Journal of Environmental Science and Engineering A 8 (2019) 131-140
doi:10.17265/2162-5298/2019.04.001

# Crude Oil Degradation Potential of Indigenous Hydrocarbonoclastic Bacterial Strain *Acinetobacter johnsonii* Firstly Isolated from Marine Sediments of Oran Port, Algeria

Faiza Bendadeche[1], Mohamed Bey Baba Hamed[2] and Sidi-Mohammed El-Amine Abi Ayad[1]

*1. Laboratory of Aquaculture and Bioremediation (AquaBior), Department of Biotechnology, Faculty of Nature and Life Sciences, University of Oran 1 Ahmed Ben Bella, Oran 31000, Algeria*

*2. Higher School of Biological Sciences of Oran, Oran 31000, Algeria*

**Abstract:** Petroleum contaminants caused great damages to environment and human health. Among, the port of Oran is subject of pollution mainly by PAHs (Polycyclic Aromatic Hydrocarbons) as a result of the large flow of ships. Thus bioremediation by indigenous microorganisms is an important means for their reduction and elimination. In the present work, a hydrocarbonoclastic bacterium strain SP49F2 was considered, firstly isolated from the contaminated marine sediments and seawater at the port of Oran (Algeria), using Bushnell-Hass mineral salt medium, and identified on the basis of morphological and biochemical characteristics and molecular tools by analysis of partial 16S rRNA gene sequence, using the Basic Local Alignment Search Tool program on the data base of NCBI (National Centre for Biotechnology Information), and the EzBioCloud 16S rRNA database. Kinetic of growth of this isolate on crude oil during 20 days of culture was studied at temperature 25 °C, 3% (*w*/*v*) of NaCl concentration and pH 7, at 140 rpm (Revolutions Per Minute). Strain SP49F2 was identified molecularly as *Acinetobacter johnsonii*, and might support high concentrations of crude oil (up to 10%, *v*/*v*). Results of growth kinetic on crude oil as sole energy and carbon source by the isolate strain showed that the stationary phase was attained at day 12. Thus, train *Acinetobacter johnsonii* SP49F2 could efficiently utilize crude oil as its sole carbon and energy source, and could be used as a wonderful native biological alternative for the bioremediation of the port of Oran, and marine area polluted by petroleum hydrocarbons, as an eco-friendly efficacy degrader, and may be suitable for biotechnological applications.

**Key words:** Crude oil, *Acinetobacter johnsonii*, bioremediation, marine sediments, 16S rDNA.

## 1. Introduction

Among the environmental pollutants, the PAHs (Polycyclic Aromatic Hydrocarbons) are the most common, presenting mutagenic, toxic and carcinogenic properties [1]. When compared to the other chemicals of ecological concern, petroleum products such as gasoline, diesel, or lubricants are widely used. However, through oil spills accidents, and leaks during fuel production, exploration, transport, and storage, petroleum-based products are scattered into the environment [2].

Petroleum hydrocarbons present serious risks to the health of all living organisms in the world. For the remediation and management of petroleum hydrocarbons, various methods such as chemical, physical, and biological are presently usable. Between these methods, bioremediation is considered as an interesting substitution for removing petroleum hydrocarbons from contaminated areas, as an economical, cost-effective and eco-friendly method [2, 3].

There are hundreds of species of fungi, archaea and bacteria that can degrade oil. Hydrocarbon-degrading

**Corresponding author:** Faiza Bendadeche, Ph.D., main research field: biotechnology and bioremediation.

microorganisms are ubiquitous, but represent only a small part of the microbial communities [4]. It has been confirmed that the application of microorganisms is more appropriate, provides practical means for cleaning-up of contamination by petroleum hydrocarbons from harbors and marine environments, especially by indigenous microorganisms [5, 6]. In addition, native hydrocarbons-degrading bacteria proliferating in contaminated area as marine sediments are better adapted and more suitable for restoring the hydrocarbon contamination in sea [6, 7].

The aim of the present work is to isolate indigenous hydrocarbon-degrading bacterial strains from contaminated marine sediments and seawater of the port of Oran (Algerian coast), with the capability to utilize crude oil, and to study the firstly isolated bacterial strain SP49F2, with its outstanding growth rate on crude oil as carbon and energy source.

## 2. Material and Methods

### *2.1 Sampling Site Localization and Samples Collection*

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

### *2.2 Procedure of Enrichment, Isolation and Selection of Hydrocrbonoclastics Bacteria*

For the isolation of hydrocarbon-degrading bacteria a synthetic BHMS (Bushnnell-Haas Mineral Salt) medium was used [8]. Sterilization of the BHMS medium was performed by autoclaving at 121 °C for 20 min, after adjusting pH at 7.2. Then, sterile crude oil (from the Hassi-Messaoud oil Refinery, Algeria) was added to cool BHMS medium as sole source of carbon and energy. Sterilization of crude oil was carried out using 0.22 μm filter membrane.

