



Archives of Ecotoxicology

Journal homepage: <https://office.scicell.org/index.php/AE>



Hydrocarbon-Degrading Bacterial Strain *Pseudomonas mendocina* Newly Isolated from Marine Sediments and Seawater of Oran Harbor (Algerian Coast)

Faiza Bendadeche-Medjahed^{a*}, Mohamed Bey Baba Hamed^b, Sidi-Mohammed El-Amine Abi Ayad^a

^a Laboratory of Aquaculture and Bioremediation (AquaBior), University of Oran 1 Ahmed Ben Bella, Faculty of Nature and Life Sciences, Department of Biotechnology, B.P 1524, El M'Naouer, 31000, Oran, Algeria.

^b Higher School of Biological Sciences of Oran, 31000, Oran, Algeria.

Article info

Received 7 February 2020
Revised 1 June 2020
Accepted 3 June 2020
Published online 3 June 2020

Regular article

Keywords:

Crude oil
Pseudomonas mendocina
Bioremediation
Marine sediments
16S rDNA

Abstract

Contamination by petroleum hydrocarbons causes serious dangers to human health and the environment, whether by accidental or chronic contamination. Due to the large flow of ships, the commercial harbor of Oran is subject to pollution particularly by polycyclic aromatic hydrocarbons. For that, bioremediation by indigenous microorganisms is the most important method to eliminate or decrease this contamination. In the present paper, hydrocarbon-degrading bacterium strain SP57N has been studied, newly isolated from contaminated marine sediments and sea water from the harbor of Oran (Northwestern-Algeria), using of Bushnell-Hass salt medium (BHSM). The strain SP57N was Gram-negative, oxidase negative, catalase negative, motile, Rod-shaped bacteria, identified molecularly as *Pseudomonas mendocina* based on partial 16S rDNA gene sequence analysis, using the BLAST program on National Centre for Biotechnology Information (NCBI) and the EzBioCloud 16S rDNA databases. This isolate could growth on high concentrations of crude oil (up to 10 %, v/v). The effects of some culture conditions such as temperature, NaCl concentration and pH on growth rate of strain SP57N on crude oil as the sole carbon and energy source were studied. In addition, growth kinetic of this isolate on crude oil during 20 days of culture at 140 rpm, under optimal culture conditions was considered. The results showed maximum growth rate at temperature 25°C, 3% (w/v) of NaCl concentration and pH 7. Results of growth kinetic on crude oil as sole carbon and energy source showed that the stationary phase was attained at day 12. Thus, *Pseudomonas mendocina* SP57N had effectively hydrocarbon-degrading potential, and could be used as an efficacy degrader to initiate a biological eco-friendly method for the bioremediation of the hydrocarbon pollution on the port of Oran, and marine environment.

1. Introduction

Marine environments harbour a constant microbial seed that can be shaped by changes in environmental conditions including contamination by petroleum components. Oil spills are a major source of oil pollution in the marine environments, even though they are spilled in small but continuous discharges of hydrocarbons from transportation and recreational activities. Therefore, prokaryotic communities are well pre-adapted to oil pollution, and several microorganisms exposed to this xenobiotics have developed an active degradation response (Acosta-González and Marqués 2016). The rate of oil-contamination in sediments is significantly reduced by natural processes over a long duration. Therefore, various methods have been studied to achieve rapid and complete removal of oil from sediments (Agarwal and Liu 2015). Also

In-situ bioremediation is particularly considered as one of the most effective and sustainable means to clean up oil-contaminated sediments. However, they can be exploited more efficiently if their multifactorial environmental and pollution

parameters are known, even their metabolism, physiology and ecology (Mapelli et al. 2017).

Amongst petroleum hydrocarbons, the polycyclic aromatic hydrocarbons (PAHs) are the major common xenobiotic pollutants presenting serious risks to human health and all living organisms, become a most important concern, due to their bioaccumulation, ubiquitous in nature, having mutagenic, toxic and carcinogenic properties (mnif et al. 2017; Patel et al. 2018).

Different remediation technologies are available to remove PAHs from polluted environments. Conventional physical and chemical treatment methods are incapable to provide sustainable technology, because of their diverse disadvantages. The recently developed bioremediation approach is an alternative technology to effectively remediate the polluted by PAHs in environment (Patel et al. 2018).

Bioremediation become an interested eco-friendly, economical and cost-effective substitution for removing petroleum hydrocarbons from contaminated environment (Logeshwaran et al. 2018; Tiralerdpanich et al. 2018). Various microorganisms such as fungi, archaea and bacteria have

*Corresponding author: faizabendadeche@yahoo.com

capacity to degrade oil and petroleum hydrocarbons-degrading (**Atlas and Hazen 2011**). In addition, indigenous hydrocarbonoclastic bacteria living in contaminated environment, such as marine sediments, are more suitable and better adapted for restoration of the hydrocarbon contamination in sea (**Capello et al. 2007; Acosta-González and Marqués 2016**).

The commercial port of Oran (northwestern Algeria), an important port economically, is actually polluted essentially by HAPs due to a large flow of ships, hence the need to carry out an eco-friendly and cost effective technique for its decontamination and remediation.

The aim of the present work is to isolate indigenous hydrocarbonoclastic bacterial strains from contaminated marine sediments and seawater at the port of Oran, having to capability grown on and utilize crude oil, and to study the newly isolated hydrocarbons-degrading bacterial strain SP57N, with its interesting potential growth on crude oil as carbon and energy source.

2. Material and methods

2.1 Localization of sampling site and samples collection

Samples of mixed seawater and marine sediment were collected, a few millimeters above the surface of the sediments, at - 40 m depth, from the port of Oran (Algerian coast) (latitude: 35°42'44"N; longitude: 0°38'28"W), in October 2013, and were immediately transported to the Aquaculture and Bioremediation laboratory (AquaBior).

2.2 Enrichment, isolation and selection of hydrocarbon-based bacteria

From samples of mixed seawater-marine sediment, the isolation of hydrocarbon-degrading bacteria was isolated out using a Bushnell Hass synthetic mineral (BHSM) medium and crude oil (from the Hassi-Messaoud refinery, Algeria) as the sole source of carbon and energy, at 4%, 6%, 8% then 10% of crude oil (v/v) subsequently according to the modified protocol of **Mehdi and Giti (2008)**. Each subculture was incubated for 72 hours at 25°C and 140 rpm Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany). From the product of the last subculture, containing 10% (v/v) crude oil, inocula were seeded in BHSM agar medium, supplemented with 10 % (v/v) of crude oil, and incubated at 25°C during 7 days. The purification of the different colonies was carried out by successive subcultures on nutrient agar medium. Purity of cultures is analyzed morphologically, macroscopically and microscopically (Gram stain). Pure cultures with highest and outstanding visible growth rate and crude oil degradation on BHSM medium supplemented with 10% (v/v) of crude oil were selected, and stored at -20°C until use.

