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ORIGINAL ARTICLE

Utilizing *Streptomyces bungoensis* as a biocontrol agent to prevent wheat infection caused by *Fusarium clumorum*

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Abstract

Fusarium species substantially threaten wheat harvests, resulting in considerable losses. Applying chemical fungicides to address fungal diseases may adversely affect the environment and human health. Therefore, the development of novel environmentally sustainable fungicides is imperative. This work isolated fifteen *Streptomyces* (St) isolates from soil and subsequently identified them using morphological, biochemical, and molecular techniques. Four St isolates (St1, St2, St3, and St4) were chosen for their pectinase and antifungal activities as alternative fungicides against *Fusarium clumorum* infection in wheat. The sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electropherogram of four *Streptomyces bungoensis* isolates exhibited 35.7% polymorphic protein bands. In contrast, discontinuous polyacrylamide gel electrophoresis (DISC-PAGE) analysis of pectinase isozymes revealed seven bands with differing densities, of which five polymorphic bands constituted 71.4%. Random amplified polymorphic deoxyribonucleic acid analysis by polymerase chain reaction (RAPD-PCR) revealed polymorphism among four isolates by identifying eight polymorphic amplified DNA fragments, representing 94% of the total. The St1, St3, and St4 isolates produced unique DNA fragments of 127, 575, and 950 base pairs. The phylogenetic tree indicates that the four isolates are closely linked within a single group. The St4 was chosen for the in vivo greenhouse trial due to its antifungal properties, superior protein content, and pectinase activity. Administering St4 (100 µg/ml) diminished the pre-emergence of root rot disease by 87%; concurrently, it decreased the incidence and severity of crown and root rot disease (CRD) by 81% and 90%, respectively. The *Fusarium* infection decreased shoot weight by 33%, although root weight remained consistent with the control group. The grain yield rose by 10% in healthy wheat plants and did not impact FC-infected plants treated with *Streptomyces*. The weight of 1000 grains rose by 40% relative to the control group. The St4 was established to regulate the levels of total chlorophyll, carotenoids, and antioxidant content in wheat infected with *F. clumorum*. The results indicated that St4 can be considered a creative, environmentally sustainable, and safe alternative fungicide.

Keywords Antifungal activities · Alternative fungicides · Fungal infection · Pectinase · *Streptomyces* · Wheat

Introduction

Microbiological diversity is a promising domain for using possible secondary metabolites that have significant implications in synthetic biology; it provides new genes and biochemical pathways that may employed to produce antibiotics, enzymes, and other essential chemicals (Clardy et al. 2009). Actinomycetes are autonomous microorganisms present in many habitats. The soil is the main home of actinomycetes and is the most active environment for interacting with other organisms (Singh and Dubey 2018). *Streptomyces* represent a significant population of actinomycetes, distinguishing themselves among all actinomycete species in the soil. One investigation identified *Streptomyces* species as necessary for antibiotic production (Agadagba 2014; Alshamsi et al. 2024). These organisms are extensively studied due to their capacity to produce secondary metabolites with considerable market value. They are screened to

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identify additional bioactive compounds (Mathew et al. 2020; Alkaabi et al. 2022; Donald et al. 2022).

Actinomycetes are Gram-positive, filamentous, heterotrophic, saprophytic microorganisms that substantially impact the breakdown of organic materials and possess a high concentration of G+C (Muazi Alenazi et al. 2023). Their filamentous topology may be branched to create a durable mycelium. The mycelium may not always remain intact, as it can break into rod- or sphere-shaped pieces. When actinomycetes reach maturity, they multiply rapidly, forming a dense network of mycelium that spreads on and beneath the agar. The mycelium growing on the surface is called aerial mycelium, while the mycelium buried beneath the substrate is called substrate mycelium (Farag et al. 2024).

Most known antibiotics, including antifungal, anticancer, and immunosuppressive agents, are certified to be produced by filamentous actinomycetes (Sapkota et al. 2020). Actinomycetes are the source of more than two-thirds of all antibiotics used in medicine. Additionally, natural chemicals derived from actinomycetes are used to create various anticancer, immunosuppressive, anti-inflammatory, and antiviral drugs (Selim et al. 2021). *Streptomyces* species are generally categorized as either independent or reliant on a host. These free-living species can also be found in aquatic and terrestrial environments. A broad spectrum of eukaryotes, including plants, insects, marine organisms, and even specific tissues of mammals and humans, can serve as hosts for *Streptomyces* (Katti et al. 2022).

Emerging antibiotic-resistant organisms provide a formidable challenge in the current international landscape. There is an urgent need to identify new microorganisms that produce efficacious secondary metabolites resistant to antibiotics (Selim et al. 2021). To overcome this difficulty, it is imperative to devise innovative methodologies to search for new compounds. One such phenomenon is the "Renaissance of Antibacterial Discovery from Actinomycetes." The exploration of distinct biological spaces and advanced isolation methods for previously unidentified genera of Actinomycetes may lead to the identification of novel gene pools and, subsequently, new products (El-Tarabily 2006; Rohilla et al. 2021).

The study of genetic diversity and functional variations in cultivable actinomycetes has advanced the cultivation of these microorganisms, isolated from the rhizosphere of diverse plant groups in two separate alpine zones (Das et al. 2021). Isolates from this region are primarily associated with *Streptomyces* species and demonstrate antibiotic activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* (Jaroszewicz et al. 2021).

Actinomycetes isolated from the rhizosphere soils of sugarcane were examined for their antibacterial and antifungal properties (Wang et al. 2021). Numerous colonies emerged upon plating, demonstrating inhibitory action against the growth of Gram-positive *Staphylococcus aureus*, while *E. coli* remained unaffected (Chang et al. 2021). Characterization of actinomycetes obtained from alluvial soils in Punjab revealed their antibacterial and antifungal capabilities, including their ability to inhibit the growth of various Gram-positive and Gram-negative bacteria, as well as fungi (Dorchenkova et al. 2022). TLC analysis identified the antibacterial component as an alcohol, phenol, or steroid (Ghanem et al. 2022).

The *Fusarium* species, including *Fusarium graminearum*, *F. culmorum*, and *F. pseudograminearum* induce diseases in cereal crops through different parts of the plant: seed, root, crown, seedling, basal stem, and spike decay (Yin et al. 2020; Zhao et al. 2022). Rot diseases are significant economic diseases affecting cereal crops in many grain-producing regions worldwide, especially arid environments, and significantly reduced the yield by exceeding 50% (El-Saadony et al. 2021; Kleczewski and Geisler 2022; Saad et al. 2023).

The agricultural advancements of the green revolution era dramatically increased wheat production, solidifying its position as a cornerstone of global food security (IDRC 2021). Wheat and wheat products provide approximately 550 kcal per person per day. This is expected to increase to 3000 kcal per person per day by 2050, as the global population is forecast to grow by over 2 billion compared to 2018 (Alexandratos and Bruinsma 2012; FAOSTAT 2021). The increasing population, higher daily calorie intake, and projected growth of the biofuel industry are expected to drive a steady rise in wheat demand until 2050 (Grote et al. 2021).

Ahn et al. (2023) assessed the virulence and survival characteristics of various *Fusarium* species infecting wheat, utilizing the seed inoculation techniques involving the placement of seeds on inoculated filter paper and seedling injection methods, which consists of the process of either adding spores suspension to the stem or placing an agar plug mycelium next to the lower part of the seedling (Bouaicha et al. 2022). Applying chemical fungicides to combat rot disease in cereals raises important concerns regarding the impact on the environment and human health. Several research efforts focus on investigating the potential of natural compounds and secondary metabolites for treating fungal diseases (Poole and Arnaudin 2014; Singh et al. 2021).

Streptomyces, the most prolific antibiotic producers, have received less attention as biocontrol agents against *Fusarium* diseases and plant diseases in general (Newitt et al. 2019). *Fusarium* diseases, particularly those caused by the vascular pathogen *Fusarium oxysporum*, pose significant

challenges due to their difficulty in control using microbial antagonists (Rahman et al. 2021). Research on *Streptomycetes* as biocontrol agents has remained in more experimental stages, with limited testing of various *Streptomyces* spp. strains under diverse conditions (Bubici 2018). *Streptomyces bungoensis* (BF26) is stated as an antifungal agent against *Alternaria sesame*, *Fusarium oxysporum*, and *Rhizoctonia solani* in vitro (Elshamy 2022).

