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Isolation and identification of endophytic *Streptomyces* strain and its antifungal activity against root-pathogenic fungi from watermelon roots

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Abstract

Watermelon production is frequently constrained by soil-borne fungal diseases, particularly those caused by *Fusarium fujikuroi*, which have been shown to severely impair plant growth and productivity. The objective of this study is to isolate, identify, and evaluate the antifungal potential of *Streptomyces* strains associated with watermelon-growing soils and to explore their functional traits related to biological control. A total of 22 actinomycete isolates were recovered and screened for chitinolytic and antifungal activities, leading to the selection of three promising strains designated NVC-TL3, strain 4, and NVC-TX. Morphological characterization and 16S rRNA gene sequencing identified these isolates as *Streptomyces cyaneochromogenes* NVC-TL3, *Streptomyces toxytricini* strain 4, and *Streptomyces lydicus* NVC-TX. All strains exhibited extracellular chitinase activity and significantly inhibited the growth of *F. fujikuroi* under in vitro conditions. Among them, *S. lydicus* NVC-TX demonstrated the strongest antagonistic activity, achieving 74.9% mycelial growth inhibition after 7 days of incubation. The efficacy of the substances was further confirmed by greenhouse experiments, which demonstrated a reduction in disease severity to 14.9% and a significant enhancement in plant vigor in comparison to the pathogen-inoculated control. The study revealed substantial functional variation among phylogenetically distinct *Streptomyces* isolates and demonstrated a close association between chitinolytic capacity and disease suppression. These findings contribute to the existing body of knowledge concerning the biodiversity of *Streptomyces* associated with watermelon and highlight the potential of *S. lydicus* NVC-TX as a promising biological control agent. The application of this strain could contribute to the reduction of dependence on synthetic fungicides, thereby promoting environmentally sustainable disease management, and supporting healthier and more productive watermelon cultivation systems.

Keywords: Streptomyces lydicus; biological control; *Fusarium fujikuroi*; chitinolytic activity; watermelon rhizosphere

1. Introduction

Watermelon (*Citrullus lanatus* L.) is a horticultural crop of significant economic importance, cultivated extensively in tropical and subtropical regions. The fruit is highly valued for its nutritional composition, including vitamins, minerals, antioxidants, lycopene, and citrulline, which contribute to human health and disease prevention [1]. In many developing countries, the cultivation of watermelon also plays a crucial role in supporting farmer livelihoods and rural economies. Despite the increasing global demand and expansion of cultivation areas, watermelon production is severely affected by various biotic stresses, particularly soilborne diseases that reduce yield and fruit quality [1,2]. Among the diseases that impact watermelon cultivation, those caused by *Fusarium* species are considered a significant constraint. Members of this fungal genus are widely distributed in agricultural soils and are responsible for destructive diseases such as wilt, root rot, and vascular infections in numerous crop species [3–5]. In watermelon, *Fusarium* infections have been shown to cause degradation of root tissue, impaired nutrient uptake, stunted growth, and plant death, resulting in significant economic losses [6,7]. Recently, *Fusarium fujikuroi* has emerged as a significant phytopathogen capable of infecting several economically valuable crops [8]. This fungus has the capacity to colonize root tissues and produce phytotoxic metabolites that disrupt normal physiological processes in plants [9]. The development of disease in intensive watermelon production systems is further accelerated by favorable environmental conditions, including high soil moisture, warm temperatures, and poor aeration [10,11].

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The management of soilborne fungal diseases has conventionally relied on the utilization of chemical fungicides and soil fumigants. Despite the capacity of these chemicals to temporarily suppress pathogen populations, their continuous application may cause environmental pollution, pathogen resistance, and adverse effects on beneficial soil microorganisms [12]. Increasing concerns regarding food safety and environmental sustainability have prompted the development of eco-friendly alternatives for disease management [13-15]. Consequently, the utilization of biological control through the implementation of beneficial microorganisms has gained considerable attention as a sustainable strategy for the mitigation of plant pathogens [16]. Endophytic microorganisms, which colonize internal plant tissues without causing harm, are recognized for their ability to enhance plant growth and protect plants against pathogens [17]. These beneficial microbes can promote plant health through multiple mechanisms, including the production of antimicrobial compounds, competition for nutrients and ecological niches, the induction of systemic resistance, and the regulation of plant hormonal balance [16]. Endophytes, being naturally associated with host plants, frequently strong adaptability and persistence within plant tissues [17]. Among the beneficial microorganisms, species belonging to the genus *Streptomyces* are of particularly importance due to their remarkable capacity to produce diverse bioactive secondary metabolites [17,18]. These filamentous actinobacteria are commonly found in soil and plant-associated environments and are renowned for producing of antibiotics, antifungal compounds, siderophores, lytic enzymes, and volatile organic compounds [19]. In agricultural systems, several *Streptomyces* strains have demonstrated notable antagonistic activity against fungal pathogens. This attribute renders them promising candidates for the applications of biological control [20,21].

Endophytic *Streptomyces strains* isolated from plant tissues are of particular value due to their adaptation to the internal plant environment. Their capacity to colonize roots and vascular tissues has facilitated close interaction with host plants and direct suppression of invading pathogens [22]. As demonstrated in previous studies, endophytic *Streptomyces* have been shown to inhibit fungal pathogens through various mechanisms including the degradation of fungal hyphae, the disruption of cell membranes, and the inhibition of spore germination [23,24]. In addition to their antifungal properties, these bacteria may also promote plant growth by producing phytohormones and improving nutrient availability [25,26]. However, the diversity and functional potential of endophytic *Streptomyces* associated with watermelon roots remain insufficiently studied, particularly under local agricultural conditions [27,28]. The present study therefore sought to isolate and identify endophytic *Streptomyces* strains from watermelon roots and evaluate their antifungal activity against root pathogenic fungi under both laboratory and greenhouse conditions. Molecular identification was performed by means of 16S rRNA gene sequencing, while the antifungal potential of the selected isolates was assessed through in vitro antagonistic assays and greenhouse experiments. The findings of this study may contribute to the development of sustainable biological control strategies for the management of fungal diseases in watermelon cultivation.

2. Materials and Methods

2.1. Isolation of streptomyces strains

Samples of Rhizosphere soil were collected from with healthy watermelon plants in Can Tho. Soil samples exhibiting a high degree of adhesion to the roots was collected at a depth of 5–10 cm, a region where microbial activity and beneficial actinomycete populations are typically abundant [29]. The entire plants were carefully extracted, and the rhizosphere soil was aseptically transferred into sterile polyethylene bags. The samples were then transported to the laboratory and air-dried at room temperature for 24 hours purposely to suppress the growth of rapidly growing bacteria and fungi [29,30]. The dried soil samples were subjected to a process of homogenization, followed by sieving to remove any debris. Subsequently, 1 g of each sample was suspended in sterile distilled water and serial dilutions ranging from 10^{-5} to 10^{-7} were prepared. For the isolation of actinomycete, 100 µL aliquots from appropriate dilutions were spread onto Gause I agar medium and incubated at temperatures ranging from 28 to 35 °C for a period of between 5 and 7 days. Colonies exhibiting morphological characteristics typical of actinomycetes were purified through repetitive subculturing until contamination-free isolates were obtained. A total of 22 isolates were initially recovered, of which 17 Gram-positive isolates were retained after Gram staining. These isolates were then screened for chitinolytic and antifungal activities, leading to the selection of three promising isolates, designated NVC-TL3, NVC-TX, and NVC-4, for further characterization and subsequent analyses [15,29,30].

2.2. Morphological characterization of three selected strains

Pure actinomycete isolates were subcultured on Gause I agar medium to obtain cultures free from contamination. Colonies exhibiting typical actinomycete characteristics, including filamentous growth, distinct pigmentation, and earthy odor, were selected for further purification and identification [31,29]. Morphological characterization was performed based on aerial mycelium color, substrate mycelium color, and diffusible pigment production in accordance to the classification system of El-Naggar et al. [32]. The spore chain morphology and spore surface characteristics were examined after 7 and 14 days of incubation utilizing Gram staining and light microscopy. Gram staining was conducted following the method as described by Chuong [33]. In summary, bacterial smears were heat-fixed, stained with gentian violet, treated with Lugol's iodine, decolorized with 95% ethanol, and counterstained with safranin. Gram-positive isolates retained the purple color, whereas Gram-negative bacteria appeared pink. Colony morphology and stained samples were observed under a light microscope with an oil immersion objective at 100× magnification. Purified isolates with well-developed colonies were subsequently preserved on agar slants at a temperature of 4 °C for further studies [34,35].

