ISOLATION AND CHARACTERIZATION OF A NEW *Saccharothrix* STRAIN AHO23 WITH ANTIMICROBIAL ACTIVITY FROM AN UNEXPLOITED ALGERIAN SAHARAN REGION

Sid Ahmed SAADI[*,**], Atika MEKLAT[*,***], Salim MOKRANE[*], Hafsa YAICHE ACHOUR[*,****], Michael D. HOLTZ[*****], Hans-Peter KLENK[******], Noureddine BOURAS[*,*******]

[*] *Ecole Normale Supérieure de Kouba, Laboratoire de Biologie des Systèmes Microbiens (LBSM), Vieux Kouba, Alger, Algeria*
[**] *Université Kasdi Merbah- Ouargla, Faculté des Sciences de la Nature et de la Vie, BP 511 Ouargla 30000, Algeria*
[***] *Université Saâd Dahleb, Faculté des Sciences de la Nature et de la Vie, Département de Biologie et Physiologie Cellulaire, Blida, Algeria*
[****] *Ecole Supérieure des Sciences de l'Aliment et des Industries Agroalimentaires (ESSAIA), Beaulieu, Oued Smar, Alger, Algeria*
[*****] *Field Crop Development Centre, Alberta Agriculture and Forestry, 5030 - 50 Street, Lacombe, Alberta T4L1W8, Canada*
[******] *School of Natural and Environmental Sciences, Newcastle University, Ridley Building 2, Newcastle upon Tyne, NE1 7RU, United Kingdom*
[*******] *Université de Ghardaia, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Département de Biologie, Ghardaïa 47000, Algeria*
Correspondence author: Noureddine Bouras, Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Vieux Kouba, Alger, Algeria, e-mail: noureddine_bouras@yahoo.fr

Abstract. Actinobacteria originated from extreme ecosystems remains the most interesting source of new antibiotics. An actinobacterial strain, named AHO23, was isolated from Ahaggar region soil in Algeria, using the dilution agar plating method with chitin-vitamins B medium supplemented with nalidixic acid and actidione. The morphological characteristics showed that strain AHO23 belonged to the genus *Saccharothrix*. Analysis of the 16S rRNA gene sequence of this strain AHO23 showed 98.95% of similarity with the closely related species *Saccharothrix xinjiangensis* NBRC 101911^T. AHO23 exhibited a strong activity against Gram-negative bacteria (15-36 mm), as well as good activity against Gram-positive bacteria (29-34 mm). *Candida albicans* (ATCC10237) was also inhibited (20 mm). Moreover, AHO23 presented a strong activity against mycotoxigenic and phytopathogenic fungi (21-36 mm), with the highest activity (36 mm) against *Fusarium culmorum*. Bioactive metabolites from AHO23 culture on ISP2 medium were extracted using four organics solvents: *n*-hexane, dichloromethane, ethyl-acetate and *n*-butanol. The highest antibacterial activities were detected in the *n*-butanol extract.

Key words: Actinobacteria; *Saccharothrix*; Ahaggar soil; 16S rRNA gene; antimicrobial activity.

INTRODUCTION

Actinobacteria are prokaryotic organisms. They are Gram-positive bacteria which exhibit variable morphological growth characteristics [3]. Actinobacteria are producers of broad spectrum bioactive secondary metabolites and represent about 70-80% of all the isolated antimicrobial compounds [30]. About 23,000 antibiotics have been discovered from microorganisms and of these around 10,000 of them have been isolated from Actinobacteria [39].

The genus *Saccharothrix* [20] belongs to the family *Pseudonocardiaceae* [10]. This genus currently contains twenty-one species and two subspecies [24, 38]. Many *Saccharothrix* strains produce secondary metabolites which are active against phytopathogenic microorganisms, toxigenic fungi, and drug-resistant pathogens [37].

The Algerian Saharan soils contain an abundance of Actinobacteria, and several rare genera have been discovered. The genus *Saccharothrix* was identified in many of these soil samples [8, 33, 42]. Several new antibiotics have been produced by these *Saccharothrix* strains, such as dithiolopyrrolones [8, 21] and anthracycline [41]. Furthermore, more undiscovered strains and their antibiotics likely remain to be identified in Algerian Saharan soils.

This research was conducted to isolate and identify, by means of morphological and molecular tools, a new *Saccharothrix* strain from an Algerian Saharan soil. Fermentation on different culture media and extraction of antimicrobial activity were also studied.

MATERIALS AND METHODS

Sampling, strain isolation and maintenance

Soil were collected at different depths (5 to 20 cm) from Ahaggar region (Tamanrasset) which is located in the south of Algeria (1900 km from Algiers). Samples were stored in sterile containers and transferred to the laboratory for analysis. The serial dilution method was used for Actinobacteria isolation according to Hayakawa and Nonomura (1987) [13]. One milliliter of each dilution was spread over the surface of chitin-vitamin B (CHV) agar plates supplemented with the antifungal actidione (80 mg/mL) and the antibacterial nalidixic acid (25 mg/mL). The plates were incubated at 30°C for 20 days. An isolated colony named AHO23 was streaked in a new plate containing ISP2 medium (International *Streptomyces* Project 2) [36] to obtain a pure culture and then was preserved at 4°C. ISP2 medium is composed of 4 g yeast extract, 10 g malt extract and 4 g glucose in 1 liter of distilled water, at pH: 7.2.

