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ORIGINAL ARTICLE

Characterization of three native *Streptomyces* isolates that inhibit the growth of fluconazole-resistant *Candida* spp strains.

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Abstract

Most fungal infections are caused by species of the *Candida* genus, particularly *C. albicans*. The increasing number of strains developing resistance to antifungals, resulting in treatment failures, underscores the urgency of finding new antifungal agents. Since many bacteria of the genus *Streptomyces* produce molecules that inhibit fungal growth, this work aimed to evaluate the antifungal activity of three native isolates obtained from a rhizosphere and an artisanal composting system. Based on 16S RNA gene sequences, as well as biochemical and morphological traits, we identified *S. globisporus*, *S. bacillaris*, and *S. cavourensis* as the species most closely related to the S1H, S40, and S41 isolates, respectively. These species have been reported to produce antifungal compounds. The inhibition of *Candida* by antagonistic activity increased with longer *Streptomyces* incubation times, with no differences observed between *Candida* species. Few studies have simultaneously evaluated the inhibitory activity of *Streptomyces* isolates against different *Candida* strains. In this study, the isolates inhibited the growth of *C. albicans*, *C. krusei*, *C. guilliermondii*, *C. glabrata*, and *C. lusitaniae*, including strains resistant to fluconazole.

Keywords: actinobacteria, antagonism, antifungal activity, antimicrobial-resistant fungi, pathogenic yeasts, native microorganisms

1. Introduction

There is growing concern about the increasing number of reports of pathogenic and opportunistic microbial species, including those resistant to multiple antibiotics. Yeasts of the *Candida* genus are the leading cause of fungal infections worldwide and are highly prevalent opportunistic pathogens, particularly affecting individuals with weakened immune systems [1]. Each year, approximately 1.565.000 people develop *Candida* bloodstream infections or invasive candidiasis, resulting in 995.000 deaths (a mortality rate of 63.6%) [2]. Species such as *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei* are the main fungal pathogens. Their prominence is attributed to characteristics such as dimorphism, biofilm formation, and production of hydrolytic enzymes [3–5].

The incidence and distribution of fungal infections caused by *Candida* species differ by region and country based on factors such as climatic and sanitary conditions, antibiotic use, and individual susceptibility [6]. According to Motoa *et al.* [7], members of the *Candida* genus were among the most frequent causes of infections in Intensive Care Units of 20 third-level hospitals in Colombia

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between 2010 and 2013. Most mycoses (94.5%) reported in hospitalized patients were caused by *Candida* spp., with incidences of 48.3 % for *C. albicans*, 38.6 % for *C. tropicalis*, and 28.5 % for *C. parapsilosis*. These numbers exceed those reported in developed countries and other Latin American countries [8].

The global prevalence of infections caused by *C. krusei* has remained between 1.7 % and 3.2 % for many years. Despite this relatively low number, this species is associated with a high mortality rate (40 %-58 %) and exhibits low susceptibility to standard antifungal therapies [9]. Similarly, clinical isolates of *C. glabrata*, a prevalent source of candidemia in high-income countries, also exhibit reduced susceptibility to fluconazole [10]. *C. guilliermondii*, another species evaluated in this study, is a rare source of candidemia, with a low incidence (1 % - 3 %) and mortality rates ranging between 3.4 % and 66.6 %, particularly among immunocompromised individuals, transplant recipients, and critically ill patients. However, due to its resistance or reduced susceptibility to antifungal agents, the *C. guilliermondii* complex has been identified as a re-emerging pathogen in high-risk patient populations [11]. Finally, *C. lusitaniae* has been reported to be responsible for 19.3 % of fungemia cases in immunocompromised patients and 1.7 % of candidiasis cases in the general population. This species can quickly develop *in vivo* resistance to antifungal agents like amphotericin B and cause meningitis, peritonitis, and urinary tract infections in addition to fungemia [12].

Infections caused by *Candida* are treated with different drug classes, including polyenes, pyrimidines, echinocandins, and azoles, with itraconazole, fluconazole, voriconazole, and posaconazole being the most prescribed treatments [13]. As a result of the widespread and prolonged use of antifungal drugs, *Candida* species—particularly *C. albicans*, *C. glabrata*, and *C. parapsilosis*—have developed adaptations that contribute to resistance, leading to therapeutic failures associated with mortality rates that can reach an incidence of 78 % [8, 14]. This challenge has promoted efforts to identify new treatment and control strategies, including new molecular techniques for early diagnosis [15], combination of antifungal therapies [13], and the exploration of novel drugs or bioactive molecules [15, 16].

