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ORIGINAL ARTICLE

Organic soil-derived *Saccharopolyspora thermophila* for enhancing plant growth and controlling *Fusarium* wilt disease in tomato

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Abstract

This study investigated the effects of *Saccharopolyspora thermophila*, a plant growth-promoting actinomycete (PGPA), on tomato plants (Suvarna 22) and its potential to mitigate Fusarium wilt disease. *Saccharopolyspora thermophila* was isolated from organic farm soil and molecularly identified through 16S rRNA sequencing. Seed treatment with *S. thermophila* significantly enhanced germination rates (83% vs 59% in control) and seedling vigor (625 vs 192 in control). Treated plants exhibited improved growth parameters, including increased plant height (83.3%), chlorophyll content (88.2%), and fruit yield (66.7%). Under pathogen challenge, *S. thermophila*-treated plants showed reduced disease incidence (37% vs 92% in untreated plants). Biochemical analyses revealed that *S. thermophila* treatment enhanced antioxidant enzyme activities (CAT, SOD, APX, POD) and maintained higher chlorophyll content under pathogen stress. The treatment also moderated pathogen-induced increases in lipid peroxidation and proline levels, indicating improved stress tolerance. Interestingly, *S. thermophila* treatment counteracted pathogen-induced starch depletion and moderated sucrose accumulation, suggesting a complex interplay in plant metabolism regulation. These findings demonstrated the potential of *S. thermophila* as a PGPA to enhance tomato plant growth, productivity, and resilience against Fusarium wilt disease. The study provides insights into the physiological and biochemical mechanisms underlying PGPA-mediated plant growth promotion and disease resistance, offering promising avenues for sustainable agricultural practices.

Keywords: anti-oxidant enzymes, chlorophyll, *Fusarium oxysporum,* lipid peroxidation, PGPA, *Saccharopolyspora thermophila*, sucrose, starch

Introduction

In the 21st century, sustainable agriculture faces two major challenges: providing food for an expanding global population and maintaining healthy soil for future crop production (Glick 2012; Silva *et al.* 2022). The global demand for agricultural production is projected to increase by up to 70% by 2050 (Giller *et al.* 2021), necessitating strategies that ensure optimal plant performance and the development of economically important crops (Zhang *et al.* 2024). Chemical inputs such as fertilizers and pesticides have proven crucial for increasing crop yields; however, their overuse poses serious dangers to human and environmental health (Tudi *et al.* 2021; Brunelle *et al.* 2024). The accumulation of these substances can lead to environmental issues such as eutrophication and the introduction of hazardous compounds, compromising long-term sustainability and food safety (Zhan *et al.* 2021; Devlin and Brodie 2023). In response, there is a growing trend

towards more ecological and food-conscious agricultural systems, with producers and consumers seeking sustainable alternatives that reduce waste and minimize environmental impact (Mo *et al.* 2023).

Under dry and semi-arid conditions, the tomato, which belongs to the order Solanales and family Solanaceae (*Solanum lycopersicon* L.), is a highly prized vegetable crop. While important for food production, tomato plants are susceptible to diseases caused by various agents, notably *Fusarium oxysporum* f. sp. *lycopersici*. *Fusarium oxysporum*, which belongs to the order Hypocreales and family Nectriaceae can reduce yields by up to 84% (Šimkovicová *et al.* 2024). The pathogen's virulence is influenced by environmental factors such as rainfall, temperature, humidity, and nutrients (Velásquez *et al.* 2018; Chai *et al.* 2023). The soilborne fungal pathogen *F. oxysporum* causes vascular wilt in tomato plants. The fungus survives for years on crop wastes and infects roots through wounds or root tips. It overwinters in soil as chlamydospores or mycelium. Once inside, it produces mycotoxins like fusaric acid and disrupts water transfer by colonizing the xylem vessels, which makes wilting worse. Lower leaves first show signs of yellowing and one-sided withering, usually in the hottest portion of the day. Vascular tissues turn brown as the illness worsens, which causes defoliation, stunted development, and plant death. Even with sufficient soil moisture, *F. oxysporum* causes permanent collapse because it selectively clogs xylem arteries, in contrast to other wilt diseases (Srinivas *et al.* 2019). Integrated disease management strategies incorporate biological approaches, chemical treatments, and cultural practices (Wharton and Diéguez-Uribeondo 2004; Abhayashree *et al.* 2016; Gowtham *et al.* 2018).

Plant growth-promoting actinomycetes (PGPA) and plant growth-promoting rhizobacteria (PGPR) are microorganisms associated with plant roots that enhance growth and induce resistance against phytopathogens (Backer *et al.* 2018; Fatmawati *et al.* 2020; de Andrade *et al.* 2023; Brunelle *et al.* 2024). These microbes trigger innate immunity in plants, providing a sustainable method of disease management (Brunelle *et al.* 2024). Research on PGPA efficacy in comparison to PGPR in inducing resistance to invasive pathogens is still limited (Jiao *et al.* 2021; Upadhayay *et al.* 2023; Sahoo *et al.* 2024; Zhang *et al.* 2024).

PGPA contribute to soil fertility, which in turn stimulates plant growth and enhances defense responses against pathogen infections (AbdElgawad *et al.* 2020). Their primary mechanism for preventing infection involves root colonization, which also facilitates nutrient absorption (Silva *et al.* 2022). PGPA can solubilize phosphates and produce growth-promoting compounds such as indole acetic acid (IAA), siderophores, cellulases, and chitinases, contributing to improved plant growth (Boukhatem *et al.* 2022; Koul *et al.* 2022).

Due to PGPA beneficial effects on agriculture, researchers are focused on utilizing these microbes to activate induced systemic resistance (ISR) in plants, enhancing growth and disease resistance (Kaari *et al.* 2022; Torres-Rodriguez *et al.* 2022). ISR involves the formation of protective compounds in plant cell walls that hinder pathogen entry (Nawrocka *et al.* 2018). PGPA also promote the accumulation of defense-related enzymes linked to resistance against phytopathogens (Silva *et al.* 2022).

Despite the proven benefits of plant microbiomes, there remains a significant gap between in vitro trials and field efficiency, particularly regarding the commercialization of bio-input products. While the potential of PGPA has been acknowledged, specific studies on their use in tomato plants to enhance growth and resistance to *Fusarium* wilt are limited. The biochemical mechanisms by which actinomycetes, like *Saccharopolyspora thermophila* of order Actinomycetales and family Pseudonocardiaceae, promote growth and induce resistance against *F. oxysporum* in tomato plants are not fully understood. *Saccharopolyspora thermophila* is nonmotile, Gram-positive, aerobic, and non-acid-alcohol-fast, and produces a widely branched, colorless mycelium to buff substrate mycelium that breaks up into rod-shaped parts after 4–5 days at 45°C. ISP medium 2 (yeast malt agar) and modified Sauton's and oatmeal agars yield good growth. Long, hooked to flexuous chains of four to six smooth-surfaced, vesicular spores (0 ± 7, 1 ± 1, 0 ± 85, 1 ± 5 μm) transport an abundant aerial mycelium (Lu *et al.* 2001). The primary objective of this study was to investigate the potential of organic soil-derived *S. thermophila* actinomycete in enhancing tomato plant growth and controlling *Fusarium* wilt. It was hypothesized that *S. thermophila* actinomycete can effectively promote growth and induce resistance against *F. oxysporum* through the production of growth-promoting compounds, activation of resistance, and modulation of plant defense-related enzymes. This study aimed to contribute to the understanding of plant-microbe interactions in sustainable agriculture, provide insights into the interplay between plants, beneficial microorganisms, and pathogens, as well as propose future research and product development.

Materials and Methods

Collection of plant material

Throughout the investigation, Suvarna 22 (S_{22}) tomato seed samples that are prone to Fusarium disease were obtained from the Indian Institute of Horticultural Research (IIHR) in Bengaluru, Karnataka, India.

Soil sampling

The organically rich site was selected adjacent to Sira, Tumakuru District of Karnataka State, India (Latitude: 13.7485522, Longitude: 76.9102812), an entirely organic farm with no cultivar introduction and unaltered soil. With an annual rainfall of 39 inches, 46% of which is humid, two types of soil were collected: one mulched and one unmulched. Both were rich in organic content. The farm has employed natural, organic, and agroforestry practices for the last two decades, resulting in enriched soil with high organic content. A soil sample was collected from the mulched rhizosphere area beneath Areca trees, the predominant flora on the farm, at a depth of 10 cm below the surface. The sample, enclosed in sterile polythene bags, was sieved with a 10 mm sieve to remove debris and was subsequently used throughout the experiment.

Isolation and actinomycete

To isolate actinomycete, 1 g of dry soil was suspended in 10 ml of distilled water and vortexed for 2 min to ensure proper mixing. Serial dilutions (1 : 10) were then prepared in duplicate for further analysis. From each dilution, 1 ml was spread onto actinomycete isolation agar (AIA) (HiMedia, India) containing cycloheximide (50 μg · ml^{-1}) and nystatin (50 μg · ml^{-1}) to suppress fungal growth, as well as chitin agar and starch casein agar (HiMedia, India). The plates were labelled and incubated at 28°C for 7 to 14 days. After 14 days, colony counts were taken for each dilution, and the plates were examined under a microscope to assess colony morphology. Richness, evenness, and diversity indices were calculated based on the variety of observed colonies, using metrics such as the Shannon-Weaver and Simpson's indices. Individual colony types were selected using sterile forceps and streaked onto glucose yeast extract agar (GYEA) (HiMedia, India) plates to obtain pure isolates for further study (Shirling and Gottlieb 1966; Trivedi *et al*. 2012; Malviya *et al.* 2013; Kämpfer *et al.* 2014). Morphological characteristics such as colony characteristics, color of aerial and substrate mycelium and pigment production were studied by inoculating the isolate in various media such as glycerol yeast extract agar, oatmeal agar, nutrient agar and yeast malt agar (HiMedia, India) and incubated at 30°C for 7–15 days (Baskaran *et al*. 2011).