Morphological and cultural characteristics

The cultural characteristics observed were the color of the aerial and substrate mycelia, sporulation and pigment production, they were determined using the ISCC-NBS color name chart [16]. The morphological characters were observed using light microscope (Motic B1 Series). All these characteristics were observed on ISP2, ISP4 and CHV agar media incubated at 30°C for 14 days.

Physiological characters

The physiological tests performed to characterize the AHO23 strain are the utilization of carbohydrates

as carbon source and degradation of tyrosine according to Gordon et al. (1974) [11]. Reduction of nitrate and degradation of starch and gelatin were examined as described by Marchal and Bourdon (1973) [26]. Tolerance to NaCl (5%, w/v) was determined as well on ISP2 medium.

16S rRNA gene amplification and phylogenetic analysis

DNA from strain AHO23 was isolated using the protocol described by Li et al. (2007) [23]. The 16S rRNA gene was amplified by PCR with the primers 10-30F (5'-GAGTTTGATCCTGGCTCA-3') and 1500R (5'-AGAAAGGAGGTGATCCAGCC-3') [32]. PCR amplification was carried out in a final volume of 30 µL containing 50 ng of DNA, 0.5 µM of each primer, 1X PCR buffer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP and 1U *Taq* DNA polymerase (SilverStar). The PCR conditions were as follows: initial denaturation at 96°C for 4 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 52°C for 1 min and extension at 72°C for 2 min, and then final extension at 72°C for 10 min. PCR products were then checked by 1% agarose gel electrophoresis, they were stained with safe view. Sequencing was performed by Genewiz company, Ltd. (Takeley, UK).

The sequence obtained was aligned with others sequences available from EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) [[40] using ClustalW in MEGA 7.0 software [19, 22]. Evolutionary distance matrices were generated as described by Jukes and Cantor (1969) [15]. A phylogenetic tree was constructed using neighbor-joining method based on bootstrap values of 1000 resamplings [34].

Antagonistic activity of the isolated strain

The antagonistic activity of strain AHO23 was determined by agar plug diffusion method on ISP2 medium against filamentous fungi: *Aspergillus carbonarius* (M333), *A. westerdijkiae* (ATCC 3174), *A. flavus* (NRRL 3251), *Fusarium culmorum*, *F. proliferatum*, *Umbelopsis ramanniana* (NRRL 1829), *Penicillium glabrum* and *Penicillium expansum*; pathogenic yeast: *Candida albicans* (ATCC10237) and nine bacteria: sensitive *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA 639c), *Listeria monocytogenes* (ATCC 13932), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *E. coli* E52, *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* serovar Typhi (ATCC 14028) and *Acinetobacter baumannii*. All these test microorganisms were obtained from LBSM (Laboratoire de Biologie des Systèmes Microbiens, Algiers).

Fermentation parameters and antimicrobial production by AHO23 strain

Fermentation was conducted on ISP2 medium. An inoculum of three agar plugs (6 mm diameter, obtained from 10 days old culture) were added to a 250 mL flask containing 50 mL of ISP2 medium. The flasks were shaken on a rotary shaker (New Brunswick Scientific Co., NJ, USA) (250 rpm) for two days. This pre-culture was used to inoculate 100 mL of ISP2 medium in 500 mL flasks. The flasks were then shaken at 250 rpm at 30°C for 10 days. pH and cell dry weight (DCWs) were measured on samples collected every 24h according to Bouras (2005) [7]. Strain AHO23 was screened for its antimicrobial activity every 24h during 10 days of fermentation, using the well agar diffusion method of Aszalos (1986) [2]. This activity was screening against *Escherichia coli* (ATCC 8739), *Listeria monocytogenes* (ATCC 13932) and methicillin resistant *Staphylococcus aureus* (MRSA 639c). Mueller Hinton (MH) agar plates (10 g/L of agar) was inoculated by the target-microorganism. Thereafter, wells of 10 mm diameter were made in the agar plates, and they were filled with 100 µL of the cell free supernatant of AHO23. Plates were then incubated at 4°C for 2h. Inhibition zones were measured after 24h of incubation at 30°C.

