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ORIGINAL PAPER

Actinobacteria isolated from wastewater treatment plants located in the east-north of Algeria able to degrade pesticides

Oumeima Boufercha[1,2,3] · Irina S. Moreira[4] · Paula M. L. Castro[4] · Allaoueddine Boudemagh[3]

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Abstract

The pollution of water resources by pesticides poses serious problems for public health and the environment. In this study, *Actinobacteria* strains were isolated from three wastewater treatment plants (WWTPs) and were screened for their ability to degrade 17 pesticide compounds. Preliminary screening of 13 of the isolates of *Actinobacteria* allowed the selection of 12 strains with potential for the degradation of nine different pesticides as sole carbon source, including aliette, for which there are no previous reports of biodegradation. Evaluation of the bacterial growth and degradation kinetics of the pesticides 2,4-dichlorophenol (2,4-DCP) and thiamethoxam (tiam) by selected *Actinobacteria* strains was performed in liquid media. Strains *Streptomyces* sp. ML and *Streptomyces* sp. OV were able to degrade 45% of 2,4-DCP (50 mg/l) as the sole carbon source in 30 days and 84% of thiamethoxam (35 mg/l) in the presence of 10 mM of glucose in 18 days. The biodegradation of thiamethoxam by *Actinobacteria* strains was reported for the first time in this study. These strains are promising for use in bioremediation of ecosystems polluted by this type of pesticides.

Keywords *Actinobacteria* · Biodegradation · Pesticides · *Streptomyces* sp. · Wastewater treatment plant

Highlights

- Physico-chemical evaluation revealed efficiency of performance of WWTPs of east-north of Algeria.
- *Streptomyces* was the dominant genus of the *Actinobacteria* isolated from the WWTPs.
- The *Actinobacteria* strains were able to grow in 9 among the 17 pesticides tested.
- Isolated strain *Streptomyces* sp.ML was able to degrade 45% of 50 mg/l 2,4-DCP.
- Isolated strain *Streptomyces* sp. OV was able to remove 84% of 35 mg/l tiam.

Introduction

The increase of the world population and the rapid development of industrialization led to the use of chemical molecules called pesticides (Meleiro Porto et al. 2011). They are defined as a substance or mixture of substances used in agriculture, public health and industry to control pests. Pesticides are widely used throughout the world. Annually, up to 5.6 billion pounds of pesticide active

✉ Oumeima Boufercha
boufercha.oumeima@yahoo.com

Irina S. Moreira
ismoreira@ucp.pt

Paula M. L. Castro
plcastro@ucp.pt

Allaoueddine Boudemagh
boudemagh.allaoueddine@yahoo.fr

1 Laboratory of Microbiological Engineering and Applications, University of Brothers Mentouri, Constantine 1, Chaâbat Erssas Campus, Ain El Bey Road, 25000 Constantine, Algeria

2 Laboratory of Molecular and Cellular Biology, University of Brothers Mentouri, Constantine 1, Chaâbat Erssas Campus, Ain El Bey Road, 25000 Constantine, Algeria

3 Department of Microbiology, Faculty of Natural and Life Sciences, University of Brothers Mentouri, Constantine 1, Ain El Bey Road, 25000 Constantine, Algeria

4 Universidade Católica Portuguesa, CBQF–Centro de Biotecnologia e Química Fina –Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

ingredients are used in agriculture to improve the yield and quality of crops (Doolotkeldieva et al. 2018). However, excessive use of pesticides can have adverse effects on the environment and human health. The reports of the United Nations estimate that only 1% of applied agricultural pesticides are retained by the target organism, and as a result they are found in the air, surface and groundwater, sediment, soil and vegetables (Rodríguez et al. 2020).

Pesticides can enter natural aquatic environments through discharges from manufacturing plants, during application, by runoff, leaching, drainage, or accidental spills (Narushima et al. 2014). Wastewater treatment plants (WWTPs) are the inevitable places where most toxic compounds are delivered. Although pesticides are the most incriminated class of organic pollutants in WWTPs, their impacts and behaviors have not been adequately clarified (Köck-Schulmeyer et al. 2013). Pesticides end up contaminating various environmental compartments if they are not completely removed in these plants. Several conventional treatment methods are available to remove pesticides from wastewater, such as photodegradation, adsorption, filtration and oxidation. These physical–chemical methods are costly and can produce undesirable by-products that require additional investment to remove (Ji et al. 2016). In contrast, bioremediation is a biological method for the remediation of contaminated sites, via which microorganisms degrade or transform hazardous organic contaminants into less hazardous and/or non-hazardous substances, is potentially an efficient, reliable, cost-effective and environmentally friendly approach (McGuinness and Dowling 2009). It has been reported that many bacteria and fungi are capable of degrading pesticides. Despite their high metabolic potential, *Actinobacteria* remain less exploited than other microorganisms for the biodegradation of these pollutants.

Actinobacteria are ubiquitous bacteria in nature, due to their physiological and metabolic variability, these organisms have aroused great interest for several biotechnological applications. They play an important ecological role in the recycling of substances and the elimination of complex organic matter, such as pesticides, which makes them the most appreciated candidates for bioremediation (Alvarez et al. 2012). In the present study, physico-chemical parameters of the influent and effluent of the three WWTPs located in the east-north of Algeria receiving domestic, agricultural and industrial wastewater were determined to evaluate their overall performance. The main aims of the present study were to isolate *Actinobacteria* strains from these WWTPs and to assess their ability to degrade pesticides of environmental concern. Degradation kinetics of 2,4-DCP and tiam by promising isolates was assessed.

Materials and methods

Chemicals

The pesticides used in this study were provided by the Cooperative of Cereals and Dry Vegetables of the city of Constantine, manufactured by Bayer CROP SCIENCE AG (Table S1), except for 2,4-DCP which was obtained from Sigma-Aldrich (Steinheim, Germany) with a purity of > 98%. The chemical structure is presented in Fig. S1.

Acetonitrile used for high performance liquid chromatography (HPLC) was of chromatographic grade obtained from Merck (Darmstadt, Germany). Trifluoroacetic acid 99% was purchased from Sigma-Aldrich (Steinheim, Germany). For the supply of ultrapure water (18.2 Mcm, organic carbon 4 g/l), a Milli-Q Gradient A-10 device (Millipore) was used. All other chemicals and reagents used in this study were of analytical grade (Sigma-Aldrich Chemie, Steinheim, Germany; Merck, Darmstadt, Germany).

Sampling

Raw wastewater, treated wastewater and activated sludge from aeration tank samples were collected from three WWTPs located in the east-north of Algeria, namely: the Ibn Ziad WWTP (City of Constantine), Oued El Athmania and Sidi Merouane WWTPs (City of Mila) (Table S2). For the isolation of *Actinobacteria*, wastewater was collected in sterile borosilicate glass vials of 250 ml. Plastic vials were used for samples for physicochemical analysis. Samples were transported in a refrigerated enclosure (at about 4 °C).