Commercial products containing *Streptomyces* species have often yielded inconsistent results across trials. Five field biocontrol trials utilizing *Streptomycetes* species demonstrated reductions in Fusarium wilt between 0 and 55% in cucumber, revealing a 1.3-fold increase in yield (Coque et al. 2020; Taha et al. 2021). The chitinolytic activity of *Streptomyces* species plays a crucial role in *Fusarium* disease biocontrol and growth promoters (Goudjal et al. 2016).

Previous studies identified endophytic *Streptomyces* strains as volatile organic compound producers, biocontrol-mediating antibiotics, and metabolites (Cordovez et al. 2015). Bioformulation, an essential component of biocontrol agent application, has been insufficiently addressed in experimental research (Shaikh and Sayyed 2014). Future investigations should explore the biocontrol efficacy of well-designed streptomyces consortia or microbial synthetic communities, potentially in optimized formulations, and the role of *Streptomyces* in reducing mycotoxin levels in grains (McLaughlin et al. 2023).

In this study, we screened and identified *Streptomyces species* based on the morphological, isozyme, pectinase activity, protein content, and DNA fingerprint. The best isolate of four *Streptomyces bungoensis* isolates was selected based on the antifungal activity. Then, it was applied as an alternative fungicide to manage *Fusarium* infection in wheat while monitoring its morphological and physicochemical parameters.

Materials and methods

Isolation of *Streptomyces* isolates

Different media were used to isolate antibiotic-producing *Streptomyces* isolates, i.e., inorganic salt starch agar (ISSA), oatmeal agar, and starch casein media (Kuster and Williams 1964). 3 g of soil were collected from the root rhizosphere of potato plants, mixed with sterile water (100 ml) in a conical flask, shaken well, and left without shaking for 30 min to precipitation. The necessary dilution (up to 10^{-5}) was made with this original solution. 100 µL of the dilution was used for inoculating 15 ml of ISSA medium at 45–50 °C in Petri plates, and the plates were incubated at 30 °C (Shirling and Gottlieb 1966; Hossain and Rahman 2014).

Observation was monitored at 5–7 days intervals to detect any colony surrounded by a clear zone of inhibition. The isolated *Streptomyces* isolates were purified through repeated plating. Streaking on ISSA and oatmeal agar was used for these purposes. For further analysis, the purified isolates were re-cultured onto starch casein agar (Yekkour et al. 2012; Njoroge et al. 2018).

Characterization of *Streptomyces* isolated

Cell morphology and developmental features

14-day-old cultures of *Streptomyces* isolates were grown on the following media: oat-meal agar to examine their cultural features (Küster 1959), yeast extract-malt extract agar (Pridham et al. 1958), glycerol-asparagine agar (Pridham and Lyons Jr 1961), starch- nitrate agar (Tadashi 1975), glucose-asparagine agar (Waksman 1961), nutrient-agar (Shirling and Gottlieb 1966), and sucrose- nitrate agar and glucose-nitrate agar (Waksman 1961). Observations were monitored at 7, 14, and 21 days at 30 °C after the inoculation according to the aerial mycelium morphology, color, presence of soluble pigment, and reverse color (Kusuma et al. 2021).

The microscopic examination was conducted using the coverslip technique, as described in Radhakrishnan and Varadharajan (2022), Daigham and Mahfouz (2020), and Sharma et al. (2014). The organization of spores on mycelium was observed using a high-power objective under controlled lighting conditions. In order to directly examine entire spores, carbon-coated grids were applied to the surface of mature aerial growth using a transmission electron microscope (TEM). A substantial quantity of spores were attached to the carbon film and were visible using a JEOL-JEM 1010 TEM operating at 80 kV.

Melanin pigment production

The *Streptomyces* isolates were incubated on pigment-promoting peptone yeast extract iron agar (ISP-6) tyrosine agar (Gordon and Smith 1955) and tryptone-yeast extract broth (Pridham et al. 1958) to induce pigment production. The melanin pigment was formed after four days of incubation at 28 °C. The occurrence of brown or dark brown melanin pigments in the early stages of plant growth generally suggests active melanin production.

Antimicrobial activity of selected *Streptomyces* isolates

The antimicrobial activity of *Streptomyces* isolates was estimated using the cork-borer method: the spore suspensions of *A. niger* TCC16404 and *Fusarium culmorum* ATCC16430 were inoculated into Dox agar plates. Also, two bacterial

strains, *E. coli* ATCC7839, and *Staphylococcus aureus* ATCC6538, were inoculated on plate count agar plates.

The cork-borer punched four wells for the *Streptomyces* isolates on the medium surface. The plates were incubated at 25 °C for 72 h (fungi), 30 °C (yeast), and for 48 h and 37 °C (bacteria) for 24 h. The microbial growth inhibition zones study reflects the presence of bioactive compounds in *Streptomyces* inoculum (Zhou et al. 2024).

Total protein determination

Standard bovine serum albumin (20–100 µg/ml) was prepared. Standard solution (200 µl) was taken, mixed with 1 ml of alkaline copper sulfate reagent, and 100 µl of folin-ciocalteu reagent were added to each tube and incubated for an hour. The *Streptomyces* extract (obtained by centrifugation at 3100×*g* for 15 min) was diluted, and a known sample volume was treated with alkaline copper and folin-ciocalteu reagents. The OD values for all the samples were observed at 660 nm (Lowry et al. 1951; Saad et al. 2015) using bovine serum albumin (BSA) as a reference protein.

SDS-PAGE electrophoresis

Streptomyces protein isolate (50 µl) was mixed with 50 µl of a loading sample buffer (LSP) and then heated for 5 min at 95 °C to be denatured. The protein was negatively charged by SDS 10%, which is a constituent in the LSP solution. The protein samples were loaded in the well of stacking gel, and the SDS-polypeptide complexes moved across 12% polyacrylamide gel in proportion to the polypeptide size. Electrophoresis was run at 150 volts for two gels until the marker dye, bromophenol blue (BPB), reached the gel's end. After the run, the gels were stained for 2 h in a 0.1% (w/v) coomassie brilliant blue R-250 in a 10% glacial acetic acid solution and 40% methanol. The stained gels were destined using the dye-free solution (Saad et al. 2021; Sayed-Ahmed et al. 2022).

Discontinuous polyacrylamide gel electrophoresis (DISC-PAGE) was used to determine pectinase isozymes (Smith et al. 1969). Each of the four *S. bungoensis* isolates was prepared by combining them with an equal volume of loading sample buffer. DISC-PAGE was performed on 10% (W/V) slab gel, and pectinase enzyme samples were loaded into the gel wells. Electrophoresis was conducted at 150 volts/two gels until the marker dye BPB reached the gels end. The gel was incubated in the dark at 37 °C for complete staining. The gels were examined with the software Alpha Ease FC 4.0 (King and Laemmli 1971; Tewfike and Yassin 2015).

Molecular diversity by PCR

Genomic DNA isolation: Following the method described by Owen and Borman (1987), the DNA of the four *Streptomyces* isolates was isolated from tissues. RAPD-PCR analysis: Four *Streptomyces* isolates were subjected to DNA fingerprinting using 15 primers from the Operan RAPD 10 mer kits. PCR was used to amplify the DNA of each isolate (50 ng) using 25 µl of randomized mixes under the following conditions: A gelatin concentration of 0.01%; 1–2 mM of $MgCl_2$; 10 mM of Tris-HCl (pH 8.0); -5 units of Taq polymerase (Promega Co., USA); 50 mM of KCl; 200 mM of dNTPs; 200 ng of oligonucleotide primer; 2 and 1 x Taq polymerase buffer (Embley and Stackebrandt 1994).

According to Weisburg et al. (1991), the DNA thermal cycler (MWG-BIOTHCK primus) was built explicitly for amplification. Perform one cycle at a temperature of 72 °C for 4 min, followed by 40 cycles at 34 °C for 20 s, 5 °C for 1 min, and 72 °C for 2 min. Then, perform one cycle at 72 °C for 5 min, followed by 40 °C for 10 min indefinitely. The amplification products were resolved by electrophoresis across a 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) in a 1X TBE buffer at 95 volts. A 100-base pair DNA ladder was employed to establish a molecular size benchmark. Photomicrographs of PCR products were taken using a Gel Documentation System (BIO-RAD 2000) under UV light. The detected bands were scored as either 1 or 0 for their presence or absence across isolates, and a binary matrix was created for subsequent NISYS software analysis.