To enrich culture, 2 mL of mixed seawater and marine sediments (from 40 m depth), after decantation, was taken and added to 100 mL BHMS, with 2% (*v*/*v*) of crude oil, in 500 mL Erlenmeyer flask, and incubated at 25 °C and 140 rpm (Revolutions Per Minute) in a shaker incubator (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany), for 7 days. For subsequent subculture, 1 mL of product of the enrichment culture was inoculated in fresh BHMS medium, then from the product of this last subculture, 1 mL was transferred to a series of four supplementary serial subcultures successively with 4%, 6%, 8% then 10% of crude oil (*v*/*v*) as sole carbon and energy source on BHMS medium. Every subculture was incubated for 72 h at 25 °C and 140 rpm. From the product of the last subculture flask, of 10% (*v*/*v*) of crude oil, inoculums were streaked out into BHMS agar medium plate, with addition of 10% (*v*/*v*) of crude oil, and incubated for 7 days at 25 °C. Purification of different colonies phenotypically was carried out in nutrient agar medium. Morphologically distinct pure cultures with higher 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 Physiological and Biochemical Characteristics*

The physiological and biochemical typical characteristics of strain SP49F2, such as Gram staining, the oxidase activity (established by kit oxidase test (Sigma-Aldrich, Germany)), respiratory type (meat-liver agar), motility (Mannitol mobility medium), the catalase activity (defined by bubble formation in a 3% (*w*/*v*) solution of hydrogen peroxide), citrate utilization test (Simmons' Citrate agar), TSI (Triple-Sugar-Iron) test, and the ability to growth in Chapman agar medium, Schubert medium, *SS* (*Salmonella-Shigella*) agar medium and BCPL (Brain-Heart Infusion Broth), were systematically analyzed according to Holt, et al. [9]. All biochemical tests above were carried out in triplicate.

*2.4 Molecular Identification*

2.4.1 DNA Extraction

From grown cells, genomic DNA of strain SP49F2 was extracted using an EasyPure® Bacteria Genomic DNA Kit (TransBionovo Co., Ltd., China), according to the manufacturer's instructions. The extracted DNA was stored at -20 °C until analysis.

2.4.2 16S rRNA Gene Sequencing and Phylogenetic Analyses

PCR (Polymerase Chain Reaction) amplification of the 16S rRNA gene was conducted using DNA template sample with the universal primers, forward primer ben27F (5'-AGAGTTTGATCCTGGCTC-3') and reverse primer ben1492R (5'-GGTTACCTTGTTACGCTT-3'), synthesized by Sigma (Germany). The PCR reaction mixture, with a total volume of 50 µL, contained the following: 1 µL of DNA template (40 ng), 1 µL of ben27F (25 µM), 1 µL of ben1492R (25 µM), 2.0 µL of dNTP (2.5 mM), 2.0 µL of $MgCL_2$ (50 mM), 5 µL of 10× buffer solution (20 mM), 0.5 µL of taq DNA polymerase (5 $UL^{-1}$), and $ddH_2O$. The PCR programmer was: 95 °C for 5 min; 35 cycles, 94 °C for 1 min denaturation, 55 °C for 1 min annealing and 72 °C for 2 min extension; and final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel and then partially sequenced by Genewiz. Sequence similarity was sought utilizing the alignment method and on the EzBioCloud 16S rRNA database [10], using reference sequences, and the NCBI (National Centre for Biotechnology Information) database, using published 16s rDNA sequences. Data analysis of the partial 16S rDNA gene sequence was performed using MEGA (Molecular Evolutionary Genetics Analysis) software package Version 7.0 [11], and using neighbor-joining methods the phylogenetic tree was carried out.

2.4.3 Nucleotide Sequence Submission

The partial 16S rDNA gene sequence of the hydrocarbonoclastic bacteria strain SP49F2 was submitted to the NCBI-GeneBank under the accession number MK334629.

*2.5 Kinetic of Growth on Crude Oil at Optimal Culture Conditions of the Isolate Bacteria*

The strain SP49F2 was grown on BHMS medium with 2% of crude oil (*v*/*v*) as sole source of carbon and energy, under optimal cultivation conditions (studied previously), such as 3% (*w*/*v*) of NaCl, pH 7 and temperature 25 °C for about 20 days on an orbital shaker at 140 rpm (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany). Cultures were carried out in 250 mL flasks containing 50 mL of BHSM medium (Fig. 4), in triplicate. The growth rate of the isolate strain was estimated indirectly by measuring the OD (Optical Density 600 nm) with spectrophotometer (Perkin Elmer Lambda 35 UV/Vis Spectrometer, USA).

## 3. Results

*3.1 Isolation of Hydrocarbonoclastic Bacteria*

Hydrocarbonoclastic strains were enriched and separated by culturing contaminated seawater and marine sediment sampled from the port of Oran, Algeria, using enrichment cultures and dilution subcultures with increasing concentrations of crude oil (2%-10%, *v*/*v*). These isolates showed variable growth rates on the BHMS medium, supplemented with 2%-10% (*v*/*v*) of crude oil as the sole carbon and energy source (data not shown). Amongst, strain SP49F2 possessed remarkable growth rate, and efficiency of crude oil utilization, as sole carbon and energy source, and could support high concentrations of crude oil (up to 10%, *v*/*v*).

