Research article

Antagonistic activity of native *Streptomyces* spp. against ESBL -and KPC- producing Enterobacteriaceae and MRSA

Actividad antagónica de *Streptomyces* spp. nativas frente a Enterobacteriaceae productoras de BLEE y KPC, y a MRSA

Víctor Manuel Osorio[1], Alejandro Mejía[2], Elizabeth Correa[3], Ana María Ochoa[4], Ana Mercedes Rada[5], José Gregorio Martínez[6]

Abstract

Introduction. The search for microorganisms producing antimicrobial compounds represents a strategy to address the global crisis of antibiotic resistance. Identification of bacteria of the genus *Streptomyces* with antagonistic activity against resistant bacteria is the initial step for the subsequent recovery of effective antibacterial metabolites for the inhibition of these resistant organisms. **Objectives.** Recognize promising sources to isolate filamentous bacteria able to inhibit clinical isolates, including ESBL- and KPC- producing Enterobacteriaceae and MRSA. **Methods.** Actinobacteria were isolated from rhizospheres and a compost system. MRSA, *Escherichia coli,* and *Klebsiella pneumoniae* clinical isolates were chosen to address antagonism tests. Antibacterial activity was recorded by a cross-streak method. *Streptomyces* isolates were characterized according to International *Streptomyces* Project and the native isolates with antimicrobial activity were identified by molecular techniques. **Results.** Nine isolates of actinobacteria with activity against resistant-antibiotic bacteria were obtained, two from the avocado rhizosphere, four from a living fence and three from a composting system. Two of the isolates showed activity against all the tested antibiotic-resistant bacteria. Molecular taxonomic identification found *S. jumonjinensis,*

1. Grupo de Investigación Biociencias. Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia.
ORCID: https://orcid.org/0000-0002-9134-2713

2. Semillero de Investigación de la Facultad de Ciencias de la Salud – SIFACS. Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia.
ORCID: https://orcid.org/0000-0002-9530-2209

3. Grupo de Investigación Biociencias. Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia. Grupo de Investigación en Micología Médica y Experimental. Corporación para Investigaciones Biológicas – CIB. Medellín, Colombia.
ORCID: https://orcid.org/0000-0001-7659-7518

4. Semillero de Investigación de la Facultad de Ciencias de la Salud – SIFACS. Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia.
ORCID: https://orcid.org/0000-0002-2661-9632

5. Grupo de Investigación Biociencias. Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia.
ORCID: https://orcid.org/0000-0002-9624-4776

6. Grupo de Investigación Biociencias. Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia. Medellín, Colombia.
ORCID: https://orcid.org/0000-0002-0731-4358

Correspondencia: victor.osorio@colmayor.edu.co

Received: 05/06/2025
Accepted: 09/07/2025

S. bacillaris, S. prasinus, S. microflavus, and *S. cadmiisoli* as putative species for native isolates. **Conclusions.** *Streptomyces* with antibacterial activities against ESBL and KPC-producing Enterobacteria and MRSA have been isolated and the potential of rhizospheres and compost systems for obtaining antibiotic-producing bacteria was validated. Native isolates exhibited common traits for *Streptomyces*; although NCBI's Blast did not show a resolution to identification, EzBioCloud 16S-based identification was able to accurately detect the identity of the isolates down to the species level.

Keywords: Actinobacteria, antibacterial agents, drug resistance, Extended-Spectrum Beta-Lactamases producers, MRSA, soil microbiology.

Resumen

Introducción. La búsqueda de microorganismos productores de compuestos antimicrobianos representa una estrategia para enfrentar la crisis global de la resistencia a los antibióticos. La identificación de bacterias del género *Streptomyces* con actividad antagónica contra bacterias multirresistentes es el paso inicial para recuperar metabolitos antibacterianos efectivos para inhibir estos organismos. **Objetivos.** Reconocer fuentes promisorias para aislar bacterias filamentosas capaces de inhibir aislamientos clínicos incluyendo Enterobacteriaceae productoras de BLEES y KPC, y MRSA. **Métodos.** Se aislaron actinobacterias de rizosferas y un sistema de compostaje. Se eligieron aislamientos clínicos de MRSA, *Escherichia coli*, and *Klebsiella pneumoniae* resistentes para realizar las pruebas de antagonismo. La actividad antibacteriana se registró mediante un método de estrías cruzadas. Los aislamientos de *Streptomyces* se caracterizaron según el Proyecto Internacional *Streptomyces* y se identificaron mediante técnicas moleculares. **Resultados.** Se obtuvieron nueve aislados de actinobacterias con actividad contra bacterias resistentes, dos de la rizosfera de aguacate, cuatro de un cerco vivo y tres de un sistema de compostaje; dos mostraron actividad contra todas las bacterias resistentes evaluadas. La identificación taxonómica molecular encontró *S. jumonjinensis, S. bacillaris, S. prasinus, S. microflavus* y *S. cadmiisoli* como especies putativas para los aislados nativos. **Conclusiones.** Se aislaron *Streptomyces* antagónicas contra Enterobacterias productoras de BLEE y KPC, y MRSA, validando el potencial de las rizosferas y los sistemas de compostaje para obtener bacterias productoras de antibióticos. Los aislados nativos presentaron características de *Streptomyces*; aunque el Blast de NCBI no mostró una resolución suficiente, EzBioCloud detectó la identidad de los aislados hasta especie.

Palabras clave: Actinobacterias, agentes antibacterianos, resistencia a medicamentos, bacterias productoras de betalactamasas de espectro extendido, MRSA, microbiología del suelo.

Introduction

The dissemination and the continuous increase of resistance even to last-resort antibiotics is expanding due to the misuse/overuse of existing antibacterial drugs and the slow introduction of new antimicrobial agents to the market. Antimicrobial resistance in bacteria represents a worldwide public health challenge because the resulting infections are associated with high mortality and morbidity, very limited treatment options, prolonged hospital stay and entail a cost to the public health systems up to 1.6 % of the Gross Domestic Product (1). In 2024, WHO published the list of bacteria for which new antibiotics are urgently needed, including extended-spectrum beta-lactamase (ESBL) producing Enterobacterales, carbapenem resistant Enterobacterales (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA), and proposed to promote investment policies for the development of new antimicrobials as one objective to confront antibiotic resistance (2).