Measurement of physico-chemical parameters

The physico-chemical parameters analyzed in wastewater samples were: temperature, pH, Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD5) and Chemical Oxygen Demand (COD). The analysis of these parameters was carried out according to the techniques recommended by Rodier et al. (2009).

Isolation of *Actinobacteria*

Selective media used for the isolation of *Actinobacteria* were: AF (Kitouni 2007), Czapek-dox modified (Soler et al. 2018), ISP4 (the International Streptomyces Project No. 4) (Silini et al. 2016) and Olson (Bensultana et al. 2010). The antibiotic nalidixic acid (20 µg/ml) and the antifungal cycloheximide (50 µg/ml) were added to the isolation media (Goodfellow et al. 1996). Incubation was performed at 30 °C for 3 weeks. After growth, the *Actinobacteria* colonies were

identified by their characteristic macroscopic aspects, followed by microscopic analysis. The *Actinobacteria* isolates were purified on ISP2 medium and preserved in ISP2 at 4 °C. Long-term preservation of the spores in the presence of 50% glycerol was also carried out (Rachedi et al. 2018).

Phenotypic identification of isolated *Actinobacteria*

Actinobacteria isolates were studied morphologically on ISP2 medium according to the technique recommended by Shirling and Gottlieb (1966). The effect of temperature (37 °C and 45 °C) on growth, tolerance to various pH (5, 6, 7, 9, 10) and growth in the presence of various concentration of NaCl (2, 5, 9, 15%) (w/v) were carried out. The biochemical characteristics: production of catalase (Li et al. 2016), amylase (Harir et al. 2017), cellulase (El-Naggar et al. 2014), pectinase (Hankin et al. 1971), gelatinase and caseinase (Minotto et al. 2014), tyrosinase (Raval et al. 2012), esterase (Sierra 1957) and lecithinase (Nitsch and Kutzner 1969) have been examined. The ability to use different carbon substrates (D-arabinose, D-fructose, D-glucose, D- mannitol, myo-inositol, sucrose, D-xylose) and different organic acids (sodium acetate, sodium oxalate and sodium succinate) was tested on ISP9 medium (Pridham and Gottlieb 1948). The assimilation of different nitrogen sources (L-cysteine and L-serine) was studied on the basal medium (Williams et al. 1983).

Molecular identification

Molecular identification was performed by sequencing of the 16S rRNA gene. Genomic DNA extraction and further amplification by polymerase chain reaction (PCR) was performed as described elsewhere using universal bacterial 16S rRNA primers 27F and 1492R (Amorim et al. 2014). PCR products were purified and sequenced by Eurofins genomics (Konstanz, Germany) using universal bacterial 16S rRNA primer (27F) (Moreira et al. 2021). Identification and phylogenetic classification was performed using the BLAST software at the National Centre of Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). The partial 16S rRNA gene sequences were submitted to the GenBank database and the microbial strains were deposited to DSZM German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

Phylogenetic analysis was performed to determine the taxonomic position of the strains. For that, 16S rRNA gene sequences were aligned with reference sequences available in the GenBank/EMBL/DDBJ database. The phylogenetic tree was constructed with the MEGA software (version 5.1) using the neighbour-joining method (Kimura two-parameter distance optimized criteria).

Selection of pesticide-tolerant *Actinobacteria*

A qualitative study was performed to select *Actinobacteria* capable of using each pesticide as a single carbon source. The qualitative screening consisted in evaluating the growth of *Actinobacteria* on a mineral salt solid medium (MSM) (Cycoń et al. 2011b). Stock solutions of the pesticides were sterilized by filtration through 0.22 µm filters. Each pesticide (50 mg/l) was separately added, as a single carbon source, to the MSM plates, which were inoculated with isolated *Actinobacteria* strains. At the same time, MSM plates supplemented with 1% of glucose were inoculated in order to evaluate the maximum growth of the strains, and MSM without any addition of carbon source were established as biotic controls. MSM supplemented with each pesticide without bacterial inoculation were used as abiotic control. The prepared plates were incubated at 30 °C for 21 days in the dark. After incubation, isolates showing growth were tested on increasing concentrations of the pesticides up to 500 mg/l.

Biodegradation of tiam and 2,4-DCP by selected *Actinobacteria*

Tiam and 2,4-DCP were selected for biodegradation studies in liquid media. Degradation experiments were performed in 250 ml volume flasks containing 75 ml of MSM. These flasks were inoculated with spore suspension at OD = 0.1 at wavelength (λ = 620 nm).

Tiam and 2,4-DCP were added separately to the flasks at a final concentration of 35 and 50 mg/l, respectively. The flasks were then incubated under agitation at 130 rpm at a temperature of 30 °C, for 30 days. Non-inoculated and inoculated flasks with bacteria and without pesticide were used as abiotic and biotic controls, respectively. Each experiment was performed in triplicate and all flasks were protected from light. Experiments in similar conditions and with addition of 10 mM of glucose or 5,9 mM of sodium acetate were established, to evaluate the effect of a supplementary carbon source. To evaluate the growth and degradation kinetics of tiam, samples were collected at the beginning of the experiment and after 18 days of incubation, while for 2,4-DCP, samples were collected periodically at regular intervals of 0, 7, 14, 21, 30 days. The rate of degradation of 2,4-DCP by the different isolates was calculated according to the following equation: $C = C_0 e^{-kt}$, where C_0 is the initial concentration of 2,4-DCP, K is the degradation rate constant. The biodegradation half-life of 2,4-DCP ($t_{1/2}$) was calculated using the formula: $t_{1/2} = \ln 2/k$. Growth was monitored by measuring the optical density at 620 nm (OD 620 nm), using a UNICAM- Hλeios spectrophotometer.

Analytical methods

HPLC analysis

After centrifugation of the cultures at 8000 g for 10 min at 4 °C, 1 ml of supernatant from each culture was taken to determine the residual concentration of 2,4-DCP and tiam. The samples were analyzed by high performance liquid chromatography (HPLC) with Beckman Coulter System Gold 126 equipment, using a reversed phase 250e4 HPLC Cartridge LiChrospher 100 RP-18 column (Merck). The mobile phase consisted of acetonitrile: water acidified at pH 2.0 with trifluoroacetic acid (60:40). A volume of 20 µl was injected at a flow rate of 0.8 ml/min. The λmax was 250 nm (tiam) and 286 nm (2,4-DCP).

Chloride analysis

For chloride analysis, biomass was previously removed by centrifugation at 8000 g for 10 min. The chloride concentration in the samples supernatant was determined using the colorimetric method previously described (Iwasaki et al. 1956).

Results

Physico-chemical characterization

The wastewater treatment plants of Ibn Ziad, Oued El Athmania and Sidi Merouane treat wastewater of various origins: industrial, agricultural and domestic wastewater. The treatment system adopted in these WWTPs is that of activated sludge. The physico-chemical parameters of the raw wastewater, the aeration basin and the treated wastewater of the three WWTPs are shown in Table 1.

