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SACCHAROTHRIX SP. ABH26, A NEW ACTINOBACTERIAL STRAIN FROM ALGERIAN SAHARAN SOIL: ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITY

Abdelhadi Lahoum[1], *Noureddine Bouras*[*1,2], *Khaoula Bouznada*[1], *Michael D. Holtz*[3], *Cathrin Spröer*[4], *Hans-Peter Klenk*[4], *Nasserdine Sabaou*[1]

Address(es): Dr. Noureddine Bouras (Ph.D)
[1]Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, B.P. 92, 16 050 Kouba, Algiers, Algeria. Phone number +213 21297511.
[2]Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, BP 455, Ghardaïa 47000, Algeria.
[3]Field Crop Development Centre, Alberta Agriculture and Rural Development, 6000 C & E Trail, Lacombe, Alberta, T4L 1W1, Canada.
[4]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany.

*Corresponding author: noureddine_bouras@yahoo.fr

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ABSTRACT

A new strain of actinobacteria, designated ABH26, was isolated from a Saharan soil in the Adrar region (Algeria), by the dilution agar plating method using a chitin-vitamins B medium supplemented with polymyxin and penicillin. The morphological studies showed that this strain represents a member of the *Saccharothrix* genus. Phylogenetic analysis showed that this strain had 16S rRNA gene sequence similarities ranging from 97.63% (with *Saccharothrix violaceirubra* NBRC 102064^T) to 99.86% (with *Saccharothrix xinjiangensis* NBRC 101911^T). Furthermore, strain ABH26 presented a strong activity against mycotoxigenic and phytopathogenic fungi including *Aspergillus carbonarius* (M333), *A. flavus* (NRRL 3251), *A. westerdijkiae* (ATCC 3174), *Fusarium oxysporum* f. sp. *lini* (Fol) and *F. solani* (Fsol). Additionally, the strain exhibited an important antimicrobial activity against many strains of the pathogenic yeast *Candida albicans* (M2, M3 and IPA200) and against methicillin resistant *Staphylococcus aureus* (MRSA 639c). Thus, four solvents (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol) were used for the extraction of produced antibiotic compounds. The highest antimicrobial activities were obtained using the butanolic extract. The thin layer chromatography (TLC) method showed two bioactive spots, named HAD1 and HAD2, which were reveled negatively by using chemical revelators (ninhydrin, naphtoresorcinol-sulfuric acid, ferrous iron chloride and formaldehyde-sulfuric). These results indicated the absence of amine group, sugar, hydroxamic acid, phenol and aromatic compound.

Keywords: Actinobacteria, *Saccharothrix*, 16S rRNA, antimicrobial activity, TLC, chemical revelators

INTRODUCTION

Actinobacteria are Gram-positive bacteria with C+G ≥ 55% (**Bergey's Manual, 2012**). These microorganisms have a proven capacity to produce secondary metabolites including various biologically active compounds such as antitumor (**Chang *et al*., 2011**), antioxidant (**Karthik *et al*., 2013**), immunosuppressive and antifungal agents (**Berdy, 2005**). **Subramani and Aalbersberg (2012)** estimated that approximately 70% of microbial secondary metabolites have been obtained from the actinobacteria, especially the genus *Streptomyces* from which a large number of antibiotics used in human therapy were obtained. Because of the increasing resistance of pathogenic microorganisms and toxicity of some antibiotics **(Berdy, 1989)**, many researches around the world have focused on the isolation of new antibiotics. Rare actinobacteria present an excellent resource for the discovery of novel interesting antibiotics **(Lazzarini *et al*., 2001)**. Therefore, various methods are applied for selective isolation of rare taxa. One of strategies used, is the exploration of extreme habitats, which are distributed on a limited basis in the natural environment. In these habitats, the microorganisms developed a specialized metabolism system to adapt to extreme conditions.
The genus *Saccharothrix* is among the most interesting rare actinobacteria. Since its first description **(Labeda *et al*., 1984)**, it has been shown to produce a vast diversity of different metabolites, including the antiviral agent fluvirucin from *Saccharothrix mutabilis* (R869-90) **(Tomita *et al*., 1991)** and the antibacterial agent saccharomicin from *Saccharothrix espanaensis* (NRRL 15764^T) **(Singh *et al*., 2000)**. The Algerian Saharan soils, exposed to an arid climate, constitute one of the most attractive sources of several rare actinobacteria genera such as *Actinomadura*, *Actinopolyspora*, *Amycolatopsis*, *Nocardiopsis*, *Saccharopolyspora* and *Saccharothrix* **(Sabaou *et al*., 1998)**. The presence of *Saccharothrix* genus has been detected in many samples of Algerian Saharan soils **(Sabaou *et al*., 1998; Zitouni *et al*., 2004a, 2005; Boubetra *et al*., 2013)**. It has also been found that *Saccharothrix* strains isolated from these soils produce new antibiotics, such as dithiolopyrrolones **(Lamari *et al*., 2002; Bouras *et al*., 2008; Merrouche *et al*., 2011)**, anthracyclines **(Zitouni *et al*., 2004b)** and chloramphenicol **(Aouiche *et al*., 2012)**. These promising results emphasize the need to continue the research in this direction.
During our ongoing research for new strains producing potential metabolites from Saharan soil samples, we described the isolation and identification, by conventional and molecular methods, of a new actinobacterial strain. In addition, we interpreted the production, extraction and partial purification of its antimicrobial compounds.

