EFFECTIVE REMOVAL OF ALKANES AND POLYCYCLIC AROMATIC HYDROCARBONS BY BACTERIA FROM SOIL CHRONICALLY EXPOSED TO CRUDE PETROLEUM OIL

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Abstract. AramcoS2 and AramcoS4 are two bacterial strains that were isolated from oil-contaminated soil and able to degrade crude petroleum. The gas chromatography-mass spectrometry (GC-MS) technique was used to identify residues of petroleum after biodegradation. The two isolates were able reduce the concentration of long-chain n-alkanes of C10 to C20; (n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, n-octadecane n-nonadecane, and n-eicosane) on average by 77% of the original concentration. They were also able to degrade polycyclic aromatic hydrocarbons (PAHs) including biphenyl, naphthalene, and anthracene on average by 67% of the original concentration within 7 and 14 days of incubation at 30ºC, pH=6.8±0.2. AramcoS2 and S4 were classified as Actinobacteria based on the 16S rRNA gene sequences (GenBank accession numbers are; AramcoS2; MN142506 and AramcoS4; MN142551). They should be of great practical significance both in the bioremediation of soil contaminated with petroleum and the bio-treatment of oil spills on surface water.
Keywords: *n-alkanes, GC-MS, PAHs degradation, microorganisms, pollutants*

Introduction

Biodegradation is the breakdown of organic substances by microbes to their final products as water and carbon dioxide. Petroleum is an excellent source of energy and materials for petrochemical industries. It is composed of different hydrocarbons mixed with non-hydrocarbons. The most common hydrocarbon molecules are n-alkanes or paraffin (22%), cycloalkanes or naphthenes (50%), and 17% PAHs (Moubasher et al., 2015). Contamination with petroleum severely affects both human and environmental health (Bacosa and Inoue, 2015). Petroleum hydrocarbons (n-alkanes and PAHs) are prevalent organic contaminants (Lee et al., 2018; Huang et al., 2019) usually produced as a result of pyrolysis of organic compounds or incomplete combustion. PAHs have mutagenic, and carcinogenic and cause endocrine system problems (Dutta et al., 2017). The carcinogenic effect of those organic pollutants on body organs appears after prolonged periods of exposure (Hernández et al., 2017; Koual et al., 2019). Short and

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long-chain alkanes and PAHs are the most common components of petroleum and many microorganisms utilize these components as carbon and energy sources (Adetutu et al., 2015; Ahmad et al., 2015). Despite their hydrophobicity, long-chain alkanes and PAHs can be degraded by microorganisms but at a slow rate (Chen et al., 2017a).

The discovery of new genera and species of microorganisms that can degrade petroleum is the core of petroleum microbiology. Biodegradation is a widely applied technology to reduce or eliminate petroleum hydrocarbons from affected soils. It has been applied with other processes to remediate both n-alkanes and PAHs contaminants. However, the Gulf area, particularly Saudi Arabia, is considered one of the largest petroleum producers; Saudi Arabia is the second-largest oil reservoir globally, hence, more prone to petroleum pollution. Therefore, biodegradation and bioremediation are useful methods to remove petroleum pollutants from the environment. The lack of information about oil-degrading microorganisms in the eastern region of Saudi Arabia motivated us to run this research. This study aimed to study the indigenous bacteria that degrade petroleum oil and reduce its contamination of soil and water.

Materials and methods

Chemicals

Crude petroleum was obtained from Aramco Company (Saudi Arabia). Hexadecane and octadecane from BDH chemicals (England), toluene from Sigma-Aldrich Chemical Co. (Germany), biphenyl from Scharlau (Spain), naphthalene and anthracene from Lobachemie (India), and bacterial medium (mineral salt medium; MSM) from Merk KGaA, Darmstadt (Germany).