The pH values upstream and downstream of the three plants were slightly alkaline, while the samples from the aeration basins were characterized by highly alkaline pH values. The samples were taken in the humid season, so the temperature values vary between 11 °C and 20°C for the samples collected. The TSS of the wastewater is significantly reduced after treatment compared to the influent of the facility with an estimated reduction of 71%, 88% and 98% respectively at the WWTP of Ibn Ziad, Oued El Athmania and Sidi Merouane. COD and BOD5 concentrations became much lower at the outlet of Ibn Ziad, Oued El Athmania and Sidi Merouane WWTP with a percentage reduction of 90%, 96% and 94% in terms of COD and 67%, 93% and 91% in terms of BOD5.

Isolation and identification of *Actinobacteria*

After 21 days of incubation at 30 °C, *Actinobacteria* colonies were recognized by morphological aspects (presence of aerial and substrate mycelium and Gram positive staining). Isolates were purified by streaking on ISP2 medium (Fig. S2). According to morphological analysis of colonies and sequencing of the 16 sRNA genes, a total of 13 *Actinobacteria* strains were isolated from the three WWTPs (Table S3). The cultural and microscopic characteristics of the isolates were described by referring to the Bergey's manual of bacteriology (Goodfellow et al. 2012) (Table 2). All the isolates were Gram-positive and presented in the form of filaments except for the isolate MM. The color of the aerial and substrate mycelium as well as the color of the spore and its morphology and ornamentation varied according to strains. The results of the physiological and biochemical assays are shown in Table S4. All isolates were able to grow at 37 °C with an optimal growth temperature equal to 30 °C, at pH 6- 10 with an optimum equal to 7. All tolerate the concentration of 2% and 5% NaCl. The characterized *Actinobacteria* strains were all capable of producing amylase and caseinase and have the ability to assimilate D-glucose as a single carbon source. In this study, the majority of isolates belonged to the genus *Streptomyces* (12 strains), while the other one belonged to the genus *Micrococcus* (Table 3). The partial 16S rRNA gene sequences of the isolated strains

Table 1 Physico-chemical characterization of the collected samples

Parameter	WWTP Ibn Ziad			WWTP Oued El Athmania			WWTP Sidi Merouane		
	Raw wastewater	Aeration basin	Treated wastewater	Raw wastewater	Aeration basin	Treated wastewater	Raw wastewater	Aeration basin	Treated wastewater
pH	7,9	10,3	7,7	7,8	9,6	7,5	7,6	9,7	7,3
T C°	19,8	20,7	18,1	13,8	14,8	11,8	15,4	16,1	15,2
TSS*	258	86	74	143	24,2	17	452	36,7	6,8
BOD5*	270	410	90	210	307,3	14	310	453,6	28
COD*	480	620	47	425	524	16,1	708	889	41

* Value in mg/l

Table 2 Morphological characteristics of colonies formed by *Actinobacteria* isolates on ISP2 medium and their type strains

WWTP Ibn Ziad					
Isolate/ closely related type strains	Aerial mycelium	Substrate mycelium	Spore	Diffusible pigment	References
AC	Grey	Bleu	Grey, spira, hairy	None	
Streptomyces speibonae strain PK-Blue	Grey	Bleu	Grey, spira, hairy	None	(Meyers et al. 2003)
AE	Khaki grey	Mahogany brown	Grey, smooth, warty, hairy	None	
Streptomyces heliomycini strain NBRC 15899	N	N	Grey, smooth, warty, hairy	None	(Goodfellow et al. 2012)
AG	Cream	Dark reddish brown	Yellow, rectus flexibilis, smooth	Dark reddish Brown	
Streptomyces gougerotii strain NBRC 13043	N	N	N	N	N
ML	Greyish to light grey	Brownish to brown	Grey, spira, smooth	Dark brown	
Streptomyces collinus strain NBRC 12759	N	N	Grey, spira, smooth	None	(Goodfellow et al. 2012)
MM	/	/	No spore, yellow smooth convex colony	None	
Micrococcus luteus strain NCTC 2665	N	N	N	N	N
YO	Yellow	Brownish yellow	Yellow, rectus flexibilis, smooth	Brown	
Streptomyces cavourensis strain NRRL 2740	N	Brown	Rectus flexibilis, smooth	Moderate to strong Brown	(Kaaniche et al. 2020)
WWTP Oued El Athmania					
Isolate/ closely related type strains	Aerial mycelium	Substrate mycelium	Spore	Diffusible pigment	References
OA	White–gray	Yellow orange	Silver grey, spira, smooth	Yellow	
Streptomyces thinghirensis strain S10	White–gray	Yellow	Spira, smooth	Yellow	(Loqman et al. 2009)
OF	White brown leathery	Cream to brown	Yellow, rectus flexibilis, smooth	Brown	
Streptomyces sampsonii strain ATCC 25495	N	N	Yellow, rectus flexibilis, smooth	N	(Goodfellow et al. 2012)
OV	White–gray	Brown	Grey, spira, smooth	None	
Streptomyces chartreusis strain NBRC 12753	White to aqua gray	Bamboo to light olive gray	Light ivory, spiral, spiny	None	(Sripreechasak et al. 2013)
SG	Beige cream	Brown	Yellow, rectus flexibilis, smooth	None	
Streptomyces bacillaris strain NBRC 13487	N	N	Yellow, rectus flexibilis, smooth	None	(Goodfellow et al. 2012)

Table 2 (continued)

WWTP Sidi Merouane					
Isolate/ closely related type strains	Aerial mycelium	Substrate mycelium	Spore	Diffusible pigment	References
OB	Dark grey	Greenish brown	Grey, spira, spiny	Brown	
Streptomyces iakyrus strain NBRC 13401	N	N	Grey, spira, spiny	N	(Goodfellow et al. 2012)
OH	Grey	White	Grey, straight, smooth	None	
Streptomyces brevispora strain BK160	N	White	Straight, smooth	None	(Zucchi et al. 2012)
OT	Focussed grey/ smoked black	Brown/grey	Greyish white, spira, rough	Brown	
Streptomyces malaysiensis strain ATB-11	Dark grey	Brown	spira, rough	Brown	(Al-Tai et al. 1999)