MATERIALS AND METHODS

Soil sampling and strain isolation

Nine soil samples were collected from three Algerian Saharan regions (Adrar, Béni-Abbès and Hoggar) and analyzed to isolate four genera of rare actinomycetes (*Saccharothrix*, *Nocardiopsis*, *Actinomadura* and *Nonomuraea*). In total, 11 actinobacterial strains were isolated. Six strains (including ABH26) with the same *Nocardiopsis* or *Saccharothrix* morphology and five strains with the same *Actinomadura* or *Nonomuraea* morphology were purified. The actinobacterial strain ABH26 was isolated from a Saharan soil collected in Adrar (Southern Algeria, latitude 27°52'N, longitude 0°17'W, altitude 282 m). The physico-chemical analysis showed that this soil is slightly alkaline (pH = 7.7), unsalted (electrical conductivity = 0.7 mS cm^{1-}) and contains a low proportion of carbon (0.62%). One gram of dry soil was suspended in 9 ml of sterile deionized water. Serially diluted sample was prepared and aliquots (0.1 ml) of each dilution were plated on chitin-vitamins B-agar medium recommended for isolation of rare actinobacteria **(Hayakawa and Nonomura, 1987)**. The medium was supplemented with 80 mg/L of cycloheximide to inhibit development of invasive fungi. Tow antibacterial agents were also added, polymyxin and penicillin, each at 25 mg/L. The plates were incubated at 30°C for three weeks. During the incubation period, all colonies were examined directly by light microscopy

(Zeiss). One colony was isolated, designated ABH26, that presented micromorphological characteristics of *Saccharothrix*. After isolation and purification, the strain was preserved on yeast-malt-extract agar **(Shirling and Gottlieb., 1966)**.

Morphological and cultural characteristics

Morphological and cultural characteristics of strain ABH26 were determined on the International *Streptomyces* Project media: yeast extract-malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch (ISP4) **(Shirling and Gottlieb., 1966)** and the Bennett medium. After growing at 30°C for 14 days, morphological characteristics were observed by the naked-eye and by using a light microscopy. The ISCC-NBS color name chart **(Kelly and Judd, 1976)** was used to determine the colors of areal mycelium, substrate mycelium and diffusible pigments.

Physiological characteristics

Utilization of carbohydrates as sole carbon source, degradation of hypoxanthine, tyrosine, citrate and lactate were examined as described by **Gordon *et al.* (1974)**, while degradation of starch and production of nitrate reductase were evaluated according to the methods of **Marchal and Bourdon (1973)** and **Marchal *et al.* (1978)**, respectively. Temperature range (25-52°C), tolerance to NaCl (0-7%, w/v) for growth were determined on ISP2 medium.

DNA extraction, PCR amplification and 16S rRNA sequencing

Strain ABH26 was grown in ISP2 broth, and genomic DNA was extracted with a DNA extraction kit (Master Pure Gram-Positive DNA Purification kit, Epicentre Biotechnologies) as described by **Li *et al.* (2007)**. PCR amplification of the 16S rRNA gene sequence of strain ABH26 was performed as described by **Rainey *et al.* (1996)** by using two universal primers: 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3'). The PCR amplification was conducted using a thermocycler (STRATAGENE RoboCycler Gradient 96) in a 50 µl containing 1.25 U of Taq DNA polymerase, 1 µl (500 ng) of purified DNA, 1 × PCR buffer (10 mM of Tris–HCl, 50 mmol of KCl, pH 9.0 at 25°C), 1.5 mmol of $MgCl_2$, 200 µmol of each dNTP and 1 µmol of each primer. The amplification cycle consisted of three steps: an initial denaturation at 98°C for 3 minutes, after which *Taq* DNA polymerase was added, followed by 30 amplification cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min, and a final extension step of 72°C for 10 min. The PCR product was detected by agarose gel electrophoresis and was visualized by ultraviolet (UV) fluorescence after ethidium bromide staining. PCR products were purified with a PCR product purification kit (Qiagen). The PCR products were sequenced using the same primers as above and an automated sequencer (model 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. The sequence obtained was compared for similarity with the reference species in the public sequence databases and with the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) **(Kim *et al.*, 2012)**, a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains.