Soil samples and site description

Soil samples (4 samples) were collected at different depths; 5, 15, 25, and 35 cm from the head well of petroleum aquifers at Uthmaniyah (Aramco). It is located around 35 km south-west of Al-Ahsa city, in the desert, geo-position 25°21'52"N, 49°33'55"E, with 83.3 mm of annual precipitation, the average temperature in summer between 35°C and 45°C, and in winter between 5°C and 25°C.

Enrichment of petroleum-degrading bacteria

The procedure of Parthipan et al. (2017) was followed. Soil samples were introduced separately to a standard bacterial medium (MSM) at 1 gm soil/100 ml, pH 6.8±0.2 in 250 ml Erlenmeyer flasks with continuous shaking at 30ºC and 120 rpm. The medium was autoclaved and amended with a 1% (v/v) filter-sterilized (0.22 μm syringe filter) petroleum as the sole carbon and energy source. A control sample of 1% petroleum in MSM was used. After a series of three subcultures, inoculums were streaked out on MSM containing agar, and the phenotypically different colonies were characterized.

Scanning electron microscopy (SEM) of AramcoS2 and AramcoS4 isolates

The bacterial cells were directly applied onto the copper grid, allowed to dry for 2 hours then examined under a scanning electron microscope (TECHNAI10-Philips).

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Sequencing of 16S rRNA

Genomic DNA from a single colony of each isolate was used separately as a template for PCR amplification of the 16S rRNA gene in the presence of the universal primers 27F and 1492R. The PCR reaction was carried out as follows; one cycle of DNA denaturation at 94°C for 45 s, 35 cycles at 94°C for 45 s, 55°C for the 60 s for annealing of primers, and 72°C for 2 min as an extension step. These cycles were followed by a final elongation step at 72°C for 5 min. Sequencing of DNA was performed using the Big Dye terminator sequencing kit in a 3730xl DNA analyzer (Applied Biosystems, USA) using the universal primer pair 785F and 907R.

Construction of phylogenetic trees

The sequences obtained from the 16S rRNA were edited and assembled using GENETYX software (GENETYX, Tokyo, Japan). The assembled sequences were used in BLASTN for the search of similar sequences on the NCBI (The National Center of Biotechnology Information) database. The most similar sequences were retrieved and applied to MEGA7 software to build phylogenetic relationship trees using the neighbor-joining method and bootstrap support based on 1000 replicates. The evolutionary distances were computed using the Kimura 2-parameter method (Kumar et al., 2016).

Biodegradation of petroleum

Petroleum residues were identified by chemical analysis of samples following the procedure of Zhang et al. (2011). Four experimental sets were prepared of 100 ml MSM supplemented with 1% crude oil for; a) AramcoS2, b) AramcoS4, c) mixed culture of AramcoS2 + AramcoS4, and d) a control set without bacteria. All four experimental sets were incubated at 30°C, pH=6.8±0.2 with continuous shaking at 150 rpm for 7 and 14 days. The rate of petroleum biodegradation was monitored by extraction and measurement of the concentration of petroleum residues using the GC-MS technique. Five milliliters of each treatment were extracted with 5 ml chloroform and shaken in a closed conical flask for 3 hours; (1:1; v/v). The organic layer was collected using a separation funnel. The obtained organic extract was analyzed in the Shimadzu GC-MS instrument, model QP2010 SE equipped with Rx-5 Sil MS (30 mm length X 0.25mm ID X 025 µm film thickness) capillary column (Li et al., 2016). Analysis of 1 µl of each extract was performed in GC-MS using the following parameters: temperature of injector, 250°C; starting temperature of the oven, 40°C (hold for 1min), then adjusted to 230°C at a rate of 10°C min^{-1}. The inlet setting was the split-less mode. The line of GC-MS transfer was held at 200°C. Highly pure helium was used as a carrier gas with a flow rate of 1 ml min^{-1}. Lab Solution software (Shimadzu, Japan) was used to control the system and to report the analytical data.