*3.2 Identification of Stain SP49F2*

Phylogenetic relationships between strain SP49F2 and other representative strains of the genus *Acinetobacter* were established. For that, about 1,500 pb of the 16S rDNA gene were amplified using universal primers by PCR (Fig. 1), then partial fragment of 987 pb from this gene was sequenced (GenBank ID:

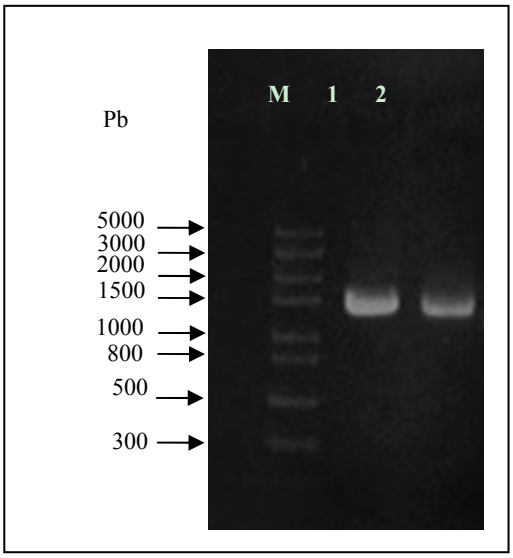



**Fig. 1 Electrophoretis of the 16S rDNA gene PCR product of the bacterial strain SP49F2 on 1.5% agarose. Lanes 1 and 2: 16S rDNA; M: Trans5K DNA Marker.**

MK334629). This continuous sequence was analyzed using the Basic Local Alignment Search Tool program (www.ncbi.nih.gov/blast) by the GenBank database on the NCBI and the EzBioCloud database in EzTaxon. Sequence similarity calculation using alignment by BLAST on NCBI database revealed that the partial 16S rDNA gene sequence of the isolate strain SP49F2 belonged to *Acinetobacter* genus, with a high sequence similarity, of 100% with *Acinetobacter johnsonii* XBB1 (CP010350.1), and 100% with *Acinetobacter johnsonii* strain ATCC 17909 (NR_117624.1), and using alignment by EzBioCloud sequences, it had 99.90% of similarity with *Acinetobacter johnsonii* CIP 64.6 (APON01000005). Afterward, two phylogenetic trees were carried out using similar 16S rDNA sequences (Figs. 2a and 2b), using the Neighbor-Joining Method, based on the two programmes of alignement (EzBioCloud and NCBI databases).

The phylogenetic trees indicated that the bacterial strain SP49F2 was related to species *Acinetobacter johnsonii* (Figs. 2a and 2b). For that, the strain SP49F2 was identified as *Acinetobacter johnsonii*.

The isolate strain SP49F2 was found to be Gram negative, strict aerobic, motile, coccobacilli-shaped bacteria (Fig. 3a). The colonies on nutrient agar plate were whitish, smooth, circular, convex, opaque and > 0.5 mm in diameter after incubation for 24 h at 25 °C (Fig. 3b). Biochemical characteristics results of strain SP49F2 are regrouped in Table 1.

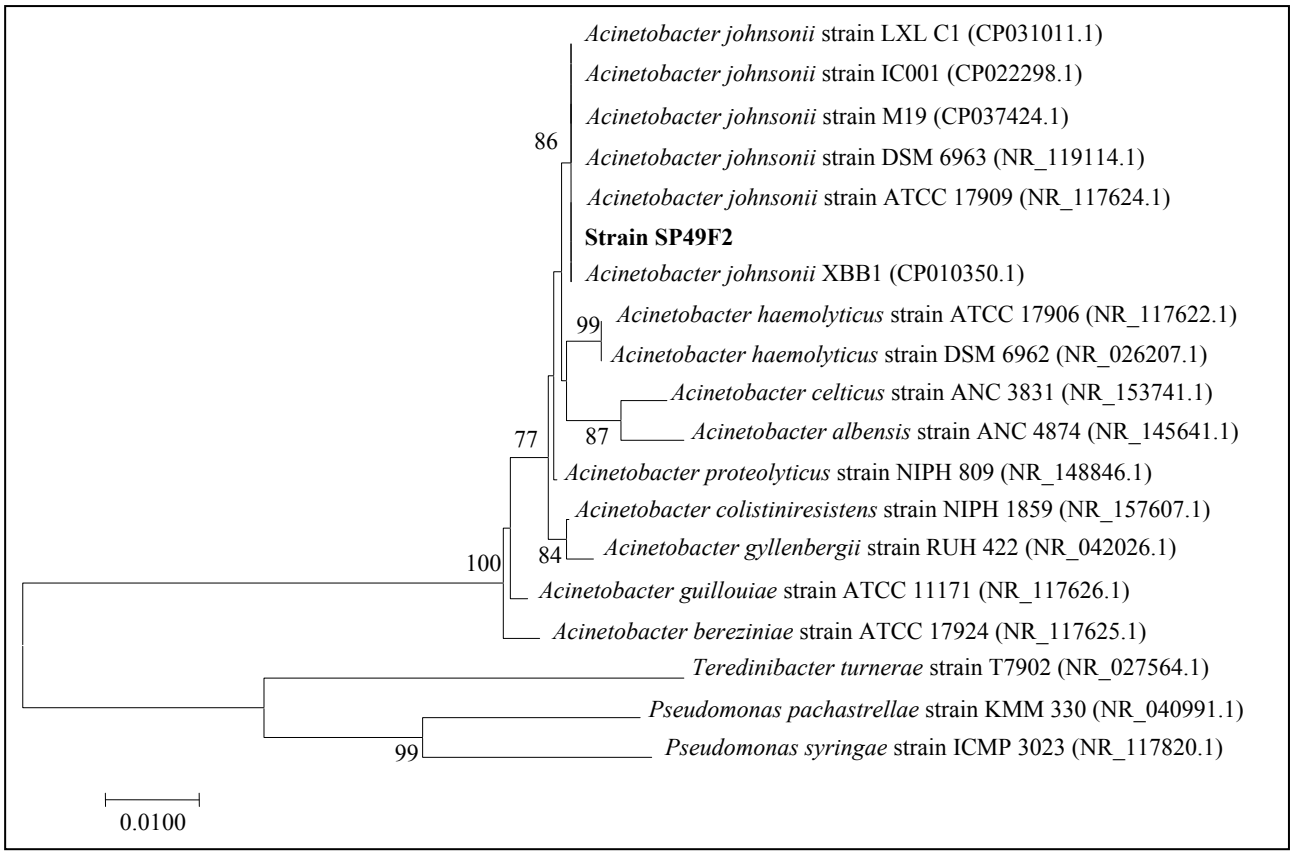
### *3.3 Growths Kinetic of the Hydrocarbonoclastic of the Isolated Bacterial Train*

