



Biocontrol of *Streptomyces* isolates from Puerto Eten barren soils against *Fusarium* and *Cercospora* species

Biocontrole de isolados de *Streptomyces* de solos áridos de Puerto Eten contra espécies de *Fusarium* e *Cercospora*

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ABSTRACT

This study aimed to determine the biocontrol potential of *Streptomyces* isolates obtained from Puerto Eten barren soils against *Fusarium* spp. and *Cercospora* spp. Potential *Streptomyces* isolates from barren soils in Lambayeque were selected using actinomycete-oriented cultural, morphological and Gram-staining criteria, and were subsequently characterised by macroscopic, microscopic and 16S rRNA-based analyses. The isolates were evaluated through paired confrontation assays against *Fusarium* spp. and *Cercospora* spp., followed by extraction of crude bioactive compounds and comparison with a benzimidazole-based commercial fungicide in well diffusion assays. In the paired confrontation assay, relative inhibition ranged from 62.22% to 72.14% for *Fusarium* spp. and from 65.31% to 70.17% for *Cercospora* spp. In the well diffusion assay, crude extracts produced relative inhibition values of 66.64% to 70.00% for *Fusarium* spp. and 65.31% to 70.17% for *Cercospora* spp., while the fungicide control produced the highest inhibition. The isolates JCH7 and JCH3 showed positive lipase, protease, chitinase and cellulase activity, whereas JCH5 lacked chitinase activity. The 16S rRNA results related JCH7 and JCH3 to *Streptomyces mutabilis*, but their similarity values support a close relationship rather than a definitive species-level assignment. The JCH5 sequence showed an inconclusive low similarity and requires resequencing. Overall, the three isolates showed in vitro biocontrol potential against both fungal genera, with the strongest evidence based on inhibition and enzymatic activity.

Keywords: Puerto Eten barren soils; Paired confrontation assay; Antifungal metabolites; Hydrolytic enzymes; 16S rRNA identification.

RESUMO

Este estudo teve como objetivo determinar o potencial de biocontrole de isolados de *Streptomyces* obtidos de solos áridos de Puerto Eten contra *Fusarium* spp. e *Cercospora* spp. Os isolados potenciais de *Streptomyces* provenientes de solos áridos de Lambayeque foram selecionados por critérios culturais, morfológicos e coloração de Gram orientados para actinomicetos, e posteriormente caracterizados por análises macroscópicas, microscópicas e baseadas no gene 16S rRNA. Os isolados foram avaliados por ensaios de confronto pareado contra *Fusarium* spp. e *Cercospora* spp., seguidos da extração de compostos bioativos brutos e comparação com um fungicida comercial à base de benzimidazol por ensaio de difusão em poço. No confronto pareado, a inibição relativa variou de 62,22% a 72,14% para *Fusarium* spp. e de 65,31% a 70,17% para *Cercospora* spp. No ensaio de difusão em poço, os extratos brutos produziram inibição relativa de 66,64% a 70,00% para *Fusarium* spp. e de 65,31% a 70,17% para *Cercospora* spp., enquanto o controle fungicida apresentou a maior inibição. Os isolados JCH7 e JCH3 apresentaram atividade positiva de lipase, protease, quitinase e celulase, enquanto JCH5 não apresentou atividade de quitinase. Os resultados de 16S rRNA relacionaram JCH7 e JCH3 a *Streptomyces mutabilis*, mas os valores de similaridade sustentam uma relação próxima e não uma atribuição definitiva em nível de espécie. A sequência de JCH5 apresentou baixa similaridade inconclusiva e requer novo sequenciamento. Em conjunto, os três isolados demonstraram potencial de biocontrole in vitro contra ambos os gêneros fúngicos, com maior sustentação nos resultados de inibição e atividade enzimática.

Palavras-chave: Solos áridos de Puerto Eten; Ensaio de confronto pareado; Metabólitos antifúngicos; Enzimas hidrolíticas; Identificação por 16S rRNA.

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1 INTRODUÇÃO

Fungal phytopathogens remain a major constraint to crop productivity because they reduce stand establishment, damage vegetative tissues and favour post-harvest deterioration. Global assessments estimate mean yield losses close to 17% across major crops, while pre- and post-harvest losses caused by fungal diseases can each approach 20% under favourable epidemiological conditions (Savary et al., 2019; Steinberg; Gurr, 2020). These losses persist despite routine chemical protection because pathogen survival structures, broad dispersal pathways and climate-dependent infection cycles allow recurrent disease outbreaks. For this reason, the search for locally adapted biological control agents has become a relevant strategy for reducing disease pressure while limiting dependence on synthetic fungicides (Torres-Rodriguez et al., 2022; Xiao et al., 2021).

