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The capacity of *Actinobacteria* isolated from arid ecosystems in the bioremediation of soils polluted by λ-cyhalothrin-pesticide

Karima BENSOUICI[1,3], Lamia OULMI[2],
Allaoueddine BOUDEMAGH[3]*, Mahmoud KITOUNI[2]

[1]*Abbes Laghrour-Khenchela University, Faculty of Natural and Life Sciences, Department of Ecology and Environment, Khenchela, Algeria; bensouici.karima@univ-khenchela.dz*
[2]*Frères Mentouri Constantine 1 University, Faculty of Natural and Life Sciences, Laboratory of Microbiological Engineering and Application, Constantine, Algeria; oulmi.lamia@umc.edu.dz; mahmoud.kitouni@umc.edu.dz*
[3]*Frères Mentouri Constantine 1 University, Faculty of Natural and Life Sciences, Laboratory of Molecular and Cellular Biology, Constantine, Algeria; allaoueddine.boudemagh@umc.edu.dz (*corresponding author)*

Abstract

The insecticide lambda cyhalothrin has been used in many crops for many years in the arid soils of the Algerian Sahara and in other countries. The urgency of the use of different biological techniques is necessary for depollution, to compensate the devastating effects of this insecticide. *Actinobacteria* have very large capacities of degradation because of their very important metabolic potential. The exploration of extreme ecosystems such as chotts and saline soils which has not been studied sufficiently is a promising approach to give native *Actinobacteria* with a high adaptive capacity to hot climates and high levels of arid soil salinity. In this work, several *Actinobacteria* were isolated on three selective media from the soils of Chott Melghir and El-Oued, in the northeast of the Algerian Sahara. The study of their growth capacities on lambda-cyhalothrin taken as the sole source of carbon and nitrogen is tested on minimum media. The isolate S1 and SO11 are the most effective in the biodegradation of the insecticide. They can live at temperatures ranging from (15 °C to 55 °C) and in neutral to alkaline pH. The lambda-cyhalothrin degradation tests showed that the selected isolates could grow on this insecticide as a sole source of carbon and nitrogen at maximum concentrations of 3 to 4 g/L. These two bacteria are assigned to *Streptomyces* genus. This study shows that *Streptomyces* are excellent agents for the bioremediation of arid soils contaminated by this insecticide.

Keywords: *Actinobacteria*; lambda-cyhalothrin; Melghir; pyrethroid; saline soil

Introduction

Agriculture in arid soils is booming in Algeria and many African countries since several years now. The soils of these hot and dry regions are subject to all kinds of aggressions like anarchic use of various phytosanitary products. Currently, excessive and uncontrolled use over large areas of these chemicals has led to pollution of various segments of the environment. These molecules are likely to leave their application sites and are therefore

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considered as very dangerous pollutants to human and animal health. According to Ardley (1999), of the total pesticides applied in agriculture, only 0.1% reaches their targets and the rest directly affects the environment.

The commercial insecticide of the family of synthetic pyrethroids is called "Karate" has been used for several years in Algerian Saharan agriculture. Karate is an insecticide that mainly contains lambda-cyhalothrin ($C_{23}H_{19}ClF_3NO_3$) as an active product. Since the 2000s, the use of insecticides from the pyrethroid family has increased considerably, following the decrease in the use of organophosphates compounds. This insecticide specifically targets a wide range of insects, such as aphids, butterfly larvae and disease-carrying insects. It is very toxic to fish, invertebrate aquatic organisms, bees and humans (Maund *et al*., 1998; Bibi *et al.,* 2014; Lu *et al.,* 2019). In addition, many studies have shown that pyrethroids can have toxicity to mammalian reproduction (Abdallah *et al*., 2010). They are neurotoxic and cause endocrine disturbances (Wolansky and Harrill 2008; Zhao *et al.,* 2008; Elhalwagy *et al.,* 2015; Bordoni *et al*., 2019).

All the harmful effects of this insecticide on the environment and on human and animal health are accentuated in certain situations such as overdoses, accidental exposures and the lack of targeted application methods. In addition, lambda-cyhalothrin has a number of resistance characteristics that give it persistence in nature. This insecticide is very stable in light at temperatures below 220 °C and in water at pH 7 and pH 9 (Hart, 1984). Because of its high hydrophobic property, this insecticide binds strongly to silica particles and to organic matter, which allows it to persist in soils and to reach different water ecosystems and even groundwater. The results of some studies have shown that pyrethroids can have many harmful effects on soil biology; which involves qualitative and quantitative changes in the telluric microflora. These ecological imbalances involve changes in the different activities of enzymes; which can affect plant growth and soil fertility (Cycon and Piotrowska-Seget, 2016).

In recent years, the preservation of land and aquatic capital has been a major concern for researchers in this field. The challenge is to clean up the soil and waters by using inexpensive and low-polluting techniques. Thus, researchers devised various methods to eliminate this pollution. Biological treatments using the metabolic capacities of microorganisms are the easiest and most economical compared to conventional physicochemical processes. They allow the cleaning of the soils and the different water environments by a very well-known phenomenon called bioremediation.

It should be noted that salinity levels have increased considerably in recent years due to the drastic climatic changes observed. It has been confirmed that high salt concentrations increase the toxic effect of pyrethroid pesticides on different aquatic organisms (Segarra *et al*., 2021). There is therefore an urgent need to find halophilic or halotolerant microorganisms capable of growing in these ecosystems and biodegrading these pesticides. Various microorganisms have capacities for biodegradation of pesticides. The microbes involved in the biodegradation of pyrethroid insecticides, are limited to several bacteria and some fungi (Bhatt *et al.,* 2019; Birolli *et al*., 2019; Hu *et al.,* 2019; Majid *et al.,* 2023). However, despite their importance in pesticide degradation, the use of *Actinobacteria* in the biodegradation of pyrethroides is very negligible. The need to test these bacteria by oriented screening, in this kind of biodegradation is of great importance. The *Actinobacteria* are known to have a wide varied enzymatic heritage and have shown significant ability to degrade the most difficult to biodegrade phytosanitary molecules (Mc Carthy and Williams, 1992; Fuentes *et al.,* 2010). These bacteria are ubiquitous and possess remarkable resistance and adaptation capabilities to the most extreme conditions (Cross, 1980; Goodfellow and Williams, 1983; Hocinat and Boudemagh, 2015).

