A NEW *Streptomyces* STRAIN AHA8 FROM DESERT SOIL: ISOLATION, TAXONOMIC IDENTIFICATION, AND ANTAGONISTIC PROPERTIES

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Abstract. Actinobacteria from extreme environments represent a valuable source of novel bioactive secondary metabolites with potential antimicrobial applications. The aim of this study was to isolate, identify, and evaluate the antimicrobial potential of a newly isolated strain of actinobacteria. AHA8 strain, was isolated from an Algerian desert soil sample in the Ahaggar region (Tamanrasset) by the dilution-agar plating method using a chitin-vitamins B medium supplemented with nalidixic acid and actidione. Morphological characterization indicated that this strain belonged to the genus *Streptomyces*. Phylogenetic analysis based on the 16S rRNA gene showed that the strain is closely related to *Streptomyces djakartensis* NBRC 15409^T (99.57% sequence similarity). Strain AHA8 showed very strong activity against pathogenic staphylococci, including *Staphylococcus aureus* (MRSA 639c), as well as against other Gram-positive bacteria: *Bacillus subtilis* (ATCC 663) and *Listeria monocytogenes* (ATCC 13932). Additionally, the strain exhibited a moderate activity against mycotoxigenic and phytopathogenic fungi, including *Aspergillus carbonarius* (M333), *A. westerdijkiae* (ATCC 3174), *Fusarium culmorum* (Fc), and *Umbelopsis ramanniana* (NRRL 1829). Almost the same results were observed for Gram-negative bacteria and the pathogenic yeast *Candida albicans* (M3). Therefore, four solvents (*n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol) were used to extract the bioactive secondary metabolites produced by the AHA8 culture on a synthetic starch medium. The highest antibacterial activity was obtained with the butanolic and ethyl acetate extracts. These findings demonstrate that the desert-derived strain AHA8 represents a promising source of antimicrobial secondary metabolites and supports further studies aimed at the purification and characterization of its bioactive compounds.

Keywords: 16S RNA gene; Actinobacteria; Algerian Saharan soil; Antimicrobial activity; *Streptomyces*.

INTRODUCTION

The phylum Actinomycetota, formerly known as Actinobacteria, was reclassified and renamed based on recent taxonomic revisions [42]. They are Gram-positive bacteria with high GC-containing genomes [19]. This phylum of bacteria has been extremely useful to the pharmaceutical industry due to their seemingly unlimited capacity to produce secondary metabolites with diverse chemical structures and biological activities [12, 46]. The genus *Streptomyces* was first described by Waksman and Henrici [51]. After a period of classification and reclassification, this genus currently consists of 1436 species and 87 subspecies with validly published names [35]. The exploration of unexplored biotopes and habitats from extreme environments is one of several research programs established to obtain new strains and potentially new bioactive secondary metabolites [40, 52].

The Algerian Saharan soils, characterized by an arid climate, are among the richest and most diverse ecosystems for isolating new Actinobacterial strains [8, 39, 44]. Many new strains of *the Streptomyces* genus have been isolated from samples of Algerian Saharan soils. Furthermore, it has been found that *Streptomyces* strains isolated from Algerian desert soils produce new antibiotics, such as saquayamycins A and C [2], hydroxamic acid derivatives [53], actinomycin D [50], 2,4-di-tert-butylphenol [5], furanone derivative [16], oligomycins A and E [27], and β- and γ-rubromycin [6].

In the present study, we describe the taxonomy of a new *Streptomyces* strain isolated from Algerian Saharan soil, which exhibits interesting antimicrobial activity. Fermentation on different culture media and the extraction of antimicrobial activity are also reported.

MATERIALS AND METHODS

Strain isolation and maintenance

The actinobacterial strain AHA8 was isolated from a Saharan soil sample collected from the Ahaggar region (GPS: 22°45'36.02N, 5°51'10.39E) (Tamanrasset, Southern Algeria), by serial dilution agar plating method using chitin-vitamin B agar [22]. The medium was supplemented with actidione (80 mg/L) to prevent growth of micro-fungi, and nalidixic acid (25 mg/L) to inhibit the growth of Gram-negative bacteria. The plates were incubated at 30°C for 20 days. A pure colony was isolated, designated AHA8, and maintained at 4°C on ISP2 (International *Streptomyces* Project 2) medium slants composed of 4 g yeast extract, 10 g malt extract, and 4 g glucose in 1 liter of distilled water, at pH: 7.2 [47].

