Biotechnology Reports 43 (2024) e00852

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Research Article

Antarctic Streptomyces: Promising biocontrol agents for combating *Fusarium oxysporum* f. sp. cubense

Jeffrey Vargas Perez [a,*], Lizette Serrano [a], Rafael Viteri [a], Daynet Sosa [a,c], Christian A. Romero [a,b], Nardy Diez [a,c,1]

[a] ESPOL Polytechnic University, Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador

[b] Universidad Bolivariana del Ecuador, UBE, Carrera de Enfermería, Km 5.5 vía Durán-Yaguachi, Durán, Ecuador

[c] ESPOL Polytechnic University, Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias de la Vida (FCV), Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador

ARTICLE INFO

Keywords:
Psychrophiles
Streptomyces
Arbitrary unit
MIC
Fusarium wilt
Antarctic

ABSTRACT

Fusarium wilt of Banana (FWB) caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) poses a significant threat to the banana industry, with current inadequate control measures. This study evaluated the antifungal potential of nine *Streptomyces* strains isolated from Antarctic soil samples, using Casein-Starch media to stimulate the production of antifungal compounds. The inhibition spectrum against Foc was assessed under laboratory conditions using the well diffusion on Mueller-Hinton agar, with antifungal activity measured in arbitrary units (AU/mL) and minimum inhibitory concentration (MIC) tested using ethyl acetate extracts. Among the nine isolates, K6 and E7 were closely related to *Streptomyces polyrhachis* and *Streptomyces fildesensis*, exhibited significant antifungal activity, with K6 and E7 showing 320 and 80 AU/mL, and MIC values of 250 and >500 ppm, respectively. These findings highlight K6 and E7 as potential biocontrol agents against Foc, offering new avenues for sustainable Fusarium wilt management in banana cultivation.

1. Introduction

Fusarium oxysporum f. sp. *cubense* (Foc) is responsible for the most destructive disease, causing *Fusarium* wilt of Banana (FWB). This disease significantly impacts the global banana planting industry and is highly aggressive towards Cavendish cultivars. It is projected that the Fusarium Wilt Tropical Race 4 (Foc RT4) will affect 17 % of the banana crop areas by 2040, resulting in a loss of 36 million tons of production and an economic loss of US$ 10 billion [1]. Foc was first reported in Asia in the 1900s and spread to banana production regions in the Middle East, India, Africa, and Europe. Recently, it was reported in America, specifically in the northeast part of Colombia in 2019 [2]. Bananas (*Musa* spp.) are the most important crop worldwide and play a significant role in ensuring food security as a staple food, source of nutrition, and employment for millions of people [3,4]. In recent years, owing to the COVID-19 pandemic, severe weather conditions, the spread of plant diseases, and the alarming discovery of Foc TR4 in Latin America (LATAM), global export quantities dropped by 1.5 million tonnes in 2021 [5].

Efficient chemical control treatment for FWB is currently lacking, and the practice of monoculture for bananas, coupled with genetic uniformity, creates an ideal scenario for the development and spread of this epidemic. Biocontrol agents offer ecologically friendly alternatives for sustainable agricultural practices, replacing chemical pesticides that adversely affect the environment and human health [6]. These agents present numerous benefits in production, enhancing plant growth and yields. Microbial biostimulants, for instance, facilitate nutrient absorption and potentiate plant protection [7]. Biopesticides also play a crucial role in suppressing and managing phytopathogens and insects [8,9].

A recent investigation discovered antifungal mechanisms using *Streptomyces*, providing new alternatives to control and mitigate this plague [10]. *Streptomyces* secondary metabolites are produced during the stationary phase. These molecules are not necessary for their development, but they provide initial protection in the colonization process, offering UV protection, heavy metal retention, and antimicrobial activity [11]. *Streptomyces spp.* strains exhibited inhibition of

* Corresponding author.
E-mail addresses: jefdavar@espol.edu.ec (J.V. Perez), ndiez@espol.edu.ec (N. Diez).
[1] Have contributed equally as corresponding author.

https://doi.org/10.1016/j.btre.2024.e00852
Received 11 March 2024; Received in revised form 8 August 2024; Accepted 15 August 2024
Available online 21 August 2024

Fusarium sp. in vitro, with the secretion of chitinase identified as the likely cause of this antimicrobial activity. The interaction with plant tissues activates plant growth promoters, effectively inhibiting plant pathogens [12]. The broad-spectrum antifungal activity of the secretion can be attributed to the presence of molecules such as indole-3-acetic acid, siderophores, chitinase, β-1,3-glucanase, lipase, and urease [13]. *Streptomyces sichuanensis* exhibits a broad-spectrum antifungal activity against Foc TR4. The n-butanol extract contains terpenoids, esters, acids, macrolides, and thiopeptide, which induce apoptosis in Foc TR4 cells [14,15].

Previous studies have found that Antarctic actinomycetes such as *Streptomyces, Arthrobacter*, and *Rhodococcus* exhibit antifungal activity against phytopathogens such as *Fusarium oxysporum, Botrytis sp., Rhizoctonia solani*, and *Phytophthora infestans* [16]. *Streptomyces* species such as *Streptomyces huiliensis sp. nov*. have been used as biocontrol agents, presenting solid antagonistic activity against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4). It was reported that the ethyl acetate extracts reached a minimum inhibitory concentration of 6.25 μg/mL. These affect the spores and mycelia of Foc TR4, causing irregularity in the cell, reduction of the cell wall, and rupture of the nucleus, vacuole, and mitochondria, leading to low production of mycelia [17]. *Streptomyces sp.* presents similar behavior isolated from marine coral, whose fermentation broth applied to banana seedlings inoculated with Foc TR4 increases their resistance to the infection and inhibits the normal development of the fungus [8]. Furthermore, the application of the fermentation broth in the seedlings caused an increase in the chlorophyll content, stem diameter, leaf area, plant height, and leaf thickness, indicating that the fermentation broth acts as a growth promoter [18].

The Antarctic continent is the most significant natural reserve in the world. Only less than 1 % of the Antarctic continent (14,200,000 Km^2) is ice sheet-free. This Ice-free zone includes coastal, mountain, and maritime zones where biological activity occurs, and the soils of these zones were the objects of research in the last decade due to the population of diverse microorganisms in their phylogeny and functionality [19]. King George Island is the largest island in the complex South Shetland archipelago, with a length of 95 km and 25 km width and 90 % of its surface covered by glaciers [20]. The average annual temperature is below one °C in the coastal zone and decreases in the continent's interior [21]. Biological activities have developed despite extreme environmental conditions. The microbial biomass in Antarctic soil is generally low, with fungi contributing between 77 and 99 % of the total [22].

Antarctic bioactive compounds play a crucial role in modifying the chemical structure, resulting in the forming hydrophobic, hydrophilic, and charged residues that contribute to molecule's stability [23]. This Kingdom was represented in Antarctica by Ascomycota, Zygomycota, and Basidiomycota [24]. Acidobacteria, Actinobacteria, and Bacteroidetes are the dominant bacteria phyla [25]. There is some controversy regarding Actinomycete distribution in Antarctic soil. Some authors claim that finding Actinomycetes in these soils is extremely rare, while others say that 80 % of isolates from the desert area belong to this genus [26]. The importance of microbes in Antarctic soil has been extensively studied by the heterogeneity of the microbial community in the different areas of the continent. Bioprospecting Antarctic microorganisms can identify promising strains that can synthesize new or novel antifungal compounds with fungicide or fungistatic properties against Foc and determine their potential use as a sustainable management strategy for synthetic fungicides. Thus, this project aims to evaluate the antifungal activity of Casein-Starch culture broths of nine *Streptomyces* strains isolated from King George Island's soil in Antarctica against Foc using the well diffusion method and the minimum inhibitory concentration (MIC) of the ethyl acetate extracts [27].

2. Materials and methods

2.1. Sampling

Sampling collection was carried out during the Chilean Antarctic Expedition No. 56/Ecuadorian Expedition No. 24 in the Antarctic summer of 2020. Twelve soil samples were collected from various locations in Fildes Bay and around the Arctowski base on King George Island. The geographic coordinates of the collected samples were recorded using a Geographic Information System (Quantum GIS 3.16.0) Fig. 1. The selection encompassed twelve specific zones, including mosses and lichens, melting areas, coastal zones, bird nesting, and areas with abundant organic matter. About 50 g of soil were collected using a metal shovel and placed in sterile plastic bags with a seal. The samples were stored at -20 °C until transportation and further processing.