Many antimicrobial molecules are obtained from cultures of bacteria of the genus *Streptomyces*. These actinobacteria are in many environments but, unlike others, *Streptomyces* has a complex multicellular development growing filaments from its germinative spores leading to a multinuclear aerial mycelium (3). For instance, tetracyclines producing species such as *Streptomyces rimosus*, *Streptomyces alboflavus*, *Streptomyces aureofaciens* and *Streptomyces vendagensis* have been isolated from soils in urbanized and industrialized areas (4). Other species such as *Streptomyces griseus*, *Streptomyces clavuligerus*, and *Streptomyces orientalis* that produce streptomycin, cephalosporins, and vancomycin, respectively, have also been found in different soils (5-7). Similarly, bacteria of the genus *Streptomyces* with antibacterial activity have been obtained from the rhizosphere of different plants, agricultural soils, and native forests (8-10).

Most species of the genus *Streptomyces* produce extracellular hydrolases that catalyze the degradation of complex substrates in soils and composting systems. A number of these compounds are found in the rhizospheres, zones that constitute environments of dynamic interactions between plants and other organisms that harbor a diverse reservoir of culturable microorganisms that can be exploited to benefit mankind (11). Although rhizosphere-associated soils may contain almost twice as many actinomycete isolates as non-rhizospheric soils (12), few studies have been found on the isolation

of filamentous bacteria from avocado tree rhizospheres (13).

Furthermore, the rhizosphere of *Swinglea glutinosa*, a shrub widely used in the construction of hedges and living fences, has been scarcely studied. The maintenance of these plants for ornamental purposes and delimitation of properties traditionally is done with the addition of fertilizers or amendments and regular pruning to maintain the shape and density of the bushes (14). These diverse management practices to these hedges probably give unique characteristics to each associated rhizosphere that allow the establishment of different microbial communities in these environments; however, reports of microbiological analysis of these soils were not found. Other promising sites for the isolation of filamentous bacteria are composting systems, mainly in a maturation stage; the isolation of bacteria from agro-industrial and vegetable waste processing systems have been described (15).

Therefore, the aim of this study was to recognize promising sources to isolate filamentous bacteria with capacity to inhibit the growth of ESBL and KPC-producing Enterobacteria and MRSA, and finally, determinate their putative identity. The results of this work constitute a preliminary step toward the discovery of antimicrobial compounds produced by native Colombian *Streptomyces* spp. isolates, active against ESBL and KPC-producing Enterobacteria and MRSA, and support their registration as potential candidates for the developing of new therapeutic agents to control bacterial infections.

Materials and methods

Samples collection

Soils of rhizospheres from an avocado tree (*Persea americana*) and a living fence (*Swinglea glutinosa)*, were collected from private land in Santa Elena, a rural district of Medellín, Colombia (latitude 6°13'N, longitude 75°29'W, altitude 2200 m). Compost samples were taken from a one-week-old artisanal organic solid waste treatment system. The collected samples were taken at a 10-20 cm depth, transported in sterile polyethylene bags to the laboratory, and stored at 4 °C.

Isolation of microorganisms

Actinobacteria were isolated by serial dilutions using glucose - yeast extract - malt extract (GYM) medium (4 g D-glucose, 4 g yeast extract, 10 g malt extract, 2 g $CaCO_3$, and 12 g agar in 1 L distilled water) (16), and starch casein nitrate medium (10 g soluble starch, 0.3 g casein, 2 g KNO_3, 2 g NaCl, 2 g K_2HPO_4, 0.05 g $MgSO_4.7H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4.7H_2O$, and 15 g agar in 1 L distilled water) (17). Both culture media were supplemented with 2.5 µg/

mL rifampicin and the pH was adjusted at 7.2. The plates were incubated at 28 °C and individual colonies corresponding to Gram-positive filamentous bacteria were subcultured on GYM agar and stored in Brain Heart Infusion (BHI) broth (Merck) with 15 % glycerol at −20 °C. All the isolates obtained were labeled and included in the microorganism collection CEMBIO of Colegio Mayor de Antioquia, Medellín, Colombia, under the framework permit for collection of specimens for non-commercial scientific research No. 1467 of the Autoridad Nacional de Licencias Ambientales with registration numbers 16D4FD0E9C3 and 16D551FC815.

Resistant bacteria

Three resistant bacterial clinical isolates and one reference strain were chosen: methicillin resistant *Staphylococcus aureus* (MRSA), ESBL- and KPC- producing *Escherichia coli* and *Klebsiella pneumoniae*, and *E. coli* ATCC 700891. The identification and antimicrobial susceptibility testing of these microorganisms were confirmed using the automated Vitek® 2 (bioMérieux, Marcy-l'Étoile, France). Susceptibilities to antimicrobial agents were profiled with results of sensitive, intermediate, and resistant according to standards for antimicrobial susceptibility testing of the Clinical and Laboratory Standards Institute (CLSI) (18).

Antibacterial assays

To select the native isolates with antibacterial activity, a cross-streak method was performed. Plates of Mueller-Hinton agar (Merck) were prepared and inoculated by a single streak in the center of the Petri dish with actinobacteria suspensions obtained from colonies formed in GYM agar; these plates were incubated at 30 °C for 10 days. Test strains MRSA, ESBL- and KPC- producing *E. coli* and *K. pneumoniae*, and *E. coli* ATCC 700891 were incubated on LB (Miller) agar at 37 °C for 24 h, and bacterial suspensions equivalent to 0.5 McFarland standard were prepared from these cultures in 0.1 % peptone water. The Mueller-Hinton plates with native isolates were seeded with test organisms by streaking perpendicular to the line of actinobacteria growth and incubated at 37 °C for 24 h. Antagonism was observed based on the inhibitory interaction between the actinobacteria and test strains; the width of each test streak was fixed at 5 mm, regions of growth and clear/inhibited areas were measured, and antibiotic activity was calculated using the equation (AWG/TSA)×100, where, AWG is the area on the streak without growth and TSA is the total streak area scored for each test streak (19).

Characterization of actinobacteria isolates

The selected isolates that showed promising antibacterial activity against resistant bacteria were morphologically and physiologically characterized according to International Streptomyces Project (ISP) and Bergey's Manual of Systematic Bacteriology (16, 20). Colonies of actinobacteria were picked up, sub-cultured on ISP-2 slants and incubated at 28 ºC for 3-4 weeks. Then, the morphological characteristics were examined by culturing isolates on different ISP media (21).