The bacterial stain SP49F2 was grown on BHMS medium, supplemented with 2% (*v*/*v*) of crude oil as sole carbon and energy source, during 20 days, on their optimal culture conditions (pH 7; temperature 25 °C and 3% (*w*/*v*) of NaCl concentration, studied previously). The growth rate of this bacterial isolate was measured at the optical density 600 nm, and the results are shown in Fig. 4. As shown in the curve, strain SP49F2 started the logarithmic growth phase from the first to 12th day, and then the stationary phase was attained at day 12.

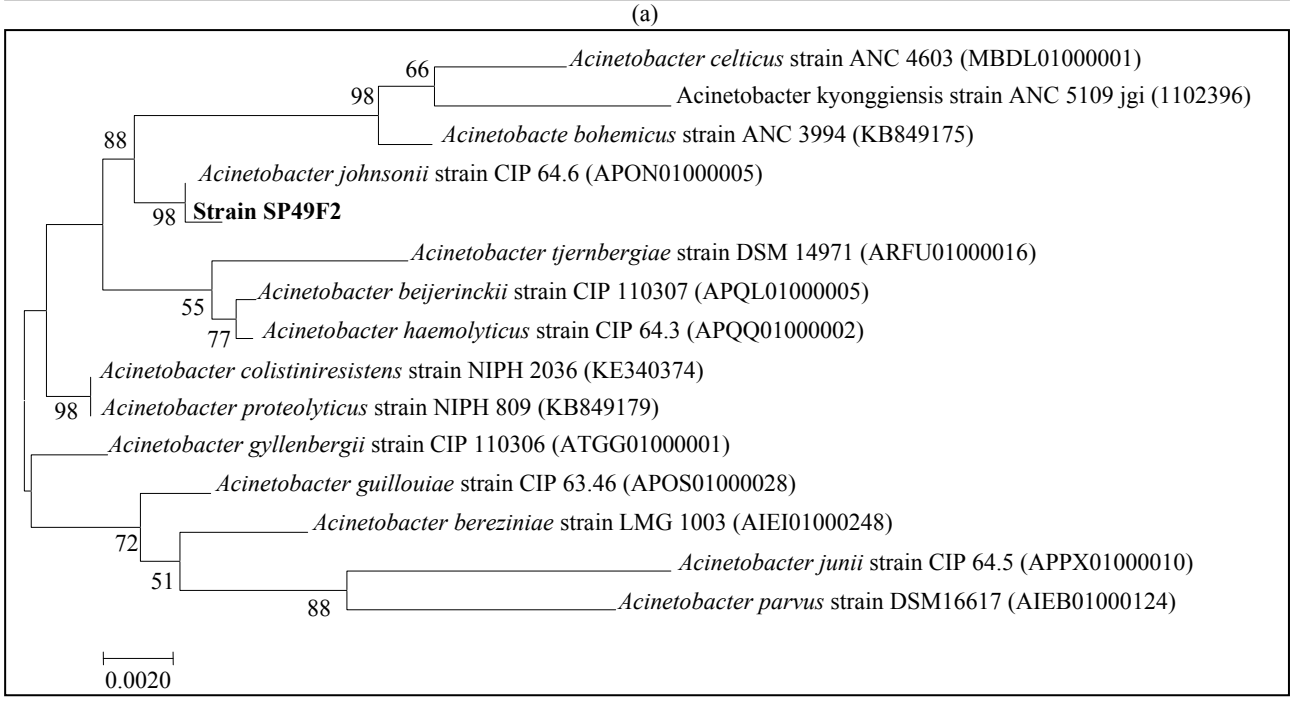
## 4. Discussion

The strain SP49F2 was one of hydrocarbonoclastic bacterium memberships to *Acinetobacter johnsonii*, firstly isolated from marine sediment and seawater from the port Oran, Algeria, having potential to develop a method to bioremediate marine environments polluted by petroleum hydrocarbons.