Bacteria of the genus *Streptomyces* are a significant source of bioactive compounds, as they produce a wide range of secondary metabolites, many of which have demonstrated potential for various pharmaceutical applications. For example, amphotericin B is naturally produced by *S. nodosus*. To date, more than 12.000 molecules synthesized by these actinobacteria have been reported, including many with antifungal activity produced by species such as *S. lavendulae, S. griseus, S. albidoflavus*, and *S. cheonanensis* [17–19].

The *Streptomyces* genus is composed of filamentous Gram-positive bacteria that are widely distributed worldwide. They thrive in various ecosystems, primarily in soils, where they are often found in association with plant roots. Previous studies, aimed at identifying native isolates with antimicrobial properties, have revealed a remarkable diversity of *Streptomyces* species in habitats such as rhizospheres, mangroves, forest reserves, and composting systems. These studies have also shown that native *Streptomyces* isolates can inhibit the growth of other microorganisms [20–23].

This growing need to discover new metabolites for treating diseases caused by both antibiotic-resistant and non-resistant microorganisms drives research into microbial bioprospecting. This approach focuses on discovering and identifying microorganisms with antimicrobial potential, serving as a first step in developing new therapeutic options for infections caused by fungi and bacteria [24, 25]. Considering Colombia's remarkable biodiversity, the limited reports of native isolates with antifungal activity, and the national policies promoting the development of new antibiotics [26], the aim of this study was to identify *Streptomyces* spp.

isolates with antifungal activity from rhizospheres and a composting system. We also sought to characterize these isolates microbiologically and confirm their ability to inhibit the growth of *Candida* spp. strains, including those resistant and non-resistant to commercial antifungals.

2. Materials and methods

2.1. Isolation of native actinobacteria

The soil sample from the rhizosphere was taken from a living fence (*Swinglea glutinosa*) located on private land in the township of Santa Elena, municipality of Medellín-Colombia (latitude 6°13'N, longitude 75°29'W, altitude 2200 m). The compost sample was taken from a one-week-old artisan organic solid waste treatment system implemented by the Colegio Mayor de Antioquia University Institution (IUCMA). Samples were collected at a depth of 5–10 cm, transported in sterile polyethylene bags, and stored at 4°C until processing. To obtain *Streptomyces* isolates, 10 g of each sample were resuspended in 100 mL of 0.9% w/v saline solution and agitated for 30 minutes at 150 rpm and 28 °C. Serial dilutions were performed and surface-plated on 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) [27]. The culture media was supplemented with 2.5 µg/mL rifampicin, and the pH was adjusted to 7.2. The plates were incubated at 28 °C until colony formation. Isolated colonies identified as Gram-positive filamentous bacteria were preserved in Brain Heart Infusion (BHI) broth (Merck) with 15% glycerol at -20 °C under the framework permit for specimen collection for non-commercial scientific research (No. 1467) issued by the Autoridad Nacional de Licencias Ambientales, with registration numbers 16D4FD0E9C3 and 16D551FC815.

2.2. Characterization of actinobacteria isolates

The native isolates were morphologically and physiologically characterized according to the International Streptomyces Project (ISP) and Bergey's Manual of Systematic Bacteriology [27,28]. Colonies of actinobacteria were subcultured on ISP-2 slants and incubated at 28 ºC for 3 weeks. Morphological characteristics were then examined by culturing the isolates on different ISP media [29]. Growth at various temperatures (20 ºC - 40 ºC) and NaCl concentrations (0.2 % - 10 %) were assessed by culturing the isolates on starch casein nitrate medium and checking the cultures 48 and 72 h after inoculation. The ability to break down different carbon sources was evaluated 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 ISP-9 medium [28].

2.3. Molecular identification of native isolates

The isolates were grown for 7 days on GYM agar at 30 °C. Bacterial colonies were inoculated into BHI broth, and cultures were incubated at 30 °C until a pellet of vegetative cells was obtained. Total genomic DNA was prepared using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. PCR amplification of the 16S rRNA gene was conducted using two primers, 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). The reaction was carried out in a total volume of 50 µL consisting of 10 µL 5X polymerase buffer, 4 µL $MgCl_2$ (25 mM), 1 µL dNTPs (10 mM), 4 µL of each primer (0.1 mM), 4 µL of template DNA, and 1.25 U of GoTaq® Flexi DNA Polymerase (Promega). The amplification reaction was performed using the following program: 30 cycles of 1 minute at 94 °C, 1 minute at 52 °C, and 1 minute at 72 °C, followed by a final incubation at 72 °C

for 10 minutes [30]. DNA extraction and PCR amplification were verified through agarose gel electrophoresis stained with EZ-Vision® In-Gel Solution 10000X (VWR) and visualized under ultraviolet light. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up Kit (Promega) and sequenced using the same PCR primers on an ABI-3500 automated sequencer, using the BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The quality of the forward and reverse nucleotide sequences was checked using Geneious software v10.0.9 [31]. We used the embedded Clustal W algorithm to perform alignments and generate consensus sequences [32]. We then subjected the consensus and reverse sequences to a BLASTn search to verify and compare them with sequences available in GenBank [33].