Morphological and cultural characteristics

Morphological and cultural characteristics of strain AHA8 were determined on ISP2, ISP4, and chitin-vitamin B agar media after growing at 30°C for 14 days. The morphological characteristics were observed by the naked eye and by using a light microscope. The ISCC-NBS color name chart [26] was used to determine the colors of aerial and substrate mycelia and diffusible pigments.

Phenotypic characteristics

Utilization of carbohydrates as a sole carbon source, degradation of tyrosine, citrate, gelatin, and lactate were examined as described by Gordon *et al.* [20], while production of nitrate reductase and degradation of starch

DOI: 10.61215/AUOFB.2026.2.03

were evaluated according to the methods of Marchal and Bourdon [37] and Marchal *et al.* [36], respectively. Furthermore, growth at 50°C and in the presence of 5% (w/v) NaCl was determined. The enzymatic profile of strain AHA8 was determined using standard biochemical tests. The activities of arginine dihydrolase, β-galactosidase, gelatinase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, and urease were evaluated according to conventional microbiological procedures [47]. Analysis are performed in three replicates.

DNA extraction, PCR amplification, and 16S rRNA sequencing

Actinobacterial strain AHA8 was grown in ISP2 broth, and genomic DNA was extracted according to the method of Li *et al.* [34]. The 16S rRNA gene was PCR amplified using an Eurogentec kit and primer pair 10-30F (5'-GAGTTTGATCCTGGCTCA-3') and 1500R (5'-AGAAAGGAGGTGATCCAGCC-3'). The PCR reactions were carried out in a final volume of 30 µL of reaction mixture containing approximately 50 ng of genomic 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 amplification cycle consisted of the following steps: 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, extension at 72°C for 2 min, and then final extension at 72°C for 10 min. Amplification products were checked by electrophoresis on 1% agarose and stained with safe view. The PCR products were sequenced by Genewiz Company, Ltd. (Takeley, UK) using the same primers as above. The sequence obtained was submitted to the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) [54], a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains.

Phylogenetic analysis

Phylogenetic analysis was conducted using MEGA 7.0 software [31]. The 16S rRNA gene sequence of strain AHA8 was aligned against neighboring nucleotide sequences using the CLUSTAL W program [32]. Evolutionary distance matrices were generated as described by Jukes and Cantor [25], and a phylogenetic tree was inferred using the neighbor-joining method of Saitou and Nei [45]. The tree topology was evaluated by a bootstrap test [17] based on 1000 re-samplings of the neighbor-joining dataset.

Antagonistic activity of strain AHA8

The actinobacterial strain AHA8 was tested for antagonistic activity against several target microorganisms using the agar plug diffusion method [41]. The filamentous fungi: *Aspergillus carbonarius* (M333), *A. westerdijkiae* (ATCC 3174), *Fusarium culmorum* (Fc) and *Umbelopsis ramanniana* (NRRL 1829) were used to evaluate the antifungal activity. The pathogenic yeast *Candida albicans* (M3) was also included in this study. The target-bacteria used were *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Acinetobacter baumannii* (S54), *Bacillus subtilis* (ATCC 663), *Listeria monocytogenes* (ATCC 13932), sensitive *Staphylococcus aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA 639c). All the target-microorganisms tested were from the LBSM (Laboratoire de Biologie des Systèmes Microbiens, Algiers) collection and were mostly pathogenic or toxigenic for humans, or phytopathogenic strains. An agar culture of the AHA8 strain was made on ISP2 medium by tight streaks on top of the solidified medium and incubated for 10 days (at 30°C). The agar cylinders were cut aseptically with a sterile cork borer (10 mm in diameter) from the AHA8 culture and deposited on the agar surface of MH (Müller-Hinton) and PDA (potato dextrose agar) plates for bacteria and micro-fungi, respectively. These semi-solid culture media were previously inoculated with target microorganisms (10^6 CFU). In the case of fungi, the inoculum was prepared from a fresh fungal culture and suspended in sterile physiological solution supplemented with Tween 20 (0.05%) to ensure proper spore dispersion before inoculation. After a 2h diffusion process at 4°C, the plates were incubated for 24 h (at 37°C) for bacteria and for 48 h (at 25°C) for micro-fungi. After incubation, the antimicrobial activity was evaluated by measuring the inhibition zone around the agar plug. Analysis were performed in triplicate.