Growth at various temperatures (20-40ºC) and NaCl concentrations (0.2-8 %) were examined by growing the isolates on starch casein nitrate medium and checking the cultures 48 and 72 h after inoculation. Urea hydrolysis was checked in a basal medium with 2 % urea by change in color from yellow to red and catalase activity was evaluated by hydrogen peroxide effervescence (21).

Utilization of different carbon sources was studied by adding 1 % filter-sterilized sugars (D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fructose, D-mannitol, I-inositol, and sucrose) to the basal medium ISP-9; D-glucose containing medium was considered as a positive control while medium without sugar was negative control (20). The amylolytic activity was verified in a medium including soluble starch (22), cellulolytic activity in a medium with carboxymethylcellulose (23), proteases production using gelatin as substrate (24) and lipolytic activity in a medium supplemented with olive oil (25).

Molecular identification

The native isolates with antimicrobial activity were incubated into BHI broth at 30 °C until a pellet of vegetative cells was obtained. The preparation of the total genomic DNA was carried out with the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. PCR amplification of the 16S rRNA gene was conducted using primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). The reaction was carried out in a 50 µL reaction volume consisting of 10 µL 5X polymerase buffer, 4 µL $MgCl_2$ (25mM), 1 µL dNTPs (10 mM), 4 µL of each primer (0.1mM), 4 µL of template DNA, and 1.25 U of GoTaq® Flexi DNA Polymerase (Promega), with 30 cycles of 1 min at 94 °C, 1 min at 52 °C, and 1 min at 72 °C, followed by another incubation at 72 ºC for 10 min (26). The quality of the extracted DNA quality and the success of PCR amplification were confirmed by agarose gel electrophoresis stained with EZ-Vision® In-Gel Solution 10000X (VWR) and subsequent visualization under ultraviolet light. PCR products were purified using the Wizard® SV Gel and PCR Clean-Up Kit

(Promega) and sequenced using the same PCR primers, in an ABI-3500 automated sequencer using the BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems), according to the manufacturer's instructions. The quality of forward and reverse nucleotide sequences was checked using Geneious software v10.0.9 (27). Alignment to create consensus sequences were performed using the embedded algorithm Clustal W (28). Next, sequences were submitted to a BLAST search for verification and comparison with those available in GenBank, discarding cross-contamination or the presence of non-bacterial taxon groups (29). Isolates sequences were then aligned and manually edited and trimmed to generate a final dataset for further identification analysis.

To this purpose, we used the new sequence analysis tool EzBioCloud, a Bioscience's curated public data and analytics portal focusing on taxonomy, ecology, genomics, metagenomics, and microbiome of Bacteria and Archaea (https://www.ezbiocloud.net/identify). The identification algorithm provides proven similarity-based searches against quality-controlled and curated databases of 16S rRNA sequences. The top-hit information for each identification is the top hit against all prokaryotic names included in the curated database. Hits against only valid prokaryotic names can be viewed for a decision about the identity. For native isolates identification, we consider a threshold identity > 97 %. BLASTn (nucleotide) from NCBI was used as an alternative method of identification, using equal parameters of threshold identity, and an E-value of zero. Likewise, when an isolate matched with multiple species/strains because they exhibited equal identity scores, the isolate was identified as "genus + sp".

Results

Isolation of actinobacteria and antimicrobial activity

A total of 34 actinobacteria isolates of different phenotypes were obtained from rhizospheres and composting samples (30). Twelve isolates from the avocado rhizosphere, 12 from the living fence rhizosphere, and 10 from the compost were recovered, and their antagonistic activities against two ESBL- and KPC- producing Gram-negative bacteria, and one methicillin resistant *S. aureus* (MRSA), Table 1, were evaluated.

Table 1. Antibiotic sensitivity profiles of clinical isolates used in antagonism tests

Test bacteria	SAM	TZP	FOX	OX	CAZ	CRO	FEP	DOR	ETP	IPM	MEM	AK	CN	CIP	E	VA	TE	TGC	SXT
S. aureus (MRSA)	–	–	R	R	–	–	–	–	–	–	–	–	–	R	S	S	S	–	S
E. coli (ESBL/KPC)	R	R	R	–	R	R	R	R	R	R	R	S	S	S	–	–	–	S	–
K. pneumoniae(ESBL/KPC)	R	R	R	–	R	R	R	R	R	R	R	S	S	S	–	–	–	S	–
E. coli (ATCC 700891)	S	S	S	–	S	S	S	S	S	S	S	S	S	S	–	–	–	S	–

CR: carbapenem-resistant. Beta-lactam: ampicillin/sulbactam (SAM), piperacillin/tazobactam (TZP), cefoxitin (FOX), oxacillin (OX), ceftazidime (CAZ), ceftriaxone (CRO), cephepime (FEP), doripenem (DOR), ertapenem (ETP), imipenem (IPM), meropenem (MEM). Aminoglycosides: amikacin (AK), gentamicin (CN). Quinolone: ciprofloxacin (CIP). Macrolide: erythromycin (E). Glycopeptide: vancomycin (VA). Tetracyclines: tetracycline (TE), tigecycline (TGC). Mixed: trimethoprim/sulfamethoxazole (SXT). R: resistant. S: susceptible. – : not evaluated.

Antimicrobial activities were proportional to clear areas for the growth of different test bacteria. In some cases, it presented a decrease in growth with faint and translucent streaks for the test bacteria. All antibiotic-resistant bacteria were inhibited by some native actinobacteria isolates; four native isolates inhibited ESBL- and KPC-producing *K. pneumoniae,* and seven inhibited ESBL- and KPC- producing *E. coli*. *E. coli* (ATCC 700981) was inhibited by six native isolates; no actinobacteria isolated from avocado rhizosphere and only one from composting system inhibited the growth of *K. pneumoniae,* Table 2.

Table 2. Antimicrobial activity of native actinobacteria isolates

Native isolate	Test bacteria			
	S. aureus (MRSA)	*E. coli* (ESBL/KPC)	*K. pneumoniae* (ESBL/KPC)	*E. coli* ATCC 700891
Avocado rhizosphere				
S4E	28	0*	0	0*
S8E	0	100	0	100
Living fence rhizosphere				
S1H	100	100	57	100
S4H	17	0	0	0
S10H	0	17	14	100
S13H	0	17	14	0*
Composting system				
S34	100	100	23	100
S40	100	100	0	100
S41	63	100	0	100

Antibiotic activity was calculated using the equation (Area without growth) × 100 / (Total streak area). CR: carbapenem-resistant. *: Faint and translucent streaks.