2.3 Study of physiological and biochemical characteristics the isolated bacterial strain SP57N

Physiological and chemical characteristics of the SP57N strain (carried out in triplicate) was determined, including Gram staining, the oxidase activity [kit oxidase test from (Fluka)], the catalase activity, motility, respiratory type, triple-sugar-iron test (TSI test), Citrate utilization test, and the ability to growth, BCPL medium, Chapman agar medium, King A agar medium, King B agar medium, and SS agar medium were systematically analyzed according to **Bergey's Manual of determination for bacteriology (Holt et al. 1998)**.

2.4 Partial 16S rRNA gene sequencing and phylogenetic analyses

Genomic DNA extraction of strain SP57N was carried out using an *EasyPure*® Bacteria Genomic DNA Kit (TransBionovo Co., Ltd., China), according to the manufacturer's instructions. DNA template sample was used for PCR amplification of the 16S rRNA gene, using universal primers, forward primer ben27F: 5'-AGAGTTTGATCCTGGCTC-3'; and reverse primer ben1492R: 5'-GGTTACCTTGTTAGGCTT-3', synthesized by Sigma (Germany). In total volume of 50 µl, mixture of the PCR reaction contained: 2 µL of DNA template (20 ng), 1µL of ben27F (25 µM), 1 µL of ben1492R (25 µM), 2.0 µL of dNTP (2,5mM), 2.0 µL of MgCl₂ (50 mM), 5 µL of 10X buffer solution (20 mM), 0.5 µL of taq DNA polymerase (5 UL⁻¹), and ddH₂O. The PCR program was: 95°C for 5 min; 35 cycles, 94°C for 1 min denaturation, 55°C for 1min annealing and 72°C for 2 min extension; and final extension at 72°C for 10 min. For analysis of the PCR products electrophoresis was carried out on 1.5% agarose gel, and then partially sequenced by Sanger sequencing services laboratory (GENEWIZ, Inc., South Plainfield, NJ, USA). Sequence similarity research was required using the alignment method on the EzBioCloud 16S rRNA database (**Yoon et al. 2017**), using reference sequences, and the National Biotechnology Information Center NCBI database, using published 16S rDNA sequences. Subsequently, phylogenetic trees was carried out using data analysis of 16S rDNA gene sequences by MEGA software package Version 7.0 (**Kumar et al. 2016**), using neighbor-joining methods.

2.5 Nucleotide sequence submission

The partial 16S rDNA gene sequence of the hydrocarbon-degrading bacteria strain SP57N was submitted to the NCBI-Gene Bank under the accession number MK825733

2.6 Investigation of culture parameters effects on growth rate of the bacterial isolate on crude oil

The optimum growth rates of the strain SP57N were investigated using different culture parameters such as temperature, NaCl of concentration and pH, using 50 ml BHSM medium, in 250 ml sterile flasks, with 2% (v/v) of crude oil, at 140 rpm shaking rate (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany), for about 48 h. All tests were performed in triplicate, and the analysis of variance (ANOVA) is conducted to analyze the data with significance levels less than 0.05 using the statistical analysis tool STATISTICA (Version 10).

2.7 Effect of temperature

The bacterial strain SP57N was inoculated aerobically into BH medium at different temperatures (20°C, 25°C, 30°C, 37°C and 40°C) at pH 7. After incubation, the growth rate was assessed by measuring optical density (OD 600 nm) with spectrophotometer (Perkin Elmer Lambda 35 UV/Vis Spectrometer, USA).

2.8 Effect of salt concentration

For the effect of the NaCl concentration test on the growth rate of strain SP57N, the concentration of NaCl in the medium was adjusted to 0%, 3%, 6%, 9%, 12% and 15% (w/v) at 25°C and pH 7, and the growth was investigated after 48 h of incubation indirectly by measuring the turbidity (OD 600 nm) with spectrophotometer (Perkin Elmer Lambda 35 UV/Vis Spectrometer, USA).

2.9 Effect of pH

The isolate bacterial strain SP57N was inoculated with different pH of the medium, (6.0, 7.0, 8.0 and 9.0), at 25°C. The pH was adjusted with 1 M NaOH or 1 M HCl. After incubation, the growth rate was measuring by the optical density (OD 600 nm) with spectrophotometer (Perkin Elmer Lambda 35 UV/Vis Spectrometer, USA).

2.10 Turbidimetric analysis of growth kinetic of the bacterial isolate on crude oil at optimal culture conditions

For the study of the growth kinetic on crude oil the bacterial strain SP57N, it was grown under optimal cultivation conditions in BHSM medium with 2% of crude oil (v/v) as sole source of carbon and energy, based on the results of these experiments, such as 3% (w/v) of NaCl, pH 7 and temperature 25°C on a orbital incubator shaker at 140 rpm (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany), for about 20 days. Cultures were performed in 50 ml of BHSM medium, on 250 ml flasks, in triplicate. Estimation of the growth rate of the isolate strain was carried out indirectly using turbidimetric analysis by measuring the optical density with spectrophotometer (Perkin Elmer Lambda 35 UV/Vis Spectrometer, USA), at 600 nm.

3. Results

3.1 Isolation and selection of hydrocarbonoclastic bacteria

Hydrocarbon-degrading bacteria were enriched then isolated from polluted marine sediments and seawater collected at the harbor of Oran (Algeria), using enrichment cultures and successive subcultures, with increasing crude oil concentrations (2-10 %, v/v). These isolates showed variable growth rates on the BHSM medium, supplemented by crude oil as sole source of carbon and energy (data not shown). Among these strains, the SP57N strain had remarkable and outstanding growth rate on crude oil as it sole carbon and energy source, at concentrations up to 10 % (v/v).