Extraction and Recovery of Antibacterial Compounds

The isolated strain was cultured in 100 mL of ISP2 medium in 500 mL flasks under fermentation conditions, they were kept on a rotary shaker (250 rpm) at 30°C for 5 days. After fermentation, the medium was harvested and centrifuged to remove cell debris. Antibacterial compound was recovered from the filtrate by solvent extraction method. Four organic solvents were tested: *n*-hexane, dichloromethane, ethyl-acetate and *n*-butanol. Each solvent was added to the filtrate in the ratio of 1:1 (v/v). The organic phase was separated from the aqueous phase under reduced vacuum (Rotavapor R-210, Buchi, Switzerland) and the obtained extracts were dissolved in 1 mL of methanol. These extracts were used to determine the antibacterial activity.

The antibacterial activity was determined by paper disk method on ISP2 medium (12 g/L agar) against *Escherichia coli* (ATCC 8739), *Listeria monocytogenes* (ATCC 13932) and methicillin resistant *S. aureus* (MRSA 639c), 80 µL of each extracts were pipetted onto a paper disk (6 mm, Pasteur Institute). Disks were then sterilized by exposing them to hood UV (CACHAN 94230) for 45 min. The plates were incubated at 30°C for 24h and the inhibition zone were expressed as diameter (mm).

Statistical analysis

Statistical analysis was carried out using SPSS 26.0 software. One-way ANOVA was used in this study, with a confidence interval of 95%. The data were represented as mean values ± standard deviation of triplicate experiments.

RESULTS

Cultural characteristics are presented in Table 1. Morphological and cultural properties were observed on ISP2, ISP4 and CHV media. AHO23 exhibited a very good growth on ISP2 medium with the production of brownish red soluble pigment. However, on ISP4 and CHV media the growth is moderate to good with

the production of medium orange and light pink pigments, for ISP4 and CHV media, respectively. The color of aerial mycelium was beige on ISP2, pink to orange-pink on ISP4 and pink to medium pink for CHV medium. Whereas, substrate mycelium was whitish pink on CHV, orange to brownish orange on ISP4 and very dark reddish brown on ISP2.

The aerial mycelium on ISP2 and ISP4 is very irregular, anarchic, fragmented to irregular spores, with the presence of spore chains. Otherwise, on CHV medium the aerial mycelium is anarchic with very irregular spores.

The physiological features are depicted in Table 2. To identify an unknown strain to species it is useful to determine the different carbon sources that can be utilized for growth by culturing it on media containing different sugars. Among nine carbon sources tested, strain AHO23 uses five of them L-arabinose, lactose, L-rhamnose, D-sucrose and D-xylose, with negative results for the other carbon sources: raffinose, methyl α-D-glucose, D- sorbitol and melibiose. Strain AHO23 has the ability to hydrolyze gelatin, starch and tyrosine, as well as the ability to produce nitrate reductase. The strain is not able to grow at 5% NaCl (w/v).

Table 1. Cultural characteristics of AHO23 strain on different media after 14 days of incubation

Agar medium	Growth	Production and color of: AHO23		Soluble pigment
		Aerial mycelium	Substrate mycelium	
ISP2	+++	+ beige	Very dark reddish brown	++++ brownish red
ISP4	++	++ pink to orange-pink	Orange to brownish orange	++ medium orange
CHV	+ to ++	++ pink to medium pink	whitish pink	± light pink

Legends: +: weak, ++: moderate, +++: strong, CHV: chitin-vitamin B agar.

Table 2. Physiological characteristics of the strain AHA23 in comparison with the most closely related species *Saccharothrix xinjiangensis* NBRC 101911^T

Test	*Saccharothrix* sp. AHO23	*Saccharothrix xinjiangensis* NBRC 101911^T
Carbon source utilization:		
L-arabinose	+	+
Lactose	+	+
Raffinose	-	+
L-rhamnose	+	+
D-sucrose	+	+
D-xylose	+	+
Methyl α-D-glucose	-	+
D- sorbitol	-	-
Melibiose	-	+
Hydrolysis of:		
Gelatin	+	Nd
Starch	+	+
Tyrosine	+	-
Nitrate reduction	+	-
Growth in the presence of:		
5% NaCl (w/v)	-	-