2.2. Isolation of Antarctic microorganisms

During the study, 12 soil samples were processed following the isolation protocol described by Pulschen et al., 2017 [28]. Serial dilutions of each sample (100 μL) were plated on Nutrient Broth Agar (NBA) diluted at a ratio of 1:10 [0.8 g/L BD DIFCO™ Nutrient Broth, 10 g/L BD BACTO™ Agar]. Then, the plates were incubated at 15 °C under dark conditions for 15 days [27]. After incubation, the colony-forming units (CFU) were counted for each dilution and replicated. Finally, colonies with a distinctive morphotype were transferred to fresh NBA plates using a sterile toothpick and incubated under the same conditions described above. The initial actinomycetes selection was based on macroscopic growth characteristics such as texture, form, substrate, and aerial mycelium [29]. The color of mycelium and the production of diffused pigment were determined by the mobile app Color Grab V3.9.2 LOOMATIX (www.loomatix.com) to identify the Hex code. Additional microscopic characterization was performed using the Gram staining technique [30]. The selected isolates were transferred to Petri dishes containing Muller-Hinton agar and incubated under the same conditions described above [31].

2.3. Bioprospection of antimicrobial compounds

To produce antimicrobial secondary metabolites, the carefully selected Streptomyces strains, chosen for their potential in antimicrobial production,were inoculated (1×10^4 cell/mL) in a modified Casein-Starch broth [3 g/L soluble starch, 3 g/L of glucose, 5 g/L of yeast extract, 5 g/L of tryptone, 1.2 g/L of potassium nitrate (KNO_3), 1.2 g/L dipotassium phosphate (K_2HPO_4), 0.03 g/L magnesium sulfate ($MgSO_4$), 0.012 g/L calcium carbonate ($CaCO_3$), 0.005 g/L iron sulfate II ($FeSO_4$)] [32]. Culture broths were incubated under continuous agitation at 120 rpm for 15 days at 15 °C. Then, the fermented broth was centrifuged at 5000 rpm for 15 min at 4 °C, and the resulting free cell supernatant (FCS) was collected. The agar-well diffusion method described by Danilovich et al., (2018) [27], was performed using Muller-Hinton agar (MHA) plates for the initial antimicrobial screening. A sterile punch was used to make a 0.6 mm diameter well, and 100 μL of each fermented broth was added to the wells. A 0.6 mm circular slice of agar with a *Fusarium oxysporum* f. sp. *cubense* (Foc) race 1, from CIBE's Microbial Culture Collection (EC15-E-GM1, EC40-M-GM2, EC35-G-GM1, EC19–2R-GM3) and obtained from banana plants exhibiting Fusarium wilt symptoms, was placed in the center of the plate, and then incubated for seven days at 27 °C. Clear inhibition zones (halo) around the sample wells indicated the presence of antifungal compounds. The strains that exhibited inhibition against Foc were chosen for molecular identification and evaluation of their antifungal properties.

2.4. Evaluation of antifungal activity

The antifungal activity of the fermented broth was detected using the

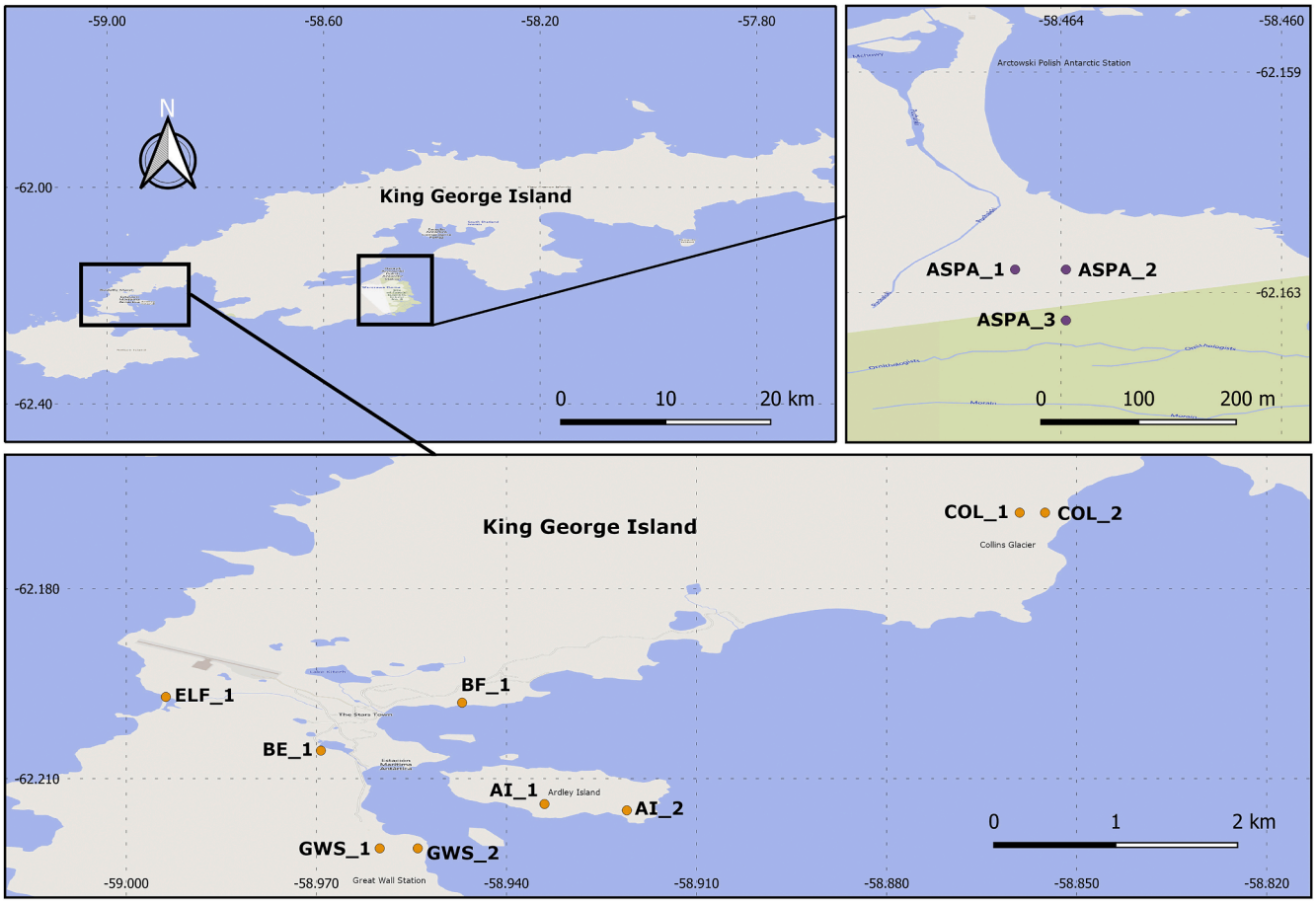

Fig. 1. Sampling site locations on King George Island.

microdilution method [33] to determine the antifungal agent's minimum inhibitory concentration (MIC). AlamarBlue® dye solution (Invitrogen, cat# DAL1025) was used to evaluate the viability of fungal cells post-treatment. AlamarBlue® is a cell viability reagent containing resazurin, a blue dye that is reduced to the pink and fluorescent compound resorufin by metabolically active cells. The degree of this reduction is directly proportional to the number of living cells, making it a reliable indicator of cell viability. The reduction process was measured and expressed as the percentage reduction of AlamarBlue® (%RAB).

A two-fold serial dilution of the fermented broth was prepared in Mueller-Hinton broth (MHB). A volume of 100 µL of each dilution was dispensed into individual wells of a 96-well microtiter plate, along with its respective replicate. Each well was inoculated with 10 µL of a fungal cell suspension containing 1 × 10^4 cells/mL of *Fusarium oxysporum* f. sp. *cubense* (Foc), strain EC15-E-GM1, following the protocol described by Al-Hatmi et al. [33]. Sterile MHB inoculated with Foc was included as a positive growth control (C+), and fenpropidin, a known antifungal agent, was used at a concentration of 15 µg/µL as a negative control (C-) to ensure fungal inhibition. The plates were incubated at 27 °C for 72 h. After incubation, AlamarBlue® reagent was added to each well at a final concentration of 10 % (v/v). The plates were further incubated for an additional 4 h to allow the reduction of resazurin by viable cells. Fluorescence, indicating the reduction of AlamarBlue®, was measured using a UV-Vis spectrophotometer (BioTek Synergy HTX) set on an excitation wavelength of 530 and an emission wavelength of 590 nm.

