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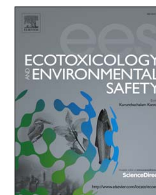

Petroleum degradation by endophytic *Streptomyces* spp. isolated from plants grown in contaminated soil of southern Algeria

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ABSTRACT

Petroleum hydrocarbons are well known by their high toxicity and recalcitrant properties. Their increasing utilization around worldwide led to environmental contamination. Phytoremediation using plant-associated microbe is an interesting approach for petroleum degradation and actinobacteria have a great potential for that. For this purpose, our study aimed to isolate, characterize, and assess the ability of endophytic actinobacteria to degrade crude petroleum, as well as to produce plant growth promoting traits. Seventeen endophytic actinobacteria were isolated from roots of plants grown naturally in sandy contaminated soil. Among them, six isolates were selected on the basis of their tolerance to petroleum on solid minimal medium and characterized by 16S rDNA gene sequencing. All petroleum-tolerant isolates belonged to the *Streptomyces* genus. Determination by crude oil degradation by gas chromatorgraph-flame ionization detector revealed that five strains could use petroleum as sole carbon and energy source and the petroleum removal achieved up to 98% after 7 days of incubation. These isolates displayed an important role in the degradation of the n-alkanes (C_6-C_{30}), aromatic and polycyclic aromatic hydrocarbons.

All strains showed a wide range of plant growth promoting features such as siderophores, phosphate solubilization, 1-aminocyclopropane-1-carboxylate deaminase, nitrogen fixation and indole-3-acetic acid production as well as biosurfactant production. This is the first study highlighting the petroleum degradation ability and plant growth promoting attributes of endophytic *Streptomyces*. The finding suggests that the endophytic actinobacteria isolated are promising candidates for improving phytoremediation efficiency of petroleum contaminated soil.

1. Introduction

Petroleum is a complex mixture of different hydrocarbon and non-hydrocarbon compounds, it represents the major source of energy and the primary raw material for industry and daily life (Riazi, 2005).

A huge quantity of petroleum hydrocarbons enters the environment whether accidentally or due to the human activities causing a common problem in the world due to their toxicity and destructive properties to the natural ecosystems (Moliterni et al., 2012).

Algeria has very important petroleum production refineries. The principal petroleum extraction companies are situated in the south, Ouargla city. Therefore, it is one of the regions exposed to petroleum soil contamination.

Among a variety of strategies used for hydrocarbon remediation, includes physicochemical methods which are expensive and destructive for the environment (Bisht et al., 2014). Today, phytoremediation is recognized as a promising technology that has attracted worldwide attention because it is an efficient, environmentally friendly and cost-effective approach (Li et al., 2012). It is used in the field of remediation of various environmental contaminants such as petroleum hydrocarbons, herbicides, explosives and heavy metals. This technology can be enhanced by the use of endophytic microbes to clean organic and

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inorganic contaminants in soil and water (Pilon-Smits, 2005).

Endophytic microorganisms are those that live inside plant tissues, without causing any harm to host plants or environments (Tan and Zou, 2001). Current efforts are now focused on the endophytic bacteria with the ability to degrade organic contaminants and/or improve plant growth (Khan et al., 2013). Several studies have reported many endophytic bacteria which present an important role in degrading different hydrocarbon compounds. Among these microorganisms are Gram negative bacteria such as *Pseudomonas* and *Brevundimonas,* (Peng et al., 2013; Phillips et al., 2008; Zhang et al., 2014) and Gram positive bacteria, including actinobacteria (Kukla et al., 2014; Singh and Sedhuraman, 2015).

Endophytic actinobacteria recovered from healthy surface-disinfected plant tissues play a significant role in the cleavage of complex polymers including organic and inorganic toxics into more readily assimilable nutrients (Doumbou et al., 2001). They are also well-known producers of a wide range of plant growth promoting activities (PGP) (Subramaniam et al., 2016), which become promising candidates to enhance the phytoremediation of polluted soils. Bacteria from the *phylum Actinobacteria* seem to have the potential to use different metabolic pathways (Polti et al., 2011).

Furthermore, the genera *Streptomyces* is known for the production of more than 60% of bioactive metabolites, they were usually explored for antimicrobial activities (Goldman and Green, 2009). There are a few reports which indicate that *Streptomyces* isolated from soil has the ability for hydrocarbons degradation (Ferradji et al., 2014). No information is available so far about the action of endophytic *Streptomyces* in the phytoremediation of petroleum-impacted soils.

Because of the potential role of actinobacteria to use different complex compounds, the study of the hydrocarbonoclastic actinobacteria associated with plants from contaminated soil may provide valuable information about the potential economic and environmental benefits of using endophytic actinobacteria in phytoremediation. To the best of our knowledge, this work provides a novel insight that describes endophytic *Streptomyces*, their petroleum degradation potential, their PGP features and emulsification ability. Therefore, the objectives of this study were (1) the isolation and the identification of petroleum-degrading endophytic actinobacteria from selected Saharian plants (2) the evaluation of their ability to use and degrade Algerian crude oil petroleum (3) the assessment of PGP features and (4) the evaluation of emulsification capacity.

