INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 5, p. 45-56, 2016

RESEARCH PAPER OPEN ACCESS

Antifungal activity of *Streptomyces* sp.14 strain isolated from Ouargla (Southeast of Algeria): identification, production and characterization of the active substance

Sabrina Bouaziz[*1,2], Abdelaziz Messis[2], Azzeddine Bettache[2], Mohammed Didi Oueld El Hadj[1], Et Said Benallaoua[2]

[1]*Department of Biological Science, Faculty of Science of Nature and Life, Laoboratory of Protection of Ecosystems in Arid and Semi-arid Zone, University Kasdi Merbah Ouargla, Ouargla, Algeria*

[2]*Faculty of Science of Nature and Life, Laboratory of Applied Microbiology, University of Bejaia, Bejaia, Algeria*

Key words: Antifungal activity, Sahara soil, *Streptomyces*, Production kinetics

http://dx.doi.org/10.12692/ijb/9.5.45-56 Article published on November 21, 2016

Abstract

This work aims to study the antifungal activity of strain encoded I4. The strain was isolated from Saharan soil of Ouargla region (Southeast of Algeria). The identification of the isolate I4 was carried out on the basis of morphological, biochemical and physiological criteria whose taxonomy suggested that this isolate belonged to *Streptomyces* genus. The antifungal activity by the agar cylinder method on solid medium revealed that isolate I4 showed strong activity against various pathogenic fungi as well as gram-positive and gram-negative bacteria, hence the best activity observed against *Aspergillus niger*2CA936 with an inhibition zone of 30 mm in diameter. The production kinetics of the antibiotic ware made on M2 liquid medium. The optimal activity was achieved at the end of exponential growth phase and beginning of decline phase, in the fifth day of incubation. The antibiotics secreted by the strain I4 were hydrophobic, and more extractable by apolar solvents, it were revealed by bioautography and chemical development. The result obtained showed the presence of a single active zone, chemical developers suggested that the active molecule contains carbohydrate residues not of polyenic nature.

* **Corresponding Author:** Sabrina Bouaziz ✉ bz.sabrine@yahoo.fr

Introduction

During the last decade, mycoses have increased severely and are classified in fourth position in nosocomial infections (Beck-Sagué and Jarvis, 1993). Serious diseases affecting humans, caused by several species of fungi and yeasts are enumerated, among which include mycotoxicoses caused by genus *Fusarium* and *Aspergillus*(Drouhet, 1978), which the last are responsible of most invasive fungal infections and nearly 90% of human mycoses, namely *A. fumigatus, A. flavus, A. niger, A. nidulans and A. terreus*(Carle, 2003 ; Couturaud, 2004).Other pathologies namely mycoallergies caused by genus *Penicillium* and *Mucor* and superficial or deep fungal infections caused by *Candida albicans*, *Microsporum*, *Trichophyton*(Drouhet, 1978).

In the early 20th century, it was discovered and developed molecules intended to fight against parasitic fungi of humans and animals, such as potassium iodideused orally in the treatment of sporotrichosis, heavy metal salts, metalloids, sulfur and phenolic derivatives, dyes ... to treat certain superficial mycoses (Hamoir *et al.*, 2001). Currently the studies are oriented towards the research and the use of biomolecules obtained from microorganisms which have antimicrobial activities.

According to Berdy *et al.*(1987), antifungals represent nearly 40% of the antibiotics synthesized by all microorganisms, from which the antibacterial range is much more important than those of antifungals (Di Domenico, 1999), more than half of these antifungals has also antibacterial activities (Berdy *et al.*, 1987). These molecules are mainly synthesized by Actinobacteria or fungi (Breton *et al.*, 1989). Antifungals available currently in therapy do not gather the ideal antibiotic criteria (Lacroix *et al.*, 2003). That is why it is indispensable to be oriented towards research of new non-toxic antifungals.

Actinobacteria represents a useful biological source of antimicrobials against fungi and pathogenic bacteria, and about 70% of active molecules of microbial origin, particularly antibiotics, are produced by this group of microorganisms (Okami and Hotta, 2007). Among the methods used to increase the probability of obtaining new antibiotics of actino-bacterial origin, there is the exploitation of extreme environments (arid and saline soils, etc.) and the least exploited habitats and also aim actinobacteria so-called 'Rare' (Boudjella *et al.,* 2007).

The objective was to study the antifungal activity, identification of a actinobacterium strain, follow up growth and production kinetics as well as a test of active substance characterization.

Materials and methods

Sampling

The isolation of the strain was carried out from arid soils of Ouargla region, southeastern Algeria from palm grove of the Faculty of Natural and Life Sciences at Kasdi Merbah University. Soil samples were collected according to the technique of Pochon and Tardieux (Pochon and Tardieux, 1962). The sample was taken in rigorous aseptic conditions. Transferring samples to the laboratory was carried out at room temperature.

Physico-chemical analyses of soil

The physicochemical analyses of soil are performed according to the method of Aubert(1978), Table 1.