2.4. *Candida* strains and clinical isolates

We used the strains *C. krusei* ATCC 14243, *C. guilliermondii* ATCC 6260, *C. glabrata* ATCC 2001, and *C. albicans* ATCC 90028, along with two clinical *C. albicans* strains (CA1 and CA2) and a *C. lusitaniae* isolate, all obtained from the Faculty of Health Sciences microorganism collection, to evaluate antifungal activity. The yeasts were preserved in BHI broth with glycerol at -20 °C and were reactivated by incubation in Sabouraud agar (Merck) for 48 hours at 37 °C before the antagonism assays. We confirmed the identification and sensitivity to antifungals using the Vitek® 2 automated system with YST and AST-YS08 cards, respectively (see **Table 1**). Sensitivity was interpreted as sensitive, intermediate, or resistant. Fluconazole resistance was further confirmed by broth microdilution following the procedures of the Clinical and Laboratory Standards Institute [34].

2.5. Direct antagonism assays

We performed a cross-streak method to verify the antifungal activity of native isolates. BHI agar (Merck) plates were inoculated with a single streak of actinobacteria suspensions, obtained from colonies formed on GYM agar, at the center of the Petri dishes. The dishes were then incubated at 30 ºC for 2, 4, or 7 days. Yeasts were incubated on Sabouraud agar at 37 °C for 24 h, and suspensions were prepared from these cultures in 0.1 % peptone water, adjusting the optical density at 600 nm to between 0.08 and 0.1. The plates with native isolates were seeded with test organisms by streaking them perpendicular to the line of actinobacteria growth and incubating them at 37 °C for 24 h. Antagonism was assessed by examining the regions of growth and the clear or inhibited areas along each test streak. Inhibitory activity was calculated using **Eq. 1**., where AWG represents the area on the streak without growth and TSA represents the total streak area scored for each test streak [35] (more information in Suppl. 1). The results are expressed as mean values ± SD. We used the Shapiro-Wilk test to confirm the normality of the data and the Kruskal-Wallis test to determine differences between samples. Statistical analysis was performed using IBM SPSS Statistics software v.25. with differences of $p < 0.05$ considered statistically significant.

$$\%I = \frac{AWG}{TSA} \times 100, \tag{1}$$

Table 1. *Candida* spp. susceptibility profiles

Candida	FLU	VOR	CAS	MIF	FCT
C. krusei ATCC 14243	R (32)	S	I	S	R
C. guilliermondii ATCC 6260	S (1)	S	S	S	S
C. glabrata ATCC 2001	NR (32)	S	-	S	S
C. albicans ATCC 90028	S (0.25)	S	S	S	S
C. albicans CA1	R (8)	I	S	S	S
C. albicans CA2	R (32)	I	S	S	S
C. lusitaniae	S (1)	S	S	-	S

R: resistant; S: sensitive; I: intermediate. NR: not required. –: not evaluated. FLU: fluconazole; VOR: voriconazole; CAS: caspofungin; MIF: micafungin; FCT: flucytosine. In parentheses, Minimum Inhibitory Concentration (MIC) in µg/mL, confirmed by broth microdilution.

2.6. 96-well plate antimicrobial assay

Cultures of the *Streptomyces* isolates were grown in BHI broth. The inocula were prepared to a 1 McFarland turbidity standard from colonies obtained on GYM agar after six days of incubation. The inoculated broths were incubated at 30 °C for five days, shaking at 150 rpm, and centrifuged at 10.000 rpm for 10 minutes to obtain the supernatants. The process was performed in triplicate. *Candida* suspensions were prepared in Sabouraud broth with an optical density at 600 nm adjusted to between 0.08–0.1 as previously indicated. Each measurement, except for the positive controls, was performed in triplicate by adding 80 µL of Sabouraud broth, 10 µL of supernatant, and 10 µL of yeast suspension to each well. Fluconazole (64 µg/mL) was used as a positive control. A yeast growth control was conducted in the same broth. The plates were incubated at 37 °C for 48 hours and the optical density was measured at 630 nm using an MR-96A (MINDRAY) microplate reader. Inhibition was calculated based on the maximum growth observed in Sabouraud broth for each *Candida* isolate.