3.2 Identification of stain SP57N

The phylogenetic relationship of the hydrocarbon-bacterial bacterial strain SP57N, and the other representative strains of the genus *staphylococcus*, was determined. For that, after amplification of approximately 1,500 bp fragments of 16S rDNA (**Figure 1.**), this amplified rDNA was partially sequenced. Analysis of the continuous sequence thus determined of 928 bp (GenBank ID: MK825733) by the BLASTn alignment program, on the NCBI database, and on the EzBiocloud database, reveals the belonging of the strain SP57N to the species *Pseudomonas mendocina*. Indeed, the calculation of the similarity of the sequences, using the BLASTn program on the basis of NCBI data, showed that the partial sequence of the 16S rDNA gene of the strain SP57N had a similarity score of 98.92 % with the strain *Pseudomonas mendocina* NK-01 (CP002620.1), and 98.60 % with the strain *Pseudomonas mendocina* strain IITR46 (MF321766), and the strain *Pseudomonas mendocina* NCIB 10541 (NR_043421.1). While by analysis on the EzBiocloud database, we recorded a similarity rate of 97.90% with the strain *Pseudomonas mendocina* NBRC 14162 (BBQC01000018). Subsequently, two phylogenetic trees are constructed using similar 16S rDNA sequences from the two databases (**Figure**

2a,b). The results of the phylogenetic analysis show that the bacterial strain SP57N was related to the species of *Pseudomonas mendocina* (**Figure 2**). The phylogenetic trees indicated that the bacterial strain SP57N was related to *Pseudomonas mendocina* species (**Figure 2a,b**). For that, the strain SP57N was identified as *Pseudomonas mendocina*.

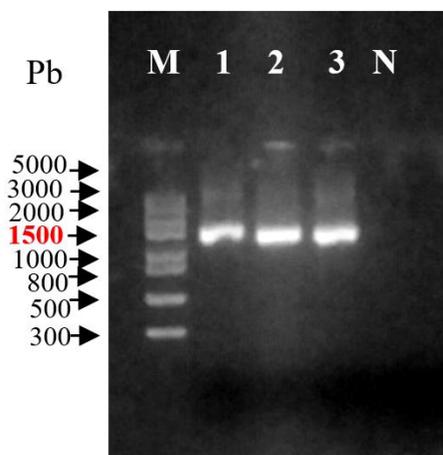


Figure 1. Electrophoresis of the PCR product of the bacterial strain SP57N 16S rDNA gene on 1.5% agarose. Lanes 1, 2 and 3: Amplified 16S rDNA; M: Trans5K DNA marker, N: negative control.

The isolate strain SP57N was found to be Gram negative, strict aerobic, motile, Rod-shaped bacteria (**Figure 3c**). The colonies on nutrient agar plate were translucent greenish-beige, Smooth, brilliant, convex, with irregular margin and 1-2 mm in diameter after incubation for 24 h at 25°C (**Figure 3a,f**), and approximately 3-4 mm in diameter after incubation for 48 h at 25°C (**Figure 3b**). The results of the biochemical characteristics of the SP57N strain are shown in **Table 1**.

3.3 Effects of culture conditions on the growth rate of the hydrocarbon degrading bacterial isolate on crude oil

To understand the environmental characteristics effects on the growth rate of the isolate strain SP57N on crude oil as sole source and energy source, factors such as temperature, NaCl concentration, and pH were studied.

Effects of different temperature in growth rate of strain SP57N on crude oil as sole carbon and energy source are illustrated in **Figure 4a**. The results in the curve indicate that this strain could utilize crude oil at temperature 20°-37°C, and it maximum growth rate was at temperature 25°C.

Results of effect of different concentration of NaCl on growth rate of the isolate strain SP57N on crude oil as sole carbon and energy source are showed in **Figure 4b**. As it can be seen from the curve, the optimum NaCl concentration for maximum growth rate of strain SP57N was 3% (w/v). However, this bacterial strain could tolerate high salt concentrations (up to 15%, w/v). Results obtained by effect of different pH of the medium on growth rate of strain SP57N in crude oil as sole carbon and energy source are represented in **Figure 4c**. This curve demonstrated that the strain SP57N had optimum growth rate at pH 7, and might grow at pH 6-9.

