Research Article

Dual Function of *Streptomyces* sp. KKU215 in Biocontrol of Bacterial Wilt and Growth Promotion of Chili

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Abstract

Bacterial wilt, caused by *Ralstonia pseudosolanacearum*, poses a serious threat to chili production. This study investigated the dual potential of *Streptomyces* sp. KKU215 as both a biocontrol agent against this pathogen and a plant growth-promoting bacterium for chili. The research involved *in vitro* antagonism assays, effects on chili seedling growth through seed priming, and effects in the biocontrol of bacterial wilt and growth promotion in pot experiments. *In vitro* assays confirmed that *Streptomyces* sp. KKU215 effectively inhibited *R. pseudosolanacearum* growth within 2 days, producing a clear zone with a diameter of 32.20 mm. Priming chili seeds with *Streptomyces* sp. KKU215 for 30 min significantly enhanced seedling growth 30 days after seeding, with increases in shoot length (14.21 vs. 12.25 cm), total length (21.05 vs. 18.63 cm), fresh weight (16.04 vs. 12.40 g), and dry weight (1.88 vs. 1.20 g), accompanied by a denser root system and larger leaves compared to the control. Most notably, in pot experiments, application of *Streptomyces* sp. KKU215 significantly suppressed bacterial wilt, achieving a disease severity reduction from 87.50% in the control to only 16.67% in the treated plants. *Streptomyces* sp. KKU215-treated seedlings showed enhanced growth, with enhanced growth, reaching maximum values for shoot length (28.26 cm), root length (24.93 cm), fresh weight (7.56 g), and dry weight (0.89 g). These findings highlight *Streptomyces* sp. KKU215 is a highly promising dual-function agent for sustainable chili cultivation. Further validation in field trials and the development of a stable formulation are essential next steps for practical application.

Keywords: Actinomycetes; Biological control; Bird chili; Plant growth-promoting bacteria; *Ralstonia pseudosolanacearum*

1. Introduction

Biological control of plant diseases is increasingly recognized as an effective strategy for managing plant diseases [1, 2]. In contrast, chemical control often leaves harmful residues in agricultural products and negatively impacts the environment. Moreover, continuous use of chemicals may lead to resistance in plant pathogens [3]. Therefore, biological control is an alternative that helps reduce dependence on agricultural chemicals, decreases toxic residues in products, and supports ecological balance [4]. Biological control of plant diseases using bacteria is an effective alternative for preventing and reducing the severity of plant pathogen infections [5]. Important bacterial genera involved in this process include *Bacillus* spp. [6], *Pseudomonas* spp. [7], and *Streptomyces* spp. [8], which can suppress pathogen growth through several mechanisms, such as the production of antimicrobial compounds, competition for nutrients, induction of systemic resistance in plants, secretion of lytic enzymes, and biofilm formation. Additionally, these bacteria enhance plant nutrient availability by increasing nitrogen availability through nitrogen fixation and ammonia production, improving phosphate availability through solubilization of insoluble phosphates, and synthesizing phytohormones, thereby making these nutrients more accessible for plant uptake [9].

Chili (*Capsicum annuum*) is an economically important crop at both household and industrial levels in many countries, including Thailand. However, its cultivation is often severely affected by bacterial wilt caused by *Ralstonia pseudosolanacearum* and *R. solanacearum*, with reported disease incidence ranging from 20% to 50% [10], which can

significantly reduce chili yield and lead to substantial economic losses, particularly for smallholder farmers [11]. Therefore, developing effective disease management strategies is urgent, and biological control methods represent an interesting and promising approach for managing this disease. In a previous study, the research team isolated *Streptomyces* sp. KKU215 from botanical garden soils [12]. The soil sample was collected in 2019 from the botanical garden of Sakon Nakhon Rajabhat University, Thailand. Given the broad-spectrum antagonistic properties of the genus *Streptomyces*, members of the genus have been reported to suppress plant pathogens and promote plant growth, suggesting that *Streptomyces* sp. KKU215 may possess similar potential. However, previous studies have not investigated these potential effects for *Streptomyces* sp. KKU215. Therefore, we hypothesize that this isolate exhibits beneficial effects on plant health, including pathogen suppression and growth promotion. The objectives of this study were to evaluate the antibacterial activity of *Streptomyces* sp. KKU215 against *R. pseudosolanacearum*, investigate the effect of *Streptomyces* sp. KKU215 seed priming on chili seedling growth, and assess the potential of *Streptomyces* sp. KKU215 in biocontrol of bacterial wilt and promotion of chili growth through pot experiments.