Nd: not determined

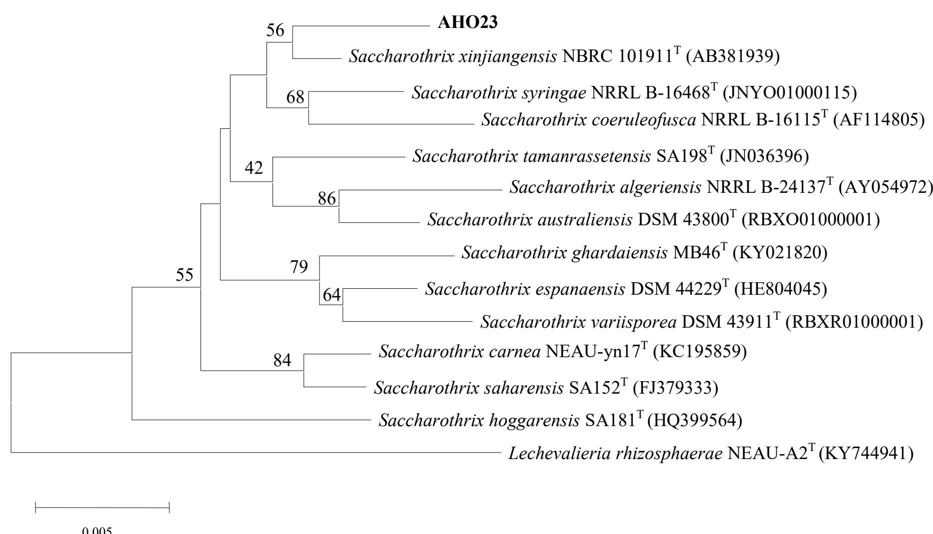

Figure 1. Phylogenic tree based on 16S rRNA gene sequences showing the position of AHO23 and its related species of the genus *Saccharothrix*. Numbers at nodes indicate percentages of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. Bar, 0.005 substitutions per nucleotide position. *Lechevalieria rhizosphaerae* NEAU-A2^T was used as outgroup. Numbers in the parentheses indicate GenBank accession numbers.

Physiological differences among strain AHO23 and the closest neighbors in the genus *Saccharothrix* are listed in Table 2. These differences concern the utilization of raffinose, methyl α-D-glucose and melibiose which are negative for the strain AHO23 and positive for *Saccharothrix xinjiangensis* NBRC 101911^T. It is the same for the hydrolysis of tyrosine and the nitrate reduction which are positive for AHO23 strain and negative for the strain *S. xinjiangensis* NBRC 101911^T.

The amplification product of the 16S rRNA gene, of approximately 1460 bp, was sequenced and aligned with other strains from EzTaxon-e server. Phylogenetic analysis revealed that the AHO23 strain is a member of the genus belongs to *Saccharothrix* and showed 98.95% of similarity with *Saccharothrix xinjiangensis* NBRC 101911^T. A phylogenetic tree was obtained by the neighbor-joining method (fig. 1). The GenBank /EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AHO23 is LC586937.

In comparison, the strain *Saccharothrix xinjiangensis* NBRC 101911^T is differentiated from strain AHO23 by some phenotypic properties, it has a greyish white aerial mycelium and pinkish buff substrate mycelium.

The antagonism assay showed broad spectrum antagonistic potential of strain AHO23 against the various microorganisms tested (Table 3). All bacteria tested were inhibited except *Salmonella enterica* serovar Typhi (ATCC 14028). This antibacterial activity was strong (≥ 30) against *Escherichia coli* E52, *Bacillus subtilis* (ATCC 663), *Staphylococcus aureus* (S), *Listeria monocytogenes* (ATCC 13932) and *Escherichia coli* (ATCC 8739). Moderate activity (15-29 mm) was detected against *Staphylococcus aureus* (MRSA 639c), *Acinetobacter baumannii* (S54) and *Pseudomonas aeruginosa* (ATCC 9027).

AHO23 showed an antifungal activity against all the target-fungi, with a strong activity for *Fusarium culmorum* (36 mm), *Umbelopsis ramanniana* NRRL 1829 (30 mm) and *Penicillium glabrum* (30 mm). A moderate inhibition (20-25 mm) was detected for *Aspergillus carbonarius* (M333), *Aspergillus westerdijkiae* (ATCC 3174), *Aspergillus flavus* (NRRL 3251), *Fusarium proliferatum* and *Penicillium expansum*. AHO23 inhibits also *Candida albicans* (ATCC10237) (20 mm).

It was observed that the antibacterial metabolites produced by the actinobacterium AHO23 against *Staphylococcus aureus* (MRSA 639c), *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 13932) started in early growth phase (1st day of incubation) and reached the maximum on the 5th day of fermentation. During fermentation pH varied between 7.35 to 8.17, difference was significant ($p < 0.05$). Biomass increased to reach the maximum (4.17 g/L) on the 5th day of incubation and then decreased slightly (Fig. 2). However, the change was significant until the 10th day of fermentation ($p < 0.05$).

After five days of fermentation culture filtrate was extracted by four organic solvents: *n*-butanol, ethyl-acetate, dichloromethane and *n*-hexane. Organic phases obtained after liquid-liquid extraction were tested against *Staphylococcus aureus* (MRSA 639c), *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 13932). *n*-Butanol and acetate-ethyl were found to be the best solvents for the extraction of maximum antibacterial metabolites. The inhibition zones are illustrated in Figure 3 and Table 4.