Technical isolation

An actinobacterium strain labeled I4 was isolated from a Saharan soil sample of Ouargla region (latitude: 31°56'57" North, longitude: 5°19'30" East and altitude relative to sea level: 138 m). The isolation was carried out by the method of suspensions-dilutions.Ten gramme of soil sample was diluted in 90 mL of water containing 9 g/l NaCl and vortexed for 10 min at room temperature. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were prepared. 100 µL of 10^{-3}, 10^{-4} and 10^{-5} dilutions were spread in triplicates on the surface of five different media. The media used were: M2 (Williams and Kuster 10 $g.L^{-1}$ starch 0.3 $g.L^{-1}$ casein, 2 $g.L^{-1}$ KNO_3, 2 $g.L^{-1}$ NaCl, 2 $g.L^{-1}$ K_2HPO_4, 0.05 $g.L^{-1}$ $MgSO_4.7H_2O$, 0.02 $g.L^{-1}$ $CaCO_3$, 0.01 $g.L^{-1}$ $FeSO_4.7H_2O$, 1 $g.L^{-1}$ glucose, 15 $g.L^{-1}$ agar, pH 7.2. 75µg.mL-1 Nystatin were added to the media to inhibit the development of filamentous fungi. The plates were incubated at 28 °C for 2-4 weeks.

Purification and conservation of strain

The isolated strain of Actinobacteria was purified, then kept and stored at 4°C on the same isolation medium.

Antimicrobial activity on solid medium

The antimicrobial activity of the purified actinobacterium isolate (I4) was tested against several pathogenic and phytopathogenic microorganisms according to the method of agar cylinders (Patel and Brown, 1969).Many germs were used, including eight filamentous fungi: *Aspergillus carbonarius* A731C,*Aspergillus niger* 2CA936, *Aspergillus favus* NRRL, *Aspergillus ochraceus* NRRL 3174, *Aspergillus flavus* AFB1, *Aspergillus parasiticus* CB5, *Fusarium polyferatum and Mucor rammanianus* NRRL 1829. Two gram positive bacteria: *Staphylococcus aureus* ATCC 25923 and *Bacillussubtilis* ATCC 6633, And Two Gram negative bacteria: *Proteus mirabilis* ATCC 13047 and *Pseudomonas aeruginosa* ATCC 27853. The Two dishes were kept at 4°C for 2 hours for the diffusion of antimicrobial substances. The inhibition zones were measured (in mm) after 48 h of incubation at 28°C for fungi and after 24 h of incubation at 37°C for bacteria. The test was performed in triplicate.

Identification of the actinobactéria strain

Cultural and morphological characteristics

The morphological characteristics were determined on the culture media recommended by Shirling and Gottlieb (1966): ISP1 (tryptone-yeast extract-agar), ISP2 (yeast extract-malt extract-glucose-agar), ISP3 (oatmeal-agar) and ISP4 (starch-inorganic salts-agar), ISP5 (Glycerol-asparagine-agar), ISP6 (Peptone-yeast extract-ferric salt-agar) and ISP7 (Tyrosine-agar) as well as ISP9.

Determination of cell components

Chemical analysis of cell constituents was carried out by the determination of the isomer of diaminopimelic acid (LL orDL form) and the presence of glycine by themethod ofBecker *etal*. (1964).

The identification of cellular sugars and parietal mycolic acid was performed according to the methods described by Lechevalier and Lechevalier(1970).

Physiological and biochemicalcharacteristics

Biochemical characterization was based on production of melanoid pigments, the use of different carbon sources: glucose, arabinose, fructose, cellibiose, galactose, lactose, maltose, mannitol, ribose, rhamnose, xylose and mannose (Shirling and Gottlieb, 1966), the use of different fatty acid sources: acetate, citrate, lactate, benzoate, oxalate, pyruvate and sodium succinate, degradation of different organic compounds namely tyrosine, milk casein (Gordon *et al*., 1974), starch, tween 80, the catalase production (Marchal *et al*., 1987) and the use of amino acids as the only nitrogen source: arginine, histidine and lysine (Gordon *et al*., 1974). The strain was examined for its ability to grow at different temperatures (10°C to 45°C), at different pH (5 to 10) and different NaCl concentrations (5% to 15%) (Athalye *et al*., 1985).

Kinetics of growth and antibiotic production

The kinetics of the antimicrobial activity of the isolate I4 was conducted in M2 liquid medium. Pre-cultures of three days were first prepared in tubes from mycelial fragments scraped from Petri dishes then incubated in ten days. Based on these sloping cultures of selected strains we added 2 ml of sterile distilled water and scrap the medium surface with a sterile pipette. One to three ml of the suspension was used to inoculate Erlenmeyer flask of 500 ml containing 100 ml of liquid medium. Incubation was carried out in a shaking incubator at 28°C for 10 days at 240 rpm. The development of antimicrobial activity was determined and monitored daily for 10 days by the well diffusion method (150 ml of culture filtrate per well of 6 mm in diameter) against *Aspergillus niger*2CA936 and *Aspergillus ochraceus* NRRL 3174. The evolution of pH and dry weight of mycelium was evaluated by the method of Pfefferle *etal*. (2000).

Selection of extraction solvent and antimicrobial activity of organic extract

The extraction of the active substance from the culture filtrate was carried out after shaking culture of 240 rpm at 28°C on M2 liquid medium, the culture filtrate was recovered by centrifugation. Three solvents were used: n-hexane, dichloromethane and ethyl acetate. To determine the best solvent (Valanarasu, 2009) of extraction, a volume of culture filtrate was extracted with an equal volume of each solvent. The organic extracts and the remaining aqueous phases were concentrated to dryness and recovered in 0.5 ml of methanol and 0.5 ml of distilled water respectively, to test their activity by the paper disk method (disks of 6 mm in diameter) against *Aspergillus niger* 2CA936 and *Aspergillus ochraceus* NRRL 3174. The reading of the results was carried out by measuring the diameter of the inhibition zones around wells after 48 h of incubation at 28 °C.