Biodegradation of pure n-alkanes and PAHs

To examine the degradability of isolates, 125 ppm of each pure n-alkane (n-hexadecane and n-octadecane), toluene, or PAHs (biphenyl, naphthalene, and anthracene) were injected into separate vials that contained 100 ml of MSM and freshly harvested cells of AramcoS2 or/and AramcoS4. Treatments were kept in a dark incubator at 30ºC and shaking at 150 rpm. Samples were collected after 7 and 14 days for analysis (Prakash et al., 2014). Three milliliters of each n-alkane, toluene, and PAHs were extracted with an equal volume of hexane for 30 min. The organic phase was collected

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using a separation funnel and then analyzed using the same GC-MS instrument mentioned in the former section and the same parameters according to Li et al. (2016).

Results and discussion

Isolation and preliminary identification of petroleum-degrading bacteria

Twenty bacterial isolates were able to grow during enrichment cultivation in MSM amended with 1% of petroleum as an energy and carbon source. Two isolates (AramcoS2 and AramcoS4 for easy recognition) were able to degrade up to 80% of petroleum under experimental conditions. The colonies of AramcoS2 appeared creamy in color, opaque and Gram-positive. Cells are non-motile, coccoid, and grow rapidly and optimally between 28-30ºC, measuring 0.88 µm in length and 0.35 µm in diameter. Whereas AramcoS4 formed small colonies, white in color, opaque, Gram-positive, slowly growing bacilli, and the cell measures 1.85 µm in length and 0.5 µm in diameter (*Fig. 1*).

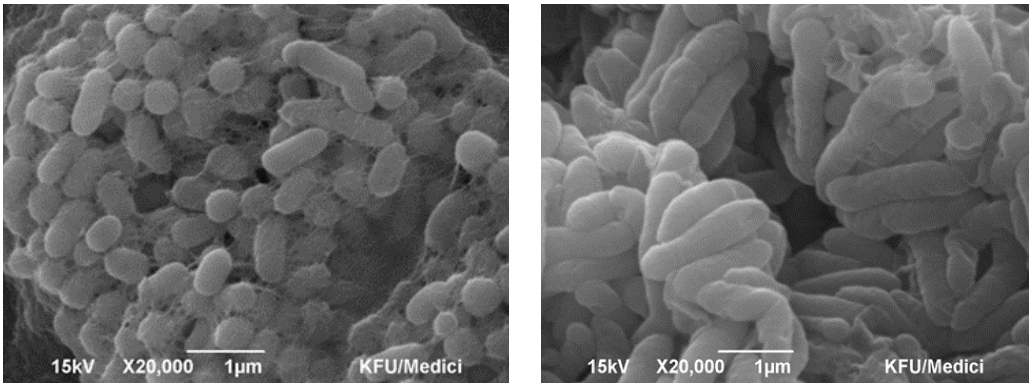

Figure 1. *Scanning electron microscope images of a pure culture of bacterial isolate AramcoS2 (left) and AramcoS4 (right)*

Sequencing and phylogeny using 16S rRNA

Based on the results of aligned sequences and phylogenetic relationships (*Fig. 2*), AramcoS2 was found similar (99.99%) to Georgia daeguensis strain 2C6-43 (NR 117960.1) of the phylum Actinobacteria, a 4-chlorophenol (aromatic hydrocarbons)-degrading bacterium isolated from activated sludge during industrial wastewater treatment (Chen et al., 2017b). In addition, the gene for AramcoS2 was similar to that of G. soli strain C1-25c-3 (JX517240.1), and G. ferrireducens strain S61 (MG786638.1). All of these strains belong to the phylum Actinobacteria, which includes members well-known to degrade cyclic contaminant hydrocarbons (Chen et al., 2014, 2017a, 2020; Hu et al., 2020).