Molecular identification of actinomycete

DNA was extracted from the culture, and its quality was assessed using a 1.0% agarose gel, which displayed a prominent band indicative of high-molecular-weight DNA. The 16S rRNA gene fragment was amplified with the 16SrRNA-F (F243; GGATGAGCCCGCGGCCTA) and 16SrRNA-R (R513GC: gc.-CGGCCGCGGCT-GCTGGCACGTA) primers, yielding a distinct PCR product of 1150 bp upon agarose gel resolution. The PCR product underwent purification to eliminate any contaminants. Following this, forward and reverse sequencing reactions were performed with the same primers using the BDT v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) on an ABI 3730xl Genetic Analyzer. A consensus sequence for the 16S rRNA gene was created from the forward and reverse sequences using alignment software Clustal W. The sequencing was carried out at Barcode BioSciences Company in Bangalore, India, an ISO 9001:2015 certified facility. The 16S rRNA gene sequence was then analyzed using BLAST against the NCBI GenBank database. The sequences with the highest identity scores were selected for alignment with the Clustal W software. A distance matrix and phylogenetic tree were subsequently constructed using MEGA 11 Software (Kimura 1980; Kumar *et al.* 2018).

Preparation of inducer conidial suspension

Pure cultures of selected *S. thermophila* were mass multiplied on potato dextrose agar (PDA) plates for 10 to 15 days. After the incubation period, 10 ml of sterile distilled water (SDW) was added, and the conidia were gently removed using a sterile brush under aseptic conditions. The conidial suspension was then filtered through two layers of sterilized cheesecloth to remove any impurities and centrifuged in a Centrifuge 5400 R (Eppendorf, CA, USA). The resulting pellet was resuspended in SDW, and the concentration of the conidial suspension was adjusted to 1×10^8 conidia per ml using a haemocytometer (HiMedia, India) for accurate measurement, following the procedure outlined by Murali *et al.* (2013).

Seed treatment with *Saccharopolyspora thermophila*

The S_{22} tomato seeds were treated with a *S. thermophila* conidial solution after they had been thoroughly surface sterilized on a 100 × 17 mm (10 cm) Petri plate with 2% sodium hypochlorite, then with 70% ethanol and then with SDW for 2 min. As part of the treatment strategy, each seed was coated separately in a conidial mixture that contained the pellet and supernatant *S. thermophila*. Next, to aid in the inducer's penetration, the treated seedlings were put in an incubator shaker that was adjusted at 1000 rpm and 25°C for three and six hours, respectively. The *S. thermophilla* treated seeds were used in further research experiments after being meticulously air-dried under aseptic conditions following the specified incubation times. The susceptible seeds in the control group were treated

only with SDW, whereas the seeds in the experimental group were treated with *S. thermophilla* (Naziya *et al*. 2020).

Evaluation of *Saccharopolyspora thermophila* seed treatment on seed germination and seedling vigor of tomato

To determine the percentage of germination, the susceptible tomato seeds treated with *S. thermophila* and SDW were equally spread across three layers of wet sterile blotter discs on Petri plates (100 × 17 mm). In order to evaluate the vigor of the seedlings, an additional batch of treated and untreated seeds underwent the between paper method (Abdul-Baki and Anderson 1973). Equations 1 and 2 were used to calculate the percentages of seed germination and seedling vigor after these treatments were incubated for 14 days at 25 ± 2°C and 90% relative humidity:

$$\text{Percent seed germination} = \frac{\text{Total number of seed germination}}{\text{Total number of seeds plated}} \times 100 \quad (1)$$

$$\text{Vigor index} = \frac{\text{Mean root length (cm)} + \text{Mean shoot}}{\text{Length percentage seed germination}} \quad (2)$$

Evaluation of *Saccharopolyspora thermophila* seed treatment on plant growth parameters of tomato

The evaluation was conducted in a controlled greenhouse setting to examine the effects of SDW treatment versus *S. thermophila* treatment on tomato plant development parameters. Plastic pots, 22.86 cm in diameter and 2.5 l capacity, containing autoclaved potting soil of a 3 : 1 mixture of soil and manure, were used. The plants were grown 7 days in a greenhouse with carefully regulated temperatures (maintained at 25 ± 2°C) and 90% relative humidity. The first set of plants was carefully uprooted to measure vegetative growth parameters, including plant height, fresh and dry shoot weight, and total chlorophyll content. Concurrently, the second group of plants was kept to assess reproductive growth characteristics, which included tracking the number of days to flowering, weighing the fresh fruit, and counting the fruits produced. Ninety days after seeding, these reproductive characteristics were evaluated (Naziya *et al*. 2020).

Preparation of pathogen inoculum

The *Fusarium oxysporum* f. sp. *lycopersici* used in this study was collected from a farmer's field in Belaguma, Tumakuru District, Karnataka, India (Latitude: 13.3523089, Longitude: 77.1423955). The sample was obtained from tomato plants exhibiting significant vascular discoloration and symptoms of Fusarium wilt in the field. Following Koch's postulates, the pathogen had been identified in a previous study and reisolated from deliberately infected plants (Aydi Ben Abdallah *et al.* 2016). Samples were taken from diseased stems and surface-sterilized plants to remove contaminants. The tissue was then placed on a selective media like PDA. After fungal growth, single spores or hyphal tips were transferred to fresh media for purification. PCR was used to confirm its identity. The isolate was cultured for 7 days at approximately 25°C on PDA medium (HiMedia, India) (Aydi-Ben-Abdallah *et al*. 2020). The 15-day-old *F. oxysporum* culture, grown on PDA medium, was flooded with 1 ml of SDW, and conidia were aseptically extracted using a sterile brush. A final concentration of 5×10^5 conidia · ml^{-1} was achieved by filtering the resulting conidial suspension through two layers of sterilized muslin fabric, as described by Sharma *et al*. (2010).

Evaluation of PGPA on wilting disease protection of tomato under greenhouse conditions

The PGPA treated and control tomato seeds which had previously been sterilized were sown in plastic pots (22.86 × 22.86 cm in diameter) containing red soil/ manure/ at a 3 : 1 ratio. The pots were watered regularly and maintained under greenhouse conditions (25 ± 2°C). The 14-day-old seedlings were challenge inoculated with a conidial suspension of *F. oxyporum* (4.5×10^5 conidia · ml^{-1}) till runoff. The challenge--inoculated plants were arranged in a randomized complete block design and maintained under greenhouse conditions (25 ± 2°C, 90% relative humidity (RH)). A treatment consisted of 4 pots: 1) control treated with SDW (C), 2) control plants treated with *F. oxyporum* (CP), 3) *S. thermophila* treated (I) and 4) *S. thermophila* and *F. oxyporum* treated (IP). The percent disease index (PDI) was then calculated using a scale, where 0 represented no symptoms and 9 indicated lesions covering more than 51% of the leaves, according to the methods described by Mayee and Datar (1986), equations 3 and 4:

$$\text{Disease incidence [\%]} = \frac{\text{Number of infected plants}}{\text{Total number of plants evaluated}} \times 100 \quad (3)$$

$$\text{PDI} = \frac{\sum \text{Rating number} \times \text{No. of plants with the rating}}{\text{Total No. of plants} \times \text{Highest rating}} \quad (4)$$

Effect of PGPA seed treatment on biochemical changes in tomato

Sampling of seedlings

Susceptible S_{22} tomato seeds treated with SDW and *S. thermophila* were subjected to the sandwich method and incubated at 25 ± 2°C for 14 days. After incubation, the seedlings were carefully removed without damaging the roots. Fusarium wilt resistance in tomato plants was assessed through four distinct treatments which were as follows.

Treatment 1: Control (C) – Seedlings were mock-inoculated with SDW, with samples collected at 0, 3, 6, 12, and 24 hours post-inoculation (hpi).

Treatment 2: Control + *F. oxysporum* Pathogen (CP) – Fourteen-day-old seedlings were inoculated with *F. oxysporum* at a concentration of 10,000 spores · ml^{-1}, with samples collected at 0, 3, 6, 12, and 24 hpi.

Treatment 3: *S. thermophila* Induced (I) – S_{22} seeds were soaked in a cell-free extract of *S. thermophila* for 6 h, employing blot drying under constant shaking before sampling at 0, 3, 6, 12, and 24 hpi.

Treatment 4: *S. thermophila* Induced + *F. oxysporum* Pathogen Inoculated (IP) – Fourteen-day-old seedlings raised from inducer-treated seeds were inoculated with *F. oxysporum* at a concentration of 10,000 spores · ml^{-1}, with samples collected at 0, 3, 6, 12, and 24 hpi.

All seedlings were stored at –80°C until further use (Chakraborty *et al*. 2017; Naziya *et al*. 2020).

Antioxidant enzyme assays

Preparation of enzyme extracts

During each phase of seed germination, various enzymes were systematically measured. For the extraction of crude enzyme, 0.25 g of plant material was homogenized in a chilled mortar and pestle with 5 ml of ice-cold 50 mM phosphate buffer (pH 7.8) (HiMedia, India). The resulting supernatant, obtained after centrifugation for 10 min at 10,000 rpm at 4°C, was immediately used for assays of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD). Enzyme activity was normalized to the sample's fresh weight (Havir and McHale 1987).