Fusarium spp. are soil-borne facultative parasites with persistent chlamydospores, high saprophytic survival and broad host range, including crops of high economic value (Balthazar et al., 2022; Dean et al., 2012; Li et al., 2024; Lv et al., 2018). Infection generally starts at the root surface, followed by penetration of epidermal and cortical tissues, colonisation of the xylem and obstruction of water and nutrient flow. The disease process produces chlorosis, vascular browning, wilting, leaf loss and plant death, especially under warm and humid conditions that favour fungal growth and host stress (Lv et al., 2018; Xu et al., 2020). These traits make *Fusarium* spp. difficult to manage through cultural practices alone and justify the evaluation of antagonistic microorganisms able to inhibit mycelial growth.

Cercospora spp. are polycyclic foliar pathogens from Mycosphaerellaceae, with repeated conidial production during the crop cycle and rapid dissemination in humid, warm environments (Bakhshi et al., 2025; Kreth et al., 2025). Infection leads to chlorotic and necrotic leaf spots that reduce photosynthetic area, accelerate defoliation and impair yield formation. Reported losses for susceptible crops can reach 50% to 61%, depending on host susceptibility, inoculum pressure and weather conditions (Bakhshi et al., 2025; Kumar et al., 2020). Because new conidia may be produced within days after infection, *Cercospora* spp. require control tools that act early on mycelial development and sporulation (Irfan et al., 2025; Lawal et al., 2025).

Conventional management relies on resistant varieties, certified seeds, crop sanitation, sowing-date adjustment and fungicide applications. These measures reduce disease incidence but often show limited long-term consistency when pathogen populations persist in soil or crop residues (El-Akshar et al., 2025). Intensive

fungicide use also increases selection pressure for resistant populations and raises environmental concerns. In this context, *Streptomyces* has practical relevance because members of this genus produce antibiotics, siderophores, volatile organic compounds and hydrolytic enzymes that can restrict fungal growth, damage cell walls and compete for nutrients (Luo et al., 2025; Torres-Rodriguez et al., 2022; Zakalyukina et al., 2024).

Arid and barren soils are promising reservoirs of *Streptomyces* isolates because low organic matter, limited water availability and intense microbial competition favour stress-tolerant lineages with distinct biosynthetic capacities. Studies on actinobacteria from desert and semi-arid habitats have reported antimicrobial activity and metabolite profiles associated with adaptation to oligotrophic conditions (Cuervo et al., 2023; Goodfellow et al., 2018; Luo et al., 2025). The barren soils of Puerto Eten, located in the coastal arid zone of Lambayeque, represent an underexplored source of local isolates for biological control.

Despite these advances, few studies have evaluated *Streptomyces* isolates from Puerto Eten barren soils as antagonists of *Fusarium* spp. and *Cercospora* spp. This gap is relevant because local isolates may express traits adapted to the edaphic conditions from which they were obtained. Therefore, this study aimed to determine the biological control potential of *Streptomyces* isolates obtained from Puerto Eten barren soils against *Fusarium* spp. and *Cercospora* spp. under in vitro conditions.

2 MATERIALS AND METHODS

2.1 SOIL SAMPLING AREA

Soil samples were collected from barren soils located in Puerto Eten, Lambayeque, on the northern coast of Peru. The area is characterised by arid coastal conditions, sparse vegetation and exposed sandy-loam surfaces with low organic matter, which supports the selection of stress-tolerant actinomycetes (Hoyos-Alayo, 2026a, b, 2025). The sampling points were selected because the site showed limited plant cover and edaphic conditions compatible with the ecological niche targeted for *Streptomyces* isolation. Figure 1 shows the geographic location and the visual condition of the sampling area.



Figure 1. Soil sampling site in Puerto Eten, Lambayeque, Peru, used for the isolation of *Streptomyces* isolates from barren soil.

Source: the authors (2025)

2.2 PHYSICOCHEMICAL CHARACTERISTICS OF THE SOIL AT THE ISOLATION SITE

The sampling site corresponded to a barren sandy-loam soil, with an electrical conductivity of $24.1 \mu\text{S cm}^{-1}$, pH 6.8, 0.2% organic matter, 5.6 ppm phosphorus, 275 ppm potassium, and 0.5% CaCO_3 . Soil samples were collected from five points at a depth of 5 cm, after removing superficial debris. Approximately 1 kg was obtained per sampling point using sterile tools and placed in sterile polyethylene bags at 4°C until processing.

2.3 ISOLATION AND SELECTION OF STREPTOMYCES ISOLATES

At each sampling point, 100 g of soil were mixed with 900 mL of sterile distilled water to prepare the initial suspension. Serial dilutions were prepared up to 10^{-3} , and 100 μL from the final dilution were spread on starch casein agar (SCA) supplemented with nystatin ($50 \mu\text{g mL}^{-1}$). Plates were incubated at 28°C for seven days. After incubation, colonies were not assumed to belong to *Streptomyces* solely on the basis of growth on SCA. Slow-growing, dry, powdery, leathery or chalky colonies with aerial mycelium and actinomycete-like morphology were selected and purified by repeated streaking on fresh SCA. Gram staining and microscopic observation were used as exclusion criteria; Gram-positive, filamentous and branched isolates were retained, whereas colonies with *Bacillus*-like morphology or Gram-negative reaction were discarded.