AramcoS4 showed high homology with the genus Arthrobacter sp. HS-NOB2 1427 (KF668242.1) (100%), A. nicotianae strain VITNJ6 (KM047491.1), and Glutamicibacter nicotianae strain FL95 (KY849352.1). Arthrobacter is well-known to degrade phenone and acetophenone (Chen et al., 2014). *A. citrus*, isolated from an organic waste-rich site, is an aerobic bacterium able to respire phenols as a sole carbon source. Recently, Zhihengliuella sp. ISTPL4 was found to efficiently degrade and detoxify phenanthrene (Mishra et al., 2020). The Actinobacteria is capable of living on limited nutritional resources, is resistant to drought, and has great potential to degrade alkanes and PAHs

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under such conditions. This fact is in full agreement with AramcoS2 and AramcoS4 isolates since they were collected from the eastern desert of Saudi Arabia where the environment is dry and nutritional resources are limited.

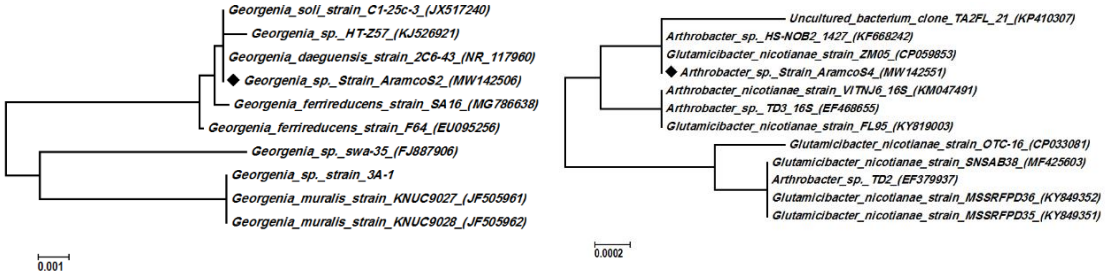

Figure 2. *Evolutionary relationships of AramcoS2 (left) and AramcoS4 (right) bacterial isolates*

Biodegradation of petroleum

The analysis of control samples showed that the most abundant fractions in petroleum are saturated hydrocarbons, mainly C10 to C20 alkanes; n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, n-nonadecane, and n-eicosan. After 7 or 14 days of incubation, the petroleum degradation was checked in the three treatments (AramcoS2, AramcoS4, and AramcoS2 + AramcoS4) along with the control. The obtained chromatograms and peak areas from the control and treatments were used to identify each compound of C10-C20 hydrocarbons. When the obtained data from the treatments were compared to the control, it was found that both isolates were able to degrade n-alkane hydrocarbons effectively after 7 days of incubation at 30ºC. In general, AramcoS2 isolate was able to degrade n-decane, n-dodecane, n-tridecane, and n-tetradecane by more than 85%, while it was able to degrade the long chains of n-pentadecane, n-hexadecane, n-heptadecane, n-nonadecane, and n-eicosan by more than 75%. AramcoS4 was able to degrade n-decane by 85%, but reduced n-undecane, n-dodecane, n-tridecane, and n-tetradecane by 65-68% and reduced long-chain alkanes n-pentadecane, n-hexadecane, n-heptadecane, n-nonadecane, and n-eicosan by 56-71%. The degradation efficiency seems to decrease with the increase of the alkane chain. The results indicated that AramcoS2 is stronger in the biodegradation of long-chain alkanes than AramcoS4. Several studies documented the biodegradation of crude oil /oily sludge using a single or consortium of microorganisms (Hamzah et al., 2013; Chen et al., 2017a).

The mixed culture of AramcoS2 + AramcoS4 reduced the peak areas of n-decane, n-undecane, n-dodecane, n-tridecane, and n-tetradecane by 72% and 58%, 65%, 53%, and 51%, respectively. Similar to the separate isolates, the degradation efficiency of the mixed isolates was lower for long-chain saturated alkanes (C15, 32%; C16, 22 %; C17, 16%; C19, 13%, and C20, 11%). Heavier hydrocarbons usually express resistance to biodegradation. Several studies have documented that long-chain alkane hydrocarbons need more degradation time than short ones when a consortium of microorganisms was used (Chen et al., 2014, 2017b; Li et al., 2016). Based on the results above, it can be stated that AramcoS2 isolate is a potential candidate tool in the field of biodegradation and soil restoration from petroleum contamination.