Determination of catalase

CAT activity was assessed using a reaction solution (3 ml) composed of 1.9 ml of phosphate buffer (pH 7.0), 1 ml of hydrogen peroxide (H_2O_2) (HiMedia, India) and 0.1 ml of enzyme extract. The enzymatic reaction was initiated by the addition of the enzyme extract. Catalase activity was quantified by measuring the decrease in absorbance at 290 nm over a 1 min period following the introduction of H_2O_2. This methodology follows the approach outlined by Havir and McHale (1987), where H_2O_2 has an extinction coefficient of 4.32 $cm^2 \cdot \mu mol^{-1}$. The unit of catalase activity was determined using the formula described by Aebi (1984).

Determination of superoxide dismutase

The SOD activity was measured by investigating the suppression of nitro blue tetrazolium (NBT) photochemical reduction at 560 nm. A 3 ml reaction mixture was prepared, consisting of 50 mmol · l^{-1} phosphate buffer (pH 7.8) (HiMedia, India), 0.1 mmol · l^{-1} EDTA (HiMedia, India), 16.7 µmol · l^{-1} riboflavin (HiMedia, India), 75 µmol · l^{-1} NBT, 13 mmol · l^{-1} methionine (HiMedia, India), and enzyme extract. Riboflavin (HiMedia, India) was the last component added, initiating the reaction when the tubes were exposed to two 9 W fluorescent lamps. After 15 min, the reaction was terminated by removing the sample from the light source. The most significant reduction in NBT and consequently the highest absorbance at 560 nm was observed in an illuminated blank lacking protein using a UV–VIS Spectrophotometer (BioSpectrometer Kinetic, Eppendorf, CA, USA). SOD activity was calculated by dividing the sample absorbance by the blank absorbance to determine the percentage of inhibition. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT photochemical reduction by 50%, as described by Giannopolitis and Ries (1977).

Determination of ascorbate peroxidase

The APX (EC 1.11.1.1) activity was determined by measuring the reduction in absorbance at 290 nm due to the oxidation of ascorbic acid. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) (HiMedia, India), 0.5 mM ascorbic acid (HiMedia, India), 0.1 mM EDTA, 0.1 mM H_2O_2, 0.1 ml of enzyme extract, and water to make a total volume of 3.0 ml. The reaction was initiated by the addition of 0.1 ml H_2O_2. The decrease in absorbance was monitored using a spectrophotometer, and the enzyme activity was calculated based on the reduction in ascorbic acid concentration, determined through a standard curve generated from known ascorbic acid concentrations (Nakano and Asada 1980; Sarker and Oba 2018).

Determination of peroxidase

The activity of POD was measured using a modified method of Rao *et al*. (1995). The reaction mixture consisted of 50 mM O-methoxyphenol (HiMedia, India), 100 mM phosphate buffer (PBS, pH 7.0), 40 mM H_2O_2, and 0.1 ml of enzyme extract. The reaction was initiated by adding the enzyme extract and allowed to run for 10 min. An increase in absorbance at 470 nm (extinction coefficient: 26.6 $mM^{-1} \cdot cm^{-1}$), corresponding

to the formation of tetraguaiacol from H_2O_2, was observed, as described by Poli *et al.* (2018).

Determination of total chlorophyll content

To calculate chlorophyll content, 0.5 g of leaf tissue was frozen in liquid nitrogen and then homogenized with a mortar and pestle. The homogenized samples were transferred to test tubes with lids, and 10 ml of 80% acetone (HiMedia, India) was added to extract the chlorophyll. The test tubes were wrapped in aluminum foil and left at room temperature overnight. The extract was then centrifuged for 5 min at 3000 rpm, and the pellet was discarded while the supernatant was retained. The absorbance of the supernatant was measured at wavelengths of 663 nm and 646 nm, corresponding to the primary absorption maxima of chlorophyll *a* and *b*, respectively, using the extinction coefficients reported by Porra, Thompson and Kriedemann (1989). Chlorophyll concentrations were expressed as µg chlorophyll per gram of dry sample ($\mu g \cdot g^{-1}$). The amount of chlorophyll was calculated using standard formulas.

Determination of lipid peroxidation

The quantification of malondialdehyde (MDA) is a key method for assessing lipid peroxidation, which is an important indicator of oxidative stress in plant tissues. In all samples, lipid peroxidation was evidenced by the presence of reactive 2-thiobarbituric acid (TBA) (HiMedia, India) metabolites, primarily MDA. For the analysis, 0.2 g of tissue was homogenized in 2 ml of 0.25% TBA solution prepared in 10% trichloroacetic acid (TCA) (HiMedia, India). The extract was heated at 95°C for 30 min and then rapidly cooled. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant was measured at wavelengths of 532 nm and 600 nm. The absorbance at 600 nm was subtracted to correct for non-specific turbidity. The extinction coefficient used to calculate lipid peroxidation levels was 155 $mM^{-1} \cdot cm^{-1}$, and the results were expressed as $\mu mol \cdot g^{-1}$ of MDA, as described by Pandey *et al.* (2010).

Determination of proline

The measurement of proline content is crucial in plant studies, as it serves as a key indicator of stress responses. Proline functions as an osmoprotectant, accumulating in plant cells under environmental stress conditions such as drought, salinity, or extreme temperatures. The determination of proline content in the samples was performed using the method outlined by Bates *et al.* (1973), with modifications based on Cagampang and Rodriguez (1980). In this method, 50 mg of plant tissue was extracted by adding 4 ml of cold 3% sulfosalicylic acid solution (HiMedia, India), followed by shaking for 30 min. The homogenate was then filtered, and the supernatant was collected. In a tube containing 0.5 ml aliquots of the supernatant, 50 µl of 6M orthophosphoric acid (HiMedia, India) and 1 ml of ninhydrin (HiMedia, India) reagent were added, and mixed thoroughly. Subsequently, 1 ml of ice-cold acetic acid (HiMedia, India) was introduced. The tube was incubated for 20 min in a boiling water bath, followed by 20 min in an ice bath, and finally allowed to stand for 20 min at room temperature. The absorbance of the solution was measured at 520 nm against a blank, and a proline standard curve was also recorded at this wavelength.

Determination of sucrose and starch

Determination of sucrose

Monitoring sucrose levels provides insights into a plant's carbohydrate metabolism and its ability to manage environmental stressors. To determine the sucrose content, 5 ml of 80% ethanol was added to 50 mg of the leaf sample and boiled for 10 min at 90°C. The supernatant was carefully decanted into a 100 ml volumetric flask following an additional 10 min of centrifugation at 3,000 rpm. This extraction procedure was repeated twice, and the supernatants from both extractions were combined. The combined supernatant was then adjusted to the required volume, diluted with distilled water, and thoroughly mixed. Next, 0.10 ml of 5% phenol reagent (HiMedia, India) was added, followed by 1 ml of 96% sulfuric acid (HiMedia, India). The resulting mixture was well mixed and allowed to react at room temperature for 10 min. Using a spectrophotometer, the absorbance of the solution was measured at a wavelength of 490 nm. To calculate the total soluble sugar content in each sample and express it as a percentage, a standard curve for sucrose was constructed (Nielsen 2010; Ramalakshmi *et al.* 2014; Viel *et al.* 2018; Landhäusser *et al.* 2018).

Determination of starch

Measuring starch content provides valuable insights into a plant's energy storage and metabolic activities. Starch serves as a primary carbohydrate reservoir, and its quantification aids in understanding the plant's ability to regulate energy utilization, growth, and responses to environmental factors. To eliminate starch, 0.2 g of frozen tissue sample was dissolved in 80% hot ethanol (HiMedia, India). The resulting residue was subjected to centrifugation for 8 to 10 min at 3,000 rpm. The residue was then repeatedly washed with hot 80% ethanol (HiMedia, India) using anthrone reagent (HiMedia, India) until no color was produced, after which it was dried over a water bath. Next, 2.5 ml

of water and 3 ml of a 52% perchloric acid (HiMedia, India) extract were added to the residue. The mixture was centrifuged at 0°C for 20 min at 3,000 rpm and the supernatant was carefully preserved. This extraction procedure was repeated with fresh perchloric acid. After centrifugation and cooling, a 0.2 ml aliquot of the supernatant was pipetted out and diluted with water to a final volume of 1 ml. To prepare the standard, 1 ml of each working standard (0.2, 0.4, 0.6, 0.8, and 1 ml) was placed in separate tubes, and 2 ml of anthrone reagent was added to each tube. The absorbance of the resulting solutions was measured at 630 nm after heating for 10 min in a boiling water bath (Hansen and Møller 1975; Nielsen 2010; Viel *et al*. 2018).

Statistical analysis

All values reported in this work are the mean of at least three independent experiments. The mean ± SE and the exact number of experiments are given in legends. The significance of differences between the control and each treatment was analyzed using T test and one-way ANOVA (Tukey's HSD) with the statistical package GraphPad Prism 10.00. Data normality and variance homogeneity were verified prior to analysis.