2.3. Molecular identification of three selected strains

The morphological and biochemical characteristics of the bacterial isolates were evaluated with reference to the procedures as outlined by Antido et al. [36]. To achieve a more accurate taxonomic identification, molecular characterization was conducted utilizing 16S rRNA gene sequencing. Genomic DNA was extracted from pure bacterial cultures by means of a QIAGEN genomic DNA extraction kit for Gram-positive bacteria in adherence to the manufacturer's instructions. The amplification of nearly full-length 16S rRNA gene fragments

was achieved through the universal primer pair RP1 (5′-AGAGTTTGATCATGGCTCAG-3′) and FD2 (5′-ACGGTTACCTTGTTACGACTT-3′). The process of PCR amplification was conducted in a final reaction volume of 50 µL containing 25 µL Taq master mix (Genet Bio, Seoul, South Korea), 2 µL of each primer (10 µM), 2 µL template DNA (50 ng μL^{-1}), and 19 µL nuclease-free water. The thermal cycling conditions included an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. The purification of PCR products was conducted using the QIAGEN PCR purification kit and the sequencing was performed in both directions with the same primers. The obtained sequences were then compared with reference sequences in the GenBank database using the NCBI BLAST algorithm. Multiple sequence alignment was performed using CLUSTALW [37]. Sequence editing and quality assessment were conducted with software program Chromas (Technelysium Pty Ltd., Australia). Phylogenetic relationships were inferred using reference 16S rRNA gene sequences, including *Streptomyces toxytricini* strain 4 (GenBank: PX830249.1; bases 1–1395), *Streptomyces griseoruber* strain NVC-TL3 (GenBank: PX986382.1; bases 1–1193), and *Streptomyces lydicus* strain NVC-TX (GenBank: PX982647.1; bases 1–1151). A phylogenetic tree was subsequently constructed using MEGA X software [38] based on the Neighbor-joining method with bootstrap analysis of 100 replications.

2.4. Determination of Chitinolytic Activity of Actinomycete Isolates

The evaluation of the chitinolytic activity of the actinomycete isolates was conducted employing the colloidal chitin agar method as previously described by Hsu and Lockwood [39] with slight modifications. Colloidal chitin was prepared from commercial chitin powder by subjecting it to treatment with concentrated HCl followed by repeated washing with sterile distilled water until neutral pH was obtained. The screening medium comprised colloidal chitin (5 g L^{-1}), K_2HPO_4 (0.7 g L^{-1}), KH_2PO_4 (0.3 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.01 g L^{-1}), $ZnSO_4$ (0.001 g L^{-1}), $MnCl_2$ (0.001 g L^{-1}), agar (20 g L^{-1}), and distilled water (1000 mL), adjusted to pH 7.0 prior to the process of sterilization [40]. Actinomycete isolates were spot-inoculated onto the surface of colloidal chitin agar plates and subsequently incubated at temperatures ranging from 28 to 30 °C for a period of 5 to 7 days. The assessment of chitinolytic activity was conducted based on the formation of a clear hydrolysis zone surrounding the colonies, which is the indication of chitin degradation. The diameter of the clear zone and colony growth were measured, and the chitinolytic index was calculated as the ratio of total halo diameter to colony diameter [41]. Isolates demonstrating larger hydrolysis zones were considered to possess more potent chitin-degrading activity and were selected for further experiments.

2.5. Collection of diseased samples and isolation of F. fujikuroi

A total of five watermelon root samples exhibiting the symptoms of root rot, plant wilting, basal stem rot, and occasionally infection spreading to the stem and fruit were randomly collected from watermelon fields in Thoi Lai commune, Can Tho City, Vietnam. Typically, watermelon roots infected by Fusarium exhibit symptoms such as brown necrotic lesions, discoloration of vascular tissues (xylem region), and potential extension of infection to the root crown or the lower stem near the soil surface. The root cortex frequently becomes easily detached, while tissues surrounding the vascular bundles may thicken or degrade, forming hollow cavities. The samples were collected and placed in sealed plastic bags prior to be transported to the laboratory within 4 hours of collection [41]. In the laboratory, root regions exhibiting visible indications of disease were selected for processing. Tissue segments measuring approximately 2 mm × 2 mm were excised from the boundary between healthy and infected tissues. These were then washed under running tap water to remove any adhering debris. The segments were subjected to a process of surface sterilization with ethanol followed by 2% sodium hypochlorite (NaOCl) for 2 minutes and rinsed three times with sterile distilled water. The infected tissue suspension was then serially diluted up to 10^{-7} and spread onto potato dextrose agar (PDA) medium to isolate root-infecting *Fusarium* fungi [42]. Following incubation under light conditions at room temperature for 3–5 days, the emerging fungal colonies were aseptically subcultured to obtain pure isolates. Preliminary identification of the fungal isolates was performed based on standard taxonomic keys and morphological characteristics, including colony appearance, mycelial pigmentation, and spore morphology. These characteristics indicated that the isolates belonged to the genus Fusarium. Following morphological characterization, the purified isolates were maintained on PDA slants and stored at a temperature of 4 °C for subsequent antagonistic assays [43].

2.6. Antagonistic activity of isolated actinomycetes against Fusarium fungi

This experiment was conducted with the objective of screening and selecting three isolates of actinomycete that demonstrated strong chitinolytic activity and pronounced antagonistic effects against the root pathogen Fusarium for further investigations. The study was arranged in a completely randomized design (CRD) with three replications on PDA medium. Antifungal activity was determined by measuring the inhibition zone, defined as the distance between the advancing margin of the fungal mycelium and the well that contained the actinomycete suspension. The evaluation of antagonistic potential of the selected actinomycete isolates against Fusarium was conducted through a combination of agar well diffusion and dual-culture techniques on PDA plates. A pathogenic Fusarium isolate obtained from diseased watermelon roots was inoculated at the center of each Petri dish and incubated for a duration of 24 hours to allow initial mycelial growth. Subsequently, sterile wells (8 mm in diameter) were aseptically punched at a distance of 2 cm from the fungal inoculum [44]. Each well was filled with 100 µL of actinomycete suspension (10^9 CFU mL^{-1}) prepared from ISP2 broth cultures grown on a rotary shaker at 150 rpm. Plates inoculated solely with Fusarium served as untreated controls [45, 46].

The inhibition zone diameter was measured after incubation at a temperature of 28 °C for a period of 7 and 14 days. The percentage of fungal growth inhibition (GI) was calculated through the following equation [47]. The diameter of the inhibition zone was measured recorded after incubation at 28 °C for 7 and 14 days. The percentage of growth inhibition (GI) of

Fusarium was calculated according to the following Eq. (1) [47].

$$GI\ (\%) = (D - D1)\ D^{-1} * 100 \quad (1)$$

where D is the colony diameter (mm) of the fungal control (without actinomycetes) at the same observation time, and D_1 is the colony diameter (mm) of the fungus in the treatment plates containing actinomycetes at the same observation time.

2.7. Antagonistic activity of actinomycete isolates against Fusarium sp. under greenhouse conditions

The watermelon cultivar Hac My Nhan F1 AFTA NP414 (Tan Nong Phat Company, Vietnam) was utilized in all greenhouse pot experiments. Prior to sowing, seeds were surface-sterilized with 75% ethanol for 30 seconds, followed by five rinses with sterile distilled water. The seeds were then sown in sterilized soil that had undergone two autoclaving at 121 °C for 30 minutes. Seedlings at the 2–3 true-leaf stage (10–15 days after sowing) were selected for the experiments, as this developmental stage is highly susceptible to *Fusarium* infection [48]. The greenhouse experiment was conducted using a completely randomized design (CRD) comprising five treatments, each with four independent replicates. Each replicate consisted of three watermelon plants cultivated in plastic pots (120 mm diameter × 105 mm height), resulting in a total of 12 plants per treatment. The following treatments were administered: NT1, plants inoculated with *Fusarium fujikuroi* without the application of actinomycete for the purpose of pathogen control; NT2, *F. fujikuroi* in combination with *Streptomyces cyaneochromogenes* NVC-TL3; NT3, *F. fujikuroi* in combination with *Streptomyces toxytricini* strain 4; NT4, *F. fujikuroi* in combination with *Streptomyces lydicus* NVC-TX; and NT5, non-inoculated and untreated plants (healthy control). All pots were cultivated under greenhouse conditions and randomly arranged throughout the experimental period. The severity of disease was evaluated within 14 days after the inoculation of inoculation on all experimental units. The resulting data were then utilized to evaluate the biocontrol efficacy of the tested actinomycete strains.

2.8. Experimental procedure

The three *Streptomyces* isolates that were selected on the basis of their strong antagonistic activity against *Fusarium* spp. that were prepared from cultures grown in liquid ISP2 medium at 150 rpm for 5–7 days. The actinomycete suspensions were adjusted to a final concentration of 10^8 CFU mL^{-1}. Similarly, *Fusarium* spore suspensions were cultivated in PDB medium and adjusted to 10^6 CFU mL^{-1} [49].