3. Results and Discussion

3.1. Actinobacteria isolation

Using the isolation methodology, we were able to recover bacteria with characteristic traits of the genus *Streptomyces*. We studied two isolates obtained from a composting system (S40 and S41) and one isolate recovered from the rhizosphere of a live fence (S1H). Microscopic observations revealed Gram-positive, rod-shaped, extensively branched filaments with spores formed on aerial hyphae for all native isolates. Previous similar studies have reported the isolation of actinobacteria from different types of composts and amended soils using culture media with similar compositions to those used in this study [36,37]. Although filamentous bacteria isolated from garden soils have been reported [38], we found no studies reporting the presence of *Streptomyces* in the rhizosphere of plants used as living fences.

3.2. Identification of native bacteria

Macroscopic examinations of cultures showed that all of them formed dry, opaque, raised colonies with rough surfaces and irregular margins (**Fig.1**). The aerial mass and substrate mycelium were predominantly yellowish-white, varying based on the culture medium (see **Table 2**). The isolates obtained from the composting system produced melanin, as well as soluble pigments in the ISP-2 and ISP-5 media (see **Table 3**). All the native isolates grew in culture media with a maximum of 2 % NaCl and assimilated all the evaluated sugars. Isolate S1H could hydrolyze urea. None of the isolates grew at temperatures below 20 °C but two isolates (S40 and S41) grew at 40 °C.

The color of the colonies, along with the other biochemical characteristics evaluated in this study, are insufficient to classify the native isolates into a specific *Streptomyces* species. Molecular tools are required for precise identification. A BLAST analysis of partial 16S rRNA gene sequences confirmed that the three native isolates belong to the genus *Streptomyces* and present an identity percentage above 99% when compared to the most closely related species (see **Table 4**). The S1H isolate was closely related to *S. globisporus* subsp. *globisporus*. Some traits reported for this subspecies, such as the color of the spore mass in certain culture media, the absence of melanoid pigments, and the use of the evaluated sugars coincide with those recorded for S1H. However, *S. globisporus* subsp. *caucasicus* and *S. globisporus* subsp. *flavofuscus*, which are known to have antifungal activity, show greater tolerance to NaCl [27]. Isolates S40 and S41 showed identical traits in this work, but they were identified as *S. bacillaris* and *S. cavourensis*, respectively. This confirms that morphologic and biochemical properties alone are insufficient to assign a single species to isolates of the genus *Streptomyces*. The 16S rRNA gene sequences of the native isolates matched multiple species/strains with identical identity scores and E-values. This indicates that RNA-based methods do not provide sufficient resolving power to identify *Streptomyces* strains at the species level; these analyses must be complemented with other phylogenetic studies [41].

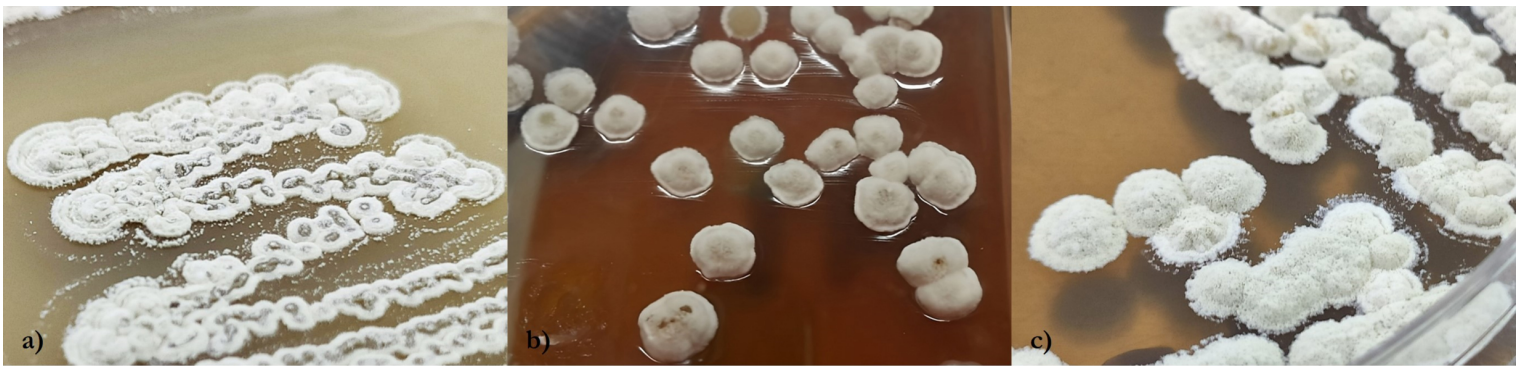

Figure 1. Colonies of native Streptomyces isolates. Cultures were grown on GYM agar and incubated at room temperature for at least 10 days. a. S1H, b. S40, c. S41.

Table 2. Colony color of native actinobacteria isolates grown in different culture media.