2. Materials and Methods

2.1 Streptomyces sp. KKU215 culture and spore preparation

The strain was cultured on nutrient agar (NA; Himedia^{TM}) at 37 °C for 10 days. The NA medium contained 0.30% (w v^{-1}) beef extract, 0.50% (w v^{-1}) peptone, and 1.50% (w v^{-1}) agar, with pH adjusted to 7. To prepare the spore suspension, sterile distilled water was added to the culture plate, and spores were gently scraped off using a sterilized stainless steel spatula. Spore concentration was determined using a hemacytometer and adjusted to 1×10^7 spores mL^{-1} with sterile distilled water.

2.2 R. pseudosolanacearum culture and cell preparation

R. pseudosolanacearum was derived from the Plant Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand. It was cultured in Yeast extract Peptone Glucose (YPG) broth containing 0.70% (w v^{-1}) yeast extract, 0.70% (w v^{-1}) peptone, and 0.50% (w v^{-1}) glucose, with the pH adjusted to 7. The culture was incubated in a shaker at 160 rpm and 28 °C for 2 days. The cells were collected by centrifugation at 5,635 × g for 10 min at 4 °C, and the pellets were washed three times before being resuspended in sterilized distilled water to achieve a final optical density of 0.80 at 600 nm.

2.3 Evaluation of antibacterial activity of Streptomyces sp. KKU215

The antibacterial activity assay was performed by the double-layer agar method according to Trinidad-Cruz *et al*. [13]. *Streptomyces* sp. KKU215 was streaked onto NA plates and incubated at 37 °C for 5 days to allow full growth of the strain. A culture of *R. pseudosolanacearum* with an optical density (OD) of 0.80 was then mixed at 5% (v v^{-1}) into molten YPG agar (added 2.00% (w v^{-1}) agar), which was maintained at approximately 45 °C to avoid heat damage to the cells. The mixture was poured over the NA plates containing the grown *Streptomyces* sp. KKU215 colonies. After solidification, the plates were incubated at 37 °C for 2 days. A clear zone around the colonies of *Streptomyces* sp. KKU215 was observed. This clear zone indicates the area where the growth of the *R. pseudosolanacearum* is inhibited. The total diameter of the clear halo was measured, spanning the entire clear area, including the central *Streptomyces* sp. KKU215 colony (expressed in mm).

2.4 Evaluation of Streptomyces sp. KKU215 seed priming on chili seedling growth

The healthy Bird chili (cultivar Jinda Red) seeds were surface sterilized with a 1% sodium hypochlorite solution for 2 min and washed three times with sterilized distilled water. A total of six trays served as the experimental units. Three trays were assigned to the control group, and the other three were assigned to the *Streptomyces* sp. KKU215 treatment. Peat moss, sterilized by autoclaving at 121 °C for 20 min using a steam pressure sterilizer twice, was used as the planting medium. For the *Streptomyces* sp. KKU215 treatment, the sterilized chili seeds were soaked in 20 mL of spore suspension (1×10^7 spores mL^{-1}) for 30 min, while seeds for the negative control were soaked in 20 mL of sterile distilled water for 30 min. Each tray was then sown with twenty seeds. Thirty days after seeding, shoot and root lengths were measured using a standard ruler. Fresh and dry weights of all seedlings per tray were pooled and recorded with a digital balance; for dry weight measurement, samples were oven-dried at 70 °C until a constant weight was achieved [14].