Partial sequence analysis of the 16S rDNA gene (978 bp) confirmed the identification as *Acinetobacter johnsonii* specie, with a similarity of 100% with *Acinetobacter johnsonii* XBB1 (CP010350.1), and 100% with *Acinetobacter johnsonii* strain ATCC 17909 (NR_117624.1), using BLAST alignment on NCBI database, and 99.90% of similarity with *Acinetobacter johnsonii* CIP 64.6 (APON01000005), using BLAST alignment on EzBioCloud database. In addition, the morphological characteristics of the strain SP49F2 were very similar to those of the *Acinetobacter johnsonii* reported previously. The results of the biochemical characteristics of the SP49F2 strain are shown in Table 1.




(a)



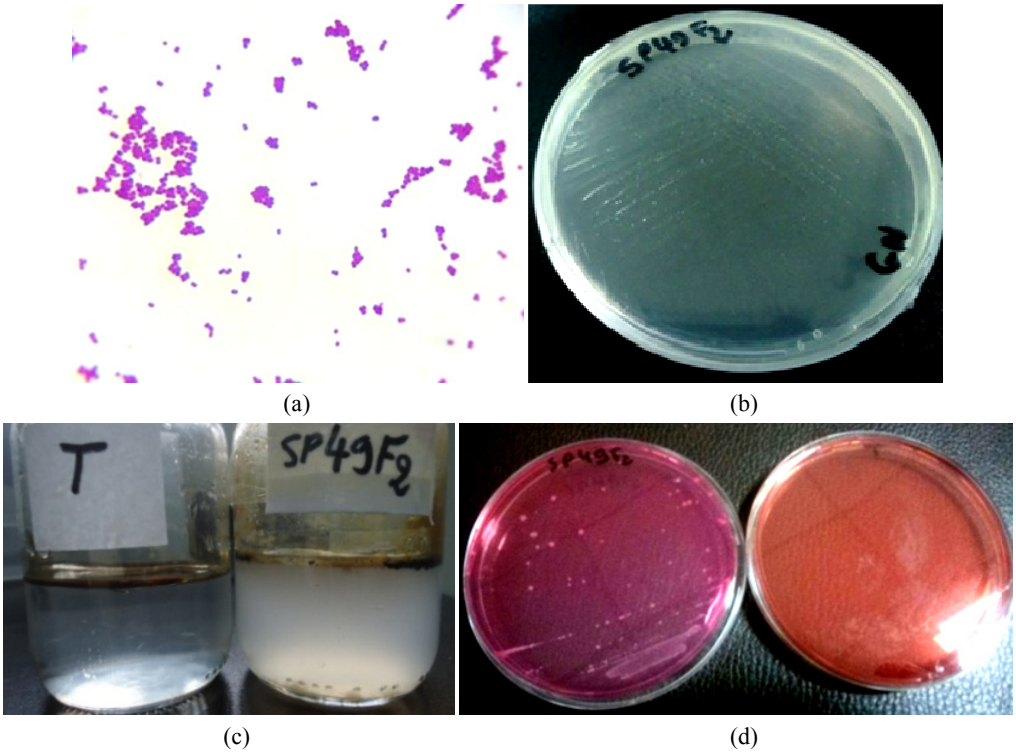

(b)

**Fig. 2 Phylogenetic trees of the 16S rDNA sequence of the SP49F2 strain and the closest-related species: (a) From the NCBI database; (b): From EzBioCloud database. The scale bar represents the sequence divergence. GenBank accession numbers are indicated in parenthesis. Calculations were carried out using the neighbor Neighbor-Joining method (bootstrap = 1,000) using the software MEGA 7.0.**

**Table 1 Morphological and biochemical characteristics of the isolated bacterial strain SP49F2.**

| Characteristics | Results |
|---|---|
| Gram straining | - (Fig. 3a) |
| Shape | Coccobacilli (Fig. 3a) |
| Oxidase test | - |
| Catalase test | + |
| Motility | + |
| Mannitol | - |
| Respiratory type | Strict aerobic |
| TSI test | K/K |
| $H_2S$ production | - |
| Gas production | - |
| Citrate utilization | - |
| Chapman | + (Fig. 3d) |
| *SS* | - |
| Lactose | - |
| Glucose | - |
| Saccharose | - |
| Indole | - |

Notes: "+", positive reaction; "-", negative reaction.




(a) (b)

(c) (d)

**Fig. 3 Colonies growth and cells morphological aspects of the bacterial strain SP49F2 on different culture medium; (a): Gram negative bacterium of strain SP49F2 (10 × 100); (b): Colonies of the strain SP49F2 on the nutrient agar plate after 24 h of incubation; (c): Colonies of the strain SP49F2 on the Chapman medium agar plate after 24 h of incubation; on the right: Chapman medium agar without culture (control); (d): Growth of the strain SP49F2 on BHMS with 2% (*v*/*v*) of crude oil as sole carbon and energy source after 7 days of incubation, on the left, BHMS with 2% (*v*/*v*) of crude oil without culture (control).**