In vivo experiment

Giza wheat cultivar 171 grains were sterilized using NaOCl, washed with sterilized distilled water to eliminate surface pathogens, and then air-dried at room temperature (Parsons and Munkvold 2012). The sterilized grains were immersed in 100 ml of *Streptomyces* inoculum (0, 25, 50, and 100 µg/ml) for 24 h (Xue et al. 2017). The control was water. The treated grains dried, and then five grains were sowed in pots containing healthy or infected soil.

The water application of *Streptomyces* was used to irrigate all bots every 15 days. The disease parameters were monitored through the experimental period of three months, including pre-damping-off, post-damping-off, damping-off, and healthy survival (El-Saadony et al. 2021).

Morphological parameters

The growth characteristics include dry weights (g) and plant height (cm). The fresh and dry weight of the root system was separated from the pot and cleansed using tap water. The stems and roots were dried in an oven set at 65 °C

for 5 h until a consistent weight (DM) was achieved. The dimensions of 1000 grains were measured in terms of length (cm), number (cm), and weight (g) (El-Saadony et al. 2021).

Pigments and antioxidant enzymes

Pigments

The pigments (chlorophyll and carotenoids) content was evaluated by Nagata and Yamashita (1992) and El-Saadony et al. (2024). One gram of fresh leaves was extracted with 100 ml of 80% aqueous acetone (v/v). The extract was filtered, and the volume was adjusted to 100 ml using 80% acetone. The optical density of the plant extract was measured using a spectrophotometer at three wavelengths (470, 649, and 665 nm). Pigment concentrations were calculated using the following Eq.

$$Chlorophyll\ (a+b) = 6.45\ (Abs\ 665) + 17.72\ (Abs\ 649) \quad (1)$$

$$\text{Carotenoids} = 1000 \times \text{O.D470} - 1.82\ \text{Ca} - 85.02\ \text{Cb}/198 \quad (2)$$

Antioxidant enzymes

Catalase activity Catalase activity was assessed following the procedure of He et al. (2020). One g of fresh leaves was suspended in a mixture of phosphate buffer (PB, pH 7), EDTA, and urea (10 mL), then blended for 10 min under cold conditions; the enzymatic suspension was centrifuged (8500×*g*, 15 min, 4 °C), the supernatant was obtained. 0.1 ml of the supernatant was mixed with 0.5 ml of hydrogen peroxide (H_2O_2) and 2.5 ml of PB (pH 5). The absorbance was read at 240 nm using a spectrophotometer three times at one-minute intervals to monitor the breakdown of H_2O_2 by catalase. One unit of catalase was calculated by a 0.01 increase in absorbance/min. The measured OD values were converted to catalase activity (U/mL/DW).

POD activity The peroxidase activity was measured following the Hussein et al. (2019). One g of fresh leaves was suspended in a mixture of PB (pH 7), EDTA, and PVPP (10 ml), then blended for 10 min under cold conditions; the enzymatic suspension was centrifuged (8500×*g*, 15 min, 4 °C) 0.1 ml of enzymatic supernatant was added to a solution of 3% guaiacol in PB (2.5 ml) and incubated for 30 min. Subsequently, 0.5 ml of H_2O_2 was added, and the absorbance was read at 470 nm. Peroxidase content (U/mL/DW) was estimated.

SOD activity The activity of SOD was measured according to He et al. (2020) at 560 nm. The initial reaction mixture was 0.1 ml of the enzymatic supernatant mixed with 1 ml of PB (pH 5), 0.3 mL of methionine (22 µM), and 0.1 m of NBT (20 µM), then diluted with 1 ml of distilled water. This reaction mixture was left in daylight for 15 min to initiate the photochemical reduction of nitroblue tetrazolium (NBT) to superoxide radicals.

Following the UV exposure, 100 µl of 0.6 M riboflavin (a substrate for SOD) was added to the reaction mixture. One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required to inhibit the reduction of nitroblue tetrazolium (NBT) by 50%. The amount of reduced NBT to determine SOD activity was estimated at 560 nm.

Statistical evaluation

A one-way analysis of variance (ANOVA) was employed to assess the variability among the triplicate data means. The post-hoc test, LSD, was employed to compare the data means. The statistical analysis was conducted using SPSS 23.0 software at $p < 0.05$.

Results

Isolation and screening of *Streptomyces*

Fifteen *Streptomyces* species (S1, S2, S3, S4,… S15) were isolated from cultivated rhizosphere soil with potato plants and collected from different locations. Four *Streptomyces* isolates (S_1, S_3, S_{12} & S_{14}) were selected out of 15 *Streptomyces* species, which showed distinct pectinase activity 2.15, 1.85, 2.15 and 2.45 U/g growth, respectively.

Identification of isolated *S. bungoensis*

The distinct four selected *Streptomyces* isolates were identified according to cultural growth, morphological (substrate mycelia, aerial mycelia color and color reserve in media), melanin pigment, and antibiosis characteristics based on Bergey's Manual (1989). The four isolates (S_1, S_3, S_{12} and S_{14}), namely St1, St2, St3, and St4, can be identified as *Streptomyces bungoensis*. On the nutrient agar medium, the four selected *St. bungoensis* colonies, St_1, St_2, St_3, and St_4, exhibited growth rate variation, and the color colonies showed variable diffusible pigment in the medium.

The culture growth of the experimental isolates was differentiated into four colors according to the sporulated aerial mycelium; the colors were green, yellowish, pale brownish

and dark greyish series. The four isolates exhibited varying growth rates on various media (Table 1): glucose asparagine agar, glycerol nitrate agar, sucrose nitrate agar and nutrient agar media. The four isolates showed diffused pigment in each experimental media.

The light microscope and TEM images for the isolates supported the biochemical tests, demonstrating varied morphological growth, distinct conidiophores, and no sporangium production (Table 2; Fig. 1). The spore chain varied among spiral short, long, open long, open short, and long, which were distinguishable in Fig. 2. The spore mass also varied among isolates (yellow-brownish, yellowish-white, pale brownish & dark greyish). Additionally, their surfaces were spiky, smooth and hairy. The morphology of conidia spores varied (barrel and oval shape) (Table 2).

Antimicrobial activity

Table 3 shows that St1 and St4 isolates showed considerable antifungal and antibacterial activities against the different

Table 1 Cultural characteristics of *Streptomyces bungoensis* isolates

Media	Cultural	*Streptomyces bungoensis* isolates			
		St1	St2	St3	St4
Starch nitrate agar	Growth	++++	+++	++++	++++
	Aerial mycelium color	Dark black grayish	Yellowish white	Dark brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Brownish-yellow	Dark greenish yellow	Dark greenish
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Glycerol nitrate agar	Growth	+	++	++++	+++
	Aerial mycelium color	Dark grayish	Yellowish white	Dark brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale yellowish	Dark greenish yellow	Dark green
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Inorganic starch agar	Growth	+++	++++	++++	++++
	Aerial mycelium color	Dark grayish	Pale brownish	Brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale greenish	Dark brownish yellow	Greenish
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Nutrient agar	Growth	+	++	++	V
	Aerial mycelium color	Dark grayish	Pale yellowish	Yellowish grayish	White
	Substrate mycelium color	Pale greenish	Pale yellowish	Pale yellowish	Pale yellowish
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Glucose asparagine agar	Growth	+	++	+++	++
	Aerial mycelium color	Dark grayish	Brownish grayish	Dark brownish grayish	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale yellowish	Yellowish brown	Dark green
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Yeast malt agar	Growth	++	++	++	+++
	Aerial mycelium color	Greenish grayish	Pale brownish grayish	Pale brownish yellow	Dark greenish white
	Substrate mycelium color	Yellowish green	Pale yellowish	Olive greenish yellow	Dark green
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Glucose nitrate agar	Growth	++	++	+++	++++
	Aerial mycelium color	Dark olive grayish gray	Pale brownish grayish	Pale brownish yellow	Dark greenish
	Substrate mycelium color	Pale greenish	Pale Yellowish grayish	Dark yellowish	Dark green black
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Glycerol asparagine agar	Growth	++	+++	+++	+++
	Aerial mycelium color	Dark olive greenish gray	Pale brownish gray	Pale brownish yellow	Dark greenish
	Substrate mycelium color	Pale greenish	Pale Yellowish grayish	Pale yellowish	Dark green
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Sucrose nitrate agar	Growth	+	+++	++++	++++
	Aerial mycelium color	Yellowish pale greenish	Pale brownish	Pale brownish yellow	Dark green
	Substrate mycelium color	Pale yellowish	Pale yellowish green	Dark greenish yellow	Greenish
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve
Oatmeal	Growth	+++	++	++	++++
	Aerial mycelium color	Dark green olive	Pale brownish grayish	Pale greenish grayish	Pale greenish
	Substrate mycelium color	Pale greenish	Pale yellowish green	Pale yellowish	Dark green
	Diffusible pigment	¯ve	¯ve	¯ve	¯ve