2.5 Evaluation of Streptomyces sp. KKU215 on biocontrol of bacterial wilt and promotion of chili growth in a pot experiment

The pot experiment was conducted under open-field conditions from December 2024 to January 2025. The key environmental parameters observed during this period were the average maximum temperature of 29.50 °C and the average minimum temperature of 15.50 °C. Relative humidity measurements showed an average maximum of 89.55% and an average minimum of 42.10%. Twenty-one-day-old chili seedlings were transplanted into plastic pots (17 cm in diameter × 14 cm in height). Each pot contained 1.20 kg of sterilized peat moss. A granular chemical fertilizer (15-15-15) was incorporated as a basal application into the sterilized peat moss at a rate of 1.20 g pot^{-1} prior to transplanting. This application supplied each pot with approximately 0.18 g of nitrogen (N), 0.08 g of phosphorus (P), and 0.15 g of potassium (K). The experiment was arranged in a Completely Randomized Design (CRD) with four treatments and 10 replicates (pots) per treatment. The four treatments were as follows (Table 1). For treatments involving *Streptomyces* sp. KKU215, a 50 mL spore suspension of *Streptomyces* sp. KKU215 (1×10^7 spores mL^{-1}) was applied to the sterilized peat moss at planting and at 7, 14, 21, and 28 days after transplanting. After 42 days after transplanting, seedlings in treatments T2 and T4 were inoculated with 50 mL of *R. pseudosolanacearum* suspension (OD_{600} = 0.80, approximately 3×10^8 CFU mL^{-1}) around the roots. For treatments not receiving a specific microbe (either *Streptomyces* sp. KKU215 or *R. pseudosolanacearum*), an equal volume of sterile distilled water was applied at the corresponding time points. The disease index (DI) and plant growth parameters (shoot and root lengths, fresh and dry weights) were measured 56 days after transplanting. Disease severity was assessed using the disease scoring (ds) system of Wu *et al*. [15], with the following scale: 0 = no symptoms; 1 = wilting on 1 – 25% of leaves; 2 = wilting on 26 – 50% of leaves; 3 = wilting on 51 – 75% of leaves and 4 = wilting on 76 – 100% of leaves or plant death. The disease index (DI) was calculated using the formula: DI = ∑(number of diseased plants in this index × ds) / (total number of plants investigated × highest ds) × 100.

Table 1 Summary of the four treatment groups.

Treatment	*Streptomyces* sp. KKU215	*R. pseudosolanacearum*
T1	Inoculated	Not treated
T2	Inoculated	Treated
T3	Not inoculated	Not treated
T4	Not inoculated	Treated

2.6 Statistical analysis

The effect of seed priming with *Streptomyces* sp. KKU215 on chili seedling growth was analyzed by an independent samples t-test. For the pot experiment, differences among the four treatment groups were evaluated using one-way ANOVA, followed by Tukey's HSD post-hoc test for pairwise comparisons. Statistical significance was determined at $p < 0.05$ for all tests. All analyses were carried out with SPSS software (version 29).

3. Results and Discussions

3.1 Effect of Streptomyces sp. KKU215 against R. pseudosolanacearum

The high level of inhibition achieved by *Streptomyces* sp. KKU215 against *R. pseudosolanacearum in vitro*, as evidenced by the clear zone (32.20 mm) observed within 2 days (Fig. 1), suggests the organism produces potent antibacterial secondary metabolites [16, 17]. This result aligns with previous studies indicating that *Streptomyces* species are major producers of bioactive compounds effective in controlling bacterial wilt disease, specifically, the formation of a clear zone typically implies the production and diffusion of extracellular antibiotics (e.g., cell-wall degrading enzymes) that actively suppress the growth of *R. solanacearum* [18].

Fig. 1 Inhibition zone of *Streptomyces* sp. KKU215 against *Ralstonia pseudosolanacearum*.