2. Materials and methods

2.1. Sample collection

Ouargla city is known for sandy soil and arid to a hyperarid climate with mean rainfall of 100 ± 50 mm a^{-1} (Hamdi-Aissa et al., 2004). Specimens of healthy plants obtained from different areas of Ouargla, Algeria (Supp. Fig. 1), were used to isolated endophytic actinobacteria. The plants were selected based on their growth in petroleum contaminated soil. One specimen of each plant was collected. *Helianthemum lippii* (Hl) was collected from Haoudh el-Hamra (31° 53′ 38.1″N 6° 0′ 6.3″E), *Zygophyllum album* (Za) from the north base of oil refinery of Hassi Messouad (31° 39′ 43.6″N 6° 3′ 21.2″E) and *Bassia mauricata* (Bm) from Haoud Berkaoui (31° 50′ 16.0″N 5° 3′ 8.7″E). To the best of our knowledge, there is no record that these plants have been previously studied in order to isolate endophytic actinobacteria.

2.2. Isolation of endophytic actinobacteria

Five healthy root samples were harvested from each plant. The roots were washed in tap water to remove adhering soil particles. Tissue surfaces were sterilized by sequential immersion in 70% (*v/v*) ethanol for 5 min, 0.9% (*v/v*) of Sodium hypochlorite (NaClO) for 20 min, then, the root samples were divided aseptically into thin discs (0.2–0.5 cm) and soaked into 10% (*w/v*) of sodium bicarbonate ($NaHCO_3$) solution for 10 min in order to reduce the opportunity for emergence of endophytic fungi from the tissue, followed by washing with sterile distilled water for three times to remove surface sterilization agents (Verma et al., 2009). Finally the roots were placed onto different media, Humic acid-vitamin B (HV) (Hayakawa and Masayuki, 1987), yeast extract-casein hydrolysate (YECD) (Coombs and Franco, 2003), tap water-yeast extract agar (TWYE) (Crawford et al., 1993) and starch-casein agar (SC) (Kuster and Williams, 1964). All media were amended with nystatin (100 μg mL^{-1}) and nalidixic acid (50 μg mL^{-1}) to suppress the growth of fungi and gram-negative bacteria, respectively. The inoculated plates were incubated at 28 °C for 4–8 weeks. To confirm the surface disinfection process was successful, 100 μL of water from the final rinse was plated out on Petri-plates of yeast extract-mal extract agar and were incubated at 28 °C for 4 weeks. No contamination was found.

Isolates from *Helianthemum lippii, Zygophyllum album* and *Bassia mauricata* were named as Hlh, Zah and Bmb respectively, followed by a number.

2.3. Screening for crude oil petroleum biodegradation

A qualitative assay was performed to evaluate the tolerance of the isolates to Algerian crude petroleum according to the protocol of Benimeli et al. (2003). Rectangular troughs were cut in the center of minimal medium (MM) agar (containing in g L^{-1}: L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}H_2O$, 0.01; glucose, 10.0) and filled with crude petroleum. Petroleum was sterilized by filtration (0.22 μm). The strains were inoculated by streaking them perpendicular to the troughs; Petri dishes were sealed with parafilm to avoid petroleum evaporation. After 7 days of incubation at 30 °C, microbial growth was evaluated and used as a qualitative parameter. Growth control was carried out using a medium without crude oil petroleum. Qualitative analysis of growth in presence of petroleum was systematized using 4 categories: from no-growth strain (no-petroleum -tolerant strain) to very good growth (highly-petroleum -tolerant strain) (Benimeli et al., 2003). The petroleum tolerant strains were selected for further assays.

The ability of the selected strains to degrade petroleum was evaluated in a liquid medium. The strains were cultivated in malt-yeast extract medium in a rotary shaker at 180 rpm for three days at 30 °C. Biomass was harvested by centrifugation (10,000 for 10 min at 4 °C), washed twice with sterile distilled water, culture suspensions were homogenized using a 10 cc syringe with 21 G1 1/2needle. The homogenized culture was inoculated at a final concentration of 0.01 g L^{-1} in 20 mL of liquid MM (without glucose) supplemented with 1% of sterile crude petroleum. Previously, the protein concentration of the homogenized culture was determined as explained below. In two milliliters sterile Eppendorf tube containing 1.3 g of glass beads (425–600 μm), 1.5 mL of the homogenized culture was added for the cell lysis. The process included six cycles of high shaking using a vortex for 10 min and cooling for 5 min between each shaking (Bélanger et al., 2011). Protein quantification was determined in the supernatant according to (Bradford, 1976). Inoculated MM with glucose as carbon source was used as growth control, and uninoculated MM was used to evaluate the abiotic loss of petroleum. Cultures were incubated at 220 rpm for 7 days at 30 °C. All experiments were performed in triplicate. The biomass was estimated after centrifugation (10,000 rpm for 10 min at 4 °C) by washing the pellets with sterile distilled water and drying to constant weight at 105 °C. The supernatants were used to determine residual petroleum concentration, from the inoculated and uninoculated flasks (Benimeli et al., 2007).