Table 3. Antimicrobial activity of strain AHO23 by the disk diffusion method on ISP2 medium

Target-microorganism	Inhibition zone (mm)	
	***Saccharothrix* sp. AHO23**	***Saccharothrix tamanrassetensis* DSM 45947** Bakour Keramane et al. (2020) [17]
Gram positive bacteria		
Staphylococcus aureus (MRSA 639c)	29 ± 2.0	36 ± 1.2
Staphylococcus aureus	33 ± 2.0	Nd
Listeria monocytogenes (ATCC 13932)	30 ± 1.0	15 ± 1.3
Bacillus subtilis (ATCC 6633)	34 ± 1.0	23 ± 0.7
Gram negative bacteria		
Escherichia coli (ATCC 8739)	30 ± 2.0	Nd
Escherichia coli (E52)	36 ± 1.0	0
Pseudomonas aeruginosa (ATCC 9027)	15 ± 1.0	Nd
Salmonella enterica serovar Typhi (ATCC 14028)	0	Nd
Acinetobacter baumannii	24 ± 2.0	14 ± 0
Filamentous fungi		
Aspergillus carbonarius (M333)	24 ± 2.0	17 ± 0.6
Aspergillus westerdijkiae (ATCC 3174)	23 ± 1.0	13 ± 0.7
Aspergillus flavus (NRRL 3251)	21 ± 2.0	13 ± 0.7
Fusarium culmorum	36 ± 1.5	8 ± 4
Fusarium proliferatum	24 ± 2.0	0
Umbelopsis ramanniana (NRRL 1829)	30 ± 2.0	29 ± 0.7
Penicillium glabrum	30 ± 0.5	8 ± 4
Penicillium expansum	25 ± 2.0	9 ± 4.4
Yeast		
Candida albicans (ATCC10237)	20 ± 1.0	Nd

Nd: not determined; All data were the mean value ± SD, $n = 3$.

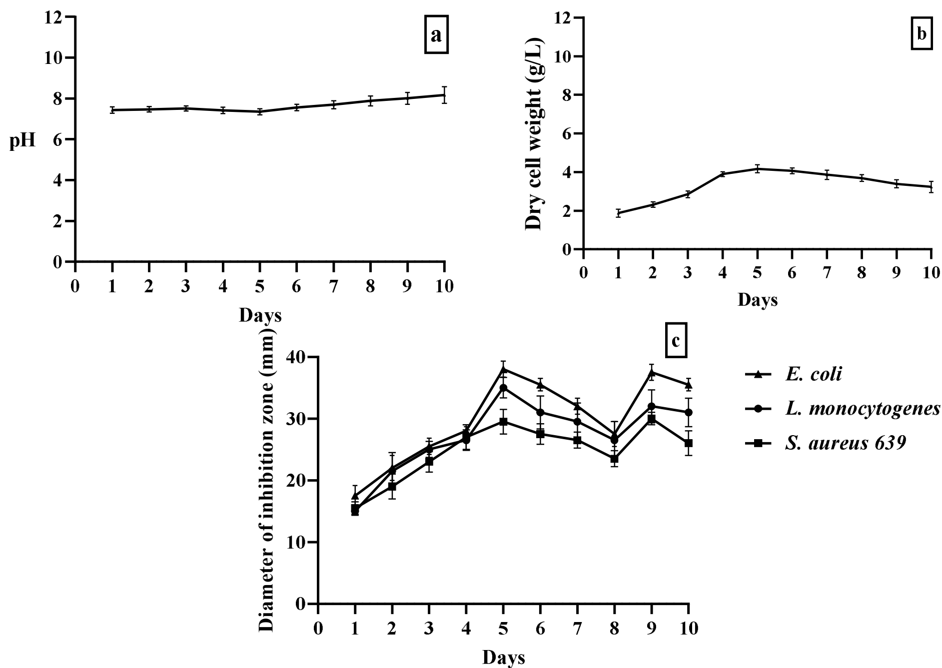

Figure 2. (a) Kinetics of pH, (b) dry cell weight and (c) antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA 639c) (■), *Escherichia coli* (ATCC 8739) (▲) and *Listeria monocytogenes* (ATCC 13932) (●). Values are mean ± SD, *n* = 3

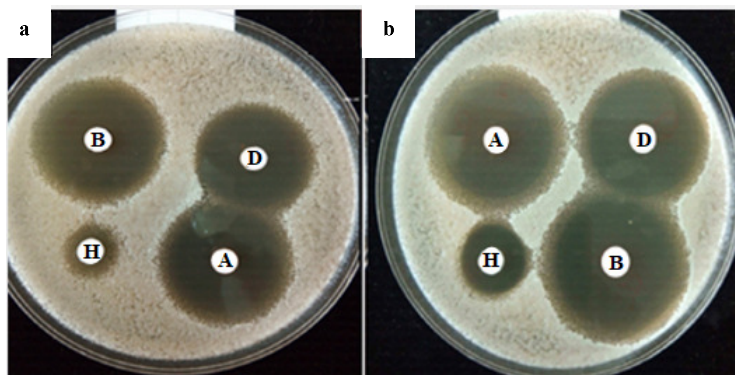

Figure 3. Antibacterial activities of dichloromethane (D), *n*-hexane (H), ethyl-acetate (A) and *n*-butanol (B) extracts against : **(a)** *Listeria monocytogenes* (ATCC 13932) and **(b)** *Escherichia coli* (ATCC 8739) by paper disk method.