Kinetics of pH, biomass, and antimicrobial production by AHA8

During the fermentation time course on the starch synthetic medium (SS medium), antimicrobial activity was evaluated against three target bacteria. SS medium contained in 1 liter of distilled water, 10 g starch, 2 g NaCl, 1 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $MgSO_4$ $7H_2O$, 2 g $CaCO_3$, and 2 g $(NH_4)_2SO_4$ [7]. The final pH of each medium was adjusted at 7.2, and the starch was autoclaved separately and added aseptically to the remaining medium before inoculation. Each 250 mL Erlenmeyer flask (containing 50 mL of each medium) was inoculated with 6 agar plugs (6 mm in diameter) using a sterile cork borer (from a 10-day culture ageing growing on ISP2 medium) at 30°C for 48 h on a rotary shaker (New Brunswick Scientific Co., NJ, USA) at 250 rpm. This pre-culture was then homogenized, and 3 mL was used to inoculate 100 mL of the same medium in a 500 mL Erlenmeyer flask. These cultures were incubated under the same conditions (30°C, 250 rpm) for 10 days.

Culture broth samples were taken every 24 h of fermentation. The pH value of the fermentation broth was measured with a pH meter (HANNA TH112).

The dry cell weights (DCWs) were measured according to Bouras *et al.* [7]. The dry cell weights (DCWs) were determined by centrifuging (Sigma mini-centrifuges, 1-14) 4 mL of homogenized culture broth in pre-weighed 2 Eppendorf tubes for 10 min at 16 000 *g*. The pellet was washed three times with HCl (0.35 mM) to remove the remaining $CaCO_3$, followed by distilled water. The Eppendorf tubes containing the pellet were

DOI: 10.61215/AUOFB.2026.2.03

dried at 105°C for 24 h, cooled in a desiccator, and weighed. The results were expressed as grams per liter.

The antagonistic activity against *Escherichia coli* (ATCC 8739), *Listeria monocytogenes* (ATCC 13932), and methicillin-resistant *Staphylococcus aureus* (MRSA 639c) was recorded every 24 h for 10 days of fermentation using the agar diffusion method (well technique). Each well, 10 mm in diameter, made in MH agar plates (10 g/L of agar) previously inoculated with target-microorganisms (10^6 CFU), was filled with 100 µL of the cell-free supernatant from AHA8 and was maintained for 2 h at 4°C. Inhibition zones (diameter in mm) were recorded after 24 h of incubation at 30°C. Analysis were performed in triplicate.

Production, extraction, detection, and chemical characterization of antimicrobial compounds

The strain AHA8 was cultivated in three 500 mL Erlenmeyer flasks, each containing 100 mL of liquid SS medium, and incubated at 30°C, under constant agitation on a rotary shaker (250 rpm). The extraction of bioactive secondary metabolites was performed on the day of maximal production (day 5 of fermentation). The fermentation broth (300 mL) was centrifuged (Refrigerated centrifuge, Jouan E96) at 5000 *g* for 20 min at 25°C to remove the mycelium. The cell-free supernatant was divided into four equal volumes of 60 mL, and each was extracted with an equal volume of four organic solvents: n-hexane, dichloromethane, ethyl acetate, and n-butanol to test effectiveness. The organic extracts were evaporated to dryness under vacuum on a Rotavapor R-210 (Buchi, Switzerland). The resulting dry extracts were dissolved in 1 mL of methanol and subjected to a biological assay (paper disk of 6 mm in diameter, Institute Pasteur) against *Escherichia coli* (ATCC 8739), *Listeria monocytogenes* (ATCC 13932) and methicillin-resistant *S. aureus* (MRSA 639c). The disks received 60 µL of each extract and were placed on ISP2 medium (12 g/L agar) inoculated with the target bacteria. Inhibition zones were measured as diameters after incubation at 30°C for 24 hours. A paper disk containing an identical volume of methanol was used as a control.

Statistical analysis

The results presented in this work are expressed as the standard deviation of the mean of three repetitions. The mean were compared using IBM SPSS statistics software version 27.0, one-way ANOVA analysis of variance, followed by HSD of Tukey-Kramer test was used in this study. A $p \leq 0.05$ value was considered statistically significant.