The percentage reduction of AlamarBlue® (%RAB) was calculated using the equation described by Al-Nasiry et al. [34]. Antifungal activity was expressed in arbitrary units per milliliter (AU/mL). One arbitrary unit (AU) was defined as the inverse of the highest dilution showing antifungal activity (%RAB), divided by the milliliters of diluted broth used (AU = 1/dilution/mL of the amount of fermented broth used), indicating fungal inhibition [35,36].

2.5. Extraction of active compounds

The fermented broth was centrifuged at 12,000 rpm for 30 min at 10 °C to separate the pellet. The resulting supernatant was mixed with ethyl acetate (EtOAc) in a 1:1 ratio. The EtOAc layer was separated and concentrated using a rotary evaporator at 40 °C, and the resulting extract was dried in a vacuum centrifuge at 1400 rpm for 30 min at 45 °C. The dry crude extract obtained was weighed and stored until further analysis for Minimum Inhibitory Concentration (MIC) evaluation, expressed in parts per million (ppm) [37,38].

2.6. Evaluation of minimum inhibitory concentration (MIC)

The dry crude extract obtained, 5.2 mg of K6 and 3.7 mg of E7, was dissolved in dimethyl sulfoxide (DMSO) for a final concentration of 5000 mg/L, equivalent to 5000 ppm. Serial twofold dilution was made in Muller-Hinton broth to obtain concentrations ranging from 2000 to 0.977 ppm, with a final DMSO concentration of 6 %. This serial dilution was evaluated following the microdilution method [33].

A *Fusarium* cell suspension was added to the wells, and the plate was incubated at 27 °C for 72 h. AlamarBlue® was added to evaluate cell viability, and the MIC values were determined by measuring the percentage reduction of AlamarBlue® (%RAB) and calculating the IC50. All experiments were conducted in triplicate following established protocols.

2.7. Molecular identification and phylogenetic analysis

The strains that exhibited antagonistic activity were incubated in MHB for 5–7 days at 15 °C and centrifuge at 12,000 rpm for 10 min at 10 °C. Genomic DNA was extracted from bacterial isolates using a modified protocol [39] and then stored at -20 °C until used. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal eubacterial primer set 27F (5′AGAGTTTGATCCTGGCTCAG 3′) and 1492R (5′ CGGTTACCTTGTTACGACTT 3′). PCR was performed in a standard 25 μL reaction with 1 μL of DNA template (20 ng/μL), 12.5 μL of GoTaq® Master Mix (Promega Corporation), 1 μL of each 10 μM primer, and 9.5 μL of water. Initial denaturation at 96 °C for 5 min was followed by 35 cycles of denaturation at 96 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. There was a final extension at 72 °C for 3 min. The PCR product was analyzed on a 1.8 % (w/v) agarose gel by electrophoresis. The PCR products were purified and sequenced at Macrogen Inc. (Seoul, South Korea). BLASTn was used to compare the sequenced data to the NCBI's nucleotide sequence database. The phylogenetic trees were generated in MEGA 11 [40]. Utilizing closely related reference species, the phylogenetic tree was constructed, incorporating an *Atinokineospora xionganensis* HBU206404 (MN582987.3) and *Micrococcus lylae* DSM20315 (X80750.1) sequences as outgroups. The TN93 model was applied the *Streptomyces* alignment, including gamma distribution (G) and the proportion of invariable sites (I). For the Streptomycetaceae align, the GTR (General Time Reversible) model was applied, supplemented with gamma distribution (G) and the proportion of invariable sites (I).

2.8. Data analysis

The IC50 values of the percentage of reduction of AlamarBlue (AB) for the nine isolates were analyzed using linear regression analysis via an internet tool (MLA-"Quest Graph IC50 calculator" by AAT Bioquest, Inc. [41], https://www.aatbio.com/tools/ic50-calculator). Subsequently, variance analysis (ANOVA), Kruskal-Wallis analysis with a significance level of 5 % ($p < 0.5$), and least significant difference tests were conducted using Infostat software [42]. For each treatment replicate, standard error values were recorded.

3. Results

3.1. Microbial loads and isolated Antarctic microorganisms

Macroscopic and microscopic characterization facilitated the isolation of 77 strains from a pool of 162 cultivable ones, yielding 49 species upon molecular identification. Among these, actinomycetes comprised 51 isolates (66.23 % of the total isolates). Samples from the Great Wall Station and Arctowski Base, specifically GWS_2 and ASPA_3, respectively, exhibited the highest isolate counts. Despite GWS_2 showing a lower UFC count than ASPA_3, it showcased a greater species diversity. Conversely, sample AI_2 from Ardley Island, despite having the second-highest UFC count, only yielded three isolated species. The areas with the highest counts of microorganisms in all samples were Henryk Arctowski Base (ASPA), Ardley Island, and Collins Island, with loads of 6E + 06, 4E + 06, and 2E + 05 CFU/mL, respectively. Notably, these regions exhibited the presence of mosses and lichens, indicative of a rich microbial environment, with prior research correlating the abundance of microorganisms to the presence of plant life and organic matter content.

3.2. Antifungal activity of Antarctic actinomycete isolates

This study unveils significant findings on the antifungal activity of 21 actinomycete isolates, a remarkable 41.18 % of the total screened. Notably, nine of these isolates maintained their biological activity upon re-testing, demonstrating potential as antifungal agents, Fig. 2. These isolates, sourced from diverse and unique Antarctic locations, exhibited

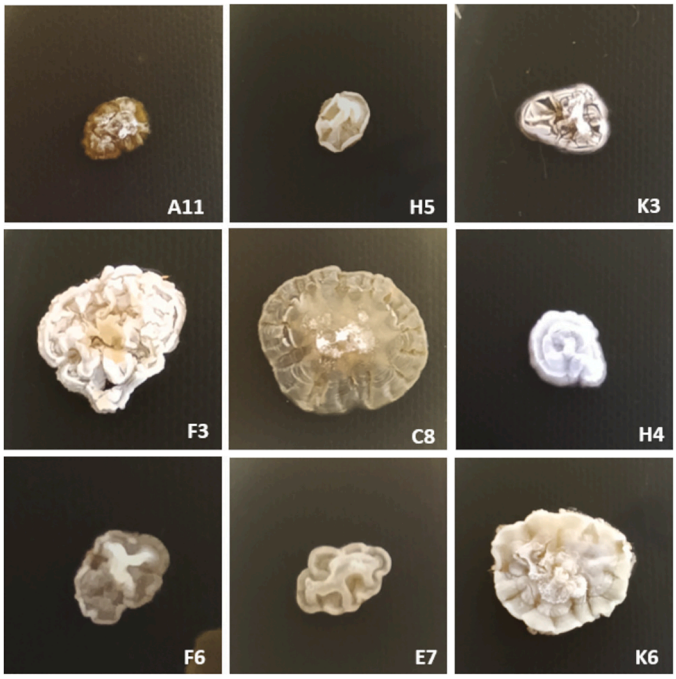

Fig. 2. Antarctic *Streptomyces* exhibiting inhibitory activity against *Fusarium oxysporum f.sp.* cubense.

significant inhibition of *Fusarium oxysporum* f. sp. *cubense (Foc)* mycelial growth. The isolates from Escudero Base (E7) and Great Wall Station (K6) show promising potential as high antifungal activity biocontrol agents.

The microdilution assay revealed that the fermented broth effectively inhibited the growth of Foc cells, as evidenced by the oxidized AlamarBlue reagent turning blue. The Kruskal-Wallis test applied to the IC50 values in arbitrary units per milliliter (AU/mL) of nine Streptomyces fermented broths showed significant variation among the isolates ($H = 24.52$, $p < 0.0019$), indicating differences in antifungal potency. Isolate K6 demonstrated the highest inhibitory potency with an IC50 value of 614.12 ± 2.33 AU/mL, followed by isolate E7 with an IC50 value of 280.83 ± 9.2 AU/mL. Other isolates, such as A11, H5, K3, F3, C8, H4, and F6, exhibited progressively higher IC50 values, indicating relatively lower antifungal potency (Fig. 3A).