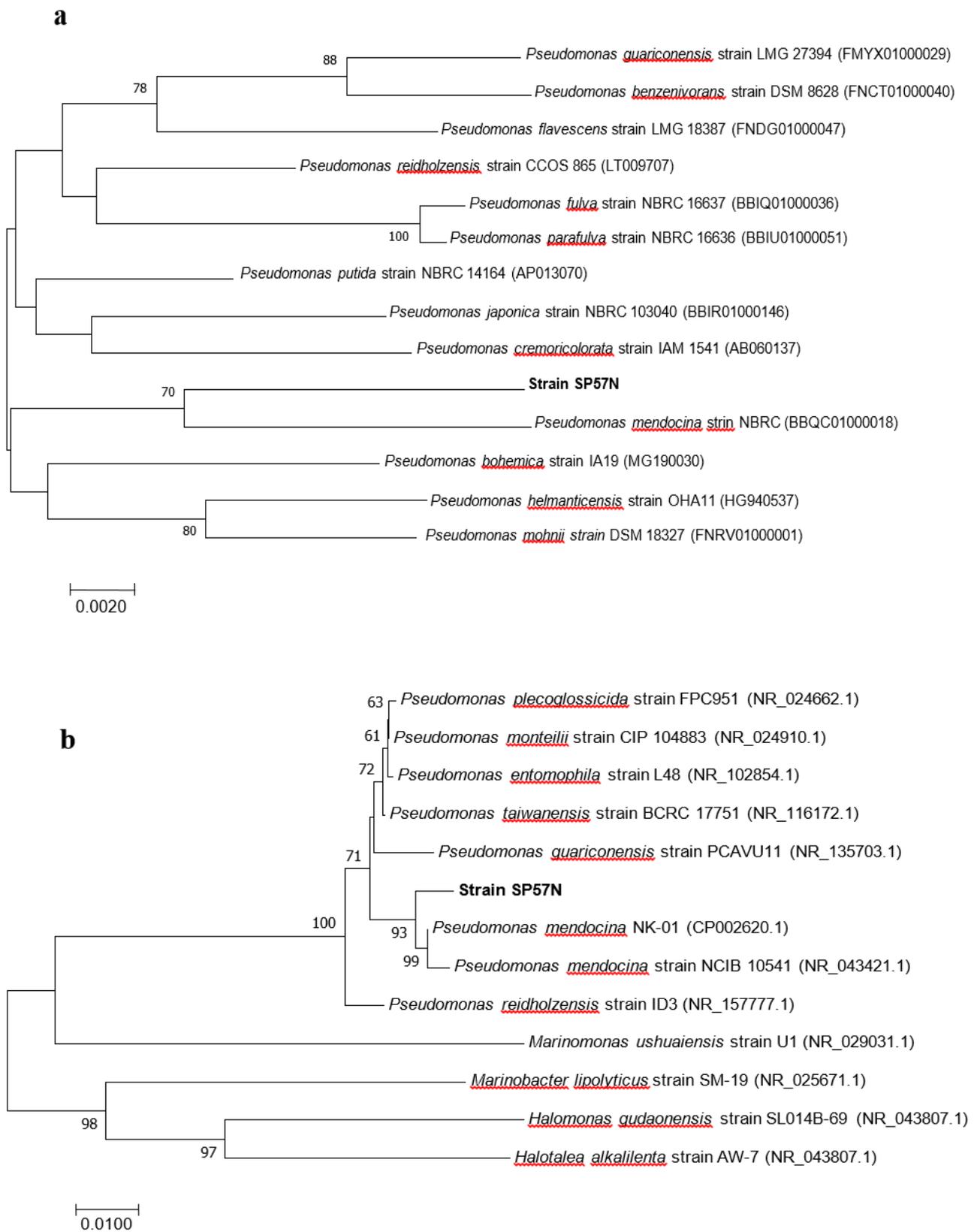


Figure 2. Phylogenetic trees of the 16S rDNA sequence of the SP57N strain and the closest-related species carried out using the software MEGA 7.0; (a) From the NCBI database, (b): From EzBioCloud database, using the neighbor Neighbor-Joining method (bootstrap = 1000); The scale bar represents the sequence divergence. GenBank accession numbers are indicated in parenthesis.

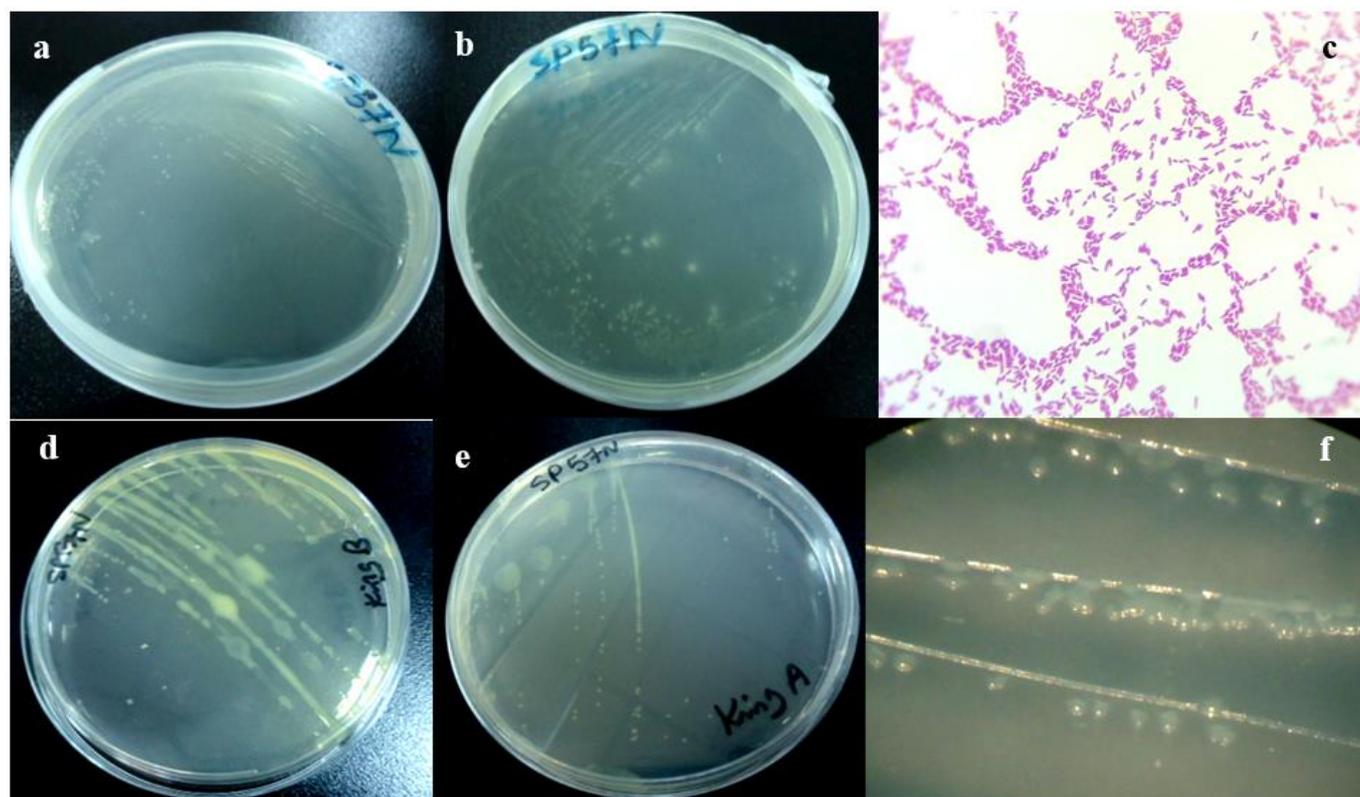


Figure 3. Colonies growth and cells morphological aspects of the bacterial strain SP57N on different culture medium; (a): Colonies of the strain SP57N on the nutrient agar plate after 24h of incubation; (b): colonies of strain SP57N on the nutrient agar plate after 48h of incubation; (c): Gram negative bacterium of strain SP57N (10 x 100); (d): Colonies of the strain SP57N on the King B medium agar plate after 24h of incubation; (e): Colonies of the strain SP57N on the King A medium agar plate after 24h of incubation; (f): Colonies of strain SP57N on the nutrient agar plate after 24h of incubation observed with Binocular magnifier (x 20).

Table 1. Morphological and biochemical characteristics of the isolated bacterial strain SP57N.