Isolate	ISP-2	ISP-3	ISP-4	ISP-5
Aerial mass				
S1H	light orange yellow	yellowish white	yellowish white	yellowish white
S40	yellowish white	yellowish white	yellowish white	yellowish white
S41	yellowish white	yellowish white	yellowish white	yellowish white
Substrate mycelium				
S1H	light yellow	moderate yellow	yellowish white	yellowish white
S40	yellowish white	yellowish white	yellowish white	brilliant yellow
S41	moderate orange yellow	yellowish white	yellowish white	pale greenish yellow

ISP-2, ISP-3, ISP-4, ISP-5: Culture media prepared according to the International *Streptomyces* Project [23]. Colors were recorded using the ISCC-NBS Color System [39, 40].

Table 3. Culture and physiological characteristics of native actinobacterial isolates.

Characteristic	Isolate		
	S1H	S40	S41
Melanin in ISP-7	–	+	+
Soluble pigment			
ISP-2	–	brown	brown
ISP-3	–	–	–
ISP-4	–	–	–
ISP-5	–	brown	brown
Hydrolysis of urea	+	–	–
Temperature range (°C)	20-37	20-40	20-40
NaCl tolerance	2%	2%	2%
Use of sugars			
D-Glucose	+	+	+
D-Xylose	+	+	+
L-Arabinose	±	+	+
L-Rhamnose	+	+	+
L-Fructose	+	+	+
D-Mannitol	+	+	+
I-Inositol	±	+	+
Sucrose	+	+	+

ISP-2, ISP-3, ISP-4, ISP-5, ISP-6, ISP-7: Culture media prepared according to the International *Streptomyces* Project [28]. (+): positive. (–): negative. (±): uncertain.

Table 4. BLAST analysis of partial 16S rRNA gene sequences for native *Streptomyces*

Isolate	Closely related species	Strain	GenBank accession	% identity	E-value
S1H	*S. globisporus*	PPT	OQ297178.1	100.00	0.0
S40	*S. bacillaris*	PA02	OQ559126.1	100.00	0.0
S41	*S. cavourensis*	QT227	MT072125.1	100.00	0.0

3.3. Inhibitory activity

The native actinobacteria inhibited the growth of all *Candida* strains. This inhibitory activity increased with longer incubation times of the *Streptomyces* before streaking the test strains. After 7 days of incubation, all the isolates completely inhibited the growth of the test fungi. Additionally, 76 % of the antagonism assays evaluated after 4 days of incubation showed a complete inhibition of *Candida* growth (see **Table 5**). We found significant differences in the percentage of inhibition of different *Candida* species based on the incubation time. All species presented the lowest value after 2 days, except for *C. glabrata,* which did not exhibit any differences across days. This confirms that the incubation time affects the accumulation of antifungal compounds produced by *Streptomyces* in the medium. However, for some isolates, such as S1H, two days were sufficient to produce compounds that completely inhibited the *Candida* growth.

A similar work reported inhibition levels of up to 80 % of *C. albicans* growth due to direct antagonism after 7 days of incubation of native actinobacteria. However, this inhibition was verified in Mueller-Hinton agar, which is less enriched than BHI agar. This could be a limitation, as actinobacteria may not accumulate antifungal compounds with the same efficiency in Mueller-Hinton agar as in BHI agar [35]. In another study, five *Streptomyces* isolates were obtained from soil samples, but only one of them, consistent with *S. griseoflavus*, inhibited *Candida* sp. growth through antagonism by perpendicular streaks with a zone of inhibition of 30 mm, similar to those obtained in our work (data not shown). The same study also showed that the composition of the culture medium affects the inhibitory activity of the *Streptomyces* isolates [38].

Table 5. Inhibition of *Candida* strains by native Streptomyces in an antagonism assay

Test organism	2 days			4 days		
	S1H	S40	S41	S1H	S40	S41
C. krusei	100.00 ± 0.0	93.33 ± 11.5	90.67 ± 8.3	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
C. guilliermondii	100.00 ± 0.0	0.00 ± 0.0	26.67 ± 11.5	100.00 ± 0.0	24.00 ± 4.0	20.00 ± 0.0
C. glabrata	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
C. albicans	100.00 ± 0.0	0.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
C. albicans CA1	100.00 ± 0.0	60.00 ± 0.0	62.67 ± 4.6	100.00 ± 0.0	86.67 ± 11.5	100.00 ± 0.0
C. albicans CA2	100.00 ± 0.0	86.67 ± 11.5	65.33 ± 2.3	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
C. lusitaniae	100.00 ± 0.0	6.67 ± 11.5	0.00 ± 0.0	100.00 ± 0.0	56.00 ± 14.4	25.33 ± 4.6

Mean values ± Standard deviation (SD) are shown.