Characterization tests ofactive substance (s)

Bioautography

Twenty five microliters of a sample from the best production medium extracted with best solvent were deposited on the silica gel plate.

Solvent systems used for migration are: B.A.W: N-butanol-Acetic acid-Water (6 :2 :2, v/v/v) and B.A.W.M (N-butanol-acetic acid-Water, Modify (6 :1,5 :2, v/v/v), A.M: ethyl acetate-methanol (100 :15, v/v) and E.A.W: ethanol-ammonia-water (40 :30 :30, v/v/v) A.W: acetonitrile-water (50 :50, v/v). After development, the plates were covered with Saboraud medium containing 8 g/l of agar, previously inoculated with the target germ *Aspergillus niger*2CA936. The reading of the results consists in detecting zones of inhibition of target germs and calculating their front ratio (FR).

Chemical revelations

The chemical revelations were performed in parallel with bioautographies on chromatograms developed in the same conditions. The chromatograms were observed with the naked eye and under ultraviolet light (245 nm), as well as other developers were used (Lee and Hwang, 2002) namely: aniline-diphenylamine, ninhydrin, ferric iron chloride ($FeCl_3$) and the formaldehyde-H_2SO_4.

Results and discussion

This study was conducted to highlight the presence of new strains of actinobacteria with antimicrobial activity in Ouargla region (Algerian Sahara).

Table 1. Physico-chemical and Biochemical Analyzes of Soil Samples.

Physico-chemical and biochemical parameters	Methods used
pH	Potentiometric method (Aubert, 1978)
Electrical conductivity	Conductimetric method (Aubert, 1978)
Texture	Granulometric method (Aubert, 1978)
Total limestone	Calcimeter of Bernard (Aubert, 1978)
Humidity	Water content (Aubert, 1978)
Total carbon	Anne's method (Aubert, 1978)
N (%)	Kjeldahl's method (Aubert, 1978)
Organic matter (O. M.)	Anne's method (Aubert, 1978)
Ca^{+2} from extract 1/5	Complexometry (Aubert, 1978)
Na^{+}	Flame spectrophotometer (Aubert, 1978)
K^{+}	Flame spectrophotometer (Aubert, 1978)
Mg^{+2}	Complexometry (Aubert, 1978)
Cl^{-}	Argentometric method of Mohr (Aubert, 1978)
SO_4^{-2}	Gravimetric method (Aubert, 1978)
HCO_3^{-}	Titrimetry with H_2SO_4(Aubert, 1978)

The soil samples studied come from palm grove in the Faculty of Biological Sciences (designated Pal) at Kasdi Merbah University, Ouargla.

The physicochemical analysis of the soil revealed variable results of parameters, Table 2.

Table 2. Physico-chemical analysis of palm grove soil.

Physico-chemical parameters	Pal
pH	7,76
Electrical conductivity EC. at 25°C (dS/m)	14,30
Texture (%)	Fine Sandy
Total limestone (%)	12,04
Humidity (%)	12,34
C.O (%)	1,3
N (%)	0,098
M.O (%)	2,23
Ca^{+2} from extract 1/5	0,725
Na^{+}	0,6
K^{+}	0,56
Mg^{+2}	9,75
Cl^{-}	2
SO_4^{-2}	0,202
HCO_3^{-}	1,2

The Palm grove soil is slightly alkaline (pH of 7.76), unsalted (C.E. 14.30 ms/cm), it has a fine sandy texture, with limestone rate (12.04%) and low humidity (12.34%) as well as a total carbon content of 1.3% and a low nitrogen content (0.098%).

It is the case of soil moderately rich in organic matter (2.23%), and which contains minerals such as Ca^{+2} (0,725C mol/kg), Na^{+} (0.6 cmol / kg), K^{+} 0.56 cmol/Kg, and Mg^{+} 2 9.75 cmol/Kg, Cl^{-2} (meq / L) of SO_4^{-2} 0.202 (meq/L) and HCO_3^{-} with 1.2 (meq/L).

Table 3. Antimicrobial activity of the strain I4 (mm).

Target microorganismes			Test strain I4 (mm)
Fungi		*Aspergillus carbonarius* A731C	14
		Aspergillus niger 2CA936	30
		Aspergillus flavus NRRL	08
		Aspergillus ochraceus NRRL 3174	21
		Aspergillus flavus AFB1	00
		Aspergillus parasiticus (CB5)	13
		Fusarium polyferatum	18
		Mucor romaninus NRRL 1829	14
Bacteria	Gram positive	*Bacillus subtilis* ATCC 6633	18
		Staphylococcus aureus ATCC 25923	15
	Gram negative	*Pseudomonas aeruginosa* ATCC 27853	10
		Proteus mirabilis ATCC 49452	12

The composition of the soil, in particular limestone rate, strongly influences the quantitative and qualitative distribution of microorganisms especially actinobacteria (Sabaou *et al.*, 1998). The distribution of actinobacteria in the soil depends essentially on several factors, such as the availability of nutrients, temperature, humidity, soil type, sampling season and climate (Oskay, 2009).