Isolates S1H and S34 inhibited the growth of all test bacteria. Only two of the 12 native isolates obtained from the avocado rhizosphere (16.6 %), four of the 12 isolates obtained from living fence rhizosphere (33.3 %), and three of the 10 isolated from a composting system (30 %) showed activity against clinical MRSA and ESBL- and KPC- producing *K. pneumoniae* and *E. coli*.

Morphological and physiological characteristics of native isolates

Microscopic observations showed Gram-positive rod-shaped branching filaments for all native isolates. Macroscopic examinations of cultures indicated that isolates formed dried, opaque, and raised colonies with rough surfaces and irregular margins, Figure 1.

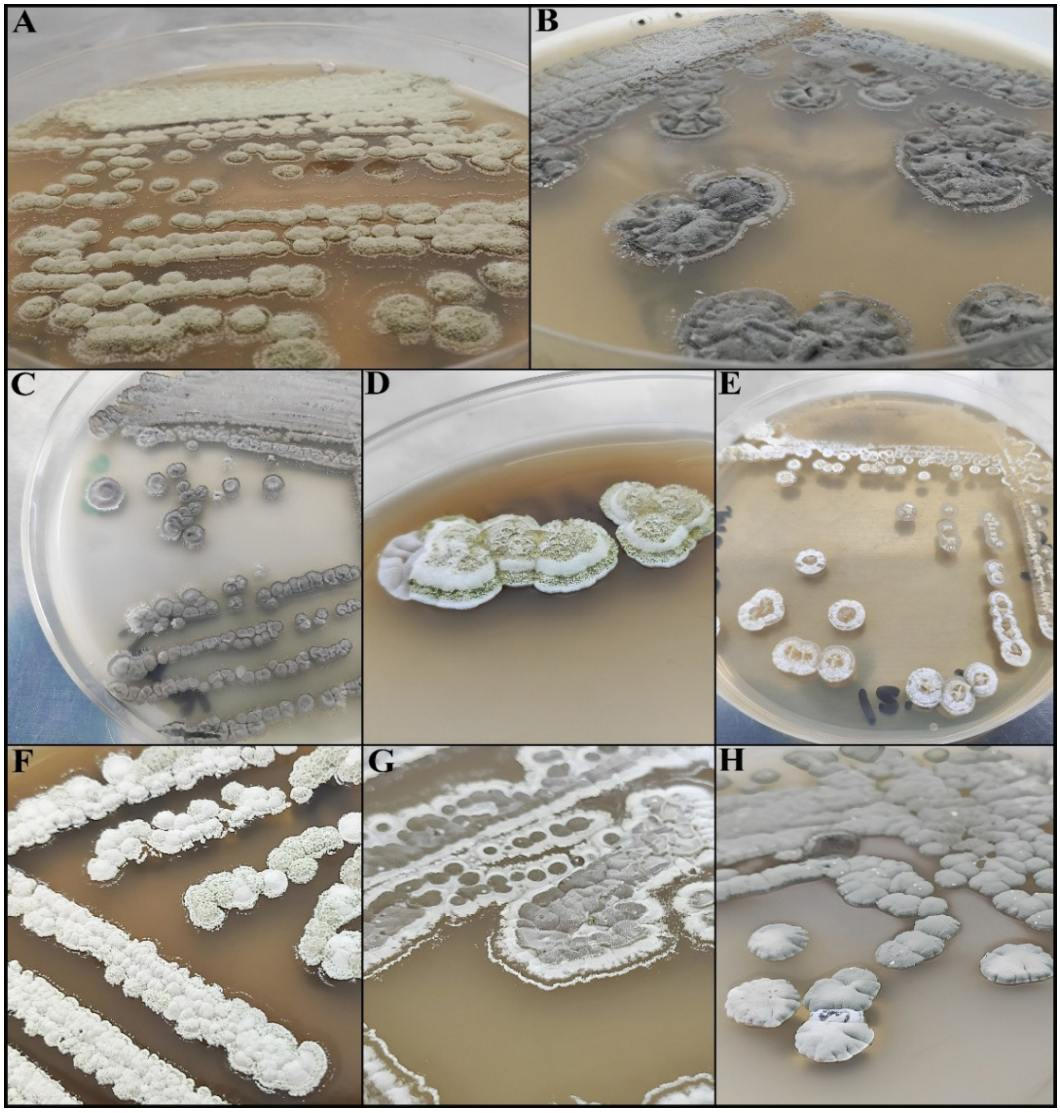

Figure 1. Colonies of native isolates obtained from rhizospheres and composting system. Cultures were made on GYM agar and incubated for at least 10 days at room temperature. A: S1H, B: S8E, C: S4H, D: S40, E: S10H, F: S41, G: S13H, H: S34.

Colonies of antagonistic isolates had different colors depending on the culture medium, but it was more common yellowish white and light olive gray aerial mass, and yellowish white and pale greenish yellow substrate mycelia, Table 3.

Table 3. Colony color of native actinobacteria isolates with antimicrobial activity

Native isolate	Aerial mass color				Substrate mycelium color			
	ISP-2	ISP-3	ISP-4	ISP-5	ISP-2	ISP-3	ISP-4	ISP-5
S4E	YG	OG	LOG	OG	LY	YW	YW	YW
S8E	YG	OG	LOG	LOG	LY	YW	YW	YW
S1H	LOY	YW	YW	YW	LY	MY	YW	YW
S4H	YG	OG	LOG	LOG	LY	PGY	MB	YW
S10H	W	LOG	YW	LOG	MOY	PGY	PGY	YW
S13H	LOG	LOG	PGY	YG	MOY	YW	LB	YW
S34	PGY	YG	PGY	YG	PGY	PGY	PGY	YW
S40	YW	YW	YW	YW	YW	YW	YW	BY
S41	YW	YW	YW	YW	MOY	YW	YW	PGY

ISP-2, ISP-3, ISP-4, ISP-5: culture medium according to International *Streptomyces* Project (20). Colors were recorded according to ISCC-NBS Colour System (31-32). BY: brilliant yellow, LB: light brown, LOG: light olive gray, LOY: light orange-yellow, LY: light yellow, MB: moderate brown, MOY: moderate orange yellow, MY: moderate yellow, OG: olive gray, PGY: pale greenish-yellow, W: white, YG: yellowish gray, YW: yellowish white.