The inhibition of three *Candida* strains showed no differences between the native isolates. However, four *Candida* strains were significantly less inhibited by at least one of the *Streptomyces* isolates (**Fig.2**). This behavior highlights the diverse interactions between the organisms studied, demonstrating both overall susceptibility and varying levels of susceptibility to different compounds produced by native isolates across *Candida* strains. Although few works have evaluated the inhibition of different *Candida* strains by *Streptomyces* isolates or the compounds they produce, Mendes [42] reported that extracts from the culture of at least seven native actinobacteria inhibited the growth of *C. albicans*, *C. glabrata*, and *C. krusei* to different degrees. Another study reported that extracts produced by *Streptomyces* sp. exhibited similar inhibitory activity against *C. albicans* and *C. krusei* [43]. A third study found that 37 % of the actinomycetes evaluated showed inhibitory activity against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, with inhibition percentages ranging between 27 % and 87 % [44].

The 96-well plate assay revealed differences in the inhibition caused by the crude extracts produced by the three *Streptomyces* isolates in liquid culture medium (**Fig.3**). The S1H isolate completely inhibited the growth of the seven *Candida* strains, showing significantly higher activity than the S40 and S41 isolates. The activity of S40 and S41 differed against *C. krusei* (resistant to fluconazole and flucytosine) and the clinical isolate of *C. lusitaniae* (with no reported antifungal resistance). Studies have shown that pure compounds isolated from fermentation broths of native *Streptomyces* strains obtained from fertile soils can inhibit the growth of different *Candida* species, even those with acquired or innate resistance to multiple antifungals [45]. Although this study focused on crude extracts, the high inhibition percentages observed suggest the potential for isolating similar bioactive molecules.

The species *S. bacillaris*, corresponding to isolate S40, has been reported to produce compounds with inhibitory activity against certain enzymes of different wild-type strains of *C. albicans*, although the *Streptomyces* strain tested in those studies was obtained from marine sediments, unlike S40 [46]. Several studies have confirmed the inhibitory activity of strains of *S. cavourensis* isolated from different soil samples against *C. albicans* and other fungi [47–49]. Finally, the antifungal activity of native isolates of *S. globisporus*, the species most closely related to the S1H isolate, was confirmed. The S1H isolate demonstrated the greatest inhibition of *Candida* growth in this study [42, 50].

We could not identify a relationship between the inhibitory activity of *Streptomyces* and the fluconazole resistance observed in the *Candida* strains used in this study. Two resistant strains (*C. krusei* and *C. albicans* CA2) were indistinctly inhibited by all the native isolates, while one resistant strain (*C. albicans* CA1) was more strongly inhibited by isolate S1H. Both the resistant and non-resistant strains showed greater inhibition with extended actinobacteria incubation times. However, *C. krusei*, which has innate resistance to fluconazole [51], showed considerable inhibition even with native isolates incubated for two days.