Lee and Hwang(2002) reported the influence of humidity on the diversity of actinobacteria. They showed the presence of the genera *Micromonospora*, *Dactylosporangium* and *Streptosporangium* in soils where humidity varies between 2,0% to 9,0%, and in particular the presence of *Actinomadura* and *Nocardioform* in soils where humidity varies from 13,1 to 20,0%.

Table 4. Analysis of cell constituents of the isolate I4, amino acids and sugars by ascending thin layer chromatography.

cell constituents	Developer	Spots color	front report (RF)	compounds cell Identification
Amino acids	Ninhydrine	S_1 : purple spot	Rf= 0,65	Acide LL-diaminopimélique
		S_2 : pink purple spot	Rf=0,82	Glycine
sugars	Diphenylamine-aniline	S_1 : blue spot	Rf= 0,15	ND
		S_2 : blue spot	Rf= 0,19	ND

ND : Not determined.

Antimicrobial activity on solid medium

The table 3 reported theantimicrobial activity of the I4 strain. The strain has a significant activity against fungi and bacteria. Indeed, activity was high against*Aspergillus niger* 2CA936and *Aspergillus ochraceus* ATCC NRRL 3174 with inhibition zones of 30 and 21 mm in diameter respectivly. And a mean activity against *Fusarium polyferatum,Aspergillus carbonarius* A731C, *Mucor romaninus* NRRL 1829 and *Aspergillus parasiticus* (CB5) with 18 mm, 14 mm and 13 mm respectively. A low activity was observed against *Aspergillus flavus* NRRL with 08 mm of inhibition zones diameter. No activity was observed against *Aspergillus flavus* AFB1. The isolate I4 has activity against a Gram positive and Gram negative bacteria. In the literature was described a broad spectrum of antibacterial action and antifungalof *Streptomyces* isolates (Thakur *et al.*, 2007; Valanarasu, 2009; Duraipandiyan *et al.*, 2010). Some were even active against fungi pathogenic for human and multi-resistant to antibiotics (Kumar and Kannabiran, 2010), or against insect larvae (Valanarasu, 2010).

Sabaou *et al.*(1998) showed that 11.18% of all actinobacteria isolated from the Algerian Sahara have an antifungal activity. The work of Hilali*et al.*(2002) have also showed during an initial screening of 85 actinobacteria strains isolated from several environmental media (soil, water and marine sediments) that only 18 have an antifungal activity against *Fusarium graminearum* and *Fusarium culmorum*. In Algeria, several works on actinobacterium were able to isolate the *Streptomyces* strains that have important antibacterial and especially antifungal activities against several pathogenic microorganisms (Boughachiche *et al.*, 2005; Badji *et al.* 2005; Aouiche *et al.*, 2012 and Toumatia *et al.*, 2014).

Identification of actinobacteria strain studied

Cultural and morphological characteristics

The isolate I4 has a better growth on the media ISP2, ISP3 and ISP6, on average ISP4, ISP5, low growth was noted on ISP1, ISP7 and ISP9 media. Sporulation is good on the medium ISP2 and ISP3, low and absent on ISP1, ISP6, ISP7 and ISP9.Aerial mycelium is grey. The substrate mycelium was brown-reddish.

Table 5. Physiological characteristics of the strain I4.

Properties	I4
Catalase Production	+
Productionof Melanin Pigment	-
Degradation of:	
Starch	+
Milk casein	-
Tyrosine	-
Tween 80	+
Use of Sugars:	
glucose	+
arabinose	-
fructose	+
cellobiose	+
galactose	+
lactose	+
maltose	+
mannitol	+
ribose	-
rhamnose	+
xylose	+
mannose	+
Use of Fatty Acids:	
Sodium citrate	+
Sodium benzoate	-
Sodium acetate	+
Sodium pyruvate	+
Sodium oxalate	+
Sodium lactate	+
Growth in the presence of NaCl:	
5%	+
8%	+
11%	+
15%	+
Growth at T °C :	
10°C	+
15°C	+
20°C	+
25°C	+
35°C	+
40°C	-
45°C	-
50°C	-
Growth at pH :	
5	+
6	+
7	+
8	+
9	+
10	+
Use of Amino Acids :	
Lysine	+
Histidine	+
Arginine	+

No diffusible pigment is secreted by the strain I4.The isolate I4 has a subtracte mycelium very dense and thick substrate, it is composed of unfragmented ramified filaments, headed by aerial mycelium producing chains of spiral spores.

Chemo-taxonomic analysis

The isolate I4 contains in cell wall the LL-isomer of the diamino-pimelic acid as well as the presence of glysine in their cell wall and the absence of glycine. Abcence of the specific sugars in the hydrolyzate. The morphological and chemical results allow linking the isolate I4 to the genus *Streptomyces* according Lechevalier and Lechevalier (1970) (Table 4).

Physiological characteristics

The results of the physiological characteristics were enumerated in Table 5.

Strain I4 can degrade a wide variety of organic compounds such as starch, Tween 80, citrate, acetate, pyruvate, oxalate and lactate sodium, but it does not degrade the milk casein, tyrosine and sodium benzoate. She is able to use most of the carbohydrate compounds as sole carbon source except for arabinose and ribose. She growth on an optimal temperature of 28 ° C and pH 7, but she can not growth in temperatures above 35 °C. The strain I4 may growth in different NaCl concentration of 5% to 15%, as it uses lysine, histidine and arginine as the sole nitrogen source.