RESULTS

Identification and characteristics of the strain AHA8

Strain AHA8 showed good growth on ISP4 and chitin-vitamin B agar media, and moderate growth on ISP2 medium. The color of the aerial mycelia is gray to bluish (or brownish) gray on tested media; substrate mycelia are bright orange to yellow-orange and produce soluble pigment on used culture media. The morphological and cultural characteristics of strain AHA8 are given in Table 1.

Strain AHA8 formed a whitish, gray to bluish gray aerial spore mass on ISP2 medium (Fig. 1A) and produced hook-like sporophores that extended to form spirals, with more than 10 spores per chain (Fig. 1B). Sclerotia, endospores, and synnemata were not observed. The cultural and morphological features of strain AHA8 were in agreement with those typically described for members of the genus *Streptomyces* [43].

The phenotypic characteristics of strain AHA8 are given in Table 2. The characterisation revealed that this strain can utilise all tested carbon sources, except D-sorbitol and hydrolysed gelatin. Citrate, starch, and tyrosine were hydrolyzed, whereas lactate was not. In addition, the strain produced nitrate reductase. Growth of the strain was observed in medium supplemented with 5% (w/v) NaCl and also at 50°C. The strain exhibited positive activities for arginine dihydrolase, β-galactosidase, gelatinase, lysine decarboxylase, tryptophan deaminase, and urease, whereas ornithine decarboxylase activity was absent.

The phylogenetic analysis using the 16S rRNA gene sequence (1422 base pairs) confirmed that AHA8 strain belonged to the genus *Streptomyces*. The sequence was deposited in GenBank /EMBL/DDBJ under the accession number MT626100. The highest similarity level was 99.57% with *Streptomyces djakartensis* NBRC 15409^T. The phylogenetic tree, obtained by applying the neighbor-joining method, illustrated in Figure 2 showed that the AHA8 strain forms a distinct phylogenetic position with closely related species of the genus *Streptomyces*. However, our strain could be distinguished from *S. djakartensis* by phenotypic traits, including the color of aerial mycelium (white to beige in *S. djakartensis*) and the utilization of fructose, D-xylose, and cellulose [24].

Antimicrobial activity

The results of the primary screening for antimicrobial activity of strain AHA8 showed a statistically significant difference ($p<0.05$). *Streptomy-*

Table 1. Macro-morphological characteristics of strain AHA8 on different culture media after 14 days of incubation

Agar medium	Growth AHA8	Production and color of:		Soluble pigment
		Aerial mycelium	Substrate mycelium	
ISP2	++	+ to ++ Gray to bluish gray	Bright orange	Bright orange
ISP4	+++	+++ Gray to brownish gray	Yellow orange	Bright yellow to yellow orange
CHV	+++	++ Gray to bluish gray	Yellow orange	Bright yellow

Note: +: weak, ++: moderate, +++: strong, –: no production, CH-V: chitin-vitamin B agar.

DOI: 10.61215/AUOFB.2026.2.03

ces sp. AHA8 showed an antifungal activity against all the micro-fungi and yeasts tested. The antimicrobial activity was strong (> 30 mm) against *Staphylococcus aureus* (MRSA 639c), *Staphylococcus aureus* (MSSA) and *Bacillus subtilis* (ATCC 663); strong to moderate (21-30 mm) against *Listeria monocytogenes* (ATCC 13932), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Acinetobacter baumannii* (S54), *Aspergillus carbonarius* (M333), *Aspergillus westerdijkiae* (ATCC 3174), *Fusarium culmorum* (Fc) and *Umbelopsis ramanniana* (NRRL 1829); and moderate (10-20 mm) against *Candida albicans* (M3) (Table 3).

Figure 1. Macro-morphology and micro-morphology of *Streptomyces* sp. AHA8. (A) Strain grown on ISP2 medium for 14 days at 30°C; (B) spore chains of strain under light microscopic view, *bar* 100 µm.

Table 2. Phenotypic characteristics of the strain AHA8 in comparison with the most closely related species *Streptomyces djakartensis* NBRC 15409^T (AB184657).

