BRIEF REPORT

Streptomyces sp. 6E3 ANTIMICROBIAL ACTIVITY ISOLATED FROM MINERAL CONCENTRATE

Angela Ampuero [1,a], Rosario Rojas [2,b], Candy Ruiz [2,c], Jasmin Hurtado [1,d]

[1] Laboratorio de Biotecnología Ambiental, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru.

[2] Unidad de Investigación en Productos Naturales, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru.

[a] Pharmaceutical chemist; [b] Physician, PhD in Medical Chemistry and Pharmacognosy; [c] chemist, magister in Chemistry; [d] microbiologist, Science doctor

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ABSTRACT

The objectives of this study were to determine the antimicrobial activity of a culture of *Streptomyces* sp. 6E3 isolated from minerals against different pathogenic strains, to produce an extract and to estimate the minimum inhibitory concentration (MIC) of the fractions against methicillin-resistant *Staphylococcus aureus* (MRSA). *Streptomyces* sp. 6E3 showed antimicrobial activity primarily against *Staphylococcus aureus* (*S. aureus*). Five of the six fractions presented antimicrobial activity and the most effective gave a MIC of 0.88 ug / mL against *S. aureus* ATCC 33862, 0.44 ug / mL against *S. aureus* ATCC 43300 and 1.76 ug / mL vs. a S. aureus MRSA strain. *Streptomyces* sp. 6E3 has an antimicrobial potential against S. aureus strains resistant to methicillin and non-resistant, being of interest carrying out of more studies on its active metabolites.

Keywords: Streptomyces; Minerals, Anti-infective agents; Cell extracts; Bacteria; *Staphylococcus aureus*; Biological Products; Microbial Sensibility Tests (Source: MeSH NLM).

INTRODUCTION

Antibiotic resistance is one of the most important threats to global health, food security and national development. Currently, different alternatives are being developed to combat microbial strains that present multi-resistance, and one of them is the search for new secondary metabolites with antimicrobial bioactivity.

Among the microorganisms that produce bioactive compounds, *Streptomyces* are the most important genus of bacteria and produces compounds such as polyketide, peptides and polyketide-peptide hybrids, all of which have been characterized with different biological activities as antibacterial, antifungal and anticancer [(1)].

Approximately 60% of all known antibiotics against Gram-positive and Gram-negative bacteria have been isolated from *Streptomyces*, including tetracycline, daptomycin and chloramphenicol [(2)]. Lately, with the purpose of finding new metabolites with antimicrobial activity from these microorganisms *(Streptomyces*), the isolation of these bacteria has begun to be conducted from little explored environments such as the sea, plants [(3,4)] and even minerals [(5)].

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Correspondence to: Jasmin Hurtado; Av. Honorio Delgado 430, San Martin de Porres. Lima, Peru; jasmin.hurtado@upch.pe

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In Peru's marine environments, species with antibacterial activity have been found in strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant E*nterococcus faecalis* [6] and also a strain capable of acting against pathogenic strains resistant to beta-lactams [7].

This study aims to determine the antimicrobial activity of cultures and metabolic extracts of *Streptomyces* sp. 6E3 against different pathogenic enterobacteria, such as *Staphylococcus* aureus and *Candida sp.*

KEY MESSAGES

Motivation for the study: The increasing microbial resistance due to the indiscriminate use of antibiotics, requires the development of new alternatives, such as the use of microorganisms isolated from poorly studied environments.

Main findings: It has been possible to identify a strain of Streptomyces 6E3 isolated from minerals with the capacity to inhibit methicillin-resistant *Staphylococcus aureus.*

Implications: Obtaining microorganisms that could become an alternative treatment would help solve the public health problem we are facing.