Kinetics of growth and antibiotic production

The kinetics of growth, antibiotic production and the pH evolution, made on M2 medium, were illustrated in Fig. 01. Growth kinetics of the strain I4 revealed rapidly growth reaching 1,9 g/l. During the first four days. The biomass growth Gradually, until it reaches its maximum on the fifth day with 2,86 g/l, then it decreased to 1,43 g/l and continues to declined until the end of the incubation.

The antifungal activity of the strain I4 against Aspergillus Niger 2CA936 and Aspergillus ochraceus ATCC NRRL 3174 was detected by the method of wells. The production of antimicrobial metabolites appeared from the fourth day of incubation.

It was maximal at the fifth day for the both strains with inhibition zones of 22 mm and 15.5 mm in diameter, compared to Aspergillus *Niger* and *Aspergillus ochraceus* respectively. The highest production of antibiotics by strain I4 occurs during the stationary phases, as in the case of the majority of microorganisms. During the incubation, it was reported an initial pH of 7.2, decreasing to 6.8 in the second day of incubation, increasing to 7 and 8.2 in the fifth and sixth day respectively, then it decreases to 6.2 at the end of incubation (Fig. 1.).

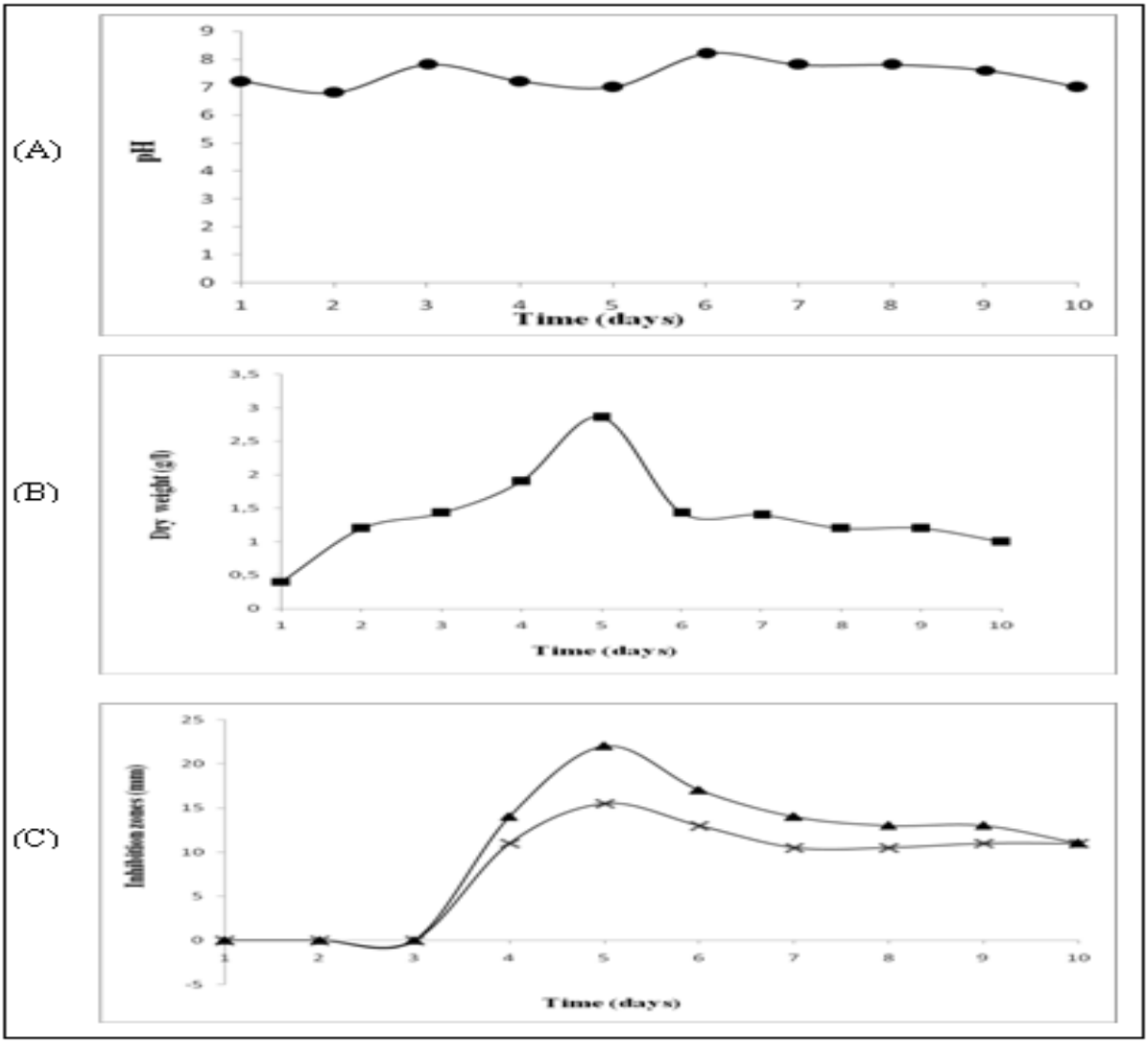

Fig. 1. Kinetics of pH evolution (A : filled cercle), growth (B : filled square) and antifungal activity of the strain I4 on the M2 medium (C: *Aspergillus Niger* 2CA936 (filled triangle)and *Aspergillus ochraceus* ATCC NRRL 3174 (cross).

