Sains Malaysiana 55(3)(2026): 461-474
http://doi.org/10.17576/jsm-2026-5503-09

Streptomyces sp. KKU215: Biocontrol of Bacterial Wilt, Tomato Growth Promotion, and Spore Production Optimization

(*Streptomyces* sp. KKU215: Biokawalan Layu Bakteria, Penggalak Pertumbuhan Tomato dan Pengoptimuman Penghasilan Spora)

AROON WONGJIRATTHITI[1], CHANANKARN SAENGPRASAN[2] & SUWAPHA SAWIPHAK[1,*]

[1]*Program of Biology, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000, Thailand*
[2]*Program of Mathematics and Statistics, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000, Thailand*

Received: 1 June 2025/Accepted: 19 February 2026

ABSTRACT

The development of microbial biocontrol agents and plant growth promoters as alternatives to chemical pesticides is crucial for sustainable agriculture. This study investigated the potential of *Streptomyces* sp. KKU215 for controlling bacterial wilt caused by *Ralstonia solanacearum,* promoting tomato growth, and enhancing spore production. The strain exhibited multiple beneficial traits, including the production of protease, chitinase, lipase, cellulase, and amylase enzymes, as well as siderophores and biofilm. It also demonstrated capabilities for nitrogen fixation, ammonia production, and indole-3-acetic acid (IAA) synthesis. *Streptomyces* sp. KKU215 significantly ($p < 0.05$) promoted the growth of 30-day-old tomato seedlings in tray trials by enhancing shoot and root lengths, fresh and dry weights, and leaf size, while also significantly ($p < 0.05$) reducing the bacterial wilt disease index and improving overall growth in pot trials. Among tested media, modified Wakimoto's and nutrient agar supported the highest levels of spore production after 20 days of incubation. The Plackett–Burman design showed peptone, potato, and incubation time as significant factors enhancing spore production. Subsequently, optimization using response surface methodology with Box–Behnken design determined the optimal conditions of 7 g/L peptone, 700 g/L potato infusion equivalent, and 20 days of incubation, resulting in a significant ($p < 0.05$) increase (46%) in spore yield. The findings of this study suggest that *Streptomyces* sp. KKU215 could serve as a sustainable alternative to harmful chemicals, contributing to enhanced crop yield and quality while supporting the development of industrial-scale biopesticide production for disease management and plant growth promotion in agricultural systems.

Keywords: Actinobacteria; biocontrol agents; Box–Behnken design; plant growth promoters

ABSTRAK

Pembangunan agen biokawalan mikrob dan penggalak pertumbuhan tumbuhan sebagai alternatif kepada racun perosak kimia adalah penting untuk pertanian lestari. Penyelidikan ini mengkaji potensi *Streptomyces* sp. KKU215 untuk mengawal layu bakteria yang disebabkan oleh *Ralstonia solanacearum*, menggalakkan pertumbuhan tomato dan meningkatkan penghasilan spora. Strain ini menunjukkan pelbagai ciri bermanfaat, termasuk penghasilan enzim protease, kitinase, lipase, selulase dan amilase, serta siderofor dan biofilem. Strain ini juga menunjukkan keupayaan untuk mengikat nitrogen, penghasilan ammonia dan sintesis asid indol-3-asetik (IAA). *Streptomyces* sp. KKU215 dengan ketaranya ($p < 0.05$) menggalakkan pertumbuhan anak benih tomato berusia 30 hari dalam ujian dulang dengan meningkatkan panjang pucuk dan akar, berat segar dan kering dan saiz daun di samping mengurangkan indeks penyakit layu bakteria dengan ketara ($p < 0.05$) dan meningkatkan pertumbuhan keseluruhan dalam ujian pasu. Antara media yang diuji, media Wakimoto yang diubah suai dan agar nutrien menyokong tahap penghasilan spora tertinggi selepas 20 hari pengeraman. Reka bentuk Plackett-Burman mendedahkan pepton, kentang dan masa pengeraman sebagai faktor penting untuk meningkatkan penghasilan spora. Seterusnya, pengoptimuman menggunakan metodologi permukaan tindak balas dengan reka bentuk Box-Behnken telah menentukan keadaan optimum 7 g/L pepton, 700 g/L setara infusi kentang dan 20 hari pengeraman menunjukkan peningkatan ketara ($p < 0.05$) sebanyak 46% dalam penghasilan spora. Hasil kajian ini menunjukkan bahawa *Streptomyces* sp. KKU215 boleh berfungsi sebagai alternatif yang mampan kepada bahan kimia berbahaya, menyumbang kepada peningkatan hasil dan kualiti tanaman di samping menyokong pembangunan pengeluaran biopestisid berskala industri untuk pengurusan penyakit dan penggalak pertumbuhan tanaman dalam sistem pertanian.