Agriculture in arid regions is very unusual, because of the particular climatic characteristics that change during the seasons and even from day to night in a considerable way. In summer, the temperatures, range from 50 to 60 °C in the morning to reach 0 to 3 °C during the night (Perret, 1935). The concentration of sodium chloride is very high, so these soils are considered very saline (Meklat *et al.,* 2011). In these ecosystems, we register a weakness and irregularity of precipitation, high evaporation and high luminosity.

Bioremediation techniques for these extreme ecosystems require the use of native microorganisms with adequate adaptive systems. According to our knowledge, studies of the biodegradation of lambda-cyhalothrin

by halophile *Actinobacteria* that resist to high temperatures are very rare; they are non-existent in Algeria. There is therefore a necessity to select efficient native strains; able to adapt to the extreme conditions of aridity of the Saharan climate and to degrade this very toxic pesticide. Thus, the main objective of this work is to isolate *Actinobacteria* and to study their roles to use this insecticide for their growth; as the only source of carbon and nitrogen. For this purpose, we chose the soils of Chott Melghir and that of the city of El-Oued for their very important degrees of salinity and temperature.

Materials and Methods

Isolation of Actinobacteria

Samples and sampling sites

Three soil samples were collected using the (Pochon and Tardieux, 1962) method, from the two study sites. The first collection location is the Chott Melghir also called chott Melrhir. This ecosystem is a salt lake located in the northeast of the Algerian Sahara. Administratively, it is part of the wilaya of El-Oued, daira of Reguiba, commune of Hamraïa. It covers an area of 551,500 square kilometers. This Chott is located about 300 km from the sea at -35 m altitude. It is therefore the lowest point of Algerian geography. It was declared a UNESCO world site in 2003 and protected by the Ramsar convention. Because of its high evaporation, it regularly becomes a salt desert. The second sampling site is the agricultural soil of the city of El-Oued. The soil samples were collected from a private farm located about 3 km from this town, which grows several vegetables. For over 15 years, this farmer has been using several synthetic insecticides that belong to pyrethroids family, especially Karate. The samples from both sampling sites were placed in sterile bottles, transported to the laboratory, and stored in the refrigerator at 4 °C until use (Figure 1).

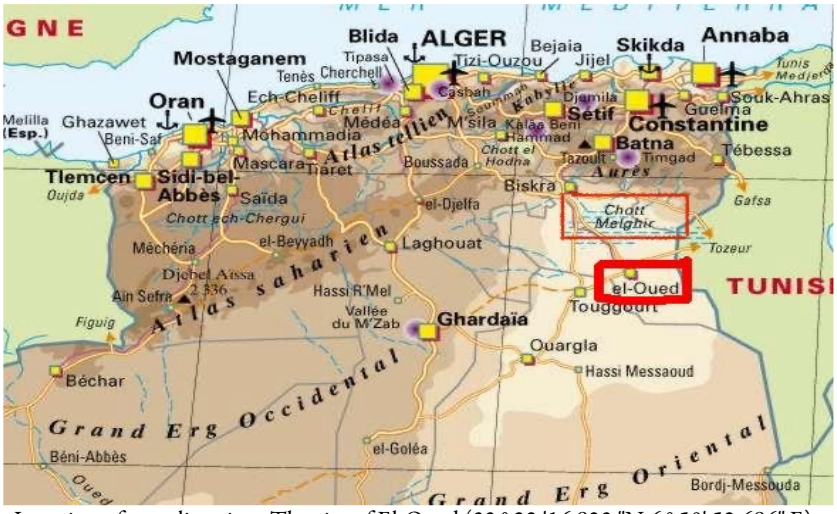

Figure 1. Location of sampling sites: The city of El-Oued (33 ° 22 '16.823 "N 6 ° 50' 52.686" E) and Chott Melghir (34°15′00″N, 6°19′00″E)
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Isolation and purification of Actinobacteria

Isolation was carried out in three selective isolation media: AIA (Uzel *et al.,* 2011), Starch Casein Agar (SCA) (Mohseni *et al.*, 2013) and ISP2 (Shirling and Gottlieb, 1966) with 10 g/ml nalidixic acid, 50 g/ml nystatin and 14% NaCl added to each to eliminate unwanted microorganisms (Gram-negative bacteria and fungi). The Antibiotic solutions have been sterilized by membrane filtration type Millipore (0.22 m porosity)

and are added aseptically to isolated environments. Mother suspensions were prepared after depositing 1 gram of each soil sample into test tubes containing 9 ml of sterile physiological water and vigorously stirred by a vortex for about 5 minutes. Decimal dilutions of up to 10^{-6} are performed for all soil samples in physiological water (9 g/L of NaCl). Inoculation is carried out by spreading 0.1 ml of each dilution over the surface of the isolation medium. The dishes are incubated at 30 °C and observed daily for 30 days. The colonies obtained were purified by successive sub-culturing on the SCA medium by the streak method and then incubated at 30 °C for 7 days until purification.

Morphological characterization and conservation of Actinobacteria

Macroscopic observation

In order to highlight the characteristic aspects of *Actinobacteria*, macroscopic observations of the colonies were made using a binocular (Leica DMLS) at 10x magnification after 7, 14, 21 days of incubation (Williams and Cross, 1971).

Microscopic observation after Gram staining

Microscopic observation was carried out after Gram staining, according to the conventional technique. This technique also provides information on the appearance and arrangement of the hyphae.

Electron microscope observation

The morphology of the mycelia and the spore chains of the performing *Actinobacteria* were observed under a scanning electron microscope. These observations were made in Scientific and Technical Research Center and Physico-Chemical Analysis (CRAPC), (Ouargla-Algeria) (*http://www.crapc.dz*).