Phylogenetic analysis

Phylogenetic analyses were conducted using the program Molecular Evolutionary Genetics Analysis version 5, MEGA5 **(Tamura *et al.*, 2011)**. The 16S rRNA sequence of strain ABH26 was aligned using the CLUSTAL W program **(Larkin *et al.*, 2007)** against corresponding nucleotide sequences of representatives of the *Saccharothrix* genus retrieved from EzTaxon-e server. Evolutionary distance was generated as described by **Jukes and Cantor (1969)** and a phylogenetic tree was inferred by the neighbor-joining method **(Saitou and Nei, 1987)**. Tree topologies were evaluated by bootstrap analysis **(Felsenstein, 1985)**, based on 1000 re-samplings of the neighbor-joining dataset.

Antimicrobial assay

The target microorganisms used for determining the antimicrobial spectrum of the new actinobacterial strain were the filamentous fungi: *Aspergillus carbonarius* (M333), *A. flavus* (NRRL 3251), *A. westerdijkiae* (ATCC 3174), *A. parasiticus* (CBS 100926), *Fusarium culmorum* (Fc), *F. equiseti* (Fe), *F. graminearum* (Fg), *F. oxysporum* f. sp. *albedinis* (Foa), *F. oxysporum* f. sp. *radicis lycopersici* (Forl), *F. solani* (Fsol), *F. sporotrichioides* (Fs), *Rhizoctonia solani* (AG3) and *Umbelopsis ramanniana* (NRRL 1829). The isolates of pathogenic yeast *Candida albicans* (M2, M3 and IPA200) were isolated from diseased patients in two hospitals located in Algiers (hospital Bachir Mentouri and Institut Pasteur d'Alger). The target bacteria *Bacillus subtilis* (ATCC 4226), *Escherichia coli* (E52), *E. coli* (E195), *Klebsiella pneumonia* (E40) and methicillin resistant *Staphylococcus aureus* (MRSA 639c) were also used to search for antibacterial activity. The target microorganisms without a collection number were from our laboratory collection. The streak assay method was used for primary screening of antimicrobial activity. The strain was first cultivated in a straight line on ISP2 medium at 30°C for 10 days. At the end of the incubation period, the target microorganisms were seeded in streaks perpendicular to the cultured actinobacterium cultivated. Inhibition distance between target microorganisms and the actinobacterial strain were measured after incubation at 30°C for 24 hours for bacteria and yeasts, and 48 hours for filamentous fungi.

Kinetics of antimicrobial production, pH and biomass

The producing strain ABH26 was cultured on a rotary shaker (250 rpm, 30°C) for 48 hours in a 250 ml Erlenmeyer flask containing 50 ml of ISP2 liquid medium. Three ml of the seed culture was transferred into a 500 ml Erlenmeyer flask containing 100 ml of the same medium. The aliquots were collected each day by centrifuging (Sigma mini-centrifuges) 4 ml of homogenized culture broth in Eppendorf tubes for 10 min at 16000 ×*g*. The centrifugate was used for determining the evolution of biomass and the supernatant was used to determine pH and antimicrobial activity. The antagonistic activity against *S. aureus*, *C. albicans*, *F. culmorum* and *U. ramanniana* was evaluated every 24 hours during 12 days of fermentation by the agar diffusion method (well technique). Wells (10 mm in diameter) made in the ISP2 agar plates (12 g/L of agar) received 100 µl of the cell-free supernatant samples. After incubation, zones of inhibition (mm in diameter) were recorded.