Maximum inhibition was observed at 320 AU/mL for isolate K6 and 80 AU/mL for isolate E7, while the remaining isolates showed inhibition levels below 40 AU/mL (Fig. 4A). Isolate K6 exhibited a significant increase in AlamarBlue reduction with increasing concentrations of the fermented broth. At concentrations ranging from 20 to 320 AU/mL, reduction percentages ranged from 0.65 % to 1.89 %, indicating potent inhibition of Foc growth. At 640 AU/mL, the reduction reached 60.83 %, and at higher concentrations (1280 AU/mL and above), it reached 99–100 %, indicating no inhibition. Similarly, isolate E7 showed a reduction percentage ranging from 1.75 % to 55.51 % at 20–320 AU/mL concentrations, rising to 91.73 % at 640 AU/mL, highlighting notable inhibition by K6 and E7 even at lower concentrations (Fig. 3B).

Additionally, analysis of the active extract's Minimum Inhibitory Concentration (MIC) revealed oxidized AlamarBlue presence at 250 ppm for K6 and 1000 ppm for E7 (Fig. 4B). The IC50 estimate for K6 was 222.86 ± 5.2 ppm, while for E7 it was 914.25 ± 19.5 ppm. These values illustrate notable differences between the isolates, with K6 showing higher inhibitory activity than E7. The decrease in activity observed with the chemical extraction method suggests the differential composition of active compounds in the fermented broths of the two isolates. The principal characteristic of the colony and optimal growth temperatures for the K6 and E7 are summarized in the Table 1.

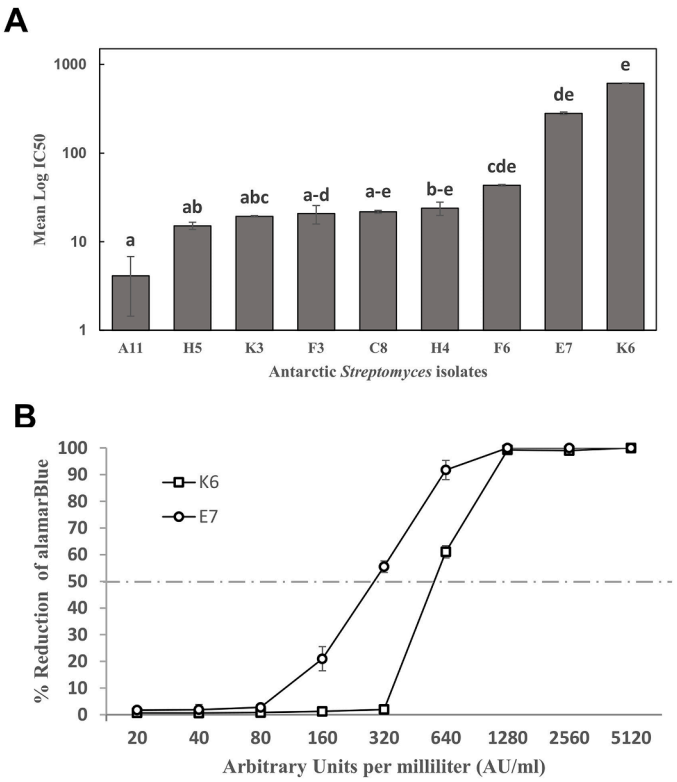

Fig. 3. A. Variability in Antifungal Potency Among Streptomyces Isolates: The graph illustrates the mean units per milliliter (AU/mL) of Streptomyces fermented broths among different isolates. The Kruskal-Wallis test indicates significant variability among the isolates, suggesting differences in antifungal potency. B. AlamarBlue percent reduction was measured at various fermented broth dilutions. Each line represents the K6 and E7 isolates. Foc spores were incubated for 72 h. Error bars indicate the standard deviation of% RAB measured.

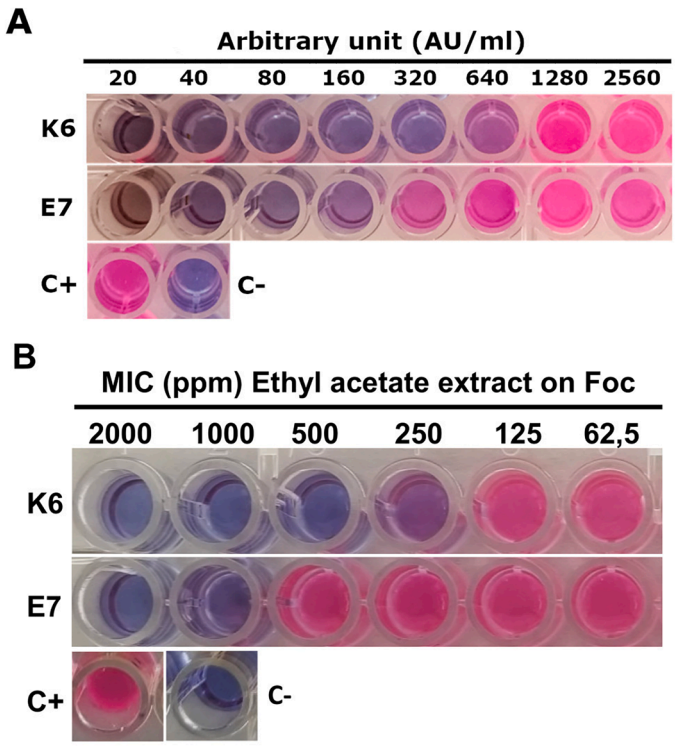

Fig. 4. Isolation and antifungal activity of Streptomyces sp. K6 y Streptomyces sp. E7. Control positive (C+) and Control negative (C-). A. Cell viability test of the broth using AlamarBlue reagent over 72 h. The reduction of Resazurin oxide (blue) to resorufin (pink) indicates the cell viability of Foc TR4 incubated in each Arbitrary unit (AU/mL) tested. B. Minimum inhibitory concentration (MIC) of the bioactive compounds in the ethyl acetate extracts.

3.3. Molecular identification and phylogenetic analysis

The partially complete 16S rRNA gene sequences of the selected strains (H5, E7, F6, K3, A11, and C8) were aligned with those of closely related strains retrieved from the NCBI database. Strains H5, E7, A11, F6, and K3, exhibited the highest similarities to *Streptomyces fildesensis* (98.41, 99.56, 98.85, 99.11, 99.71, 99.63, and 99.20 %, respectively) (Fig. 5A); F3 and K6 showed 97.04 and 96.98 % genetic similarity to *S. polyrhachis* (Fig. 5B); C8 has 99.57 % similarity to *S. xanthophaeus*; and H4 to *Embleya hialina* with 98.48 %. The phylogenetic analysis showed that the strains K3 and A11 were closely related to *S. fildesensis*, forming a distinct clade (Fig. 5B), while H5 and E7, formed a separate clade, like strain F6. On the other hand, strain H4 clustered with *Embleya spp.*, whereas K6 and F3 showed the largest phylogenetic distance to the *Streptomyces* strains used for this analysis. Further genetic characterization using other gene markers (i.e. atpD, gyrB, recA, rpoB, and trpB) is required to better understand the phylogenetic relationship between the selected strains.

The isolates were deposited at CIBE's Microbial Culture Collection (CCM-CIBE)", CIBE-ESPOL, Ecuador, and their respective accession numbers are listed in Table 2.

4. Discussion

Actinomyces are distributed in nature, forming a large part of the microbial population of soil, aquatic ecosystems, marine environments and salt marshes [7], exhibiting resilience to extreme conditions (i.e. high temperatures, high salt concentrations, low pH) [43,44]. Despite

Table 1
Colony characteristics of the antifungal producer actinomycetes.

Isolate	IG [a]	AM [b]	DP [c]	MIC [d]	IC50[e]	OGTR[f]
K6	*Streptomyces polyrhachis*	White	Dark brown: Orange	1000 ppm	914,25 ± 19,5 ppm	28 °C [55]
E7	*Streptomyces fildesensis*	White	Dark brown: Orange	250 ppm	222,86 ± 5,22 ppm	4 - 15 °C [56,57]

[a] Identified genera. [b] Color of aerial mycelium. [c] Diffusible pigment. [d] Minimum inhibitory concentration for Foc. [e] Inhibitory concentration (IC50) of EtOAc extract. [f] Optimal growth temperature reported.