Conservation of Actinobacteria isolates

The isolates are stored on the SCA medium in inclined agar at a temperature of 4 °C. This preservation requires a sub-culturing every two months for the maintenance of the strains. The spores of *Actinobacteria* isolates are incubated in 50% of glycerol and then frozen at -18 °C for long storage (Boudemagh *et al.,* 2005).

Selection of isolates using Karate for their growth

The mineral salts medium (MSM1) is used to determine the ability of *Actinobacteria* isolates to use Karate insecticide as the only source of carbon. It is composed in (g/L) of: Na_2HPO_4, 2.1; $MgSO_4$, 0.01; $CaCl_2$, $2H_2O$, 0.1; $FeSO_4$, $7H_2O$, 0.001; $CuSO_4$, 0.04; Na_2MoO_4, 0.002; $(NH_4)_2SO_4$, 1.0; Agar, 15 (Bano and Musarrat, 2004), supplemented with 14 % NaCl. The pH is adjusted to 7.2 -7.4 and then autoclaved at 121 °C for 15 min. This minimum medium is composed of mineral salt and mineral nitrogen and contains no carbon source. Insecticide Karate is the missing source of carbon. Each *Actinobacteria* isolate was sown with streaks tightened on the surface of a Petri dish containing the minimum medium MSM1, added with a concentration of 0.1% of the insecticide, previously sterilized through a 0.22 µ porosity filtration membrane. A second medium, (MSM2) is composed like MSM1 supplemented with 1 % glucose and without $(NH_4)_2SO_4$, makes it possible to evaluate the growth capacity of *Actinobacteria* on 0.1 % of sterile insecticide, taken as the sole source of nitrogen. The minimal medium MSM3 was prepared as MSM1, but without $(NH_4)_2SO_4$ and with Karate at a concentration of 0.1% as the only source of carbon and nitrogen. The positive control composed of MSM1 medium plus 1% glucose and 0.1% of the insecticide as well as the negative control (without bacteria) are used for all these experiments as control samples. All dishes are incubated for 14 days at 30 °C in the dark. Degradation of the pesticide results in significant growth of colonies on the media (Bano and Musarrat, 2004; Tamil Kumar and Syed Jahangir, 2018).

Growth of efficient isolates in liquid culture

The isolates that show growth on solid medium were tested in 250 ml Erlenmeyer flasks, containing 100 ml of liquid MSM1 medium supplemented with 14% NaCl and 0.1% of the insecticide. Sterile glass beads have been added to break the pellets of certain kinds of *Actinobacteria*, which interfere with their measurement of optical density (OD). The cultures are incubated in the dark in a water bath set at 30 °C with shaking at 120 rpm. After 7 days, the optical density of the isolates was measured at 620 nm by a spectrophotometer of the Jenway 7509 brand, in order to measure the growth of each bacterium.

Physiological characterization of selected isolates

Temperature effect

The best performing isolates were selected and cultured in Erlenmeyer of 250 mL flasks, according to the same protocol described in the "Growth of efficient isolates in liquid culture section". Optic density was measured after incubation at different temperatures (15 °C, 30 °C, 37 °C, 45 °C, and 55 °C).

Effect of pH

Different values of pH (6, 6,5, 7, 7,5, 8 and 9) were tested in Erlenmeyer of 250 mL volume. The crops and the measurement of growths were carried out according to the same protocol described in "Growth of efficient isolates in liquid culture section".

Effect of sodium chloride

Different concentrations of 2.5 %, 5 %, 14 %, 20 %, 25 % and 30 % (w/v) of NaCl were tested in 250 ml Erlenmeyer flasks according to the same protocol described in "Growth of efficient isolates in liquid culture section" (Shirling and Gottlieb, 1966).

Tolerance of isolates at different concentrations of Karate

The resistance of performing *Actinobacteria* to different concentrations of the insecticide was studied on solid medium in petri dishes, containing MSM1 medium and concentrations of 0,2, 0,3, 0,5, 1, 2, 3, 4, 5 and 6 g/L of the pesticide. These insecticide-enriched media were streaked and then incubated at 30 °C for 14 days. Dishes without pesticides and others without bacteria were used under the same conditions, respectively as positive and negative controls. The results are declared positive when the number of colonies is large compared to the controls. The absence of bacterial growth indicates a negative result.

Genus identification of performing isolates

Some *Actinobacteria* have characteristic cultural, morphological and colony-like features, which aid in the rapid identification of these bacteria at the genus level (Shirling and Gottlieb, 1966).

According to Lechevalier (1989), several genera belonging to *Actinobacteria* such as *Streptomyces Streptoverticillium Micromonospora*, *Microbispora* and many others, are easily identified by adequate microscopic observations, which make it possible to see the arrangement of the chains of spores, the appearance of aerial mycelia and those of the substrate (Lechevalier, 1989; Lechevalier, 1994; Silini *et al.,* 2016).

This technique consists of gently inserting a sterile cover glass into the ISP2 medium so that it forms a 45-degree angle with the middle surface. A drop of bacterial inoculum is deposited on the cover glass in contact with the medium. After 14 days of incubation at 30 °C, it is carefully removed from the medium, bringing with it the aerial mycelium and that of the substrate. The cover glass is then placed on a slide and examined under an optical microscope (G × 100) (Shirling and Gottlieb, 1966; Holt *et al.,* 1994).

Statistical analysis

Each experiment was carried out in triplicate. SE represented as error bars in figures. The comparison of means was performed using a one-way analysis of variance (ANOVA), utilizing Tukey's Honestly Significant Difference (HSD) post-hoc test to compare the mean values. The differences are considered significant at $p < 5\%$. Statistical analysis was performed using IBM SPSS 20.0 Statistics Premium Grad Pack software.

Results

Isolation and enumeration of Actinobacteria

After 30 days of incubation, 10 colonies with the characteristic appearance of *Actinobacteria* were isolated from the chott Melghir and 18 from the soil of El-Oued (Table 1). According to this study, the SCA medium is best indicated for the isolation of these bacteria from these two sites, followed by the AIA medium. The ISP 2 medium, however, allows the isolation of a limited number of *Actinobacteria* (Table 1).