2.4. Petroleum characterization using gas chromatography (GC-FID)

The hydrocarbons from the culture supernatants were recovered through a liquid-liquid extraction process: 10 mL of pentane was added

to 10 mL of supernatant, after shaking, the mixture was decanted and the organic phase was recovered. The method recovery efficiency was 94%. Then, 5 mL of each sample was mixed with 1 g of Na_2SO_4 (99.99%) to dry them (EPA, 2012). Next, samples were filtered and the recovered liquid phase was analyzed using a chromatograph Varian 3800 GC, with FID detector, and capillary column VF-5 ms (30 m, 0.25 mm, 0.2523 µm). The injector and detector temperatures were 200 °C and 300 °C, respectively. The run parameters were: 45–100 °C, increasing 5 °C min^{-1}, then a second ramp from 100 to 275 °C increasing 8 °C min^{-1}. The final temperature (275 °C) was keeping during 5 min. Internal control was performed with cyclohexanone. The control was prepared with 1 µL of cyclohexanone and 5 mL of pentane. In addition, 1 µL of cyclohexanone was added to each sample. The concentration of cyclohexanone was determined and the recovery was calculated at 80–90%. Calibration was performed with the original petroleum at a concentration of 1%, 2% and 5%.

2.5. Screening of endophytic actinobacteria for plant growth promoting metabolites production

All isolates were cultivated on malt-yeast extract medium for two days at 180 rpm and 30 °C. 10 µL of washed culture at a final concentration of 0.01 g L^{-1} was used to determine PGP characteristics of the strains.

Phosphate solubilization ability was determined in PVK medium according to (Nautiyal, 1999). The production of indole-3-acetic acid (IAA) was determined by inoculating the strain on 10 mL of liquid MM containing 2 mg mL^{-1} of L-tryptophan and incubated at 30 °C with shaking at 180 rpm for five days at darkness (Khamna et al., 2010). The amount of IAA was determined by a colorimetric method, by mixing 1 mL of culture supernatant with 1 mL of Salkowski reagent (Glickmann and Dessaux, 1995), followed by 30 min incubation in the dark. Colour intensity was determined as A540 using a spectrophotometer. The concentration of IAA was quantified using IAA standard curve.

Growth on N-free medium was determined using a method described by Franco-Correa et al. (2010). 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was carried out by streaking the isolated on MM agar (without L-asparagine) containing 3 mM of ACC per liter (MM-ACC agar) as a sole nitrogen source. The plates were incubated at 28 ± 2 °C in the dark for 7 days. Growth and sporulation of the strains on MM-ACC agar were taken as an indicator of the efficiency of selected isolates to utilize ACC and to produce ACC deaminase (El-Tarabily, 2008).

Siderophores production was screened according to the method described by Schwynan and Neilands (1987), which is based on the chelation of the iron by the siderophore producing a change in the colour of the dye of blue to orange.

2.6. Screening methods for biosurfactant

Production of biosurfactant was determined by hemolytic activity (Carrillo et al., 1996) and the emulsifying activity was carried out on LB (containing in g L^{-1}: peptone, 10.0; yeast extract, 5.0; NaCl, 10.0; glucose, 1.0), after 7 days of incubation at 30 °C. The emulsification index (E24) was calculated by using the following equation $E24 =$ (Height of emulsion formed / Total height of solution) × 100 (Kukla et al., 2014).

2.7. Phylogenetic analyses of selected strains

The identification of isolates was carried out by the sequencing of a fragment of the gene 16S rDNA. The isolates were grown in 20 mL of ISP2 (containing in g L^{-1}: glucose 4.0; Malt extract, 10.0; yeast extract, 4.0) at 30 °C and 180 rpm for 24–48 h. Biomass was harvested by centrifugation (10,000 rpm for 5 min). DNA extraction was carried out using genomic DNA extraction kit (Promega, USA) according to the protocol supplied by the manufacturer. The DNA was used as a template for PCR amplification, the primers used were 27 f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and p1492r (5′-TAC GGC TAC CTT GTT ACG ACT). All amplification products were checked by electrophoresis on 1% agarose gels.

The amplified fragments were purified and sequenced by Macrogen (Korea). The 16S rDNA gene sequences of actinomycete strains have been deposited in GenBank.

Sequences belonging to the same species or closely related species of type strains, available through the public databases, were aligned and a similarity matrix was calculated using MEGA 7 programme package (Kumar et al., 2016). The phylogenetic analysis was carried out using MEGA 7 software by the neighbor-joining method with 1000 bootstrap (Saitou and Nei, 1987) and plotted with the MEGA 7 software package.

2.8. Statistical analysis

Data analysis was conducted using the R (3.2.2) program for windows. Data were represented as the mean ± standard deviation (SD) of the triplicates samples.

3. Results and discussion

3.1. Isolation and selection of petroleum degrading endophytic actinobacteria

Endophytic actinobacteria have been isolated from different kind of plants, including medicinal plants and crop (Coombs and Franco, 2003; Qin et al., 2009). In the present study, seventeen endophytic actinobacteria were isolated from the internal tissue of surface-sterilized roots of plants growing at sites contaminated by crude petroleum. The surface sterilization protocol was checked by spreading out the last washing water of root samples on ISP2 medium. After 15 days of incubation, no growth was observed, revealing that the surface sterilization protocol was effective to remove the epiphytic microorganisms and the obtained isolates can be considered to be true endophytic actinobacteria.