3.2 Effect of Streptomyces sp. KKU215 seed priming on chili seedling growth

Priming chili seeds with a spore suspension of *Streptomyces* sp. KKU215 for 30 min resulted in significantly enhanced seedling growth 30 days after seeding compared to the control group treated with sterile distilled water. Specifically, the *Streptomyces* sp. KKU215-treated seedlings exhibited significantly greater shoot length (14.21 cm vs. 12.25 cm) and total length (21.05 cm vs. 18.63 cm) (Fig. 2(a)). Furthermore, the biomass was also significantly increased, with the treated group showing significantly higher fresh weight (16.04 g vs. 12.40 g) and dry weight (1.88 vs. 1.20 g) compared to the control (Fig. 2(b)).

In contrast, while the average root length of the treated seedlings was slightly greater than that of the control group (6.84 cm vs. 6.38 cm), this difference was not statistically significant (Fig. 2(a)). However, despite the lack of statistical significance in length, visual assessment revealed clear morphological improvements in the treated plants (Fig. 2(c)). The *Streptomyces* sp. KKU215-primed seedlings developed a visibly denser and more extensive root system and exhibited larger leaves. Conversely, the control plants were smaller overall, with poorly developed roots and smaller leaves.

The mechanisms by which microorganisms promote seedling growth through seed biopriming include their ability to synthesize phytohormones, which play a crucial role in early plant development. Research has confirmed that *Streptomyces* spp. can produce auxins, particularly indole-3-acetic acid (IAA), in high amounts, which is associated with the stimulation of root system development [19]. This mechanism directly supports our observation of a denser and more extensive root system, even though the increase in overall root length was not statistically significant. Moreover, seed biopriming with plant growth-promoting bacteria (PGPB) stimulates various metabolic processes that lead to enhanced seedling vigor, such as increased leaf size, higher root density, and overall improved seedling growth [20]. A clear example is provided by Hijab *et al*., who reported that seed priming by soaking in bacterial suspensions of *Bacillus velezensis* (ATCC 6051), *Pseudomonas fluorescens* (ATCC 13525), and *Serratia marcescens* (ATCC 13880) significantly increased radicle and plumule lengths [21]. Our experimental results clearly reflect these findings, showing that seed biopriming with *Streptomyces* sp. KKU215 significantly promotes chili seedling growth, especially in terms of shoot length, total length, and biomass (fresh and dry weights), while also promoting a denser root system. These findings suggest the strong potential of *Streptomyces* sp. KKU215 as PGPB, suitable for application in sustainable chili seedling production.

