TUOMS PRESS

Research Article

CrossMark

Polycyclic Aromatic Hydrocarbons Degradation by Aquatic Bacteria Isolated from Khazar Sea, the World's Largest Lake

Vida Ebrahimi[1,2] , Shirin Eyvazi[3], Soheila Montazersaheb[2], Parivar Yazdani[2], Mohammad Amin Hejazi[4], Vahideh Tarhriz[2*] , Mohammad Saeid Hejazi[1,2*]

[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

[2]Molecular Medicine Research Center, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.

[3]Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

[4]Branch for the Northwest and West Region, Agriculture Biotechnology Research Institute of Iran (ABRII), Tabriz, Iran.

Abstract

Background: Aquatic microorganisms have an important role in the bioremediation of environmental pollutants. Polycyclic Aromatic Hydrocarbons (PAHs) are described as dangerous pollutants that can bind covalently to the nucleic acids, causing mutations. Therefore, they have carcinogenic and toxic properties. Also, are involved in diseases such as asthma, lung dysfunction, and chronic bronchitis. This study aimed to isolate and characterize aquatic bio-degrading bacteria from the world's largest lake, Khazar, with the ability to use PAHs as only carbon source.

Methods: Samples were taken from the estuary of Siah Rud River (Mazandaran province, Iran) and Fereydunkenar beach leading to isolation of twenty-three bacteria on marine agar and sea water media. The isolates were cultured on separate ONR7a medium, each supplemented with only one PAH; as the sole carbon source; including naphthalene, phenanthrene, and anthracene.

Results: Eleven bacterial isolates were able to grow on supplemented media: TBZ-E1, TBZ-E2, TBZ-E3, TBZ-S12, TBZ-S16, TBZ-E20, TBZ-SF2, TBZ-F1, TBZ-F2, TBZ-F3 and TBZ2. These isolates belong to *Alteromonas*, *Marivivens*, *Pseudoalteromonas*, *Vibrio*, *Shewanella*, *Photobacterium*, *Mycobacterium* and *Pseudomonas* genera. The qualitative analysis showed that the consortium of isolates TBZ-F1, TBZ-F2, TBZ-F3, TBZ-SF2, and TBZ2 displayed the highest degradation rate for phenanthrene and naphthalene. Naphthalene, phenanthrene, and anthracene were potently degraded by TBZ2 and TBZ-SF2 and accordingly were subjected to measure degradation potential of mentioned PAHs.

Conclusion: The bacterial isolates of Caspian lake have a critical duty in biodegradation of PAHs. These isolates are representative samples of the bacterial population of this lake, participating in the purification process of this habitat.

Introduction

Aquatic microorganisms are very important due to their roles in materials decomposition, food chains, and biogeochemical cycling.[1] The beneficial effects of bacteria derive from their role in the carbon, oxygen, and/or nitrogen cycles, which are important for the organism's survival and their associations with other living systems. Therefore, isolation and identification of effective microorganisms are needed to determine how they interact in an ecosystem.[2] Since 1900, all of marine sciences have developed greatly. Unfortunately, most of marine bacteria are left unknown and also there is not enough information about known bacteria based on their applications.[3] It can be due to their small sizes, limited range of morphologies and more importantly the difficulties in obtaining pure cultures that can be representative of natural populations.[1] Today, molecular approaches such as 16s rRNA gene sequencing are used as genetic markers for the dominant bacterial species in natural microbial populations.[3]

PAHs are a group of priority pollutants present at high concentrations in many industrial contaminated sites.[4] They enter the environment from numerous routes including fossil fuel or other organic material burning, the accidental spilling of processed hydrocarbons and oils, coal liquefaction and gasification, or organic oil seepage and surface run-offs from forest/brush fires and natural geologic processes.[5] PAHs have widespread environmental

distribution and have potential for causing cancer in human.[6] Some of low-molecular-weight PAHs are acutely toxic and higher-molecular-weight PAHs are genotoxic.[7] Based on toxicity, carcinogenicity, and widespread distribution, the U.S. Environmental Protection Agency has listed 16 PAHs as priority pollutants.[8] PAHs are considered more stable and less degradable than many other organic compounds.[4] Given the harmful effects of PAHs along with their stability, it is very important to develop ways to remove PAHs from the environment. Bioremediation is the utilization of microorganisms to convert hazardous organic materials into harmless substances, like carbon dioxide and water. Bioremediation has a lot of advantages over thermal and some physicochemical techniques, such as its low cost.[4] For all this reasons, isolation and characterization of bacteria with the ability to degrade PAHs is indeed in demand. This study aimed to isolate and identify aboriginal aquatic bacteria which are adaptive in the most environmental pollution region of Khazar (Caspian) sea with the ability to use PAHs as only carbon source. In addition, most important question is whether the native environmental bacteria have the ability to remedy of lakes· pollution?

Materials and Methods
Water sample collection
Two water samples were collected from the estuary of Siah Rud River (36°39′05.2″N 52°19′15.9″E) (Mazandaran province, Iran) and Fereydunkenar Beach (36°41′50.1″N 52°33′28.9″E) (Fereydunkenar County, Mazandaran Province, Iran). The samples were then transported to the laboratory of pharmaceutical biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences. Isolation of aquatic bacteria was performed on marine agar and sea water media. Marine agar medium contains (per liter): peptone, 5.0 g; yeast extract, 1.0 g; Fe^{3+}-citrate, 0.1 g; NaCl, 19.45 g; $MgCl_2$ (dried), 8.8 g; Na_2SO_4, 3.24 g; $CaCl_2$, 1.8 g; KCl, 0.55 g; $NaHCO_3$, 0.16 g; KBr, 0.08 g; $SrCl_2$, 34.0 mg; H_3BO_3, 22.0 mg; sodium silicate, 4.0 mg; NaF, 2.4 mg; $(NH_4)NO_3$, 1.6 mg; Na_2HPO_4, 8.0 mg; agar 15.0 g and pH was adjusted to 7.6 ± 0.2 as described by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) medium 2216. Sea water medium was prepared as described by DSMZ medium 246 which contains (per liter): beef extract, 10.0 g; peptone, 10.0 g; agar, 20.0 g; tap water, 250 ml; artificial sea water: 750 ml. Artificial sea water consist of NaCl, 28.13 g; KCl, 0.77 g; $CaCl_2.2H_2O$, 1.6 g; $MgCl_2.6H_2O$, 4.80 g; $NaHCO_3$, 0.11 g; $MgSO_4.7H_2O$, 3.5 g. About 10 μL aliquots from the samples were spread onto plates. The plates were incubated at 30 °C for a week. To obtain a pure culture, the colonies were cultured in marine broth and stored at -70 °C in marine broth supplemented with 30% (v/v) glycerol.[9] Gram staining was performed and the result was checked by KOH lysis test technique.[10,11]