2.4 ISOLATION OF PHYTOPATHOGENIC FUNGI

Fusarium spp. and *Cercospora spp.* were isolated from symptomatic plant tissues obtained from diseased crop material maintained in the laboratory collection. *Fusarium spp.* was recovered from root tissues showing necrosis and vascular

discoloration, whereas *Cercospora* spp. was recovered from leaf lesions with chlorotic and necrotic margins. Because the host plant species was not included in the available field record, the isolates are reported by symptomatic tissue type and fungal genus. Tissue fragments from lesion edges were surface-disinfected in 1% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water, dried on sterile filter paper and plated on potato dextrose agar (PDA). Plates were incubated at 25 °C for seven days. Genus-level identification was based on colony morphology and microscopic characteristics of conidia, conidiophores and mycelial structures. Pure cultures were obtained through single-spore isolation and maintained on PDA for the confrontation assays.

2.5 PAIRED CONFRONTATION ASSAY BETWEEN STREPTOMYCES ISOLATES AND THE PHYTOPATHOGENS *Fusarium* spp. AND *Cercospora* spp.

The three *Streptomyces* isolates (JCH3, JCH5 and JCH7) were evaluated for antagonistic activity using the paired confrontation technique. Spore suspensions of each *Streptomyces* isolate were streaked along one side of PDA plates, while 5 mm mycelial plugs from seven-day pure cultures of *Fusarium* spp. or *Cercospora* spp. were placed on the opposite side. Plates were incubated at 28 ± 2 °C for seven days. Fungal controls were maintained on PDA without bacteria. The assay followed a completely randomized design with three independent replicates per treatment and pathogen. The relative inhibition (RI) of radial growth was calculated using the following equation (1):

$$RI = \frac{Dc - Dt}{Dc} \times 100 \quad (1)$$

Where: RI = relative inhibition (%), Dc = diameter of the negative control (mm), and Dt = diameter of the treatment (mm).

2.6 MORPHOLOGICAL AND GRAM-STAINING CHARACTERISATION OF THE THREE STREPTOMYCES ISOLATES

For the morphological characterisation of the three *Streptomyces* isolates, the following traits were assessed: colony texture, aerial mycelium colour, diffusible pigment formation, hyphal septation, and the presence and arrangement of substrate and aerial mycelia. Gram staining was included as a preliminary criterion to support the selection of actinomycete-like isolates before molecular analysis.

2.6.1 Macroscopic Characterisation

After eight days of incubation on oat agar, the colony morphology of each isolate was evaluated, focusing on colony texture, aerial mycelium colour, substrate pigmentation and production of diffusible pigments into the medium.

2.6.2 Microscopic Characterisation

Each *Streptomyces* isolate was cultured on oat agar supplemented with 0.1% nystatin. Sterile cover slips were placed at a 45° angle on the agar surface inside Petri dishes and incubated at 25 °C for 15 days. After incubation, cover slips were mounted on microscope slides and observed under a light microscope to document hyphal septation, branching pattern, aerial mycelium and substrate mycelium.

2.6.3 Gram Staining

Gram staining was performed from seven-day cultures grown on SCA. A purple Gram-positive reaction together with filamentous and branched cells was used as supportive evidence for actinomycete-like selection. These results were interpreted together with colony morphology and 16S rRNA analysis rather than as a stand-alone taxonomic criterion.

2.7 GENETIC CHARACTERISATION OF *Streptomyces* VIA 16S rRNA AMPLIFICATION BY PCR

2.7.1 Nucleic Acid Extraction

Genomic DNA was extracted from purified *Streptomyces* isolates using an optimised in-house protocol developed for filamentous actinobacteria.

2.7.2 Polymerase Chain Reaction (PCR)

Amplified region: 16S rRNA. Primers used: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Amplification conditions: Pre-denaturation: 96 °C for 2 min; Denaturation: 95 °C for 1 min; Annealing: 56 °C for 30 s; Extension: 72 °C for 3 min; Number of cycles: 35.

2.7.3 Sequencing

PCR products were sequenced by Macrogen (Chile).

2.7.4 Bioinformatic Analysis

Software and databases used: Ugene (v10.7) and EzBioCloud (<https://www.ezbiocloud.net/>). Analyses performed: sequence quality control and curation, assembly, generation of consensus sequences, and identification of the closest homologues based on sequence similarity.

2.8 BIOCHEMICAL ASSAYS FOR ENZYMATIC ACTIVITY

To assess the enzymatic potential of the isolates, qualitative plate assays were performed for chitinase, protease, lipase and cellulase activity. Results were recorded as positive (+) when a visible hydrolysis halo was observed and negative (–) when no halo was detected under the incubation conditions.

2.8.1 Chitinase Activity

Minimal medium was prepared from stock solutions containing: (1) K_2HPO_4 and KH_2PO_4 (30 g L^{-1}); (2) $MgSO_4 \cdot 7H_2O$ (15 g L^{-1}); and (3) $CaCl_2$, $FeCl_3$ and $NaCl$ at concentrations of 5 g L^{-1} , 0.6 g L^{-1} and 5 g L^{-1} , respectively. The medium was supplemented with 1% colloidal chitin. Control plates were prepared using minimal medium supplemented with 10 g L^{-1} glucose. Cultures were incubated at 22 °C for 5-8 days, after which hydrolysis halos were recorded as evidence of chitinase activity.