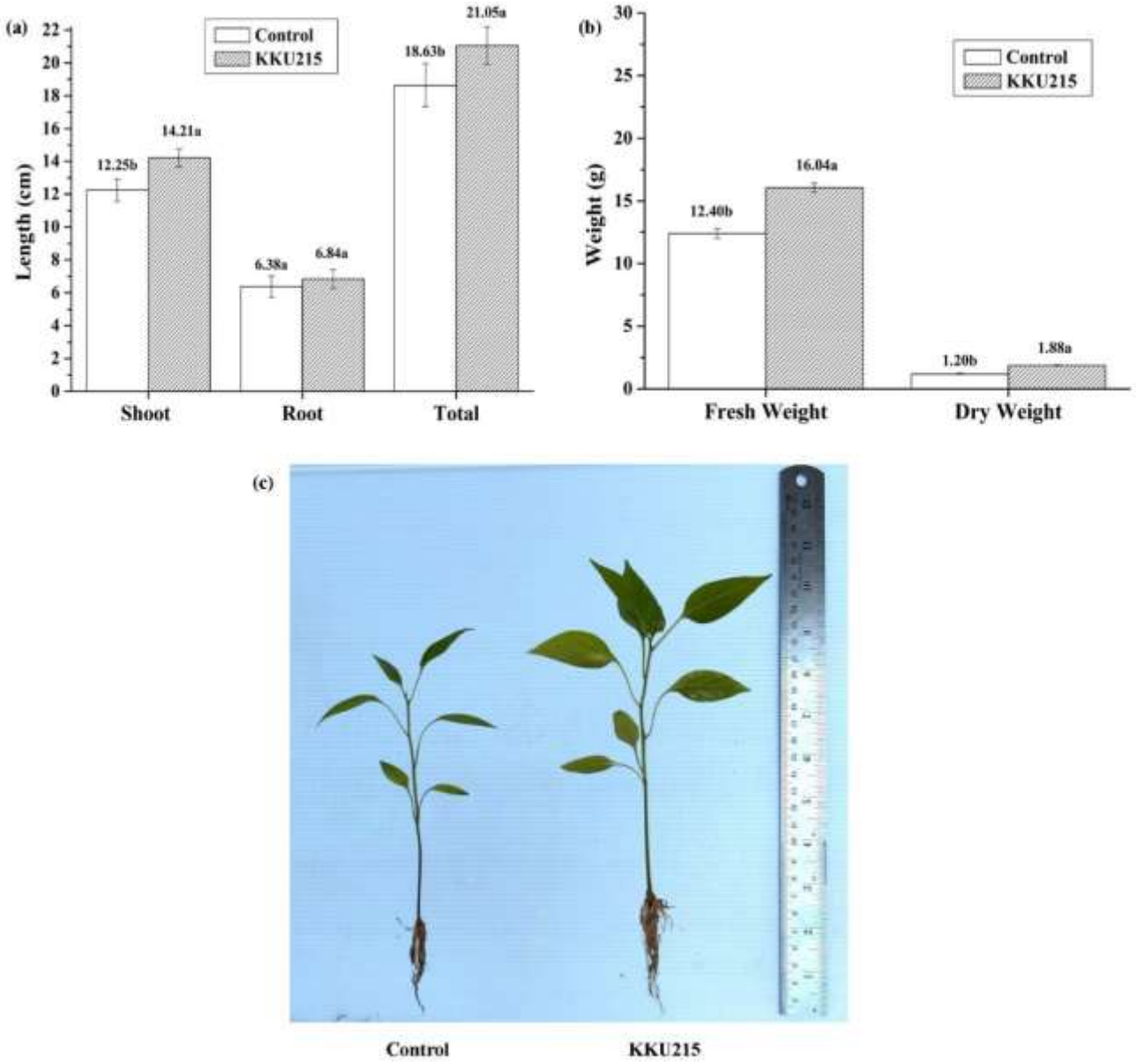

Fig. 2 Effects of *Streptomyces* sp. KKU215 on the growth of chili seedlings thirty days after seeding in trays experiment. (a) shoot, root, and total lengths, (b) fresh and dry weights, and (c) chili seedlings photographs. Means within the same parameter followed by different letters (a, b, c) are significantly different by independent samples t-test at $p < 0.05$.

3.3 Effects of Streptomyces sp. KKU215 on biocontrol of bacterial wilt and promotion of chili growth in a pot experiment

Application of *Streptomyces* sp. KKU215 (T2) significantly suppressed bacterial wilt, reducing the DI to 16.67% compared to the pathogen-only control (T4), which exhibited a severe DI of 87.50% (Fig. 3(a)). Notably, while not completely eliminating the symptoms, the DI of the T2 treatment was statistically indistinguishable from that of the healthy control treatments (T1 and T3), which both had a DI of 0%. This strong biocontrol effects may be attributed to the ability of *Streptomyces* spp. to suppress plant pathogens. It has been reported that these gram-positive bacteria are well known for producing various antimicrobial compounds, such as antibiotics and hydrolytic enzymes, which can inhibit soil-borne pathogens like *R. pseudosolanacearum* [22, 23]. In addition, *Streptomyces* may compete with pathogens in the rhizosphere through competitive exclusion, by colonizing the root zone and utilizing available resources more efficiently, thereby preventing pathogen establishment [24]. Furthermore, *Streptomyces* can induce systemic resistance (ISR) in plants through

various mechanisms, such as producing cyclic lipopeptides (CLPs), which act as elicitors to enhance the plant's ability to resist pathogenic infections [25].