Table 1. Number of *Actinobacteria* isolated from the soils of Melghir chott and El-Oued

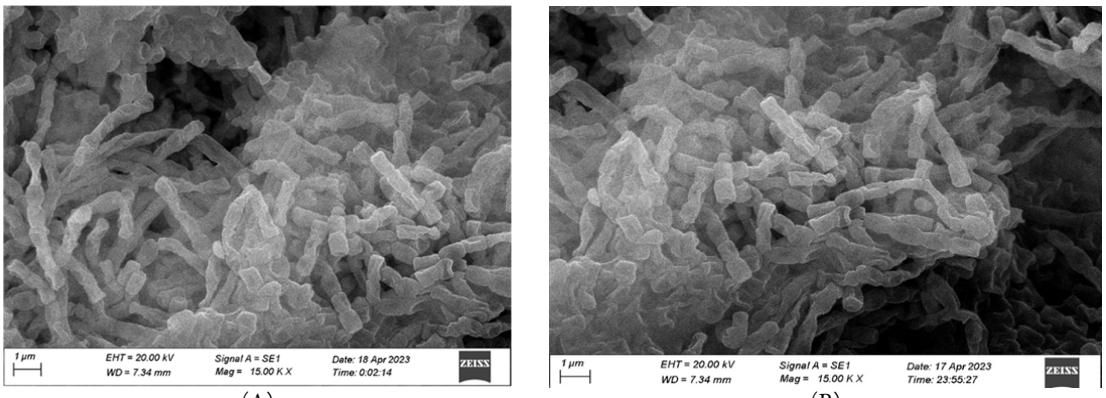
Site	AIA medium	SCA medium	ISP medium
Melghir chott soil	3	6	1
El-Oued soil	5	10	3

Morphological characterization of isolates

After 21 days of incubation, the colonies obtained appear dry, rough, in multiple colors with smooth or indented contours that strongly adhere to the agar. They are of average sizes with a diameter that does not exceed millimeters, powdery or not, regular or not, flattened or domed. Microscopic observation after Gram staining indicates a mostly filamentous appearance and a positive Gram stain for all isolated bacteria. Some bacteria keep their filamentary appearance, others fragment into a cocci or a stick.

Observation under an electron microscope of the S1 isolate shows straight spore chains, composed of a variable number of spore rods. Isolate SO11 presents the same micro-morphological characteristics (Figure 2).

The combination of all these culture characteristics and the macro and micro-morphological characters indicate that these isolates belong to *Actinobacteria*.



(A) (B)

Figure 2. Scanning electron microscope appearance of isolates S1 (A) and SO11 (B)

Growth of Actinobacteria on the insecticide Karate

The obtained results are collated in Tables 2 and 3. If the bacteria are unable to use the substrates, their growth is either weak or completely absent, otherwise the strains exhibiting good growth are considered

positive. The results show that the isolates (S1, S2, and S6) which come from the soil of Melghir have shown substantial growth on the medium M1, M2 and M3. This growth is equivalent to that of the positive control. These isolates are therefore able to live on the Karate insecticide as a sole source of carbon and nitrogen (Table 2). For these same bacteria, the insecticide is used in a weaker way when it is used only as the sole source of carbon. These isolates which come from this very harsh ecosystem therefore possess the enzymatic equipment necessary for the degradation of this xenobiotic. The rest of the *Actinobacteria* isolates (S3, S4, S5, and S10) grow moderately on this pollutant.

Table 2. Growth of *Actinobacteria* in Melghir soil on different media

Isolate	NC	PC	MSM1	MSM2	MSM3
S1	-	++	++	++	++
S2	-	++	++	++	++
S3	-	+	-	+	+
S4	-	+	-	+	+
S5	-	+	-	+	+
S6	-	++	++	++	++
S7	-	+	-	+	-
S8	-	+	-	+	-
S9	-	+	-	+	-
S10	-	+	+	+	+

MSM: Minimum mineral salt medium; NC: negative control; PC: positive control;
++: Substantial growth; +: moderate growth; -: negative growth.

The isolates S7 and S9 show no growth on Karate. Regarding the soil of El-Oued, the situation is completely different because the number of *Actinobacteria* capable of growing on this insecticide is higher. Indeed, the growth of six isolates (SO2, SO4, SO5, SO7, SO10 and SO11) compared to that of the positive control, is substantial. The rest of the *Actinobacteria* show either moderate or completely negative growth. We believe that this high number of positive *Actinobacteria*; is justified by the adaptation of the majority of these isolates to the presence of this pesticide in agricultural soil. Thus, these strains can be excellent candidates for the bioremediation of arid and saline soils contaminated by this insecticide. The absence of growth in all the negative controls proves that the degradation of Karate in these study conditions requires the intervention of bacteria.

Table 3. Growth of *Actinobacteria* from El-Oued soil on different media

Medium Isolate	NC NC	PC PC	MSM1 MSM1	MSM2 MSM2	MSM3 MSM3
S01	-	+	+	+	+
S02	-	++	++	++	++
S03	-	+	+	+	+
S04	-	++	+	++	++
S05	-	++	++	++	++
S06	-	+	-	+	-
S07	-	++	++	++	++
S08	-	+	+	+	+
S09	-	+	-	+	-
S010	-	++	+	++	++
S011	-	++	+	++	++
S012	-	+	-	+	+
S013	-	+	+	+	+

S014	-	+	+	+	+
SO15	-	+	-	+	-
SO16	-	+	-	+	-
SO17	-	+	-	+	-
SO18	-	+	-	+	-

MSM: Minimum mineral salt medium; NC: negative control;
PC: positive control; ++: Substantial growth; +: moderate growth; -: negative growth.

Growth of efficient isolates in liquid medium

The growth of the isolates was evaluated by measuring the optical density in a liquid medium. It appears that the isolates S1 and SO11 are the best performing among the 9 isolates showing good growth on solid medium (OD 7.8 and 7.5 respectively, $p<0.05$) (Figure 3). The first isolate is from Melghir chott, the second is from El-Oued soil.