Production, extraction, detection and chemical characterization of antimicrobial compounds

The strain ABH26 was cultivated in three 500 ml Erlenmeyer flasks each containing 100 ml of liquid ISP2 medium, and incubated at 30°C, under constant agitation of 250 rpm. The extraction of bioactive compounds took place on the day of optimal production (at day 8 of fermentation). The fermentation broth (300 ml) was centrifuged at 8000 rpm for 20 min at 25°C to remove the mycelium. The cell-free supernatant was divided into four equal volumes of 60 ml, and then each was extracted with an equal volume of four organic solvents: *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The organic extracts were evaporated to dryness under vacuum on a Rotavapor R-205 (Buchi, Switzerland). The residues were dissolved in 1 ml of methanol and subjected to biological assay (paper disk of 6 mm in diameter, Institute Pasteur) against *S. aureus*, *C. albicans* and *U. ramanniana*. The disks received 60 µl of each extract and were placed on the ISP2 medium (12 g/L agar) inoculated with target microorganism. Inhibition zones were expressed as diameter and measured after incubation at 30°C for 24 hours for *S. aureus* and *C. albicans*, and for 48 hours for *U. ramanniana*. A paper disk containing an identical volume of methanol was used as control. After that, 20 × 20 cm silica gel plates (Merck Art. 5735, Kiesselgel 60HF 254-365) were prepared, and then activated at 105 °C for one hour. A volume of 60 µl of each organic extract was spotted on these plates, which were developed with system solvent: ethyl acetate/methanol (100:15 v/v). The developed TLC plates were air-dried overnight at 40°C to evaporate all solvent. The separated compounds were visualized with the naked-eye as colored spots and also under ultraviolet (UV) at 254 (absorbance) and at 365 nm (fluorescence). For detecting the active spots, we used the method of **Betina (1973)** known as bioautography. Three TLC plates were placed in plastic bioassay dishes (23 × 23 × 2.2 cm^3) and overlaid with 100 ml/plate of ISP2 medium (containing 7 g/L of agar) seeded with *S. aureus*, *C. albicans* and *U. ramanniana*, separately. After the incubation period, clear areas due to inhibition of microorganism growth indicated the location of antimicrobial compounds on the TLC plates. The retention factor (RF) values of each active spot were recorded. The antimicrobial compounds were revealed on silica gel plates using the chemical revelators: ninhydrin, naphtoresorcinol-sulfuric acid, ferrous iron chloride and formaldehyde-sulfuric acid as reported by **Boudjella *et al.* (2006)**.

RESULTS

Strain ABH26 formed well-branched substrate mycelia without fragmentation on agar media. Arial mycelium is well developed and fragmented anarchically, often with zigzag into long chains of non-motile and elongated spores with different sizes, on ISP2, ISP3, ISP4 and Bennett medium (figure 1).

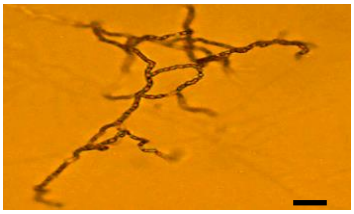

Figure 1 Micromorphology with light microscopy of spore chains of strain ABH26 grown on ISP2 for 10 days at 30 °C, bar 10 µm.

Endospores, sclerotic granules and synnemata were not observed. ABH26 showed a good growth on ISP2, ISP3 and Bennett, and moderate growth on ISP4 medium. The color of the aerial mycelia is white to pale yellow-pink on most media; substrate mycelia are orange to yellow-brown and produce an orange soluble pigment on ISP2 but pale brown on Bennett medium. The morphological and cultural characteristics of stain ABH26 are given in Tab 1.

Table 1 Macromorphological characteristics of strain ABH26 on different media after 14 days of incubation

Agar medium	Growth	Production and color of:		Soluble pigment
		Aerial mycelium	Substrate mycelium	
ISP2	+++	+ white	Pale orange	Orange
ISP3	+++	++ to +++ yellow-pink	Orange	–
ISP4	+ to ++	+ to ++ white-yellow	Pale orange	–
Bennett	+++	+ to ++ yellow-pink	Yellowish gray	Moderate brown

+: weak, ++: moderate, +++: strong, –: no production.

The physiological studies revealed that the strain ABH26 utilized L-arabinose, D-fructose, D-glucoside, D-glucose, D-lactose, D-mannose, D-manitol, α-methyl-D- glucoside, raffinose, L-rhamnose, D-sucrose and D-xylose as sole carbon source, but not adonitol, inositol, melibiose as can be seen from the physiological characters given in Tab 2. Citrate, starch and tyrosine were hydrolyzed, but acetate, hypoxanthine and lactate are not. The test was negative for production of nitrate reductase. Growth occurs at 28–45°C and tolerates up to 3 % (w/v) of NaCl.