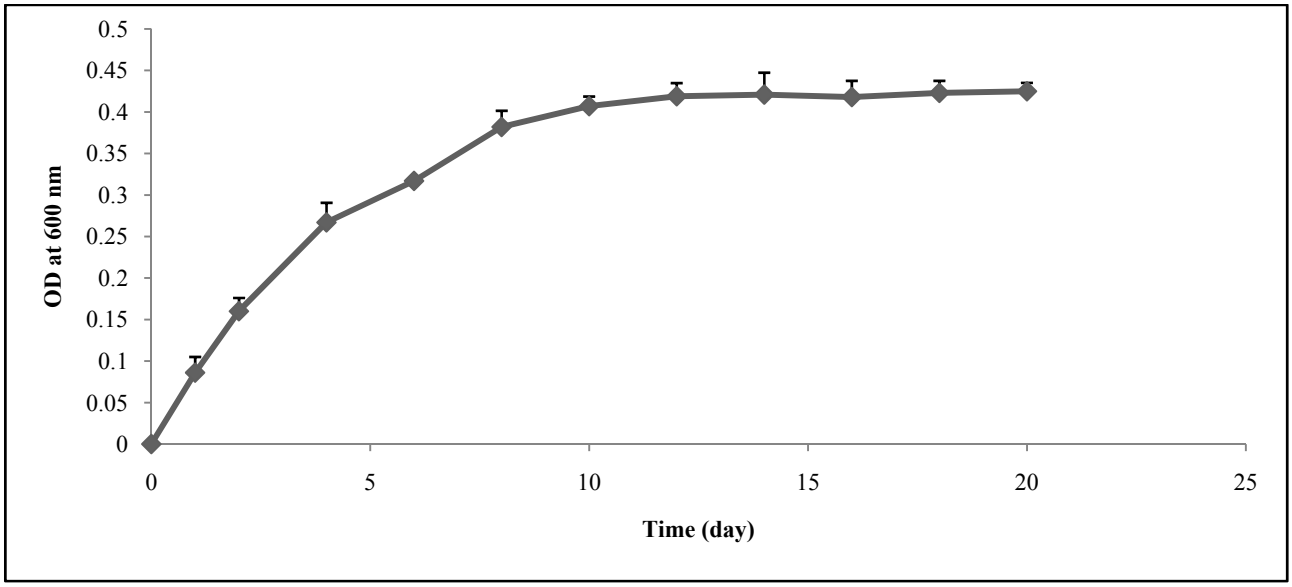



**Fig. 4 Growth kinetic of the bacterial strain SP49F2 on 2% of crude oil (*v*/*v*) as sole source of carbon and energy at temperature 25 °C, pH 7 and 140 rpm over 20 days of incubation. Bars represent standard deviation and experiments were performed in triplicate.**

From results obtained from kinetic of biodegradation of crude oil by the isolated bacterial strain in Fig. 4, the stationary phase was attained at day 12. These results indicate that this bacterial strain SP49F2 had effectively the ability to degrade and use crude oil as the sole source of carbon and energy.

Formerly, numerous strains of *Acinetobacter* sp. were informed to own their hydrocarbon-degrading aptitude [3, 12, 13]. Also, this species possessed the capability to remediate the hydrocarbon-contaminated environments [14]. Recent studies demonstrated production of biosurfactant by *Acinetobacter* sp. with potential application on hydrocarbon bioremediation [15, 16].

Other studies showed the ability of strains of *Acinetobacter* sp. to the bioremediation of polluted environments by various compounds, such as pharmaceutic compounds, presenting ubiquitous pollutants, effecting aquatic organisms and human health, including SMX (Sulfamethoxazole), SD (Sulfadiazine), SMT (Sulfamethazine), THM (Trimethoprim), TCS (Triclosan), DFC (Diclofenac) and CBZ (Carbamazepine) [17]. Also, *Acinetobacter* sp. had capability to degrade herbicide used in agriculture, including FE (Fenoxaprop-P-Ethyl) [18], and DEHP (Di-2-Ethylhexyl Phthalate), one of PAEs (Phthalic Acid Esters), representing a group of refractory and hazardous compounds blended in plastics [19]. Bhattacharya and Gupta [20] and Panda and Sarkar [21] demonstrated potential and application of *Acinetobacter* sp. in bioremediation and detoxification of heavy-metals-rich industrial wastewater and in tannery effluents such as Cr (VI).

Moreover, strains of *Acinetobacter* sp. were capable to use crude oil as a sole carbon and energy source [14, 22, 23], and were described as the most dominant genus in marine sediments [15].

Strains of *Acinetobacter johnsonii* were capable of biodegradation of organophosphate pesticide, such as malathion (*O*,*O*-dimethyl-S-[1,2-di(ethoxyl-carbonyl) ethyl] phosphoro-dithioate) used in agriculture [24], removal of inorganic impurity from waste oil and wash-down water [25], and biodegradation of fungicide, such as cyprodinil (4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine), used worldwide on agriculture as a broad-spectrum anilinopyrimidine, affecting the ecosystem, and causing slight acute toxicity as well as groundwater contamination [19, 26].

In addition, *Acinetobacter johnsonii* could biodegrade

diesel [12], and PAHs, such as pyrene, naphthalene, phenanthrene, and anthracene, and possessed catechol 2,3-dioxygenase enzyme exhibiting the higher capacity to degrade PAHs [27]. Xue, et al. [28] isolated bacterial from marine deep sea sediments and identified as *Acinetobacter johnson*.

The isolate strain *Acinetobacter johnsonii* SP49F2 from contaminated marine sediments and sea water, in our study tolerates significant concentrations of crude oil (up to 10%, *v*/*v*), because of the polluted area and the induction of developed enzymes of interest. This tolerance may reflect the evolutionary adaptation that results in the high stability, allowing bacteria with the ability to react faster than bacteria without spoilage to new sources of hydrocarbons [29].