Tests	AHA8	*Streptomyces djakartensis* NBRC 15409^T
Carbon source utilization:		
L-arabinose	+	+
Fructose	+	-
D-Glucose	+	+
Inositol	+	+
Mannitol	+	+
Raffinose	+	+
L-rhamnose	+	+
D-sucrose	+	+
D-xylose	+	-
Cellulose	+	-
D- Galactose	+	+
D- sorbitol	-	Nd
Melibiose	+	Nd
Hydrolysis of:		
Gelatin	+	Nd
Citrate	+	+
Lactate	–	Nd
Starch	+	Nd
Tyrosine	+	Nd
Nitrate reduction	+	Nd
Growth in the presence of:		
5% NaCl (w/v)	+	Nd
Growth at 50°C	+	Nd
Enzymes		
Arginine dihydrolase	+	Nd
Beta-galactosidase	+	Nd
Gélatinase	+	Nd
Lysine decarboxylase	+	Nd
Ornithine decarboxylase	-	Nd
Tryptophane deaminase	+	Nd
Urease	+	Nd

Note: – : negative, + : positive, nd: not determined.

DOI: 10.61215/AUOFB.2026.2.03

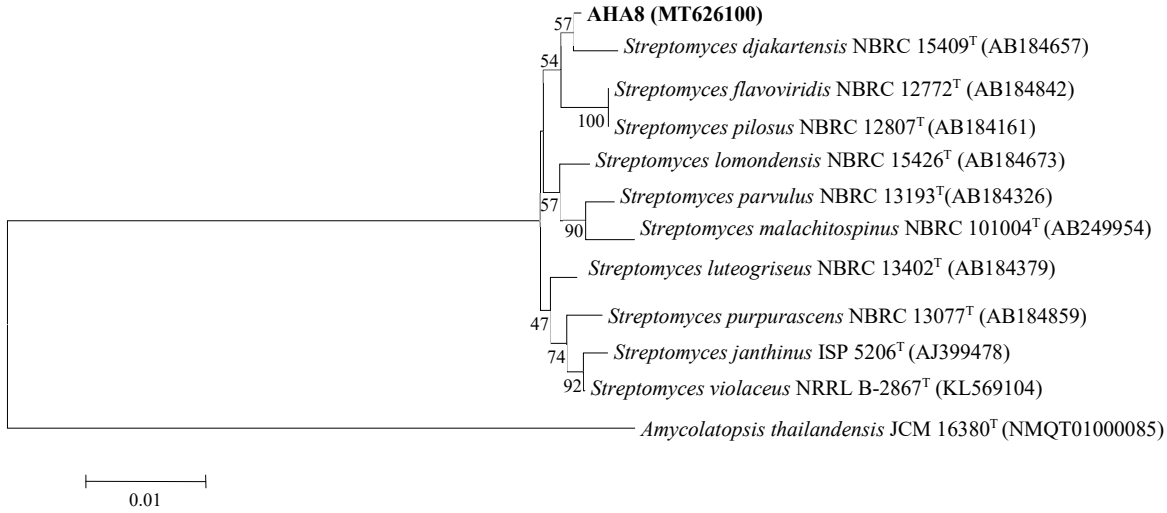

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences showing relationships among strain AHA8 and all type strain species of *Streptomyces*. Numbers at nodes indicate bootstrap support percentages based on a neighbor-joining analysis of 1000 resampled datasets. Bar, 0.01 substitutions per nucleotide position. The *Amycolatopsis thailandensis* JCM 16380T (NMQT01000085) sequence was used as the outgroup. Numbers in the parentheses indicate GenBank accession numbers.

Table 3. Antimicrobial activity of strain AHA8 by the agar plug diffusion method on ISP2 medium. Different superscript letters indicate significant differences ($p < 0.05$).

Target-microorganism	Inhibition zone (mm)
Staphylococcus aureus (MRSA 639c)	45 ± 0.66^{a}
Staphylococcus aureus (S)	38 ± 1.33^{b}
Listeria monocytogenes (ATCC 13932)	29 ± 0.33^{c}
Bacillus subtilis (ATCC 663)	39 ± 1^{b}
Escherichia coli (ATCC 8739)	28 ± 0.53^{c}
Pseudomonas aeruginosa (ATCC 9027)	21 ± 0.66^{f}
Acinetobacter baumannii (S54)	28 ± 1.33^{c}
Aspergillus carbonarius (M333)	24 ± 1.53^{e}
Aspergillus westerdijkiae (ATCC 3174)	$25 \pm 0.6^{d,e}$
Fusarium culmorum (Fc)	28 ± 1.06^{c}
Umbelopsis ramanniana (NRRL 1829)	$27 \pm 0.26^{c,d}$
Candida albicans (M3)	16 ± 1.6^{g}

pH, biomass, and antimicrobial Kinetics

During fermentation in SS broth, antibacterial activity was evaluated against *Escherichia coli* (ATCC 8739), *Listeria monocytogenes* (ATCC 13932), and methicillin-resistant *S. aureus* (MRSA 639c) using the agar diffusion method (well technique). Biological activity was observed after 24 h against the three target bacteria. The maximum antibacterial activity was after 5 days of incubation. The pH ranged from 7.12 ± 0.20 to 7.53 ± 0.18 during fermentation. Biomass increased during the first days and reached a maximum (13.5 ± 0.45 g/L) after 3 days of fermentation (Fig. 3).