Selection of the extraction solvent and antimicrobial activity of the organic extract

The activity was found better in the organic phase with hexanoic extract, against both strains of *Aspergillus Niger* and *Aspergillus ochraceus* with inhibition diameters of 27 mm and 12 mm respectively (Fig. 2.), the activity was absent in the aqueous phase and presented at mycelium. This demonstrates that the active compound secreted by the strain I4 was hydrophobic, and more extractable by apolar solvents.

Characterization tests of active substance(s)

Bioautography

The analytical thin layer chromatography of hexanoic extract, performed by different systems, allowed to

show that the system B.A.W.M. (N-butanol-Acetic acid-Water, 6: 1.5: 2, v / v / v, Modify) was the best from the point of separation (Fig. 3), compared to other systems: B.A.W, A.M, E.A.W and A.N.E. The B.A.W.M system allows to highlight eight spots, this system is adopted for the bioautography. The microbiological revelation of hexanoic extract of B.A.W.M system, allowed to highlight a single active spot against *Aspergillus niger*2CA936 noted T4, with Rf of 0.35. The bioautography of hexanoic extract revealed a single active spot which suggested the presence of a single antibiotic or a complex of antibiotics with chemical structure very similar and difficult to separate by thin layer chromatography.

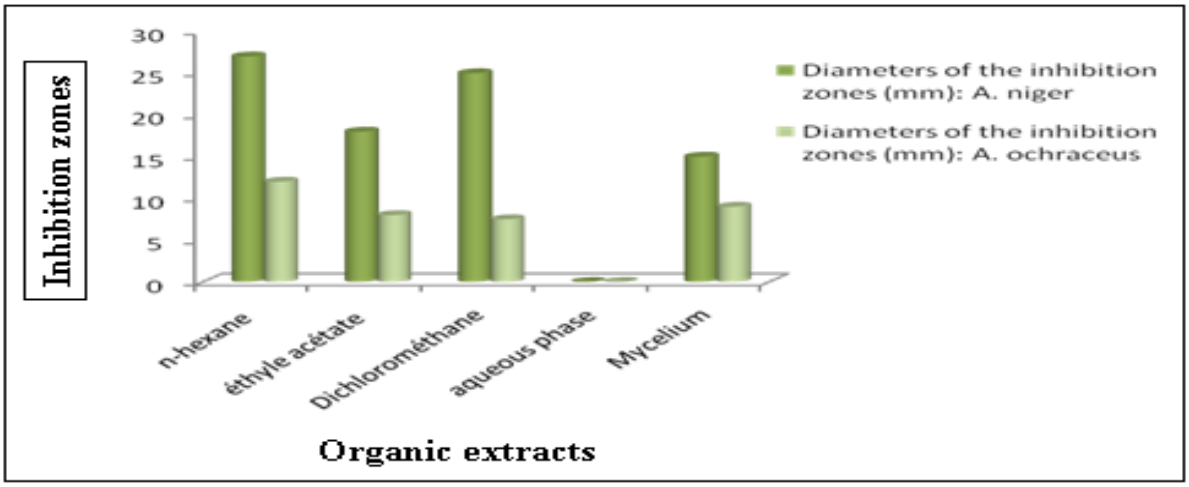

Fig. 2. Diameters of the inhibition zones of organic extracts and mycelium of the strain I4 relative to *Aspergillus Niger* 2CA936and *Aspergillus ochraceus* ATCC NRRL 3174.

Chemical revelations

Chromogenic tests have been done in parallel to bioautographies for the chemical development and this in the same previous conditions. The active substance T4 reacts positively with diphenylamine-aniline (Fig. 3.) and negatively with ferric chloride (FeCl3), ninhydrin and formaldehyde H_2SO_4. This implies that the product contains carbohydrate residues in its structure while hydroxamic acids, amines and polycyclic aromatic residues were absent. Many antibiotics varied by their chemical structure; aminosides, aromatics, polyenes, nucleoside antibiotics, their antibacterial, antifungal or antiviral activity, were secreted by the actinomycetes including the genus *Streptomyces* (Boudjella *et al.*, 2007; Duraipandiyan *et al.*, 2010).