Kata kunci: Agen biokawalan; aktinobakteria; penggalak pertumbuhan tumbuhan; reka bentuk Box-Behnken

INTRODUCTION

Ralstonia solanacearum (Rs) is a bacterium responsible for bacterial wilt (BW disease), a serious threat to tomato production worldwide, including in Thailand, causing significant yield losses (Akarapisan et al. 2021; Fonseca et al. 2024). Rs produces exopolysaccharides that obstruct xylem vessels, leading to disruption of water and mineral transport, ultimately causing plant wilting and death. Chemical control methods can reduce disease incidence but pose serious risks to human health and the environment (Nicolopoulou-Stamati et al. 2016). Moreover, current management strategies are often ineffective, as Rs can survive in soil and water for extended periods even without a host (Vailleau & Genin 2023). Hence, safer and more efficient alternatives are urgently needed.

Biological control using *Streptomyces* spp. shows promise in managing plant diseases by producing antimicrobial compounds, competing for nutrients and space, and inducing systemic resistance (Khan et al. 2023). They also promote plant growth by fixing nitrogen and producing plant hormones, ammonia, and biofilms (Ajijah et al. 2023; Li et al. 2024). Various *Streptomyces* strains have been shown to reduce BW disease and enhance plant growth (Boukaew, Chuenchit & Petcharat 2011; Devi, Sharma & Manhas 2022; Elsharkawy et al. 2015; He et al. 2024; Kaari et al. 2022a; Le et al. 2021; Ling et al. 2020; Xue et al. 2019; Zhuang et al. 2020). Given *Streptomyces* efficacy in disease suppression and plant growth promotion, this study aimed to discover novel strains for managing BW disease and enhancing tomato growth.

The novel strain of *Streptomyces* sp. KKU215 was isolated from botanical garden soils. The isolate was identified by 16S rRNA gene sequencing (Yottakot & Leelavatcharamas 2019), and the sequence was deposited in GenBank (Accession No. MK835665). Subsequently, its antagonism against *Ralstonia solanacearum* DOA-BCC1954 was tested using a double-layer assay, with preliminary results showing strong antagonistic activity (Figure S1). Building upon these preliminary findings, *Streptomyces* sp. KKU215 appears to be a distinctive strain with a robust combination of multifunctional traits and intrinsic potential for high-yield spore production, a critical requirement for commercial biopesticide development. To enable agricultural applications, it is necessary to investigate the biocontrol and growth-promoting mechanisms of KKU215 and to optimize spore production due to its high inoculum requirements. Techniques such as the Plackett-Burman (PB) design (Liu et al. 2020) and Response Surface Methodology (RSM) with the Box-Behnken (BB) design have been successfully used to optimize microbial metabolite production (Liu et al. 2025).

This study aimed to (1) investigate the characteristics related to disease control and plant growth promotion of KKU215, (2) evaluate its efficacy in controlling BW disease and promoting tomato growth, and (3) screen factors affecting spore production using PB design, followed by optimization of culture conditions for enhanced spore production through RSM with BB design. The outcomes of this study offer an alternative to harmful chemicals in enhancing crop yield and quality, as well as promoting sustainable agricultural practices.

MATERIALS AND METHODS

MICROBIAL STRAIN AND CULTURE CONDITION

Spore suspension of KKU215, which inhibits RsDOA-BCC1954, was cultured on nutrient agar (NA; Himedia™) at 37 °C for 10 days. This temperature was selected based on preliminary tests indicating optimal and rapid spore production on solid media. Sterile distilled water was added to the plates, and spores were scraped off with a sterile spatula. Spore concentration was adjusted to 1×10^7 spores/mL using a hemacytometer. This concentration was used in all experiments. Cell suspension of RsDOA-BCC1954, obtained from the Plant Protection Research and Development Office (Department of Agriculture, Thailand), was cultured in yeast extract peptone glucose (YPG) medium containing 0.7% peptone, 0.7% yeast extract, and 0.5% glucose (pH 7.0) at 28 °C for 2 days on a rotary shaker (160 rpm). Cells were harvested by centrifugation (5,635 × g, 10 min, 4 °C), washed three times with sterile distilled water, and adjusted to OD600 = 0.5.

BIOCONTROL ACTIVITY EVALUATION

Extracellular lytic enzyme production was assessed on selective media: starch agar (amylase; Hasan et al. 2017), carboxymethyl cellulose agar (cellulase; Li et al. 2020), tributyrin agar (lipase; Ilesanmi et al. 2020), skim milk agar (protease; Shaikh et al. 2023), and colloidal chitin agar (chitinase; Xia et al. 2011). A 2 µL spore suspension of KKU215 was spotted onto each medium and incubated at 37 °C. After 2 days, enzyme activity was indicated by clear zones around the colony. Siderophore production was assessed using Chromeazurol S (CAS) agar plates (Schwyn & Neilands 1987), following the method of Fahsi et al. (2021). Similarly, 2 µL spore suspension of KKU215 was spotted onto CAS agar and incubated at 37 °C for 3 days. Siderophore production was indicated by the appearance of orange zones around the colony. Biofilm formation was assessed following Jain et al. (2013). A 100 µL spore suspension of KKU215 was inoculated into 1 mL nutrient broth (NB; Himedia™) in glass tubes and incubated at 37 °C for 7 days without agitation, with an uninoculated control. After incubation, cultures were discarded, tubes washed twice with phosphate-buffered saline (pH 7.3), air-dried, stained with 0.1% crystal violet for 5 min, rinsed with sterile distilled water, and examined for staining to confirm biofilm formation.