N Not determined

Table 3 Molecular identification of *Actinobacteria* strains isolated from WWTPs

Isolate	Accession number (NCBI)	Accession number (DSMZ)	Closest organism	Accession of the closest strain	Similarity %
AC	MZ357071	DSM 113650	*Streptomyces speibonae* strain PK-Blue	NR_025212.1	99%
AE	MZ357073	DSM 113649	*Streptomyces heliomycini* strain NBRC 15899	NR_041197.1	99%
AG	MZ411470	DSM 113540	*Streptomyces gougerotii* strain NBRC 13043	NR_112610.1	100%
ML	MZ314444	DSM 113726	*Streptomyces collinus* strain NBRC 12759	NR_041063.1	99%
MM	MZ348828	DSM 113600	*Micrococcus luteus* strain NCTC 2665	NR_075062.2	99%
OA	MZ348609	DSM 113725	*Streptomyces thinghirensis* strain S10	NR_116901.1	100%
OB	MZ348825	DSM 113652	*Streptomyces iakyrus* strain NBRC 13401	NR_041231.1	99%
OF	MZ348833	DSM 113651	*Streptomyces sampsonii* strain ATCC 25495	NR_025870.2	99%
OH	MZ348836	DSM 113653	*Streptomyces brevispora* strain BK160	NR_117081.1	98%
OT	MZ348621	DSM 113545	*Streptomyces malaysiensis* strain ATB-11	NR_114497.1	99%
OV	MZ411469	DSM 113542	*Streptomyces chartreusis* strain NBRC 12753	NR_118341.1	99%
SG	MZ348824	DSM 113543	*Streptomyces bacillaris* strain NBRC 13487	NR_041146.1	99%
YO	MZ475021	DSM 113541	*Streptomyces cavourensis* strain NRRL 2740	NR_043851.1	100%

were submitted to the GenBank database under accession numbers presented at Table 3. Furthermore, to determine the taxonomic position of each strain, 16S rRNA gene sequences were aligned with reference sequences available in the GenBank/EMBL/DDBJ database and the phylogenetic tree was constructed (Fig. 1). 16S rRNA gene tree shows that most of the isolated strains are members of the genus *Streptomyces* sp., forming clusters with type strains of different species from this genus. The strain MM was identified as organism belonging to *Micrococcus* sp. Strain MM forms a cluster with *Micrococcus luteus* NCTC 2665 and *Micrococcus yunnanensis* 65004.

Pesticide-tolerant *Actinobacteria*

The ability of 13 strains of *Actinobacteria* isolated from three WWTPs located in Algeria to degrade separately various families of pesticides was evaluated assessing their growth on minimal salts medium agar plates containing the compounds at different concentrations. All *Actinobacteria* isolates showed good growth on MSM medium containing glucose, while none of the isolates were able to grow on MSM medium without any carbon source or on MSM medium added with the following pesticides: Topik 80 EC, Lambda-cythrin 25 Ec, Vapcomic, Concord 5 EC, Horizon 25 EW, Madjloul miracle, Opus, Vidan 25 (Table 4). For abiotic control test, no lysis zones or changes appear.

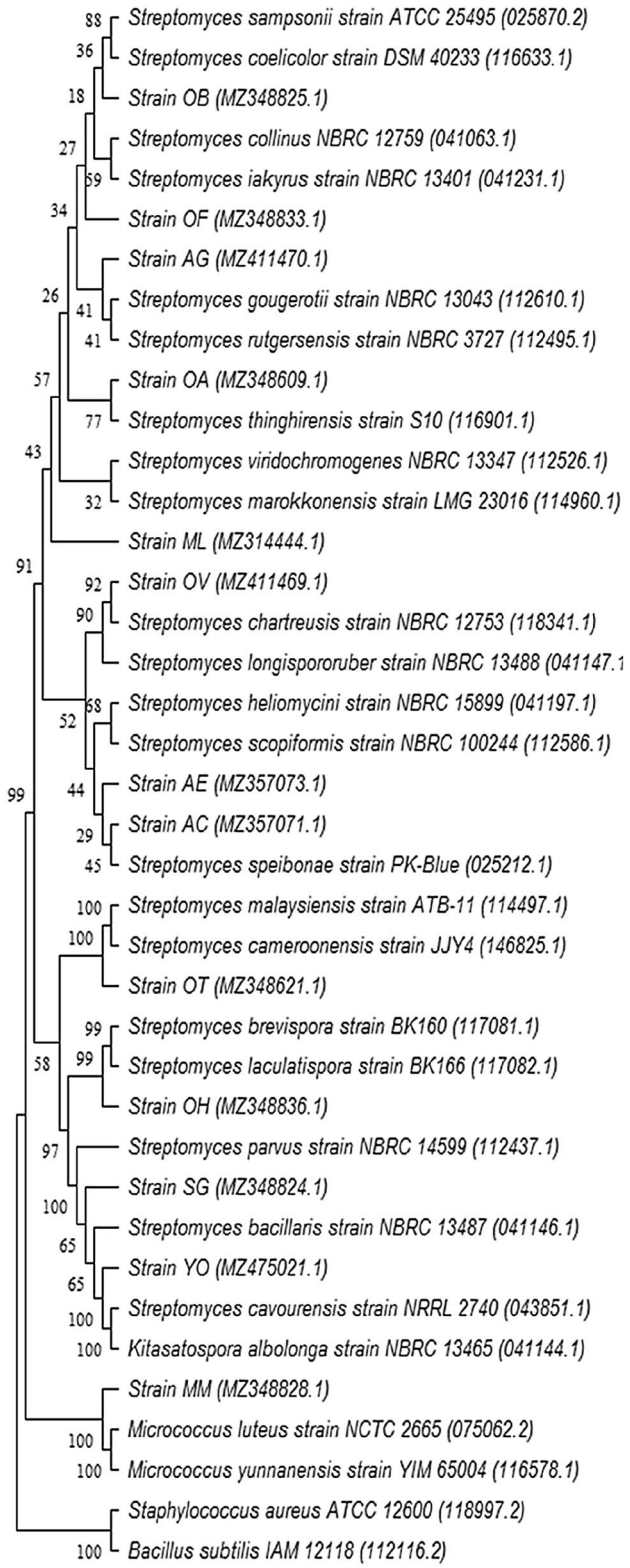

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the nearest neighbours of the isolated strains. *Bacillus subtilis* and *Staphylococcus aureus* type strains were used as outgroup. Bootstrap values (expressed as percentages generated from 1000 replicates) greater than 50% are shown at branch points. GenBank accession numbers are given in parentheses. The bar represents 0.05 substitutions per site

Biodegradation kinetics studies

Biodegradation of tiam

Based on the previous results, the *Streptomyces* strains designated as OA, OB, OH, OV and SG were able to grow on MSM containing tiam. These bacteria were tested for further characterization of their biodegradation capacities. The results presented in Fig. 2 show that in the absence of an alternative carbon substrate, strains OA, OB, OH, OV and SG presented a low degradation potential that is respectively in the order of 2, 20, 15, 11 and 19% after 30 days of incubation. The addition of glucose as a supplementary carbon source increased the degradation rate by the strain OV to 84% in 18 days, while the addition of sodium acetate increased the degradation potential of the strains OB and OH to 78 and 69%.

In the presence of co-substrate, the biomass increased proportionally with the amount of tiam degraded (Fig. 3). However, no cell growth was observed when tiam was degraded as a sole carbon source (data not shown); the same result was observed in the biotic control without any carbon source (data not shown). The cell growth of *Actinobacteria* strains is linked to the presence of an additional carbon source that was used during the degradation of tiam. Interestingly, the growth observed for the strains OB and OH with acetate and for the strain OV with glucose was much higher in the assays of tiam degradation than with the supplementary carbon sources alone (Fig. S3), revealing that the degradation of tiam is also contributing as carbon source for cell growth. In the abiotic control flasks, without bacterial inoculation, no decrease in tiam concentration was observed, revealing that the molecule did not degrade spontaneously (data not shown). This indicates that no photolytic degradation occurred under the conditions tested.