Results

Isolation of rhizosphere actinomycete

The isolation of rhizosphere actinomycete morphology unveiled the presence of *Saccharopolyspora* species in soil samples from organic farming environments. The actinomycete isolate had white colonies on all types of agars used. Ariel mass was white while the substrate mycelium was yellow and no pigments were produced (Fig. 1). The stained Gram positive and the spore chain morphology was simple rectus. This identification enriches the understanding of actinomycete diversity and ecological interactions within agricultural ecosystems

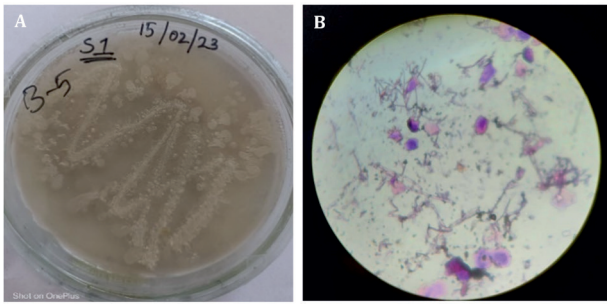

Fig. 1. A. Morphology of *Saccharopolyspora* species B. Microscopic View of *Saccharopolyspora* Species

Additionally, the identification of *Saccharopolyspora* species, which belongs to actinomycetes, highlights its potential contributions to nutrient cycling and the decomposition process (Bhatti *et al*. 2017).

Molecular identification of actinomycete

The 1113 bp 16S rRNA gene sequence (Fig. 2) of the isolate was analyzed using a BLAST search, revealing a 100% match with *S. thermophila*. Consequently, the isolate was identified as *S. thermophila* based on molecular taxonomy and phylogenetic analysis. The evolutionary relationships were determined using the maximum likelihood method in conjunction with the Tamura-Nei model. The resulting phylogenetic tree, which had the highest log likelihood score of –1874.61, is presented (Fig. 3). Initial trees were generated using

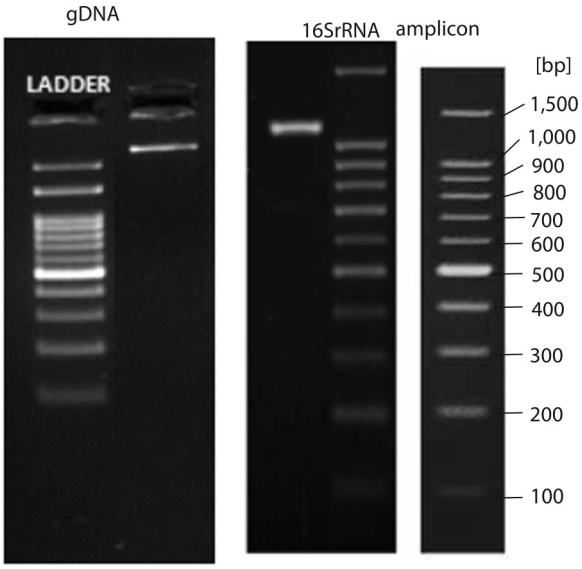

Fig. 2. gDNA and 16SrRNA amplicon QC data of *Saccharopolyspora thermophila*

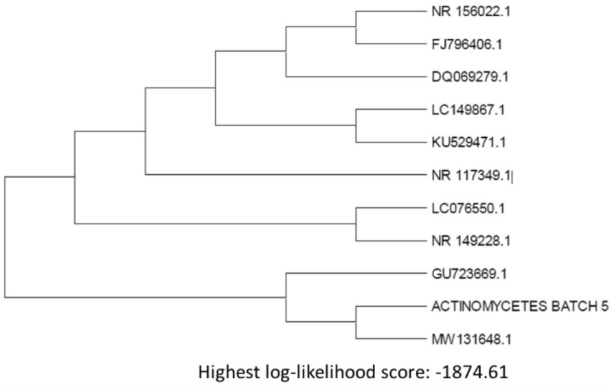

Fig. 3. Molecular phylogenetic analysis of *Saccharopolyspora thermophila*

neighbor- joining and BioNJ algorithms, which utilized pairwise distance matrices based on the Tamura-Nei model. The topology with the best likelihood score was chosen. This study analyzed 11 nucleotide sequences. Since this was a 16S rRNA analysis, codon positions were not applicable. The final dataset comprised 1101 nucleotide positions. Phylogenetic analysis was conducted using MEGA11 software (Kumar *et al.* 2018) (Fig. 3). The pairwise distance matrix shows the number of base substitutions per site between sequences, with standard error estimates provided above the diagonal. The maximum composite likelihood model (Kimura 1980) was applied to calculate substitution rates. Ambiguous positions were excluded for each sequence comparison using the pairwise deletion option, leaving 1101 positions for the analysis, which was completed in MEGA11.

Effects of *Saccharopolyspora thermophila* seed treatment on tomato seed germination and seedling vigor

The study evaluated the effects of *S. thermophila* treatment on seed germination percentage and seedling vigor at 3 and 6 h of exposure. In the control group, germination remained constant at 59.00% at both time points. In contrast, seeds treated with *S. thermophila* exhibited significantly higher germination percentages, with 80.65% at 3 h and 83.33% at 6 h, reflecting an overall increase of 22.95% in treated seeds compared to the control. Seedling vigor in the control group was 192.35 at 3 h and 193.35 at 6 h, whereas treated seeds showed markedly enhanced vigor indices of 573.25 and 625.42 at 3 and 6 h, respectively. An overall increase of 405.4 seedling vigor was found in treated seeds compared to the control. These results clearly indicate that *S. thermophila* significantly enhanced both germination rate and seedling vigor. This confirmed its potential as an effective PGPA for improving seed germination and overall plant growth.

Evaluation of PGPA seed treatment on plant growth parameters

Plants treated with *S. thermophila* reached a height of 22 cm, an 83.3% increase over the 12 cm height of control plants. Additionally, treated plants exhibited higher shoot fresh and dry weights (4.2 g and 2.6 g, respectively) than the control plants (2.7 g and 1.5 g). The total chlorophyll content in treated plants was 32 mg · g^{-1}, which was 88.2% higher than the 17 mg · g^{-1} recorded in control plants. In terms of reproductive development, treated plants flowered earlier (at 45 days), than control plants (at 52 days). *Saccharopolyspora thermophila* treated plants also produced an average of 15 fruits per plant, compared to 9 fruits in control plants, and the average fruit weight was 72 g, compared to 50 g in the control. The data demonstrated that *S. thermophila* treatment significantly improved ($p < 0.05$) both vegetative and reproductive growth parameters in tomato plants.

Effect of PGPA seed treatment on *Fusarium* wilt disease resistance in tomato under greenhouse conditions

The disease incidence (%) and percent disease index were measured under four treatment conditions: 1) control treated with SDW (C), 2) control plants treated with *F. oxyporum* (CP), 3) *S. thermophila* treated (I) and 4) *S. thermophila* and *F. oxyporum* treated (IP). In the control group, both disease incidence and percent disease indices were low, at 4% and 2%, respectively, indicating minimal disease presence. When a pathogen was introduced, disease incidence increased drastically to 92%, with a percent disease index of 83%, highlighting the significant impact of the pathogen. In the *S. thermophila* treated plants, disease incidence was reduced to 29%, with a percent disease index of 12%, showing that *S. thermophila* provided some protection against the disease (Fig. 4). However, when both *S. thermophila* and pathogen were combined, disease incidence was still relatively low at 37%, though the percent disease index dropped to 19%. This suggests that while inoculation helped reduce disease severity when the pathogen was present, it did not completely prevent disease occurrence.

Antioxidant assay

Catalase assay

In control S_{22} plants, CAT activity (Fig. 5A) increased from 0.087 units · mg^{-1} protein at 0 h to 0.267 units · mg^{-1} protein at 24 h (3.17-fold); the average increase was 1.134-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction, CAT activity decreased from 0.167 units · mg^{-1} at 0 h to 0.037 units · mg^{-1} protein at 24 h (3.17-fold); the average decrease was 0.22-fold from 0 h to 24 h. In S_{22} plants treated with the PGPA *S. thermophila,* SOD activity increased from 0.158 units · mg^{-1} protein at 0 h to 0.272 units · mg^{-1} protein at 24 h (1.72-fold); the average increase was 1.151-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction and with PGPA *S. thermophila,* CAT activity increased from 0.134 units · mg^{-1} protein at 0 h to 0.423 units · mg^{-1} protein at 24 h (1.37-fold); the average increase was 3.13-fold from 0 h to 24 h. The results demonstrated that the PGPA *S. thermophila* is able to enhance the antioxidant defense mechanism and interplay between catalase regulation, inducers, and pathogen presence in modulating catalase activity.