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Biodegradation of pure n-alkanes and PAHs

To confirm the above results, it was found that AramcoS2 and AramcoS4 could metabolize pure C16 and C18 alkanes. AramcoS2 degraded 85-90% of n-hexadecane and 80-85% of n-octadecane after 7 and 14 days of incubation (*Fig. 3a & b*), respectively. Whereas, AramcoS4 degraded about 70-80% of n-hexadecane and 40%-70% of n-octadecane during the same incubation periods (*Fig. 3c & d*).

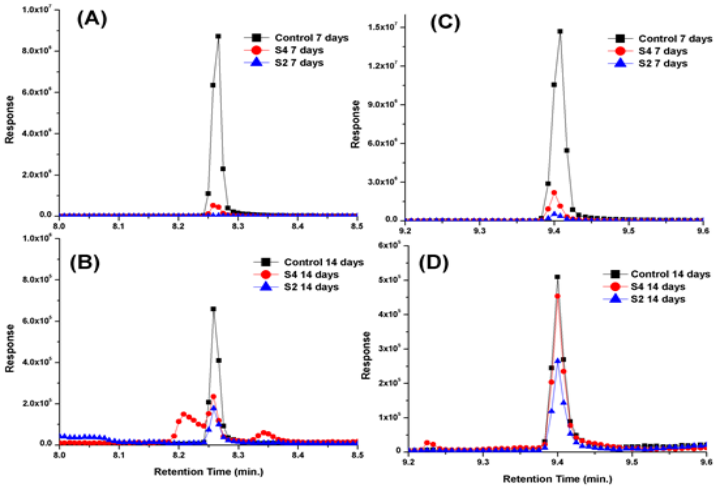

Figure 3. *n-Hexadecane (a) & (b) or n-Octadecane (c) & (d) degradation after 7 or 14 days, respectively, of incubation without or with AramcoS2, AramcoS4, or a mix of both isolates*

AramcoS2 degraded about 94-96% of the pure toluene, and 95-98% of biphenyl after 7 and 14 days of incubation, successively, and degraded 91% of naphthalene after 14 days, but there was no significant degradation of naphthalene after 7 days. While AramcoS4 degrade about 73-91% of the toluene, and 88-91% of biphenyl after 7 and 14 days of incubation, consecutively, and degraded 77% of the naphthalene after 14 days, but there was no significant degradation of naphthalene after 7 days (*Fig. 4a-d; Fig. 5a & b*).

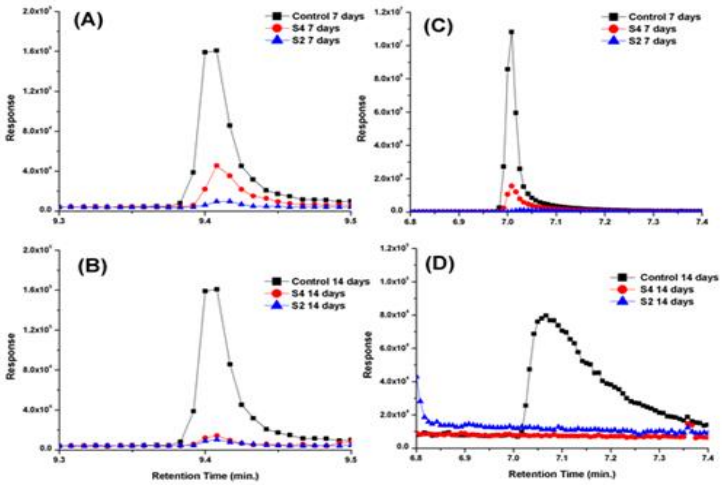

Figure 4. *Toluene (a) & (b) or Biphenyl (c) & (d) degradation after 7 or 14 days, respectively, of incubation without or with AramcoS2, AramcoS4, or a mix of both isolates*