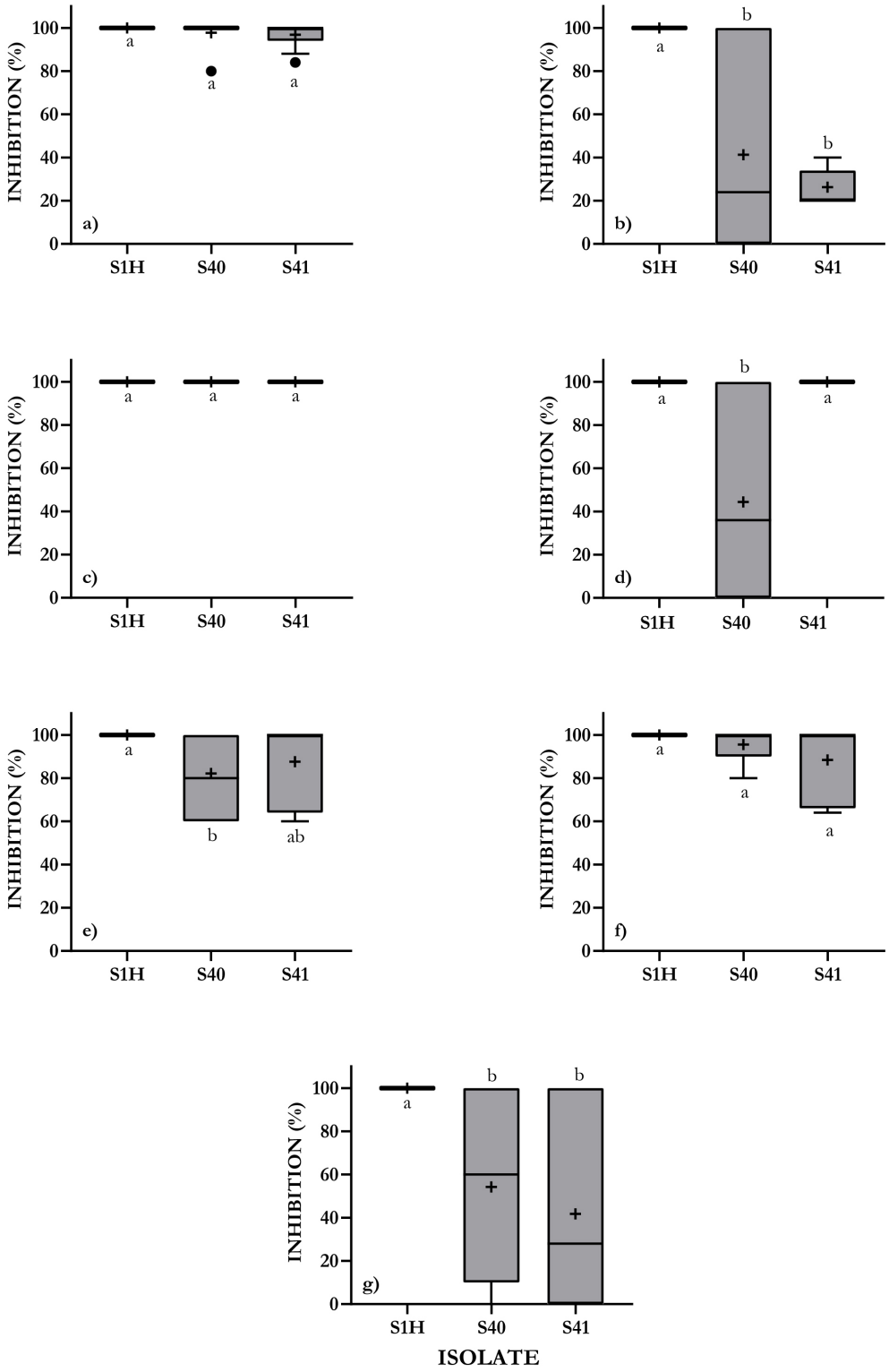

Figure 2. Inhibition of Candida spp. depending on the native isolate. Boxplots represent the median, arithmetic mean, and outliers. **a**. *C. krusei*, **b**. *C. guilliermondii*, **c**. *C. glabrata,* **d**. *C. albicans*, **e**. *C. albicans* CA1, **f**. *C. albicans* CA2, **g**. *C. lusitaniae*. Identical letters indicate no significant differences.

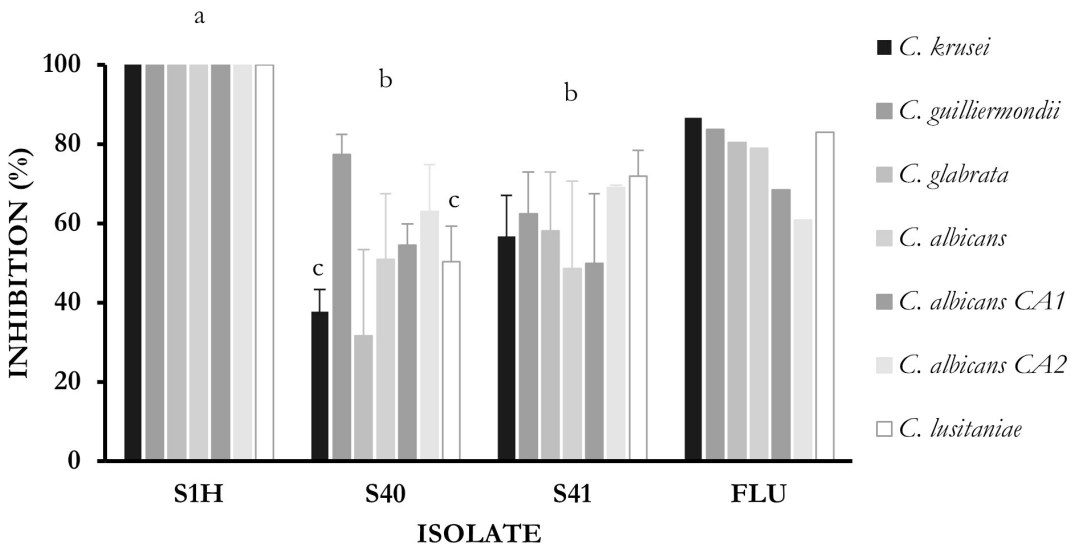

Figure 3. Inhibition of *Candida* spp. by 96-well plate antimicrobial assay depending on the native isolate. Bars represent the arithmetic mean. Identical letters indicate no significant differences. FLU: fluconazole (64 μg/mL).