2.8.2 Protease Activity

The protease medium contained 1 g peptone, 0.5 g $NaCl$, 0.5 g meat extract, 0.24 g gelatine, 1.5 g agar and 100 mL distilled water. Plates were incubated at 22 °C for 5-8 days, and hydrolysis zones were recorded as indicators of protease activity.

2.8.3 Lipase Activity

The lipase medium was prepared with 1 mL Tween 20, 1 g bactopectone, 0.5 g $NaCl$, 0.01 g $CaCl_2 \cdot 2H_2O$, 1.5 g agar and 100 mL distilled water. Plates were incubated at 22 °C for 5-8 days, and visible hydrolysis halos were recorded as indicators of lipase activity.

2.8.4 Cellulase Activity

For cellulase activity, minimal medium contained 1 g carboxymethyl cellulose (CMC), 1.5 g agar and 100 mL distilled water. Plates were incubated at 22 °C for 5-8 days, and hydrolysis halos were recorded as indicators of cellulase production.

2.9 PRODUCTION OF CRUDE EXTRACT AND EXTRACTION OF SECONDARY METABOLITES

Bioactive compound production was conducted using modified ISP-1 medium (International *Streptomyces* Project). Actinomycete cultures were reactivated at 28 °C for three days in 3 mL tryptic soy broth (TSB). The resulting suspension was used to inoculate liquid ISP-1 medium, which was incubated at 150 rpm for seven days. The cultures were centrifuged at 4000 rpm for 20 min to obtain cell-free supernatants, which were stored at –20 °C. Secondary metabolites were extracted following the method of Leal et al. (2025). After fermentation, the culture broth was centrifuged at 6000 rpm for 20 min at 4 °C. The cell-free supernatant was mixed with ethyl acetate (1:1, v/v) and shaken vigorously for two hours. The ethyl acetate phase was collected

and evaporated at 40 °C using a rotary evaporator. The resulting residue was dissolved in 10% methanol and refrigerated for later use in antifungal assays.

2.10 IN VITRO EVALUATION OF BIOACTIVE COMPOUNDS VERSUS COMMERCIAL SYNTHETIC FUNGICIDE AGAINST *Fusarium* spp. AND *Cercospora* spp.

The antagonistic activity of the crude extracts was compared with that of a commercial benzimidazole-based fungicide using the agar well diffusion method. PDA plates were prepared and allowed to solidify. Three wells (0.2 cm diameter) were bored into each plate, and 40 µL of crude bioactive extract from each *Streptomyces* isolate (JCH7, JCH3 and JCH5) was added. A 5 mm mycelial disc of each phytopathogen (*Fusarium* spp. or *Cercospora* spp.) was placed at the plate centre. All assays were conducted in triplicate. A carbendazim-based commercial fungicide (500 g L⁻¹ active ingredient) was used as the positive control. The fungicide was diluted in sterile distilled water at 1 mL L⁻¹, and 40 µL of this solution was applied per well, matching the extract volume. Sterile 10% methanol was used as solvent control when required.

2.11 STATISTICAL ANALYSIS.

Antifungal assays were arranged in a completely randomized design with three replicates per treatment. For the paired confrontation assay, each pathogen was analysed independently with isolate as the fixed factor. For the well diffusion assay, each pathogen was also analysed independently with treatment as the fixed factor, including the fungicide and negative control groups. The comparison between fungal genera was excluded because the assays were not structured as a factorial experiment. Data were expressed as mean relative inhibition ± standard deviation. Normality and homogeneity of variance were checked before analysis. One-way ANOVA followed by Tukey's test at $\alpha = 0.05$ was applied using R software. P values reported by the software as 0.000 were expressed as $p < 0.001$.

3 RESULTS AND DISCUSSION

3.1 MACROSCOPIC AND MICROSCOPIC IDENTIFICATION

Figure 2 presents three criteria used to support preliminary selection of the isolates: colony morphology, aerial mycelium development and microscopic filamentation. JCH7 formed beige to light-brown colonies with dry, folded growth and abundant aerial mycelium; JCH5 developed paler beige colonies with cracked surfaces and visible radial folds; and JCH3 showed yellowish substrate pigmentation with whitish aerial mycelium. This pigmentation and texture variability is consistent with actinobacterial colony heterogeneity reported for *Streptomyces* and related taxa, where aerial mycelium, substrate pigmentation and sporulation patterns are useful

screening descriptors but not definitive taxonomic markers (Barka et al., 2016; Goodfellow et al., 2018; Zakalyukina et al., 2024).

Microscopic observations showed branched septate hyphae and spore chains in all isolates. Gram staining was positive for JCH3, JCH5 and JCH7, supporting their preliminary selection as actinomycete-like isolates and excluding Gram-negative colonies or *Bacillus*-like morphotypes. This interpretation follows the usual actinobacterial screening logic, in which Gram-positive reaction, filamentous growth and aerial mycelium guide isolate selection before molecular confirmation (Barka et al., 2016; Olanrewaju; Babalola, 2019). Because morphology and Gram reaction alone do not provide definitive genus-level identification, these traits were interpreted together with the 16S rRNA results.