Biochemical tests
Biochemical tests were performed for isolates: TBZ-SF2, TBZ-F1, TBZ-F2, TBZ-F3 and TBZ2 using *biomerieux* API 20E and API 20NE kits. Assimilation tests were performed by the aid of API 20NE kit and fermentation/oxidation tests of some sugars were done by API 20E kit. Additionally, Oxidase and Catalase tests, Growth at various NaCl concentrations, temperatures from 5 up to 50 °C at intervals of 5 °C and pH 4.0 to 12.0 0 (using increments of 1 pH unit) were also carried out on lab-made marine broth medium. To determine the optimal growth on NaCl concentration, temperature and pH, samples were measured by UV absorbance at 600-nanometer wavelength (Optical Density (OD) in 600 nm) after 48 hours. The pH values were adjusted using buffer system including 0.1 M citric acid/0.1 M sodium citrate for pH 4.0–5.0; 0.1 M KH_2PO_4/0.1 M NaOH for pH 6.0–8.0; 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 for pH 9.0–10.0; 0.05 M and $Na2HPO_4$/0.1 M NaOH for pH 11.0-12.0.

16S rRNA gene sequences
To conduct genetic characterization, DNA was extracted from isolates according to the method described by Corbin *et al.* (2001) with some modifications.[12] For phylogenetic analysis based on the 16S rRNA gene sequence, the 16S rDNA was targeted for amplification by polymerase chain reaction (PCR) in the presence of forward 16F 5'- AGAGTTTGATCCTGGCTCAG-3', and reverse 16R for Gram-negative bacteria; 5'- ACGGCTACCTTGTTACGACTT-3' and reverse 16R for Gram-positive bacteria; 5'-TACCTTGTTAGGACTTCACC-3' primers.[13] The program for PCR was: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, primer annealing at 55°C for 55 sec and extension at 72°C for 85 sec and completed by the final extension at 72°C for 10 min.[14]
The amplified fragments were purified with Roche kit (Germany) followed by, sequencing with forward and reverse primers by Macrogen Company (Korea). The 16S rRNA sequencing results were analyzed using Chromas Pro software (ChromasPro 2.1, Technelysium Pty Ltd, Tewantin QLD, Australia). Phylogenetic trees were constructed using the neighbor-joining method in MEGA version 7 software package.[15]

Qualitative bio-degradation Tests
Stock concentrated-solutions of each PAH (naphthalene, phenanthrene, and anthracene) (Merck-Schuchart, Germany) were prepared in acetone with a concentration of 1 mg/ml and sterilized using 0.22 μm filters. The capability of isolates to use of PAHs as the sole sources of carbon, ONR7a medium with naphthalene or phenanthrene or anthracene was prepared. For this purpose, ONR7a medium was prepared based on three solutions, containing: solution "1" with the volume of 500 ml is consisted of NaCl, 22.79 g; Na_2SO_4, 3.98 g; KCl, 0.72 g; NaBr, 83.00 mg; $NaHCO_3$, 31.00 mg; H_3BO_3, 27.00 mg; NaF, 2.60 mg; NH_4Cl, 0.27 g; $Na_2HPO_4.7H_2O$, 89.00 mg;

TAPSO, 1.30 g, 12 g of agarose (Sigma) (pH=7.6), solution "2" with the volume of 450 ml is consisting $MgCl_2$. $6H_2O$, 11.18 g; $CaCl_2$. $2H_2O$, 1.46 g; $SrCl_2$. $6H_2O$, 24.00 mg and solution "3" with the volume of 50 ml composed of $FeCl_2$. $4H_2O$, 2.00 mg/ml. The solutions were autoclaved separately and cooled about 50ºC before mixing.[16] The acetone stock solutions were diluted with cool ONR7a medium before solidification and the final concentration of each PAH was 200 ppm.[17,18] Besides, pure ONR7a medium was tested as negative control plates and plates with glucose (200 ppm) were served as positive controls.

Quantitative bio-degradation Tests
Based on qualitative analysis, the isolated TBZ1 and TBZ2 strains showed to have high degradation potential. Therefore, quantitative biodegradation tests were carried out in ONR7a broth medium containing 50 ppm of naphthalene, phenanthrene or anthracene for these isolates. For this aim, 2 ml of bacterial suspension with OD=0.6 in 600 nm were added in 17.8 ml of ONR7a broth medium along with 200 μl of PAH stock solution as sole carbon source. Two series of control Erlenmeyer flasks were prepared for this study: the bacterium-free media and the PAH-free culture media.

The flasks were incubated on 100 rpm shaking incubator at 30 ºC for 6 days. Spectroscopic scanning of the samples were carried out by using spectrophotometer (UNICO UV-1200) with λ_{max} of 311 nm for naphthalene, 316 nm for phenanthrene and 345 nm for anthracene.[19] Before spectroscopic measurement, PAHs were extracted from culture medium in 5 ml of cyclohexane.[20] PAH residual concentration was calculated based on calibration curve equation. All experiments were done at least in triplicates. To make sure that the decline in PAH concentration is completely contributed to bacterial growth due to PAHs consumption and biodegradation, not to cell surface adsorption or accumulation, bacterial growth was checked by OD measurement at 600 nm during examinations.

Statistical analysis
Results of quantitative tests and measurements were analyzed by SPSS Statistics 23 based on ANOVA and Tukey's post hoc tests and Excel 2016 softwares.