Table 4. Antimicrobial activity of strain AHO23 by the disk diffusion method on ISP2 medium

Target-microorganism	Zone of inhibition (mm) obtained by using different solvents			
	n-Butanol	Ethyl-acetate	Dichloromethane	*n*-Hexane
S. aureus (MRSA 639c)	34 ± 2.0	28 ± 2.0	27 ± 2.0	13 ± 2.2
E. coli (ATCC 8739)	47 ± 2.0	45 ± 1.3	43 ± 2.0	19 ± 1.6
L. monocytogenes (ATCC 13932)	42 ± 2.7	41 ± 1.3	36 ± 2.0	14 ± 2.7

Values are mean ± SD, *n* = 3

DISCUSSION

Actinobacteria are one of the most explored microorganisms among prokaryotes. They are promising candidates for the production of antibiotics with medicinal and pharmaceutical importance [3, 35]. Algerian Saharan soil have proven to be an interesting source of *Saccharothrix* strains, where several species and novel antibiotics have been identified, e.g., *Saccharothrix algeriensis*, *S. saharensis*, *S. hoggarensis* and *S. tamanrassetensis* [4-6, 42].

The use of CH-V medium for *Saccharothrix* strain isolation is recommended by several authors [1, 31]. This specific culture medium promotes sporulation which makes their detection easier [9] and contain chitin as sole carbon source which is difficult to be degraded by non actinobacterial microorganisms (N. Sabaou, unpublished data).

Actinobacteria species identification cannot be determined only by 16S rRNA data, phylogeny as well as cultural and physiological proprieties were used to determine a possible species [35].

On the basis of micromorphological characteristics, strain AHO23 was assigned to the genus *Saccharothrix*. Some physiological proprieties of strain AHO23, with associated cultural proprieties, were found to be distinguished from the type strain *Saccharothrix xinjiangensis* NBRC 101911^T [14], its close phylogenetic neighbor. The 16S rRNA gene sequence of AHO23 strain was compared with those of other *Saccharothrix* species. The similarity level with the most closely related species *Saccharothrix xinjiangens* NBRC 101911^T is 98.95%. Studies on the gene encoding for 16S rRNA and DNA-DNA hybridization have shown that there is a probability of about 50% to have a new species when similarity percentage is around 99.5% [18, 27]. Furthermore, the results of 16S rRNA gene sequence of the strain AHO23 of *Saccharothrix* were below or approximately the same as the similarities between closely related species within this genus, such as the similarities exhibited between *Saccharothrix isguenensis* and *S. ecbatanensis* (99.8% similarity) [8, 29]. Therefore, it is possible that the strain AHO23 is new species, but it should be confirmed by DNA-DNA hybridization with the closest related strains.

Our results showed that strain AHO23 has a broad spectrum of antibacterial and antifungal activity. The same results were found by Lu et al. (2018) [25] and Merrouche et al. (2017) [28] for other species of *Saccharothrix*. The highest antibacterial activity was recorded against Gram-negative bacteria, these characteristics is rarely reported in Actinobacteria. All the Gram-negative bacteria tested have been inhibited except *Salmonella enterica* serovar Typhi which is resistant. Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria, because they have a largely impermeable cell wall [12]. However, the activity of *Saccharothrix* species against Gram-positive bacteria has been widely published [4, 33, 37, 42].

Several works have raised the importance of the antagonistic properties of the genus *Saccharothrix*. Antagonistic potential of AHO23 could be due to the secretion of diffusible antibacterial and antifungal metabolites. *Saccharothrix* species are known to produce several antibiotics of great structural diversity and with an interesting action. Numerous bioactive complex chemical compounds from different families have been reported, such as dithiolopyrrolone derivatives, heptadecaglycosides [37], anthracyclines [41], phenicols [1] and chitinase [9].

The findings of the present study conclude that the Algerian Saharan soils are a promising source of actinobacterial strains. New regions in the Algerian Saharan deserve to be investigated for their Actinobacteria diversity. On the other hand, strain AHO23 deserves to be further investigated as a promising antibacterial agent, especially against other Gram-negative bacteria and fungi.

Acknowledgments. The authors wish to gratefully acknowledge the late Prof. Nasserdine SABAOU (1956-2019) for his great contribution in this study.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Aouiche, A., Sabaou, N., Meklat, A., Zitouni, A., Bijani, C., Mathieu, F., Lebrihi, A., (2012): *Saccharothrix* sp. PAL54, a new chloramphenicol-producing strain isolated from a Saharan soil. The Journal of Antibiotics. 28(3): 943-951.

[2] Aszalos, A., (1986): Modern Analysis of Antibiotics (Drugs and the Pharmaceutical Sciences). CRC Press, 1st Edition 568 p.

[3] Bhatti, A.A., Haq, S., Bhat, R.A., (2017): Actinomycetes benefaction role in soil and plant health. Journal of Microbial pathogenesis. 111: 458-467.