The number of isolates in the three plants was quite different (Table 1). The endophytic community may be influenced by the pollutant concentration in the soil and the host plant species (Peng et al., 2013). Also, we observed that the isolation efficiency was influenced by the culture medium used. In the present study, the majority of isolates were obtained on TWYE (47.05%) followed by HV (35.29%), then YECD medium (11.76%) (Table 1). However, HV was the only medium that allowed isolating actinobacteria from the three plants. Starch casein agar yielded the lowest number of isolates (5.88%). Previous research has shown that low-nutrient media permit the growth and sporulation of actinobacteria before fast growing fungi mask slow growing actinobacteria (Coombs and Franco, 2003; Qin et al., 2009).

Many endophytic bacteria have exhibited hydrocarbon and metal tolerance (Babu et al., 2013; Peng et al., 2013). Qualitative assay of growth in the presence of petroleum indicated that all isolated actinobacteria were tolerant to 20% of crude petroleum oil (Supp. Fig. 2).

Moreover, the strains Hlh1, Hlh8, Hlh9 and Zah8 have exhibited similar growth in both contaminated and uncontaminated medium,

Table 1
Number of endophytic isolates of actinobacteria recovered from the roots of the three collected plants.

Plants Medium	*Helianthimum lippi* (Hl)	*Zygophyllum album* (Za)	*Bassia mauricata* (Bm)	Total (%)
HV	4	1	1	35.29
TWYE	7	1	–	47.05
YECD	2	–	–	11.76
SCA	1	–	–	5.88

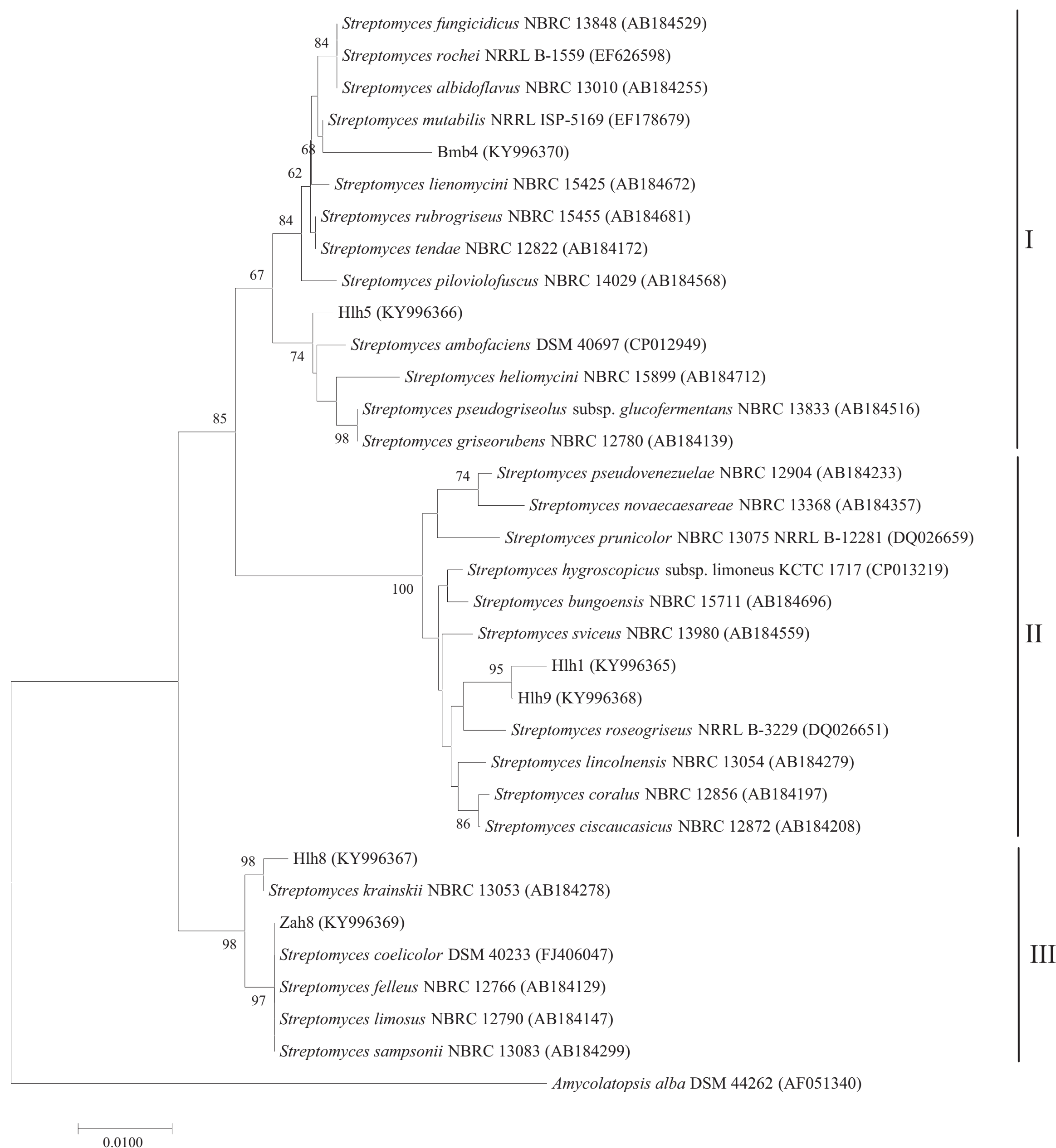

Fig. 1. Phylogenetic tree of selected actinobacteria inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Jukes-Cantor method. Evolutionary analyses were conducted in MEGA7. Accession numbers of 16s rDNA sequences are given in parentheses.