Results
Twenty three bacterial isolates were obtained from water samples of Siah Rud River and Fereydonkenar beach which were isolated from sea water medium and marine medium. Among these, 13 isolates were able to clarify insoluble PAHs from ONR7a broth medium. These isolates were picked for 16S rRNA sequencing. To find out the phylogenetic position of each isolate, the 16S rRNA gene sequences were analyzed based on NCBI and EZtaxon data banks and finally, the respective phylogenetic tree was constructed (Figure 1). These isolates contain TBZ-E1 (MK480663) (TBZ-E2 = TBZ-E4), TBZ-E3, TBZ-S12 (MK480664), TBZ-S16 (MK480662), TBZ-E20, TBZ-SF2, TBZ-F1

(MK479299), TBZ-F2 (MK480612) (=TBZ1), TBZ-F3 (MK480666) and TBZ2 (KX712072). The results indicated that strain TBZ2 belongs to the genus *Pseudomonas* and its closely related type strain is *P. oleovorans* subsp. Lubricantis $RS1^T$ (98.65% similarity).[21] TBZ2 is recently introduced as *Pseudomonas khazarica* as a novel species.[22]

Strain TBZ-SF2 showed 100% similarity in 16S rRNA sequence with *S. haliotis* JCM 14758^T.[23] Strain TBZ-F1 belongs to the genus *Shewanella* and its closely related type strain is *S. algae* JCM 21037^T (99.66% similarity).[24] Strains TBZ-F2 and TBZ1 are members of the genus *Photobacterium* and their closely related type strain is *P. ganghwense* DSM 22954^T (99.65% similarity).[25] Strain TBZ-F3 belongs to the genus *Microbacterium* and its closely related type strain is *M. esteraromaticum* DSM 8609^T (99.88% similarity).[26] Strain TBZ-E1, TBZ-E2, and TBZ-E4 belong to the genus *Alteromonas* and their closely related type strain is *A. litorea* (99.86%). Strain TBZ-E3 belongs to the genus *Marivivens* and its closely related type strain is *M. donghaensis* AM-4^T (100% similarity).[27] Strain TBZ-S12 belongs to the genus *Pseudoalteromonas* and its closely related type strain is *P. flavipulchra* NCIMB 2033^T (99.86% similarity).[28] Strain TBZ-S16 belongs to the genus *Vibrio* and its closely related type strain is *V. diazotrophicus* NBRC 103148^T (99.86% similarity).[29] Strain TBZ-E20 belongs to the genus *Pseudomonas* and its closely related type strain is *P. chengduensis* MBR^T(100% similarity).[30](Table 1)

Based on qualitative biodegradation tests, five bacterial isolates had the remarkable ability to use naphthalene, phenanthrene or anthracene as sole carbon sources.

These strains were subjected to biochemical API 20E and API 20NE tests. Besides, Lab-made tests including oxidase and catalase tests, growth at different temperatures, pHs and concentrations of NaCl were performed and the results of these tests are summarized in Table 2 and Table 3, respectively.

Qualitative Biodegradation Tests demonstrated that stains TBZ-F2 and TBZ2 are more efficient than others in terms of consuming and degradation of naphthalene, phenanthrene or anthracene. So these strains were subjected to quantitative biodegradation tests. Bacterial growths for two isolates were analyzed in 6 days based on OD measurement in λ = 600nm. Growth curves are shown in Figures 2 and 3, and the results of naphthalene, phenanthrene, and anthracene bio-degradation tests are shown in Figures 4 and 5. The comparison of OD (λ = 600 nm) and growth curves in each day of the experiment reveals that there is a significant difference between tests and PAH-free control groups (p-value ≤ 0.05). Moreover, mean concentrations of residual PAHs in each day for tests and bacterium-free control groups were also significantly different analysis (p-value ≤ 0.05).

Discussion
Aromatic compounds comprise almost 65% of chemical wastes. Most of the aromatic compounds possess mutagenic and toxic properties.[31] PAHs are very stable pollutants
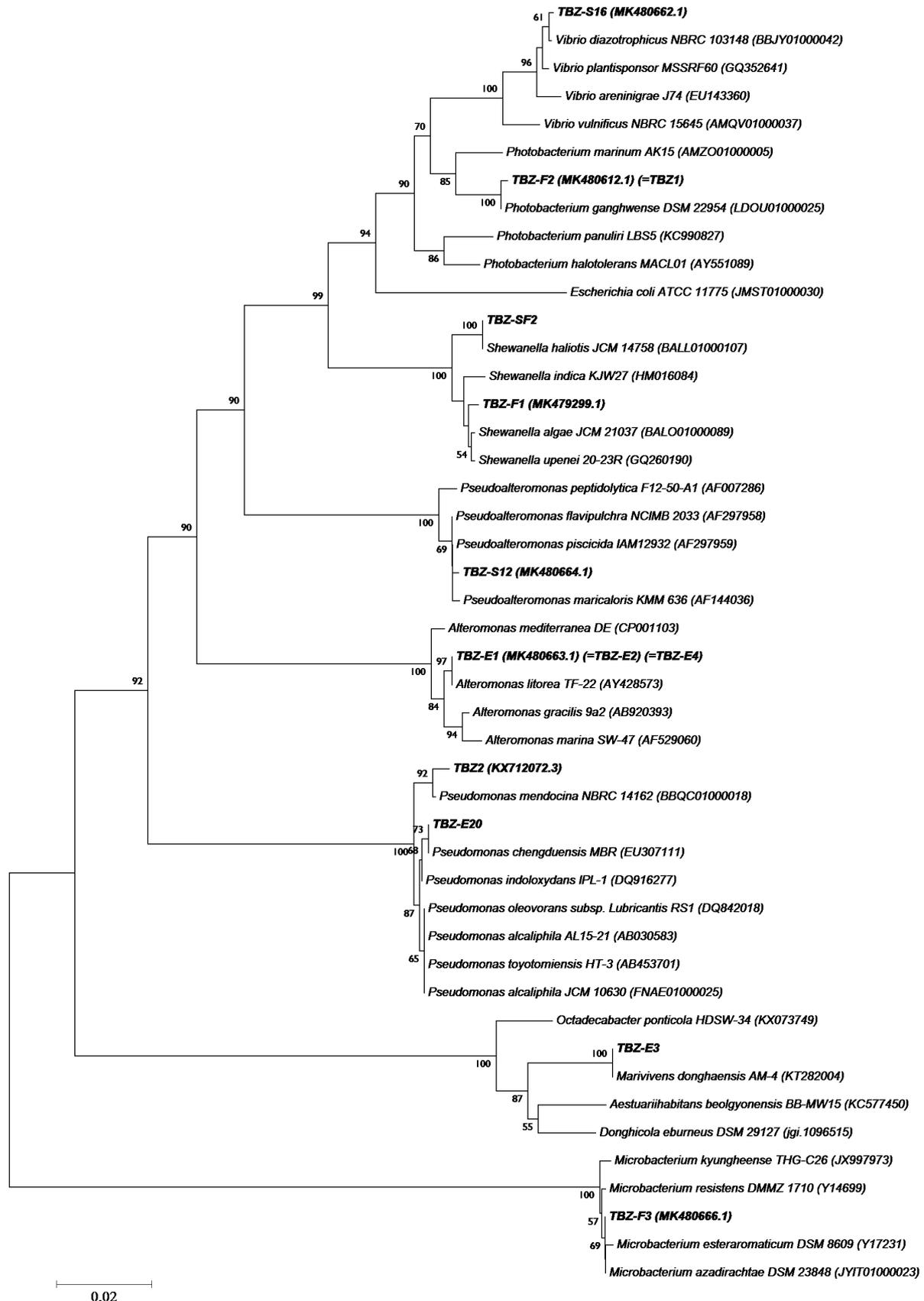
Figure 1. Neighbor-joining phylogenetic tree derived from 16S rRNA gene sequence data showing the positions of 13 isolated bacteria including TBZ-E1 (TBZ-E2 = TBZ-E4), TBZ-E3, TBZ-S12, TBZ-S16, TBZ-E20, TBZ-SF2, TBZ-F1, TBZ-F2 (=TBZ1), TBZ-F3 and TBZ2 among related bacteria. Numbers at branch points are bootstrap percentages based on 500 replicates. Bar, 0.02 changes per nucleotide position.