Antarctica's harsh environment, King George Island's soil (KGI) supports the development of diverse Actinomycete communities, especially in areas with high microbial richness such as moss-covered regions and those near bird habitats [45,46]. Conversely, zones near the Collins Glacier have lower microbial counts [47]. Over decades, discovering secondary metabolites from Actinomycetes (ASMS) has played a crucial role in human medicine and biotechnology, particularly for antimicrobial compounds [11]. Antarctic actinomycetes are a valuable reservoir of novel biosynthetic genes with the potential to synthesize new structurally diverse bioactive molecules with antimicrobial, phytotoxic, and antitumoral activities [48]. However, many gene clusters remain cryptic and require biological elicitation to activate silent pathways for new secondary metabolite synthesis [49,50]. *Streptomyces* has been widely employed as a biological control agent against Foc due to its potential for producing novel metabolites that inhibit fungal growth and stimulate plant defense mechanisms [51], a study reported that 48 % of the tested Antarctic Actinomycetes strains, from the genera *Streptomyces sp.* and

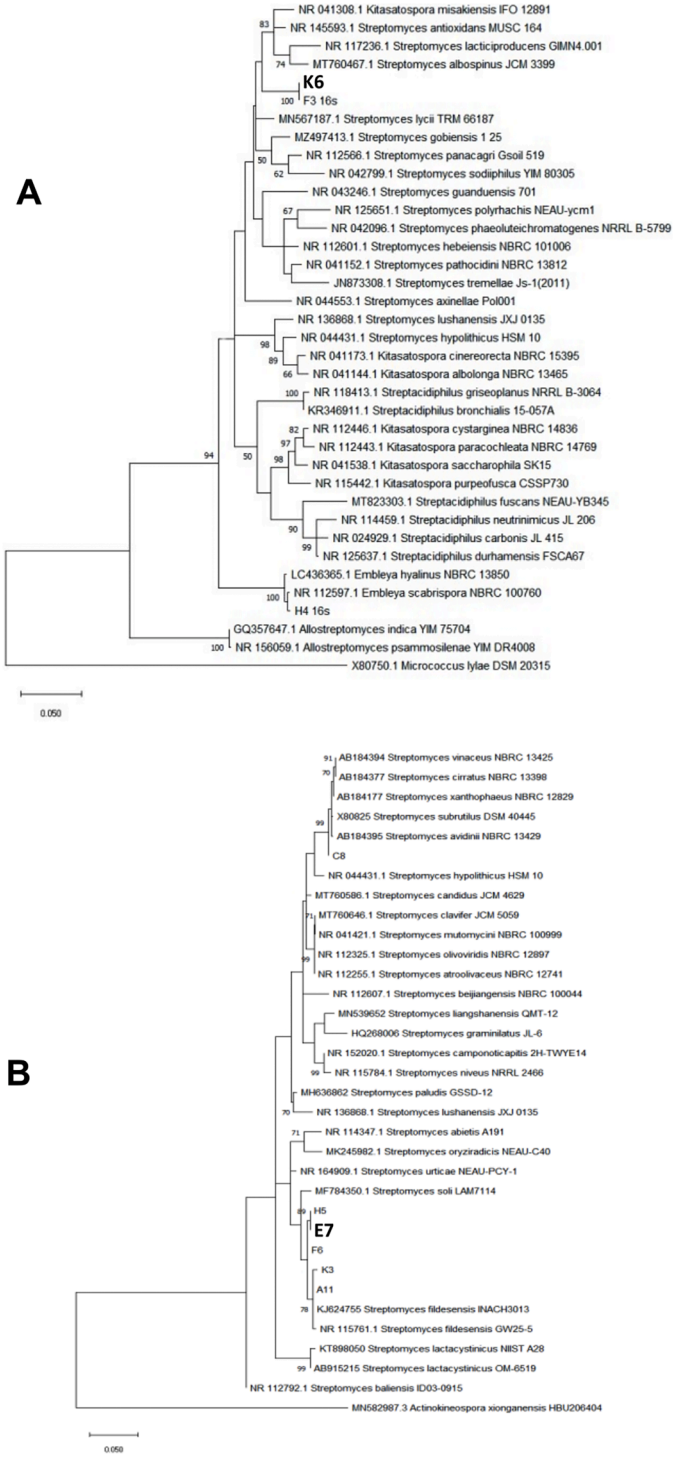

Fig. 5. Phylogenetic tree of Antarctic Streptomyces isolates, highlighting their evolutionary relationship with different Streptomyces species. The tree was constructed based on the comparison of 16S rRNA sequences. A. The maximum likelihood phylogenetic tree was generated using the GTR+G + I (General time reversible model with gamma distribution, and proportion of invariable sites) showing the relationship between the selected strains (K6, F3, and H4) and other genera of the family Streptomycetaceae; the numbers displayed at the branch points indicate bootstrap percentages derived from 500 replications, with only values above 70 % shown. B. A maximum likelihood phylogenetic tree constructed using the TN93+G + I (Tamura-Nei model with gamma distribution, and proportion of invariable sites), it shows the relationship between the selected strains (C8, H5, E7, F6, K3, and A11) and other species of the genus Streptomyces; numbers at branch points indicate bootstrap percentages (based on 500 replications); only values above 50 % are shown.

Table 2
Molecular identification of the *Streptomyces* isolates.

Isolate code	GenBank accession number	Closer taxon (16SrARN)	Percent identity (%)
A11	OQ623321	***Streptomyces fildesensis***	100
C8	OQ623320	***Streptomyces nojiriensis***	99,91
E7	OQ623319	***Streptomyces fildesensis***	99,4
F3	OQ623318	***Streptomyces polyrhachis***	96,91
F6	OQ623323	***Streptomyces fildesensis***	100
H4	OQ623316	***Streptomyces scabrisporus***	99,13
H5	OQ623317	***Streptomyces fildesensis***	99,11
K3	OQ623315	***Streptomyces sp.***	99,85
K6	OQ623322	***Streptomyces polyrhachis***	96,98

Arthrobacter sp., were able to inhibit the growth of Foc [16].

Recent reports highlight *Streptomyces* crude extracts and fermented broths to exhibit antifungal activity against Foc 4 in banana plants [10, 17]. Cyclic peptides, peptide derivatives, linear peptides, and macrocycles are a group of secondary metabolites biosynthesized by this genus where ulleungamide, legonimide, somamycins, and polyoxin D have shown potent fungicide activity [52,53]. The mechanism of action of antifungal compounds targets specific enzymes, disrupting ergosterol synthesis, affecting membrane stability, and inhibiting mitochondrial respiration, catalyzed by cytochrome B, leading to an energy deficit by reducing ATP production. This inhibition further alters DNA by blocking topoisomerases necessary for DNA maintenance, replication, and transcription [54,55]. Incubating Foc cells with *Streptomyces* secondary metabolites affected mycelium metabolism, leading to decreased sugar and protein content, which was reduced by 28.6 % and 29.1 %, and increasing N-acetylglucosamine concentration; the process triggers a gradual accumulation of Reactive Oxygen Species (ROS) in the hypha, causing plasma membrane destruction, mitochondrial dysfunction, and cell apoptosis [10].

Among the tested isolates, strains K6 and E7 displayed the highest activity against F. oxysporum f. sp. cubense mycelium growth. Molecular identification revealed that strain K6 shares 96.98 % genetic identity with *Streptomyces polyrhachii*, known for its symbiotic relationship with edible Chinese black ants (*Polyrhachis vicina Roger*) [56], that provides nutritional resources and protection against fungal pathogens. *S. polyrhachii* has no Antarctic counterpart and does not report antifungal activity against *Fusarium* strains. Strain E7 shows 99,4 % genetic similarity to *Streptomyces fildensensis,* which produces ethyl acetate crude extracts with potent antimicrobial activity, with a reported MIC value of 15.6 μg/mL against Gram-positive pathogens [57,58]. Previous studies have shown that *S. fildensensis* cultured in M1 broth inhibited *F. oxysporum* mycelium growth by 75 % [16].

Antarctic *Streptomyces sp.* strains growing on M1 broth inhibited the growth of *F. oxisporum* at a low concentration of 1.56 %. Y. Wei et al. [59] reported MIC values of 3.125 ppm against *F. oxysporum* species in a crude extract of *Streptomyces* sp. isolated from soil and rhizosphere. The decrease in our crude extract's activity could be attributed to the decomposition of active molecules during extraction [60]. Previous studies have noted a decline in activity upon re-cultivation in Streptomyces [27]. Reductions in antifungal production may be associated with laboratory culture conditions where biosynthetic gene clusters (BGCs) in *Streptomyces* are either not expressed or expressed at low levels due to variations in culture media composition [61]. Changes in nutrients, pH, loss of genetic information, and genetic mutations are believed to influence antimicrobial production [55].