Growth: V: Variable growth, +: Weak strong, ++: Moderate growth, +++: Strong growth, ++++: Very strong growth

Table 2 Morphological sporangium characteristics of *Streptomyces* isolates

Characteristics	Streptomyces isolates			
	St1	St2	St3	St4
Conidiophores	6 µm	4.5 µm	4 µm	3 µm
Spore mass	Dark grayish	Yellowish white	Yellow brownish	Yellow brownish
Spore surface	Hairy	Smooth	Smooth	Spiny
Spore chain	Spiral short	Spiral long	Spiral open long	Spiral long
Spore shape	Oval	Barrel	Barrel	Oval
* Dimension	12×17 mm	7×20 mm	8×10 mm	10×13 mm

Magnification 10,000×

* Width x length

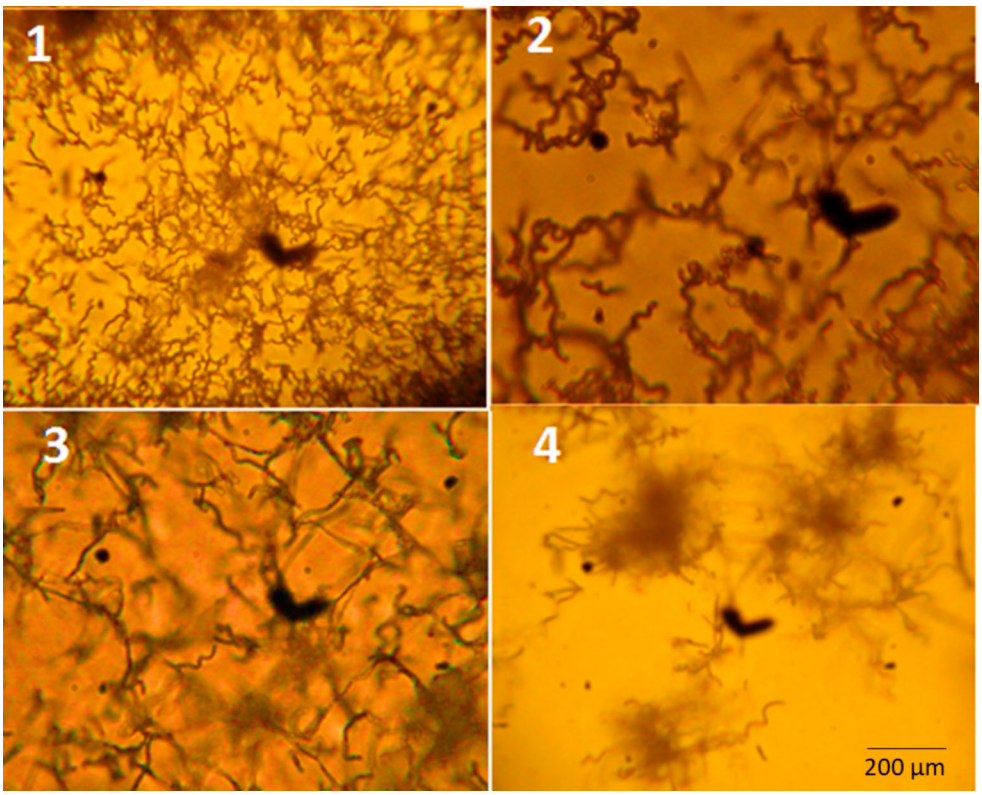

Fig. 1 Spore chains of St4 isolates on a light micrograph of (×200)

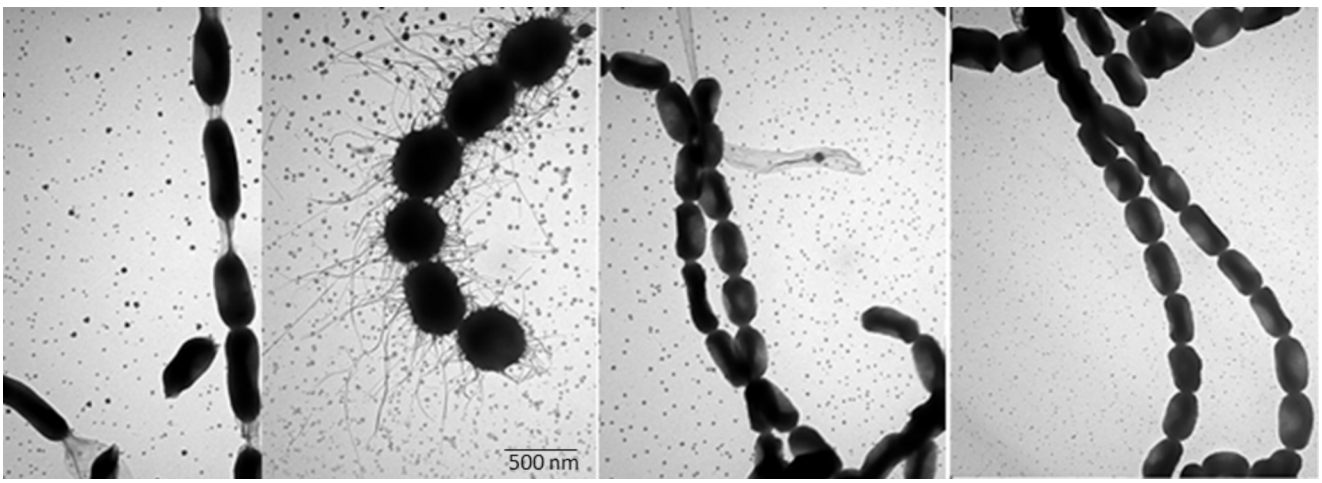

Fig. 2 Spore surface of St4 isolates on electron micrograph of (×15.000)

Table 3 Antimicrobial activity of *S. bungoensis* isolates

Tested organism	St_1		St_2		St_3		St_4	
	Relative inhibition	IZDs (mm)	Relative inhibition	IZDs (mm)	Relative inhibition	IZDs (mm)	Relative inhibition	IZDs (mm)
Fusarium clumorum	58.6 ± 0.9a	40 ± 0.3a	41.4 ± 0.8a	28 ± 0.5ab	51.4 ± 0.2a	35 ± 0.3a	71.4 ± 0.9a	50 ± 0.6a
Aspergillus solani	42.8 ± 0.7b	30 ± 0.5b	42.8 ± 0.2a	30 ± 0.8a	42.8 ± 0.4b	30 ± 0.8b	57.1 ± 0.3b	40 ± 0.2b
Staphylococcus aureus	21.4 ± 0.5c	15 ± 0.8c	14.2 ± 0.5b	20 ± 0.2b	14.2 ± 0.6c	10 ± 0.2c	28.6 ± 0.7c	20 ± 0.3c
Escherichia coli	18.6 ± 0.2d	13 ± 0.2d	-ve	-	-ve	-	18.6 ± 0.6d	13 ± 0.8d

Data are presented mean ± SD; lowercase letters in each column indicate a significant difference ($p < 0.05$)

Table 4 Protein content and pectinase activity in four *Streptomyces* isolates

Streptomyces isolates	Protein content (mg/g)	Total activity (µ)	Specific activity (µ/mg)
St_1	505 ± 2.1b	2250 ± 9.5b	4.45 ± 0.2b
St_2	470 ± 3.6c	2075 ± 11.3c	4.41 ± 0.5b
St_3	450 ± 2.8d	1970 ± 12.5d	4.38 ± 0.6c
St_4	515 ± 3.3a	2520 ± 10.3a	4.89 ± 0.7a

Data are presented mean ± SD; lowercase letters in each column indicate a significant difference ($p < 0.05$)

pathogens: *Fusarium culmorum*, *Aspergillus niger*, *Staphylococcus aureus*, and *Escherichia coli.* St4 showed the highest inhibition zones against the tested microorganisms, especially fungi, where *Fusarium clumorum* and *Aspergillus niger* were more susceptible to St4, i.e., 50 and 40 mm with relative inhibition of 71.4 and 57.1%; meanwhile, *Staphylococcus aureus*, and *Escherichia coli* showed lower inhibition zones (20 and 13 mm). St1 recorded second place in the inhibition zones and relative inhibition percentage. No inhibition zones were observed against *E. coli* in St2 and St3 isolates.