Biodegradation of 2,4-DCP

Due to its high solubility in water, high sorption potential and persistence in the aquatic environment (Gaya et al. 2010), 2,4-DCP was chosen to further investigate its degradation by three isolated strains ML, AE and AC, in liquid media. The concentration 50 mg/l of 2,4-DCP was chosen for this experiment because the three *Streptomyces* strains were able to grow at this concentration, and a higher concentration of 500 mg/l of 2,4-DCP totally inhibited growth, as revealed in agar plates experiments. This concentration is commonly used in 2,4-DCP degradation experiments (Patel and Kumar 2016; Chris Felshia et al. 2020). Figure 4 shows that the strains ML, AE and AC were able to biodegrade respectively 45%, 32%, 26% of the initial concentration of 2,4-DCP in 30 days. Taking into account the stoichiometry of the reaction, the released chloride represented 74%, 42%

Table 4 Tolerance of *Actinobacteria* isolates to the tested pesticides at different concentrations [C] (mg/l). The assimilation or not of these pesticides was assessed as being null (–), weak (–/+), moderate (+) or abundant (++) by comparing the growth of the *Actinobacteria* with that obtained on the MSM added with glucose and that obtained on the MSM without any carbon source

Pesticide	[C]	AC	AE	AG	ML	MM	OA	OB	OF	OH	OT	OV	SG	YO
2,4-DCP	50	+	+	–	+	–	–	–	–	–	–	–	–	–
	200	–	–	–	–/+	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Alphaban 20 SC	50	–	–	+	–/+	–	–	–	+	–	–	–	–	–
	200	–	–	+	–	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Diazinon	50	–	–	–	–	–	–	+	–	–	–	–	–	–
	200	–	–	–	–	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Imiguard 20% SL	50	–	–	–	–	–	–	–	+	–	–	–	+	–
	200	–	–	–	–	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Rustilan	50	–	–	–	–	–	+	–	–	–	–	–	+	–
	200	–	–	–	–	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Tiam	50	–	–	–	–	–	–/+	+	–	+	–	+	+	–
	200	–	–	–	–	–	–	–	–	–	–	–	–	–
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Aliette	50	–	+	–	+	–	+	–	–	+	–	–	+	+
	200	–	–	–	–	–	–	–	–	–	–	–	+	+
	500	–	–	–	–	–	–	–	–	–	–	–	+	+
Pelthio 70 WP	50	–	+	–	–	–	–	–	–	+	–	–	+	+
	200	–	–	–	–	–	–	–	–	–	–	–	–	+
	500	–	–	–	–	–	–	–	–	–	–	–	–	–
Teldor	50	–	–	–	+	+	–	–	–	–	–	–	–	+
	200	–	–	–	+	–	–	–	–	–	–	–	–	+
	500	–	–	–	–	–	–	–	–	–	–	–	–	+

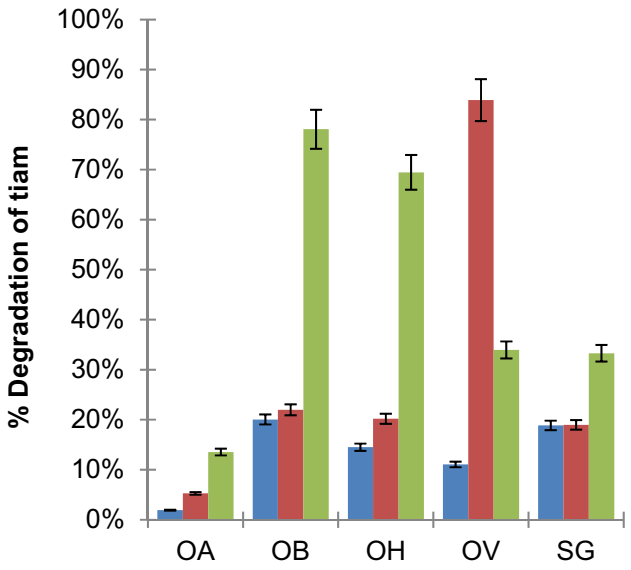

Fig. 2 Biodegradation of the commercial insecticide tiam (35 mg/l) in MSM by *Actinobacteria* strains isolated from WWTPs. (■): as sole carbon source (after 30 days of incubation), (■): in the presence of 10 mM of glucose (after 18 days), (■): in the presence of 5,9 mM of sodium acetate (after 18 days)

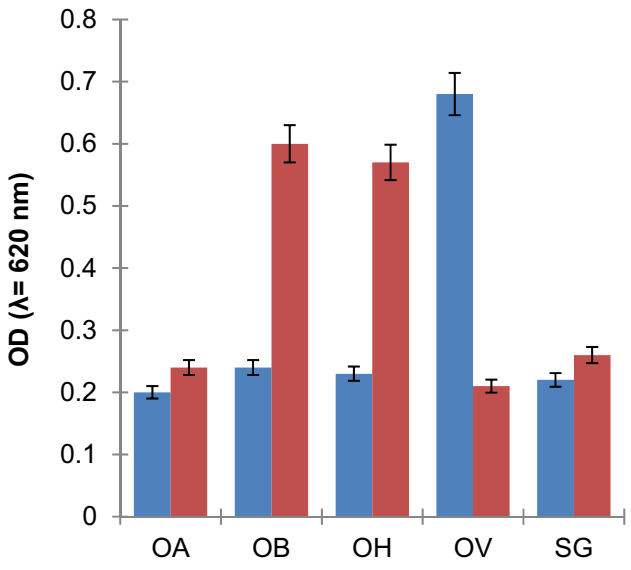

Fig. 3 Cellular growth of *Actinobacteria* strains isolated from WWTPs in MSM. (■)**:** in the presence of the commercial insecticide tiam and 10 mM of glucose (after 18 days of incubation), (■)**:** in the presence of tiam and 5,9 mM of sodium acetate (after 18 days of incubation)

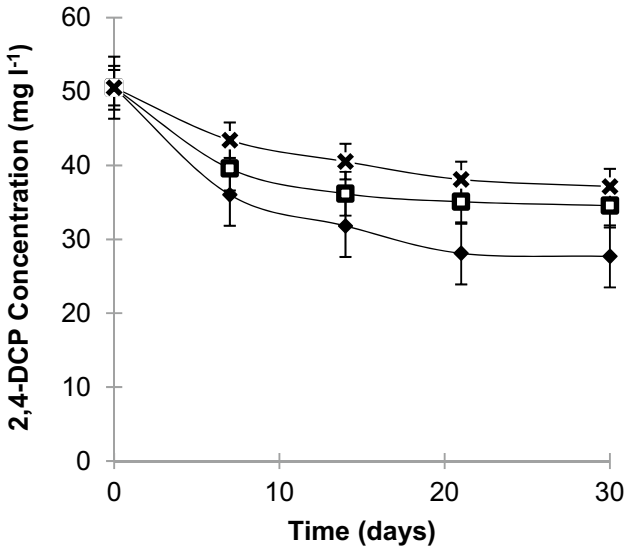

Fig. 4 Biodegradation of 50 mg/l 2,4-DCP as sole carbon source in MSM by ML (♦), AE (□) and AC (×) strains isolated from WWTPs (incubation for 30 days)