Soluble pigments on ISP-5 medium were observed only for isolates from compost system (S34, S40, S41); three isolates did not release melanin in ISP-6 or ISP-7 culture media, four isolates hydrolyzed urea, one was catalase-negative and all native isolates used all the tested sugars as the sole carbon source; besides, two isolates obtained from compost system grew at temperatures above 37 °C and only one did not show growth above 30°C, Table 4. Hydrolytic enzymes production was positive for these isolates, and none grew in cultures with NaCl concentrations of 8 % or higher.

Table 4. Cultural and physiological characteristics of native actinobacterial isolates.

Characteristic	Isolate								
	S4E	S8E	S1H	S4H	S10H	S13H	S34	S40	S41
Melanin (ISP-6/ISP-7)	−/−	−/−	−/−	−/+	−/+	+/+	−/+	+/+	+/+
Pigments (ISP-5)	−	−	−	−	−	−	Violet	Brown	Brown
Hydrolysis of urea	−	−	+	−	+	+	+	−	−
Catalase activity	+	±	+	±	+	±	+	+	−

Characteristic	Isolate								
	S4E	S8E	S1H	S4H	S10H	S13H	S34	S40	S41
Use of sugars									
D-Glucose	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	±	+	±	+	+	+	+
L-Rhamnose	±	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+
I-Inositol	±	±	±	±	±	±	+	+	+
Sucrose	±	+	+	+	+	+	+	+	+
NaCl tolerance									
0.2 %	+	+	+	+	+	+	+	+	+
2 %	+	+	+	+	+	+	+	+	+
4 %	+	+	±	±	−	±	±	±	±
6 %	±	+	±	−	−	±	−	±	−
8 %	−	−	−	−	−	−	−	−	−
Temperature range (°C)	20-30	20-37	20-37	20-37	20-37	20-37	20-37	20-40	20-40

(+): positive. (±): doubtful. (−): negative. ISP-5, ISP-6, ISP-7: culture medium according to International Streptomyces Project (20).

Molecular identification of native actinobacteria

After assembling bidirectional reads of 16S rRNA, we obtained a 1038 bp matrix containing nine unique haplotypes, one per isolate. Sequences of each isolate were submitted to Genbank under the accession number OM401309-OM401317. Results of the molecular identification using the curated database EzBioCloud (16S based ID) identified five putative species with identities ranging from 99.32 to 100%, Table 5.

Table 5. Molecular taxonomic identification of bacterial species isolated from rhizospheres and a composting system, using the 16S rRNA gene against the EzBioCloud curated database for prokaryotic diversity

Isolate	GenBank accession number	Top-hit Taxon (ezBiocloud curated database)	Identity (%)
S40	OM401309	*Streptomyces jumonjinensis*	99.65
S41	OM401310	*Streptomyces bacillaris*	99.32
S34	OM401311	*Streptomyces prasinus*	99.83
S4E	OM401312	*Streptomyces microflavus*	99.59
S8E	OM401313	*Streptomyces microflavus*	100
S1H	OM401314	*Streptomyces microflavus*	99.84
S4H	OM401315	*Streptomyces microflavus*	99.68
S10H	OM401316	*Streptomyces microflavus*	100
S13H	OM401317	*Streptomyces cadmiisoli*	100

After the BLASTn-based identification, all the nine isolates were identified as *Streptomyces* sp., because they matched with multiple species/strains that exhibited equal identity scores and E-values.

Discussion

This study was undertaken to highlight the presence of actinobacteria of genus *Streptomyces* in two rhizospheres and one home composting system, to select native isolates capable of inhibiting the growth ESBL- and KPC- producing Enterobacteriaceae and MRSA, and to explore their identification through microbiological and molecular approaches. The isolation of bioactive strains from these non-clinical environments reinforces the potential of underexplored ecological niches as reservoirs of microorganisms with antimicrobial capabilities. The antagonistic activity observed in some *Streptomyces* isolates against antibiotic resistant clinical strains supports their relevance as candidates for the discovery of new antimicrobial agents

Several native *Streptomyces* isolates were recovered from sampling sites in compost and rhizospheres in the district of Santa Elena, a rural zone of Medellín, Colombia. Bioecologically, this district is in the low montane per-humid forest life zone, with an average annual rainfall of 2500 mm and temperature of 14.7 °C; this district has mainly non-flooded soils, with good moisture retention and high fertility indices, suitable for the development of forestry and agroforestry systems and isolation of actinobacteria (33).

Few studies have described the isolation of actinobacteria from soils associated with avocado (*P. americana*) trees and none have evaluated the activity of these native isola-

tes against bacteria pathogenic to humans, being this the first study driving this last question. For instance, Trinidad-Cruz *et al.* (13) showed that of 41 isolates of actinobacteria obtained from the rhizosphere of an avocado tree var. Hass, 12 (29 %) showed activity against *Xanthomonas* sp., exhibiting similar percentages of isolates with antimicrobial activity to those obtained in this study (32 %). Otherwise, isolates of actinobacteria from rhizospheres of *S. glutinosa* or other plants that are used as living fences are not reported.

In this study, the percentage of native isolates from *S. glutinosa* with activity against antibiotic-resistant bacteria (33.3 %) was higher than *P. americana* (16.6 %), which could suggest interactions in the soil microbiota encouraged by the traditional fertilization and the agroforestry management applied to these living fences (34).

Other soils and rhizospheres have been more studied to obtain filamentous bacteria with different results on antimicrobial activity. According to Thakur *et al.* (8), 110 *Streptomyces* isolates were recovered from five soils at protected forest areas, 59 % inhibiting the growth of at least one tested bacteria; this high percentage may be due to the assays were carried out using bacteria with no reported antibiotic-resistance. In the same way, Sharma *et al.* (21) reported the obtention of 134 isolates of actinobacteria from different soils, and 38 % showed antibacterial activity against strains with some mechanism of resistance to antibiotics.