For NT1, 20 mL of *Fusarium* spore suspension (10^6 CFU mL^{-1}) was applied uniformly to the soil surface of each pot containing a two-week-old watermelon seedling. In NT5, instead of the fungal suspension, sterile distilled water was applied. For NT2–NT4, 20 mL of each actinomycete suspension (10^8 CFU mL^{-1}) was applied separately to the rhizosphere of 15-day-old watermelon seedlings according to the respective isolate. Following a 24-hour period, 20 mL of *Fusarium* spore suspension (10^6 CFU mL^{-1}) was inoculated into the soil near the roots. Each treatment comprised three plants, which were replicated four times [50]. The severity of disease was evaluated two weeks after inoculation using a 0–5 disease rating scale that was based on leaf wilt symptoms. A value of 0 indicates a healthy plant; whereas a value of 1 indicates ≤10% wilting. A value of 2 indicates 11–20% wilting, a value of 3 indicates 21–50% wilting, a value of 4 indicates 51–100% wilting, and a value of 5indicates a dead plant. The Disease Severity Index (DSI) and biological control efficacy were calculated according to the method outlined by Al-Mutar et al. [51] and Hanson et al. [52]:

Disease severity was assessed visually 14 days after the inoculation of *Fusarium fujikuroi* based on the presence of leaf wilting symptoms and the overall plant condition of the plant. The evaluation of individual plants were conducted using a 0–5 disease severity scale, and the Disease Severity Index (DSI) was subsequently calculated according to the following Eq. 2.

$$DSI\ (\%) = [\Sigma\ (d \times n) / (D \times N)] \times 100 \quad (2)$$

In this equation, “d” denotes the disease rating, “n” is the number of plants assigned to a given rating class, “D” is the maximum disease rating (5), and “N” is the total number of plants evaluated in each treatment. The mean DSI value of the four replicates was then utilized for the purpose of statistical analysis.

The biocontrol efficacy (BCE) of each actinomycete treatment was determined through the following Eq. 3.

$$BCE\ (\%) = [(DSI_{(control)} - DSI_{(treatment)}) / DSI_{(control)}] \times 100 \quad (3)$$

In this equation, “$DSI_{(control)}$” represents the disease severity index of the pathogen-inoculated control treatment, and “$DSI_{(treatment)}$” represents the disease severity index of the corresponding actinomycete-treated treatment. It is posited that higher BCE values indicate greater suppression of Fusarium wilt symptoms and enhanced disease control performance.

2.9. Statistical analysis

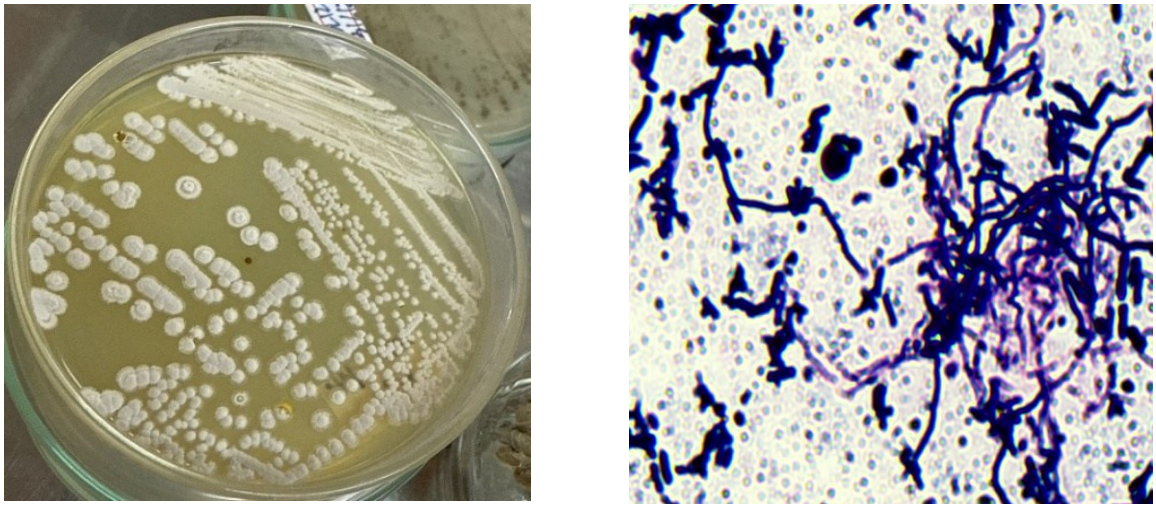
All experiments were conducted in a completely randomized design with three replications. The analysis of the was undertaken using Statgraphics Centurion XVI software, and the treatment means were compared by analysis of variance (ANOVA) followed by Duncan’s multiple range test at $p \leq 0.05$.

3. Results and discussion

3.1 Molecular isolation of three Streptomyces strains

The colony morphology and microscopic observations presented in Fig. 1 revealed that strain NVC-TL3 exhibited the characteristic features of the genus *Streptomyces*. When cultivated on Gause I agar medium, the isolate produced dry, powdery colonies with well-developed aerial mycelia and irregular colony margins (see Fig. 1(a)). These morphological characteristics are commonly associated with *Streptomyces* species and reflect their capacity to yield abundant spores and filamentous structures. Microscopic examination further demonstrated a dense network of branched substrate and aerial hyphae, which are the distinctive taxonomic features of filamentous actinomycetes (Fig.1(b)). The extensive mycelial development suggests active physiological growth and adaptation to the culture medium. It has been established through earlier studies that the presence of well-developed aerial mycelia and spore-forming structures in *Streptomyces* are

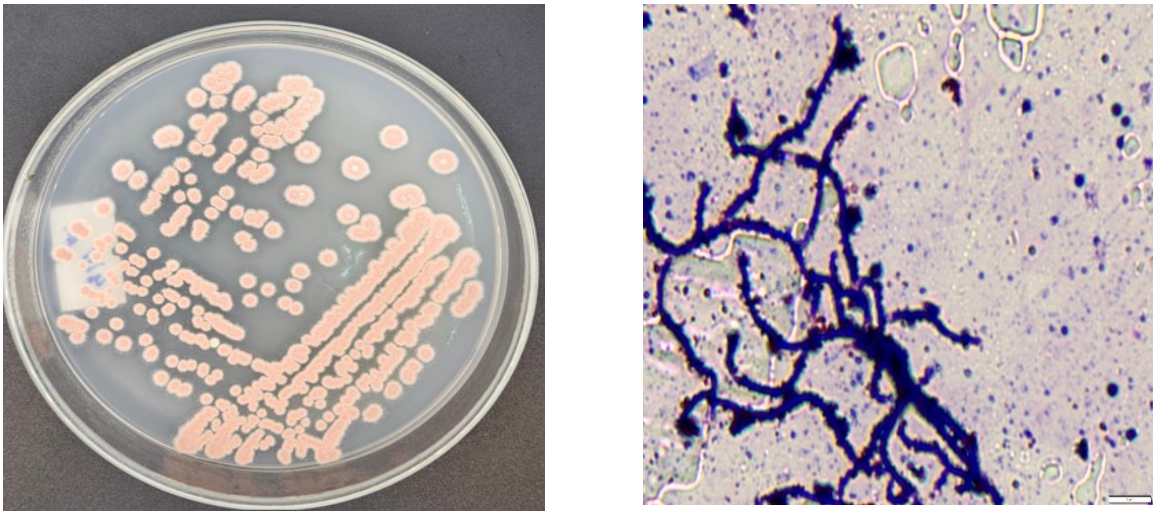
frequently associated with the synthesis of diverse secondary metabolites, encompassing antibiotics and antifungal compounds. Therefore, the morphological characteristics observed for strain NVC-TL3 not only support its preliminary identification as a member of the genus *Streptomyces* but also indicate its potential as a promising biological control agent against plant pathogenic fungi. These findings provide a significant basis for subsequent molecular identification and evaluation of its antimicrobial activities [53].