THE STUDY

Experimental study to analyze the antimicrobial activity of the *Streptomyces* sp. 6E3 strain. This strain was isolated from minerals and identified previously, phenotypically and genetically [5]. The strains confronted with *Streptomyces* sp. 6E3 were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, Proteus mirabilis ATCC 12453, *Salmonella typhimurium* ATCC 25241, *Shigella sonnei* ATCC 25931, *Candida albicans* ATCC 90028, *Staphylococcus aureus* ATCC 43300 (MRSA) and *Staphylococcus aureus* (MRSA), from Instituto de Medicina Tropical Alexander Von Humboldt (Lima).

The production phase was determined by means of the antimicrobial activity test, in which the double-layer method of Singh *et al*. was used. [8] Cultures of Streptomyces sp. 6E3 sown in the center of the XGAL agar from 3, 5, 7 and 10 day growth plates were compared to strains of *Escherichia coli* ATCC 25922 and Staphylococcus aureus ATCC 33862. During these growth stages, colony growth was observed as well, both microscopically and macroscopically.

We used the double layer method of Singh *et al*. to screen the antimicrobial activity of the strain [8]. The experiment was carried out twice against each pathogenic strain. For the extracts and fractions, the agar diffusion method modified by Rojas *et al.* was used [9].

Ethyl acetate solvent was used for the preparation of the extracts. Thirty XGAL plates were sown and, with the aid of a micropipette, 3 ml of the solvent was added to the culture. The cells moistened by the solvent and the supernatant were swept and collected in Falcon tubes. The content from the mentioned tubes was homogenized with ultrasonic agitation for 15 minutes at room temperature. The tubes with the lysed cells were centrifuged for 10 minutes at 5000 rpm. The supernatant was then removed and the ethyl acetate solvent, evaporated, first in a rotavapor with a pressure of less than 250 millibars and in a water bath at 40 °C, and then with a nitrogen gas stream, until 147 mg of extract was obtained, which was kept at -20 °C until use.

A reverse-phase thin-layer chromatography (TLC Silicagel 60 RP18 from Merck®) with a mobile phase of acetonitrile (water in a ratio of 2:1) was performed on this extract to observe the number of compounds under a UV lamp at 254 nm and 366 nm. In addition, an antibiogram was performed by the diffusion method with discs to observe whether the desired antimicrobial effect was still present.

We used a glass column packed with silica gel RP60 from Merck®, pre-washed with methanol for the extract fractionation. 50 mg of the extract were dissolved in 400 uL ethyl acetate. The mobile phases were 20 mL of acetonitrile and water in ratios of 1:1 (v/v), 1:2, 6:4, 7:3, 8:2, 9:1 and 10:0. The fractions were collected in test tubes up to a volume of 4 mL and were subjected to reverse-phase thin-layer chromatography using an acetonitrile mobile phase: water, in a ratio of 3:1.

The chromatographies were observed under UV light of 366 nm. The fractions that presented similar chromatographic profiles were collected and the solvent was removed by means of the rotary evaporator. Finally, six fractions named A, B, C, D, E and F were obtained. A thin layer chromatography and an antibiogram, according to the disc diffusion method, were conducted again on the six fractions, to observe which of the fractions had the antimicrobial effect.

The method of Wiegan *et al.* [10] was used to determine the minimum inhibitory concentration (MIC). As positive control, vancomycin was used for MRSA strains, and penicillin for *Staphylococcus aureus*, which ranged from 120 to 0.06 ug/mL. The negative control was a Mueller Hinton broth inoculated with a standard strain; the blank one was a sterile broth. The six fractions were dissolved in 400 uL dimethyl sulfoxide (DMSO) to prepare stock solutions, and make the respective dilutions. The amounts of the fractions used to make the stock solutions

were as follows: 0.5 mg of the B fraction; 0.5 mg of the C fraction; 0.5 mg of the D fraction; 2 mg of the E fraction; and 1 mg of the F fraction.