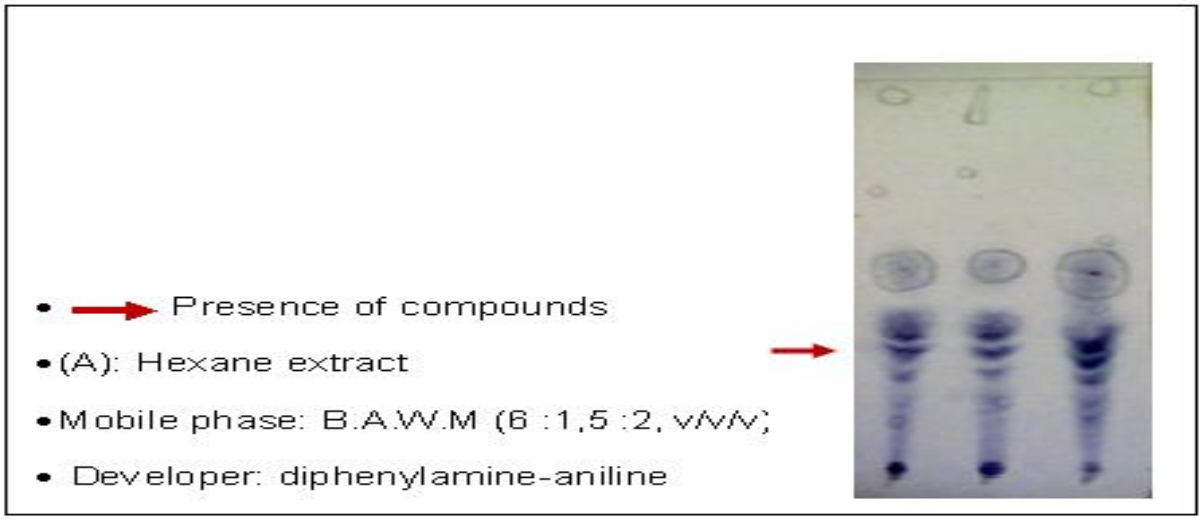

Fig. 3. Identification of bioactive compounds from *Streptomyces sp.* I4 by Thin layer chromatography (TLC).

The results obtained may suggested that our active substance has not a polyenic nature which was extracted by n-butanol and very slightly soluble in water. These results were interesting because the polyene molecules were undesirable in programs screening for new antifungal molecules due to problems related to their toxicity, their instability and their poor solubility in water (Drouhet et Dupont, 1987; Gupte *et al.*, 2002).

All the results and preliminary data obtained have allowed us to conclude that the antibiotic produced by the strain I4 contains carbohydrate residues.

References

Aouiche A, Sabaou N, Meklat A, Zitouni A.,Mathieu F, Lebrihi A. 2012. Activité antimicrobienne de *Streptomyces sp*. PAL111 d'origine saharienne contre divers microorganismes cliniques et toxinogènes résistants aux antibiotiques.Journal De Mycologie Médicale, 1-10. http://dx.doi.org/10.1016/j.mycmed.2011.12.077.

Athalye M, Goodfellow M, Lacey J, White RP. 1985. Numerical classification of Actinomadura and Nocardiopsis.International Journal of Systematic and Evolutionary Microbiology **35**, 86-98. http://dx.doi.org/10.1099/00207713-35-1-86.

Aubert G. 1978. Relation entre le sol et cinq d'étricacées dans le Sud Est de la France. Oecologia plantarum **13(3),** 253-269.

Badji B, Riba A, Mathieu F, Lebrihi A, Sabaou N. 2005. Activité antifongique d'une souche d'Actinomadura d'origine saharienne sur divers champignons pathogènes et toxinogènes. Journal de Mycologie Médicale **15,** 211-219. http://dx.doi.org/10.1016/j.mycmed.2005.07.001

Becker B, Lechevalier MP, Gordon RE, Lechevalier HA. 1964. Rapid differentiation between Nocardia and Streptomyces by paper chromatography of Whole cell hydrolysates. Journal of Applied Microbiology **12,** 421-430.

Beck-Sagué C, Jarvis WR. 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980-1990. The Journal of Infectious Diseases **167,** 1247-1251.

Berdy J, Aaszalos A, Mc Nitt KL. 1987. CRC Handbook of antibiotic compounds. Vol. XIII. Microbial metabolites. Boca Raton. Part 1, 2, 3. Florida, USA. CRC Press.

Betina V. 1973. Bioautography in paper and thin layer chromatography and its scope in the antibiotic field. Journal of Chromatography A **78,** 41-51. http://dx.doi.org/10.1016/S0021-9673(01)99035-1

Boudjella H, Bouti K, Zitouni A, Mathieu F, Lebrihi A, Sabaou N. 2007. Isolation and partial characterization of pigment-like antibiotics produced by a new strain of Streptosporangium isolated from an Algerian soil. Journal of Applied Microbiology **103,** 1364-5072. http://dx.doi.org/10.1111/j.1365-2672.2006.03280.x

Boughachiche F, Reghioua S, Oulmi L, Zerizer H, Kitouni M, Boudemagh A, Boulahrouf A. 2005. Isolement d'actinomycetales productrices de substances antimicrobiennes à partir de la sebkha de Ain Mlila. Sciences & Technologie C **23,** 5-10.

Breton A, Theilleux J, Sanglier JJ, Vobi G. 1989. Organismes producteurs: biologie, taxonomie et écologie. In: Larpent, J.P., SanglierJ.J., Ed. Biotechnologie des antibiotiques. Masson, Paris, 33-70.

Carle S. 2003. Les antifongiques dans le traitement des infections invasives. Pharmactuel **36,** 25-41.

Couturaud F. 2004. Aspergillus et poumon. *Revue* Française d'Allergologie et d'*Immunologie* Clinique **44,** 83-8. http://dx.doi.org/10.1016/j.allerg.2003.10.017

Di Domenico B. 1999. Novel antifungal drugs. Current Opinion *in* Microbiology **2,** 509-15. http://dx.doi.org/10.1016/S1369-5274(99)00009-0.

Drouhet E. 1978. Antifungal agents. Antibiotics and Chemotherapy **25,** 253-88.

Drouhet E, Dupont B. 1987. Evolution of antifungal agents: past, present and future. Reviews of Infectious Diseases **9,** 4-16.