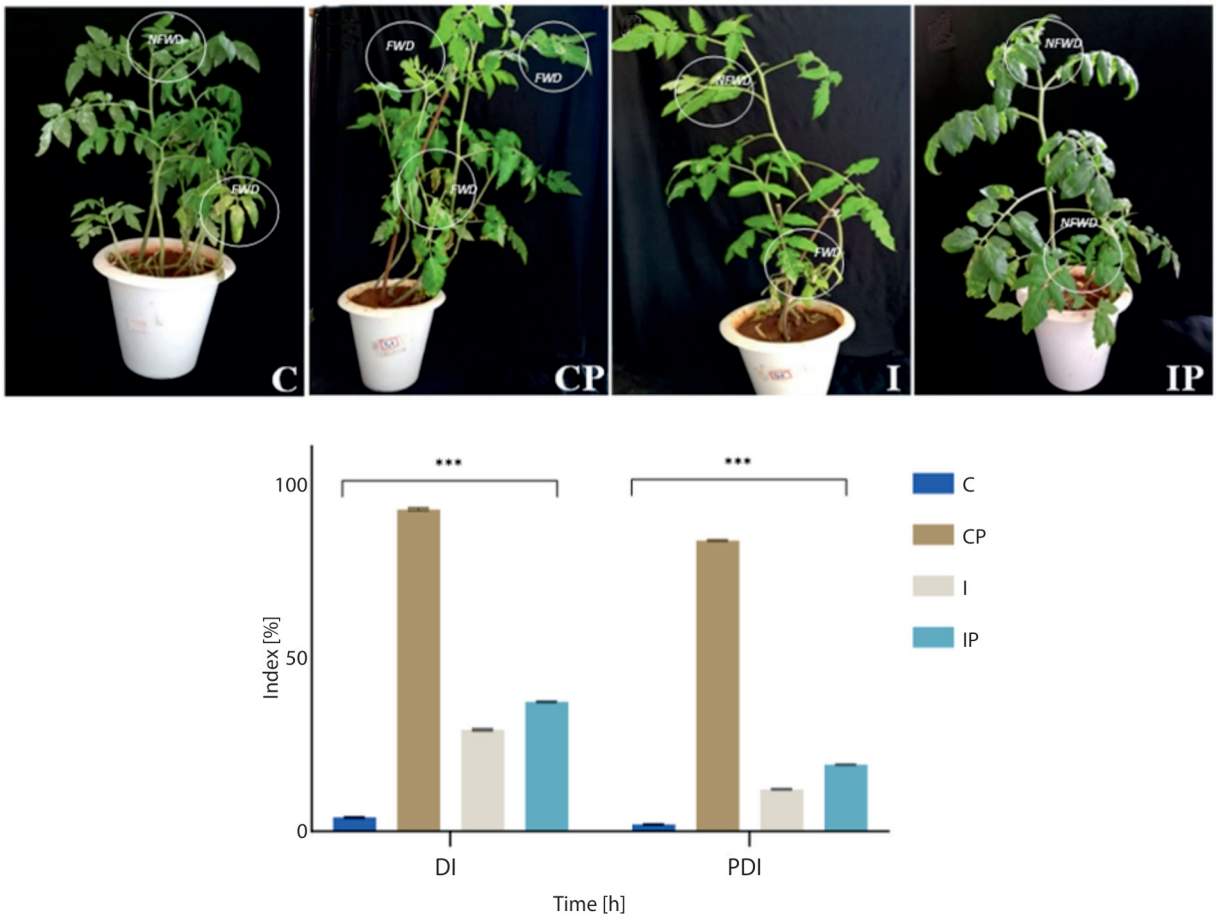

Fig. 4. Effect of PGPA treatment to wilt disease in S_{22} tomato seedlings under four distinct treatments: control treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Disease is labelled as FWD, fusarium wilt disease and NFWD, no fusarium wilt disease. The graph shows the disease index (DI) and percent of disease index (PDI) in all four treatments. Data represent the means ± SE of three separate experiments. A star indicates significant differences at ***$p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$

Superoxide dismutase assay

In control S_{22} plants, SOD activity (Fig. 5B) increased from 1.20 units · mg^{-1} protein at 0 h to 3.72 units · mg^{-1} protein at 24 h (3.08-fold); the average increase was 2.11-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction, SOD activity decreased from 1.69 units · mg^{-1} protein at 0 h to 0.57 units · mg^{-1} protein at 24 h (0.34-fold); the average decrease was 1.06-fold from 0 h to 24 h. In S_{22} plants treated with the PGPA *S. thermophila*, SOD activity increased from 3.80 units · mg^{-1} protein at 0 h to 5.24 units · mg^{-1} protein at 24 h (1.38-fold); the average increase was 4.17-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction and with PGPA *S. thermophila,* SOD activity increased from 3.92 units · mg^{-1} protein at 0 h to 6.81 units · mg^{-1} protein at 24 h (1.73-fold); the average increase was 4.75-fold from 0 h to 24 h. The results demonstrated that the PGPA *S. thermophila* can enhance the antioxidant defense mechanism, making the plant more resistant to stress conditions and diseases.

Ascorbate peroxidase assay

In control S_{22} plants, APX activity (Fig. 5C) increased from 0.85 units · g^{-1} protein at 0 h to 1.10 units · g^{-1} protein at 24 h (1.29-fold); the average increase was 1.07-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction, APX activity decreased from 0.95 units · g^{-1} protein at 0 h to 0.63 units · g^{-1} protein at 24 h (0.66-fold); the average decrease was 0.91-fold from 0 h to 24 h. In S_{22} plants treated with the PGPA *S. thermophila*, APX activity increased from 0.98 units · g^{-1} protein at 0 h to 1.45 units · g^{-1} protein at 24 h (1.47-fold); the average increase was 1.11-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction and with PGPA *S. thermophila*, APX activity increased from 1.25 units · mg^{-1} protein at 0 h to 1.91 units · mg^{-1} protein at 24 h (1.52-fold); the average increase was 1.11-fold from 0 h to 24 h.

Peroxidase assay

In control S_{22} plants, POD activity (Fig. 5D) increased from 0.18 units · mg^{-1} protein at 0 h to

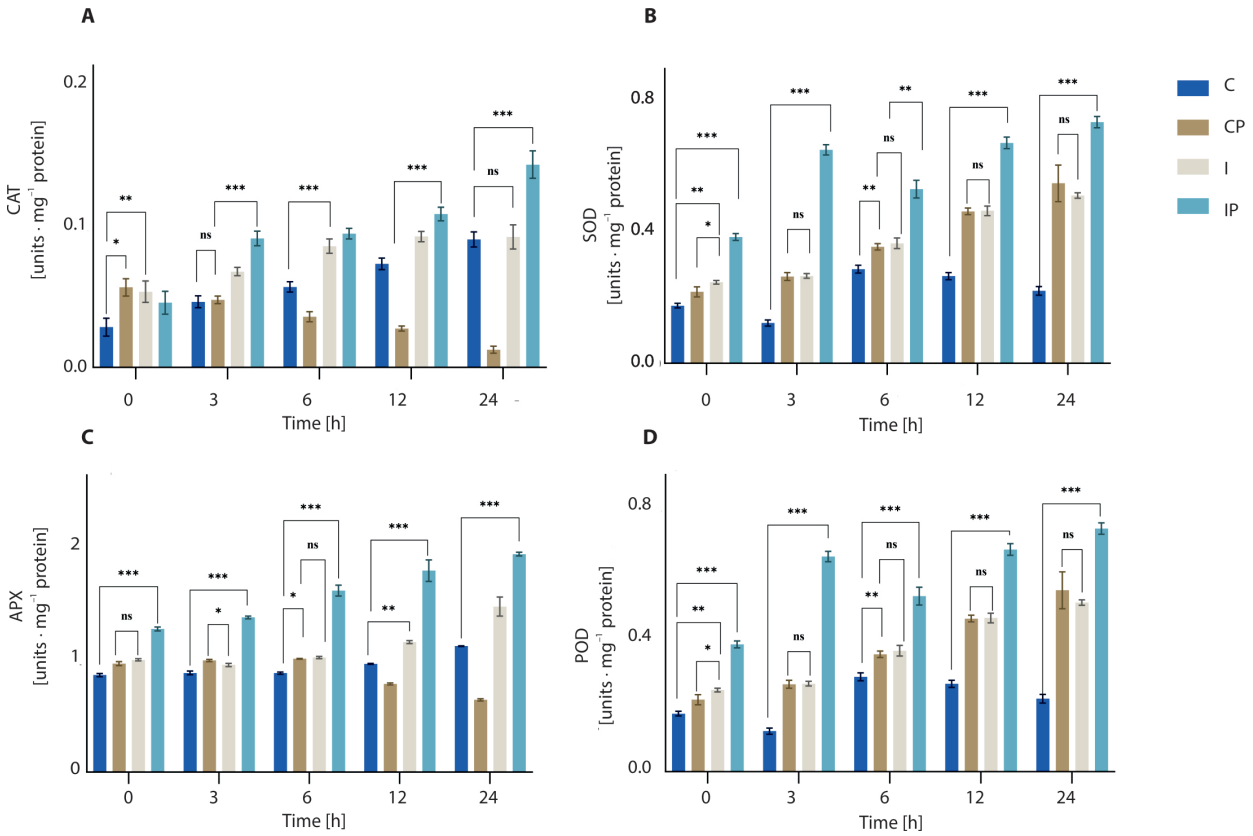

Fig. 5. The activity of CAT (A), SOD (B), APX (C) and POD (D) activities in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. A star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

0.22 units · mg^{-1} protein at 24 h (1.25-fold change); the average increase was 1.20-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction (CP), POD activity decreased from 0.22 units · mg^{-1} protein at 0 h to 0.55 units · mg^{-1} protein at 24 h (2.51-fold); the average decrease was 1.26-fold from 0 h to 24 h. In S_{22} plants treated with the PGPA *S. thermophila* (I), POD activity increased from 0.25 units · mg^{-1} protein at 0 h to 0.51 units · mg^{-1} protein at 24 h (2.06-fold); the average increase was 1.21-fold from 0 h to 24 h. In S_{22} plants treated with a pathogen *F. oxysporum* for disease induction and treated with PGPA *S. thermophila,* POD activity increased from 0.39 units · mg^{-1} protein at 0 h to 0.74 units · mg^{-1} protein at 24 h (1.90-fold); the average increase was 1.22-fold from 0 h to 24 h. The differences in POD activity between the treatments suggest that the PGPA may have had a protective effect against the pathogen, possibly by inducing a defense response in the plant.

