sediments. *Microb. Cell Fact*., **7**, 1-16.
7. Medina-Bellver, J.I., Marin, P., Delgado, A., Rodriguez-Sanchez, A., Reyes, E., Ramos, J.L., and Marque, S. (2005) Evidence for *in situ* crude oil biodegradation after the Prestige oil spill. *Environ. Microbiol*., **7**, 773–779.
8. Shafiee, P., Shojaosadati, S.A., and Charkhabi, A.H. (2006) Biodegradation of polycyclic aromatic hydrocarbons by aerobic mixed bacterial culture isolated from hydrocarbon polluted soils. *Iran. J. Chem.*

Eng., **25**, 73-78.
9. Fernanda, M., Mariana, G., and Siu Mui, T. (2014) Capacity of aromatic compound degradation by bacteria from amazon dark earth. *Diversity*, **6**, 339-353.
10. Mrozik, A., Piotrowska-Seget, Z., and Labuzek, S. (2003) Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. *Pol. J. Environ. Stud*., **12**, 15-25.
11. Alam Khan, J., and Rizvi, S.H.A. (2011) Isolation and characterization of microorganism from oil contaminated sites. *Adv. Appl. Sci. Res*., **2**, 455-460.
12. Haines, J.R., Wrenn, B.A., Holder, E.L., Strohmeier, K.L., Herrington, R.T., and Venosa, A.D. (1996) Measurement of hydrocarbon-degrading microbial populations by a 96-wellplate most-probable-number procedure. *J. Ind. Microbiol*., **116**, 36-41.
13. Hassanshahian, M., Ahmadinejad, M., Tebyanian, H., and Kariminik, A. (2013) Isolation and characterization of alkane degrading bacteria from petroleum reservoir waste water in Iran (Kerman and Tehran provenances). *Mar. Pollut. Bull*., **73**, 300-305.
14. Brown, E.J., and Braddock, J.F. (1990) Sheen screen, a miniaturized most-probable number method for enumeration of oil -degrading microorganisms. *Appl. Environ. Microbiol*., **56**, 3895-3896.
15. Chen, L., Li, G., and Zu-Liang, C. (2010) Biodegradation of naphthalene by strain *Bacillus fusiformis (BFN). J. Hazard. Mater*., **182**, 771-777.
16. Shamsuzzaman, K.M. and Barnsley, E.A. (1974) The regulation of naphthalene oxygenase in *Pseudomonads. J. Gen. Microbiol*., **83**, 165-170.
17. Holt, J.G., Krieg, NR., Sneath, P.H., Staley, J.T., and Williams, S.T. (1994) Bergey's manual of determinative bacteriology. Williams and Wilkins: Baltimore, pp. 75-85.
18. Mette, H.N., and Neils, B.R. (2002) Denaturing gradient gel electrophoresis (DGGE) approaches to study the diversity of ammonia-oxidizing bacteria). *J. Microbiol. Methods*, **50**, 189–203.
19. Troussellier, M., Got, P., Mboup, M., Corbin, D.,Giuliano, L., Cappello, S., and Bouvy, M. (2005) Daily bacterioplankton dynamics in a sub-Saharan estuary (Senegal River, West Africa): a mesocosm study. *Aquat. Microb. Ecol*., **40**, 13-24.
20. Maidak, B.L., Olsen, G.J., Larsen, N., Overbeek, R., McCaughey, M.J. and Woese, C.R. (1997) The RDP (ribosomal database project). *Nucleic Acids Res*., **25**, 109-110.
21. Yakimov, M.M., Cappello, S., Crisafi, E., Tursi, A ., Savini, A ., Corselli, C ., Scarfi, S., and Giuliano, L. (2006) Phylogenetic survey of metabolically active microbial communities associated with the deep-sea coral *Lophelia pertusafrom* the Apulian plateau, Central Mediterranean Sea. *Ocean Res*., **53**, 62-75.
22. Rosenberg, M., Gutnick, D., and Rosenberg, E. (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. *FEMS Microbiol. Lett*., **9**, 29-33.
23. Pruthi, V., and Cameotra, S. (1997) Rapid identification of biosurfactant-producing bacterial strains using a cell surface hydrophobicity technique. *Biotechnol. Tech*., **11**, 4-7.
24. Nnamchi, CI., Obeta, J.A.N., and Ezeogu, L.I. (2006) Isolation and characterization of some polycyclic aromatic hydrocarbon degrading bacteria from Nsukka soils in Nigeria. *Int. J. Environ. Sci. Tech.*, **3**, 181-190.
25. Khazi, M., Aravindan, R., and Viruthagiri, T. (2010) Recent advances in the biodegradation of phenol. *Asian J. Exp. Biol. Sci.*, **1**, 219-234.
26. Zeinali, M., Vossoughi, M., and Ardestani, S.K. (2008) naphthalene metabolism in *Nocardia otitidiscaviarum* strain TSH1, a moderately thermophilic microorganism. *Chemosphere*, **72**, 905-909.
27. Kom, R., Mbawala, A., and Ngassoum, M. (2012) naphthalene biodegradation by microbial consortia isolated from soils in Ngaoundere (Cameroon). *Int. J. Environ. Sci*., **3**, 1-10.
28. Sack, U., Heinze, T.M., Deck, J., Cerniglia, C.E., Martens, R., Zadrazil, F., and Fritsche, W. (1997) Comparison of phenanthrene and pyrene degradation by different wood decaying fungi. *Appl. Envrion. Microbiol*., **63**, 3919-3925.

29. Cerniglia, C.E. (1993) Biodegradation of polycyclic aromatic hydrocarbons. *Curr. Opin. Biotech*., **4**, 331-338.
30. Leahy, J.G., and Colwel, R.R. (1990) Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev*., **54**, 305-315.
31. Daane, L.L., Harjono, I., Zylstra, G.J., and Haggblom, M.M. (2001) Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. *Appl. Environ. Microbiol*., **67**, 2683-2691.
32. Zafar, M., Kumar, S., and Kumar, S. (2010) Optimization of naphthalene biodegradation by a genetic algorithm based response surface methodology. *Braz. J. Chem. Eng*., **27**, 89-99.
33. Johnsen, A.R., Wick, L.Y., and Harms, H. (2005) Principles of microbial PAH degradation in soil. *Environ. Pollut*., **133**, 71-84.
34. Ariel, A.G., Fermin, M., and Viccelda, D. (2014) *Potential of degradation of naphthalene, phenanthrene and pyrene from sediments collected area near the Panama Canal*. Twelfth LACCEI Latin American and Caribbean Conference for Engineering and Technology, **12**, 1-11.
35. Hassanshahian, M. (2008) Synergism effects of phenol-degrading yeast and ammonia-oxidizing bacteria for nitrification in coke wastewater of Esfahan Steel Company. *Wast Manag. Res.*, **26**, 203-208.
36. Hassanshahian, M., Yakimov, M., Denaro, R., Genovese, M. and Cappello, S. (2014) Using Real-time PCR to assess changes in the crude oil degrading microbial community in contaminated seawater mesocosms. *Int. Biodeter. Biodegr.*, **93**, 241-248.
37. Hassanshahian, M. and Mohamadian, J. (2011) Isolation and characterization of *Halobacterium salinarum* from saline lakes in Iran. *Jundishapur J. Microbiol*., **4**, S59-S65.