Characteristics	Results
Gram staining	- (Fig. 3c)
Shape	Rod-shaped (Fig. 3c)
Oxidase test	-
Catalase test	-
Motility	+
Mannitol	-
Respiratory type	Strict aerobia
TSI test	K/K
Lactose	-
Saccharide	-
Glucose	-
Indole	-
H ₂ S production	-
Gas production	-
Citrate utilization	+
Chapman	-
SS	+
King A	+ (Fig. 3f)
King B	+ (Fig. 3d)
BCPL	-

3.4 Growths kinetic of growth the hydrocarbonoclastic isolate SP57N under optimal culture conditions on crude oil

The bacterial stain SP57N was grown on 2% (v/v) of crude oil as sole carbon and energy source in BHSM medium, for 20 days, on their optimal culture conditions (Temperature 25°C; pH 7; and 3% (w/v) of NaCl concentration). The growth rate of this bacterial isolate was estimated using turbidimetric method by measuring at the optical density 600 nm, and the results are shown in **Figure 5**. As shown in the curve, strain SP57N started the logarithmic growth phase from the first to 12th day, and then the stationary phase was attained at day 12.

4. Discussion

The SP57N strain was part of the hydrocarbon-degrading bacterium belonging to *Pseudomonas mendocina*, which was firstly isolated from marine sediments and seawater at the port of Oran (Algeria), having potential to develop a method of bioremediation to clean up petroleum hydrocarbon contamination in marine environments.

Partial sequence analysis of the 16S rDNA gene (928 pb) of isolated strain SP57N confirmed the identification as *Pseudomonas mendocina* specie, with a similarity to *Pseudomonas mendocina* specie of 98.92 % using BLASTn alignment on NCBI database, and 97.90% of similarity using BLASTn alignment on EzBioCloud database. In addition, the morphological characteristics of the strain SP57N were very similar to those of the *Pseudomonas mendocina* reported previously.

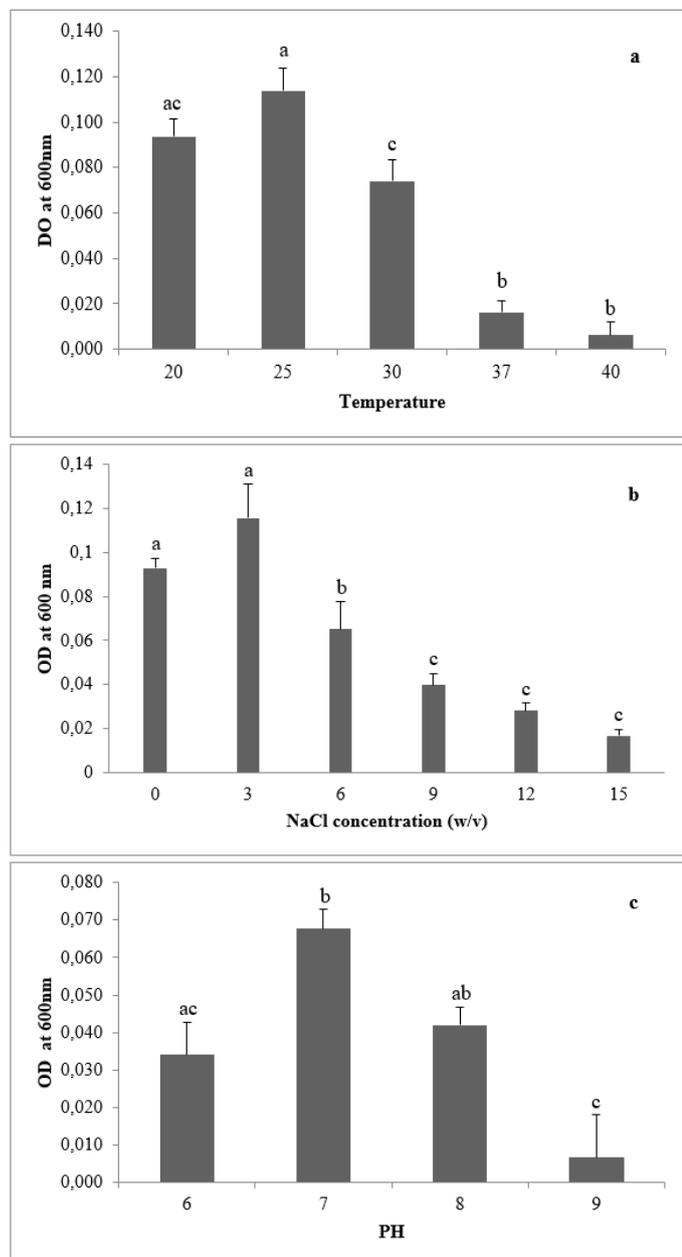


Figure 4. Effects of culture conditions on growth rate of strain SP57N in BHSM medium supplemented with 2% of crude oil (v/v) as sole source of carbon and energy, after incubation time of 48 h at 140 rpm. The histograms with different indices are significantly different ($p < 0.05$). (a): Effect of temperature; (b): Effect of medium salinity (NaCl concentration); (c): Effect of pH.

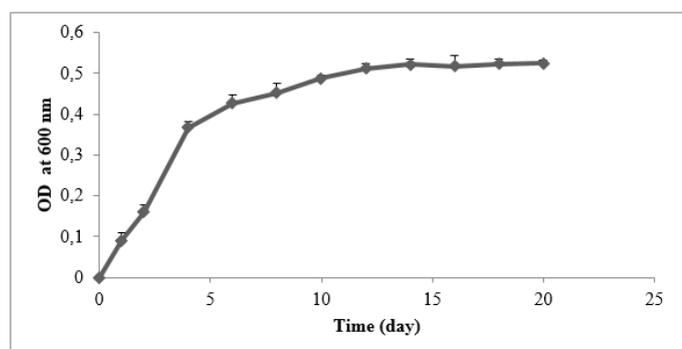


Figure 5. Growth kinetic of the bacterial strain SP57N on 2% of crude oil (v/v) as sole source of carbon and energy at optimal culture conditions and 140 rpm over 20 days of incubation. Bars

represent standard deviation and experiments were performed in triplicate.

Before contribution to bioremediation, it is very important to study effects of culture conditions on petroleum hydrocarbons degradation, since it furnishes information about the bacteria and their growth requirements.

Several bacteria have the capacity to biodegrade hydrocarbons. These microorganisms are available in sites polluted by hydrocarbons. The number and diversity of these hydrocarbonoclast bacteria are influenced by various environmental, physical and chemical factors, such as temperature, pH, salinity, and the source of carbon. Seasonal and climate change also plays a role in the number and diversity of bacteria in general and hydrocarbonoclast bacteria in particular (Acosta-González and Marqués, 2016; Kumar et al. 2019).