Numerous Actinomycete species exhibit antagonistic activity against

fungal phytopathogens. *Streptomyces huiliensis,* isolated from the rhizosphere of *Opuntia stricta,* inhibits Foc RT4 growth and spore germination with a MIC value of 6.25 μg/mL. This extract inhibited the Foc4 spore germination with an IC50 of 22.78 μg/ml [17]. Foc cells come into contact with *S. huiliensis* broth, causing severe damage to their cellular structure, potentially leading to cellular death. *Streptomyces spp*. Alter the hyphae structure of *Sclerotinia sclerotiorum,* causing significant damage [62]. GCMS– analysis of a *Streptomyces sp.* extract revealed phenolic compounds, pyrrolizidine, hydrocarbons, ester, and acid with antimicrobial properties. Bioactive molecules like fatty acid, oleic acid, and 13-docosenamide in *Streptomyces* extract might contribute to its antifungal activity [17]. Exploration of bioactive compound synthesis genes in Antarctic *Streptomyces* revealed the presence of polyketide synthase type II (PKS-II), nonribosomal peptides (NRPs), and cytochrome P450 genes (CYP) [63]. PSK_II and NRPs are associated with Citreamicins biosynthetic, antimicrobial compounds inhibiting the growth of plant pathogenic fungi such as *Fusarium solani* and *Fusarium graminearum* [64]. *S. xanthophaeus* was linked to the production of rishirilide A and lupinacidin A, displaying antimicrobial and anticancer activities [65,51].

This work highlights the potential of Antarctic Streptomyces to produce antifungal compounds to combat *Fusarium oxysporum* f. sp. *cubense* (Foc) in banana cultivars, offering a promising solution to reduce losses from Fusarium wilt. Our research identifies *Streptomyces sp.* (K6) and *Streptomyces fildesensis* (K7) as producers of antifungal compounds effective against Foc, suggesting their potential as biocontrol agents in various crops affected by this pathogen. This offers a new alternative to fungicides for biocontrol in banana crops. Previous studies reveal the high potential of these isolates to produce specific natural products, such as antibiotics, pigments, or toxins.

Our work focuses on utilizing the metabolites secreted in the fermented broth of Streptomyces rather than directly using the Antarctic strains in the field. This approach avoids the use of Antarctic microorganisms in the field, as they would be affected by the temperatures outside their reported optimal growth parameters. These psychrophilic molecules can withstand extreme conditions such as low temperature, high salinity, radiation, and resistance to organic solvents, in addition to the cold-adaptive activity, which offers significant advantages over molecules isolated from mesophilic microorganisms [66–68]. The importance of psychrophilic enzymes in industrial applications is underscored by their involvement in research and development projects by companies. These strains have the potential to produce antifungals from inexpensive carbon sources such as starch, generate patents, and make significant contributions to various industries. Future research is essential for optimizing metabolite production, characterizing bioactive compounds, and verifying the genetic identity of *Streptomyces sp.* (K6) and *Streptomyces fildesensis* (K7), thereby enhancing their application as sustainable biocontrol agents in agriculture.

5. Conclusion

Antarctic *Streptomyces* offers a vast repertoire of bioactive compounds with great potential to develop novel antifungal therapies. Therefore, the discovery of two antifungal-producing bacteria from King George Island, namely, *Streptomyces* sp. K6 and *Streptomyces fildesensis* E7 represents an important advancement in the fight against fungal phytopathogens. The isolated strains exhibited a great capacity to inhibit the mycelial growth of *Fusarium oxysporum* f.sp. *cubense* under *in vitro* conditions. The antifungal activity shown by these strains highlights their potential as novel biocontrol agents for the treatment of *Fusarium* wilt of Banana (FWB). These results significantly contribute to the ongoing efforts in the banana industry to mitigate the impact of *Fusarium* wilt and establish sustainable management strategies to protect banana crops. This study emphasizes the importance of investigating extreme and underexplored environments as a source of novel bacterial strains with potential used in biotechnological applications. Further studies are being carried out to evaluate the biological activity of the identified antifungal compounds under field conditions.

Funding

The study's sampling was supported by the Instituto Oceanográfico y Antártico de la Armada (INOCAR) and the Instituto Antártico Chileno (INACH) for the expedition to Antarctica. In addition, ESPOL financially supported the experiments and use of the Ecuadorian Center for Biotechnology Research (CIBE) in the Proteomic and Protein Biochemistry area.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used GRAMMARLY in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

CRediT authorship contribution statement

Jeffrey Vargas Perez: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lizette Serrano:** Writing – review & editing, Methodology, Formal analysis. **Rafael Viteri:** Writing – review & editing, Methodology, Formal analysis. **Daynet Sosa:** Supervision, Project administration. **Christian A. Romero:** Writing – review & editing, Supervision, Methodology. **Nardy Diez:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jeffrey Vargas Perez reports administrative support, equipment, drugs, or supplies, and travel were provided by Instituto Oceanográfico y Antártico de la Armada. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The sequences of the nine *Streptomyces* strains were deposited in the GenBank database under the accession numbers provided in Table 2. The data that has been used is confidential.

Acknowledgments

We thank Instituto Antártico Ecuatoriano (INAE) and Instituto Antártico chileno (INACH) for the logistics and mobility to Antártida. A special thanks to all the crew for their assistance during the sampling and processing of samples in the laboratories of the Chilean Antarctic research base Prof. Julio Escudero during the Chilean Antarctic Expedition No. 56/Ecuadorian Expedition No. 24, Antarctic summer of 2020. This project complies with the regulations outlined in the FRAMEWORK CONTRACT FOR ACCESS TO GENETIC RESOURCES OF THE SCIENTIFIC RESEARCH PROJECT No. MAATE-DBI-CM-2022-0236.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2024.e00852.

References

[1] C. Staver, D.E. Pemsl, L. Scheerer, L. Perez Vicente, M. Dita, Ex ante assessment of returns on research investments to address the impact of Fusarium wilt tropical race 4 on global banana production, Front. Plant Sci. 11 (Jul. 2020), https://doi.org/10.3389/FPLS.2020.00844.

[2] F.A. García-Bastidas, et al., First report of Fusarium wilt tropical race 4 in cavendish bananas caused by Fusarium odoratissimum in Colombia, https://doi.org/10.1094/PDIS-09-19-1922-PDN 104 (3) (Dec. 2019), https://doi.org/10.1094/PDIS-09-19-1922-PDN.

[3] B.O. Olivares, J.C. Rey, D. Lobo, J.A. Navas-Cortés, J.A. Gómez, B.B. Landa, Fusarium wilt of bananas: a review of agro-environmental factors in the Venezuelan production system affecting its development, Agron 11 (5) (May 2021) 986, https://doi.org/10.3390/AGRONOMY11050986. *2021, Vol. 11, Page 986*.

[4] A.K. Justine, N. Kaur, Savita, P.K. Pati, Biotechnological interventions in banana: current knowledge and future prospects, Heliyon. 8 (11) (Nov. 2022) e11636, https://doi.org/10.1016/J.HELIYON.2022.E11636.

[5] FAO, Banana Market Review – Preliminary Results 2021, 2022. Rome,Accessed: Jul. 13, 2022. [Online]. Available, https://www.fao.org/3/cb9411en/cb9411en.pdf.

[6] D.K. Jaiswal, S.J. Gawande, P.S. Soumia, R. Krishna, A. Vaishnav, A.B. Ade, Biocontrol strategies: an eco-smart tool for integrated pest and diseases management, BMC. Microbiol. 22 (1) (Dec. 2022) 1–5, https://doi.org/10.1186/S12866-022-02744-2/METRICS.

[7] G. da C. Silva, I.T. Kitano, I.A. de F. Ribeiro, P.T. Lacava, The potential use of actinomycetes as microbial inoculants and biopesticides in agriculture, Front. Soil Sci. 2 (Feb. 2022) 833181, https://doi.org/10.3389/FSOIL.2022.833181.

[8] J. Wang, et al., Biological control of Fusarium oxysporum f. sp. cubense tropical race 4 in banana plantlets using newly isolated Streptomyces sp. WHL7 from marine soft coral, Plant Dis. 106 (1) (Jan. 2022), https://doi.org/10.1094/PDIS-06-21-1275-RE.

[9] G. Bubici, M. Kaushal, M.I. Prigigallo, C.G.L. Cabanás, J. Mercado-Blanco, Biological control agents against Fusarium wilt of banana, Front. Microbiol. 10 (APR) (Apr. 2019) 445720, https://doi.org/10.3389/FMICB.2019.00616/BIBTEX.

[10] Y. Duan, et al., Antifungal mechanism of Streptomyces ma. FS-4 on Fusarium wilt of banana, J. Appl. Microbiol. 130 (1) (Jan. 2021) 196–207, https://doi.org/10.1111/JAM.14784.