Table 2 Physiological characteristics of the strain ABH26 in comparison with the most closely related species *Saccharothrix xinjiangensis* NBRC 101911^T

Tests	ABH26	*Saccharothrix xinjiangensis* NBRC 101911^T
Carbon source utilization:		
L-arabinose	+	+
Melibiose	–	+
α-Methyl-D-glucoside	+	+
D-lactose	+	+
Raffinose	+	+
L-rhamnose	+	+
D-sucrose	+	+
D-xylose	+	+
Hydrolysis of:		
Citrate	+	–
Lactate	–	–
Starch	+	+
Tyrosine	+	–
Nitrate reduction	–	–
Growth in the presence of:		
5 % NaCl (w/v)	–	–
Growth at 50 °C	–	+

– : negative, + : positive.

Through 16S rRNA sequence analysis, an amplified fragment of 1438 base pairs was obtained and compared with sequences of the reference species of bacteria contained in EzTaxon-e server. Phylogenetic analysis showed that strain ABH26 had 16S rRNA gene sequence similarities ranging from 97.63% (with *Saccharothrix violaceirubra* NBRC 102064^T) to 99.86% (with *Saccharothrix xinjiangensis* NBRC 101911^T). The phylogenetic tree obtained by applying the neighbor-joining method is illustrated in figure 2. The GenBank /EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ABH26 is KJ572276.

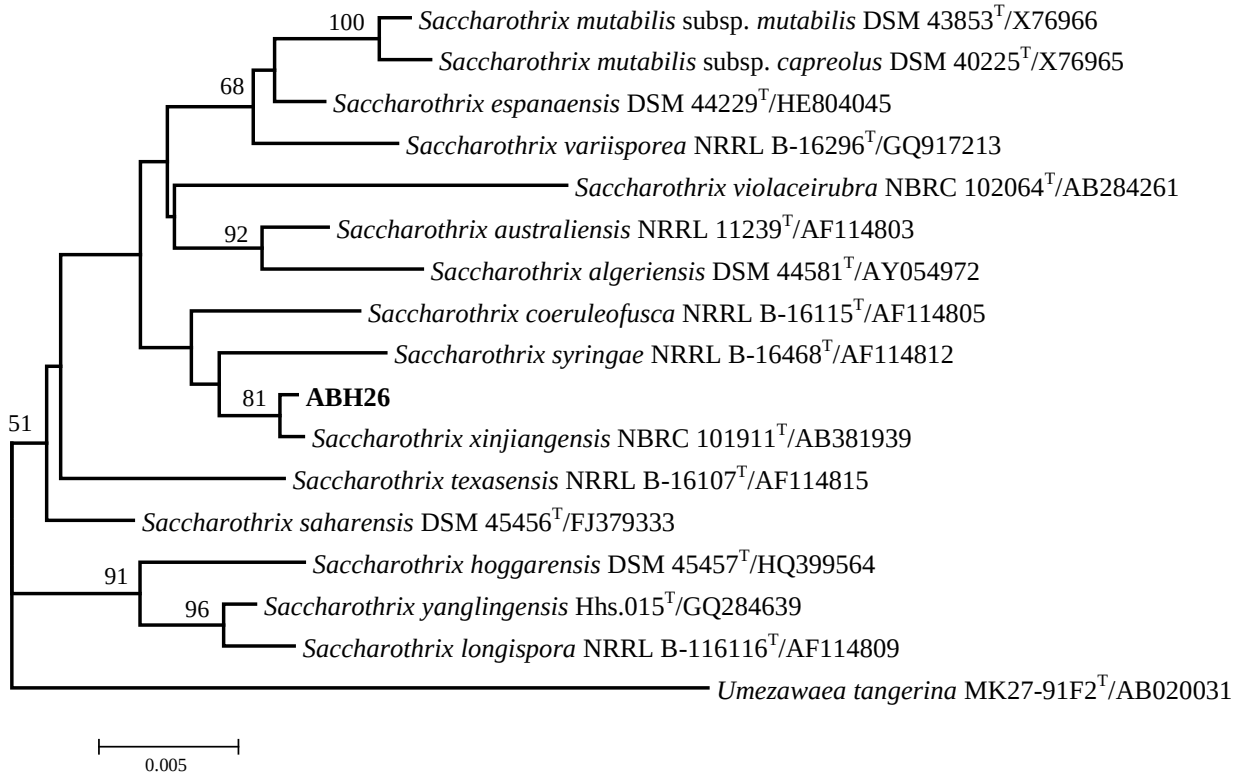

Figure 2 Phylogenic tree based on 16S rRNA gene sequences showing relationships among strain ABH26 and all type strain species of *Saccharothrix*. Numbers at nodes indicate percentages of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets: only values above 50% are given. Bar, 0.005 substitutions per nucleotide position.