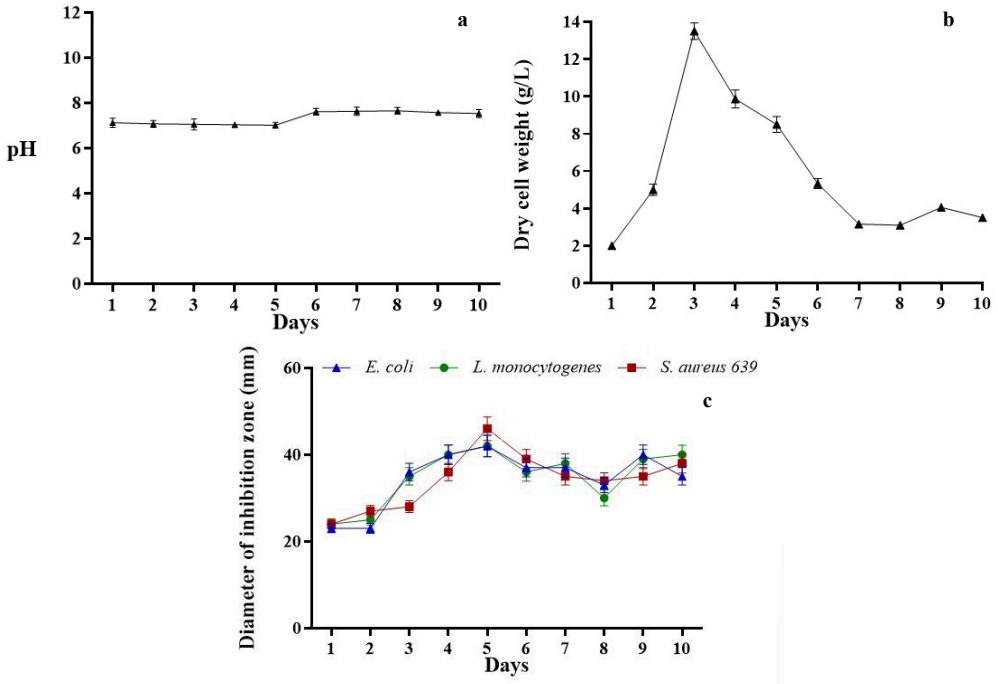

Figure 3. Kinetics of pH, dry cell weight, and antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA 639c) (■), *Escherichia coli* (ATCC 8739) (▲), and *Listeria monocytogenes* (ATCC 13932) (●).

DOI: 10.61215/AUOFB.2026.2.03

After five days of fermentation, the antimicrobial activity of four organic solvents (*n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol) was evaluated by the paper disk method against *Staphylococcus aureus* (MRSA 639c), *Escherichia coli* (ATCC 8739), and *Listeria monocytogenes* (ATCC 13932). The highest antibacterial activity was observed with the butanolic and ethyl acetate extracts, followed by dichloromethane, compared to the methanol control, which did not exhibit a growth-inhibitory zone. The inhibition zones differed significantly among the solvent extracts for each target microorganism ($p < 0.05$). The resulting inhibition zones are illustrated in Figure 4 and Table 4.

DISCUSSION

Actinobacteria are considered the most attractive source of natural bioactive secondary metabolites, particularly the genus *Streptomyces*, which produces over two-thirds of the clinically useful natural antibiotics [23, 30]. Many of these molecules have found important therapeutic applications [1, 18].