Total chlorophyll content

Total chlorophyll content plays a crucial role in plants, as it is directly related to their photosynthetic capacity and overall productivity (Li *et al.* 2018). Biotic stress caused by *F. oxysporum* in plants leads to a decrease in total chlorophyll content. In control S_{22} plants, the total chlorophyll content was found to be an average of 4.75 mg · g^{-1} FW across all time intervals. However, pathogen infection to S_{22} plants led to decreased chlorophyll content from 4.45 mg · g^{-1} FW at 0 h to 3.23 mg · g^{-1} FW at 24 h (0.72-fold), with an average decrease of 0.92-fold from 0 h to 24 h likely due to the stress imposed by the pathogen and hence the loss of photosynthesis. Interestingly, when S_{22} plants were treated with the plant PGPA *S. thermophila*, a significant increase in total chlorophyll content was observed. It increased from 4.39 mg · g^{-1} FW at 0 h to 6.17 mg · g^{-1} FW at 24 h (1.40-fold change), with an average increase of 1.09-fold from 0 h to 24 h. This suggests that the PGPA had a beneficial effect on maintaining chlorophyll and even increased its photosynthetic capacity. The most notable finding is that when the S_{22} plants were first treated with the PGPA and then inoculated with the pathogen, the chlorophyll content did not decrease to the levels observed in the pathogen-treated control plants. Instead, the total chlorophyll content was maintained at

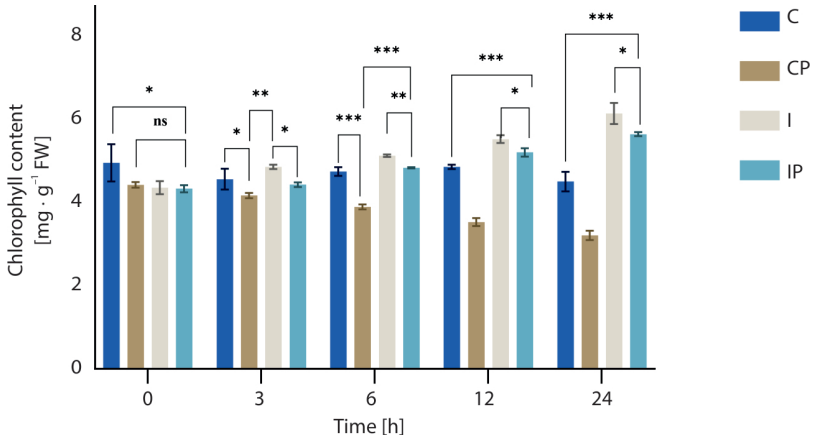

Fig. 6. The total chlorophyll content in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control plants treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. FW – fresh weight; a star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

4.46 mg · g^{-1} FW at 0 h and increased to 5.68 mg · g^{-1} FW at 24 h, a 1.30-fold change (Fig. 6). This indicates that the PGPA helped the plant to better withstand the biotic stress imposed by the pathogen, allowing the plant to maintain its chlorophyll and photosynthetic capacity, thereby allowing the plant to remain healthy even after pathogenic disease induction.

Lipid peroxidation

Lipid peroxidation (LPO) level, measured in terms of malondialdehyde (MDA) content, was observed in control S_{22} plants under various experimental settings throughout time. The average total chlorophyll content was determined to be 10.45 µmol · g^{-1} FW for all time intervals. However, in the control group subjected to pathogen inoculation, MDA levels increased gradually from 11.37 µmol · g^{-1} FW at 0 h to 44.90 µmol · g^{-1} FW at 24 h (3.94-fold), with an average increase of 1.44-fold from 0 h to 24 h, indicating oxidative stress induced by the pathogen. Furthermore, in PGPA *S. thermophila* treated S_{22} plants the MDA concentration was slightly lower than MDA levels in control plants and was found to be 9.13 µmol · g^{-1} FW at 0 h to 9.14 µmol · g^{-1} FW at 24 h (1.0-fold), with an average increase of 1.0-fold from 0 h to 24 h. This group showed slightly lower MDA levels than the control plants, suggesting a potential protective effect of the inducer against oxidative stress. Interestingly, in the inducer treated + pathogen inoculated group, MDA levels initially increased slightly compared to pathogen induced group and were found to be 12.64 µmol · g^{-1} FW at 0 h to 16.30 µmol · g^{-1} FW at 24 h 1.28-fold, with an average increase of 1.07-fold from 0 h to 24 h, possibly indicating a protective response induced by the combination of the inducer treatment and pathogen inoculation (Fig. 7). Overall, the results suggest that the inducer treatment may mitigate oxidative stress caused by pathogen infection.

Proline concentration

Proline levels were assessed in plant samples subjected to various treatments and pathogen inoculation over a 24-hour period. In the control S_{22} plants, the values ranged from 1.41 µmol · g^{-1} FW at 0 h and 3h to 1.82 µmol · g^{-1} FW at 24 h, indicating a slight increase over time. Conversely, in the control group exposed to a pathogen, proline concentration increased steadily over time, starting at 1.75 µmol · g^{-1} FW at 0 h and reaching 5.44 µmol · g^{-1} FW at 24 h, suggesting a significant elevation in response to pathogen presence. Additionally, in S_{22} plants treated with PGPA *S. thermophila*, proline concentration showed a moderate increase from 1.49 µmol · g^{-1} FW at 0 h to 1.89 µmol · g^{-1} FW at 24 h. However, in plants treated with both the *pathogen F. oxysporum* for disease induction and PGPA *S. thermophila*, a marginal increase in proline levels was observed, starting at 1.53 µmol · g^{-1} FW at 0 h and gradually rising to 1.94 µmol · g^{-1} FW at 24 h (Fig. 8). This combinational response suggests plant defense activation and stress responses. These results demonstrate the significance of proline as a vital component of plant defense systems and show that the application of PGPA lowered the levels of amino acids, especially proline, in stressed plants.

Sucrose and starch content

Sucrose content

The experiment investigated the effects of a pathogen *F. oxysporum* and a PGPA *S. thermophila* on sucrose accumulation in plants over a 24-hour period. The control plants maintained a relatively stable sucrose

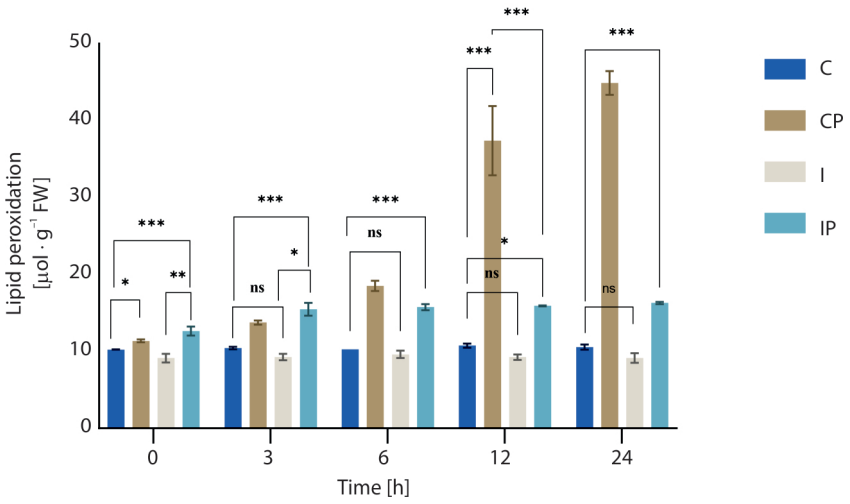

Fig. 7. Lipid peroxidation (LPO) expressed as malondialdehyde (MDA) content in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control plants treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. FW – fresh weight; a star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

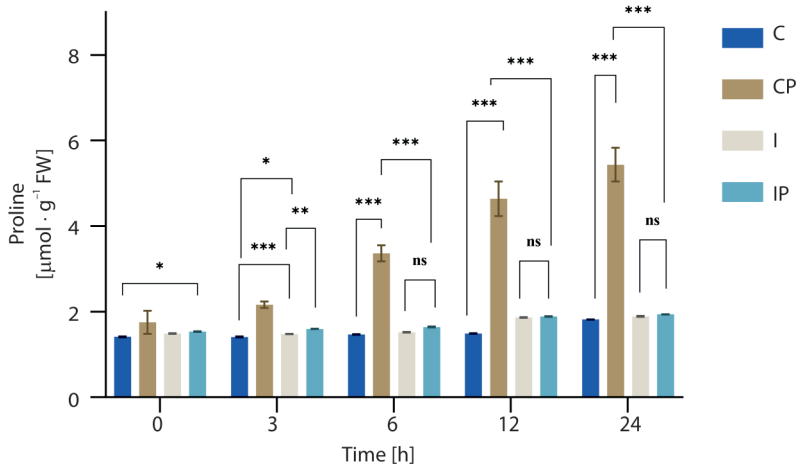

Fig. 8. The proline concentration in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control plants treated with SDW (C), control plants treated with *F. oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. FW – fresh weight; a star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

content throughout the experiment, ranging from 32.00 to 33.33 mg · g^{-1} FW. Plants treated with the pathogen alone exhibited a steady increase in sucrose content, rising from 37.33 mg · g^{-1} FW at the initial time point to 58.33 mg · g^{-1} after 24 h, suggesting the pathogen induced increased sucrose accumulation. In contrast, plants treated solely with the growth-promoting actinomycete showed sucrose levels similar to the control group, ranging from 31.67 to 34.00 mg · g^{-1} FW, indicating that this treatment did not significantly impact sucrose levels. Interestingly, plants exposed to both the pathogen and the actinomycete displayed an intermediate response, with sucrose accumulation increasing over time but reaching only 40.33 mg · g^{-1} FW after 24 h (Fig. 9). This level, lower than the pathogen-only treatment, suggests a potential interaction where the presence of the growth-promoting actinomycete moderated the pathogen's effect on sucrose induction.