The microplate was incubated at 37 °C for 24 hours. The results were obtained by two methods: a) triphenyltetrazolium chloride was used, in which the color change of the microwell contents to red indicated microbial growth, and b) the microplate was placed in an ELISA reader, in which only turbidity was measured. The MIC was found by means of absorbance versus concentration curves, according to the methodology of Devienne *et al.* [11]

Descriptive statistical analysis was performed to analyze the results of antimicrobial activity tests by the double layer and disc diffusion method.

RESULTS

Antimicrobial activity was observed in the 7- and 10-day cultures, which was related to the macroscopic observation of a reddish substance in the colony, while microscopically spores were observed, indicating the end of its mycelial phase (Figure 1).

In screening with *Staphylococcus aureus* ATCC 33862, *Staphylococcus aureus* ATCC 43300 (MRSA) and *Staphylococcus aureus* clinical strain (MRSA), inhibition halos from 21.5 mm to 42 mm in diameter were observed. All other strains were found to have inhibition halos of 1.5 mm to 3 mm, so the three Staphylococcus aureus strains were continued.

An extract of 141.9 mg was obtained, from which six fractions named A, B, C, D, E and F were obtained and six chromatographic spots numbered 1, 2, 3, 4, 5 and 6, respectively, could be identified. The thin layer chromatography performed on the fractions corroborated the presence of the same chromatographic spots present in the raw extract (Figure 2).

The antibacterial activity of the fractions was determined and inhibition halos of 8 to 15 mm were observed against *Staphylococcus aureus* strains. The D fraction showed the best activity, as well as a lower MIC unlike the rest of the fractions (Table 1 and Figure 3). The A fraction did not show any activity with the disk diffusion method, which is why the MIC was not determined

DISCUSSION

Bioactive compounds are mostly produced as secondary metabolites and some of them may also be pigments [12]. Many *Streptomyces* produce pigmented compounds with antimicrobial activity, such as the antibiotics actinorhodin (13) and roseoflavin [14]. The reddish coloration observed in the seven-day cultures may be related to the production of some antimicrobial compound produced by this bacterium.

The Streptomyces strain used in this investigation is of mineral origin, specifically from a concentrate of arsenopyrite [5]. It is evident that mining environments are very important for the search of strains with potential bioactivity, the same has been demonstrated in a study, in which Pleurostomophora sp. isolated from minerals produced anti-inflammatory compounds [15].

Using the double-layer method (Table 1) it was observed that the Streptomyces strain, although it showed inhibition of the gram-negative strains' growth, greater activity was ob-