Table 1. Closest known bacteria to various isolates from Caspian Sea based on 16S rRNA gene sequence.

| Isolate	Number of isolates	Accession number	Nearest relative	Genus	Similarity (%)
TBZ2	1	KX712072	*P. oleovorans subsp. Lubricantis*	*Pseudomonas*	98.65
TBZ-SF2	1	HM016086	*S. haliotis*	*Shewanella*	100.00
TBZ-F1	1	MK479299	*S. algae*	*Shewanella*	99.66
TBZ-F2, TBZ1	2	MK480612	*P. ganghwense*	*Photobacterium_*	99.65
TBZ-F3	1	MK480666	*M. esteraromaticum*	*Microbacterium*	99.88
TBZ-E1, TBZ-E2, TBZ-E4	3	MK480663	*A.litorea TF-22*	*Alteromonas*	99.86
TBZ-E3	1	KT282004	*M. donghaensis AM-4*	*Marivivens*	100
TBZ-S12	1	MK480664	*P. flavipulchra NCIMB 2033*	*Pseudoalteromonas*	99.86
TBZ-S16	1	MK480662	*V. diazotrophicus NBRC 103148*	*Vibrio*	99.86
TBZ-E20	1	EU307111	*P. chengduensis MBR*	*Pseudomonas*	100

Table 2. Comparison of the characteristics of isolated bacteria based on API 20E and API 20NE.

Characteristics	Isolate TBZ2	Isolate TBZ-SF2	Isolate TBZ-F1	Isolate TBZ-F2	Isolate TBZ-F2 (=TBZ1)
Hydrolysis of:					
Arginine	+	-	-	-	+
Esculin	-	+	+	+	+
Production of:					
Indole	-	-	-	-	+
Acetoin[1]	-	-	-	-	-
H_2S	-	+	+	-	-
Enzymes:					
Urease	-	-	-	-	+
Tryptophane deaminase	+	-	-	-	-
Gelatinase	±[2]	+	+	-	+
Lysine decarboxylase	-	-	-	-	-
Ornithine decarboxylase	-	+	+	-	-
β-Galactosidase (Ortho-Nitro Phenyl-ßDGalactopyranosidase)	-	-	-	-	+
β-galactosidase (Para-Nitro Phenyl-ßDGalactopyranosidase)	-	-	-	±	+
Fermentation / Oxidation of:					
D-Glucose	+	-	-	-	+
D-Mannitol	+	-	-	-	+
Inositol	-	-	-	-	+
D-Sorbitol	-	-	-	-	±
L-Rhamnose	-	-	-	-	+
D-Sucrose	-	-	-	-	+
D-Melibiose	-	-	-	-	+
Amygdalin	-	-	-	-	±
L-Arabinose	-	-	-	-	+
Assimilation of:					
Glucose	+	-	-	-	±
Arabinose	-	-	-	-	±
Mannose	+	-	-	-	w[3]
Mannitol	+	-	-	-	w
N-Acetyl-Glucosamine	-	+	+	-	w
Maltose	-	-	-	-	w
Potassium Gluconate	+	-	-	-	+
Capric acid	+	+	+	-	+
Adipic acid	-	-	-	-	-
Malate	+	+	+	-	±
Trisodium Citrate	+	-	-	-	+
Phenylacetic acid	-	-	-	-	+

[1] Voges–Proskauer or VP test, [2] ± means doubtful, [3] weakly positive

Table 3. Comparison of the characteristics of isolated bacteria based on lab-made tests.