Total protein determination

The protein content differed among the four isolates, where it was 505, 470, 450, and 515 mg/g for St1, St2, St3, and St4 isolates, respectively. Pectinase activity varied among four isolates. It was 2250, 2075, 1970, and 2520µ for St1, St2, St3, and St4 isolates respectively (Table 4). The St4 showed the highest pectinase activity and protein content.

SDS PAGE analysis

The electropherogram of four *S. bungoensis* isolates revealed 41 protein bands, 8 (19.5%), 11 (62%), 7 (17%), and 8 (19.5%) with different molecular weights ranging from 100 to 20 KDa for St1, St2, St3, and St4, respectively (Table 5), The molecular weight, density and number of bands differ among the four isolates where some protein bands present or/and absent in some isolates (Table 5; Fig. 3). The polymorphism analysis among four isolates revealed six monomorphic bands (typical protein bands in all isolates) represented 42.9%, 6 Polymorphic bands (specific protein bands among isolates) represented 35.7), and three unique

Table 5 Protein bands of *Streptomyces bungoensis* isolates detected using SDS PAGE

MW protein marker (KDa)	St_1	St_2	St_3	St_4	Polymorphism
100	++	+++	++	+++	Monomorphic
90	++	+++	++	+++	Monomorphic
87	–	+++	+	+++	Polymorphic
85	+	+	+	+	Monomorphic
80	–	++	–	–	Unique
78	++	+	++	+	Monomorphic
75	+	–	–	+	Polymorphic
72	++	++	–	-	Polymorphic
63	–	+	–	+	Polymorphic
56	+	+	++	-	Polymorphic
50	+	–	–	–	Unique
40	–	+	–	–	Unique
35	+	+	+	+	Monomorphic
25	+	+	+	+	Monomorphic
% Protein bands	8	11	7	8	34
	*(19.5) %	*(62) %	*(17) %	*(19.5) %	

*% = percent of protein band. Polymorphism: Monomorphic = common protein band. Polymorphic = specific protein band. Unique = protein marker. Protein band density: - = absent, + = week, ++= moderate +++= high

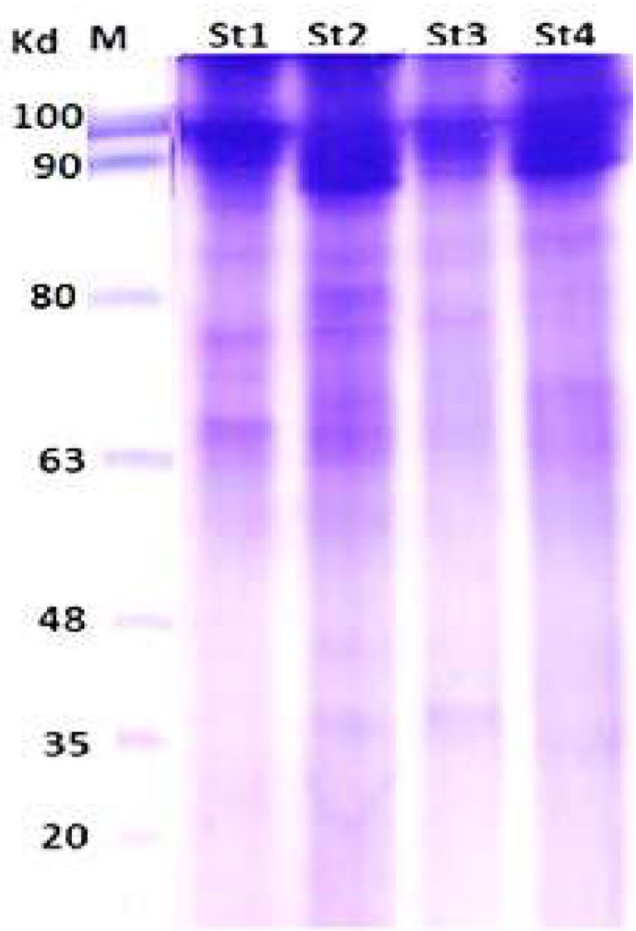

Fig. 3 SDS-PAGE 12% electrophoresis stained showing the protein bands for four *S. bungoensis* isolates, St_1, St_2, St_3, and St_4 using leader 100 KDa marker

protein bands (marker protein) accounted for 21.43%. In the SDS PAGE, St1 showed unique bands at 50 kDa, St2 isolate showed unique bands at 80 and 40 KDa, while the other molecular weights showed polymorphic bands among four *S. bungoensis* isolates.

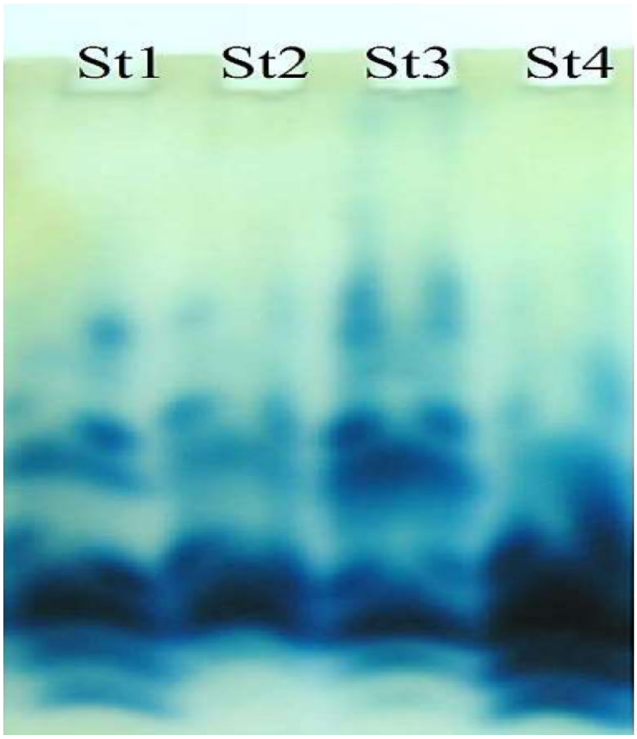

Fig. 4 DISC-PAGE (10%) illustrating pectinase isozymes of four *S. bungoensis* St1, St2, St3, and St4

The examination of pectinase isozymes in *S. bungoensis* is conducted by assessing the degree of activity, relative mobility, and band number.

Table 6; Fig. 4 show that the DISC-PAGE results revealed seven bands with varying densities. Among the isolates, two monomorphic bands recorded 28.5% of the samples, while the remaining isolates exhibited five polymorphic bands, accounting for 71.4% of the samples. The percentages of isozymes' bands for each isolate were as follows: 5 bands (71.4%) for St1, five bands (71.4%) for St2, four bands (57.1%) for St3, and five bands (71.4%) for St4. Furthermore, there were variations in the activity and specific

Table 6 DISC-PAGE banding patterns of pectinase isozymes of four *S. bungoensis* isolates

Rf	*S. bungoensis* isolates								Polymorphism
	St_1		St_2		St_3		St_4		
	Density	%	Density	%	Density	%	Density	%	
1.7	+	8	-	-	+	15	-	-	Polymorphic
1.9	-	-	+	15	-	-	+++	35	Polymorphic
2.5	+	13	-	-	++	40	+++	25	Polymorphic
3.0	+	25	++	25	++	25	++	12	Monomorphic
3.4	++	33	+++	55	++	20	+	15	Monomorphic
3.7	-	-	++	10	-	-	+	13	Polymorphic
4.0	+++	21	+	5	-	-	-	-	Polymorphic
Total	5 (71.4%)		5 (71.4%)		4(57.1%)		5 (71.4%)		19

Density of pectinase isozymes bands: ++++ Very strong band, +++ Strong band, ++ Moderate band, + Weak band, & - Absent band, Polymorphism: Unique = Genetic marker, Polymorphic = Specific polypeptide, Monomorphic = Common polypeptide, *Rf* Relative Front

activity of the enzymes among the four isolates, as seen in Table 2; Fig. 2.