Numerous culture media were used for selective isolation of rare actinobacteria, for example, chitin-vitamins B, which contains chitin as a unique source of carbon and nitrogen. This substrate is better degraded by actinobacteria than by other bacteria. Generally, rare bacteria are auxotrophic and non-prototrophic, and consequently, B vitamins were added to stimulate growth. Many auditors reported the performance of the chitin-vitamin B medium for isolating new strains of rare Saharan actinobacteria, producing potential new antibiotics [2, 9, 13, 14]. The strain *Streptomyces* sp. AHA8 was obtained on chitin-vitamins B agar supplemented by nalidixic acid as a selective agent. Based on its morphological characterization, this Saharan strain belongs to the genus *Streptomyces*.

The 16S rRNA sequence of strain AHA8 was compared with sequences of validated *Streptomyces* type strains. The similarity level was 99.57% with *Streptomyces djakartensis* NBRC 15409^T, which is the most closely related species. Meier Kolthoff *et al.* [38] reported that the DNA-DNA hybridization should be mandatory only if 16S rRNA gene sequence is above a similarity percentage of 98.2%. However, Kim *et al.* [29] showed that 98.65% 16S rRNA gene sequence similarity can serve as a threshold for differentiating two closely related bacterial species. On the other hand, it is interesting to note that very high 16S rRNA gene sequence similarities have been found between the type strains of validly published *Streptomyces* species, as exemplified by *Streptomyces Artemisiae* YIM 63135 and *S. armeniacus* NBRC 12555^T (99.9%) [55] and *Streptomyces* sp. MJM 10778 and *S. misionensis* NBRC 13063^T (99.9%) [33], *Streptomyces yangpuensis* fd2-tb and *S. amritsarensis* JCM 19660^T (99.9%) [48]. Although the strains showed a high 16S rRNA gene sequence similarity to closely related species, the authors emphasized that one should not rely solely on 16S rRNA sequences to define species status. By performing DNA–DNA hybridization, they demonstrated that the strains' genomic DNA was sufficiently distinct from that of the type strain, confirming that they represent a novel species. Therefore, in our case, this possibility cannot be ruled out, especially since our strain AHA8 exhibits several differences in morphological and physiological characteristics, for example, the color of aerial mycelium and the utilization of fructose, D-xylose, and cellulose. DNA-DNA hybridization experiments between strain AHA8 and the closely related species *S. djakartensis* NBRC 15409T, or whole-genome sequencing, should determine the originality of this Saharan strain or its assignment to the *S. djakartensis* species.

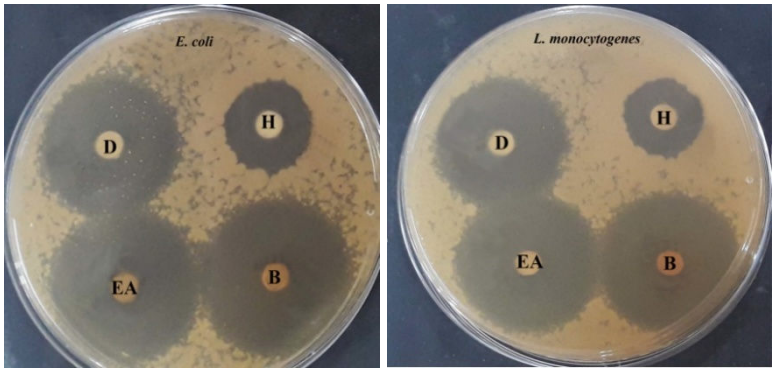

Figure 4. Antibacterial activities of dichloromethane (D), *n*-hexane (H), ethyl-acetate (E.A), and *n*-butanol (B) extracts against *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 13932) by the paper disk method.

Table 4. Antimicrobial activity of organic extracts of *Streptomyces* sp. AHA8 culture against *Staphylococcus aureus* (MRSA 639c), *Escherichia coli* (ATCC 8739) and *Listeria monocytogenes* (ATCC 13932).

Target-microorganism	**Zone of inhibition (mm) obtained by using different solvents**			
	n-butanol	Ethyl acetate	Dichloromethane	*n*-hexane
S. aureus (MRSA 639c)	31 ± 0.8^{a}	29 ± 0.3^{b}	27 ± 0.5^{c}	14 ± 0.8^{d}
E. coli (ATCC 8739)	$30 \pm 0.4^{a,b}$	32 ± 0.4^{a}	29 ± 0.9^{b}	15 ± 1.2^{c}
L. monocytogenes (ATCC 13932)	35 ± 0.3^{a}	34 ± 0.6^{a}	31 ± 1^{b}	18 ± 1.4^{c}

Different superscript letters within the same row indicate statistically significant ($p < 0.05$).