Characteristics	Isolate TBZ2	Isolate TBZ-SF2	Isolate TBZ-F1	Isolate TBZ-F3	Isolate TBZ-F2 (= TBZ1)
Oxidase test	+	+	+	+	+
Catalase test	+	+	+	+	+
Temperature range (°C)	10- 55°C (optimum 30 °C)	10-42°C (optimum 37 ºC)	10-40°C (but not at 4 °C)	4-37°C (but very week at 4 °C, optimum 30°C)	10-45°C (optimum 35°C)
pH range	4-10 (optimum 6-7)	5-11 (optimum 7)	5-11 (optimun 7.5)	5-10 (optimun 7-7.5)	5-11 (optimum 8-9)
NaCl concentration (%)	0-8.5% (w/v) (optimum 2-2.5%)	0-10% (w/v) (optimum 4%)	0-6% (w/v) (optimum 2-2.5%)	0-6.5% (w/v) (optimum 2-2.5%)	1–7% (w/v) (optimum 2%)
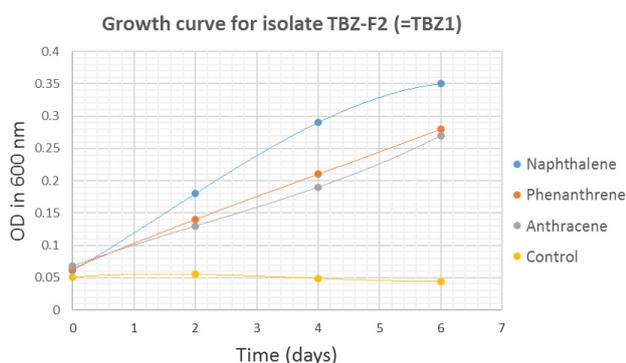
Figure 2. Growth curve for isolate TBZ-F2 (=TBZ1) based on OD measurement in λ = 600 nm in presence of naphthalene, phenanthrene or anthracene as sole carbon source and without carbon source in control group.
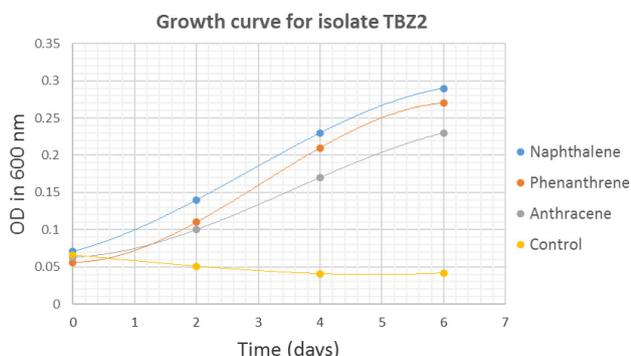
Figure 3. Growth curve for isolate TBZ2 based on OD measurement in λ = 600 nm in presence of naphthalene, phenanthrene or anthracene as sole carbon source and without carbon source in control group

due to having benzene rings. In fact, by increasing the number of benzene rings, the stability of PAHs increases and microbial degradation become more difficult.[32] Five isolates were able to use PAHs as sole carbon source and consequently, were subjected to PAH degradation potential analysis. These isolated strains belonged to the genera *Shewanella*, *Photobacterium*, *Mycobacterium*, and *Pseudomonas*.

Based on other studies on PAH degrading microorganisms, most of anaerobic nitrate-reducing and sulfate-reducing bacteria have the potential to degrade PAHs. The greatest number of PAH-degrading bacteria belong to the genus *Pseudomonas*.[33]

Kim *et al.* compared genome sequences of two pyrene-degrading *Mycobacterium* strains, isolated from Han river.[34] In a study, Tahseen *et al.* enhanced *Pseudomonas putida* degradation potential of crude oil by gamma-ray irradiation.[35] Rathour *et al.* demonstrated that a bacterial strain belonging to genus *Shewanella*, isolated from Pongong lake is capable of pyrene degradation.[36] Liu *et al.* introduced *Photobacterium* strains as polycaprolactone (PCL) degrading isolates of Yangtze river.[37]

Multiple studies reported the sensitivity of *Photobacterium phosphoreum* to PAHs. Therefore, *P.phosphoreum* is used for toxicity prediction of PAH contaminations in luminescence inhibition test (Microtox test).[38] In the present study, Photobacteium ganghwence is introduced as PAH-degrading bacteria. Non-toxic effect of these materials on bacterial growth may be due to low molecular weights and simple structures of these compounds.[39] Although, more researches are required to identify producing metabolites from these PAHs and toxicity of them.

Arbabi *et al.* performed bio-treatment of phenanthrene with two indigenous bacterial mixed cultures, isolated from petroleum contaminated sites in Iran, for 20 weeks in the solid-phase reactor and could effectively remove phenanthrene.[40] Mardani *et al.* used genetically manipulated Pseudomonas putida for oil bio-degradation in spiked soil and could significantly increase biodegradation of PAHs in the recombinant group.[41] Tarhriz *et al.* isolated and introduced *Tabrizicola aquatica* as the novel genus of aquatic bacteria from qurugol Lake in the mountains region of Azerbaijan which can adsorb heavy metals.[42] Elyasifar *et al.* isolated halophilic bacterial belonged to *Bacillus subtilis* and *Virgibacillus olivae*, from Dagh Biarjmand and Haj Aligholi salt deserts in Iran with potential to produce antibacterial and antifungal agents.[43]