The antimicrobial activity of strain ABH26 against target microorganisms is shown in Tab 3. ABH26 presented an important activity against all filamentous fungi and yeasts tested. The strain exhibited a strong activity against the Gram-positive bacterium *Bacillus subtilis* (ATCC 4226), a moderate activity against *Staphylococcus aureus* resistant to methicillin (MRSA 639c). However, almost no activity was observed against Gram-negative bacteria *Escherichia coli* (E52), *E. coli* (E195) and *Klebsiella pneumonia* (E40).

Table 3 Antimicrobial activity of strain ABH26 by the streak assay method on ISP2 medium

Target microorganisms	Inhibition zone (mm)
Aspergillus carbonarius (M333)	33
Aspergillus flavus (NRRL 3251)	21
Aspergillus westerdijkiae (ATCC 3174)	20
Aspergillus parasiticus (CBS 100926)	17
Fusarium culmorum (Fc)	28
Fusarium equiseti (Fe)	24
Fusarium graminearum (Fg)	24
Fusarium oxysporum f. sp. *albedinis* (Foa)	27
Fusarium oxysporum f. sp. *radicis lycopersici* (Forl)	22
Fusarium solani (Fsol)*	27
Fusarium sporothrichioides (Fs)	26
Rhizoctonia solani (AG3)**	28
Umbelopsis ramanniana (NRRL 1829)	32
Candida albicans (M2)	22
Candida albicans (M3)	30
Candida albicans (IPA200)	23
Bacillus subtilis (ATCC 4226)	22
Escherichia coli (E52)	0
Escherichia coli (E195)	0
Klebsiella pneumonia (E40)	0
Staphylococcus aureus (MRSA 639c)	15

*: Moroccan isolate, **: Canadian isolate.

During the time course of fermentation on ISP2 broth, the antimicrobial activity was evaluated against *Umbelopsis ramanniana* (NRRL 1829), *S. aureus* and *Candida albicans* (M3) using the agar diffusion method (well technique). The biological activity started after 2 days against the *S. aureus* and against *U. ramanniana*; however, the activity against *C. albicans* was detected on day 5 of fermentation. The maximum antifungal activity was decreased after 7 days; while, the maximum activity against the bacterium and yeast was located on day 8. The pH varied between 6.1 and 8.2 during the fermentation. The biomass increased during the first days and reached the maximum after 4 days, then decreased after 5 days of fermentation (figure 3).

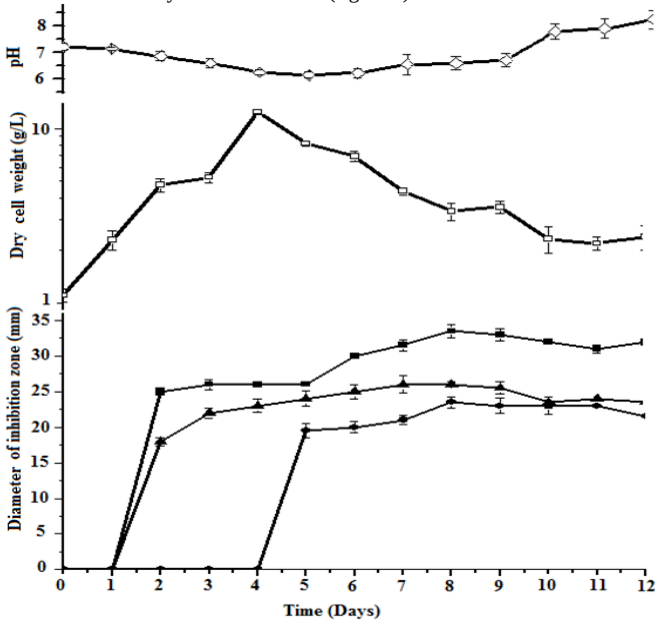

Figure 3 Kinetics of pH, dry cell weight and antimicrobial activity against *S. aureus* (MRSA639c) (■), *U. ramanniana* (NRRL 1829) (▲) and *Candida albicans* (M3) (●).

After 8 days of fermentation, the antimicrobial activity of different organic solvents (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol) was evaluated by paper disk method against *S. aureus*, *C. albicans* and *U. ramanniana*. The higher antimicrobial activities were extracted by the butanolic extract, followed by dichloromethane and ethyl acetate in comparison to methanol control which did not manifest any growth inhibitory zone. However, no activity was observed on the *n*-hexane extract. The resulting inhibition zones are illustrated in figure 4 and Tab 4.