DOI: 10.61215/AUOFB.2026.2.03

Species of *Streptomyces* exhibit significant antagonistic potential against bacterial and fungal pathogens through the production of antibiotics, lytic enzymes, and other bioactive compounds [4]. The *Streptomyces* strain AHA8 exhibited inhibitory activity against both Gram-positive bacteria and mycotoxigenic and phytopathogenic fungi, with particularly pronounced activity against *Staphylococcus aureus* (MRSA 639c), followed by *Bacillus subtilis* (39 ± 1 mm) strain and the sensitive *S. aureus* (38 ± 1.33 mm). This strong antagonism is consistent with Streptomyces's known ability to produce antibacterial metabolites that diffuse into the medium, effectively inhibiting Gram-positive bacteria, including multidrug-resistant strains. The obtained results showed that the antibacterial activity against Gram-positive bacteria is better than against Gram-negative bacteria. Generally, Gram-negative bacteria are more resistant to antibacterial compounds than Gram-positive bacteria. Several studies showed that the double membranes (outer cell membrane in Gram-negative bacteria) contains many protective mechanisms against antibiotics [21]. Moreover, the increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids that can readily spread through bacterial populations [10, 11].

Fungal targets displayed differential sensitivity, with filamentous species being moderately inhibited while the yeast *Candida albicans* showed minimal susceptibility. This trend suggests that the strain's metabolites preferentially affect organisms with filamentous growth and may target specific components of fungal cell walls or growth pathways. Several recent studies support the view that Saharan soils are a rich reservoir of *Streptomyces* strains with significant antagonistic potential against a broad range of pathogenic microorganisms. The extreme conditions of the Sahara, characterized by high temperatures, intense UV radiation, and severe aridity, appear to select for microorganisms with unique adaptations and enhanced secondary metabolism, often reflected in strong antimicrobial activities. For example, strains isolated from different Algerian Saharan regions have exhibited pronounced antimicrobial activities, with some showing broad-spectrum inhibition against both bacteria and fungi in cross-streak and agar diffusion assays. In one study, a Saharan *Streptomyces fimbriatus* isolate demonstrated high inhibitory activity against multiple bacterial and yeast pathogens, highlighting the potent biocontrol capacity of desert actinomycetes [28]. Similarly, other Saharan *Streptomyces* strains have been shown to produce bioactive compounds, such as saquayamycins, with documented antibacterial and antifungal effects, underscoring the chemical diversity of metabolites derived from desert actinomycetes. Many published papers have already demonstrated the richness and biodiversity of *Streptomyces* strains in the desert soils in Algeria. These studies have led to the discovery of several novel interesting antibiotics, such as, saquayamycins from *Streptomyces* sp. PAL114 [2], di-(2-ethylhexyl) phthalate from *Streptomyces* sp. G60 [15], actinomycin D from *Streptomyces* sp. IA1 [50], vineomycin A1 and chaetoglobosin A by *Streptomyces* sp. PAL114 [3], hydroxamic acid derivative from *Streptomyces* sp. WAB9 [53], 2,4-di-tert-butylphenol from *Streptomyces* sp. G61 [5], oligomycins A and E [27], mzabimycins A and B from *Streptomyces* sp. PAL114 [49]. This study indicated that the Algerian desert soils are rich and diversified in actinobacteria with interesting antagonistic properties. Together, these findings contextualize our results and reinforce the potential of Saharan *Streptomyces* isolates as a source of novel antimicrobial agents. Overall, these findings indicate that the strain AHA8 produces a spectrum of antimicrobial compounds with selective activity, supporting its potential for further exploration in the development of novel antibacterial and antifungal agents. Further studies focusing on the isolation, characterization, and chemical identification of the metabolites produced by the *Streptomyces* AHA8 strain are warranted to fully explore its therapeutic potential.

Acknowledgements. The authors wish to thank the late Prof. Nasserdine Sabaou (1956-2019) for his significant contribution to this paper. This work could not exist in anything like its current form without his contribution.

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

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DOI: 10.61215/AUOFB.2026.2.03

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Received: April 12, 2026
Accepted: July 20, 2026
Published Online: July 22, 2026
Analele Universităţii din Oradea, Fascicula Biologie
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
Oradea University Press
https://www.bioresearch.ro/revistaen.html
https://doi.org/10.61215/auofb.2026.2.03