(a) (b)

Fig. 1. Morphological characteristics of *Streptomyces cyaneochromogenes* strain NVC-TL3 cultured on Gause I agar medium. (a) Colony morphology showing well-developed aerial mycelia; (b) Microscopic observation at 100× magnification showing branched hyphal structures characteristic of *Streptomyces*

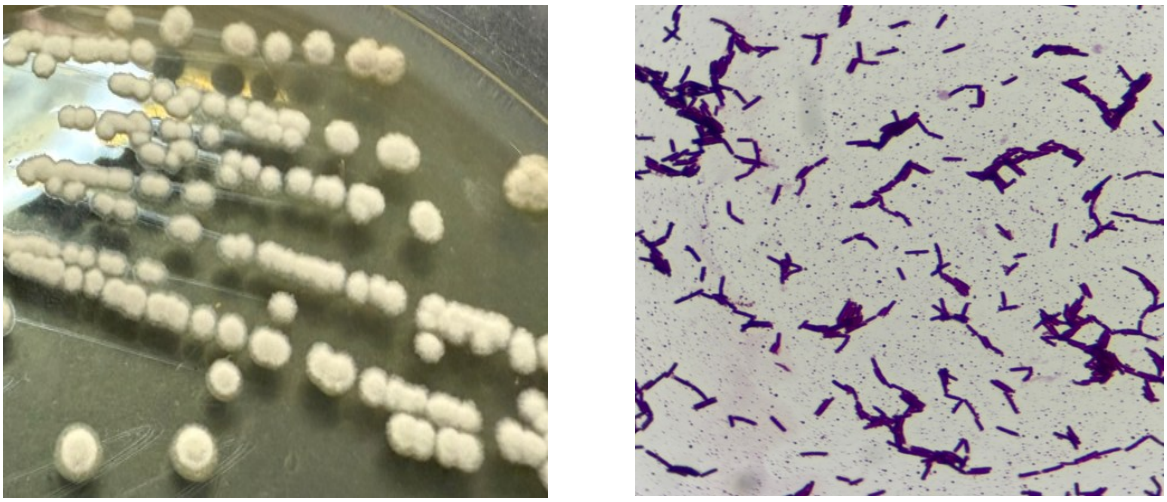
Fig. 2 illustrates the colony morphology and microscopic characteristics of *S. toxytricini* strain 4 grown on Gause I agar medium. The isolate developed compact, dry, and chalky-white colonies with abundant aerial mycelia that covered the colony surface (Fig. 2(a)). Such morphological traits are typical of members of the genus *Streptomyces* and indicate active sporulation and robust vegetative growth under laboratory conditions. Microscopic examination revealed an extensive network of filamentous hyphae with pronounced branching patterns (Fig. 2(b)), a defining feature of filamentous actinomycetes. The well-organized mycelial structure suggests that there is efficient nutrient acquisition and adaptation to the growth medium. Furthermore, extensive hyphal development in Streptomyces species is habitually associated with the biosynthesis of secondary metabolites, including antibiotics, antifungal compounds, and extracellular enzymes. Therefore, the observed morphological characteristics provide substantial evidence to support the taxonomic affiliation of strain 4 to the genus Streptomyces. Furthermore, these characteristics indicate its potential as a promising source of bioactive metabolites for agricultural and biotechnological applications. These observations provide a morphological basis for subsequent molecular identification and functional characterization of the isolate [53,54].



(a) (b)

Fig. 2. Morphological characteristics of *Streptomyces toxytricini* strain 4 grown on Gause I agar medium. (a) Colony morphology with well-developed aerial mycelia; (b) Light microscopic image at 100× magnification showing the branched hyphal network typical of *Streptomyces* genera

Fig. 3 presents the morphological features of *S. lydicus* strain NVC-TX cultivated on Gause I agar. The isolate formed distinct, dry, and opaque colonies with a dense aerial mycelial layer covering the colony surface (Fig. 3(a)). Such colony characteristics are typical of *Streptomyces* genera and indicate vigorous vegetative growth and active differentiation under culture conditions. Microscopic examination at 100× magnification revealed a complex network of filamentous hyphae with extensive branching patterns (Fig. 3(b)), confirming the filamentous nature of the isolate. The presence of well-developed aerial and substrate mycelia is considered a key diagnostic trait for members of the genus *Streptomyces*. Moreover, the highly branched hyphal system increases the surface area available for nutrient absorption and is typically associated with enhanced production of secondary metabolites. It is suggested that these morphological attributes suggest that strain NVC-TX possesses strong physiological adaptability and may have considerable potential for synthesizing bioactive compounds that could be useful in biological control and sustainable agricultural application [53,54].



(e) (f)

Fig 3. Morphological characteristics of *S lydicus* strain NVC-TX cultured on Gause I agar medium. (e) Colony morphology exhibiting abundant aerial mycelial development; (f) Microscopic observation at 100× magnification showing branched filamentous hyphae characteristic of *Streptomyce*

The phylogenetic tree based on 16S rRNA gene sequences revealed that the three isolates belonged to distinct clades within the genus *Streptomyces* (Fig. 4). The strain NVC-TL3 demonstrated a high degree of genetic similarity with *S. lydicus* ATCC 25470, as evidenced by a high bootstrap value, indicating strong phylogenetic relatedness and genetic similarity. This clustering suggests that NVC-TL3 may share similar biological and antagonistic characteristics with previously reported *S. lydicus* strains. Strain 4 formed a stable branch with *S. toyocaensis* strain NBRC 15428, a finding supported by high bootstrap confidence, thus demonstrating its close evolutionary relationship within this lineage. Concurrrently, the NVC-TX strain, which was grouped with *S. griseoruber* strain NBRC 12873, formed a distinct subcluster that was separated from the other isolates. The phylogenetic distances observed among the three strains indicate considerable genetic diversity despite their classification within the same genus. Overall, the tree topology confirmed that the isolates represent *Streptomyces* lineages that are distinct from one another in terms of their phylogeny. The phylogenetic relationships observed among the isolates are consistent with the high genetic diversity commonly reported within the genus *Streptomyces*. The clustering of NVC-TL3 with *S. lydicus* is particularly significant, given the frequent association of this species with antimicrobial activity and biological control potential. Similarly, the close association of strain 4 with *S. toyocaensis* suggests possible similarities in metabolic capacity and secondary metabolite biosynthesis. The separation of NVC-TX into an independent branch related to *S. griseoruber* further highlights the genetic heterogeneity of the isolates. High bootstrap support values have been demonstrated to strengthen the reliability of the phylogenetic placement and support the molecular identification of the three strains [55,56].

3.2 Molecular characterization of F. fujikuroi

Fig. 5(a) illustrates the morphological characteristics of *F. fujikuroi* exhibiting the rapid radial expansion of the fungal colony grown on Potato Dextrose Agar (PDA) with abundant aerial mycelium. The colony surface exhibited a cottony and fluffy appearance, forming a dense network of hyphae with a white to light pink coloration. The colony margin was irregular and filamentous, indicating an active hyphal growth. As the culture matured, slight pigmentation developed towards the center of the colony, a phenomenon that is commonly observed in many *Fusarium* species. Microscopic observation at 100× magnification (Fig 5(b)) revealed hyaline and septate hyphae forming a branched mycelial structure. The presence of characteristic spindle-shaped macroconidia was observed, typically characterized by slightly curved, tapered ends and the presence of several septa. These spores were produced from specialized conidiogenous cells found along the hyphae. The presence of elongated macroconidia and septate hyphae is a distinctive feature associated with fungal species belonging to the genus *Fusarium.* Phylogenetic analysis based on rDNA sequences demonstrated that the studied strain is clearly affiliated with the genus *Fusarium*.