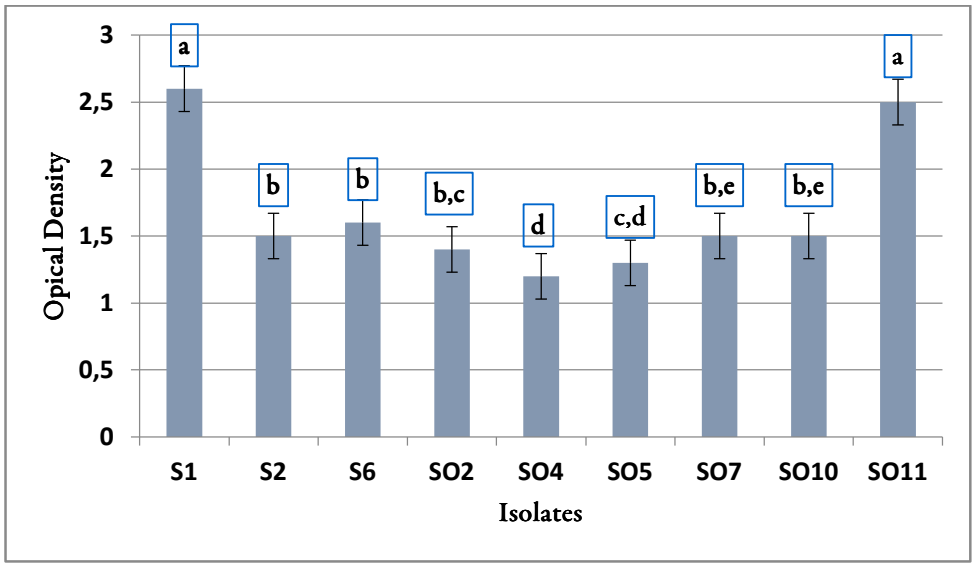


Figure 3. Values of the optical densities of the different isolates in liquid medium
Results followed by the same letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$). Error bars represent (SEM).

Physiological characters of the selected isolates

Temperature effect

The two isolates selected in this study (S1 and SO11) are those which show substantial growth in Karate, among all the isolates obtained. These two bacteria can not only live at high temperatures; but also, at average ones (30 and 37 °C, $p<0.05$) and even at low temperatures (15 °C). They are not therefore, thermophiles obliged. However, these two bacteria prefer high temperatures (45 °C and 55 °C, $p<0.05$) (Figure 4). This result is very important, because it indicates that our strains are capable of living in the presence of the insecticide Karate, in arid soils of the Sahara, known for its high heat in the morning and low temperatures at night. These results are even more interesting because they show that these extreme ecosystems offer bacteria with great capacity of adaptation to very wide temperature ranges. Contrary to works using mesophiles, bacterial representatives do not tolerate large temperature fluctuations. This is the case, for example, with the work of Lin *et al.* (2011), on the biodegradation of cypermethrine (a pesticide in the perythroids family). The results of this work show that the capacity for biodegradation of mesophilic *Streptomyces* decreases by 90% to 10% in temperatures above 34 °C.

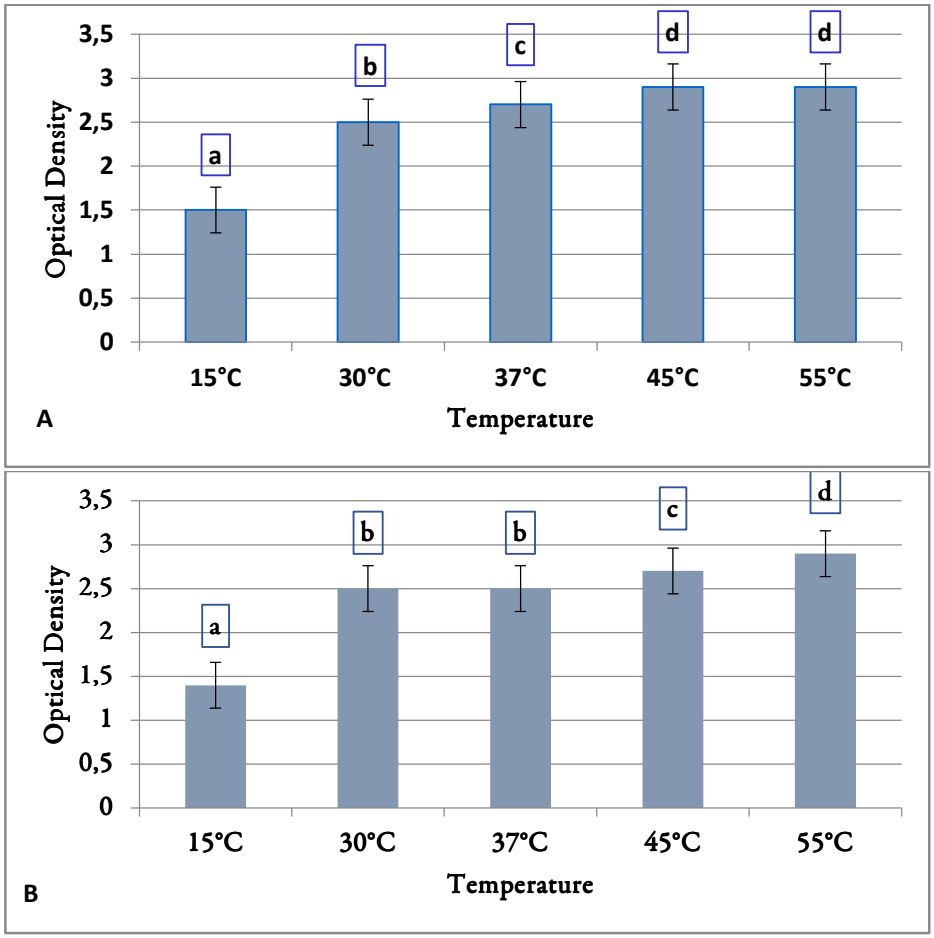


Figure 4. Effect of temperature on microbial growth (**A**: isolate S1, **B**: isolate SO11)
Results followed by the same letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$).