thus, they were considered highly petroleum tolerant strains; whereas Hlh5 and Bmb4 were displayed a little growth in comparison to the growth observed in the uncontaminated medium (low-petroleum tolerant strains). The remaining strains displayed a very low tolerance. This could probably be because of the presence of some toxic volatile hydrocarbons which affect their growth, as reported previously by (Leahy and Colwell, 1990) who reported that some volatile petroleum hydrocarbon compounds could influence microbial growth.

The petroleum concentrations used in the qualitative screening assured the selection of high tolerance strains. Although all endophytic isolates were able to grow in the presence of petroleum, some isolates showing a strong tolerance, therefore, the best six strains were selected for further assays.

3.2. Phylogenetic analysis of endophytic actinobacteria

The taxonomic position of the selected strain was elucidated through phylogenetic analysis using their 16S rDNA sequences (Fig. 1).

A phylogenetic tree showed that all the strains found in this study belong to *Streptomyces* genera. Furthermore, previous studies reported that among actinobacteria, *Streptomyces* is the most frequently isolated genera (Qin et al., 2011). Furthermore, (Goudjal et al., 2016) indicated that Saharian native plants in Algeria were rich in *Streptomyces.* The overall 16S rDNA G + C content was ranged from 58.8 to 59.9 mol%.

The 16S rDNA sequences were compared to the corresponding sequences of 28 culture collection strains, and *Amycolatopsis alba* was used as an outgroup. According to the phylogenetic analysis, the strains were associated with three clusters. Inside the cluster I, the isolate Bmb4 was closely associated to *Streptomyces mutabilis* and *S. rochei*, with 98.9% and 98.6% of identity, respectively. The isolate Hlh5 was highly related to *Streptomyces ambofaciens* (99.6%) and *Streptomyces pseudogriseolus* subsp. *glucofermentans* (99.4%). Within cluster II, the isolates Hlh1 and Hlh9 were closely associated among them with 99.5% of identity; also, they showed 98.5% and 99.0% of identity with

Streptomyces sviceus, respectively. In cluster III, the isolate Hlh8 was closely associated to *Streptomyces krainskii* (99.8%). The isolate Zah8 showed 100% of identity with four *Streptomyces* species: *S. coelicolor*, *S. felleus*, *S. limosus* and *S. sampsonii.* Although they showed a high identity, further work needs to be done to assign species. Moreover, none of the strains that related to the new isolates found in this study showed the ability to utilize petroleum.

It was not possible to perform an ecological analysis on the diversity of the endophytic actinobacteria found in these plants because there are no previous reports on this subject. However, there have been some reports regarding the diversity and ecology of the endophytic bacterial population from plants grown in contaminated soil and the use of them to degrade organic pollutants (Kukla et al., 2014; Peng et al., 2013). In addition, there is no study on endophytic *Streptomyces* isolated from other plants growing in areas contaminated with crude petroleum.

3.3. Determination of petroleum degradation ability

The petroleum biodegradation by the actinobacteria was evaluated in MM liquid medium supplemented with 1% of Algerian light petroleum (10,000 ppm) as sole carbon and energy source. It was evaluated by the biomass produced and the petroleum degradation after 7 days of incubation.

According to the results of the GC-FID analysis, five strains could effectively utilize and degrade petroleum at different rates (Fig. 2). The highest petroleum removal was observed by *Streptomyces* sp. Hlh9 and *Streptomyces* sp. Zah8 (from 10,000 ppm to 250 and 1443 ppm, respectively). *Streptomyces* sp. Hlh8 and *Streptomyces* sp. Hlh1 could degrade 85.70% and 57.14% of petroleum respectively, while *Streptomyces* sp. Bmb4 degraded 8.91% of petroleum. Biomass production ranged from 0.18 to 0.43 g L^{-1} among the different strains (Fig. 2). No variations of petroleum concentration were observed in uninoculated control, so there was no proof of noticeable contribution of abiotic loss during the petroleum degradation.

Numerous studies have reported the use of endophytic bacteria for the biodegradation of organic pollutants in the environment (Khan et al., 2013). However, experimental work presented here provides, for the first time, a systematic screening on endophytic *Streptomyces* able to degrade petroleum.

Kukla et al. (2014) reported other genera of endophytic actinobacteria, including *Rhodococcus* and *Microbacterium* for their potential hydrocarbon degradation ability. Furthermore, endophytic *Nocardiopsis* able to degrade diesel was isolated from *Hibiscus rosasinensis* (Singh and Sedhuraman, 2015).