Several aerobic naphthalene-degrading bacteria account for 86.9% of the hydrocarbon-degrading microorganisms found in gasoline-contaminated aquifers.[44] For the first time, Ben Said *et al.* described the ability of *Shewanella oneidensis* species to degrade fluoranthene and pyrene as high molecular weight and, phenanthrene as a low molecular weight PAH.[45] Several *Mycobacterium* species
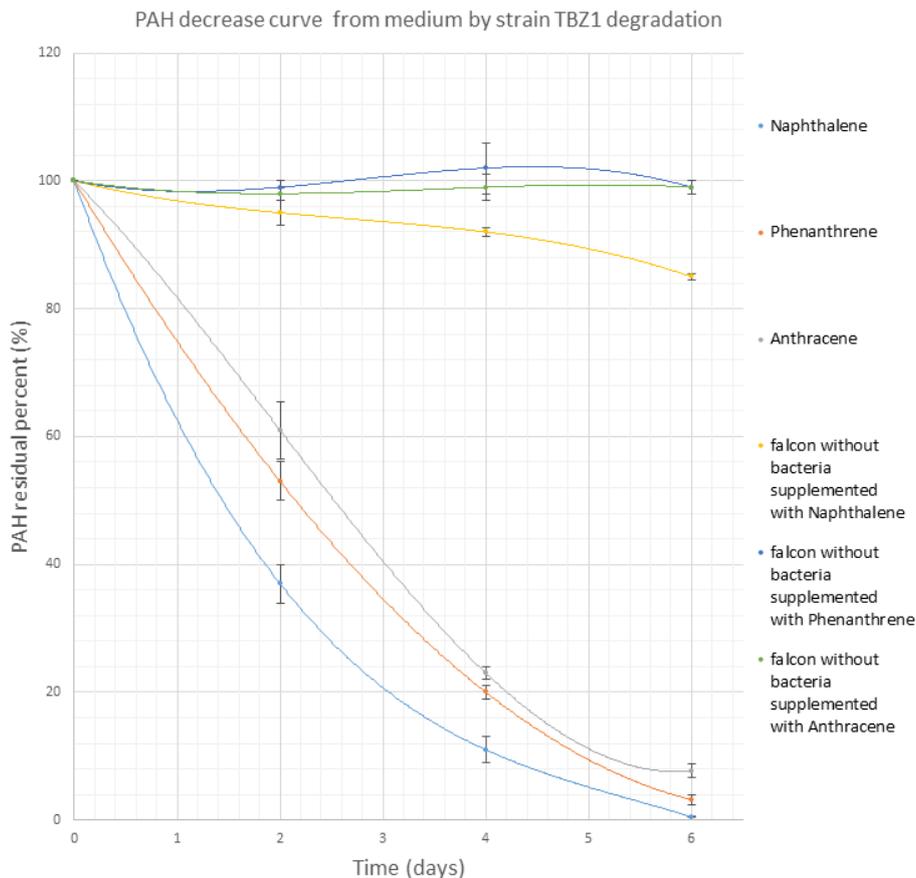
Figure 4. Naphthalene, phenanthrene or anthracene degradation curve by isolate TBZ1 in comparison with control groups.
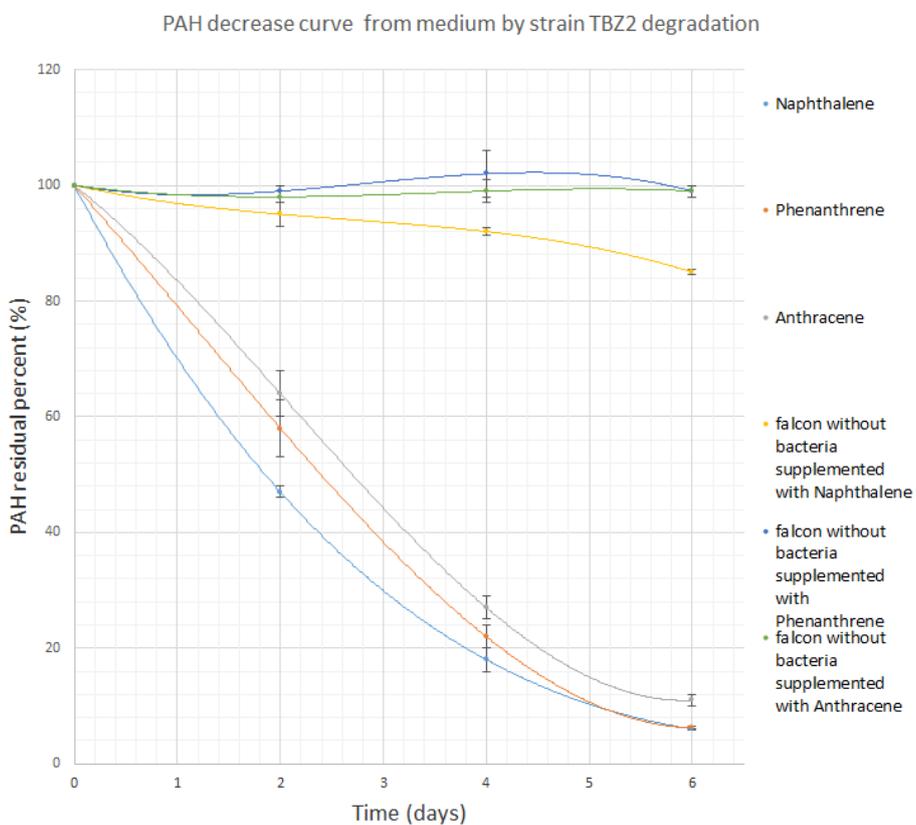

Figure 5. Naphthalene, phenanthrene and anthracene degradation curve by isolate TBZ2 in comparison with control groups.

are suggested as degradants of high-molecular-weight PAHs.[46,47] Also, genomic studies on these species were done to understand mechanisms of bacterial PAH degradation, for example, *Mycobacterium gilvum* PYR-GCK.[48]

Conclusion

The Khazar (Caspian) Sea as the largest inland mass of water is exceptional scientific interest. The human-made changes, notably those resulting from the construction of dams, shipping, fisheries and the production of petroleum and natural gas in the Khazar (Caspian) basin cause to change its complex geologic and climatic evolution. Our funding showed that the native bacterial population of Khazar (Caspian) Lake have an efficient role in biodegradation of PAHs. By separating the adaptive bacteria in the most environmental pollution region of Khazar (Caspian) sea with the ability to use PAHs as only carbon source from Khazar (Caspian) Lake, it has been inferred that part of the purification process of the lake is performed by these bacteria.

Acknowledgments

Conflict of Interest

The authors claim that there is no conflict of interest.