Beyond disease control, the application of *Streptomyces* sp. KKU215 demonstrated a potent plant growth-promoting effect, as the two treatments involving this strain (T1 and T2) consistently resulted in the highest values for all growth parameters measured (Fig. 3(b)−(c)). Specifically, in the absence of the pathogen (T1), seedlings achieved maximum shoot length (28.26 cm), root length (24.93 cm), fresh weight (7.56 g), and dry weight (0.89 g), all of which were significantly higher than the healthy control (T3). Even under pathogen attack, the application of *Streptomyces* sp. KKU215 (T2) provided significant benefits, resulting in greater shoot length, root length, fresh weight, and dry weight compared to the diseased-only control (T4). Notably, this protective effect was so strong that the T2 plants not only performed at a level statistically indistinguishable from the completely healthy control plants (T3) in terms of root length, fresh weight, and dry weight, but also exhibited significantly greater shoot length. This demonstrates the strain's remarkable ability to both maintain plant vigor and actively promote growth despite disease pressure. These findings are visually supported by the clear morphological differences observed, where both T1 and T2 plants were visibly larger and healthier than T3 and T4 plants, with the T4 plants showing severe wilting and stunting (Fig. 3(d)). This pronounced growth promotion is characteristic of PGPB. Several studies have demonstrated that *Streptomyces* spp. can significantly promote plant growth by increasing shoot and root length, as well as fresh and dry biomass [26 − 28]. For instance, a recent study on tomato, another solanaceous crop, showed that *Streptomyces* sp. SP5 substantially increased shoot length, root length, and biomass [29]. This growth enhancement may be attributed to their ability to produce plant hormones such as indole-3-acetic acid (IAA), release ammonia, and solubilize essential nutrients like potassium (K) and phosphorus (P), thereby significantly improving overall plant development [30].