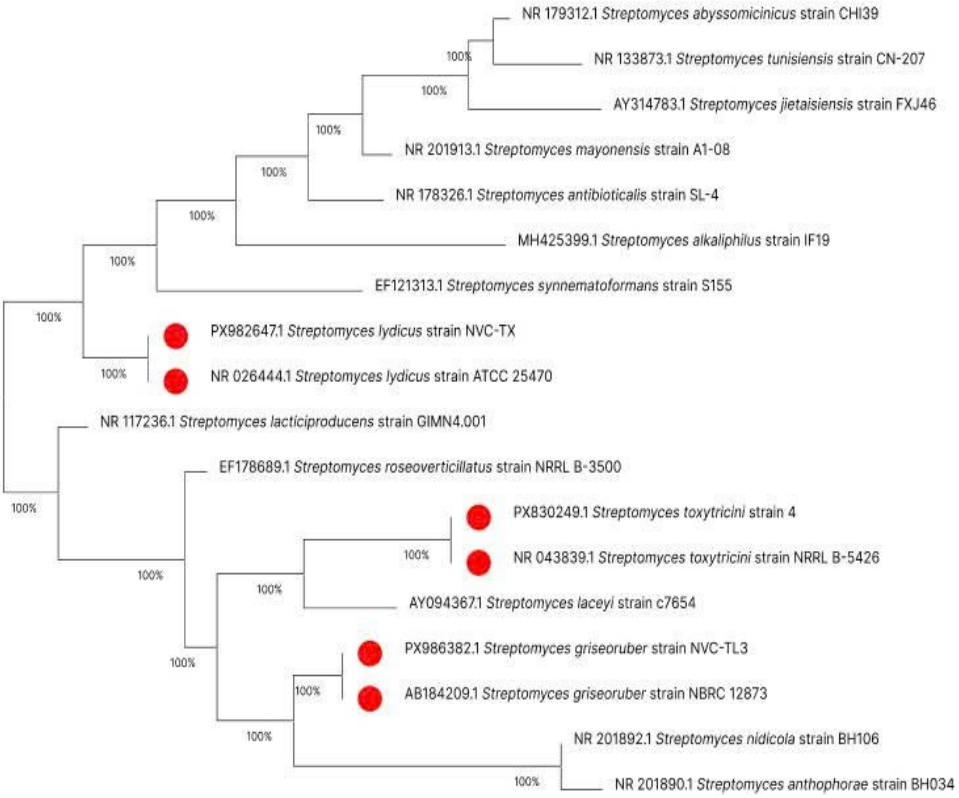
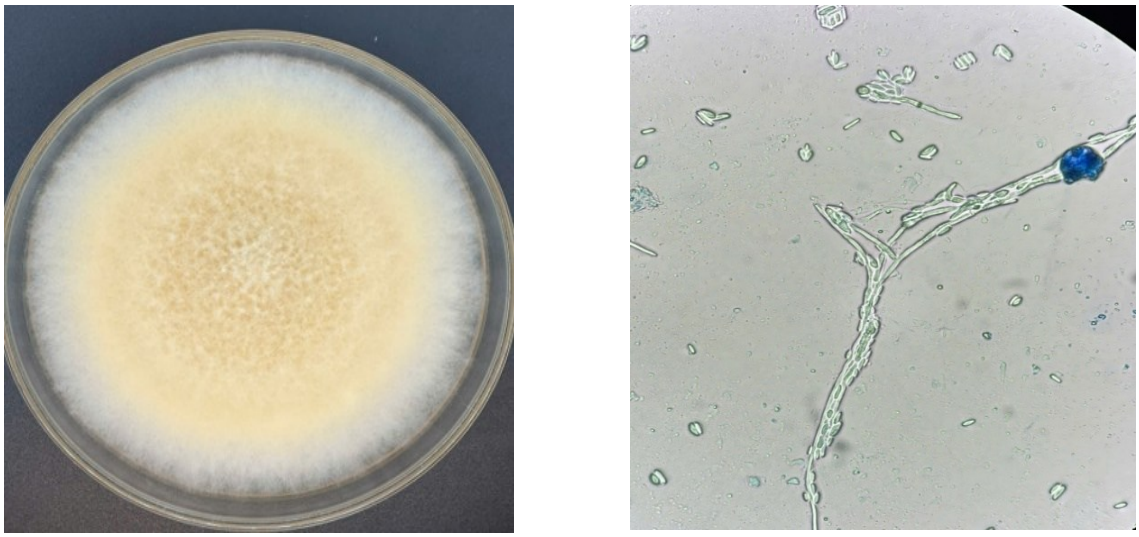


Fig. 4. Phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic relationships of three *Streptomyces* isolates with closely related reference strains retrieved from GenBank. The tree illustrates the phylogenetic positions of strains NVC-TL3, strain 4, and NVC-TX within the genus *Streptomyces*. Bootstrap values (%) based on 1,000 replications are indicated at branch nodes



(a) (b)

Fig. 5. (a) Colony morphology of *F. fujikuroi* on PDA after 7 days; (b) Microscopic observation at 100× magnification

In the phylogenetic tree (see Fig. 6), the strain clustered tightly with *F. fujikuroi* CBS 221.76, supported by a high bootstrap value of 100%. This finding indicates a highly robust and reliable relationship. This close association suggests a high degree of genetic similarity and provides a strong support for its identification as *F. fujikuroi*. The cluster containing *F. fujikuroi* is closely related to *F. annulatum* and *F. acutatum*, forming a well-supported subgroup within the *Fusarium fujikuroi species complex* (FFSC). It is evident that other species such as *F. dlaminii* and *F. nygamai* are positioned in adjacent branches, thereby reflecting moderate evolutionary divergence. Taxa exhibiting a more distant relationship, including *F. concentricum*, *F. humuli*, and *F. ipomoeae*, constitute separate clades that demonstrate robust bootstrap support (100%). Overall, the tree topology confirms the taxonomic placement of the studied strain and highlights its close evolutionary relationship within the FFSC (Fig 6).

3.3. Colony morphology and chitinolytic activity of selected Streptomyces strains

Strain NVC-TL3 (Fig. 7(a)) formed a compact colony with

dense white aerial mycelia and regular circular margins, indicating stable growth and moderate sporulation ability. In contrast, strain 4 (Fig. 7(c)) exhibited rapid radial expansion with abundant cottony mycelia and irregular colony edges, suggesting vigorous vegetative development. Conversely, strain NVC-TX (Fig. 7(e)) produced a well-developed colony characterized by dense aerial hyphae and a distinct central elevation, reflecting robust physiological adaptation and sporulation potential. The chitin hydrolysis assay revealed that all three strains were capable of degrading chitin, as evidenced by the clear halo zones surrounding colonies on chitin agar plates (Fig 7(b, d, and f)). Among them, strain 4 exhibited the largest hydrolysis zone, indicating the strongest chitinolytic activity, whereas strains NVC-TL3 and NVC-TX showed moderate but distinct halo formation. The results suggest that the selected *Streptomyces* strains possess extracellular chitinase activity, which may contribute to their antagonistic effects against fungal pathogens through degradation of fungal cell wall components.

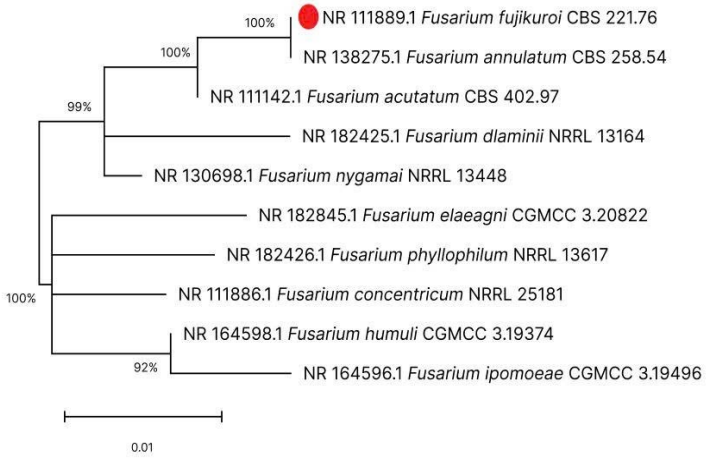


Fig. 6. Phylogenetic tree based on 16S rRNA gene sequences. Bootstrap values (100%) calculated from 1000 replicates are indicated at the branch nodes. The *F. fujikuroi* isolate obtained in this study is highlighted in red

Fig. 7. Colony morphology and chitin-solubilizing activity of three selected *Streptomyces* strains. Colony morphology of (a) strain NVC-TL3, (c) strain 4, and (e) strain NVC-TX cultured on Gause 1 medium, and chitin hydrolysis activity of the corresponding strains shown in (b), (d), and (f), respectively.

The colony morphology observed in the present study is consistent with the typical characteristics of antagonistic *Streptomyces* species reported previously. As posited by Barka et al. [57], the formation of abundant aerial mycelia and powdery colonies is commonly associated with active secondary metabolite production and enhanced biocontrol potential. In addition, the degradation of chitin is considered as a significant antifungal mechanism, given its role as a constituent of substantial proportions within the fungal cell walls. Similar findings were reported by Umar et al. [58], who demonstrated that chitinase-producing actinomycetes generated clear hydrolysis zones on colloidal chitin medium and effectively suppressed several soil-borne fungal pathogens. Furthermore, *Streptomyces* isolates exhibiting strong chitinolytic activity demonstrated greater antagonistic effects against *Fusarium* sp. [58]. Consequently, the observed chitin hydrolysis activity may represent one of several mechanisms involved in fungal suppression. Other mechanisms, including the production of antifungal secondary metabolites and competition for nutrients, were not investigated in the present study.

3.4 Antagonistic activity of three selected strains against F. fujikuroi

As depicted in Table 1, the antagonistic activity of the three *Streptomyces* strains against *Fusarium fujikuroi* increased progressively with incubation time. Among the tested isolates, strain NVC-TX exhibited the strongest inhibitory effect throughout the experiment, reaching growth inhibition (GI) values of 45.6%, 69.2%, and 74.9% after 3, 5, and 7 days, respectively. This strain was also found to yield the largest inhibition zone (SI), particularly on day 3 (14.5 mm), indicating rapid antifungal activity. Strain NVC-TL3 exhibited moderate antagonistic performance with GI values ranging from 40.6% to 73.6%, whereas strain 4 demonstrated comparatively lower levels of inhibition, particularly on day 3 (37.2%). However, strain 4 demonstrated a gradual increase in antifungal activity over time, suggesting stable inhibitory capacity during prolonged incubation. Statistical analysis revealed significant differences among treatments across all observation periods ($p \leq 0.01$). The relatively low coefficient of variation values further confirmed the reliability and consistency of the experimental data.