[11] E.S. Kim, Recent advances of actinomycetes, Biomolecules 11 (2) (Feb. 2021) 1–3, https://doi.org/10.3390/BIOM11020134.

[12] E.M. Colombo, A. Kunova, C. Pizzatti, M. Saracchi, P. Cortesi, M. Pasquali, Selection of an endophytic Streptomyces sp. strain DEF09 from wheat roots as a biocontrol agent against Fusarium graminearum, Front. Microbiol. 10 (Oct. 2019) 466992, https://doi.org/10.3389/FMICB.2019.02356/BIBTEX.

[13] L. Zhang, H. Zhang, Y. Huang, J. Peng, J. Xie, W. Wang, Isolation and evaluation of rhizosphere actinomycetes with potential application for biocontrolling Fusarium wilt of banana caused by Fusarium oxysporum f. sp. cubense tropical race 4, Front. Microbiol. 12 (Oct. 2021) 763038, https://doi.org/10.3389/FMICB.2021.763038/BIBTEX.

[14] D. Qi, et al., Biocontrol potential and antifungal mechanism of a novel Streptomyces sichuanensis against Fusarium oxysporum f. sp. cubense tropical race 4 in vitro and in vivo, Appl. Microbiol. Biotechnol. 106 (4) (2022) 1633–1649, https://doi.org/10.1007/s00253-022-11788-3.

[15] J.Y. Cha, et al., Microbial and biochemical basis of a Fusarium wilt-suppressive soil, ISMe J. 10 (1) (Jan. 2016) 119, https://doi.org/10.1038/ISMEJ.2015.95.

[16] A. Santos, K. Núñez-Montero, C. Lamilla, M. Pavez, D. Quezada-Solís, L. Barrientos, Antifungal activity screening of antarctic actinobacteria against phytopathogenic fungi, Acta Biol. Colomb. 25 (2) (2020) 353–358, https://doi.org/10.15446/abc.v25n2.76405.

[17] D. Qi, et al., Identification and antifungal mechanism of a novel actinobacterium Streptomyces huiliensis sp. nov. against Fusarium oxysporum f. sp. cubense tropical race 4 of banana, Front. Microbiol. 12 (Nov. 2021), https://doi.org/10.3389/FMICB.2021.722661/FULL.

[18] X. Li, et al., Biological control of banana wilt disease caused by Fusarium oxyspoum f. sp. cubense using Streptomyces sp. H4, Biol. Control 155 (Apr. 2021) 104524, https://doi.org/10.1016/J.BIOCONTROL.2020.104524.

[19] M. Ortiz, et al., Microbial nitrogen cycling in Antarctic soils, Microorganisms 8 (9) (Sep. 2020) 1–17, https://doi.org/10.3390/MICROORGANISMS8091442.

[20] D. Schories, G. Kohlberg, Marine Wildlife, Dirk Schories Publ, King George Island, Antarctica, 2016, p. 348.

[21] Enrique Serrano Cañadas, Espacios protegidos y política territorial en las islas Shetland del Sur (Antártida, Boletín la Asoc. Geógrafos Españoles 31 (2001) (2001) 5–21. Accessed: Jul. 30, 2022. [Online]. Available, https://www.researchgate.net/publication/28081106_Espacios_protegidos_y_politica_territorial_en_las_islas_Shetland_del_Sur_Antartida.

[22] B. Malcheva, M. Nustorova, M. Zhiyanski, M. Sokolovska, R. Yaneva, E. Abakumov, Diversity and activity of microorganisms in Antarctic polar soils, One Ecosyst. 5 (2020) 1–16, https://doi.org/10.3897/ONEECO.5.E51816.

[23] F. Pucci, M. Rooman, Physical and molecular bases of protein thermal stability and cold adaptation, Curr. Opin. Struct. Biol. 42 (Feb. 2017) 117–128, https://doi.org/10.1016/J.SBI.2016.12.007.

[24] P. Durán, et al., Occurrence of soil fungi in Antarctic pristine environments, Front. Bioeng. Biotechnol. 7 (MAR) (2019) 28, https://doi.org/10.3389/FBIOE.2019.00028/FULL.

[25] B. Cong, et al., Diversity of cultivable microbes from soil of the Fildes Peninsula, Antarctica, and their potential application, Front. Microbiol. 11 (Sep. 2020) 2182, https://doi.org/10.3389/FMICB.2020.570836/BIBTEX.

[26] C. Lamilla, M. Pavez, A. Santos, A. Hermosilla, V. Llanquinao, L. Barrientos, Bioprospecting for extracellular enzymes from culturable actinobacteria from the South Shetland Islands, Antarctica, Polar. Biol. 40 (3) (Mar. 2017) 719–726, https://doi.org/10.1007/s00300-016-1977-z.

[27] M.E. Danilovich, L.A. Sánchez, F. Acosta, O.D. Delgado, Antarctic bioprospecting: in pursuit of microorganisms producing new antimicrobials and enzymes, Polar. Biol. 41 (7) (Jul. 2018) 1417–1433, https://doi.org/10.1007/s00300-018-2295-4.

[28] A.A. Pulschen, A.G. Bendia, A.D. Fricker, V.H. Pellizari, D. Galante, F. Rodrigues, Isolation of uncultured bacteria from antarctica using long incubation periods and low nutritional media, Front. Microbiol. 8 (JUL) (Jul. 2017), https://doi.org/10.3389/fmicb.2017.01346.

[29] Q. Li, et al., Morphological identification of actinobacteria, Actinobacteria - Basics Biotechnol. Appl. (Feb. 2016), https://doi.org/10.5772/61461.

[30] R.B. Parada, E.R. Marguet, M. Vallejo, Aislamiento y caracterización parcial de actinomicetos de suelos con actividad antimicrobiana contra bacterias multidrogo-resistentes, Rev. Colomb. Biotecnol. 19 (2) (Jul. 2017) 17–23, https://doi.org/10.15446/rev.colomb.biote.v19n2.64098.

[31] F.M. Njeru, P. Ndungu, C. Bii, Characterization and antimicrobial susceptibility of actinomycetes from TB smear negative and retreatment patients in Nairobi, Kenya, J. Biosci. Med. 07 (08) (2019) 1–12, https://doi.org/10.4236/JBM.2019.78001.

[32] M. Al-Ansari, M. Kalaiyarasi, M.A. Almalki, P. Vijayaraghavan, Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from Streptomyces sp. AS11 isolated from the marine environment, J. King Saud Univ. - Sci. 32 (3) (Apr. 2020) 1993–1998, https://doi.org/10.1016/j.jksus.2020.02.005.

[33] A.M.S. Al-Hatmi, I. Curfs-Breuker, G.S. de Hoog, J.F. Meis, P.E. Verweij, Antifungal susceptibility testing of Fusarium: a practical approach, J. Fungi 3 (2) (Jun. 2017), https://doi.org/10.3390/JOF3020019.

[34] S. Al-Nasiry, M. Hanssens, C. Luyten, R. Pijnenborg, The use of Alamar blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells, Hum. Reprod. 22 (5) (2007) 1304–1309, https://doi.org/10.1093/HUMREP/DEM011.

[35] A. Ansari, R.R. Zohra, O.M. Tarar, S.A.U. Qader, A. Aman, Screening, purification and characterization of thermostable, protease resistant Bacteriocin active against methicillin resistant Staphylococcus aureus (MRSA), BMC. Microbiol. 18 (1) (Nov. 2018), https://doi.org/10.1186/S12866-018-1337-Y.

[36] R.B. Parada, E.R. Marguet, M. Vallejo, Aislamiento y caracterización parcial de actinomicetos de suelos con actividad antimicrobiana contra bacterias multidrogo-resistentes, Rev. Colomb. Biotecnol. 19 (2) (Jul. 2017) 17–23, https://doi.org/10.15446/REV.COLOMB.BIOTE.V19N2.64098.

[37] M.A. Abneuf, et al., Antimicrobial activity of microfungi from maritime antarctic soil, Czech Polar Rep. 6 (2) (2016) 141–154, https://doi.org/10.5817/CPR2016-2-13.

[38] K. Shanthi Kumari, P. Shivakrishna, A.M. Al-Attar, V.S. Ramakrishna Ganduri, Antibacterial and cytotoxicity activities of bioactive compounds from Micrococcus species OUS9 isolated from sea water, J. King Saud Univ. - Sci. 32 (6) (Sep. 2020) 2818–2825, https://doi.org/10.1016/J.JKSUS.2020.07.003.