Therefore, as an indigenous microorganism, *Acinetobacter johnsonii* SP49F2 is a very important and useful means for the bioremediation and decontamination of marine sediments such as the port of Oran.

## 5. Conclusion

In the present paper, indigenous marine hydrocarbon-degrading bacterium strain SP49F2 was identified as *Acinetobacter johnsonii*, isolated from mixed seawater and marine sediment at the port of Oran (Algeria), according to phenotypic and phylogenetic characteristics, which is adapted to polluted environment and possessed high growth capacity in crude oil as sole carbon and energy source. Therefore, the isolate strain SP49F2 could be used as a convenient degrader, to initiate an advantageous eco-friendly method for the removal of hydrocarbon contaminations in different marine environments polluted by hydrocarbons, in especially, the port of Oran, Algeria.

## Acknowledgments

The authors thank scientific group of Aquaculture and Bioremediation Laboratory (AquaBior) for their fruitful help for the development of this work.

## References

[1] Mnif, S., Chebbi, A., Mhiri, N., Sayadi, S., and Chamkha, M. 2017. "Biodegradation of Phenanthrene by a Bacterial Consortium Enriched from Sercina Oil Field." *Process Saf Environ Prot* 107: 44-53. doi: 10.1016/j.psep.2017.01.023.

[2] Logeshwaran, P., Megharaj, M., Chadalavada, S., Bowman, M., and 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: 175-93. doi: 10.1016/j.eti.2018.02.001.

[3] Tiralerdpanich, P., Sonthiphand, P., Luepromchai, E., Pinyakong, O., and Pokethitiyook, P. 2018. "Potential Microbial Consortium Involved in the Biodegradation of Diesel, Hexadecane and Phenanthrene in Mangrove Sediment Explored by Metagenomics Analysis." *Mar Pollut Bull* 133: 595-605. doi: 10.1016/j.marpolbul.2018.06.015.

[4] Atlas, R. M., and Hazen, T. C. 2011. "Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History." *Environ Sci Technol* 45 (16): 6709-15. doi: 10.1021/es2013227.

[5] Deng, M.-C., Li, J., Liang, F.-R., Yi, M., Xu, X.-M., Yuan, J.-P., et al. 2014. "Isolation and Characterization of a Novel Hydrocarbon-Degrading Bacterium *Achromobacter* sp. HZ01 from the Crude Oil-Contaminated Seawater at the Daya Bay, Southem China." *Mar. Pollut. Bull.* 83 (1): 79-86. doi: 10.1016/j.marpolbul.2014.04.018.

[6] Mohanram, R., Jagtap, C., and Kumar, P. 2016. "Isolation, Screening and Characterization of Surface-Active Agent-Producing, Oil-Degrading Marine Bacteria of Mumbai Harbor." *Mar. Pollut. Bull.* 105 (1): 131-8. doi: 10.1016/j.marpolbul.2016.02.040.

[7] Capello, S., Caruso, G., Zampio, D., Monticelli, L. S., Maimone, G., Denaro, R., Tripodo, B., Trousselier, M., Yakimov, M. M., and Giuliano, L. 2007. "Microbial Community Dynamics during Assay of Harbor Oil Spill Bioremediation: A Microscale Simulation Study." *J. App. Microbiol.* 102: 184-94. doi: 10.1111/j.1365-2672.2006.03071.x.

[8] 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." *Int Biodeterior Biodegradation* 62: 170-8. doi: 10.1016/j.ibiod.2008.01.004.

[9] Holt, S. G., Kriey, N. R., Sneath, P. H. A., Staley, J. T., and Williams, S. T., eds. 1998. *Bergy's Manual of*

*Determination for Bacteriology*. New York: Williams and Wilkins.

[10] Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. 2017. "Introducing EzBioCloud: A Taxonomically United Database of 16S rRNA Gene Sequences and Whole-Genome Assemblies." *Int. J. Syst. Evol. Microbiol* 67: 1613-7. doi: 10.1099/ijsem.0.001755.

[11] Kumar, S., Stecher, G., and Tamura, K. 2016. "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets." *Mol. Biol. Evol.* 33: 1870-4. doi: 10.1093/molbev/msw054.

[12] Lee, M., Woo, S.-G., and Ten, L. N. 2012. "Characterization of Novel Diesel-Degrading Strains *Acinetobacter haemolyticus* MJ01 and *Acinetobacter johnsonii* MJ4 Isolated from Oil-Contaminated Soil." *World Journal of Microbiology and Biotechnology* 28 (5): 2057-67. doi: 10.1007/s11274-012-1008-3.

[13] Fang, Y., Zhang, L., Wang, J., Zhou, Y., and Ye, B. 2017. "Biodegradation of Phthalate Esters by a Newly Isolated *Acinetobacter* sp. Strain LMB-5 and Characteristics of Its Esterase." *Pedosphere* 27 (3): 606-15. doi: 10.1016/s1002-0160(17)60355-2.

[14] Obafemi, Y. D., Taiwo, O. S., Omodara, O. J., Dahunsi, O. S., and Oranusi, S. 2018. "Biodegradation of Crude Petroleum by Bacterial Consortia from Oil-Contaminated Soils in Ota, Ogun State, South-Western, Nigeria." *Environmental Technology & Innovation* 12: 230-42. doi: 10.1016/j.eti.2018.09.006.