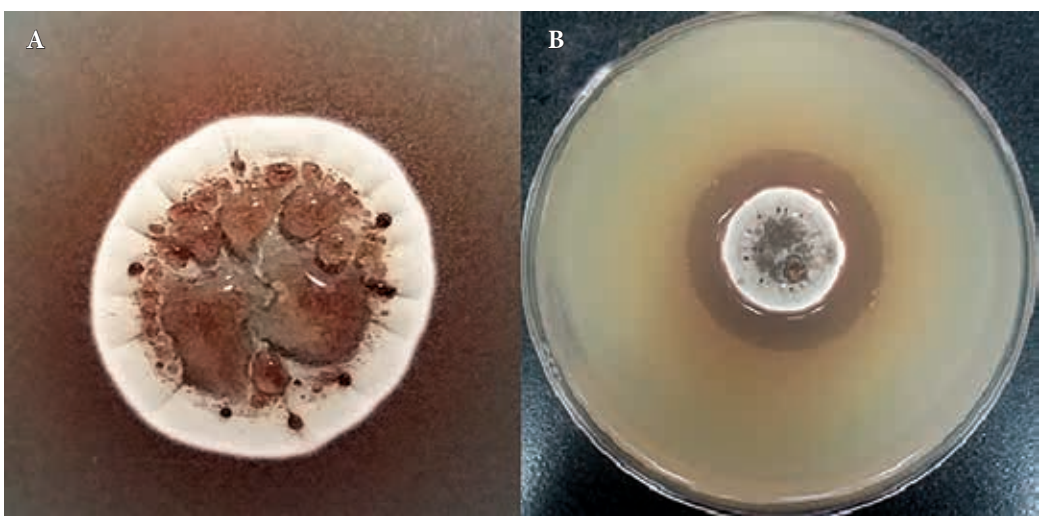

Figure 1. A) Determination of the production phase. Macroscopic observation of the *Streptomyces* sp. 6E3 colony at seven days. B) Antimicrobial activity of the strain against *Staphylococcus aureus* at seven days

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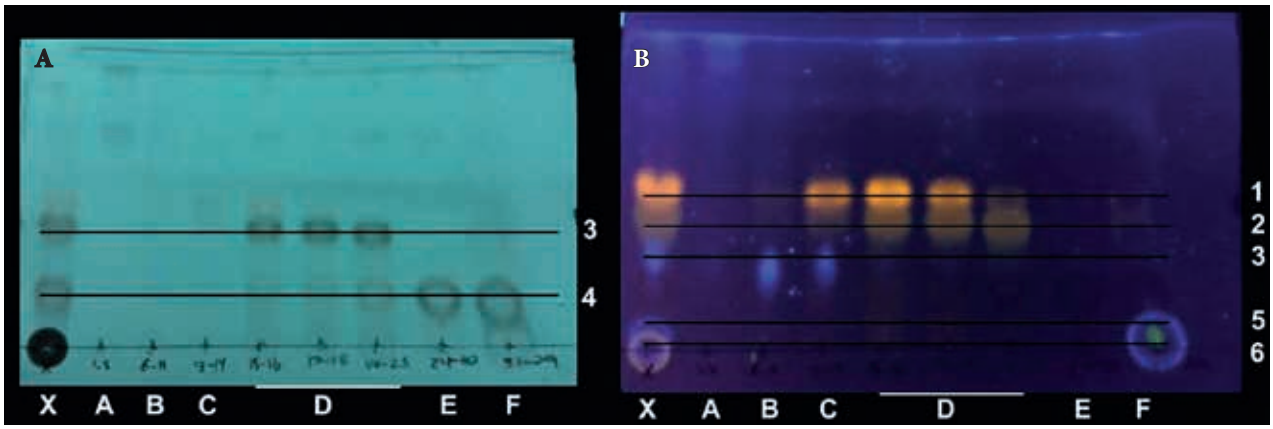


1-6: chromatographic spots; A-F: fractions; X: raw extract

Figure 2. Thin layer chromatography of the six fractions observed under UV lamp at A) 254 nm and B) 366 nm

tained against the group of gram-positive strains which were represented by the three strains of Staphylococcus aureus, including those resistant to methicillin. Streptomyces have been isolated from poorly studied environments, such as the marine environment. Strain RT 408, which produces a polycleotide [16], and coral associated *Streptomyces* [17], which inhibits gram-positive strains, including MRSA, have been isolated.

The extraction process was carried out by the surface sweeping of the culture plate, and it is possible that method facilitated recovery of the metabolites released by the bacteria into the environment or the ones contained within the cells.

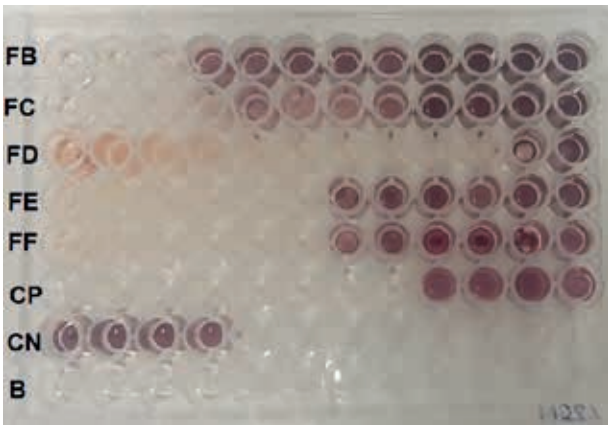
In the case of this strain, at least six chromatographic spots have been visualized by means of thin layer chromatography (Figure 2). Many of the species of the genus *Streptomyces* produce more than one metabolite depending on the product obtained, which is related to the solvent used and the carrying capacity according to its polarity and that of the compounds [18,19]. From the metabolites found, only some would have antimicrobial activity. *Streptomyces coelicolor*, in addition to producing actinorhodin, also produces other compounds with antimicrobial activity [13] and *Streptomyces violaceusniger* produces an antibacterial and antifungal compound [20].