DNA fingerprint

The DNA yield of four *S. bungoensis* isolates, for St1, St2, St3, and St4, were estimated to be 7.5, 8.2, 8.5, and 9.2 µg/g fungal tissues, respectively. The DNA purity was assessed using the 260/280 ratio, yielding values of 1.6, 1.7, 1.5, and 1.8, respectively. It was discovered that DNA quality was an excellent template for sharp and clear PCR amplification products. RAPD-PCR was used for polymorphism detection among four *S. bungoensis* isolates using 3 random primers.

The optimal amplification outcomes were determined by calculating the mean quantity of fragments per primer. The operon random primers were assessed out of 15 primers to determine their ability to generate adequate amplification products by RAPD analysis.

The three random primers were more productive and stable and provided adequate polymorphism among the four *S. bungoensis* isolates. Therefore, the primers in Tables 3, 4 and 5; Fig. 3 were chosen. The DNA extracted from four isolates of *S. bungoensis* was subjected to RAPD-PCR analysis, resulting in the identification of 44 replicated fragments (17, 14, and 13) with diverse molecular weights ranging from 1327 to 175 bp for OBA1, OBA2, and OBA3 primers, respectively (Tables 7 and 8). The DNA fragments amplified from 4 isolates varied in molecular weight, number & density.

The variation study among four isolates revealed specific DNA amplified fragments' absence or/and presence in some isolates (Table 7; Fig. 5). The polymorphism study of four isolates showed seven unique fragments, comprising 41.28%, three polymorphic amplified fragments (specific fragments) accounting for 17.64%, and seven monomorphic amplified fragments (common fragments) constituting 41.28%. St1 and St3 with (OBA1) had fragments at 1327, 575 bp, St1 and St2 with (OBA2) had fragments at 1018, 950, 200 bp, and St4 with (OBA3) had fragments at 950,350 bp (Table 8).

In vivo experiment

Disease parameters

The results of Table 9 are consistent with Table 3 in vitro antifungal results. The *Streptomyces*-treated wheat showed completely healthy plants as control with superior morphological parameters; however, the *Fusarium clumorum*-infected wheat treated with the St4 (100 µg/mL) demonstrated a survival rate of 85% of Giza 171 compared to the control group because of the substantial reduction of pre-root rot disease's emergence by 87% ($p = 0.0015$); in addition preventing the post-emergence of disease, and considerably ($p = 0.00106$) decreased the incidence and severity of root rot disease by 80 and 92%, respectively; meanwhile, 82% of CRD were reduced.

Table 7 DNA fragments amplified from DNA extracts of four *S. bungoensis* isolates by OBA1, OBA2, and OBA3 using RAPD-PCR

Isolates Ladder bp	St_1	St_2	St_3	St_4	Polymorphism
OBA1					
1327	++	-	-	-	Unique
1018	++++	++++	-	++++	Polymorphic
625	++++	++++	+++	++++	Monomorphic
575	-	-	+++	-	Unique
353	++	+	++	++	Monomorphic
200	++	++	++	++	Monomorphic
OBA2					
1018	-	-	-	+	Unique
950	-	-	-	+++	Unique
517	++	+++	+	++++	Monomorphic
425	++	++	++	-	Polymorphic
200	-	++	-	-	Unique
175	++	++	+	++	Monomorphic
OBA3					
950	-	-	-	+++	Unique
625	++	++++	++	++	Monomorphic
517	++++	+++	++++	++++	Monomorphic
425	++	++	++	++	Polymorphic
350	-	-	-	+	Unique

Unique = Genetic marker, Polymorphic (PAF) = Specific amplified fragment, Monomorphic (MAF) = Common amplified fragment, Fragment density = ++++ very strong, +++ strong, ++ Moderate, + weak & - absent fragment

Table 8 Polymorphism and genetic markers among four *S. bungoensis* isolates by DNA fingerprints

Isolates	Polymorphism				Genetic markers of isolates				
Primers	TAF	MAF	PAF	Unique	Fragment (bp)	St_1	St_2	St_3	St_4
OBA_7	6	3	1	2	1327	+	-	-	-
					575	-	-	+	-
OBA_2	6	2	1	3	1018	+	-	-	-
					950	+	-	-	-
					200	-	+	-	-
OBA_3	4	2	1	2	950	-	-	-	+
					350	-	-	-	+
Total Fragment	17	7	3	7		3	1	1	2

Polymorphic (PAF) = Specific amplified fragment, Monomorphic (MAF) = Common amplified fragment

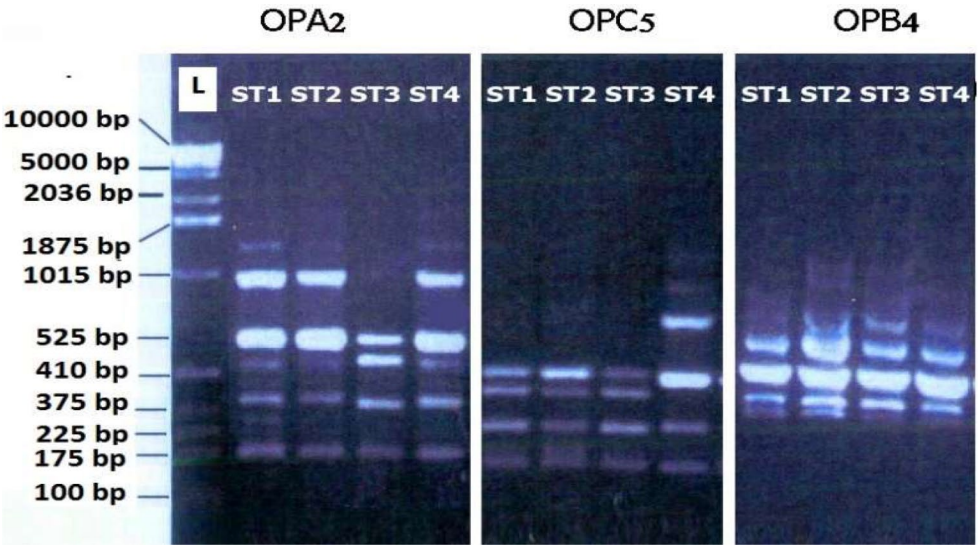

Fig. 5 Agarose gel 1% showing RAPD-PCR products amplified from DNA extracts of four *S. bungoensis* isolates St1, St2, St3, and St4. L: high DNA ladder

Table 9 The in vivo antifungal effect of St4 (water application) at three concentrations on % crown and root disease incidence and severity in Giza 171 wheat

Treatment groups	Conc (mg/l)	Disease parameters (%)					
		Pre-treatment	Post	Healthy	CRD	Incidence	Severity
Control	–	0	0	100 ± 0.0	0	0	0
Fusarium culmorum	–	26.9 ± 0.2a	35 ± 0.5a	0	45 ± 0.2a	93 ± 0.2a	85 ± 0.9a
Streptomyces	25	0	0	100 ± 0.0a	0	0	0
	50	0	0	100 ± 0.0a	0	0	0
	100	0	0	100 ± 0.0a	0	0	0
Streptomyces + *F. culmorum*	25	17.3 ± 0.9b	10.0 ± 0.9b	45 ± 0.5d	25 ± 0.8b	52 ± 0.1b	31 ± 0.2b
	50	10.4 ± 0.8c	4.3 ± 0.7c	71 ± 0.9c	18 ± 0.9c	30 ± 0.5c	12 ± 0.4c
	100	3.0 ± 0.6d	0	85 ± 0.4b	9.4 ± 0.4d	18 ± 0.6d	8.5 ± 0.6d
p value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$n = 30$, data are presented as values mean ± SE, *p* value lower than 0.05 indicate significant differences, *CRD* crown and root disease

Morphological parameters

Table 10 illustrates a significant enhancement in overall plant growth parameters due to the reduction in disease incidence and severity caused by St4 (100 µg/ml). The application of *Streptomyces* 4 at 100 µg/ml resulted in a significant increase in the dry weights of the roots and shoots in healthy plants, reaching 2.8 and 9.2 g, respectively, compared to 2.4 and 8.3 g in the control plants.