[15] Mahjoubi, M., Jaouani, A., Guesmi, A., Ben Amor, S., Jouini, A., Cherif, H., et al. 2013. "Hydrocarbonoclastic Bacteria Isolated from Petroleum Contaminated Sites in Tunisia: Isolation, Identification and Characterization of the Biotechnological." *New Biotechnol.* 30 (6): 723-33. doi: 10.1016/j.nbt.2013.03.004.

[16] Jadeja, N. B., Moharir, P., and Kapley, A. 2018. "Genome Sequencing and Analysis of Strains *Bacillus* sp. AKBS9 and *Acinetobacter* sp. AKBS16 for Biosurfactant Production and Bioremediation." *Appl. Biochem. Biotechnol.* 187 (20): 518-30. doi: 10.1007/s12010-018-2828-x.

[17] Wang, W., Chen, X., Yan, H., Hu, J., and Liu, X. 2018. "Complete Genome Sequence of the Cyprodinil-Degrading Bacterium *Acinetobacter johnsonii* LXL_C1." *Microbial Pathogenesis* 127: 246-9. doi: 10.1016/j.micpath.2018.11.016.

[18] Dong, W., Jiang, S., Shi, K., Wang, F., Li, S., Zhou, J., et al. 2015. "Biodegradation of Fenoxaprop-*P*-Ethyl (FE) by *Acinetobacter* sp. Strain DL-2 and Cloning of FE Hydrolase Gene *afe*H." *Bioresource Technology* 186: 114-21. doi: 10.1016/j.biortech.2015.03.039.

[19] Wang, J., Jiang, L.-H., Zhou, Y., and Ye, B.-C. 2017. "Enhanced Biodegradation of Di-n-butyl Phthalate by *Acinetobacter* Species Strain LMB-5 Coated with Magnetic Nanoparticles." *International Biodeterioration & Biodegradation* 116: 184-90. doi: 10.1016/j.ibiod.2016.10.024.

[20] Bhattacharya, A., and Gupta, A. 2013. "Evaluation of A*cinetobacter* sp. B9 for Cr (VI) Resistance and Detoxification with Potential Application in Bioremediation of Heavy-Metals-Rich Industrial Wastewater." *Environmental Science and Pollution Research* 20 (9): 6628-37. doi: 10.1007/s11356-013-1728-4.

[21] Panda, J., and Sarkar, P. 2011. "Bioremediation of Chromium by Novel Strains *Enterobacter aerogenes* T2 and *Acinetobacter* sp. PD 12 S2." *Environmental Science and Pollution Research* 19 (5): 1809-17. doi: 10.1007/s11356-011-0702-2.

[22] Hanson, K. G., Nigam, A., Kapadia, M., and Desai, A. J. 1997. "News & Notes: Bioremediation of Crude Oil Contamination with *Acinetobacter* sp. A3." *Current Microbiology* 35 (3): 191-3. doi: /10.1007/s002849900237.

[23] Huang, H., Bowler, B. F. J., Oldenburg, T. B. P., and Larter, S. R. 2004. "The Effect of Biodegradation on Polycyclic Aromatic Hydrocarbons in Reservoired Oils from the Liaohe Basin, NE China." *Organic Geochemistry* 35 (11-12): 1619-34. doi: 10.1016/j.orggeochem.2004.05.009.

[24] Xie, S., Liu, J., Li, L., and Qiao, C. 2009. "Biodegradation of Malathion by *Acinetobacter johnsonii* MA19 and Optimization of Cometabolism Substrates." *Journal of Environmental Sciences* 21 (1): 76-82. doi: 10.1016/s1001-0742(09)60014-0.

[25] Jiang, Y., Qi, H., Zhang, X., and Chen, G. 2012. "Inorganic Impurity Removal from Waste Oil and Wash-Down Water by *Acinetobacter johnsonii*." *Journal of Hazardous Materials* 239-240: 289-93. doi: 10.1016/j.jhazmat.2012.08.076.

[26] Chen, X., He, S., Liu, X., and Hu, J. 2018. "Biobegradation and Metabolic Mechanism of Cyprodinil by Strain *Acinetobacter* sp. from a Contaminated-Agricultural Soil in China." *Ecotoxicology and Environmental Safety* 159: 190-7. doi: 10.1016/j.ecoenv.2018.04.047.

[27] Jiang, Y., Zhang, Z., and Zhang, X. 2018. "Co-biodegradation of Pyrene and Other PAHs by the Bacterium *Acinetobacter johnsonii*." *Ecotoxicology and Environmental Safety* 163: 465-70. doi: 10.1016/j.ecoenv.2018.07.065.

[28] Xue, D., Zeng, X., Lin, D., and Yao, S. 2018. "Thermostable Ethanol Tolerant Xylanase from a

Cold-Adapted Marine Species *Acinetobacter johnsonii*." *Chinese Journal of Chemical Engineering* 37 (5): 1166-70. doi: 10.1016/j.cjche.2018.06.019.

[29] Girvan, M. S., Campbell, C. D., Killham, K., Prosser, J. I., and Glover, L. A. 2005. "Bacterial Diversity Promotes Community Stability and Functional Resilience after Perturbation." *Environ. Microbiol.* 7 (3): 301-13. doi: 0.1111/j.1462-2920.2004.00695.x.