Table 5 Rate constant (k) and half-life ($t_{1/2}$) for degradation of 50 mg/l of 2,4-DCP as sole carbon source

Strain	K (d^{-1})	$t_{1/2}$ (d)
ML	0.027 ± 0.00005	25.61 ± 0.051
AE	0.017 ± 0.000005	40.75 ± 0.017
AC	0.013 ± 0.00001	53.27 ± 0.04

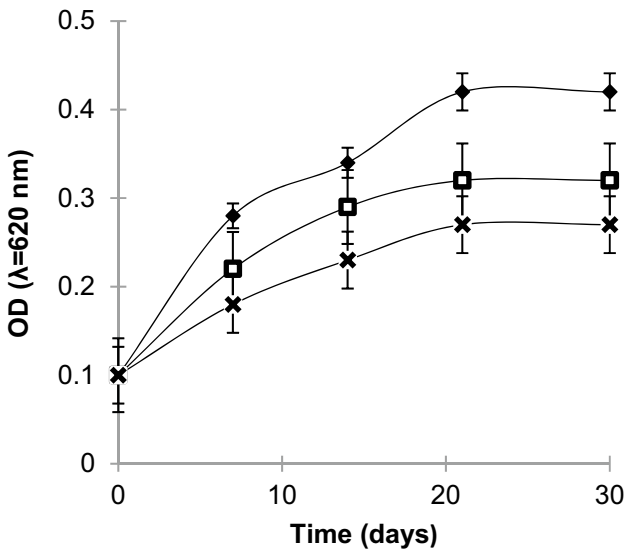

Fig. 5 Cellular growth of ML (♦), AE (□) and AC (×) strains isolated from WWTPs in MSM contain 50 mg/l of 2,4-DCP as sole source carbon (incubation for 30 days)

and 30% of the total amount of consumed substrate, respectively. The highest degradation rate of 2,4-DCP was obtained with the strain ML while the strain AC was characterized by the lowest degradation potential (Table 5). Degradation of 2,4-DCP was faster during the first 7 days (Fig. 4). The biomass increased proportionally with the degradation of 2,4-DCP and became constant when degradation stopped, indicating the use of degraded 2,4-DCP as carbon source (Fig. 5). The addition of supplementary carbon sources did not significantly improve the degradation rate of 2,4-DCP by these strains (Fig. S4 and S5, Table S5) and did not significantly improve the cell growth rate (Fig. S6 and Table S6). This indicated that the degradation was not impaired by the lack of carbon for cellular growth but probably was inhibited by the formation of toxic or non-degradable intermediary metabolites. In abiotic control flasks, no decrease in 2,4-DCP concentration or chloride release was observed, revealing that the molecule did not degrade spontaneously (data not shown), indicating that there was no abiotic degradation.

Discussion

Isolation of *Actinobacteria* from WWTPs

In this study, a range of *Actinobacteria* were isolated from three WWTPs located in the east north of Algeria, some able to degrade potent pesticides. WWTPs are decontamination systems by excellence with the aim of minimizing the risk of contamination of the receiving environment, providing a significant diversity of *Actinobacteria* metabolically adapted to the various contaminants (Hocinat 2018). In those the effluent COD values do not exceed the standards of discharge of wastewater allowed in nature in Algeria which is about 120 mg/l (JORA 2006). The results of this study are similar to previously reported (Boumediene and Abdelkader 2015; Lakhlifi et al. 2017; Olabode et al. 2020). The activated sludge from the three WWTPs carries an important organic and inorganic load which can contribute to an important biodiversity of *Actinobacteria*. Furthermore, the alkaline pH of the aeration tanks is in favor of the proliferation of *Actinobacteria* which tolerate alkaline pH (Saker 2015).

In wastewater treatment plants, *Actinobacteria* play an important role in the biological treatment process by activated sludge and contribute to the degradation of a variety of complex and recalcitrant organic compounds (El-Shatoury et al. 2004). In this study, 13 strains of *Actinobacteria* were isolated, from which 11 were isolated from the aeration basin samples. Several studies have revealed the abundance of *Actinobacteria* in activated sludge; they form flocs and play an important role in the removal of carbonaceous material and the accumulation of phosphate (Seviour et al. 2008; Agunbiade et al. 2016). A metagenomic analysis of activated

sludge indicated that *Actinobacteria* corresponded to 31% of the bacterial composition of sludge (Ibarbalz et al. 2016). *Microthrix*, *Nocardia* and *Gordonia* are the most frequently encountered genera in activated sludge (Vanysacker et al. 2014; Khairnar et al. 2014; Guo et al. 2015).

In the present study, the most dominant genus isolated through cultivation on selective media was *Streptomyces*. This result is similar to that found by Hocinat and Boudemagh (2016) at Ibn Ziad WWTP. They recovered five *Streptomyces* strains able to degrade the commercial fungicide ortiva in a total of seven isolated. Silini et al. (2016) at Oued El Athmania WWTP were only able to isolate strains from the genus *Streptomyces*. On the other hand, the isolation of *Micrococcus* in sewage treatment plants is widely reported. For example, *Micrococcus* sp. MF-1 was isolated from aminoplastic wastewater effluent capable of degrading melamine formaldehyde (El-Sayed et al. 2006); *Micrococcus yunnanensis* was isolated from pharmaceutical sludge able to degrade ibuprofen (Sharma et al. 2019). *Micrococcus luteus* AS2 isolated from industrial wastewater showed resistance against some heavy metals (Sher et al. 2020).

Ability of isolated *Actinobacteria* for biodegradation of pesticides

Most of the *Actinobacteria* isolates (12 out of 13) revealed the ability to grow in the presence of some of nine of the selected pesticides supplemented as only carbon source in agar plates. Little information is available on the interaction of microbial communities with pesticides in WWTPs systems. Although biodegradation through resistant microorganisms is the most common process for pesticide dissipation, some pesticides exert a deleterious effect on the biomass activity and metabolism of these communities (Marinozzi et al. 2013). The pesticides tested in this study are among the most commonly used pesticides in Algeria.