Duraipandiyan V, Sasi AH, Islam VIH, Valanarasu M, Ignacimuthu S. 2010. Antimicrobial properties of actinomycetes from the soil of Himalaya. Journale de Mycologie Medicale **20,** 15-20.
http://dx.doi.org/10.1016/j.mycmed.2009.11.002

Gordon RE, Barnett DA, Handarhan JE, Pang CHN. 1974. *Nocardia coeliaca, Nocardia autotrophica*, and the nocardin strains. International journal of systematic bacteriology **24,** 54-63.

Gupte M, Kulkarni P, Ganguli BN. 2002. Antifungal antibiotics. *A*pplied Microbiology and Biotechnology **58,** 46-57.
http://dx.doi.org/10.1007/s002530100822.

Hamoir J, Goret M, Mignon B, Gustin P. 2001. Actualité sur les antifongiques enregistrés en Belgique dans le cadre du traitement des dermatophytoses chez les carnivores domestiques. Annales de Médecine Vétérinaire **145,** 226-232.

Hilali L, Khattabi A, Nessarlah N, Malki A. Finance C. 2002. Isolement des nouvelles souches d'actinomycétales productrices de substances antifongiques à partir du milieu naturel Marocain. Biotechnology and Molecular Biology Reviews **2,** 49-53.

Kumar S, Kannabiran K. 2010. Antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates from pulmonary tuberculosis patients. Journale de Mycologie Medicale **20,** 101-107.
http://dx.doi.org/10.1016/j.mycmed.2010.04.005.

Lacroix C, Dubach M, Feuilhade M. 2003. Les échinocandines : une nouvelle classe d'antifongiques. *Médecine* et maladies infectieuses **33**, 183-191.
http://dx.doi.org/10.1016/S0399-077X(03)00059-3184C.

Lechevalier HA, Lechevalier MP. 1970. Composition of whole-cell hydrolysates as a criterion in the classification of aerobic actinomycetes. In: Prauser H., Ed. The Actinomycetales. Ed. Jena: Fischer G., 393-404.

Lee JY, Hwang BK. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Canadian Journal of Microbiology **48(5),** 407-417.
http://dx.doi.org/10.1139/w02-025.

Marchal N, Bourdon JL, Richard Cl. 1987. Les milieux de culture pour l'isolement et l'identification biochimique des bactéries. Doin, Paris.

Maskey RP, Li FC, Qin S, Fiebig HH, Latsch H. 2003. Chandrananimycins A~C: Production of novel anticancer antibiotics from a marine *Actinomadurasp*. Isolate M048 by variation medium composition and growth conditions. Journal of Antibiotics **56,** 622-9.

Okami Y, Hotta K. 1988. Search and discovery of new antibiotics. In: GoodfellowM., WilliamsS.T., Mordarski M.Biotechnology. Ed. Actinomycetes, Academic Press, London, 33-67.

Oskay M. 2009. Antifungal and antibacterial compounds from *Streptomyces* strains. Academic journal **8(13),** 3007-3017.

Patel JJ, Brown ME. 1969. Interactions of Azofohacfer with rhizosphere and root-surface microflora. Plant and Soil **3,** 1273-1281.

Pfefferle C, Theobald U, Gürtler H, Fiedler HP. 2000. Improved secondary metabolite production in the genus *Streptosporangium* by optimization of the fermentation conditions. Journal of Biotechnology **80,** 135-142.
http://dx.**doi**.org/10.1016/S0168-1656(00)00249-2.

Pochon J, Tardieux P. 1962. Techniques d'analyse en microbiologie du sol: la tourtourelle, Ed. Saint Mandé, 22.

Sabaou N, Boudjella H, Bennadji A, Mostefaoui A, Zitouni A, Lamari L. 1998. Les sols des oasis du Sahara algérien, source d'actinomycètes rares producteurs d'antibiotiques. Sécheresse **9,** 147-53.

Shirling EB, Gottlieb D. 1966. Methods for characterization of Streptomyces species. International Journal of Systématic Bacteriology **16**, 313-40.
http://dx.doi.org/10.1099/00207713-16-3-313

Thakur D, Yadav D, Gogoi BK, Bora TC. 2007. Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. Journale de Mycologie Medicale **17,** 242-249.
http://dx.doi.org/10.1016/j.mycmed.2007.08.00.1

Toumatia O, Yekkour A, Goudjal Y, Riba A, Coppel Y, Mathieu F, Sabaou N, Zitouni A. 2014. Antifungal properties of an actinomycin D-producingstrain, *Streptomyces* sp. IA1, isolated from a Saharan soil. Journal of Basic Microbiologie **54,** 1-8.
http://dx.doi.org/10.1002/jobm.201400202.

Valanarasu M, Duraipandiyan V, Agastian P, Ignacimuthu S. 2009. In vitro antimicrobial activity of *Streptomyces spp*. ERI-3 isolated from Western Ghats rock soil (India). Journale de Mycologie Medicale **19,** 22-8.
http://dx.doi.org/10.1016/j.mycmed.2008.12.002.

Valanarasu M, Kannan P, Ezhilvendan S, Ganesan G, Ignacimu-thu S, Agastian P. 2010. Antifungal and antifeedant activities of extracellular product of *Streptomyces* spp. ERI-04 isolated from Western Ghats of Tamil Nadu. Journale de Mycologie Medicale **20,** 290-297.
http://dx.doi.org/10.1016/j.mycmed.2010.09.001.