The phenotypic data support the selection of the isolates for antagonism tests, but they do not by themselves demonstrate biocontrol potential. Biocontrol requires functional evidence, such as inhibition of pathogen growth, enzymatic activity or metabolite production, because taxonomic placement alone does not predict antifungal performance (Torres-Rodriguez et al., 2022; Alam et al., 2022). In this study, functional evidence was obtained through the paired confrontation assay, the well diffusion assay and the enzymatic profile.

Accordingly, the macroscopic, microscopic and Gram-staining results were used as preliminary evidence for isolate selection, while the antifungal and enzymatic assays provided the functional evidence required to assess biological control potential. This separation between taxonomic screening and functional validation also avoids overstating the interpretation of Figure 2.

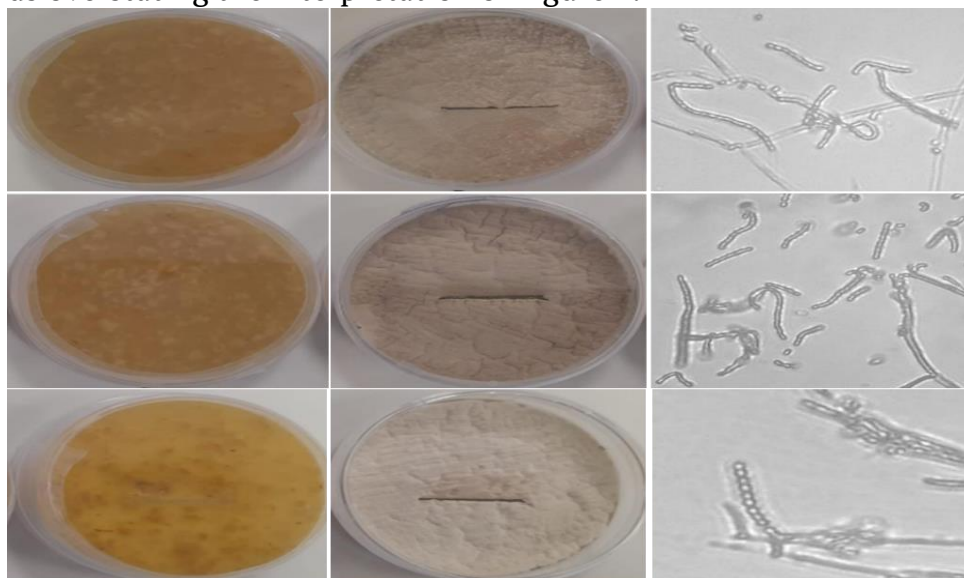


Figure 2. Macroscopic and microscopic characteristics of *Streptomyces* isolates JCH7, JCH5 and JCH3 from Puerto Eten barren soils. Rows from top to bottom correspond to JCH7, JCH5 and JCH3. The first column shows young colonies on oat agar, the second column shows mature colonies with aerial mycelium, and the third column shows branched filamentous structures and spore chains observed by light microscopy. Scale bars in micrographs = 10 μ m.

Source: the authors (2025)

3.2 DUAL CONFRONTATION ASSAY

Figure 3 presents the relative inhibition obtained in the paired confrontation assay for each fungal genus. The control did not inhibit fungal growth, whereas the three *Streptomyces* isolates reduced radial growth significantly in each independent analysis (ANOVA, $p < 0.001$; Tukey, $\alpha = 0.05$). Against *Fusarium spp.*, relative inhibition ranged from 62.22% to 72.14%, with JCH7 showing the highest mean response, followed by JCH5 and JCH3. Against *Cercospora spp.*, relative inhibition ranged from 65.31% to 70.17%, with the three isolates forming the same statistical group in Tukey's test. Similar inhibition patterns have been reported for *Streptomyces*-based biocontrol studies, where direct competition and diffusible antifungal products restrict mycelial expansion in vitro (Xiao et al., 2021; Torres-Rodriguez et al., 2022; Li et al., 2024).

These results show in vitro antagonistic activity of JCH3, JCH5 and JCH7 against both fungal genera. The data support biocontrol potential at the screening stage, but they do not identify the specific metabolites responsible for inhibition. This interpretation is consistent with the need to combine confrontation assays with enzymatic, chemical and molecular evidence when proposing *Streptomyces* isolates as biological control candidates (Barka et al., 2016; Olanrewaju; Babalola, 2019; Torres-Rodriguez et al., 2022). The enzymatic profile shown in Table 1 provides complementary evidence that hydrolytic enzymes may participate in the antagonistic response.