Given the complexity of petroleum products and environmental conditions, the study of the effect of environmental conditions on oil degradation is essential. Indeed, before contribution to bioremediation, it is very important to study effects of culture conditions on petroleum hydrocarbons degradation, since it furnishes information about the bacteria and their growth requirements.

To disclose the crude-oil-degrading conditions of strain SP57N, temperature from 20° to 40°C, NaCl concentration from 0 to 15% (w/v) and pH from 6 to 9 were investigated, as shown in (Figure 4a,b,c).

From the curve of Fig. 3a, we note that our isolate had capability to growth in a wide temperature range, from 20°C to 37°C, with maximum growth rate at 25°C. These results are according with those obtained by Chang et al. (2011).

Concerning temperature, it is responsible for controlling the nature and quantity of microbial metabolism in hydrocarbons, and diffusion rates, solubility and bioavailability. It is the main factor that influences the rate of biodegradation, by directly effecting bacterial metabolism and growth rate. It affects also the chemical and physical properties of oil (Wong et al. 2012).

Results of effect of NaCl concentration test (Figure 4b) showed that strain SP57N had maximum growth rate at 3% (w/v) of NaCl, corresponding to the salinity of seawater which is 37–39.2 ‰ in the Mediterranean Sea (Effrosynidis et al. 2018). Also, this isolate could support high concentrations of NaCl and grown in wide range of salinity (0–15%, w/v). These results are consistent with those obtained by Abou-Elela et al. (2010). According to Shiaris (1989), salinity could have a positive impact on the capability of degrading hydrocarbon. There is correlation between degree of salinity and the rate of several hydrocarbons mineralization.

Maximum growth was obtained at pH 7.0 (Figure 4c). These results consist with those obtained by Chang et al. (2011). According to Deng et al. (2014), extreme pH inhibited the biodegradation of the oil. However, in the marine environment, as in fresh water, the favorable range of pH was between 7 and 8 (Varjani and Upasani 2017).

For results obtained from kinetic of biodegradation of crude oil by the isolated bacterial strain in the optimal culture conditions (Figure 5), the stationary phase was attained at day 12. These results indicate that this bacterial strain SP57N had effectively the ability to utilize crude oil as the sole source of carbon and energy, which reflects the potential for degradation of hydrocarbons.

Formerly, several studies have shown the capacity of *Pseudomonas* sp. to degrade hydrocarbons (Nwinyi et al. 2016; Chebbi et al. 2017; Nogales et al. 2017; Ramadass et al. 2018). Also, the genus *Pseudomonas* is among the species that show a high efficiency in degrading polycyclic aromatic hydrocarbons (Lamichhane et al. 2017; Sun et al. 2019).

These microorganisms have the property of colonizing environments contaminated by hydrocarbons (Liu et al. 2019). In addition, many strains of *Pseudomonas mendocina* had the capacity to degrade various environmental pollutants (Zhang et al. 2017; Mudhoo et al. 2018). In effect, *Pseudomonas mendocina* NSYSU was able to mineralize a high concentration of pentachlorophenol (PCP) (150 mg / l) according to Kao et al. (2005), and the *Pseudomonas mendocina* strain ZAM1 degraded more than 64.5% of endosulfan after twelve days of incubation according to Mir et al. (2017).

Pseudomonas mendocina could grow in crude oil as the sole carbon and energy source (Kumari et al. 2018). In addition, this bacterial specie had effectively the ability to degrade polycyclic aromatic hydrocarbons (PAHs) (Nogales et al. 2017). Indeed, according to Barman et al. (2017), almost 98.8% and 98.6% of the degradation of acenaphthene and naphthalene respectively were recorded by *Pseudomonas mendocina* under optimal growth conditions. Degradation of naphthalene by *Pseudomonas mendocina* isolated from seawater has also been reported by Mangwani et al. (2014). This species has been reported to have the ability to degrade phenanthrene (Tian et al. 2002). *Pseudomonas mendocina* was reported to have the gene encoding the enzyme catechol dioxygenase, the key enzyme for the breakdown of PAHs (Heinaru et al. 2000; Kahlon 2016; Nogales et al. 2017).

Also, other studies have demonstrated the production of biosurfactants by the *Pseudomonas mendocina* strain, which has potential for application for the bioremediation of hydrocarbons (Kumari et al. 2018; Tripathi et al. 2019).

Consequently, the species *Pseudomonas mendocina* is a powerful and interesting means for the bioremediation of the environment contaminated by PAHs, and for biotechnological applications.

In our study, the bacterial strain SP57N isolated from contaminated marine sediments and seawater tolerated high concentrations of NaCl (up to 15%, w/v) and crude oil (up to 10%, v/v), because of the polluted environment, that induce development of enzymes of interest. This tolerance may reflect evolutionary adaptation that results in high stability in presence of hydrocarbon sources (Al-Dahash and Mahmoud 2013; Acosta-González and Marqués 2016; Joy et al. 2017).

Therefore, as an indigenous microorganism, *Pseudomonas mendocina* SP57N is a very useful biological means, as an efficient degrader, for the decontamination and bioremediation of marine sediments polluted by hydrocarbons such as the harbor of Oran.

5. Conclusion

In the present study, indigenous hydrocarbon-degrading bacterium strain SP57N was newly isolated from mixed seawater and marine sediment at the port of Oran (Algeria), and identified as *Pseudomonas mendocina*, based on their phenotypic phylogenetic characteristics, which possessed high growth capacity in crude oil as sole carbon and energy source, and excellent adaptability to salinity. Therefore, having capability to grown in the divers environmental conditions, the isolate strain SP57N could be used as a suitable degrader, to initiate an cost effective eco-friendly method for the removal of hydrocarbon contaminations in different marine environments polluted by hydrocarbons, in particularly, the harbor of Oran.

Acknowledgments

The authors are grateful to all staff members of the Pharmacognosy laboratory, A.M.U, Aligarh who assisted in the experiment.