Although *Streptomyces* sp. Hlh5 displayed petroleum tolerance in the solid medium as well as a slight growth in liquid medium, none petroleum degradation was observed. Therefore, the tolerance was not associated with petroleum degradation ability (Polti et al., 2007). Similarly, previous reports have demonstrated that some hydrocarbons are able to support the growth of actinobacteria even no degradation was observed (Bourguignon et al., 2014).

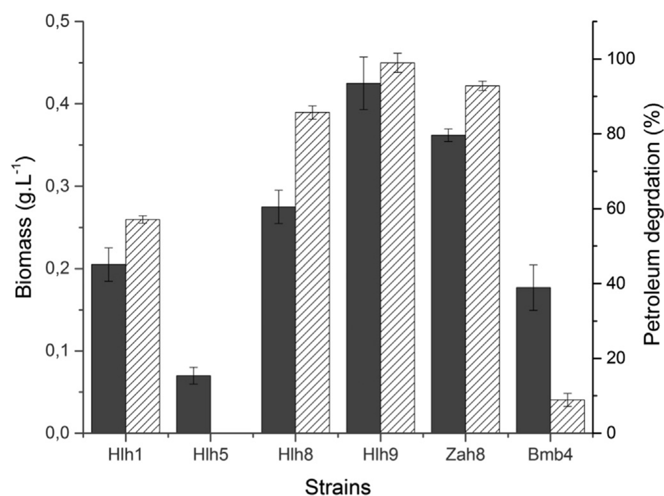

Fig. 2. () Growth of strains in MM liquid medium supplemented with 1% of crude petroleum expressed in g L^{-1}. () Degradation of petroleum by selected strains.

Table 2
Algerian crude petroleum composition and utilization of hydrocarbons by the selected isolates.

Hydrocarbons	Petroleum composition (ppm)	Petroleum degradation (%)				
		HLh1	HLh8	HLh9	Zah8	Bmb4
C6	0.01	96.37	100	100	0.00	0.00
C7	77.12	100	97.49	100	99.36	64.52
C8	82.53	91.27	100	100	99.48	20.43
C9	520.79	99.75	96.94	99.61	99.62	75.88
C10	499.03	83.63	95.17	99.34	99.73	66.08
C11	608.06	73.85	93.91	99.33	99.37	43.40
C12	641.29	95.33	95.58	99.54	97.96	33.56
C13	641.29	97.09	98.41	99.50	99.10	3.89
C14	10.73	96.41	80.00	64.45	60.00	2.30
C15	552.59	94.84	98.40	99.59	97.40	87.00
C16	497.15	100	99.24	99.29	94.50	0.00
C17	467.54	100	98.40	99.66	98.48	99.44
C18	385.44	100	98.90	99.73	94.83	0.00
C19	312.01	35.26	98.00	99.52	94.37	0.00
C20	284.24	47.91	98.72	99.58	96.35	0.00
C21	251.00	37.14	98.14	99.59	96.92	0.00
C22	219.27	24.99	98.78	99.63	97.31	0.00
C23	73.30	39.16	92.32	99.47	89.81	0.00
C24	64.91	36.46	0.00	0.00	87.03	0.00
C25	25.17	50.68	92.32	99.47	88.76	0.00
C26	43.74	61.20	98.53	100	99.47	0.00
C27	23.98	5.72	93.42	100	92.44	0.00
C28	14.53	95.43	99.10	100	94.08	0.00
C29	0.43	38.46	100	100	72.58	0.00
C30	0.49	0.00	100	20.00	8.70	0.00
Benzene	1.93	0.00	0.00	0.00	0.00	0.00
Toluene	1.13	100	0.00	0.00	85.71	0.00
p-Xylene	37.98	100	4.56	10.00	93.68	78.73
o-Xylene	35.53	100	4.69	11.20	98.63	0.00
m-Xylene	32.59	99.54	7.71	14.2	97.30	7.78
Naphthalene	34.24	90.00	65.43	98.18	75.30	13.49
2-methyl naphthalene	32.35	70.08	0.00	0.00	87.90	14.43
1-methyl naphthalene	63.35	0.00	0.00	0.00	55.12	10.28
acenaphthylene	49.96	0.00	0.00	0.00	71.42	6.32
Acenaphtalene	34.10	0.00	59.00	100	45.14	0.00
Fluorene	70.82	76.34	29.21	96.43	0.00	0.00
Pristane	542.09	52.14	90.44	99.19	78.45	0.00
Phytane	385.44	83.6	29.69	100	0.00	19.39
Phenanthrene	2.91	47.91	43.61	54.00	0.00	0.00
Anthracene	4.59	37.67	17.99	10.00	0.00	0.00
Fluoranthene	10.16	43.75	0.00	0.00	42.66	0.00
Pyrene	1.12	48.38	0.00	0.00	0.00	0.00
benzo(a) anthracene	37.77	0.00	0.00	0.00	98.57	0.00
Chrysene	31.94	69.76	0.00	0.00	99.78	0.00
benzo(b) fluoranthene	12.89	0.00	0.00	0.00	92.83	0.00
benzo(k) fluoranthene	0.88	0.00	0.00	0.00	88.80	0.00
Benzo(a)pyrene	7.22	0.00	0.00	0.00	98.35	0.00

In fact, the hydrocarbon degrading actinobacteria, as published previously, were usually isolated from soil. Balachandran et al. (2012) have described that the hydrocarbon degrading *Streptomyces* ERI-CPDA-1 was able to degrade of 98.25% of petroleum at 0.01–0.1% within 7 days at pH 7 and 1–5 g L^{-1} of NaCl. Furthermore, Barabás et al. (2001) reported that *Streptomyces* isolated from Kuwait Burgan oil soil were able to utilize n-alkanes, kerosene and crude oil as sole carbon and energy sources.