Effect of pH

The pH test shows the ability of the two isolates S1 and SO11 to grow at neutral to alkaline pHs (Figure 5). According to Feller's pH scale of soils, isolate S1 prefers slightly basic pHs (7.5 and 8, $p<0.05$). However, according to the same scale, isolate SO11, prefers neutral to slightly alkaline pH ($p<0.05$) (Feller, 1995). These two *Actinobacteria* can therefore grow in the arid soils of the Algerian Sahara (sodium or limestone), most of which tend to be slightly alkaline.

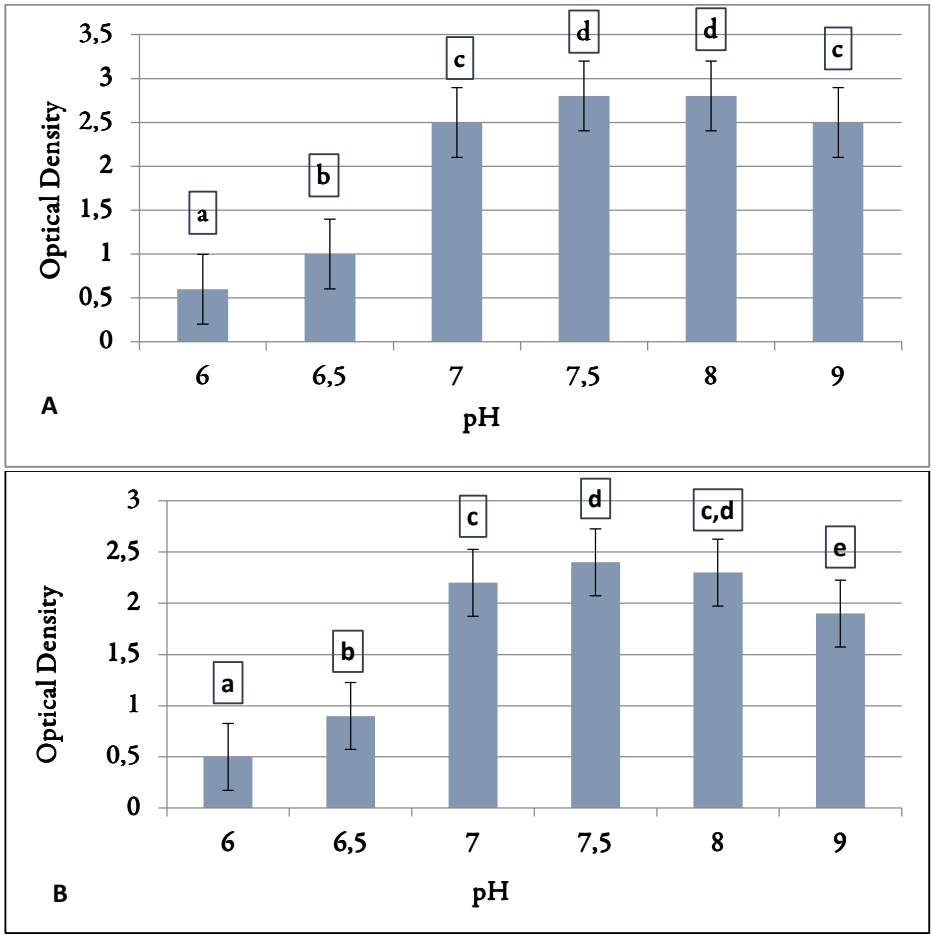


Figure 5. Effect of pH on microbial growth (**A**: isolate S1, **B**: isolate SO11)
Results followed by the same letters are not significantly different according to Tukey's post-hoc test ($p<0.05$).

Effect of sodium chloride

The two performing isolates have a different resistance capacity to NaCl. The S1 isolate from Melghir chott is much more resistant than the SO11 isolate from El-Oued. It can grow at a high percentage of NaCl (up to 30%, $p<0.05$). This high resistance is explained by the extremely salty nature of the ecosystem from which the S1 isolate originates. The SO11 isolate has a much lower resistance capacity with a maximum percentage of 14% NaCl ($p<0.5$) (Figure 6).