This study has demonstrated that organic waste composting systems are potential sites to obtain filamentous bacteria, achieving up to 30 % of isolates with activity against antibiotic-resistant bacteria. In these ecosystems, the composition of microbial communities is dynamic and mainly determined by physical-chemical factors such as temperature, pH, water content, and the C/N ratio, and these artificially modulated environments, probably generate ecological pressure to drive the production of antimicrobial compounds (35). It has been reported several actinobacteria isolates obtained in the maturation stage greater than other phases of composting; at this point, these bacteria decompose natural polymers producing easily degradable molecules that other microorganisms can consume and they will compete for nutrients with other organisms, many times inhibiting their growth due to the production of antibiotics (36). In a study by Salamoni *et al.* (37), 25 isolates of the genus *Streptomyces* were obtained from the composting of domestic organic waste, and 72 % of them inhibited at least one tested bacteria, some of them with resistance to antibiotics, even so the number of native isolates that inhibited resistant bacteria such MRSA was much lower (16 %).

Here, eight of the antagonist native isolates inhibited ESBL- and KPC- producing Enterobacteriaceae isolates; in addition, six inhibited the growth of MRSA isolate. Similar results showed that eight *Streptomyces* isolates obtained from the soil inhibited the growth of *S. aureus* resistant to oxacillin and six additional antibiotics (9).

It is important highlight that some isolates inhibited the growth of bacteria with resistance to several antibiotics. For example, isolates S4E, S1H, S4H, S34, S40, and S41 inhibited the growth of one MRSA isolate resistant to ciprofloxacin. On the other hand, in this study the isolates S1H, S10H, S13H and S34 inhibited the growth of ESBL- and KPC- producing *E. coli* and *K. pneumoniae* isolates, resistant to 10 antibiotics according to their sensitivity profile. An interesting result of this study was the finding of native isolates, such as S1H and S34, which inhibited the growth of the tested Enterobacteriaceae isolates, as well as MRSA, which emphasize the significance of these isolate as potential sources of antimicrobial compounds with the ability to inhibit the growth of different types of pathogenic bacteria.

Morphological examinations of native actinobacteria showed, as expected, colonies initially with a smooth surface, but five days later, an aerial mycelium began to develop that may appear powdery or velvety (16). Even though colors and morphologies were too variable, a distinction between *Streptomyces* species cannot be made according to these aspects, since some isolates with similar morphologies showed physiological traits that differentiate them. Additionally, in this study some tests carried out such as sugar assimilation, catalase activities, and production of hydrolytic enzymes that do not offer information useful to differentiate these isolates.

Besides, species assignment to the genus *Streptomyces* based on these and other phenotypic characteristics is difficult due to the high number of species with validly published names and the similarity in these traits between different species. For this reason, it is common to assign a species to native filamentous bacteria based on sequence analysis of the 16S rRNA gene. Since 16S RNA-based methods do not have sufficient resolving power to delimit all *Streptomyces* phenotypic species in this study, we suggest these analyses must be complemented with genomic data, so that much more information allows for increased resolution of differences, in a context of probably very recent speciation, where the 16S marker is unable to accompany changes in the phenotype, as already observed for *Streptomyces* (38).

This pattern of misidentification suggests some possible explanations. First, the molecular neutral changes do not accompany the speciation process in those species of

Streptomyces for the 16S rRNA gene, specifically when the speciation occurs by selection (39). Second, an evolutionary convergence for some examined regions of the 16S rRNA gene can be occurring (40).

Although NCBI's Blast has been a very good tool for molecular identification of many higher taxonomic groups, in this study the results do not show a resolution that would allow identification down to the species level, but only to the genus. This implies, once again, that errors occur during the identification of bacterial strains in the labs, and hence, in its derived sequences being deposited in the web repositories as GenBank (41-42). Contrary, EzBioCloud 16S-based identification, was able to accurately detect the identity of the isolates down to the species level, with higher identity values and easier decision-making, as already achieved when compared with methods based on GenBank datasets (43).

Despite the high levels of identification obtained using molecular techniques, the microbiological characterization of some isolates does not correspond with that reported in the literature for the identified species. For example, five isolates named *S. microflavus*, all of them obtained from rhizospheres of avocado and a living fence, displayed different aerial mass colors, albeit in the gray and yellow series as reported for this species; however, two of them showed melanin production, and all of them tolerated salt concentrations above 2.5%, which contradicts previous characterizations for *S. microflavus* (44-45). It has been reported in other works that *S. microflavus* inhibits the growth of Gram-positive bacteria although it does not show activity against Gram-negative bacteria (45-46), which coincides with observed inhibition for S4E and S4H isolates in this work; the S8E, S1H and S10H isolates, on the other hand, inhibited *E. coli* ATCC 700891 and ESBL- and KPC- producing *E. coli*.

Similarly, the phenotype of S41, nominally *S. bacillaris* (according molecular ID), shares characteristics such as melanin production, sugar assimilation and inhibition of Gram-positive and Gram-negative bacteria with those reported for this species in other studies, although the color of the aerial mass is predominantly yellow and not white like S41 (16, 47). Isolate S34, named *S. prasinus*, exhibited melanin production and colonies with morphologies different from those reported for this species, although its antibacterial activity against *S. aureus* strains has been confirmed by other studies (16, 48). Finally, the phenotypes for *S. cadmiisoli* and S13H are similar, but they differ in melanin production and xylose and mannitol assimilation; to date, antimicrobial activity for *S. cadmiisoli* has not been reported (49). No morphological aspects have been reported in the literature for the species *S. jumonjinensis* to verify traits found for the isolate S40.

Although all these species are Gram-positive bacteria that live primarily in soil and decaying organic matter, playing a crucial role in the decomposition of animal and plant waste, none of them have been previously identified in Colombia.

Conclusions

The inhibition of ESBL- and KPC- producing Enterobacteriaceae and by antagonistic activity of native *Streptomyces* spp. was confirmed, and rhizospheres and compost systems for obtaining antibiotic-producing bacteria was validated. Native isolates exhibited common traits for the *Streptomyces* genus and identification by EzBioCloud 16S-based algorithms detect *S. microflavus*, *S. jumonjinensis*. *S. bacillaris*, *S. prasinus* and *S. cadmiisoli* as putative species for native isolates.

Acknowledgments

The authors thank all the students of the Research Hotbed of the Faculty of Health Sciences (SIFACS for its acronym in Spanish) who collaborated in carrying out some of the experimental activities. This work was supported by the Institución Universitaria Colegio Mayor de Antioquia through the projects: "Production of compounds with antimicrobial activity by solid-state fermentation with filamentous fungi and native actinomycetes" (Grant number FCSA-CONV 2013) and "Antimicrobial activity of extracts obtained from suspensions generated by a solid-state fermentation (SSF) process (Grant number FCSA-CONV 2014).