Starch

The experiment aimed to investigate the effects of a pathogen *F. oxysporum* and a PGPA *S. thermophila* on starch accumulation in plants over a 24-hour period. The control plants maintained a relatively

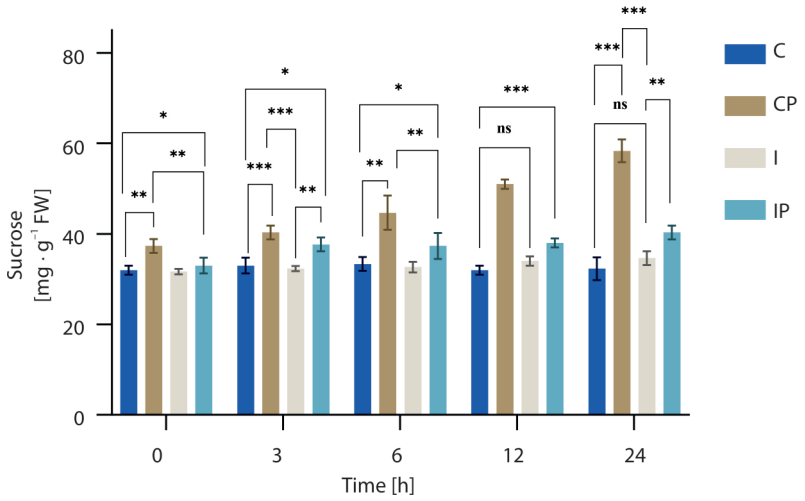

Fig. 9. The content of sucrose in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control plants treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. FW – fresh weight; a star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

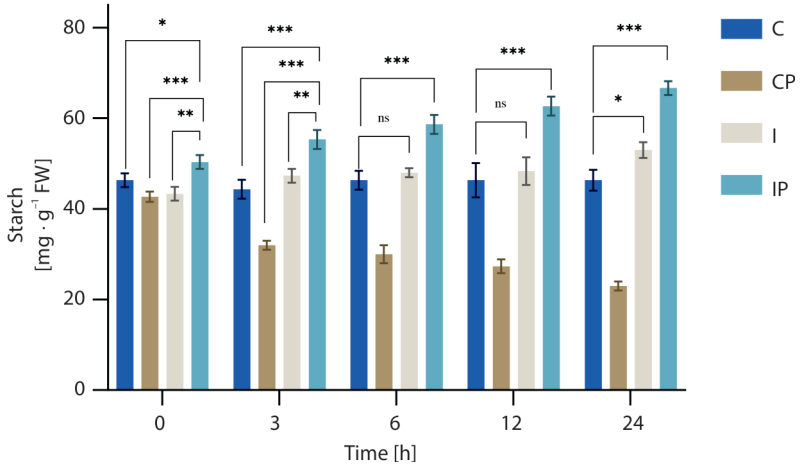

Fig. 10. The content of starch in S_{22} plants under different time points (0, 3, 6, 12 and 24 h) for control plants treated with SDW (C), control plants treated with *Fusarium oxysporum* (CP), *Saccharopolyspora thermophila*-treated (I), and *Saccharopolyspora thermophila* and *Fusarium oxysporum*-treated (IP). Data represent the means ± SE of three separate experiments. FW – fresh weight; a star indicates significant differences at $*p \leq 0.05$, $**p \leq 0.005$, $***p \leq 0.0005$ (Tukey HSD) with an ANOVA summary $p < 0.001$; ns – not significant

constant starch content of around 46.33 mg · g^{-1} FW throughout the experiment. In contrast, plants treated with the pathogen alone exhibited a steady decline in starch levels, decreasing from 42.67 mg · g^{-1} FW at the initial time point to 23.00 mg · g^{-1} FW after 24 h, indicating that the pathogen induced a reduction in starch accumulation. Interestingly, plants treated solely with the growth-promoting actinomycete showed a slight increase in starch content, rising from 43.33 mg · g^{-1} FW to 53.00 mg · g^{-1} FW over the 24-hour period, suggesting a potential promotion of starch accumulation by the actinomycete (Fig. 10). However, the most notable observation was in plants exposed to both the pathogen and the actinomycete, where a substantial increase in starch levels was observed, reaching 66.67 mg · g^{-1} FW after 24 h.

Discussion

We hypothesized that *S. thermophila* actinomycete can effectively promote growth and induce resistance against *F. oxysporum* through the production of growth-promoting compounds, activation of resistance, and modulation of plant defense-related

enzymes. This study aimed to contribute to the understanding of plant-microbe interactions in sustainable agriculture, provide insights into the interplay between plants, beneficial microorganisms, and pathogens, and propose future research and product development.

Collection of soil sample

The decision to gather soil samples from an organic farm carried profound implications for this research. Organic farming methods are renowned for their ability to improve soil health, promote biodiversity, and reduce reliance on synthetic inputs, thereby fostering a more sustainable and ecologically harmonious agricultural ecosystem (Reganold and Wachter 2016). Despite the region's semi-arid conditions and dependence on rainwater, the farm has achieved remarkable self-sufficiency through effective groundwater restoration and organic farming methods. The selected organic farm has adhered steadfastly to natural organic and agroforestry techniques for over two decades. By examining samples from this farm, the aim was to gain insights into how such practices influence soil microbial communities, thereby offering a valuable context for understanding the complex interactions between organic farming, soil health, and microbial dynamics (Sun *et al.* 2020; Suman *et al.* 2022).

Isolation and identification of actinomycete

This study has prompted further exploration into the ecological roles and potential biotechnological applications of *Saccharopolyspora* species by providing insights into sustainable agricultural practices and environmental management strategies. The studies of Barka *et al.* (2015) and Goodfellow and Fiedler (2010) emphasize the ecological significance of such actinomycete species. The molecular identification of a sample such as *S. thermophila* enhances the understanding of actinomycete diversity and ecology. Nucleotide homology and phylogenetic analysis support this identification. This identification emphasizes the significance of *S. thermophila* in plant growth promotion by producing phytohormones, e.g., the biocontrol of pathogens through the production of antibiotic compounds to inhibit the growth of various phytopathogens (Sayed *et al.* 2020). Also involved are soil fertility improvement, soil structure enhancements (Silva *et al.* 2022), and nutrient availability enhancement (Bhatti *et al.* 2017). Continued research on *S. thermophila* biology can provide valuable insights into its ecological roles and potential applications in fields like agriculture and biotechnology (Boubekri *et al.* 2022; Nazari *et al.* 2022).

Effects of *Saccharopolyspora thermophila* seed treatment on tomato seed germination and seedling vigor

The results demonstrate that *S. thermophila* treatment substantially enhanced seed germination and seedling vigor compared to the SDW treatment. The stable but relatively low germination and vigor under control conditions suggest that the seeds' natural growth potential is limited without microbial assistance. In contrast, the marked increase in both germination rate and seedling vigor following *S. thermophila* treatment indicated the strain's efficacy as a PGPA. This enhancement likely results from improved nutrient uptake, growth regulation, and possible resistance to plant stress. The findings suggest that *S. thermophila* has a strong potential for use in agricultural practices aimed at improving seed germination and promoting healthier early-stage plant growth. Previous studies found that the endophytic actinomycete *S. griseoflavus* is an IAA producer with significant potential for plant growth promotion. Co-inoculation with *Bradyrhizobium* species has shown benefits such as enhanced root elongation, nutrient uptake, and soybean yield (Soe and Yamakawa 2013; Htwe *et al.* 2019). This synergy boosts nodule formation and nitrogen fixation in soybean plants, probably due to phytohormone production by *S. griseoflavus* (Cuesta *et al.* 2012; Silva *et al.* 2022).

Evaluation of *Saccharopolyspora thermophila* seed treatment on plant growth parameters of tomato

The results clearly indicate that *S. thermophila* significantly promoted both productivity and vegetative growth in tomato plants. The observed increase in plant height, biomass, and chlorophyll content suggests that *S. thermophila* enhanced nutrient uptake and photosynthetic efficiency, contributing to overall plant growth. The acceleration in flowering time and the significant increase in fruit number and weight suggest that *S. thermophila* positively impacted productivity, improving both the yield and quality (weight, size and color) of tomato fruits. These findings highlight the potential of *S. thermophila* as a beneficial plant growth-promoting agent, likely due to its influence on plant physiology and nutrient absorption. Actinomycetes play a crucial role in promoting plant growth by modulating phytohormones and improving nutrient availability. For example, *S. olivaceoviridis*, *S. rimosus*, and *S. rochei* are known to produce substances such as auxins, gibberellins, and cytokinin-like compounds, which have a beneficial impact on plant development (Aldesuquy *et al.* 1998; Singh and Dubey 2018).

Additionally, *S. atrovirens* enhances the rhizosphere environment, improving seed quality and legume productivity by increasing nitrogen availability and supporting nitrogen metabolism (AbdElgawad *et al.* 2020).