References

1. Hiorns WD, Methe BA, Nierzwicki-Bauer SA, Zehr JP. Bacterial diversity in adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl Environ Microbiol.1997;63(7):2957-60. doi:10.1128/AEM.63.7.2957-2960.1997

2. Hannan MA, Rahman MM, Mondal MN, Deb SC, Chowdhury G, Islam MT. Molecular identification of *Vibrio alginolyticus* causing vibriosis in shrimp and its herbal remedy. Pol J Microbiol. 2019;68(4):429-38. doi:10.33073/pjm-2019-042

3. Giovannoni SJ, Britschgi TB, Moyer CL, Field KG. Genetic diversity in sargasso sea bacterioplankton. Nature 1990;345:60. doi:10.1038/345060a0

4. Wilson SC, Jones KC. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (pahs): A review. Environ Pollut. 1993;81(3):229-49. doi:10.1016/0269-7491(93)90206-4

5. Freeman DJ, Cattell FC. Woodburning as a source of atmospheric polycyclic aromatic hydrocarbons. Environ Sci Technol. 1990;24(10):1581-5. doi:10.1021/es00080a019

6. Pahlman R, Pelkonen O. Mutagenicity studies of different polycyclic aromatic hydrocarbons: The significance of enzymatic factors and molecular structure. Carcinogenesis. 1987;8(6):773-8. doi:10.1093/carcin/8.6.773

7. Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. Salmonella mutagenicity tests: Ii. Results from the testing of 270 chemicals. Environ Mutagen. 1986;8(S7):56-119. doi:org/10.1002/em.2860080803

8. Keith L, Telliard W. ES&T special report: Priority pollutants: I-a perspective view. Environ Sci Technol 1979;13(4):416-23. doi:org/10.1021/es60152a601

9. Tarhriz V, Mohammadzadeh F, Hejazi MS, Nematzadeh G, Rahimi E. Isolation and characterization of some aquatic bacteria from Qurugol Lake in Azerbaijan under aerobic conditions. Adv Environ Biol. 2011:3173-9.

10. Gerhardt P. Methods for general and molecular bacteriology: Washington, D.C.: American Society for Microbiology; 1994.

11. Gregersen T. Rapid method for distinction of gram-negative from gram-positive bacteria. Appl Microbiol Biotechnol. 1978;5(2):123-7. doi:org/10.1007/BF00498806

12. Corbin DR, Grebenok RJ, Ohnmeiss TE, Greenplate JT, Purcell JP. Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. Plant Physio. 2001;126(3):1116-28. doi:10.1104/pp.126.3.1116

13. Karlson U, Dwyer D, Hooper S, Moore E, Timmis K, Eltis L. Two independently regulated cytochromes P-450 in a *Rhodococcus rhodochrous* strain that degrades 2-ethoxyphenol and 4-methoxybenzoate. J Bacteriol 1993;175(5):1467-74. doi:10.1128/jb.175.5.1467-1474.1993

14. Tarhriz V, Hirose S, Fukushima S-i, Hejazi MA, Imhoff JF, Thiel V, et al. Emended description of the genus *Tabrizicola* and the species *Tabrizicola aquatica* as aerobic anoxygenic phototrophic bacteria. Antonie Van Leeuwenhoe. 2019;112(8):1169-75. doi:10.1007/s10482-019-01249-9

15. Kumar S, Stecher G, Tamura K. Mega7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870-4. doi:10.1093/molbev/msw054

16. Tahriz V, Hamidi A, Rahimi E, Eramabadi M, Eramabadi P, Yahaghi E, et al. Isolation and characterization of naphthalene-degradation bacteria from Qurugol Lake located at Azerbaijan. Biosci Biotechnol Res Asia. 2014;11(2):715-22. doi:10.13005/bbra/1326

17. Hassanshahian M, Boroujeni NA. Enrichment and identification of naphthalene-degrading bacteria from the Persian Gulf. Mar Pollut Bull. 2016;107(1):59-65. doi:10.1016/j.marpolbul.2016.04.020

18. Cui Z, Gao W, Xu G, Luan X, Li Q, Yin X, et al. *Marinobacter aromaticivorans* sp. Nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from sea sediment. Int J Syst Evol Microbiol. 2016;66(1):353-9. doi:10.1099/ijsem.0.000722

19. Mansouri E, Yousefi V, Ebrahimi V, Eyvazi S, Hejazi

MS, Mahdavi M, et al. Overview of ultraviolet-based methods used in polycyclic aromatic hydrocarbons analysis and measurement. Sep Sci Plus. doi:10.1002/sscp.201900077

20. Wu M, Nie M, Wang X, Su J, Cao W. Analysis of phenanthrene biodegradation by using FTIR, UV and GC–MS. Spectrochim Acta A Mol Biomol Spectrosc. 2010;75(3):1047-50. doi:10.1016/j.saa.2009.12.051

21. Saha R, Spröer C, Beck B, Bagley S. *Pseudomonas oleovorans* subsp. *Lubricantis* subsp. nov., and reclassification of *Pseudomonas pseudoalcaligenes* ATCC 17440^T as later synonym of *Pseudomonas oleovorans* ATCC 8062^T. Curr Microbiol. 2010;60(4):294-300. doi:10.1007/s00284-009-9540-6

22. Tarhriz V, Nouioui I, Spröer C, Verbarg S, Ebrahimi V, Cortés-Albayay C, et al. *Pseudomonas khazarica* sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from Khazar Sea sediments. Antonie van Leeuwenhoek. 2019; 113(4):521-32. doi:10.1007/s10482-019-01361-w

23. Kim D, Baik KS, Kim MS, Jung B-M, Shin T-S, Chung G-H, et al. *Shewanella haliotis* sp. nov., isolated from the gut microflora of abalone, haliotis discus hannai. Int J Syst Evol Microbiol. 2007;57(12):2926-31. doi:10.1099/ijs.0.65257-0

24. Simidu U, Kita-Tsukamoto, Yasumoto T, Yotsu M. Taxonomy of four marine bacterial strains that produce tetrodotoxin. Int J Syst Evol Microbiol. 1990;40(4):331-6. doi:10.1099/00207713-40-4-331

25. Park Y-D, Baik KS, Seong CN, Bae KS, Kim S, Chun J. *Photobacterium ganghwense* sp. Nov., a halophilic bacterium isolated from sea water. Int J Syst Evol Microbiol. 2006;56(4):745-9. doi:10.1099/ijs.0.63811-0

26. Takeuchi M, Hatano K. Union of the genera *Microbacterium* orla-jensen and *Aureobacterium* collins et al. In a redefined genus microbacterium. Int J Syst Evol Microbiol. 1998;48(3):739-47. doi:10.1099/00207713-48-3-739

27. Park S, Kim S, Jung YT, Yoon JH. *Marivivens donghaensis* gen. nov., sp. nov., isolated from seawater. Int J Syst Evol Microbiol. 2016;66(2):666-72. doi:10.1099/ijsem.0.000772