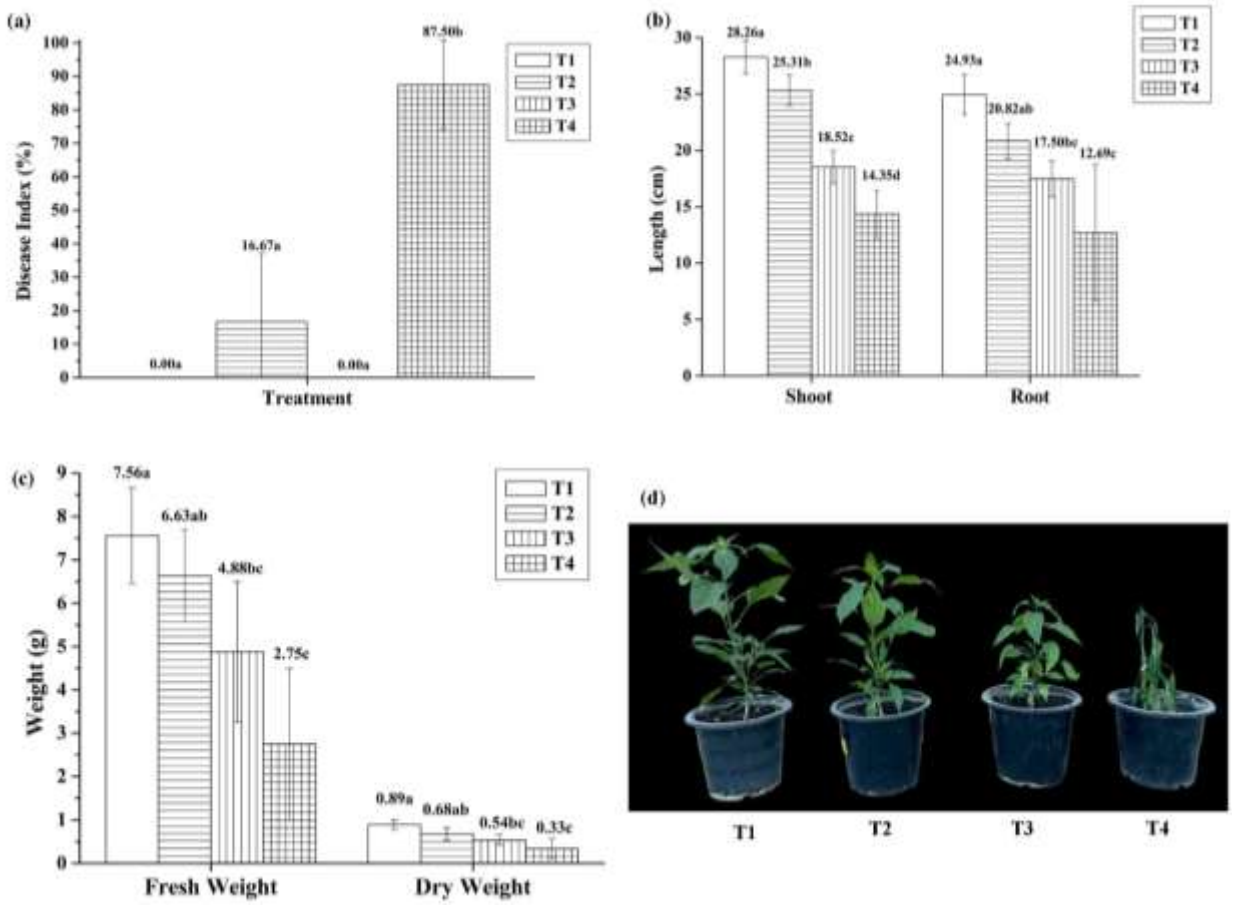

Fig. 3 Effects of *Streptomyces* sp. KKU215 on BW disease control and the growth of chili plant fifty-six days after transplanting in pot experiment. (a) disease index, (b) shoot and root lengths, (c) fresh and dry weights, and (d) chili plant photographs. Means within the same parameter followed by different letters (a, b, c) are significantly different by Tukey's HSD test following one-way ANOVA at $p < 0.05$.

4. Conclusion

The findings of this study indicate that *Streptomyces* sp. KKU215 possesses strong potential for controlling bacterial wilt and promoting chili cultivation. The strain exhibited potent antibacterial activity against *R. pseudosolanacearum in vitro*. In tray experiments, seed priming with *Streptomyces* sp. KKU215 significantly enhanced chili seedling vigor, as evidenced by increased shoot length, total length, and biomass, alongside the development of a denser root system. Furthermore, in pot experiments, the application of *Streptomyces* sp. KKU215 effectively reduced the severity of bacterial wilt while simultaneously promoting plant growth under pathogen pressure. Remarkably, the strain demonstrated powerful dual-action capabilities, showing a significant growth-promoting effect over healthy plants grown without *Streptomyces* sp. KKU215 while also providing a strong protective and growth-enhancing effect over pathogen-challenged plants grown without *Streptomyces* sp. KKU215. These results strongly suggest that *Streptomyces* sp. KKU215 is a promising candidate for development as both a biocontrol agent and a PGPB for sustainable chili production.

This study provides preliminary data on the potential application of *Streptomyces* sp. KKU215 for the biocontrol of bacterial wilt and the promotion of chili growth. However, practical application requires further elucidation of the mechanisms underlying disease suppression and plant growth promotion mediated by *Streptomyces* sp. KKU215. Moreover, comprehensive field trials are necessary to evaluate the colonization efficiency, ecological fitness, and persistence of *Streptomyces* sp. KKU215 within the rhizosphere and endophytic niches under natural environmental conditions. Furthermore, future research should focus on formulation development to create a stable and user-friendly product, as well as its compatibility with common agrochemicals used in chili cultivation.

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Data availability

The strain *Streptomyces* sp. KKU215 used in this study was officially registered and deposited in the GenBank. The strain's identity and ownership are verified by the GenBank accession number: MK835665.

The pathogenic strain *R. pseudosolanacearum* DOAC-B1954 was obtained from the Plant Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand.

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