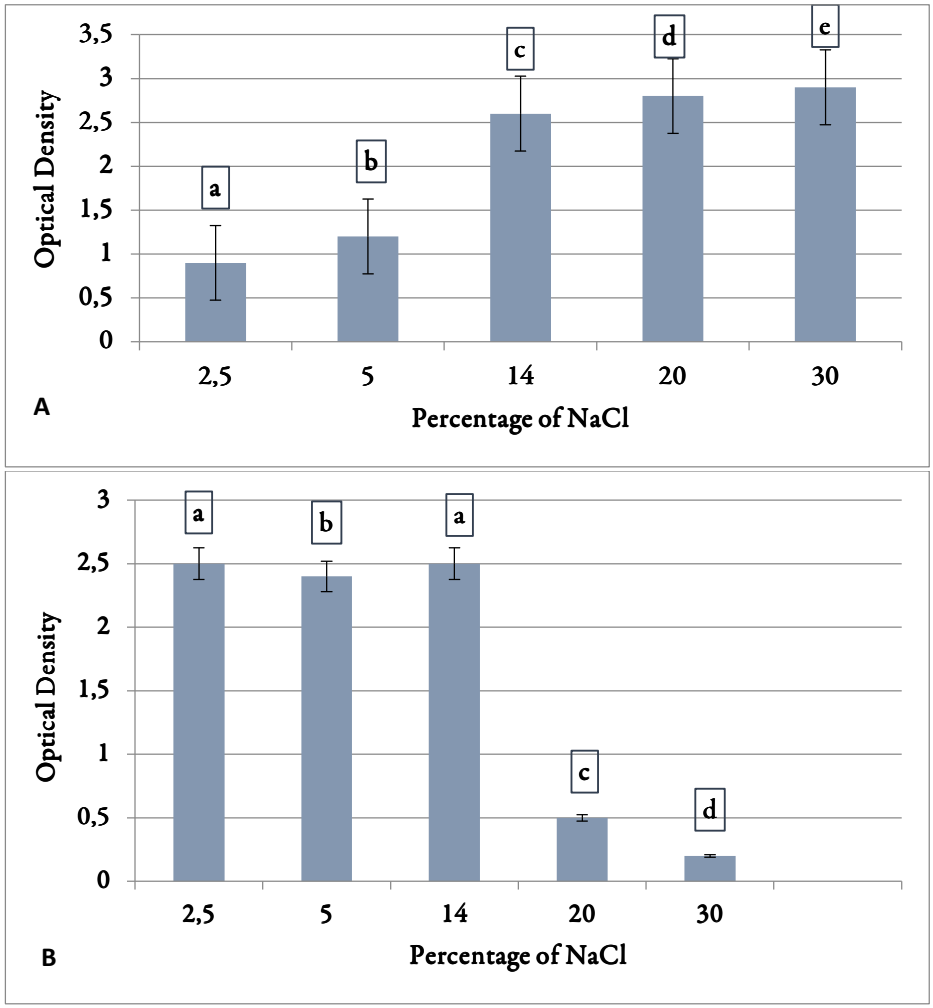


Figure 6. Effect of NaCl on microbial growth (**A**: isolate S1, **B**: isolate SO11)
Results followed by the same letters are not significantly different according to Tukey's post-hoc test ($p < 0.05$)

Based on the classification of Tang *et al*. (2003) the isolate S1 supported and only grows at very high NaCl concentrations 30 %. It is considered an extreme halophilic *Actinobacteria*. The isolate (SO11) can live in percentages of 2.5 to 14% of NaCl, it is considerate to be a moderately halophilic actinobacterium. These results indicate different requirements of these bacteria concerning NaCl. The strain S1 that comes from telluric samples from Chott requires a very high concentration of NaCl and can only live under these conditions. However, the SO11 strain, which originates from the region of El-Oued is a moderately halophilic bacterium and can live in soils that are moderately rich in sodium chloride. Therefore, these bacteria have enormous potential and can be used in bioremediation of soils with a wide range of NaCl concentrations.

Tolerance of isolates at different concentrations of Karate

Both isolates have been tested for their resistance to different concentrations of Karate Insecticide. The results reported in Table 4 indicate that the S1 isolate withstands a maximum concentration of 4 g/L. Isolate SO11 is less resistant to this pesticide with a maximum tolerance of 3 g/L (Table 4). The concentration of lambda-cyhalothrin varies considerably from an ecosystem to another depending on agricultural activities. For example, this insecticide was found at a concentration of 0.11 to 0.14 µg /L in water from agricultural watersheds in Stanislaus Counties, California (He *et al*., 2008). Residues of this insecticide were detected in

sediments procured from sites sampled in Imperial, Monterey, Stanislaus and Placer counties with a concentration ranging from 0.003 to 0.315 µg /g of dry weight (Starner *et al.,* 2008).

Table 4. Tolerance of isolates at different concentrations of Karate

Karate (g/L)	0.2	0.3	0.5	1	2	3	4	5	6
S1	++	++	++	++	++	++	++	-	-
SO11	++	++	++	++	++	++	-	-	-

++: substantial growth; -: negative growth

Genus identification of high-performance isolates

After sub culturing, the colonies of isolates S1 and SO11 appear after 2 to 3 days of incubation at 30 °C on the ISP2 medium, this characterizes fast-growing *Actinobacteria*. The colonies of these two isolates are round and powdery, with a beige color. For the two isolates, we note the production of a diffusible pigment, which completely colors the agar**.** Under an optical microscope, we observe several filaments carrying chains of straight spores. We also note the presence of conidia, which carry between six and eight spores per filament. The aerial and substrate mycelia are developed and persistent (Figure 7). These cultural macroscopic and microscopic characters, allow us to assign our two isolates to the genus *Streptomyces* (Lechevalier, 1994).

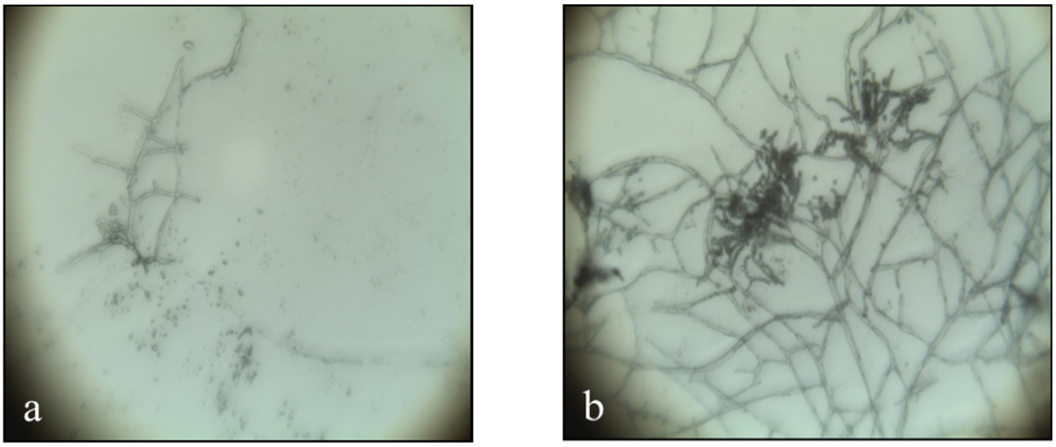


Figure 7. Aerial mycelium (a) and substrate mycelium (b) of isolate S1 at magnification (×100)

Discussion

In recent years, investigations on the use of *Actinobacteria* in the bioremediation of polluted soils by pesticides; have considerably increased (Fuentes *et al.,* 2010). This interest can be justified by the fact that these bacteria have a high capacity of resistance to the lack of water and nutrients (Mc Carthy and Williams, 1992). This characteristic therefore allows the use of these bacteria in the most arid and nutrient-poor soils. In addition, these bacteria can tolerate the presence of various inorganic pollutants in the soil such as mercury and copper (Ravel *et al*., 1998; Albarracin *et al.,* 2008). This property goes in favor of *Actinobacteria* and that is because of the ability of these microorganisms to degrade pesticides in the presence of heavy metals. The possibilities of its use in the bioremediation of polluted soils by these pollutants are not compromised. Furthermore, the production capacity of mycelium by many *Actinobacteria* (Ensign, 1992), facilitates the colonization of soil particles and helps their propagation in the most diverse recesses of telluric sites. This

important characteristic of *Actinobacteria* is very interesting in bioremediation, it avoids each time the reversals of the soils essential for other non-filamentous bacteria.