The higher response of JCH7 against *Fusarium spp.* suggests that isolate-level differences may influence antagonism under paired confrontation conditions. Such differences are plausible because *Streptomyces* isolates often differ in biosynthetic gene content, metabolite secretion, enzyme expression and capacity to produce volatile organic compounds, even when they show similar colony morphology (Cuervo et al., 2023; Alam et al., 2022; Zakalyukina et al., 2024). For *Cercospora spp.*, the overlapping statistical grouping indicates that the three isolates showed comparable inhibition under the same assay conditions.

The comparison between fungal genera was not interpreted as a factorial effect because each pathogen was analysed independently. Therefore, the results are presented as pathogen-specific evidence of relative inhibition rather than as a direct statistical comparison between *Fusarium spp.* and *Cercospora spp.* This restriction strengthens the statistical interpretation because it avoids assigning biological meaning to a comparison that was not supported by the experimental design.

The practical implication of this assay is that the three isolates merit further screening, but the results remain limited to in vitro contact conditions. Additional assays should separate direct competition, volatile-mediated inhibition and soluble metabolite effects, because these mechanisms can operate simultaneously in *Streptomyces*-pathogen interactions (Cuervo et al., 2023; Torres-Rodriguez et al., 2022).

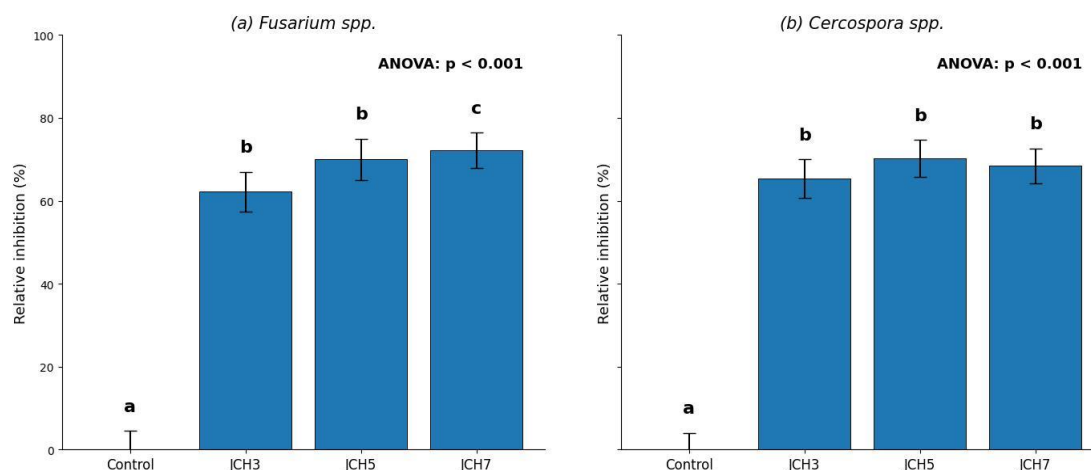


Figure 3. Relative inhibition of *Fusarium* spp. (a) and *Cercospora* spp. (b) by *Streptomyces* isolates in the paired confrontation assay. Bars represent mean $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate significant differences among treatments within each pathogen according to Tukey's test ($\alpha = 0.05$). ANOVA p values below the software reporting limit are shown as $p < 0.001$.

Source: the authors (2025)

3.3 BIOCHEMICAL CHARACTERISATION OF THE ISOLATED STRAINS

Table 1 summarises the qualitative enzymatic profile of isolates JCH3, JCH5 and JCH7. Lipase, protease, chitinase and cellulase activities were evaluated because these enzymes are associated with degradation of fungal structural components, nutrient acquisition and microbial competition. Hydrolytic enzymes are frequently discussed as part of *Streptomyces* antagonism because fungal cell walls and infected plant tissues contain chitin, proteins, glycoproteins and polysaccharides that can serve as enzymatic targets (Hamid et al., 2013; Barka et al., 2016; Torres-Rodriguez et al., 2022). All isolates showed positive lipase, protease and cellulase activity. Chitinase activity was positive in JCH3 and JCH7, but absent in JCH5.

The simultaneous detection of lipase, protease and cellulase activity across the three isolates indicates hydrolytic capacity under the assay conditions. Proteases may affect fungal proteins and glycoproteins, while cellulases can act on cellulose-containing substrates associated with infected plant tissues (Nunes et al., 2025; Zerizer et al., 2024). This result agrees with reports that *Streptomyces* isolates combine extracellular enzymes with secondary metabolites to restrict phytopathogen growth, although the relative contribution of each mechanism depends on the isolate and pathogen tested (Olanrewaju; Babalola, 2019; Torres-Rodriguez et al., 2022).

The positive chitinase reaction in JCH3 and JCH7 is relevant because chitin is a major component of the fungal cell wall. Chitin hydrolysis has been associated with cell wall weakening, hyphal deformation and increased susceptibility to other antimicrobial compounds (Hamid et al., 2013; El-Akshar et al., 2025). The absence of detectable chitinase activity in JCH5 indicates that its inhibitory effect in the confrontation and well diffusion assays may depend on mechanisms other than chitin hydrolysis under these conditions, including diffusible antifungal metabolites or volatile compounds (Cuervo et al., 2023; Alam et al., 2022).