Antioxidant ability of *Saccharopolyspora thermophila* treated plants

SOD, CAT, APX and POD are antioxidant enzymes that play a crucial role in plants during stress conditions. These enzymes help protect plants against oxidative damage by reducing the levels of superoxide and hydrogen peroxide, which are reactive oxygen species (ROS) produced in response to stress (Rajput *et al.* 2021). During drought stress, plants activate their antioxidant defense system, upregulating enzymatic antioxidants e.g., SOD, CAT, APX, and POD which protect cells from the negative effects of ROS (Cruz de Carvalho 2008). These enzymes are responsible for scavenging ROS and protecting the plant from oxidative damage caused by drought stress (Rajput *et al.* 2021). Antioxidant activity is a key defense mechanism for plant development during biotic and abiotic stress in a stressed environment because it increases the formation of hydrogen peroxide and superoxide (Saeed *et al.* 2023). The results of this research show antioxidant activity dynamics in S_{22} plants under varied conditions and time points. The interplay between inducers, pathogens, and temporal effects significantly influences antioxidant activity. Control plants exhibited steady antioxidant expression, while disease presence caused a notable decline, indicating pathogen-mediated suppression. Inducer treatment alone boosted antioxidant expression, yet the most significant increase occurred in inducer-treated plants with disease, suggesting a synergistic effect. This phenomenon possibly stemmed from defense pathway activation triggered by the inducer in disease-induced plants, leading to a robust antioxidant response. It suggests that the PGPA may enhance the plant's antioxidant defense system, which could help the plant to better cope with various stressors, including pathogen attack (Stepien and Klobus 2005; McGovern 2015). A similar mechanism was observed in *Phytophthora nicotianae* infected tobacco plants (Blackman and Hardham 2008). Other examples include *Bacillus aryabhattai* (AN30), *B. megaterium* (AN24), *B. megaterium* (AN31) and *B. megaterium* (AN35) strains infected *Oryza sativa* L. plants (Saeed *et al.* 2023). Furthermore, under heat stress settings, Chinese cabbage lowers hydrogen peroxide (H_2O_2) deposits, increases CAT enzyme activity, and promotes the production of catalase 2 (BcCAT2) (Wang *et al.* 2023). *Fritillaria taipaiensis* plants treated with three PGPA species, *Streptomyces lavendulae*, *Streptomyces fradiae* and *Streptomyces zaomyceticus*, increased the expression of antioxidant enzyme POD, SOD and CAT related genes (Kong *et al.* 2024). Another example, *T. longibrachiatum* and *B. stabilis* isolated from rhizosphere soil of *Pinus massoniana* showed an increase of SOD activity against Fusarium damping-off disease in *P. massoniana* seedlings (Yu *et al.* 2024). Therefore, strategies that enhance the plant's antioxidant defense system, such as the use of PGPA, could be a promising approach for managing plant diseases (Salehi *et al.* 2020). Hence, the increase in antioxidants activity in S_{22} plants treated with *S. thermophila* has several potential implications for plant health and resilience.

Total chlorophyll content

The results from this study indicate that chlorophyll content in plants is an important indicator of plant health and photosynthetic capacity. Treatment with PGPA *S. thermophila* increased chlorophyll content by 1.09-fold, indicating enhanced photosynthesis. Pre-treatment followed by pathogen inoculation maintained 1.30-fold higher chlorophyll levels and enhanced stress tolerance. The same results have been observed in recent scientific literature, which has also found that biotic and abiotic stresses can lead to decreases in chlorophyll content in plants (Yang *et al.* 2023). The application of PGPA, such as *Streptomyces* sp. PGPA39 to tomato plants (Palaniyandi *et al.* 2014), endophytic *Streptomyces* sp. GMKU 336 to rice plants (Jaemsaeng *et al*. 2018), and *Arthrobacter arilaitensis*, *Streptomyces pseudovenezuelae* to maize plants helps maintain the total chlorophyll content during biotic and abiotic stress conditions (Narsing Rao *et al.* 2022).

However, the use of beneficial microorganisms like PGPA to enhance chlorophyll content and stress tolerance is a promising area of research (Djebaili *et al.* 2021). Overall, these findings demonstrate the importance of chlorophyll as an indicator of plant health and the potential of PGPA to improve plant productivity even under stress conditions.

Lipid peroxidation

Lipid peroxidation, as measured by MDA levels, is a useful biomarker for assessing oxidative stress in plants (Sachdev *et al.* 2021). Results indicate that the pathogen infection increased 295.17% of MDA concentration and induced substantial oxidative stress in these plants. When the S_{22} plants were treated with the PGPA *S. thermophila*, the MDA levels remained relatively stable, suggesting that the PGPA treatment may have had a protective effect against oxidative stress in the plants. Furthermore, when the plants were first treated with the PGPA and then inoculated with the pathogen, the MDA levels increased to a lesser extent

than the pathogen-inoculated group. This indicates the PGPA treatment may have helped the plants better withstand the oxidative stress induced by the pathogen. Studies have shown that biotic and abiotic stresses, such as pathogen infections, can lead to increased lipid peroxidation and MDA levels in plants (Kazerooni *et al.* 2022; Sahu *et al.* 2022). *Streptomyces paradoxus* isolated from the rhizosphere of *Phragmites communis* enhances salt stress tolerance in soybean plants (Gao *et al.* 2022). It is possible to use beneficial microorganisms such as PGPA to mitigate oxidative stress and maintain plant health (Lopes *et al.* 2021; Jalal *et al.* 2023). The findings demonstrate the potential of PGPA to enhance plant resilience against biotic stresses by mitigating oxidative damage, which is a promising area for further research.

Proline concentration

Proline, a crucial amino acid in plants, accumulates in response to stress, aiding in osmotic balance, antioxidant defense, and metal chelation (Szabados and Savouré 2010). It plays a role in stabilizing structures, scavenging free radicals, and regulating gene expression (Kaur and Asthir 2015). Proline accumulation is linked to stress tolerance and plant development (Mattioli *et al.* 2009). The significant increase in proline concentration in plants exposed to a pathogen suggests robust activation of defense pathways upon pathogen recognition. This is consistent with previous studies indicating proline's role as a key component of plant defense against biotic stresses, where its accumulation serves as a marker of stress perception and subsequent activation of defense responses (Perea-Brenes *et al.* 2023; Koc *et al.* 2024). According to Koc *et al.* (2024), exogenously administered proline boosts antioxidant activities and strengthens rice's self-defense system against salt tolerance. Interestingly, the marginal increase in proline levels in plants treated with both the pathogen *F. oxysporum* and PGPA *S. thermophila* hints at a complex interplay between pathogen-induced stress and PGPA-mediated defense mechanisms. This combinational response indicates that the presence of the PGPA may have modulated the plant's defense response to the pathogen, resulting in a less pronounced proline accumulation than in the pathogen-only treatment.

Sucrose and starch content

Sucrose content

Plants often undergo metabolic reprogramming and alter their carbohydrate partitioning and allocation when faced with biotic stress, such as pathogen infection (Rojas *et al.* 2014). The accumulation of soluble sugars, including sucrose, is a common response to pathogen challenge, as these compounds serve multiple functions in plant defense mechanisms (Morkunas and Ratajczak 2014). Sucrose and other soluble sugars contribute to the activation of defense-related genes and the production of pathogenesis-related proteins, which are crucial for mounting an effective defense response (Berger *et al.* 2007). The observed increase in sucrose levels in the pathogen-treated plants may therefore be an indicator of the plant's attempt to activate defense mechanisms and mitigate the effects of the pathogen. Interestingly, the presence of the PGPA *S. thermophila* appeared to moderate the pathogen-induced sucrose accumulation. This observation aligns with previous findings suggesting that beneficial microorganisms, such as PGPA, can prime or induce systemic resistance in plants, enabling them to mount a more effective and efficient defense response against pathogens (Ebrahimi-Zarandi *et al.* 2022). It is noteworthy that the plants treated solely with the growth-promoting actinomycete did not exhibit significant changes in sucrose levels compared to the control plants. This suggests that the actinomycete did not elicit a strong defense response in the absence of a pathogen challenge, which is consistent with the concept of induced systemic resistance, where beneficial microbes prime the plant's defense mechanisms without activating them fully (Gupta *et al.* 2021; Ebrahimi-Zarandi et al. 2022).

Starch content

Pathogens often reprogram plant metabolism to divert resources towards defense mechanisms, leading to a decrease in starch synthesis and storage (Ramzi *et al.* 2019). This metabolic shift aims to provide energy and precursors for the production of defense-related compounds. Interestingly, the presence of the PGPA *S. thermophila* counteracted the pathogen-induced starch depletion, resulting in enhanced sucrose accumulation. This observation aligns with previous findings that beneficial microbes can modulate plant metabolism and promote growth (Warrad *et al.* 2020). Specifically, PGPA have been shown to stimulate photosynthesis, increase nutrient uptake, and influence carbohydrate partitioning, leading to improved carbon assimilation and storage (AbdElgawad *et al.* 2020; Ebrahimi-Zarandi *et al.* 2022). The synergistic effect observed when both the pathogen and growth-promoting actinomycete were present suggests a potential interaction between the two organisms in regulating plant metabolism. It is possible that the actinomycete mitigated the pathogen's impact on starch biosynthesis while also promoting starch accumulation through its growth-promoting mechanisms (Selim *et al.* 2021). Further investigations are needed to elucidate the underlying molecular mechanisms and signaling pathways involved in this synergistic interaction (Diwan *et al.* 2022).

Conclusions

This thorough investigation offers strong evidence of *S. thermophila's* effectiveness as a PGPA, showing how it can improve plant productivity, growth, resistance to pathogenic stress, and specifically, Fusarium wilt in tomato plants. Plant height, biomass, chlorophyll content, and fruit production were among the growth characteristics that *S. thermophila* considerably enhanced in addition to seed germination and seedling vigor. Furthermore, by boosting the plant's antioxidant defense system, preserving chlorophyll content, lowering lipid peroxidation, and adjusting glucose metabolism under stress, it successfully lessened the detrimental effects of *F. oxysporum* infection. These numerous advantages highlight *S. thermophila* potential in sustainable agricultural methods and present a viable substitute for chemical pesticides and fertilizers. Subsequent studies ought to concentrate on clarifying the fundamental molecular processes, examine possible mutualistic impacts with additional advantageous bacteria, and create useful applications for deployment in field settings. In light of increasing biotic and abiotic challenges, this work lays a solid basis for the creation of *S. thermophila*-based bioinoculants, which may be essential for advancing sustainable agriculture and food security.

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