4. Conclusions

The rhizosphere of a living fence and an artisanal composting system demonstrated potential as sources of native isolates of actinobacteria capable of inhibiting the growth of fungi from the *Candida* genus. We confirmed that the native isolates belong to the Streptomyces genus. Although morphological characteristics and sequence analysis were not sufficient to fully define the species corresponding with the isolates, their macroscopic and microscopic morphologies, biochemical traits, and partial 16S rRNA gene sequences identified *S. globisporus, S. bacillaris*, and *S. cavourensis* as the most closely related species. The inhibition of growth, both through direct antagonism and broth assays, did not show a specific pattern associated with the antifungal resistance of the *Candida* strains evaluated. However, the inhibitory activity was influenced by the species associated with the native isolate and the incubation time before starting the assays with *Candida*. We plan to confirm the antifungal potential of the cell-free supernatants obtained from the native Streptomyces cultivated in this study and proceed with separation and purification processes. Subsequently, we will conduct toxicity studies to ensure the potential safe application of the obtained metabolites for the treatment of diseases associated with the *Candida* genus.

5. Acknowledgements

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6. Conflict of interest

The authors declare no affiliations with or involvement in any organization or entity with a financial interest (such as honoraria, educational grants, speakers membership, employment, consultancies, stock ownership, equity interest, expert testimony, or patent arrangements), or non-financial interests (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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Caracterización de tres aislamientos nativos de *Streptomyces* que inhiben el crecimiento de cepas de *Candida* spp. resistentes a fluconazol

Resumen: La mayoría de las infecciones fúngicas son causadas por especies del género *Candida*, particularmente *C. albicans*. El aumento en el número de cepas que desarrollan resistencia a los antifúngicos, resultando en fallas terapéuticas, resalta la urgencia de encontrar nuevos agentes antifúngicos. Dado que muchas bacterias del género *Streptomyces* producen moléculas que inhiben el crecimiento de hongos, el objetivo de este trabajo fue evaluar la actividad antifúngica de tres aislamientos nativos obtenidos de una rizósfera y de un sistema de compostaje artesanal. Nos basamos en las secuencias del gen RNA 16S, junto con características bioquímicas y morfológicas, para identificar a *S. globisporus*, *S. bacillaris* y *S. cavourensis* como las especies más cercanas a los aislamientos S1H, S40 y S41, respectivamente. Se ha reportado que estas especies producen compuestos antifúngicos. Los aislamientos de *Streptomyces* inhibían con mayor efectividad el crecimiento de *Candida* por actividad antagonista después de tiempos de incubación más largos. Observamos diferencias entre las especies de *Candida*. Pocos estudios han evaluado simultáneamente la actividad inhibidora de aislamientos de *Streptomyces* frente a diferentes cepas de *Candida*. En este estudio, los aislamientos inhibieron el crecimiento de *C. albicans*, *C. krusei*, *C. guilliermondii*, *C. glabrata* y *C. lusitaniae*, incluyendo cepas resistentes a fluconazol.

Palabras Clave: Actinobacterias; Actividad antifúngica, Antagonismo; Hongos resistentes a antimicrobianos; Levaduras patógenas; Microorganismos nativos.

Caracterização de três isolados nativos de *Streptomyces* que inibem o crescimento de cepas de *Candida* spp. resistentes ao fluconazol

Resumo: A maioria das infecções fúngicas é causada por espécies do gênero *Candida*, particularmente *C. albicans*. O aumento no número de cepas que desenvolvem resistência a antifúngicos, resultando em falhas terapêuticas, destaca a urgência de encontrar novos agentes antifúngicos. Muitas bactérias do gênero *Streptomyces* produzem moléculas que inibem o crescimento de fungos. Por tanto, este trabalho teve como objetivo avaliar a atividade antifúngica de três isolados nativos obtidos de uma rizosfera e de um sistema de compostagem artesanal. Com base nas sequências do gene RNA 16S, junto com características bioquímicas e morfológicas, identificamos *S. globisporus*, *S. bacillaris* e *S. cavourensis* como as espécies mais próximas aos isolados S1H, S40 e S41, respectivamente. Essas espécies são conhecidas por produzir compostos antifúngicos. Os isolados de *Streptomyces* inibiram de forma mais eficaz o crescimento de *Candida* por atividade antagônica após tempos de incubação mais longos. Não observamos diferenças entre as espécies de *Candida*. Poucos estudos têm avaliado simultaneamente a atividade inibitória de isolados de *Streptomyces* contra diferentes cepas de *Candida*. Neste estudo, os isolados inibiram o crescimento de *C. albicans*, *C. krusei*, *C. guilliermondii*, *C. glabrata* e *C. lusitaniae*, incluindo cepas resistentes ao fluconazol.

Palavras-chave: Actinobactérias; Antagonismo; Atividade antifúngica; Fungos resistentes a antimicrobianos; Leveduras patogênicas; Microrganismos nativos.

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