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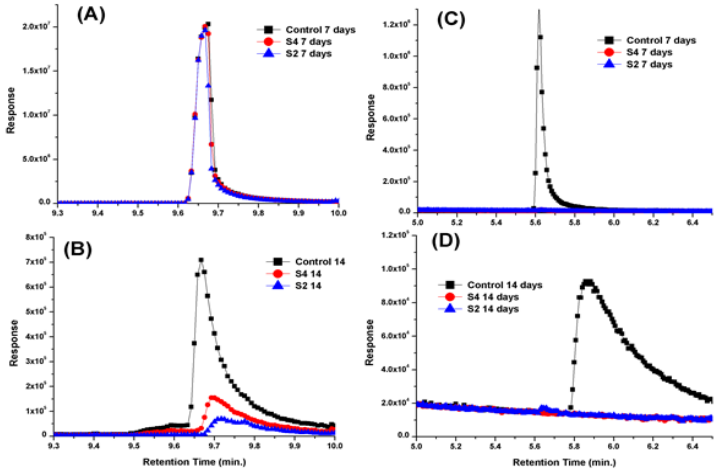

Figure 5. *Naphthalene (a) & (b) and Anthracene (c) & (d) degradation after 7 or 14 days, respectively, of incubation without or with AramcoS2, AramcoS4, or a mix of both isolates*

The degradation of anthracene by both isolates was nearly identical, i.e. 96% after 7 days and 98% after 14 days of incubation (*Fig. 5c & d*).

Therefore, the degradation of petroleum, C16, and C18 alkanes, toluene, and PAHs by the two isolates show aligned results, which emphasized that both isolates, i.e., AramcoS2 and AramcoS4 are petroleum-degrading bacteria. They can also use alkanes, toluene, and PAHs to support their metabolism. Microbial degradation of alkanes has been extensively studied, and the pathways and genes involved in the degradation of alkanes are also documented (Bian et al., 2015; Laso-Pérez et al., 2019; Wang et al., 2019; Liu et al., 2019). Monooxygenases were found to be triggered by alkanes and start to oxidize the terminal methyl group to produce alcohol that is turned into aldehyde, then to a fatty acid by continuous oxidation. β-oxidation processes the fatty acid into acetyl-CoA. The degradation of alkanes by microorganisms focused on the short and medium-chain alkanes. AramcoS2 and AramcoS4 were isolated from soil chronically exposed to petroleum spills. They were able to degrade long-chain n-alkanes of C10 to C20, toluene, and PAHs. They can lower the concentration of alkanes and PAHs. The obtained 16S rRNA gene sequences placed both isolates in the phylum Actinobacteria, a well-known degrading bacteria.

Conclusion

In conclusion, AramcoS2 and AramcoS4 are two bacterial isolates inhabiting the eastern desert soil of Saudi Arabia. They can efficiently degrade the major components of crude petroleum oil; n-alkanes and PAHs. Despite that, AramcoS2 isolates degraded crude oil with higher efficiency compared to AramcoS4 isolate. The 16S rRNA analysis of both bacteria placed these isolates in the same Actinobacteria phylum that contains members known for their ability to degrade crude oil spills along with cyclic contaminant hydrocarbons. Based on the results obtained from the current study, it is suggested that AramcoS2 and AramcoS4 isolates can be considered key players to mediate in or remove oil spills, saturated hydrocarbons, and PAHs fractions.

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Conflicts of Interests. The authors declared no conflict of interests.

REFERENCES

[1] Adetutu, E. M., Bird, C., Kadali, K. K., Bueti, A., Shahsavari, E., Taha, M., Patil, S., Sheppard, P. J., Makadia, T., Simons, K. L., Ball, A. S. (2015): Exploiting the intrinsic hydrocarbon-degrading microbial capacities in oil tank bottom sludge and waste soil for sludge bioremediation. – International Journal of Environmental Science and Technology 12: 1427-1436.