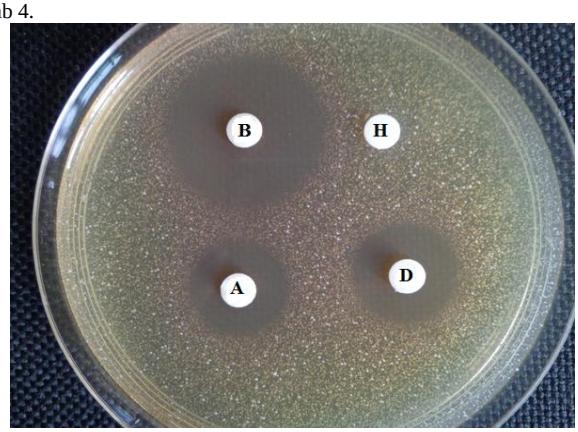

Figure 4 Antimicrobial activities of *n*-butanol (B), ethyl acetate (A), dichloromethane (D) and *n*-hexane (H) extracts against *Candida albicans* (M3) by paper disk method.

The profile of silica gel thin layer chromatography (TLC) showed the presence of several spots, but only those noted HAD1 and HAD2 (with retention factor values of 0.75 and 0.84, respectively) were active. The spot HAD1 was extracted only by *n*-butanol, and showed a yellow color under UV (365 nm); however HAD2 was extracted by ethyl acetate and dichloromethane, and showed a purple blue color under UV (365 nm). The both spots HAD1 and HAD2 exhibited a strong activity against *S. aureus*, *C. albicans* and *U. ramanniana*, though HAD1 was more active than HAD2 (data not shown).

DISCUSSION

Various methods were used for selective isolation of rare actinobacteria, for example, by dry heating of soil samples to obtain *Actinomadura* strains **(Zakharova *et al.*, 2003)**, or by freezing of marine sediment samples to get *Actinocorallia*, *Actinomadura*, *Knoellia*, *Glycomyces*, *Nocardia*, *Nonomuraea*, *Pseudonocardia*, *Rhodococcus* and *Streptosporangium* strains **(Bredholdt *et al.*, 2007)**. Particular media were also used, such as chitin-vitamins B which contains chitin as sole source of carbon and nitrogen. This substrate is better degraded by actinobacteria compared to non-mycelial bacteria. Generally, rare bacteria are auxotrophic and B vitamins were added to stimulate growth. Our group research demonstrated the high selectivity of chitin-vitamins B medium to isolate new strains of rare actinobacteria, producing new potential antibiotics **(Lamari *et al.*, 2002; Zitouni *et al.*, 2004b; Bouras *et al.*, 2008; Merrouche *et al.*, 2011; Aouiche *et al.*, 2012)**. Other studies confirmed the performance of this medium such as **Pathom-aree *et al.* (2006)** and **Zhang *et al.* (2010)**. The strain *Saccharothrix* sp. ABH26 was obtained on chitin-vitamins B agar supplemented by two selective agents (polymyxin and penicillin). On the basis of its morphological properties, this strain represents a member of the genus *Saccharothrix*. It has an aerial mycelium with anarchically fragmentation to produce long chains of non-motile and elongated spores. The 16S rRNA sequence of strain ABH26 was compared with those of other *Saccharothrix* species. The similarity level ranged from 97.63% with *Saccharothrix violaceirubra* NBRC 102064^T to 99.86% with *Saccharothrix xinjiangensis* NBRC 101911^T which is the most closely related species. According to **Stackebrandt and Goebel (1994)**, two microorganisms have less than 97% 16S rRNA similarity belong to the different species. However, DNA-DNA hybridization is used for species that share over of 97% similarity. In the genus *Saccharothrix*, many species share 16S rRNA gene similarities within the range of 97.5 to 98.9%, but have levels of DNA-DNA relatedness well below 70% (the relatedness guideline proposed by **Wayne *et al.* (1987)** for delineation of separate species). For example, the type strain *S. hoggarensis* DSM 45457^T exhibited 16S rRNA gene sequence similarity of 98.9% with the most closely related type strain *Saccharothrix longispora* NRRL B-16113^T (but shared a DNA-DNA association value of 16.05%) **(Boubetra *et al.*, 2013)**. In addition, the type strains of *S. algeriensis* DSM 44581^T and *S. australiensis* DSM 43800^T with a 16S rRNA similarity value of 98.8% share a DNA-DNA relatedness value of 55.9% **(Zitouni *et al.*, 2004a)**. Furthermore, the studied strain *Saccharothrix* sp. ABH26 differs from the closely related species *S. xinjiangensis* NBRC 101911^T not only by cultural characteristics, but also by physiological characteristics as given in Tab 2.