The residual concentration of hydrocarbon fractions was analyzed

in the supernatant of culture medium. Four strains showed the ability to degrade a variety of hydrocarbons present in the crude petroleum. Moreover, the degradation of n-alkanes and aromatic hydrocarbons were significantly different between strains (Table 2). These compounds are the most frequent organic pollutants and are the main components in the crude petroleum (Zhang et al., 2011).

Streptomyces ssp. Hlh1, Hlh8 and Hlh9, isolated from the same plant, showed a similar petroleum degradation profile, they effectively degraded n-alkanes from C_6 to C_{30}, while, the degradation of aromatic hydrocarbons was better performed by *Streptomyces* sp. Hlh1. On the other hand, *Streptomyces* sp. Zah8 displayed a very broad hydrocarbon degradation profile, being able to degrade all n-alkanes and polycyclic aromatic hydrocarbons (PAH). Wang et al. (2011) have described *Dietzia* strain with the ability to use n-alkanes, aromatic compounds, and crude oil as sole carbon sources. There have been rare reports about bacteria with the ability to degrade simultaneously n-alkanes and PAH (Zhang et al., 2011). In contrast, *Streptomyces* sp. Bmb4 degraded only n-alkanes from C_7 to C_{17}. And also, none strain was able to degrade benzene (Table 2). Efficient degradation of n-alkanes and simple aromatic hydrocarbons, such as xylene, and naphthalene were observed in all the strains.

Similarly, three *Streptomyces* recovered from soils at Mitidja plain (North of Algeria) were able to degrade aliphatic fractions (C_{11}-C_{30}) present in crude oil and naphthalene as an individual hydrocarbon (Ferradji et al., 2014). In the present study, all the strains were effective degraders of n-alkanes as well as aromatic hydrocarbons and PAH. Although, PAH are being persistent in the environment as the increase of molecular size and their slow degradation by few microbes (Wei et al., 2017), *Streptomyces* sp. Hlh1 was the only strain that showed pyrene degradation as well as the PAH having three cycles or more. Sheng et al. (2008) found a pyrene-degrading *Enterobacter* sp. isolated from *Allium macrostemon* grown in PAH-contaminated soil.

It is also demonstrated that aliphatic fractions are more susceptible to degradation than aromatic, asphaltene and resin fractions. Therefore, the biodegradation of crude oil is affected by petroleum hydrocarbons composition and environmental conditions (Yanto and Tachibana, 2013).

However, it should be indicated that all plants in this study were grown in the contaminated soil. It would thus confirm that the endophytes isolated from plants growing in petroleum contaminated sites have displayed a natural ability for potential petroleum degradation (Siciliano et al., 2001).

3.4. PGP activities and biosurfactant production

Even though plants are autotrophic organisms, they absorb and metabolize hydrocarbons, but do not depend on them as sole carbon and energy source. Therefore, bacterial endophytes play an important role in the degradation of hydrocarbons absorbed by the plants in polluted environments (Khan et al., 2013). Bacterial PGP features enhance the adaptation and the growth of plants and consequently improve phytoremediation of hydrocarbon contaminated soil (Afzal et al., 2014). In our study, the actinobacterial strains were assessed for some plant growth promotion attributes like phosphate solubilization, growth on N-free media, ACC deaminase, siderophores and IAA production. Results summarized in Table 3 showed that the isolates were able to produce at least one PGP traits tested.

The production of IAA ranged from 11 to 22 μg IAA mL^{-1} after 96 h of incubation. *Streptomyces* sp. Bmb4 was the most efficient IAA producer, with 22.8 μg IAA mL^{-1} followed by *Streptomyces* sp. Zah9 which excreted 19 μg IAA mL^{-1}. The IAA is the most important phytohormone responsible for root development (Duca et al., 2014), stimulating plant cell proliferation, plant cell elongation and induce the transcription of ACC deaminase (Glick, 2014).

Five isolates were able to secrete siderophores into Chrome Azurol S (CAS) medium, as well as, they grew on the nitrogen-free media. There

Table 3
Plant growth promoting characteristics of the isolates.