[2] Ahmad, M., Sajjad, W., Rehman, Z. U., Hayat, M., Khan, I. (2015): Identification and Characterization of Intrinsic Petrophilic Bacteria from Oil Contaminated Soil and Water. – Int. J. Curr. Microbiol. App. Sci (on-line) 4: 338-346.

[3] Bacosa, H. P., Inoue, C. (2015): Polycyclic aromatic hydrocarbons (PAHs) biodegradation potential and diversity of microbial consortia enriched from tsunami sediments in Miyagi, Japan. – Journal of Hazardous Materials (on-line) 283: 689-697. 10.1016/j.jhazmat.2014.09.068.

[4] Bian, X. Y., Mbadinga, S. M., Liu, Y. F., Yang, S. Z., Liu, J. F., Ye, R. Q., Gu, J. D., Mu, B. Z. (2015): Insights into the anaerobic biodegradation pathway of n-Alkanes in oil reservoirs by detection of signature metabolites. – Scientific Reports 5: 1-12.

[5] Chen, Y., Li, C., Zhou, Z., Wen, J., You, X., Mao, Y., Lu, C., Huo, G., Jia, X. (2014): Enhanced biodegradation of alkane hydrocarbons and crude oil by mixed strains and bacterial community analysis. – Applied Biochemistry and Biotechnology 172: 3433-3447.

[6] Chen, Q., Li, J., Liu, M., Sun, H., Bao, M. (2017a): Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. – PLoS ONE 12: 1-23.

[7] Chen, W., Li, J., Sun, X., Min, J., Hu, X. (2017b): High efficiency degradation of alkanes and crude oil by a salt-tolerant bacterium Dietzia species CN-3. – International Biodeterioration and Biodegradation (on-line) 118: 110-118. 10.1016/j.ibiod.2017.01.029.

[8] Chen, C., Chen, S., Zhang, W., Yuan, F., Yu, J., Liu, Q. (2020): Streptomyces sp. S501, a Marine Petroleum-Degrading Actinobacterium Isolated from Sediment of Yalujiang Estuary, Northern China, and Its Genome Annotation. – Current Microbiology (on-line) 77: 3643-3650. 10.1007/s00284-020-02181-2.

[9] Dutta, T., Kwon, E., Bhattacharya, S. S., Jeon, B. H., Deep, A., Uchimiya, M., Kim, K. H. (2017): Polycyclic aromatic hydrocarbons and volatile organic compounds in biochar and biochar-amended soil: a review. – GCB Bioenergy 9: 990-1004.

[10] Hamzah, A., Phan, C. W., Abu Bakar, N. F., Wong, K. K. (2013): Biodegradation of crude oil by constructed bacterial consortia and the constituent single bacteria isolated from Malaysia. – Bioremediation Journal 17: 1-10.

[11] Hernández, Á. R., Boada, L. D., Mendoza, Z., Ruiz-Suárez, N., Valerón, P. F., Camacho, M., Zumbado, M., Almeida-González, M., Henríquez-Hernández, L. A., Luzardo, O. P. (2017): Consumption of organic meat does not diminish the carcinogenic potential associated with the intake of persistent organic pollutants (POPs). – Environmental Science and Pollution Research 24: 4261-4273.

[12] Hu, J., Bao, Y., Zhu, Y., Osman, R., Shen, M., Zhang, Z., Wang, L., Cao, S., Li, L., Wu, Q. (2020): The Preliminary Study on the Association Between PAHs and Air Pollutants

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DOI: http://dx.doi.org/10.15666/aeer/2101_725733

and Microbiota Diversity. – Archives of Environmental Contamination and Toxicology (on-line) 79: 321-332. 10.1007/s00244-020-00757-4.