However, in FC-infected plants, water application of St4 did not affect the root weight but reduced the shoot weight by 40%. No sense ($p = 0.89$) was monitored in the number of tillers after treatment with St4. However, the grain yield increased by 15% in healthy wheat plants and

Table 10 The in vivo effect of St4 (water application) at three concentrations on morphological traits of Giza 171 wheat

Treatment groups	Conc (mg/L)	Morphological properties					
		Root dry weight (g)	Shoot dry weight (g)	No tiller	Spike length (cm)	No grains	1000 grains weight (g)
Control	–	2.6 ± 0.0ab	8.6 ± 0.2b	3 ± 0.1a	10.56 ± 0.6b	48 ± 0.5bc	44 ± 1.0c
Fusarium culmorum	–	0.8 ± 0.02c	3.2 ± 0.3d	2 ± 0.0b	9.7 ± 0.2c	43 ± 0.9c	36 ± 0.2d
Streptomyces	25	2.5 ± 0.1a	8.5 ± 0.4ab	3 ± 0.0a	11.3 ± 0.3a	52 ± 0.8b	48 ± 0.5b
	50	2.6 ± 0.2a	8.9 ± 0.1ab	3 ± 0.0a	11.5 ± 0.1a	53 ± 0.7ab	48.6 ± 0.6b
	100	2.7 ± 0.3a	9.2 ± 0.9a	3 ± 0.2a	11.8 ± 0.2a	55 ± 0.6a	51.0 ± 0.9a
Streptomyces + *F. culmorum*	25	2.4 ± 0.2b	4.7 ± 0.5d	2 ± 0.0b	10.4 ± 0.5b	49 ± 0.4bc	44 ± 0.2c
	50	2.51 ± 0.4b	5.5 ± 0.1c	3 ± 0.0a	10.8 ± 0.4b	51 ± 0.5b	47.1 ± 0.4b
	100	2.59 ± 0.2ab	6.8 ± 0.2c	3 ± 0.2a	11.2 ± 0.9ab	53 ± 0.4ab	48.6 ± 0.8b
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$n = 30$, data are presented as values mean ± SE, p value lower than 0.05 indicate significant differences

Table 11 The in vivo effect of St4 (water application) at three concentrations on antioxidant enzymes and pigment content of Giza 171 wheat

Treatment groups	Conc (mg/L)	Pigment content		Antioxidant enzymes (U/g)		
		Total chlorophyll	Total carotenoids	Catalase	Peroxidase	SOD
Control	–	2.2 ± 0.1b	0.75 ± 0.01b	14.5 ± 0.6c	1.5 ± 0.2c	2.7 ± 0.3c
Fusarium culmorum	–	1.3 ± 0.1c	0.55 ± 0.02d	22 ± 0.2a	2.2 ± 0.1a	3.1 ± 0.2a
Streptomyces	25	2.4 ± 0.2ab	0.81 ± 0.03b	17 ± 0.9b	1.8 ± 0.3b	2.8 ± 0.5b
	50	2.45 ± 0.5a	0.84 ± 0.04ab	15 ± 0.8c	1.4 ± 0.5c	2.5 ± 0.2c
	100	2.49 ± 0.1a	0.89 ± 0.04a	13.5 ± 0.6	1.31 ± 0.1d	2.3 ± 0.3d
Streptomyces + *F. culmorum*	25	1.4 ± 0.2c	0.62 ± 0.00 cd	18.3 ± 0.5ab	1.8 ± 0.3b	3.1 ± 0.1ab
	50	1.8 ± 0.3c	0.66 ± 0.01c	16.8 ± 0.7c	1.55 ± 0.1c	2.6 ± 0.2b
	100	2.2 ± 0.5b	0.69 ± 0.02c	15.6 ± 0.8c	1.41 ± 0.2c	2.31 ± 0.8d
p-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

$n = 30$, data are presented as values mean ± SE, p value lower than 0.05 indicate significant differences, *SOD* Superoxide dismutase

remained unchanged in FC-infected plants. The weight of 1000 grains increased by 53% compared to the control group.

Pigments content and antioxidant enzymes

The total carotenoids and chlorophyll content was higher in the St4-treated plants, as indicated in Table 11. Applying St4 to wheat increased the carotenoids and chlorophyll levels by 20% compared to the control group in healthy plants. However, there were no changes in pigments' content in FC-infected plants.

The antioxidant enzymes' activity reflects the oxidative state of living organisms. In this study, the FC infection led to a substantial increase of 45% and 31% in the activity of the antioxidant enzymes CAT, Pyx, and SOD, respectively. However, in healthy plants, these enzymes' activity remained under control. Applying St4 at 100 µg/mL to wheat significantly reduced antioxidant enzyme levels in the control group ($p = 0.0198$–0.0269).

Discussion

In this work, 15 *Streptomyces* isolates were isolated from potato rhizosphere, and four isolates were selected based on pectinase activity and antifungal activity. The four isolates were identified based on their culture growth, morphological characteristics, and color patterns. The isolates can be classified as *S. bungoensis* based on the presence or absence of sporangium, sporangiophore length, spore surface texture, shape, mass, chain arrangement, and color. These findings align with those of Barghouthi et al. (2017) and Castañeda-Cisneros et al. (2020).

The morphological variation observed among the *S. bungoensis* isolates is attributed to the distinct physicochemical properties of the soils and the varying climatic conditions of their respective locations. This study focuses on the genetic diversity of *S. bungoensis* isolates selected based on their diverse morphological characteristics and pathogenicity (Bull et al. 1992; Hamid et al. 2020; Montaño et al. 2022).

Several molecular biology techniques have been utilized to study the genetic diversity of *Streptomyces bungoensis* (Giovannoni et al. 1990; Abbas and Awwad 2007; Conn and Franco 2004). Electrophoretic protein banding patterns can

be utilized to determine its biochemical and genetic markers (Abu-Almaaty et al. 2020). It can also provide information about structural genes and their regulatory mechanisms that influence the biosynthetic pathways of the protein banding pattern (Abdelsalam et al. 1999). Gel electrophoresis is a common technique for genetic diversity analysis. The electrophoretic differences observed allelic variations in the *S. bungoensis* enzymes, which mutational events in *Streptomycetes* may cause in response to stress and changes in the loci that regulate isozyme production. (Ismail et al. 2020).

The homogenate of the four *S. bungoensis* isolates was analyzed electrophoretically for pectinase isozymes to evaluate enzyme activity. Pectinase isozymes are enzymes characterized by their shared ability to act on the pectin substrate (Shrestha et al. 2023). The group of pectinase isozymes is extensively studied in various microbes and species; thus, every band of pectinase activity indicates polypeptide chain composition. Consequently, every band on the gel corresponds to the result of a particular locus (allele). The pectinase zymograms exposed that the bands migrated randomly and were found in specific *S. bungoensis* isolates (Shrestha et al. 2023) but absent in others. The four *S. bungoensis* isolates included two bands essential for the enzyme composition. In *S. bungoensis* isolates, St_1 and St_3 bands with Rf 1.7 were seen; in St2 and St4, Rf 1.9 and 3.7 bands were observed, and in St1 and St2, Rf 3.0 band was observed, which may be the result of a gene produced in response to stress. The findings suggest that allelic mutations are present in all *S. bungoensis* isolates at this locus, indicating that this enzyme is inherited in a polymorphic manner. The observed changes in protein enzymatic patterns in this study can be attributed to genetic mutations, which align with the results of Abdelsalam et al. (1999) and Yin et al.(2021).

Furthermore, Kormanec et al. (2019) and Szafran et al. (2020) connected such alterations to chromosomal abnormalities creation, such as micronuclei, laggards and bridge breaks, which can result in genetic information loss. A mutational event in the transcriptionally repressed regulatory genes could account for the disappearance of certain bands (Wang et al. 2023). Meanwhile, new bands could be formed by a mutational event in the regulatory system of the gene(s) that activate them but are not yet expressed (Zhang et al. 2022).

Several key factors, including the number of coding genes, the homozygous or heterozygous alleles of those genes, and the size of the protein products, influence the presence of bands on the gel. In the most basic scenario, distinct electromorphs (allozymes) are identified in microorganisms inhabiting a specific salinity zone (Bhoyar et al. 2024). Since allozymes are generally inherited predominantly, the presence and quantity of the bands are determined by the number of polypeptide subunits in the active enzyme (Vigolo et al. 2022).