Strains	IAA[a]	NFB	ACC	Siderophores[b]	PS
Hlh1	11.0 ± 0.6	+	++	20.5 ± 0.7	+
Hlh5	–	+	–	20.0 ± 1.8	–
Hlh8	–	+	–	14 ± 1.40	–
Hlh9	–	+	+++	4 ± 1.41	+
Zah8	19.0 ± 1.6	–	–	ng	–
Bmb4	22.8 ± 0.6	+	++	21.0 ± 0.0	+

+: present; -: absent; ng: no growth was observed on CAS agar.
[a] In μg IAA mL^{-1} of medium. ± : standard deviation of three replicates.
[b] The halo diameter calculated by subtracting the colony diameter from the total halo size (mm).

is some evidence that siderophores production may associate with nitrogen fixation ability (Coombs and Franco, 2003). Additionally, siderophores stimulate plant growth by enhancing iron translocation from roots to shoots in the seedlings (Subramaniam et al., 2016). Tripathi et al. (2005) found that *Pseudomonas putida* KNP9 produces siderophore which increases the growth of mung bean in contaminated soil by cadmium and lead (Tripathi et al., 2005). Furthermore, only *Streptomyces* sp. Zah8 did not grow into CAS medium. These results were slightly similar to (Kukla et al., 2014) who found that the composition of the medium may not suitable or could inhibit microbial growth for some Gram positive bacteria.

Phosphate solubilization improves soil fertility through organic compounds degradation (Subramaniam et al., 2016). *Streptomyces* ssp. Hlh1, Hlh9 and Bmb4 were able to solubilize mineral phosphate producing a clear zone on screening agar plate. In fact, actinobacteria were rarely described for their ability to produce organic acid responsible of phosphate solubilization (Jog et al., 2014). These strains were also able to use ACC as sole nitrogen source. ACC deaminase producing *Streptomyces* has been previously reported by other authors (Dimkpa et al., 2009; El-Tarabily, 2008). Indeed, ACC deaminase stimulates plant growth reducing ethylene level produced by the plants under stressful conditions (Glick, 2014). Additionally, inoculation plants with petroleum degrading bacterial endophytes with ACC deaminase activity increases plant biomass, petroleum removal and therefore, improve phytoremediation of hydrocarbons contaminated soil (Afzal et al., 2012). Hong et al. (2011) found a petroleum degrading rhizobacterium *Gordonia* sp. S2RP-17 with ACC deaminase activity and siderophores that increase both total petroleum hydrocarbon removal and the growth of *Zee mays* in petroleum contaminated soil.

Streptomyces ssp. Hlh1 and Bmb4 showed all PGP traits whereas *Streptomyces* sp. Zah8 was only IAA producer. Sheng et al. (2008) have described one pyrene degrading endophytic *Enterobacter* able to produce IAA, siderophores and solubilize inorganic phosphate, demonstrating its potential to increase pyrene removal and improve plant growth in pyrene contaminated soil.

According to Afzal et al. (2014), endophytic bacteria having classic PGP and pollutant degradation activities performed better than those bacteria having only one of these activities. Moreover, the contaminant-degrading activity could be considered itself as a plant-growth promoting trait, because contaminants, in general, affect negatively the plant develop; consequently, the elimination of the toxics will benefit them (Cruz-Morales et al., 2016). In this sense, the determination of PGPs has great relevance to evaluate the actual usefulness of the endophytic actinobacteria in bioremediation. As reported in previous research, the presence of PGP traits in hydrocarbon degrading endophytic bacteria can select them as a promising resource to enhance phytoremediation of contaminated soil (Khan et al., 2013).

The major limiting factor for the biodegradation of pollutants is often their low availability. Biosurfactant-producing microorganisms can increase pollutant availability (Calvo et al., 2009). The five endophytic actinobacteria selected produced clear zones around the

colonies causing lysis of blood. However, the emulsification activity was positive only in *Streptomyces* sp. Hlh1, Hlh9 and Bmb4. The values of EI_{24} were ranged from 35% to 46%. The highest emulsification layer was formed by *Streptomyces* sp. Bmb4 (EI_{24} = 46.64%). Although Carrillo et al. (1996) recommended the hemolysis activity as a primary method to screen biosurfactant production, Youssef et al. (2004) reported that not all biosurfactant have hemolytic activity and compounds other than biosurfactants may cause hemolysis. Moreover, this method may exclude good biosurfactant producers. Thus, the hemolytic activity may not be an effective method for the screening of biosurfactant production. In this study, the production of biosurfactant was not found to be associated with the hemolysis activity. Consequently, the isolates exhibited biosurfactant production will be studied further to enhance their production in order to ensure their effect on the bioavailability of hydrocarbons.

4. Conclusion

There was no previous scientific study on *Streptomyces* from plant roots that can degrade crude petroleum. *Helianthemum lippii, Bassia mauricata* and *Zygophyllum album* were selected to investigate petroleum-degrading endophytic actinobacteria. The results of our study confirmed that it is possible to isolate endophytic actinobacteria with plant growth promoting features, as well as, petroleum degrading ability from desert plants grown naturally in sites contaminated with crude petroleum. Among seventeen isolates, five showed the ability to remove petroleum up to 98%, after 7 days in liquid medium. These strains belonged to *Streptomyces* genera. Furthermore, they were producers of various plant growth promoting features. To the best of our knowledge, this is the first report on the biodegradation of crude petroleum oil by endophytic *Streptomyces* spp. These properties open up promising perspectives for their application as potential agents for phytoremediation of a petroleum-contaminated ecological environment.

Conflict of interest

No potential conflict of interest was reported by authors in this study.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.09.013.

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