[13] Huang, Y., Pan, H., Wang, Q., Ge, Y., Liu, W., Christie, P. (2019): Enrichment of the soil microbial community in the bioremediation of a petroleum-contaminated soil amended with rice straw or sawdust. – Chemosphere (on-line) 224: 265-271. 10.1016/j.chemosphere.2019.02.148.

[14] Koual, M., Cano-Sancho, G., Bats, A. S., Tomkiewicz, C., Kaddouch-Amar, Y., Douay-Hauser, N., Ngo, C., Bonsang, H., Deloménie, M., Lecuru, F., Le Bizec, B., Marchand, P., Botton, J., Barouki, R., Antignac, J. P., Coumoul, X. (2019): Associations between persistent organic pollutants and risk of breast cancer metastasis. – Environment International (on-line) 132: 105028. 10.1016/j.envint.2019.105028.

[15] Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. – Molecular Biology and Evolution 33: 1870-1874.

[16] Laso-Pérez, R., Hahn, C., Van Vliet, D. M., Tegetmeyer, H. E., Schubotz, F., Smit, N. T., Pape, T., Sahling, H., Bohrmann, G., Boetius, A., Knittel, K., Wegenera, G. (2019): Anaerobic degradation of non-methane alkanes by "candidatus methanoliparia" in hydrocarbon seeps of the Gulf of Mexico. – mBio 10: 1-20.

[17] Lee, D. W., Lee, H., Lee, A. H., Kwon, B. O., Khim, J. S., Yim, U. H., Kim, B. S., Kim, J. J. (2018): Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea. – Environmental Pollution (on-line) 234: 503-512. 10.1016/j.envpol.2017.11.097.

[18] Li, X., Zhao, L., Adam, M. (2016): Biodegradation of marine crude oil pollution using a salt-tolerant bacterial consortium isolated from Bohai Bay, China. – Marine Pollution Bulletin (on-line) 105: 43-50. 10.1016/j.marpolbul.2016.02.073.

[19] Liu, Y. F., Qi, Z. Z., Shou, L. B., Liu, J. F., Yang, S. Z., Gu, J. D., Mu, B. Z. (2019): Anaerobic hydrocarbon degradation in candidate phylum 'Atribacteria' (JS1) inferred from genomics. – ISME Journal (on-line) 13: 2377-2390. 10.1038/s41396-019-0448-2.

[20] Mishra, A., Rathour, R., Singh, R., Kumari, T., Thakur, I. S. (2020): Degradation and detoxification of phenanthrene by actinobacterium Zhihengliuella sp. ISTPL4. – Environmental Science and Pollution Research 27: 27256-27267.

[21] Moubasher, H. A., Hegazy, A. K., Mohamed, N. H., Moustafa, Y. M., Kabiel, H. F., Hamad, A. A. (2015): Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. – International Biodeterioration and Biodegradation (on-line) 98: 113-120. 10.1016/j.ibiod.2014.11.019.

[22] Parthipan, P., Preetham, E., Machuca, L. L., Rahman, P. K. S. M., Murugan, K., Rajasekar, A. (2017): Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium Bacillus subtilis A1. – Frontiers in Microbiology 8: 193.

[23] Prakash, A., Thavaselvam, D., Kumar, A., Kumar, A., Arora, S., Tiwari, S., Barua, A., Sathyaseelan, K. (2014): Isolation, identification and characterization of Burkholderia pseudomallei from soil of coastal region of India. – Springer Plus 3: 1-10.

[24] Wang, Y., Wegener, G., Hou, J., Wang, F., Xiao, X. (2019): Expanding anaerobic alkane metabolism in the domain of Archaea. – Nature Microbiology (on-line) 4: 595-602. 10.1038/s41564-019-0364-2.

[25] Zhang, Z., Hou, Z., Yang, C., Ma, C., Tao, F., Xu, P. (2011): Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated Pseudomonas aeruginosa DQ8. – Bioresource Technology (on-line) 102: 4111-4116. 10.1016/j.biortech.2010.12.064.

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