Víctor Manuel Osorio-Echeverri contributed to conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, and writing – original draft. Alejandro Mejía-Muñoz and Elizabeth Correa-Gómez, contributed to formal analysis, and methodology. Ana María Ochoa-Aristizábal contributed to formal analysis, methodology, and visualization. Ana Mercedes Rada-Bravo contributed to formal analysis, methodology, and writing - review and editing. José Gregorio Martínez contributed to formal analysis, visualization, and writing - review and editing.

Conflict of interest statement

The authors declare having no affiliations with or involvement in any organization or entity with any financial interest.

References

1. World Health Organization. Report of the 6th Meeting: WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance with AGISAR 5-year strategic framework to support implementation of the global action plan on antimicrobial resistance (2015-2019). Seoul: WHO Press; 2015.

2. World Health Organization. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance [Internet]. Geneva: WHO Press; 2024 [accessed 4 June 2025]. Available in: https://www.who.int/publications/i/item/9789240093461

3. Procópio REL, da Silva IR, Martins MK, de Azevedo JL, de Araújo JM. Antibiotics produced by *Streptomyces*. Braz J Infect Dis. 2012; 16(5): 466-471. doi: 10.1016/j.bjid.2012.08.014.

4. Asagbra AE, Sanni AI, Oyewole OB. Solid-state fermentation production of tetracycline by *Streptomyces* strains using some agricultural wastes as substrate. World J Microbiol Biotechnol. 2015; 21: 107-114. doi: 10.1007/s11274-004-2778-z.

5. Higgens CE, Kastner RE. *Streptomyces clavuligerus* sp. nov., a b-Lactam antibiotic producer. Int J Syst Microbiol. 1971; 21(4): 326-331. doi: 10.1099/00207713-21-4-326.

6. Arora A, Nain L, Gupta JK. Solid-state fermentation of wood residues by *Streptomyces griseus* B1, a soil isolate, and solubilization of lignins. World J Microbiol Biotechnol. 2005; 21: 303-308. doi: 10.1007/s11274-004-3827-3.

7. Moellering RC. Vancomycin: A 50-Year Reassessment. Clin Infect Dis. 2017; 42(S1): 3-4. doi: 10.1086/491708.

8. Thakur D, Yadav A, Gogoi BK, Bora TC. Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. J Mycol Med. 2007; 17(4): 242-249. doi: 10.1016/j.mycmed.2007.08.001.

9. Ceylan O, Okmen G, Ugur A. Isolation of soil *Streptomyces* as source antibiotics active against antibiotic-resistant bacteria. EurAsian J Biosci. 2008; 2: 73-82.

10. Ryandini D, Pramono H, Sukanto S. Antibacterial activity of *Streptomyces* SAE4034 isolated from Segara Anakan mangrove rhizosphere against antibiotic resistant bacteria. Biosaintifika. 2018; 10(1): 117-124. doi: 10.15294/biosaintifika.v10i1.12896.

11. Dazzo F, Ganter S. Rhizosphere. In: Schaechter M, editor. Encyclopedia of Microbiology. San Diego: Academic Press; 2009. p. 335-349. doi: 10.1016/B978-012373944-5.00287-X.

12. Crawford DL, Lynch JM, Whipps JM, Ousley MA. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl Environ Microbiol. 1993; 59(11), 3899-3905. doi: 10.1128/aem.59.11.3899-3905.1993.

13. Trinidad-Cruz JR, Rincón-Enríquez G, Evangelista-Martínez Z, Guízar-González C, Enríquez-Vara JN, López-Pérez L *et al.* Actinobacteria from avocado rhizosphere: antagonistic activity against *Colletotrichum gloeosporioides* and *Xanthomonas* sp. Rev Terra Latinoam. 2021; 39: e802. doi: 10.28940/terra.v39i0.802.

14. Tello T. Dosis de gallinaza y distanciamiento de siembra de plantones de *Swinglea glutinosa* como cerco vivo y su efecto en las características agronómicas en Yurimaguas – Perú [internet] [Thesis]. Iquitos: Universidad Nacional de la Amazonía Peruana; 2015. Available in: http://repositorio.unapiquitos.edu.pe/handle/20.500.12737/4444.

15. Al-Dhabi NA, Esmail GA, Mohammed Ghilan AK, Arasu MV. Composting of vegetable waste using microbial consortium and biocontrol efficacy of *Streptomyces* sp. Al-Dhabi 30 isolated from the Saudi Arabian environment for sustainable agriculture. Sustainability. 2019; 11(23): 6845. doi: 10.3390/su11236845.

16. Kämpfer P. Order XIV. Streptomycetales ord. nov. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki K, Ludwig W, *et al.*, editors. Bergey's Manual of Systematic Bacteriology. Volume 5. The Actinobacteria, Part A and B. Athens, USA: Springer; 2012. p. 1446-1804. doi: 10.1007/978-0-387-68233-4.

17. Mackay SJ. Improved enumeration of *Streptomyces* spp. on a starch casein salt medium. Appl Environ Microbiol. 1977; 33(2): 227-230. doi: 10.1128/aem.33.2.227-230.1977.

18. Clinical and Laboratory Standards Institute – CLSI. Performance standards for antimicrobial susceptibility testing: 35th edition. Wayne, PA: CLSI; 2025.