Physico-chemial degradation of aliette fungicide was shown to generate the formation of phosphonic acid. The degradation of phosphonic acid leads to the formation of ethanol and phosphoric (Buiarelli et al. 2018). This study showed the ability of the strains, AE, ML, OA, OH, SG and YO to use the fungicide aliette (fosetyl-al) as the sole carbon source. This is the first study reporting the isolation of bacterial strains able to degrade this pesticide. Fournier et al. (2020) studied the effect of Previcur fungicide (propamocarb (47.3%) and fosetyl-al (27.7%)) on microbial communities in mesocosms. Previcur showed no effect on the bacteria, fungi and protist diversity. The analysis of indicator and keystone soil microbial showed that the bacterial community including *Actinobacteria* was little affected by the synthetic pesticide Previcur, probably related to its rapid breakdown in soil (Fournier et al. 2020).

Streptomyces sp. YO strain showed good growth on medium containing the commercial fungicide pelthio 70 WP (thiophanate methyl), while the isolates AE, OH and SG were able to grow only at the lower concentration. Only one work reported previously the biodegradation of thiophanate methyl; the strains *Enterobacter* sp. TDS-1 and *Bacillus* sp. TDS-2 isolated from soil were able to degrade 60% and 77% of the initial concentration of thiophanate methyl (50 mg/l) during 16 days (Cycoń et al. 2011a). In relation to the commercial fungicide teldor (fenhexamid), which has excellent activity against gray mold caused by *Botrytis cinerea*, very little information exists about its degradation. In the present study, the strain named YO showed the ability to use up to 500 mg/l of teldor, while the strain *Streptomyces* sp. ML showed growth only at up to 200 mg/l and *Micrococcus* sp. MM at 50 mg/l. Previous reports showed the ability of *Bacillus megaterium,* isolated by enrichment from soil, to degrade 83% of the initial concentration of 20 mg/l fenhexamid after 46 days (Abbate et al. 2007). The microbial degradation of the two fungicides thiophanate methyl and fenhexamid by *Actinobacteria* strains was reported for the first time in this work.

It is reported that many non-target organisms are overly sensitive to the toxic effects of pyrethroids. In addition, pyrethroids insecticides may affect DNA stabilization and may cause endocrine disruption, neurotoxicity, immunosuppression and carcinogenesis (Soderlund 2012). The *Actinobacteria* strain AG showed good growth on alphaban 20 SC (alpha-cypermethrin) (50 mg/l and 200 mg/l). Recently, Gür et al. (2014) reported that the strain *Stenotrophomonas maltophilia* OG2 was able to degrade alpha-cypermethrin via an ortho-cleavage pathway. Strain OG2 converted 3-phenoxybenzaldehyde to 3-phenoxybenzoic acid and oxidized phenol to muconic acid (Gür et al. 2014). Many studies have indicated that different bacterial strains have the potency to degrade cypermethrin. Some examples include: *Streptomyces* sp.HU-S-01 (Lin et al. 2011), *Bacillus* sp. strain SG2 (Pankaj et al. 2016), *Pseudomonas aeruginosa* (Gurjar 2018) and *Bacillus thuringiensis* strain SG4 (Bhatt et al. 2020).

Organophosphate insecticides are notorious for their toxicity, even at low doses, and can cause cancer (Yair et al. 2008). The isolated strain *Streptomyces* sp. OB was able to grow in the presence of the diazinon at the concentration 50 mg/l. This result is similar to the one found by Briceño et al. (2016) who showed that *Streptomyces spp* was able to use up to 32% of the initial concentration (50 mg/l) of diazinon as its sole carbon source in 96 h. Other works have shown the ability of *Serratia marcescens* DI101 and *Stenotrophomonas maltophilia* to degrade diazinon (Abo-Amer 2011; Pourbabaee et al. 2018). Zhao et al. (2020) reported that the insecticide diazinon was degraded by cytochrome P450, which was mostly involved in phase I metabolism's

oxidation and hydrolysis. Diethyl, diazoxon, and pyrimidinol were the main diazinon metabolites.

The adverse ecological effects of neonicotinoids especially on non-target organisms such as bees have attracted attention. For the insecticide rustilan (acetamiprid), the strains OA and SG isolated from activated sludge presented good growth on this. Additionally, the isolates OF and SG were able to use imiguard 20% SL (imidacloprid) as the only carbon source. Is interesting to notice that the strain SG was able to degrade the three neonicotinoids tested. Several works reported the microbial degradation of acetamiprid and imidacloprid. *Pigmentiphaga* sp. D-2 (Yang et al. 2013), *Ochrobactrum* sp. D-12 (Wang et al. 2013), *Rhodococcus* sp. BCH2 (Phugare and Jadhav 2015), *Variovorax boronicumulans* CGMCC 4969 (Sun et al. 2017) and *Streptomyces canus* CGMCC 13,662 (Guo et al. 2019) have been shown to be effective in the degradation of acetamiprid. Zhou et al. (2014) demonstrated that the enzyme nitrile hydratase of *Ensifer meliloti* CGMCC 7333 is responsible for the biotransformation of acetamiprid to the unstable N-amidoamide metabolite, which degrades to create a chlorinated pyridyl methylmethanamine molecule. Other bacteria belonging to the genera *Ochrobactrum* (Hu 2013), *Bacillus*, *Brevibacterium*, *Pseudomonas*, *Rhizobium* (Sabourmoghaddam et al. 2015) and *Mycobacterium* (Kandil et al. 2015) were capable of breaking down imidacloprid. The isolated strains OA, OB, OH, OV and SG were able to grow in the presence of tiam (thiamethoxam).

In addition to these adverse effects on bees like other neonicotinoid insecticides, thiamethoxam is considered an endocrine disruptor and is also hepatotoxic (Swenson and Casida 2013; Baines et al. 2017). Thiamethoxam has low volatility and low adsorption to soil. However, due to its high water solubility, it is considered a possible pollutant of surface waters. Thiamethoxam (225 µg/l) was detected in fresh water (Anderson et al. 2013). Therefore, it was included in the European monitoring list of emerging water pollutants (Directive 2000/60/EC). However, studies carried on its degradation remains limited. *Streptomyces* sp. OV was able to degrade 84% of the initial tiam concentration (35 mg/l) in the presence of 10 mM glucose after 18 days of incubation. The strains *Streptomyces* sp. OB and OH degrade respectively 78% and 69% of amount of tiam in presence of sodium acetate. This result is similar to that found by Pandey et al. (2009); they found that *Pseudomonas* sp. G1 isolated from soil was unable to degrade thiamethoxam as a sole carbon source, while in the presence of 10 mM glucose, 70% of initial concentration of thiamethoxam (50 mg/l) was removed during 14 days. This strain may transform the magic-nitro group (=N-NO2) of neonicotinoids imidacloprid and thiamethoxam to nitrosoguidine (IMI-I) by aldehyde oxidase enzyme activity. The strain *Pseudomonas* sp. 1G was found to convert both nitroso guanidine and the parent molecule to urea metabolites (IMI-IV) via desnitro/guanidine metabolites (IMI-III) (Pandey et al. 2009). The availability of additional carbon source increases the amount of viable cell and microbial activity (Ortíz et al. 2013). Several studies show that the degradation of pesticides is more efficient in the presence of a co-substrate and the cometabolism is a principal mechanism for the degradation of pesticides (Huang et al. 2018). For example, Zhao et al. (2009) reported that the degradation of the insecticide thiacloprid by *Stenotrophomonas maltophilia* CGMCC 1.178 improved tenfold in the presence of sucrose as carbon source. Furthermore, Anwar et al. (2009) reported that the pesticide chlorpyrifos was completely degraded by *Bacillus pumilus* C2A1 after 3 days in the presence of glucose. On the other hand, the addition of sodium acetate just enhances marginally the removal of tiam by the strains OA and SG (15% and 33%). This result was similar to that obtained by Gangireddygari et al. (2017); they found that the addition of the carbon source enhance slightly the degradation of quinalphos insecticide by *Bacillus thuringiensis*.