Similar findings were reported by Umar et al. [58], who observed that Streptomyces isolates producing extracellular enzymes and antibiotics effectively suppressed several Fusarium species. Similarly, Eichfeld et al. [59] demonstrated that chitinase-producing actinomycetes significantly reduced fungal mycelial growth through the degradation of fungal cell walls. In addition, Barka et al. [57] emphasized the significance of Streptomyces spp. as biocontrol agents, owing to their capacity to produce a wide range of bioactive metabolites with antifungal properties. Therefore, the strong inhibitory activity observed in strain NVC-TX suggests its promising potential for utilization as a biological control agent against *F. fujikuroi* in sustainable crop protection strategies.

3.5 Effects of three selected Streptomyces strains on the pathogenicity of F. fujikuroi

The greenhouse experiment demonstrated that all three *Streptomyces* strains significantly reduced Fusarium wilt

severity and improved watermelon growth in comparison with the infected control (NT1). The pathogen-inoculated control recorded the highest disease severity index (DSI) at 91.7%, with severe wilting and poor plant growth observed in Fig. 8. In contrast, *Streptomyces*-treated plants exhibited reduced disease symptoms and enhanced vegetative development.

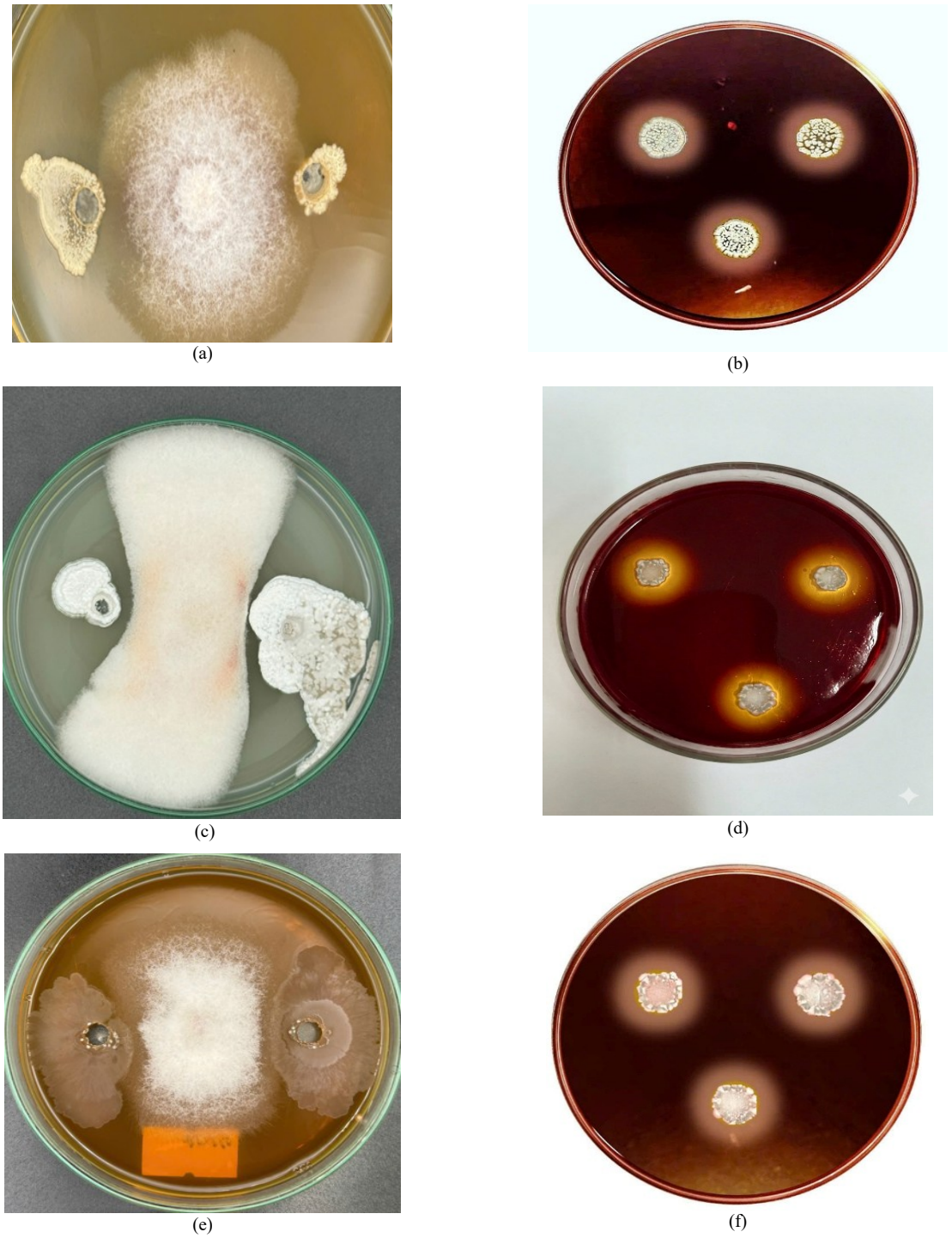


Fig. 7. Colony morphology and chitin-solubilizing activity of three selected *Streptomyces* strains. Colony morphology of (a) strain NVC-TL3, (c) strain 4, and (e) strain NVC-TX cultured on Gause 1 medium, and chitin hydrolysis activity of the corresponding strains shown in (b), (d), and (f), respectively

Table 1. Inhibitory activity of three *Streptomyces* strains against *F. fujikuroi* under in vitro conditions at different incubation periods

Strains	SI (mm)	D1 (mm)			GI (%)		
		Inoculation days					
		3	5	7	3	5	7
strain NVC-TL3	12.8±0.07^{b}	18.7±0.4^{b}	21.9±1.89^{b}	23.7±0.17^{b}	40.6±1.29^{b}	64.1±0.11^{b}	73.6±0.19^{b}
strain 4	12.6± 0.03^{b}	19.8±0.49^{a}	22.8±0.07^{a}	25.8±0.66^{a}	37.2±1.54^{c}	63.4±0.64^{b}	71.2±0.74^{c}
strain NVC-TX	14.5±0.55^{a}	18.7±0.40^{b}	21.9±1.89^{b}	23.7±0.17^{b}	45.6±2.14^{a}	69.2 ± 0.48^{a}	74.9±0.18^{a}
Ftest	**	**	**	**	**	**	**
CV(%)	9.30	8.70	8.30	12.2	14.2	4.51	15.0

Fig.8. Effects of selected *Streptomyces* strains on the growth and disease suppression of watermelon plants challenged with *Fusarium* sp. under greenhouse conditions

Table 2. Disease severity and antagonistic activity of three selected strains against *F. fujikuroi*

Treatment	DSI	AE
	(%)	
NT1 (*F. fujikuroi)*	91.7 ± 3.33[a]	Without AE
NT2 (*F. fujikuroi* + strain NVC-TL3)	30.0 ± 3.85[c]	55.1±2.57[b]
NT3 (*F. fujikuroi* + strain 4)	43.3 ± 6.67[b]	46.59c ± 4.22[c]
NT4 (*F. fujikuroi* + strain NVC-TX)	14.9 ± 3.34[d]	66.22a ± 2.72[a]
NT5 (without *F. fujikuroi and Streptomyces)*	6.67±7.70[e]	Without AE
Ftesst	**	**
CV(%)	8.23	15.9

Note: Values are presented as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences at $p \leq 0.05$. AE is percentage of antagonistic efficiency

As presented in Table 2, among the treatments, strain NVC-TX (NT4) exhibited the most pronounced biocontrol activity, resulting in the lowest DSI (14.9%) and the highest antagonistic efficiency (66.22%). The application of this strain to plants demonstrated healthier stems, greener leaves, and increased vigor. Strain NVC-TL3 (NT2) also effectively suppressed disease, reducing DSI to 30.0% with 55.1% antagonistic efficiency. In contrast, strain 4 (NT3) exhibited lower activity, characterized by a DSI of 43.3%. Overall, all isolates demonstrated biocontrol potential, particularly NVC-TX against *Fusarium* wilt.

The combined results from Table 2 and Fig. 8 confirmed that the three *Streptomyces* strains effectively suppressed *F. fujikuroi* infection and enhanced watermelon growth under greenhouse conditions. Among the isolates that were tested, strain NVC-TX exhibited the strongest biocontrol activity, as evidenced by the lowest DSI value and the highest antagonistic efficiency. This superior performance may be associated with multiple antagonistic traits commonly found in *Streptomyces* spp., including extracellular lytic enzymes and antifungal metabolites. However, these mechanisms were not examined directly in the present study and require further investigation. Similar findings were reported by Eichfeld et al. [59], positing that *Streptomyces* spp. significantly reduced soil-borne fungal diseases through antibiotic production and rhizosphere colonization. Similarly, Eichfeld et al. [59] and Zeyad et al. [60] reported that antagonistic Streptomyces strains suppressed *Fusarium* pathogens by degrading fungal cell walls and competing for nutrients. Although NVC-TL3 and strain 4 also reduced disease severity, their antagonistic effects were comparatively lower, suggesting variability in metabolite production and root colonization efficiency among the isolates. The healthier morphology and improved vigor of treated plants observed in Fig. 7 further support the role of Streptomyces in enhancing plant resistance and reducing pathogen damage under greenhouse conditions.