19. Velho-Pereira S, Kamat NM. Antimicrobial screening of actinobacteria using a modified cross-streak method. Indian J Pharm Sci. 2011; 73(2): 223-228. doi: 10.4103/0250-474X.91566

20. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol. 1966; 16(3): 313-40. doi: 10.1099/00207713-16-3-313

21. Sharma D, Kaur T, Chadha BS, Manhas RK. Antimicrobial activity of actinomycetes against multidrug resistant *Staphylococcus aureus*, *E. coli* and various other pathogens. Trop J Pharm Res. 2011; 10(6): 801-808. doi: 10.4314/tjpr.v10i6.14

22. Santos ÉR, Teles ZNS, Campos NM, Souza DAJ, Bispo ASR, Nascimento RP. Production of α-amylase from *Streptomyces* sp. SLBA-08 strain using agro-industrial by-products. Brazilian Arch Biol Technol. 2012; 55(5): 793-800. doi: 10.1590/S1516-89132012000500020

23. Prasad P, Singh T, Bedi S. Characterization of the cellulolytic enzyme produced by *Streptomyces griseorubens* (Accession No. AB184139) isolated from Indian soil. J King Saud Univ – Sci. 2013; 25: 245–250. doi: 10.1016/j.jksus.2013.03.003

24. Manivasagan P, Gnanam S, Sivakumar K, Thangaradjou T, Vijayalakshmi S, Balasubramanian T. Isolation, identification and characterization of multiple enzyme producing actinobacteria from sediment samples of Kodiyakarai coast, the Bay of Bengal. J Microbiol. 2010; 4(14): 1550–1559. doi: 10.5897/AJMR.9000470

25. Ugur A, Sarac N, Boran R, Ayaz B, Ceylan O, Okmen G. New lipase for biodiesel production: partial purification and characterization of LipSB 25-4. ISRN Biochem. 2014; 289749. doi: 10.1155/2014/289749

26. Zarith N, Zin M, Tasrip NA, Nasir M, Desa M, Kqueen CY, *et al.* Characterization and antimicrobial activities of two *Streptomyces* isolates from soil in the periphery of Universiti Putra Malaysia. Trop Biomed. 2011; 28(3): 651-660.

27. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12): 1647-1649. doi: 10.1093/bioinformatics/bts199

28. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22(22): 4673-4680. doi: 10.1007/978-1-4020-6754-9_3188

29. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3): 403-410. doi: 10.1016/S0022-2836(05)80360-2

30. Osorio-Echeverri VM, Martinez J. *Streptomyces* from rhizospheres and compost [dataset]. Mendeley Data, V1. 2022. Available from doi: 10.17632/r7pfw2kywj.1

31. Kelly KL, Judd DB. Color. Universal Language and Dictionary of Names. Washington, DC: National Bureau of Standards. 1955.

32. Centore P. ISCC-NBS Colour System [Internet]. 2016 [accessed 10 March 2025]. Available in: https://www.munsellcolourscienceforpainters.com/ISCCNBS/ISCCNBSSystem.html

33. Pérez N, Jaramillo DF, Ruiz OS, Parra LN. Caracterización de un Andisol de la cuenca alta de la quebrada Santa Elena, Oriente Antioqueño, Colombia. Rev Fac Cienc. 2017; 6(1): 24-38. doi: 10.15446/rev.fac.cienc.v6n1.60628

34. Wemheuer F, Berkelmann D, Wemheuer B, Daniel R, Vidal S, Bisseleua HB. Agroforestry management systems drive the composition, diversity, and function of fungal and bacterial endophyte communities in *Theobroma cacao* leaves. Microorganisms. 2020; 8(3): 405. doi: 10.3390/microorganisms8030405

35. Williams HTP, Lenton TM. Artificial selection of simulated microbial ecosystems. Proc Natl Acad Sci USA. 2007; 104(21), 8918-8923. doi: 10.1073/pnas.0610038104

36. Rebollido R, Martínez J, Aguilera Y, Melchor K, Koerner I, Stegmann R. Microbial populations during composting process of organic fraction of municipal solid waste. Appl Ecol Environ Res. 2008; 6(3), 61-67. doi: 10.15666/aeer/0603_061067.

37. Salamoni SP, Mann MB, Campos FS, Franco AC, Germani JC, van der Sand ST. Preliminary characterization of some *Streptomyces* species isolated from a composting process and their antimicrobial potential. World J Microbiol Biotechnol. 2010; 26: 1847-1856. doi: 10.1007/s11274-010-0366-y.

38. Kiepas AB, Hoskisson PA, Pritchard L. 16S rRNA phylogeny and clustering is not a reliable proxy for genome-based taxonomy in *Streptomyces*. Microb Genom. 2024; 10(9):001287. doi: 10.1099/mgen.0.001287.

39. Kocher TD. Adaptive evolution and explosive speciation: the cichlid fish model. Nat Rev Genet. 2004; 5(4): 288-298. doi: 10.1038/nrg1316.

40. Lai YP, Loerger TR. Exploiting homoplasy in genome-wide association studies to enhance identification of antibiotic-resistance mutations in bacterial genomes. Evol Bioinform Online. 2020; 16: 1176934320944932. doi: 10.1177/1176934320944932.

41. Vilgalys R. Taxonomic misidentification in public DNA databases. New Phytol. 2003; 160(1): 4-5. doi: 10.1046/j.1469-8137.2003.00894.x

42. Chorlton SD. Ten common issues with reference sequence databases and how to mitigate them. Front Bioinform. 2024; 4:1278228. doi: 10.3389/fbinf.2024.1278228.

43. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol. 2017; 67(5):1613-1617. doi: 10.1099/ijsem.0.001755

44. Leibniz Institut DSMZ. *Streptomyces microflavus* Krainsky. BacDive. The Bacterial Diversity Metadatabase. 2020. doi: 10.13145/bacdive15404.20241212.9.2

45. Atta HM, Yassen AM. Antimicrobial activities of a *Streptomyces microflavus* isolated from KSA: taxonomy, fermentation, extraction and biological activities. Int J Life Sci. 2015; 4(4): 260-269.

46. Cho E, Kwon O-S, Chung B, Lee J, Sun J, Shin J, *et al.* Antibacterial activity of Chromomycins from a marine-derived *Streptomyces microflavus*. Mar Drugs. 2020; 18(10):522. doi: 10.3390/md18100522

47. Chung B, Kwon O-S, Shin J, Oh K-B. Antibacterial activity and mode of action of Lactoquinomycin A from *Streptomyces bacillaris*. Mar Drugs. 2021; 19(1):7. doi: 10.3390/md19010007

48. Taechowisan T, Chuen-Im T, Phutdhawong WS. Antibacterial and anticancer properties of Endophenazines from *Streptomyces prasinus* ZO16, an endophyte in *Zingiber officinale* Rosc. Pak J Biol Sci. 2024; 27:469-478. doi:10.3923/pjbs.2024.469.478

49. Li K, Tang X, Zhao J, Guo Y, Tang Y, Gao J. *Streptomyces cadmiisoli* sp. nov., a novel actinomycete isolated from cadmium-contaminated soil. Int J Syst Evol Microbiol. 2019; 69(4). doi: 10.1099/ijsem.0.003262