The soil of El-Oued offers six *Actinobacteria* capable of growing on Karate, against only three isolated from the salty soil of chott Melghir. This number can be explained by the fact that the bacteria in these agricultural soils are in permanent contact with these phytosanitary products and can therefore develop adaptations to these molecules. The non-agricultural soils of chott, can also be a source of these bacteria which have in their genetic inheritance metabolic aptitudes of degradation of this pesticide. The reasons for this finding remain inexplicable at this level of study and deserve in our opinion further investigations in order to explain these biochemical capacities.

In Birolli's *et al.* (2019) work for example, the degradation of different enantiomers of the insecticide cyhalothrin was studied. Results have shown that the enantiomer gamma-cyhalothrin is more efficient than lambda. It is also very easily biodegraded compared to lambda-cyhalothrin by a consortium of three strains of *Bacillus*. Therefore, the protection of different plants with gamma-cyhalothrin, which can be applied at concentrations lower than those of lambda-cyhalothrin can is more efficient against insects and can be biodegraded faster in the environment, which decrease toxic effects on non-targeted organisms.

There is a great deal of literature on the involvement of other microorganisms in the biodegradation of pyrethroids. The bacteria involved in this biodegradation are: *Ochrobactrum anthropi* YZ-1, *Bacillus*, *Pseudomonas*, *Raoultella*, *Achromobacter*, *Acidomonas*, *Serratia*, *Sphingobium*, *Clostridium*, *Klebsiella*, *Mesorhizobium* sp. (S1b), *Bartonella* sp. (S2b) and *Lysinibacillus* (Zhang *et al*., 2010; Tamil Kumar and Syed Jahangir, 2018; Bhatt *et al*., 2019).

Studies conducted by Kaliamoorthi and Namasivayam show that a mobile Gram-negative bacterium isolated from clinical samples has the capacity to biodegrade five pyrethroid pesticides in 8 days. This bacterium has been assigned to *Enterobacter ludwigii.* This degradation was evaluated at more than 90% of a pesticide concentration of 100 ppm in 3% NaCl. In these same studies, the researchers demonstrated that this bacterium is also able to adapt to hypersaline conditions. The authors concluded in their investigations, that this strain can be used for the degradation of pyrethroid insecticides under saline conditions (Kaliamoorthi and Namasivayam, 2020).

However, the involvement of *Actinobacteria* in the biodegradation of this type of insecticide is very limited. It is represented only by *Rhodococcus erythropolis* (Tamil Kumar and Syed Jahangir, 2018), *Streptomyces* sp. *Streptomyces parvulus* HU-S-01 and *Brevibacterium* (Tamil Kumar and Syed Jahangir, 2018; Bhatt *et al*., 2019). According to Huang *et al.* (2018), the pesticides frequently degraded by *Actinobacteria* are aldrin, carbofuran, chlorpyrifos, diazinon and diuron.

According to our knowledge, the work of Yan Liu *et al*. (2023) constitutes the first report on a halophilic *Actinobacteria*, capable of producing a hydrolase involved in the degradation of a pyrethroid pesticide. These results are similar to ours. They also show that the saline and hypersaline sites explored in this work are a promising source of halophilic and hyperhalophilic *Streptomyces* capable of degrading the insecticide λ-cyhalothrin

Two *Actinobacteria* named (S1 and SO11) are the most efficient. The first isolate is from Melghir chott, the second is from El-Oued soil. These two isolates can not only live at high temperatures (45 °C and 55 °C) but also at average ones (30 °C and 37 °C) and even at low ones (15 °C). They are not therefore obligate thermophile *Actinobacteria*. The isolate S1 grows well at slightly alkaline pHs. The isolate SO11, prefers neutral to slightly alkaline pHs. The isolate S1 is an extreme halophilic *Actinobacteria* and can grow at a high NaCl percentage, up to 30%. However, the isolate SO11 is a halotolerant bacterium that can live at percentages of 2.5 to 14% of NaCl. The isolate S1 withstands a maximum concentration of 4 g/L and a maximum concentration of 3 g/L for isolate SO11. The preliminary identification of these two bacteria allowed us to assign them to the genus *Streptomyces*. These two *Actinobacteria* isolated from the arid and salty soils of these

two poorly explored regions have the capacity to use for their growth, the insecticide lambda-cyhalothrin as the only source of carbon and nitrogen. These bacteria also have very interesting physiological adaptive skills and, in our opinion, deserve special attention.

Conclusions

In this work, the presence of *Actinobacteria* was highlighted in saline and hot ecosystems. Eighteen isolates come from the region of El-Oued and ten from the chott of Melghir. The culture medium that allows the isolation of the greatest number of these bacteria is CSA, compared to AIA and ISP2 media. Of these isolates, 9 showed good growth on the insecticide lambda-cyhalothrin. The named *Actinobacteria* (S1 and SO11) are the most efficient, being able to tolerate a respective concentration of 4 g/L and 3 g/L of the insecticide. The S1 isolate is an extreme halophilic bacterium that can grow at a high percentage of NaCl, which reaches 30%. Isolate SO11 is a salt-tolerant bacterium that can live at percentages ranging from 2.5% to 14% NaCl. These two *Actinobacteria* are however not obligate thermophiles and can also grow at medium temperatures. These two bacteria have been assigned to the genus *Streptomyces*. These two *Actinobacteria* can be excellent bioremediation agents for agricultural soils in Saharan regions contaminated by lambda-cyhalothrin insecticide.

Authors' Contributions

K.B. validated and realized the methodology and wrote the manuscript. L.O. analyzed data, M.K. and A.B have corrected the protocol and brought an expertise to the whole manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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