[4] Boubetra, D., Sabaou, N., Zitouni, A., Bijani, C., Lebrihi, A., Mathieu, F., (2013): Taxonomy and chemical characterization of new antibiotics produced by *Saccharothrix* SA198 isolated from a Saharan soil. Journal of Microbiological Research. 168(4): 223-230.

[5] Boubetra, D., Zitouni, A., Bouras, N., Mathieu, F., Lebrihi, A., Schumann, P., Spröer, C., Klenk, H.-P., Sabaou, N., (2013): *Saccharothrix hoggarensis* sp. nov., an actinomycete isolated from Saharan soil. International journal of systematic evolutionary microbiology. 63(2): 549-553.

[6] Boubetra, D., Zitouni, A., Bouras, N., Mathieu, F., Lebrihi, A., Schumann, P., Spröer, C., Klenk, H.-P., Sabaou, N., (2013): *Saccharothrix saharensis* sp. nov., an actinomycete isolated from Algerian Saharan soil. International Journal of Systematic and Evolutionary Microbiology. 63(10): 3744-3749.

[7] Bouras, N., (2005): Régulation de la production d'antibiotiques dithiolopyrrolones chez *Saccharothrix algeriensis* NRRL B-24137. PhD Thesis, Institut National Polytechnique de Toulouse, France.

[8] Bouznada, K., Bouras, N., Mokrane, S., Chaouch, F.C., Zitouni, A., Pötter, G., Spröer, C., Klenk, H.-P., Sabaou, N., (2016): *Saccharothrix isguenensis* sp. nov., an actinobacterium isolated from desert soil. International journal of systematic evolutionary microbiology, 66(11): 4785-4790.

[9] Bouznada, K., Bouras, N., Mokrane, S., Chaouch, F.C., Zitouni, A., Pötter, G., Spröer, C., Klenk, H.P., Sabaou, N., (2017): *Saccharothrix ghardaiensis* sp. nov., an actinobacterium isolated from Saharan soil. Antonie van leeuwenhoek. 110(3): 399-405.

[10] Garrity, G.M., (2012): Bergey's manual of systematic bacteriology: Volume one: the Archaea and the deeply branching and phototrophic bacteria. Springer Science and Business Medi, 722 p.

[11] Gordon, R.E., Barnett, D.A., Handerhan, J.E., Pang, C.H., (1974): *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. International Journal of Systematic Evolutionary Microbiology. 24(1): 54-63.

[12] Gupta, V., Datta, P., (2019): Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. The Indian Journal of Medical Research. 149(2): 97.

[13] Hayakawa, M., Nonomura, H., (1987): Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. Journal of Fermentation Technology. 65(5): 501-509.

[14] Hu, Y.T., Zhou, P.J., Zhou, Y.G., Liu, Z.H., Liu, S.J., (2004): *Saccharothrix xinjiangensis* sp. nov., a pyrene-degrading actinomycete isolated from Tianchi Lake, Xinjiang, China. International Journal of Systematic Evolutionary Microbiology. 54(6): 2091-2094.

[15] Jukes, T.H., Cantor, C.R., (1969): Evolution of protein molecules. Mammalian Protein Metabolism. 3: 21-132.