These results support the functional interpretation of the antagonistic assays without assuming a specific metabolite profile. The enzyme data indicate that the isolates have traits compatible with fungal growth restriction, but chemical identification of the active compounds remains necessary to define the precise mechanism. Therefore, Table 1 should be interpreted as qualitative mechanistic support, not as proof of which enzyme or metabolite caused the measured inhibition.

Table 1. Qualitative enzymatic activity of *Streptomyces* isolates JCH7, JCH5 and JCH3 based on hydrolysis halo formation in plate assays.

Isolate	Replicate	Lipase	Protease	Chitinase	Cellulase
JCH7	1	+	+	+	+
	2	+	+	+	+
	3	+	+	+	+
JCH5	1	+	+	–	+
	2	+	+	–	+
	3	+	+	–	+
JCH3	1	+	+	+	+
	2	+	+	+	+
	3	+	+	+	+

Source: the authors (2025)

3.4 WELL DIFFUSION ASSAY

Figure 4 presents the well diffusion assay performed with crude extracts and the carbendazim-based benzimidazole control. For each pathogen, treatments differed significantly (ANOVA, $p < 0.001$; Tukey, $\alpha = 0.05$). Against *Fusarium spp.*, crude extracts of JCH3, JCH5 and JCH7 produced relative inhibition between 66.64% and 70.00%, while the fungicide control exceeded 90%. Against *Cercospora spp.*, the crude extracts produced relative inhibition between 65.31% and 70.17%, also below the fungicide control but above the negative control. These values indicate that inhibitory activity remained detectable after extraction, which is consistent with the ability of *Streptomyces* to produce soluble antifungal metabolites in addition to volatile compounds and extracellular enzymes (Cuervo et al., 2023; Alam et al., 2022; Xiao et al., 2021).

The similar response of the three extracts indicates that the recovered metabolites retained antifungal activity after extraction. Because the assay compared treatments within each pathogen independently, the results support extract-level inhibition against *Fusarium spp.* and *Cercospora spp.* without claiming a factorial comparison between fungal genera. This distinction is important because extract assays measure diffusible bioactivity in a simplified agar system and do not reproduce soil colonisation, rhizosphere competition or plant-mediated responses (Olanrewaju; Babalola, 2019; Torres-Rodriguez et al., 2022).

The fungicide control produced the greatest inhibition, which is expected for a commercial active ingredient applied as a reference treatment. Even so, the crude extracts produced measurable inhibition above the negative control, supporting their

value as preliminary candidates for further chemical characterisation and formulation tests. Crude extracts should not be considered equivalent to formulated fungicides because active-molecule concentration, stability, diffusion and purity differ between biological extracts and registered chemical products.

These data complement the paired confrontation assay and indicate that the antifungal response was associated with diffusible compounds produced by the isolates. Further work should identify the active molecules by chromatographic and spectrometric methods, assess dose-response behaviour, and test extract stability before comparing performance with commercial fungicides under greenhouse or field conditions. Such steps are required because *Streptomyces* metabolite profiles vary according to culture medium, incubation time and extraction solvent (Barka et al., 2016; Alam et al., 2022).

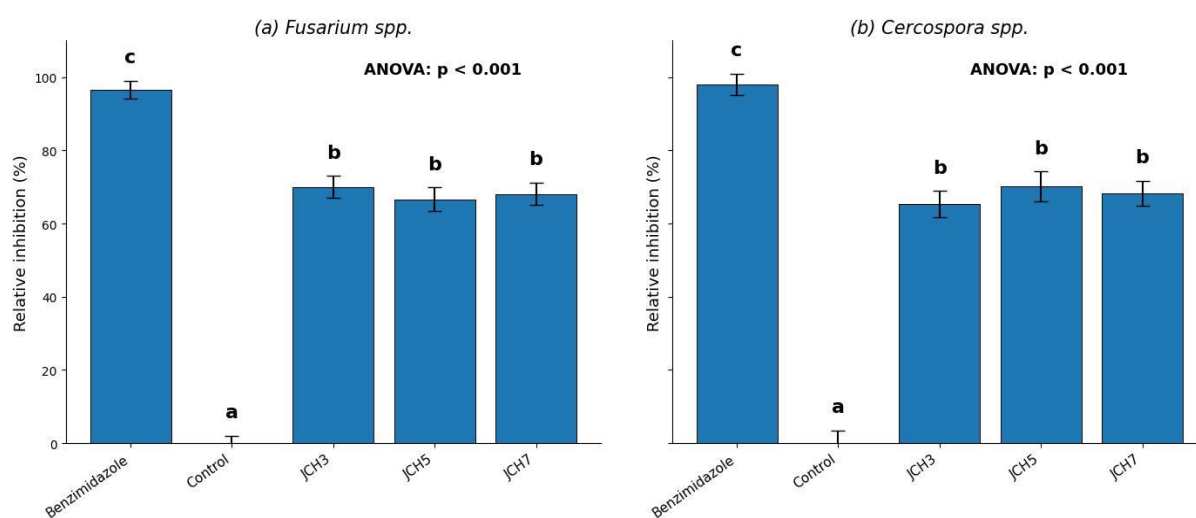


Figure 4. Relative inhibition of *Fusarium* spp. (a) and *Cercospora* spp. (b) by crude extracts of *Streptomyces* isolates in the well diffusion assay. Bars represent mean $\pm$ standard deviation ($n = 3$). Different lowercase letters indicate significant differences among treatments within each pathogen according to Tukey's test ($\alpha = 0.05$). The benzimidazole-based fungicide was included as the positive control, and ANOVA p values below the software reporting limit are shown as $p < 0.001$.