The results obtained from the fractions' MIC test show that the D fraction is the one with the highest antimicrobial activity and concentration. Two chromatographic spots in this fraction were separated, this indicates a higher con-

Table 1. Antimicrobial activity of *Streptomyces* sp. 6E3 extract fractions against *Staphylococcus aureus* strains according to the disc diffusion and microdilution method

Fractions	Disc diffusion method			Microdilution method		
	Diameter of inhibition halos (mm)			MIC (ug/mL)		
	S. aureus ATCC 33862	*S. aureus* ATCC 43300 (SARM)	*S. aureus* clinical strain (SARM)	*S. aureus* ATCC 33862	*S. aureus* ATCC 43300 (SARM)	*S. aureus* clinical strain (SARM)
Fraction A	0	0	0	ND	ND	ND
Fraction B	13	13	12	8.13	8.13	16,3
Fraction C	10	14	13	8.13	8.13	16.3
Fraction D	14	11	11	0.88	0.44	1.76
Fraction E	8	10	9	2.19	2.19	4.38
Fraction F	0	15	13	8.75	2.19	8.75
Vancomycin [a]	ND	ND	ND	ND	0.12	0.47
Penicillin [b]	ND	ND	ND	0.06	ND	ND

ND: not determined; MIC: minimum inhibitory concentration
[a] Used as a positive control for the methicillin-resistant *S. aureus* strain
[b] Used as a positive control for the *S. aureus* ATCC 33862 strain



FB-FF: fractions; CP: positive control (vancomycin); CN: negative control (Mueller-Hinton broth inoculated); B: Blank (sterile Mueller-Hinton broth)

Figure 3. Minimum inhibitory concentration of the five fractions against *Staphylococcus aureus* ATCC 43300 (SARM)

centration of active metabolites with antimicrobial activity against Staphylococcus aureus strains.

The extract from *Streptomyces* sp. ERI-3 (compound isolated from *Streptomyces* sp) has been reported to produce inhibition of *Staphylococcus aureus* with a MIC of 0.25 mg/mL [19]. SPG278 has a MIC of 256 ug/mL against *Staphylococcus aureus* (N6) and the extract of marine-related *Streptomyces* M10-77 has a MIC de 7.9 ug/mL [6]; whereas, the D fraction of *Streptomyces* sp. 6E3 produced a MIC of 0.44 ug/mL against *Staphylococcus aureus* ATCC 43300 (MRSA).

The number of test repetitions is considered as one of the limitations of the study. It could not be determined whether the antimicrobial activity of the extract was in a compound within the cell or was excreted. Since several secondary metabolites may be present in each fraction of the extract, it is not clear whether the inhibition was due to the combined action of these metabolites or just by one of them.

In conclusion, *Streptomyces sp*. 6E3 showed increased antimicrobial activity against *Staphylococcus aureus* ATCC 33862 and MRSA strains.

Authors' contribution: JH, AA, CR and RR have participated in the conception of the experimental work, design of the article, as well as its analysis, data interpretation and writing. In addition, AA executed the experimental work and collected data, and CR supported during the execution. All authors approved the final version of the article.

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Conflict of interest: All authors have none to declare.

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