Among the three selected isolates, *Streptomyces lydicus* NVC-TX was identified to consistently exhibited superior

performance in both in vitro antagonism and greenhouse disease suppression assays. This enhanced efficacy may be attributed to its stronger chitinolytic activity than that of other isolates, suggesting a greater capacity to degrade fungal cell wall components. The consistency of strain NVC-TX performance across diverse experimental conditions further indicates that its antagonistic potential is not solely dependent on direct fungal inhibition but may also involve improved adaptation and persistence in the rhizosphere environment. Although the specific mechanisms were not investigated in the present study, the enhanced disease suppression achieved by strain NVC-TX suggests that this strain may possess a more extensive repertoire of bioactive compounds or antagonistic traits than the other isolates. The observed differences among the three phylogenetically distinct Streptomyces species highlight the significance of strain-level variation in determining biocontrol efficacy, even among closely related actinomycetes.

4. Conclusion

This present study successfully isolated and characterized three indigenous *Streptomyces* strains associated with watermelon-growing soils, specifically *S. cyaneochromogenes* NVC-TL3, *S. toxytricini* strain 4, and *S. lydicus* NVC-TX. All isolates exhibited chitinolytic activity and significant antifungal effects against *F. fujikuroi* under both laboratory and greenhouse conditions, signifying their potential as biological control agents. The integration of morphological, molecular, enzymatic, and biocontrol assessments revealed considerable functional variation among the isolates, with *S. lydicus* NVC-TX that demonstrated the strongest antagonistic activity and the greatest reduction in disease severity. These findings indicate that Streptomyces associated with watermelon represent a valuable source of beneficial microorganisms for sustainable disease management. The positive correlation between chitinolytic activity and the suppression of pathogen further suggests that extracellular lytic enzymes contribute to the observed biocontrol effects. Overall, *S. lydicus* NVC-TX is a promising candidate for the development of eco-friendly biocontrol products, despite the necessity for further studies on its metabolites, genomic traits, and field performance.

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References

1. A. Manivannan, E. S. Lee, K. Han, Lee, H.-E., and D.-S. Kim, *Versatile Nutraceutical Potentials of Watermelon-A Modest Fruit Loaded with Pharmaceutically Valuable Phytochemicals*, Molecules 11(2020) 5258.
2. A. W. Sultani, M. H. Mohammadi, M. Rasoli, *Farm-level economic assessment of watermelon production in nangarhar province*, J. Nat. Sci. Rev. 3 (2025) 228–238.
3. Y. Tong, H. Du, J. Xiao, B. Zhou, X. Zheng, Y. Deng, X. Zheng, and M. Chen, *Watermelon wilt disease: Causes, harms, and control measures,* Front. Microbiol. 7 (2025)1601130.
4. Z. Li, F. He, X. Gai, H. Zhu, S. He, Y. H. Xuan, and L. Bai, *The growth, development and infection process of the plant pathogen Fusarium,* Plant Signal Behav. 20(2025) 2573097
5. K. Abeysinghe, A. Madhushan, A. M. Ismail, E. Ilyukhin, and S. S. N. Maharachchikumbura, *The Multifaceted menace of Fusarium as a plant, animal, and human pathogen*, Biology 6(2026) 453.
6. H. Lv, R. Sang, L. Huang, Lv H, Y. Long, D. Xu, M. Wang, Q. Zhang, L. Jia, Q. Ding, C. Yan, and H. Zhang, Continuous *watermelon cropping impairs plant growth by modifying soil biochemistry and rhizosphere microbial communities*, Front. Microbiol.16 (2025) 1648481.
7. M.M. Hossain, F. Sultana, M. Mostafa, M. T. Rubayet, N. J. Mishu, I. Khan, and M. G. Mostofa, *Biological management of soil-borne pathogens through tripartite rhizosphere interactions with plant growth-promoting fungi*, Appl. Microbiol. 5 (2025) 123.
8. T.I. Ekwomadu, M. Mwanza, *Fusarium fungi pathogens, identification, adverse effects, disease management, and global food security: a review of the latest research*, *Agriculture* 13(2023) 1810.
9. D. Shao, Q. Xu, X. Lv, C. Li, L. Luo, J. Xu, Y. Zhang, C. Zhao, and C. Chen, *Growth-promoting effects of VOCS produced by Trametes hirsuta and Talaromyces pinophilus on rice,* Agriculture 15(2025) 2451.
10. B. Yang, C. Yue, C. Guo, M. Zheng, X. Yao, J. Xu, S. Huang, and M. Yang, *Disease-resistant watermelon variety against Fusarium wilt by remodeling rhizosphere soil microenvironment,* BMC Microbiol. 25(2025) 350.
11. C. H. Aguilar, D. Pires, C., Cortaga, R. Peja, M. A. Cruz, J. Langres, M. C. F., Redillas, L. Galvez, and M. A. Balendres, *Harnessing legume productivity in tropical farming systems by addressing challenges posed by legume diseases*, Nitrogen 3(2025)65.
12. M. A. Cucu, R. Choudhary, V. Trkulja, S. Garg, and S. Matić, *Utilizing environmentally friendly techniques for the sustainable control of plant pathogens: a review*, Agronomy 15(2025) 1551.
13. C. V. Nguyen, T. L. K. Tran, *Molecular identification and characterization* of *Peribacillus simplex* LT4 *isolated from the roots of baby maize* (*Zea mays* L.), Nitrogen 7 (2026) 1-11.
14. N. V. Chuong, *Effect of three different nitrogen rates and three rhizosphere N2- fixing bacteria on growth, yield and quality of peanuts,* TiS. 21(2024) 7281.
15. N. Trang, and N. Chuong, *The enhancement of soil fertility and baby maize output by Streptomyces panayensis and vermicompost*, Eurasian J. Soil Sci. 14(2025) 140-148.
16. B. Espinosa-Palomeque, O. Jiménez-Pérez, R. I. Ramírez-Gottfried, *Biocontrol of phytopathogens using plant growth promoting rhizobacteria: bibliometric analysis and systematic review,* Horticulturae 11 (2025) 271.
17. A. A. Najjar, *Therapeutic potential of endophytic microbes: emphasizing both fungal and bacterial endophytes*, Appl. Microbiol. 5 (2025) 5.
18. N. V. Chuong, T. L. K. Tri, L. M. Tuan, *Assessing the superiority of Bacillus songklensis strain kca6 along with lime and cow manure to increase white bean yield in cadmium contaminated soil,* Aust J Crop Sci. 18(2024)768-774.
19. P. Kapoor, P. Joshi, M. Pal, V. Parkash, *Actinobacteria as proficient biocontrol agents for combating fungal diseases in forest plant species,* J. Basic Microbiol. 65(2025) e70030.
20. I. J. S. da Silva, T. P. Sousa, T. M. de Souza, *Streptomyces strains from amazonian sediments as plant growth promoters and biocontrol agents of anthracnose in postharvest capsicum Chinense*, Microorganisms 13 (2025) 2713.
21. N.V. Chuong, T. L. K. Tri, T. T. Liem, N. V. Thuan, T. V. T. Em, N. N. P. Trang, *Four newly identified endophytic bacillus megaterium strains with biofertilizer potential for enhancing maize growth and productivity*, TiS. 23 (2025) 11347.
22. Md. Arshad Ali, T. Ahmed, E. Ibrahim, *A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi*

and their plant growth-promoting activities, Heliyon 10(2024) e31573.