Table 4 Antimicrobial activity of *Saccharothrix* sp. ABH26 against *S. aureus* (MRSA 639c), *C. albicans* (M3) and *U. ramanniana* (NRRL 1829) by using of different organic solvents

Target microorganism	Zone of inhibition (mm) by using different solvents			
	n-hexane	dichloromethane	ethyl acetate	*n*-butanol
S. aureus	0	24	27	38
C. albicans	0	20	19	31
U. ramanniana	0	22	21	32

Strain ABH26 showed inhibitory activity against both Gram-positive bacteria and mycotoxigenic and phytopathogenic fungi. Several studies reported the production of several antimicrobial compounds by *Sacchrothrix* strains against fungi and Gram-positive bacteria, such as a phthalate derivative **(Johnson *et al*., 2012)** and tianchimycins A and B **(Wang *et al*., 2013)**. Strain ABH26 was also active against pathogenic strains of *C. albicans* (M3) which are known to be resistant to antibiotics amphotericine B, cycloheximide, itraconazole, nystatine, thioconazole and terbinafine **(Aouiche *et al*., 2012)**. The saccharomicins, produced by a strain of *Saccharothrix espanensis* (NRRL 15764), has no activity against *C. albicans* (GC 3066) **(Singh *et al*., 2000)**; however, the antibiotic saccharothrixmicine produced by another strain of *S. espanensis* (An 113) exhibited a biological activity towards *C. albicans* **(Kalinovskaya *et al*., 2010)**. The obtained results showed no activity of ABH26 against *Escherichia coli* (E52), *E. coli* (E195) and *Klebsiella pneumonia* (E40). In general, Gram-negative bacteria are more resistant to antibacterial compounds than Gram-positive bacteria. Several studies showed that the outer cell membrane in Gram-negative bacteria (double membranes) contains many protective mechanisms against antibiotics **(Gupta, 2011)**. Moreover, the increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids that can readily spread through bacterial populations **(Carattoli *et al*., 2006; Carattoli, 2009)**. For our strain *Saccharothrix* sp. ABH26, the highest activities were observed after 7 to 9 days of fermentation. It means that the strain grow firstly to form a considerable amount of biomass flowed by the production of antibiotics. Several studies reported that some actinobacterial strains produce the antibiotics in the first days of fermentation, such as arylomycine which is produced by a strain of *Streptomyces* sp. (Tü 6075) after 3 days of incubation **(Schimana *et al*., 2002)**. However, other strains produce antibiotics only after 12 days of fermentation, for instance, pradimicine S by a strain of *Actinomadura spinosa* (AA0851) **(Saitoh *et al*., 1993)**. The *n*-hexane extract showed no antimicrobial activity. This shows the non-lipid nature of the bioactive metabolite(s) produced by *Saccharothrix* sp. ABH26.

The active compounds HAD1 and HAD2 were revealed negatively with ninhydrin, naphtoresorcinol-sulfuric acid, ferrous iron chloride and formaldehyde-sulfuric. These results showed the absence of amine group, sugar, hydroxamic acid, phenol and aromatic compound. The chloramphenicol produced by *Saccharothrix* sp. (PAL54) contains aromatic and amine groups **(Aouiche *et al*., 2012)**, however, the mutactimycin PR produced by *Saccharothrix* sp. (103) contains polycyclic aromatic with amine groups **(Zitouni *et al*., 2004b)**. The type strain *S. espanaensis* NRRL 15764^T is known by its production of saccharomicins with phenol and sugars. The dithiolopyrrolone antibiotics from *S. algeriensis* DSM 44581^T contains amine functions but not sugars, hydroxamic acids, phenols and aromatic compounds. *S. xinjiangensis* NBRC 101911^T, the most closely species of the strain ABH26 produces tianchimycin A without amines, sugars, hydroxamic acids, phenols and aromatic compounds. Unlike our active compounds, the tianchimycin A has been shown to be active against Gram-negative bacteria **(Wang *et al*., 2013)**. For this, the antimicrobial compounds produced by the strain ABH26 may represent new secondary metabolites, which will be confirmed by further studies.

CONCLUSION

The results of current research revealed the taxonomic position of strain ABH26 as a member of *Saccharothrix* genus, and give support to the biotechnological use of the rare actinobacteria as source of natural products. These molecules could be beneficial on plant and human therapy. However, further studies will be necessary to determine the structure, the activity mechanism and the toxicity of these compounds.

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