Declaration of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Abou-Elela, S. I., Kamel, M. M., Fawzy, M. E. 2010. Biological treatment of saline wastewater using a salt-tolerant microorganism. *Desalination*, 250(1), 1–5. <http://dx.doi.org/10.1016/j.desal.2009.03.022>
- Acosta-Gonzalez, A., Marques, S. 2016. Bacterial diversity in oil-polluted marine coastal sediments. *Current Opinion in Biotechnology*. 38, 24–32. <http://dx.doi.org/10.1016/j.copbio.2015.12.010>
- Agarwal, A., Liu, Y. 2015. Remediation technologies for oil-contaminated sediments. *Marine Pollution Bulletin*. 101(2), 483–490. <http://dx.doi.org/10.1016/j.marpolbul.2015.09.010>
- Al-Dahash, L. M., Mahmoud, H. M. 2013. Harboring oil-degrading bacteria: A potential mechanism of adaptation and survival in corals inhabiting oil-contaminated reefs. *Marine Pollution Bulletin*. 72(2), 364–374. <http://dx.doi.org/10.1016/j.marpolbul.2012.08.029>
- Atlas, R. M., Hazen, T. C. 2011. Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History. *Environmental Science & Technology*. 45(16), 6709–6715. <http://dx.doi.org/10.1021/es2013227>
- Bahmani, N., Mirnejad, R., Arabestani, M. R., Mohajer, P., Hashemi, S. H., Karami, M., Alikhani, M. Y. 2017. Comparison of PCR-RFLP and PFGE for determining the clonality of *Brucella* isolates from human and livestock specimens. *Saudi Journal of Biological Sciences*. <http://dx.doi.org/10.1016/j.sjbs.2017.08.017>
- Barman, S. R., Banerjee, P., Mukhopadhyay, A., Das, P. 2017. Biodegradation of acenaphthene and naphthalene by *Pseudomonas mendocina*: Process optimization, and toxicity evaluation. *Journal of Environmental Chemical Engineering*. 5(5), 4803–4812. <http://dx.doi.org/10.1016/j.jece.2017.09.012>
- Cappello, S., Caruso, G., Zampino, D., Monticelli, L. S., Maimone, G., Denaro, R., Tripodo, B., Trousselier, M., Yakimov, M. M., Giuliano, L. 2007. Microbial community dynamics during assays of harbour oil spill bioremediation: a microscale simulation study. *Journal of Applied Microbiology*. 102(1), 184–194. <http://dx.doi.org/10.1111/j.1365-2672.2006.03071.x>
- Chang, C.H., Lee, J., Ko, B.G., Kim, S.K., Chang, J.S. 2011. *Staphylococcus* sp. KW-07 contains *nahH* gene encoding catechol 2,3-dioxygenase for phenanthrene degradation and a test in soil microcosm. *International Biodeterioration & Biodegradation*. 65(1), 198–203. <http://dx.doi.org/10.1016/j.ibiod.2010.11.003>
- Chebbi, A., Hentati, D., Zaghden, H., Baccar, N., Rezgui, F., Chalbi, M., Sayadi, S., Chamkha, M. 2017. Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated *Pseudomonas* sp. strain from used motor oil-contaminated soil. *International Biodeterioration & Biodegradation*. 122, 128–140. <http://dx.doi.org/10.1016/j.ibiod.2017.05.006>
- Deng M-C, Li J, Liang F-R, Yi M, Xu X-M, Yuan, J.-P., Peng, J., Wu, C.-F., Wang, J.-H. 2014. Isolation and characterization of a novel hydrocarbon-degrading bacterium *Achromobacter* sp. HZ01 from the crude oil-contaminated seawater at the Daya Bay, Southern China. *Mar pollut Bull*. 83(1), 79–86. <http://dx.doi.org/10.1016/j.marpolbul.2014.04.018>
- Effrosynidis, D., Arampatzis, A., Sylaios, G. 2018. Seagrass detection in the mediterranean: A supervised learning approach. *Ecological Informatics*. 48, 158–170. <http://dx.doi.org/10.1016/j.ecoinf.2018.09.004>
- Heinaru, E., Truu, J., Stottmeister, U., Heinaru, A. 2000. Three types of phenol and p-cresol catabolism in phenol- and p-cresol-degrading bacteria isolated from river water continuously polluted with phenolic compounds. *FEMS Microbiology Ecology*. 31(3), 195–205. <http://dx.doi.org/10.1111/j.1574-6941.2000.tb00684.x>
- Holt, S.G., Krieger, N.R., Sneath, P. H. A., Staley, J. T., Williams, S. T. 1998. *Bergey's Manual of determination for Bacteriology*. Williams and Wilkins, New York.
- Joy, S., Rahman, P. K. S. M., Sharma, S. 2017. Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. *Chemical*