23. P. Aleahmad, L. Ebrahimi, N. Mohayer, *Antifungal properties of Eucalyptus endophytic Streptomyces strains*, Sci. Rep. 16(2025) 2893.
24. L. Cai, H. Zhang, Y. Deng, W. Tian, G. Fan, X. Sun, *Antifungal activity of Streptomyces hygroscopicus* JY-22 against *alternaria alternata and its potential application as a biopesticide to control tobacco brown spot*, Agronomy 13(2023)1944.
25. M. Maciel-Rodríguez, F.D. Moreno-Valencia, M. Plascencia-Espinosa, *the role of plant growth-promoting bacteria in soil restoration: a strategy to promote agricultural sustainability*, Microorganisms 13 (2025)1799.
26. D. Palmieri, G. IaniriC. Del Grosso, *Advances and perspectives in the use of biocontrol agents against fungal plant diseases*, Horticulturae 8(2022) 577.
27. S. Gore, S. Singh, P. Swarnkar, *Endophyte mediated plant defence responses and their potential against pathogenic bacteria and fungi*, *Discov. Plants* 2(2025) 1-27.
28. L. Sena, E, Mica, G. Valè, P. Vaccino, N. Pecchioni, *Exploring the potential of endophyte-plant interactions for improving crop sustainable yields in a changing climate*. Front. Plant Sci. 15(2024)1349401.
29. N. Van Chuong, T. L. K. Tri, T. M. Vu, L. M. Tuan, T. T. Liem, N. N. P. Trang, *Isolation and identificatio*n of *Bacillus aryabhattai* M2C: *its effects with vermicompost on yield and nutrients of peanut* (*Arachis hypogaea* L.). Int. J. Microbiol. 2025(2025) 9923279.
30. A. El Karkouri, S. A. Assou, M. El Hassouni, *Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope*. Pan Afr Med J. 33 (2019) 329.
31. A. R. Ali, Y. Bahrami, E. Kakaei, S. Mohammadzadeh, S. Bouk, N. Jalilian, *Isolation and identification of endophytic actinobacteria from Citrullus colocynthis (L.) Schrad and their antibacterial properties,* Microb. Cell Fact. 21(2022) 206.
32. N.el-A. El-Naggar, N. A. Abdelwahed, W. I. Saber, A. A. Mohamed, *Bioprocessing of some agro-industrial residues for endoglucanase production by the new subsp.; Streptomyces albogriseolus subsp. cellulolyticus strain NEAE-J,* Braz. J. Microbiol. 45(2014) 743–756.
33. N. V. Chuong, *Influences of Enterobacter cloacae strain fg 5-2 and its vermicompost and nitrogen fertilizer usage efficiency on groundnut yield*, TiS. 21(2024) 8039.
34. N. Tripathi, M. Zubair, A. Sapra, *Gram Staining. [Updated 2025 Mar 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available* from: https://www.ncbi.nlm.nih.gov/books/NBK562156/
35. A. Dwiyani, Y. Retnowati, A. Sidik, *Isolation and characterisation of actinomycetes in the rhizosfer pandanus ammaryllifolius plants in Gorontalo,* Mikhayla J. Adv. Res. *2*(2025) 19–28.
36. J. W. A. Antido, F. M. M. Climacosa, *Enhanced isolation of streptomyces from different soil habitats in Calamba City, Laguna, Philippines using a modified integrated approach*, Int. J. Microbiol. 26 (2022) 2598963.
37. F.A. Rainey, N. Ward-Rainey, R.M. Kroppenstedt, "The *genus nocardiopsis represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam,* Int. J. Syst. Bacteriol. 46 (1996) 1088-1092.
38. S. Kumar, G. Stecher, M. Li, *MEGA X*: *molecular evolutionary genetics analysis across computing platforms,* Mol. Biol. Evol. 35 6 (2018)1547-1549.
39. S. C. Hsu, J. L. Lockwood, *Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil*, Appl. Microbiol. 29 (1975), 422-426.
40. A. A. Umar, A. B. Hussaini, J. Yahayya, I. Sani, H. Aminu, *Chitinolytic and antagonistic activity of Streptomyces isolated from fadama soil against phytopathogenic fungi*. Trop Life Sci. Res. 32 (2021) 25-38.
41. I. J. Seago, L. C. Marsh, K. J. Stevens, *A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma*, Ann. Bot. 96 (2005)565-579.
42. B. Kalman, D. Abraham, S. Graph, R. Perl-Treves, Y. Meller Harel, O. Degani, *Isolation and identification* of *Fusarium* spp. *the causal agents of onion* (*Allium cepa*) basal rot in northeastern Israel, *Biology* 9(2020) 69.
43. B. K. Das, V. Kumar, S. Samanta, S. Roy, A. K. Sahoo, K. Bisai, A. K. Jana, R. Chakraborty, A. Adhikari, R. C. Malick, S. K. Rathod, A. Majumder, *Molecular characterization and virulence of fungal pathogens associated with mass mortalities in hilsa Shad* (*Tenualosa ilisha*). Sci. Rep. 15 (2025) 13957.
44. A. J. Morris, T. C. Byrne, J. F. Madden, L. B. Reller, Duration of incubation of fungal cultures, J. Clin. Microbiol. 34 (1996) 1583–1585.
45. S. Ait Assou, J. Anissi, L. Dufossé, M. Fouillaud, M. Hassouni, *Inducing and enhancing antimicrobial activity of mining-soil-derived actinomycetes through component modification of bennett's culture medium*, Microbiol. Res. 16(2025) 72.
46. D. Al-Mutar, N. Alzawar, M. Noman, *Suppression of fusarium wilt in watermelon by Bacillus amyloliquefaciens DHA55 through extracellular production of antifungal lipopeptides*, J. Fungi. 9(2023) 336.
47. M. F. Hasan, M. A. Islam, B. Sikdar, *evaluation of possible biological control* of *Fusarium Concentricum* sp. *using plant extracts and antagonistic species of microbes in vitro,* F1000Res. 9 (2020)1394.
48. Y. Si, Y. Haxim, L. Wang, *Optimum sterilization method for in vitro cultivation of dimorphic seeds of the succulent halophyte suaeda aralocaspica*, Horticulturae 8(2022) 289.
49. P. Sripreechasak, A. Matsumoto, K, Suwanborirux, *Streptomyces siamensis* sp. nov., and *Streptomyces similanensis* sp. nov., isolated from Thai soils, J Antibiot. 66 (2013) 633-40.
50. D. Zhao, Y. Zhang, Z. Jin, R. Bai, J. Wang, L. Wu, Y. He, *Benzalkonium chloride and benzethonium chloride effectively reduce spore germination of ginger soft rot pathogens*: *Fusarium solani* and *Fusarium oxysporum*, J. Fungi (Basel). 10(2023) 8.
51. D. M. K. Al-Mutar, N. Noman, N. S. A. Alzawar, H. H., Li, D. Qasim, F. Song, *The extracellular lipopeptides and volatile organic compounds* of *Bacillus subtilis* DHA41 *display broad-spectrum antifungal activity against soil-borne phytopathogenic fungi*, J. Fungi (Basel) 9 (2023), 797.
52. R. K. Hanson, K. E. Morton-Bourgon, *The accuracy of recidivism risk assessments for sexual offenders: a meta-analysis of 118 prediction studies*, Psychol. Assess. 21(2009), 1–21.
53. K. O. G. de Neves, G. V. da Costa, C. C. B. Campos, C. A. Queiroz, T. F. Sousa, A. S. dos Vasconcelos, F. M. A. da Silva, M. E. B. Yamagishi, H. H. F. Koolen, G. F. da Silva, *Streptomyces amazonensis sp. nov. isolated from Madeira river sediments with genomic potential for secondary metabolite production,* Braz J Microbiol 57(2026)14.
54. K. Alam, A. Mazumder, S. Sikdar, Y. M. Zhao, J. Hao, C. Song, Y. Wang, R. Sarkar, S. IslamY. Zhang, A. Li, *Streptomyces: The biofactory of secondary metabolites*, Front. Microbiol.13(2022) 968053.
55. M. Benhadj, T. Menasria, N. Zaatout, S. Ranque, *Genomic insights and antimicrobial potential of newly Streptomyces cavourensis isolated from a ramsar wetland ecosystem*, Microorganisms 13 (2025) 576.
56. L. T., Tran, C. V. Nguyen, *Nitrogen-Fixing and Phosphate-Solubilizing Bacillus stercoris CMB2 from Baby Maize Roots,* Nitrogen, 7(2026), 38.
57. E. A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, J. P. Meier-Kolthoff, H. P. Klenk, C. Clément, Y. Ouhdouch, G. P. van Wezel, *Taxonomy, Physiology, and natural products of actinobacteria*, Microbiol. Mol. Biol. Rev. 80(2015) 1–43.
58. A. A. Umar, A. B. Hussaini, J. Yahayya, I. Sani, H. Aminu, *Chitinolytic and antagonistic activity of Streptomyces isolated from fadama soil against phytopathogenic fungi*, Trop. Life Sci. Res. 32(2021) 25–38.

59. R. Eichfeld, A. B. Endeshaw, M. J. Hellmann, T. N. B. M. Moerschbacher, A. Zuccaro, *Domain gain or loss in a fungal chitinase enables specialization towards antagonism or immune suppression*, Nat. Commun. 17 (2026) 3115

60. M. T. Zeyad, P. Tiwari, W. A. Ansari, S. C. Kumar, M. Kumar, H. Chakdar, A. K. Srivastava, U. B. Singh, A. K. Saxena, *Bio-priming with a consortium of Streptomyces araujoniae strains modulates defense response in chickpea against Fusarium wilt*, Front. Microbiol. 13 (2022) 998546.