[16] Kelly, K.L., Judd, D.B., (1976): Color: universal language and dictionary of names. 190 p.
[17] Bakour Keramane, L., Boudjella, H., Meklat, A., Bouras, N., Sabaou, N., MATHIEU, F., (2020): Antimicrobial screening of *Saccharothrix tamanrassetensis* DSM 45947 and effect of carbon and nitrogen sources on antibiotic production. Algerian Journal of Arid Environment "AJAE". 10(1).
[18] Kim, M., Oh, H.S., Park, S.C., Chun, J., (2014): Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. International Journal of Systematic Evolutionary Microbiology. 64(2): 346-351.
[19] Kumar, S., Stecher, G., Tamura, K., (2016): MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 33(7): 1870-1874.
[20] Labeda, D., Testa, R., Lechevalier, M., Lechevalier, H., (1984): *Saccharothrix*: a new genus of the Actinomycetales related to *Nocardiopsis*. International Journal of Systematic Evolutionary Microbiology. 34(4): 426-431.
[21] Lamari, L., Zitouni, A., Boudjella, H., Badji, B., Sabaou, N., Lebrihi, A., Lefebvre, G., Seguin, E., Tillequin, F., (2002): New Dithiolopyrrolone Antibiotics from *Saccharothrix* sp. SA 233. The Journal of Antibiotics. 55(8): 696-701.
[22] Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., (2007): Clustal W and Clustal X version 2.0. Bioinformatics. 23(21): 2947-2948.
[23] Li, J., Yang, J., Chen, D., Zhang, X., Tang, Z., (2007): An optimized mini-preparation method to obtain high-quality genomic DNA from mature leaves of sunflower. Genetics and Molecular Research. 6(4): 1064-1071.
[24] Li, Y.-Q., Liu, L., Cheng, C., Shi, X.-H., Lu, C.-Y., Dong, Z.-Y., Salam, N., An, D.-D., Li, W.-J., (2015): *Saccharothrix lopnurensis* sp. nov., a filamentous actinomycete isolated from sediment of Lop Nur. Antonie van Leeuwenhoek. 108(4): 975-981.
[25] Lu, Y., Wang, N., He, J., Li, Y., Gao, X., Huang, L., Yan, X., (2018): Expression and characterization of a novel chitinase with antifungal activity from a rare actinomycete, *Saccharothrix yanglingensis* Hhs. 015. Protein Expression Purification. 143: 45-51.
[26] Marchal, N., Bourdon, J.L., (1973): Milieux de culture et identification biochimique des bactéries. 505 p.
[27] Meier-Kolthoff, J.P., Göker, M., Spröer, C., Klenk, H.-P., (2013): When should a DDH experiment be mandatory in microbial taxonomy? Archives of Microbiology. 195(6): 413-418.
[28] Merrouche, R., Yekkour, A., Lamari, L., Zitouni, A., Mathieu, F., Sabaou, N., (2017): Efficiency of *Saccharothrix algeriensis* NRRL B-24137 and Its Produced Antifungal Dithiolopyrrolones Compounds to Suppress Fusarium oxysporum-Induced Wilt Disease Occurring in Some Cultivated Crops. Arabian Journal for Science and Engineering. 42(6): 2321-2327.
[29] Mohammadipanah, F., Hamedi, J., Schumann, P., Spröer, C., del Carmen Montero-Calasanz, M., Klenk, H.-P., microbiology, e., (2015): *Saccharothrix ecbatanensis* sp. nov., an actinobacterium isolated from soil. International journal of systematic. 65(12): 4544-4549.
[30] Nagpure, A., Choudhary, B., Kumar, S., Gupta, R.K., (2014): Isolation and characterization of chitinolytic *Streptomyces* sp. MT7 and its antagonism towards wood-rotting fungi. Annals of Microbiology. 64(2): 531-541.
[31] Pathom-Aree, W., Stach, J.E., Ward, A.C., Horikoshi, K., Bull, A.T., Goodfellow, M., (2006): Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. Extremophiles. 10(3): 181-189.
[32] Rainey, F.A., Ward-Rainey, N., Kroppenstedt, R.M., Stackebrandt, E., (1996): The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology. 46(4): 1088-1092.
[33] Sabaou, N., Boudjella, H., Bennadji, A., Mostefaoui, A., Zitouni, A., Lamari, L., Bennadji, H., Lefèbvre, G., Germain, P., (1998): Les sols des oasis du Sahara algérien, source d'actinomycètes, rares producteurs d'antibiotiques. Science et changements planétaires/Sécheresse. 9(2): 147-153.
[34] Saitou, N., Nei, M., (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4): 406-425.
[35] Salwan, R., Sharma, V., (2020): Molecular and biotechnological aspects of secondary metabolites in actinobacteria. Microbiological Research. 231: 126374.
[36] Shirling, E.T., Gottlieb, D., (1966): Methods for characterization of *Streptomyces* species1. International Journal of Systematic and Evolutionary Microbiology. 16(3): 313-340.
[37] Singh, M., Petersen, P., Weiss, W., Kong, F., Greenstein, M., (2000): Saccharomicins, novel heptadecaglycoside antibiotics produced by *Saccharothrix espanaensis*: antibacterial and mechanistic activities. Antimicrobial agents chemotherapy. 44(8): 2154-2159.
[38] Stackebrandt, E., Rainey, F.A., Ward Rainey, N.L., (1997): Proposal for a new hierarchic classification system, Actinobacteria classis nov. International Journal of Systematic Evolutionary Microbiology. 47(2): 479-491.
[39] Thomas, T.R.A., Kavlekar, D.P., LokaBharathi, P.A., (2010): Marine drugs from sponge-microbe association: A review. Marine drugs. 8(4): 1417-1468.
[40] Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., (2017): Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology. 67(5): 1613.
[41] Zitouni, A., Boudjella, H., Mathieu, F., Sabaou, N., Lebrihi, A., (2004): Mutactimycin PR, a New Anthracycline Antibiotic from *Saccharothrix* sp. SA 103: I. Taxonomy, Fermentation, Isolation and Biological Activities. The Journal of Antibiotics. 57(6): 367-372.
[42] Zitouni, A., Lamari, L., Boudjella, H., Badji, B., Sabaou, N., Gaouar, A., Mathieu, F., Lebrihi, A., Labeda, D., (2004): *Saccharothrix algeriensis* sp. nov., isolated from Saharan soil. International Journal of Systematic Evolutionary Microbiology. 54(4): 1377-1381.

Received: October 27, 2020
Accepted: February 26, 2021
Published Online: March 1, 2021
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House