To our knowledge, there are no reports in the literature of *Actinobacteria* strains capable of degrading thiamethoxam. In general, microbial degradation of thiamethoxam has been less reported and limited to only a few microorganisms. Zhou et al. (2013) reported that *Ensifer adhaerens* strain TMX-23 isolated from the rhizosphere degraded 21,6% of the 200 mg/l of thiamethoxam as a single carbon and nitrogen source after five days. *Bacillus aeromonas* strain IMBL 4.1 and *Pseudomonas putida* strain IMBL 5.2 degraded 45.28 and 38.23% of thiamethoxam (50 mg/l) as a sole carbon source in 15 days of incubation, respectively (Rana et al. 2015). A new study showed that the white rot fungus *Phanerochaete chrysosporium* degraded 49% and 98% of 10 mg/l thiamethoxam after 15 days and 25 days of incubation, respectively (Chen et al. 2021).

In relation to 2,4-DCP, this compound can be derived from the transformation of the herbicide 2,4-dichloro-phenoxy-acetic acid (2,4-D) (Pascal-Lorber et al. 2012). In several countries, environmental regulations stipulate that the maximum allowable concentration of phenols in industrial effluents should be less than 1 mg/l. Higher concentrations of chlorophenols have been commonly found in polluted water areas with levels from 0.15 mg/l to 200 mg/l (Angelini et al. 2011; Kusic et al. 2011). It's considered as ubiquitous xenobiotic in wastewater which required their elimination from wastewater before discharge into the environment (Quan et al. 2003).

Streptomyces sp. ML was able to degrade 45% of the supplied amount of 2,4-DCP (50 mg/l), with release of 74% of the stoichiometric chloride, as the sole carbon source within 30 days. It is known that biodegradation can be impaired by the generation of toxic intermediates (Gkorezis et al. 2016). This is confirmed in this study when the addition

of carbon source does not improve degradation and cell growth. Megharaj et al. (2014) reported that vinyl chlorine from trichloroethylene biodegradation is very toxic intermediate and inhibits the degradation of the parent compound. Biodegradation of endosulfan, a chlorinated insecticide, is usually accompanied by the formation of endosulfan sulfate, a more toxic and persistent metabolite (Kwon et al. 2005).

It has been reported that several mesophilic microorganisms are able to degrade 2,4-DCP and the bacterial metabolic pathways for 2,4-dichlorophenol degradation can occur via ortho-cleavage or the distal meta-cleavage (Arora and Bae 2014). According to Gallizia et al. (2003), microorganisms use non-halogenated phenolics more readily than chlorinated phenol because chlorine atoms make aromatic compounds less accessible to microorganisms. This is the case for *Micrococcus* sp. isolated from activated sludge, that degraded phenol up to 500 mg/l in 50 h, while 2,4-DCP revealed to be more recalcitrant. *Micrococcus* sp. removed 883 mg/g and 230 mg/g of 2,4-DCP in 10 days using initial concentrations of 100 and 200 mg/l (Gallizia et al. 2003). However, the authors verified a considerable amount of abiotic degradation on non-inoculated control flasks (330 mg/g), which may have contributed to the observed 2,4-DCP decrease. Additionally, were detected metabolites with retention time corresponding to dichlorochatecols, indicating that the degraded 2,4-DCP was not fully mineralized. Korobov et al. (2017) found that *Rhodococcus erythropolis* 17S, which was isolated from soil contaminated with phenol and its derivatives, could degrade phenol and 2,4-DCP (100 mg/l) as the only carbon source. The phenol content of the culture decreased by 55% on the fourth day, while for 2,4-DCP, 53% degradation was observed after 22 days of incubation. The authors did not evaluate chlorine removal or metabolites formation (Korobov et al. 2017). In another study, a *Bacillus* consortium was able to mineralize up to 85% of the initial concentration of 2,4-DCP (400 mg/l) during 21 days with 4,7 mM of chloride release (Herrera et al. 2008). A study conducted by Al-Khalid and El-Naas (2017) reported the removal of 2,4-DCP by a commercial strain of *Pseudomonas putida* pre-adapted to 2,4-DCP immobilized in a PVA gel matrix. It was showed that the immobilized cells degraded an initial concentration of 70.5 mg/l of 2,4-DCP with a degradation rate of 40.1 mg/l/h. In this study dechlorination was not evaluated.

Actinobacteria strains are participate fully in the activity of the microbial community in WWTP in a significant way (Polti et al. 2014). The genus *Streptomyces* is the largest part of the *Actinobacteria*, widely distributed in natural environments. The versatility of *Streptomyces* species has received considerable attention as a promising biotechnological solution for the remediation of contaminated environments (Alvarez et al. 2017). In this study 11 strains of *Streptomyces* have confirmed the ability of the genera *Streptomyces* to degrade several classes of pesticides. For the best of our knowledge, this is the first study reporting the isolation of bacterial strains able to degrade aliette.

Conclusions

Actinobacteria strains isolated from WWTPs, 11 of which belonged to the *Streptomyces* and one to *Micrococcus* genus, can grow in the presence of pesticides 2,4-DCP, aliette, alphaban 10 SC, diazinon, imiguard 20% SL, pelthio 70 WP, rustilan, teldor and tiam. Of the isolated strains, two showed a broad range of pesticides degradation potential. Namely, *Streptomyces* sp. SG was able to degrade the three neonicotinoids tested, tiam, imiguard 20% SL and rustilan, and the two fungicides aliette and pelthio 70 WP. *Streptomyces* sp. ML, the best 2,4-DCP degrading strain, also showed potential to degrade alphaban 10, aliette and teldor. The biodegradation potential of these strains indicates that they could be used par excellence in the bioremediation of ecosystems polluted by these pesticides.

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Author contributions All authors contributed to the study conception and design. Isolation and characterization of bacterial strains and biodegradation experiments were performed by Oumeima Boufercha. Deposit of bacterial strains was performed by Irina S. Moreira. The first draft of the manuscript was written by Oumeima Boufercha and Irina S. Moreira, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The 16S rRNA gene sequences of *Actinobacteria* isolated were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/).

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publication Not applicable.

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