Source: the authors (2025)

3.5 GENETIC CHARACTERISATION OF THE ISOLATED STRAINS

Figure 5 shows amplification of the 16S rRNA region for isolates JCH7, JCH3 and JCH5, with bands close to 1500 bp. This result confirms successful amplification of the expected marker but does not by itself provide species-level identification or evidence of biocontrol activity. The gel image is therefore useful as a molecular quality-control result, while taxonomic interpretation depends on sequence comparison and phylogenetic context.

Sequencing and comparison with the EzBioCloud database related JCH7 and JCH3 to *Streptomyces mutabilis*, with identity values of 98.54% and 98.30%, respectively, and 99.60% sequence completeness in both cases. These values indicate close relatedness to *S. mutabilis*, but they remain below the commonly used 98.65% to

99% range applied as a conservative warning threshold for species-level demarcation based only on 16S rRNA gene similarity (Kim et al., 2014; Yarza et al., 2014). Therefore, JCH7 and JCH3 were interpreted as *Streptomyces* isolates closely related to *S. mutabilis*, not as definitive species-level identifications.

The JCH5 sequence produced a low similarity value of 66.90%, which is inconsistent with reliable genus-level assignment using 16S rRNA. This result was therefore considered inconclusive and may reflect sequence quality problems, mixed templates or contamination during sequencing. JCH5 was retained in the biological assays as an actinomycete-like isolate, but its taxonomic identity requires resequencing and additional phylogenetic analysis before any genus-level or species-level conclusion can be stated.

The molecular data should be strengthened by a phylogenetic tree including reference *Streptomyces* species, outgroup sequences, bootstrap support values and a scale bar. Because raw sequence files were not available in the manuscript package, a reliable phylogenetic tree could not be reconstructed in this revision. For the objective of this study, the biological control interpretation rests on the inhibition assays and enzymatic profile, while molecular identification is treated as supporting evidence that still requires confirmation by resequencing, phylogenetic analysis or genome-based comparison (Kim et al., 2014; Yarza et al., 2014).

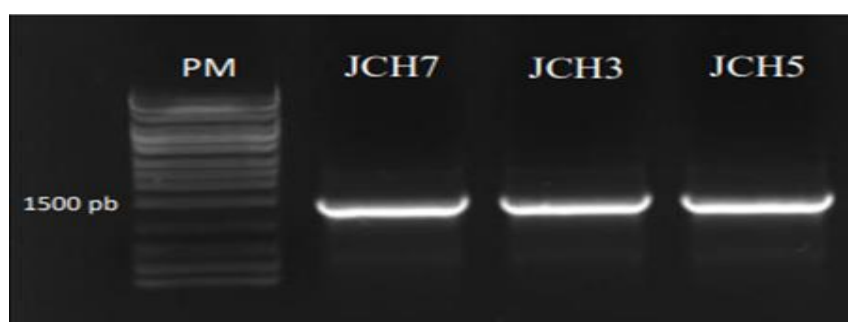


Figure 5. Agarose gel electrophoresis of 16S rRNA amplicons from the isolates. PM = molecular weight marker; JCH7, JCH3 and JCH5 = amplified products of approximately 1500 bp. The gel confirms amplification of the target region but does not provide species-level assignment.

Source: the authors (2025)

4 CONCLUSIONS

The results indicate that the three isolates obtained from Puerto Eten barren soils have in vitro biological control potential against *Fusarium* spp. and *Cercospora* spp. In the paired confrontation assay, JCH7 showed the strongest response against *Fusarium* spp., while JCH3, JCH5 and JCH7 all inhibited *Cercospora* spp. within the 65.31% to 70.17% range. In the well diffusion assay, the crude extracts of the three isolates also inhibited both fungal genera, with relative inhibition values of 66.64% to 70.00% for *Fusarium* spp. and 65.31% to 70.17% for *Cercospora* spp. These results confirm potential at the in vitro screening level, although the commercial fungicide remained the most inhibitory treatment.

The enzymatic assays support the antagonistic potential of the isolates because all showed lipase, protease and cellulase activity, and JCH3 and JCH7 also showed chitinase activity. This enzymatic profile is consistent with mechanisms associated with fungal growth restriction, especially degradation of cell-wall or structural substrates. The study evaluated only three isolates, which limits generalisation about *Streptomyces* from barren soils. Future work should include a larger number of isolates, calibrated phylogenetic analysis based on 16S rRNA or multilocus/genome data, greenhouse and field assays, and evaluation of formulation stability before recommending practical biocontrol use.

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