28. Ivanova EP, Shevchenko LS, Sawabe T, Lysenko AM, Svetashev VI, Gorshkova NM, et al. *Pseudoalteromonas maricaloris* sp. Nov., isolated from an australian sponge, and reclassification of [*Pseudoalteromonas aurantia*] NCIMB 2033 as *Pseudoalteromonas flavipulchra* sp. Nov. Int J Syst Evol Microbiol. 2002;52(1):263-71. doi:10.1099/00207713-52-1-263

29. Guerinot M, West P, Lee J, Colwell R. Vibrio diazotrophicus sp. Nov., a marine nitrogen-fixing bacterium. Int J Syst Evol Microbiol. 1982;32(3):350-7. doi:10.1099/ijsem.0.000456

30. Tao Y, Zhou Y, He X, Hu X, Li D. *Pseudomonas chengduensis* sp. Nov., isolated from landfill leachate. Int J Syst Evol Microbiol. 2014;64(1):95-100. doi:10.1099/ijs.0.050294-0

31. Sharifinia M, Mahmoudifard A, Namin JI, Ramezanpour Z, Yap CK. Pollution evaluation in the shahrood river: Do physico-chemical and macroinvertebrate-based indices indicate same responses to anthropogenic activities? Chemosphere. 2016;159:584-94. doi:10.1016/j.chemosphere.2016.06.064

32. Bauer JE, Capone DG. Effects of four aromatic organic pollutants on microbial glucose metabolism and thymidine incorporation in marine sediments. Appl Environ Microbiol. 1985;49(4):828-35.

33. Haritash A, Kaushik C. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. J Hazard Mater. 2009;169(1-3):1-15. doi:10.1016/j.jhazmat.2009.03.137

34. Kim D-W, Lee K, Lee D-H, Cha C-J. Comparative genomic analysis of pyrene-degrading mycobacterium species: Genomic islands and ring-hydroxylating dioxygenases involved in pyrene degradation. J Microbiol. 2018;56(11):798-804. doi:10.1007/s12275-018-8372-0

35. Tahseen R, Arslan M, Iqbal S, Khalid ZM, Afzal M. Enhanced degradation of hydrocarbons by gamma ray induced mutant strain of *Pseudomonas putida*. Biotechnol Lett. 2019;41(3):391-9. doi:10.1007/s10529-019-02644-y

36. Rathour R, Gupta J, Tyagi B, Kumari T, Thakur IS. Biodegradation of pyrene in soil microcosm by *Shewanella* sp. Istpl2, a psychrophilic, alkalophilic and halophilic bacterium. Bioresour Technol Rep. 2018;4:129-36. doi:10.1016/j.biteb.2018.10.004

37. Liu X, Hille P, Zheng M, Gao P, Shang K, Wei R, et al., editors. Diversity of polyester degrading bacteria in surface sediments from yangtze river estuary. AIP Conference Proceedings. 2019:AIP Publishing LLC. doi:10.1063/1.5116502

38. McConkey BJ, Duxbury CL, Dixon DG, Greenberg BM. Toxicity of a PAH photooxidation product to the bacteria *Photobacterium phosphoreum* and the duckweed *Lemna gibba*: Effects of phenanthrene and its primary photoproduct, phenanthrenequinone. Environ Toxicol Chem.1997;16(5):892-9. doi:10.1002/etc.5620160508

39. Hund K, Traunspurger W. Ecotox-evaluation strategy for soil bioremediation exemplified for a PAH-contaminated site. Chemosphere. 1994;29(2):371-90. doi:10.1016/0045-6535(94)90111-2

40. Arbabi M, Nasseri S, Chimezie A. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in petroleum contaminated soils. Iran J Chem Eng. 2009;28(3):53-9. doi:10.4314/jasem.v22i1.26

41. Mardani G, Mahvi AH, Hashemzadeh-Chaleshtori M, Naseri S, Dehghani MH, Ghasemi-Dehkordi P. Application of genetically engineered dioxygenase producing *Pseudomonas putida* on decomposition of oil from spiked soil. Jundishapur. J Nat Pharm Prod. 2017;12(3 (Supp)):e64313. doi:10.5812/jjnpp.64313

42. Tarhriz V, Akbari Z, Dilmaghani A, Hamidi A, Hejazi

MA, Hejazi MS. Bioreduction of iron and biosorption of heavy metals (Ni^{2+}, Co^{2+}, pb^{2+}) by a novel environmental bacterium, *Tabrizicola aquatica* RCRI19^T. Asian J Water Environ. 2019;16(3):73-81. doi:10.3233/AJW190035

43. Elyasifar B, Jafari S, Hallaj-Nezhadi S, Chapeland-leclerc F, Ruprich-Robert G, Dilmaghani A. Isolation and identification of antibiotic-producing halophilic bacteria from Dagh Biarjmand and Haj Aligholi salt deserts, Iran. Pharm Sci. 2019;25(1):70-7. doi:10.15171/PS.2019.11

44. Ridgway H, Safarik J, Phipps D, Carl P, Clark D. Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer. Appl Environ Microbiol. 1990;56(11):3565-75. doi:10.1128/AEM.56.11.3565-3575.1990

45. Ben Said O, Goñi-Urriza M, El Bour M, Dellali M, Aissa P, Duran R. Characterization of aerobic polycyclic aromatic hydrocarbon-degrading bacteria from bizerte lagoon sediments, Tunisia. J Appl Microbiol. 2008;104(4):987-97. doi:10.1111/j.1365-2672.2007.03621.x

46. Kanaly RA, Harayama S. Advances in the field of high-molecular-weight polycyclic aromatic hydrocarbon biodegradation by bacteria. Microb Biotechnol. 2010;3(2):136-64. doi:10.1111/j.1751-7915.2009.00130.x

47. Peng R-H, Xiong A-S, Xue Y, Fu X-Y, Gao F, Zhao W, et al. Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev. 2008;32(6):927-55. doi:10.1111/j.1574-6976.2008.00127.x

48. Badejo AC, Badejo AO, Shin KH, Chai YG. A gene expression study of the activities of aromatic ring-cleavage dioxygenases in *Mycobacterium gilvum* pyr-gck to changes in salinity and ph during pyrene degradation. PLoS One. 2013;8(2):e58066. doi:10.1371/journal.pone.0058066