- Engineering Journal*. 317, 232–241. <http://dx.doi.org/10.1016/j.cej.2017.02.054>
16. Kahlon, R. S. 2016. Biodegradation and Bioremediation of Organic Chemical Pollutants by *Pseudomonas*. *Pseudomonas: Molecular and Applied Biology*. 343–417. http://dx.doi.org/10.1007/978-3-319-31198-2_9
 17. Kao, C., Liu, J., Chen, Y., Chai, C., Chen S. 2005. Factors affecting the biodegradation of PCP by NSYSU. *Journal of Hazardous Materials*. 124(1-3), 68–73. <http://dx.doi.org/10.1016/j.jhazmat.2005.03.051>
 18. Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7). 1870–1874. <http://dx.doi.org/10.1093/molbev/msw054>
 19. Kumar, R., Mishra, A., Jha, B. 2019. Bacterial community structure and functional diversity in subsurface seawater from the western coastal ecosystem of the Arabian Sea, India. *Gene*. <http://dx.doi.org/10.1016/j.gene.2019.02.099>
 20. Kumari, S., Regar, R. K., Manickam, N. 2018. Improved polycyclic aromatic hydrocarbon degradation in a crude oil by individual and a consortium of bacteria. *Bioresource Technology*. 254, 174–179. <http://dx.doi.org/10.1016/j.biortech.2018.01.075>
 21. Logeshwaran, P., Megharaj, M., Chadalavada, S., Bowman, M., & Naidu, R. 2018. Petroleum hydrocarbons (PH) in groundwater aquifers: An overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. *Environmental Technology & Innovation*. 10, 175–193. <http://dx.doi.org/10.1016/j.eti.2018.02.001>
 22. Lamichhane, S., Krishna, K. B., & Sarukkali, R. 2017. Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: A review. *Journal of Environmental Management*. 199, 46–61. <http://dx.doi.org/10.1016/j.jenvman.2017.05.037>
 23. Liu, Q., Tang, J., Liu, X., Song, B., Zhen, M., & Ashbolt, N. J. 2019. Vertical response of microbial community and degrading genes to petroleum hydrocarbon contamination in saline alkaline soil. *Journal of Environmental Sciences*. <http://dx.doi.org/10.1016/j.jes.2019.02.001>
 24. Mangwani, N., Shukla, S. K., Rao, T. S., & Das, S. 2014. Calcium-mediated modulation of *Pseudomonas mendocina* NR802 biofilm influences the phenanthrene degradation. *Colloids and Surfaces B: Biointerfaces*. 114, 301–309. <http://dx.doi.org/10.1016/j.colsurfb.2013.10.003>
 25. Mehdi, H. and Giti, E. 2008. Investigation of alkane biodegradation using the microtiter plate method and correlation between biofilm formation, biosurfactant production and crude oil biodegradation. *International Biodeterioration & Biodegradation*. 62(2), 170–178. <http://dx.doi.org/10.1016/j.ibiod.2008.01.004>
 26. Mir, Z. A., Ali, S., Tyagi, A., Ali, A., Bhat, J. A., Jaiswal, P., ... & Oves, M. 2017. Degradation and conversion of endosulfan by newly isolated *Pseudomonas mendocina* ZAM1 strain. *3 Biotech*. 7(3). <http://dx.doi.org/10.1007/s13205-017-0823-5>
 27. Mnif, S., Chebbi, A., Mhiri, N., Sayadi, S., & Chamkha, M. 2017. Biodegradation of phenanthrene by a bacterial consortium enriched from Sercina oilfield. *Process Safety and Environmental Protection*. 107, 44–53. <http://dx.doi.org/10.1016/j.psep.2017.01.023>
 28. Mapelli, F., Scoma, A., Michoud, G., Aulenta, F., Boon, N., Borin, S., ... & Daffonchio, D. 2017. Biotechnologies for Marine Oil Spill Cleanup: Indissoluble Ties with Microorganisms. *Trends in Biotechnology*. 35(9), 860–870. <http://dx.doi.org/10.1016/j.tibtech.2017.04.003>
 29. Nogales, J., Garcia, J. L., & Diaz, E. 2017. Degradation of Aromatic Compounds in *Pseudomonas*: A Systems Biology View. *Aerobic Utilization of Hydrocarbons, Oils and Lipids*. 1–49. http://dx.doi.org/10.1007/978-3-319-39782-5_32-1
 30. Mudhoo, A., Bhatnagar, A., Rantalankila, M., Srivastava, V., & Sillanpää, M. 2018. Endosulfan removal through bioremediation, photocatalytic degradation, adsorption and membrane separation processes: A review. *Chemical Engineering Journal*. 360, 912–928. <http://dx.doi.org/10.1016/j.cej.2018.12.055>
 31. Nwinyi, O. C., Ajayi, O. O., & Amund, O. O. 2016. Degradation of polynuclear aromatic hydrocarbons by two strains of *Pseudomonas*. *Brazilian Journal of Microbiology*. 47(3), 551–562. <http://dx.doi.org/10.1016/j.bjm.2016.04.026>
 32. Patel, A. B., Mahala, K., Jain, K., & Madamwar, D. 2018. Development of mixed bacterial cultures DAK11 capable for degrading mixture of polycyclic aromatic hydrocarbons (PAHs). *Bioresource Technology*. 253, 288–296. <http://dx.doi.org/10.1016/j.biortech.2018.01.049>
 33. Ramadass, K., Megharaj, M., Venkateswarlu, K., & Naidu, R. 2018. Bioavailability of weathered hydrocarbons in engine oil-contaminated soil: Impact of bioaugmentation mediated by *Pseudomonas* spp. on bioremediation. *Science of The Total Environment*. 636, 968–974. <http://dx.doi.org/10.1016/j.scitotenv.2018.04.379>
 34. Shiaris, M. P. 1989. Phenanthrene mineralization along a natural salinity gradient in an Urban Estuary, Boston Harbor, Massachusetts. *Microbial Ecology*. 18(2), 135–146. <http://dx.doi.org/10.1007/bf02030122>
 35. Sun, S., Wang, Y., Zang, T., Wei, J., Wu, H., Wei, C., Wua H, Weib C., Li, F. 2019. A biosurfactant-producing *Pseudomonas aeruginos* S5 isolated from coking wastewater and its application for bioremediation of polycyclic aromatic hydrocarbons. *Bioresource Technology*. 281, 421–428. <http://dx.doi.org/10.1016/j.biortech.2019.02.087>
 36. Tian, L., Ma, P., Zhong, J.-J. 2002. Kinetics and key enzyme activities of phenanthrene degradation by *Pseudomonas mendocina*. *Process Biochemistry*. 37(12), 1431–1437. [http://dx.doi.org/10.1016/s0032-9592\(02\)00032-8](http://dx.doi.org/10.1016/s0032-9592(02)00032-8)
 37. Tiralerdpanich, P., Sonthiphand, P., Luepromchai, E., Pinyakong, O., & Pokethitiyook, P. 2018. Potential microbial consortium involved in the biodegradation of diesel, hexadecane and phenanthrene in mangrove sediment explored by metagenomics analysis. *Marine Pollution Bulletin*. 133, 595–605. <http://dx.doi.org/10.1016/j.marpolbul.2018.06.015>
 38. Tripathi, V., Gaur, V. K., Dhiman, N., Gautam, K., & Manickam, N. 2019. Characterization and properties of the biosurfactant produced by PAH-degrading bacteria isolated from contaminated oily sludge environment. *Environmental Science and Pollution Research*. <http://dx.doi.org/10.1007/s11356-019-05591-3>
 39. Varjani, S. J., Upasani, V. N. 2017. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. *International Biodeterioration & Biodegradation*. 120, 71–83. <http://dx.doi.org/10.1016/j.ibiod.2017.02.006>
 40. Wong, Y. S., Ong, S. A., Teng, T. T., Aminah, L. N., & Kumaran, K. 2012. Production of Bioflocculant by *Staphylococcus cohnii* ssp. from Palm Oil Mill Effluent (POME). *Water, Air, & Soil Pollution*. 223(7), 3775–3781. <http://dx.doi.org/10.1007/s11270-012-1147-z>
 41. Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol*. 67: 1613–1617. <http://dx.doi.org/10.1099/ijsem.0.001755>
 42. Zhang, S., Gedalanga, P. B., Mahendra, S. 2017. Advances in bioremediation of 1,4-dioxane-contaminated waters. *Journal of Environmental Management*. 204, 765–774. <http://dx.doi.org/10.1016/j.jenvman.2017.05.033>