The RAPD-PCR findings revealed that the primers in the study generated RAPD profiles; genetic markers with MW 1323, 250; 1018, 950 were identified in (St1); 200 (St2); 975 (St3) and 50 bp (St4), however, they were absent in the other isolates. Fortunately, the average degree of similarity among the four isolates was 26, 24, 22, and 26% for St1, St2, St3, and St4, respectively. The genetic origins of *S. bungoensis* isolates are expected to be identical. The combination of RAPD-PCR has increased the potential for polymorphism analysis by allowing tiny arbitrary nucleotide segments without prior knowledge of genes and/or genomic sequences (Bousba et al. 2020). In addition, the RAPD-PCR test has obvious proactive advantages for DNA variation detection. It is technically more superficial, less expensive, and faster than other molecular approaches (Keerthana et al. 2023). This study revealed that the RAPD-PCR approach was used to distinguish *S. bungoensis* isolates.

The examined isolates were chosen based on their distinct habitat and pathogenicity via their susceptibility characteristics and well-characterized resistance upon *S. bungoensis* exposure. One of the ten randomly examined primers yielded reproducible and inheritable stable polymorphic markers for *S. bungoensis*. Several primers generated monomorphic bands among the isolates, or the detected polymorphism could not be reproduced. In a recent study, the genetic diversity of *S. bungoensis isolates* was measured by the length of RAPD-PCR fragments allozyme (Akl and El-Masry 2024).

Crown and root rot diseases substantially impact wheat yield, particularly under conditions of high pathogen severity. Various fungal organisms often cause diseases, reducing the wheat yield and grain quality (Moya-Elizondo et al. 2015). Rot disease causes vascular constriction, which hinders water absorption and transport, thereby promoting the formation of whitehead breakouts during the filling stage. It is common for various fungal species to interact synergistically or competitively, affecting their ability to spread and cause diseases (Elsayed et al. 2023; Zhang et al. 2024).

Cereal-growing areas face significant economic challenges due to fungal infections affecting wheat. In the Pacific Northwest region, it has been shown that *Fusarium* crown rot can affect 76% of winter wheat plants, leading to significant reductions in crop yield. Approximately 18% of fields are heavily polluted (Gautier et al. 2020). *Fusarium* infections in Australia have been documented to result in yield reductions ranging from 25 to 58%, leading to a financial loss of 80 million USD (Schreuder 2021).

Rhizosphere microorganisms are of considerable importance in synthesizing various antimicrobial compounds, such as volatile organic components and metabolites, which assist in preventing diseases and promoting plant growth (El-Tarabily et al. 2009; Saeed et al. 2017; Kamil et al.

2018; Desoky et al. 2020a; El-Saadony et al. 2022; Ashry et al. 2022). These microorganism-produced inorganic compounds, such as NH_3, CO_2, and HCN, can alter the structures of aldehydes, terpenes, ketones, sulfur-containing N compounds, and ketones (Abd El-Mageed et al. 2022a, b; AbuQamar et al. 2024). Volatile organic compounds (VOCs) possess distinct physical and chemical properties, such as low molecular weight, high vapor pressure, hydrophilic components, low boiling point, and gaseous phase mobility. These characteristics allow VOCs to diffuse long distances through air and soil (Gong et al. 2022; Santos-Beneit et al. 2022).

For numerous years, organic fertilizers and bioagents have been derived from bacteria and fungi (Qiu et al. 2019). Focusing on in vitro inhibition neglects the various ecological factors that can influence the growth and survival of bio-agents in their natural habitats (Kaminsky et al. 2019; Barbuto Ferraiuolo et al. 2021). When researching alternative methods for managing plant diseases, particular pathogen-host pairings are frequently emphasized, raising concerns regarding the efficacy and generalizability of these bio-agents across diverse crop varieties and plant pathogens (LeBlanc 2022).

The production of various antibiotics is the primary means of biocontrol (El-Tarabily and Sivasithamparam 2006). Antifungal and antibacterial enzymes, including proteases, lipases, and chitinases, are present in several antimicrobial compounds. The microbial enzymes can break down fungal cells and prevent plant infections. Proteases are the most common type of antifungal enzyme (El-Tarabily 2003; Vurukonda et al. 2018).

According to the pathogens responsible for their occurrence, plant diseases are categorized as infectious or non-infectious. Non-infectious plant diseases remain localized to individual plants, while infested plant diseases can spread from an infected plant to a healthy one (Nazarov et al. 2020). Abiotic and biotic factors can both influence the development of plant diseases. Symptoms such as decay, hyperplasia (overgrowth), spotting (necrosis), bumps and wilting, mildew, distortion, discoloration, mummification, and tissue destruction distinguish plant diseases. These diseases are transmitted by various organisms, including fungi, bacteria, and viruses (Nazarov et al. 2020).

Actinomycetes are a class of microorganisms found in various ecological habitats, including sedimentary environments, air, soil, and plant detritus (Bhatti et al. 2017). Within the Actinomycetes order, the capacity of the *Streptomyces* genus to generate a wide variety of secondary compounds is a well-established trait. Antibacterial properties have been observed in secondary metabolites, which have been linked to various plant and human diseases (Viaene et al. 2016). Employing antibiotic-producing bacteria as a soil biocontrol agent offers a viable alternative to chemical antimicrobials (Gutierrez et al. 2022). *Streptomyces* is renowned for producing a diverse array of bioactive organic compounds, which have been associated with antimicrobial properties and direct or indirect influences on plant development and growth (Viaene et al. 2016). An organic compound generated by *Streptomyces yanglinensis* strain 3–10 can impede the growth of *A. flavus* and halt the production of toxins in soybeans that have been stored (Gong et al. 2022).

Streptomyces strains generate antagonistic compounds such as siderophores to prevent bacterial infections and demonstrate antibiofilm characteristics. *Streptomyces* is additionally accountable for the synthesis of a wide range of biologically active secondary metabolites, including antibiotics, antioxidants, and anticancer agents, as well as enzymes that are of industrial value (Butt et al. 2023). Microbial inoculants exhibit considerable potential to preserve agricultural sustainability (Desoky et al. 2020b; Ahmed et al. 2022; Abdelkhalik et al. 2023; Ali et al. 2024). The importance of these microorganisms in improving nutrient availability and plant health was emphasized by Qiu et al. (2019).

Bacteria that are naturally present in disease-suppressive soils impede the development of plant pathogens by generating secondary metabolites and competing for resources, such as carbon and iron (LeBlanc 2022). In agricultural biocontrol, new findings of *Streptomyces* species are garnering considerable interest. *Streptomyces* is characterized by its capability to synthesize toxins, VOCs, and antimicrobial compounds, such as antibiotics (Vurukonda et al. 2018). This mechanism enables *Streptomyces* to safeguard plants from pathogens that could cause damage. Interactions with phytopathogens can potentially suppress plants' natural defenses against pathogens. *Streptomyces* can alleviate the severity of the disease by enhancing plant productivity and stimulating elements of the plant's immune system (Olanrewaju and Babalola 2019).

Furthermore, its function as a nutrient enhancer and its impact on various factors contribute to its effect on soil fertility (Vurukonda et al. 2018). Despite their well-documented capacity to alleviate diseases caused by fungal plant pathogens and their exceptional species diversity, the commercial application of *Streptomyces* strains as bio-agents has been restricted (LeBlanc 2022). Among commercial biopesticides, Actinovate and Mycostop, which utilize *Streptomyces lydicus* WYEC108 and *Streptomyces griseoviridis* K61 respectively, are the only products granted regulatory approval for use worldwide. The challenges of commercializing these products and the discrepancies between laboratory findings and field observations delay their widespread availability (Vurukonda et al. 2018).

Conclusion

To successfully manage diseases of plants caused by phytopathogenic fungi, it is vital to identify antimicrobial substances from eco-friendly natural sources. Much research has focused on the *Streptomyces* species that is used as a biocontrol agent to fight against fungi that cause damage to plants. Streptomyces species have great promise in contemporary agricultural practices, such as biocontrol agents and biofertilizers, which may explain why they continue to be a marketable economic agent. Based on its capabilities to create IAA and siderophores, show ACC deaminase activity, solubilize mineral phosphate, pectinase activity, and fix nitrogen, *Streptomyces bungoensis* (St4) might be a promising candidate for biofertilizer development, according to this study. St4 stimulated plant defense systems, which in turn relieved wheat crown and root rot disease. During the early phases of infection with *F. clumorum*, St4 directly induced reactive oxygen species production and callose deposition. One possible strategy for inhibiting *F. clumorum* growth is to increase the activity of antioxidant enzymes. The results of this study show that St4 might be a very useful bioagent or biofertilizer in the future.

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Data availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no conflicts of interest.

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