[39] F.M. Ausubel, *Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology*, no. Sirsi) i9780471250920. 2002.

[40] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (7) (Jul. 2021) 3022–3027, https://doi.org/10.1093/MOLBEV/MSAB120.

[41] I.AAT Bioquest, "IC50 calculator." 2024.

[42] J.A. Di Rienzo, F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada, C. W. Robledo, InfoStat, Universidad Nacional de Córdoba, Córdoba, Argentina, 2011 [Online]. Available, http://www.infostat.com.ar/.

[43] A.A. Bhatti, S. Haq, R.A. Bhat, Actinomycetes benefaction role in soil and plant health, Microb. Pathog. 111 (Oct. 2017) 458–467, https://doi.org/10.1016/J.MICPATH.2017.09.036.

[44] C.Y. Chua, et al., Analysis of bacterial communities of King George and Deception Islands, Antarctica using high-throughput sequencing, Curr. Sci. 115 (9) (Nov. 2018) 1701–1705, https://doi.org/10.18520/CS/V115/I9/1701-1705.

[45] A. Yerkhova, I. Parnikoza, M. Pavlovska, H. Yevchun, Y. Prekrasna-Kviatkovska, Microbiomes of Antarctic pearlwort (Colobanthus quitensis) of the maritime Antarctic: distinct diversity and core microbes in rhizosphere and endosphere compartments of the plant, Ukr. Antarct. J. 20 (2) (2022) 212–240, https://doi.org/10.33275/1727-7485.2.2022.701.

[46] M. Bölter, H.P. Blume, D. Schneider, L. Beyer, Soil properties and distributions of invertebrates and bacteria from King George Island (Arctowski station), maritime Antarctic, Polar. Biol. 18 (5) (1997) 295–304, https://doi.org/10.1007/S003000050191. *1997 185*.

[47] M.K. Zdanowski, P. Węgleński, Ecophysiology of soil bacteria in the vicinity of Henryk Arctowski station, King George Island, Antarctica, Soil Biol. Biochem. 33 (6) (May 2001) 819–829, https://doi.org/10.1016/S0038-0717(00)00230-3.

[48] L.J. Silva, et al., Actinobacteria from Antarctica as a source for anticancer discovery, Sci. Rep. 10 (1) (Dec. 2020) 13870, https://doi.org/10.1038/S41598-020-69786-2.

[49] Á. Manteca, P. Yagüe, Á. Manteca, P. Yagüe, Streptomyces as a source of antimicrobials: novel approaches to activate cryptic secondary metabolite pathways, Antimicrob. Antibiot. Resist. Antibiofilm Strateg. Act. Methods (Mar. 2019), https://doi.org/10.5772/INTECHOPEN.81812.

[50] S.S. El-Hawary, M.H.A. Hassan, A.O. Hudhud, U.R. Abdelmohsen, R. Mohammed, Elicitation for activation of the actinomycete genome's cryptic secondary metabolite gene clusters, RSC. Adv. 13 (9) (Feb. 2023) 5778–5795, https://doi.org/10.1039/D2RA08222E.

[51] L. Zhang, et al., Biocontrol potential of endophytic Streptomyces malaysiensis 8ZJF-21 from medicinal plant against banana Fusarium wilt caused by Fusarium oxysporum f. sp. cubense tropical race 4, Front. Plant Sci. 13 (May 2022) 874819, https://doi.org/10.3389/FPLS.2022.874819/BIBTEX.
[52] H.J. Lacey, P.J. Rutledge, A. Takahashi, H.J. Lacey, P.J. Rutledge, Recently discovered secondary metabolites from Streptomyces species, Mol. 27 (3) (Jan. 2022) 887, https://doi.org/10.3390/MOLECULES27030887. *2022, Vol. 27, Page 887*.
[53] T. Rey, B. Dumas, Plenty is no plague: Streptomyces symbiosis with crops, Trends. Plant Sci. 22 (1) (Jan. 2017) 30–37, https://doi.org/10.1016/J.TPLANTS.2016.10.008.
[54] P. Borrego-Muñoz, F. Ospina, D. Quiroga, A Compendium of the most promising synthesized organic compounds against several Fusarium oxysporum species: synthesis, antifungal activity, and perspectives, Mol. 26 (13) (Jun. 2021) 3997, https://doi.org/10.3390/MOLECULES26133997. *2021, Vol. 26, Page 3997*.
[55] B. Zhao, D. He, L. Wang, Advances in Fusarium drug resistance research, J. Glob. Antimicrob. Resist. 24 (Mar. 2021) 215–219, https://doi.org/10.1016/J.JGAR.2020.12.016.
[56] C. Yu, et al., Streptomyces polyrhachii sp. nov., a novel actinomycete isolated from an edible Chinese black ant (Polyrhachis vicina Roger), Antonie Van Leeuwenhoek 104 (6) (Sep. 2013) 1013–1019, https://doi.org/10.1007/S10482-013-0021-3. *2013 1046*.
[57] K. Núñez-Montero, et al., Antarctic Streptomyces fildesensis So13.3 strain as a promising source for antimicrobials discovery, Sci. Rep. 9 (1) (Dec. 2019), https://doi.org/10.1038/s41598-019-43960-7.
[58] P.L. Lavin, S.T. Yong, C.M.V.L. Wong, M. De Stefano, Isolation and characterization of Antarctic psychrotroph Streptomyces sp. strain INACH3013, Antarct. Sci. 28 (6) (Dec. 2016) 433–442, https://doi.org/10.1017/S0954102016000250.
[59] Y. Wei, et al., A newly isolated Streptomyces sp. YYS-7 with a broad-spectrum antifungal activity improves the banana plant resistance to Fusarium oxysporum f. sp. cubense tropical race 4, Front. Microbiol. 11 (Aug. 2020) 557315, https://doi.org/10.3389/FMICB.2020.01712/BIBTEX.
[60] I. Charousová, J. Medo, E. Halenárová, S. Javoreková, Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands, J. Adv. Pharm. Technol. Res. 8 (2) (Apr. 2017) 46–51, https://doi.org/10.4103/japtr.JAPTR-161-16.
[61] K. Alam, et al., Streptomyces: the biofactory of secondary metabolites, Front. Microbiol. 13 (Sep. 2022) 3387, https://doi.org/10.3389/FMICB.2022.968053/BIBTEX.
[62] D.A.S. Gebily, G.A.M. Ghanem, M.M. Ragab, A.M. Ali, N.E. din K. Soliman, T. H. Abd El-Moity, Characterization and potential antifungal activities of three Streptomyces spp. as biocontrol agents against Sclerotinia sclerotiorum (Lib.) de Bary infecting green bean, Egypt. J. Biol. Pest Control 31 (1) (Dec. 2021) 1–15, https://doi.org/10.1186/S41938-021-00373-X/TABLES/6.
[63] M. Encheva-Malinova, et al., Antibacterial potential of streptomycete strains from antarctic soils, Biotechnol. Biotechnol. Equip. 28 (4) (Oct. 2014) 721–727, https://doi.org/10.1080/13102818.2014.947066.
[64] L.-L. Liu et al., "Genome-based analysis of the type II PKS biosynthesis pathway of xanthones in Streptomyces caelestis and their antifungal activity †," 2019, doi: 10.1039/c9ra07345k.
[65] J. Wei, X.Y. Zhang, S. Deng, L. Cao, Q.H. Xue, J.M. Gao, α-Glucosidase inhibitors and phytotoxins from Streptomyces xanthophaeus, Nat. Prod. Res. 31 (17) (2017) 2062–2066, https://doi.org/10.1080/14786419.2016.1269100.
[66] P. De Maayer, D. Anderson, C. Cary, D.A. Cowan, Some like it cold: understanding the survival strategies of psychrophiles, EMBo Rep. 15 (5) (2014) 508, https://doi.org/10.1002/EMBR.201338170.
[67] R.P.R. Metpally, B.V.B. Reddy, Comparative proteome analysis of psychrophilic versus mesophilic bacterial species: insights into the molecular basis of cold adaptation of proteins, BMC Genomics 10 (1) (Jan. 2009) 1–10, https://doi.org/10.1186/1471-2164-10-11/TABLES/6.
[68] T. Correa, F. Abreu, Antarctic microorganisms as sources of biotechnological products, Physiol. Biotechnol. Asp. Extrem. (Jan. 2020) 269–284, https://doi.org/10.1016/B978-0-12-818322-9.00020-4.