PLANT GROWTH PROMOTION TRAITS EVALUATION

Nitrogen fixation was assessed on nitrogen-free (NF) agar plates as described by Kifle & Laing (2016), by spotting 2 µL of a spore suspension of KKU215 onto the plates and incubating at 37 °C for 5 days, with colony growth indicating the ability to fix nitrogen. Ammonia production was measured following the method of Fahsi et al. (2021), in which 100 µL of a spore suspension of KKU215 was inoculated into 100 mL of 4% peptone broth and incubated at 160 rpm and 37 °C for 10 days. After incubation, 0.5 mL of Nessler's reagent was added to 2.5 mL of the culture and allowed to react for 10 min. The ammonia concentration was determined at 420 nm using a spectrophotometer and compared to a standard curve of ammonium sulfate. Indole acetic acid (IAA) production was measured following Fonseca et al. (2024). A 100 µL spore suspension of KKU215 was inoculated into NB with L-tryptophan concentrations of 1-5 mg/mL. The culture was incubated at 37 °C with shaking (160 rpm), and samples were collected every 2 days for 15 days. After centrifugation at 5,635 × g for 10 min, Salkowski's reagent was added to the supernatant and incubated in the dark for 30 min. IAA concentration was measured at 535 nm and compared to a standard curve.

SEEDLING GROWTH

This study was conducted in October 2024 in an open greenhouse with temperatures ranging from 23.2 °C to 32.4 °C and relative humidity between 55.9% and 90.5%. Tomato seeds were surface-sterilized with 1% sodium hypochlorite for 2 min, followed by three rinses with sterile distilled water. Fifteen seeds per treatment were divided into three replicates (trays), with five seeds sown in each tray. Seeds were soaked in a spore suspension of KKU215 or sterile distilled water (control) for 30 min (Hernandez-Herrera et al. 2016) before being sown in trays with 50 g of sterile peat moss without fertilizer. After 30 days, shoot and root lengths were measured with a ruler, and fresh and dry weights were determined using a digital balance.

POT EXPERIMENTS

The experiment was conducted from November to December 2024 in an open greenhouse with temperatures ranging from 18.9°C to 30.6°C and relative humidity between 48.9% and 90.7%. Twenty-one-day-old tomato seedlings were transplanted into 1.2 kg of sterile peat moss per pot, supplemented with 1.2 g of 15:15:15 fertilizer. Treatments were T1, KKU215 only; T2, KKU215+RsDOA-BCC1954 (biocontrol treatment); T3, no KKU215 and no RsDOA-BCC1954 (uninoculated control); and T4, RsDOA-BCC1954 only (pathogen control).

The pot experiment was arranged in a Completely Randomized Design (CRD) with 4 treatments and 10 replicate pots per treatment. The total number of experimental units was 40 pots, and each pot served as one experimental unit. A 50 mL KKU215 spore suspension was applied on days 0, 7, 14, 21, and 35. On day 42, 50 mL of RsDOA-BCC1954 suspension (OD600 = 0.5) was applied to the root zone. At 56 days, disease index (DI) and plant growth parameters (shoot/root lengths, fresh/dry weights) were recorded. The DI was assessed once at 56 days post-transplantation using the scale (0-4) and formula from Wu et al. (2016). The scale defined symptoms as 0 = no symptoms; 1 = 1-25% wilting; 2 = 26-50% wilting; 3 = 51-75% wilting; and 4 = 76-100% wilting or dead. DI was calculated using the formula:

$$DI = \sum(\text{number of diseased plants in this index} \times \text{di}) / (\text{total number of plants investigated} \times \text{highest di}) \times 100$$

OPTIMIZATION OF SPORE PRODUCTION

Six solid culture media were evaluated for their effectiveness in supporting KKU215 spore production. All media were prepared with 1.5% (w/v) agar, and their respective compositions (% w/v) were as follows: ATCC medium: 0.1 g tryptose, 0.1 g beef extract, 0.1 g yeast extract, 1.0 g glucose, and 0.01 g $FeSO_4$ (Dionigi et al. 1992), half-strength potato dextrose medium (½ PDA) (1.2 g potato dextrose broth powder (Difco™, France)) (Chouychai et al. 2022), inorganic salt starch medium (ISS) (1.0 g starch, 0.1 g K_2HPO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.2 g $(NH_4)_2SO_4$, 0.2 g $CaCO_3$, 0.01 g $FeSO_4$ $7H_2O$, 0.01 g $MnCl^{2} \cdot 7H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$) (Aoki et al. 2007), NA medium (2.8 g nutrient agar powder (Himedia™) (Yottakot & Leelavatcharamas 2019), glucose yeast-malt medium (GYM) (0.2 g $CaCO_3$, 1.0 g malt extract, 0.4 g yeast extract, 0.4 g glucose) (Boukaew et al. 2022), and modified Wakimoto's medium (MWM) (30 g potato, 0.5 g peptone, 2 g sucrose, 0.05 g $Ca(NO_3)_2$, 0.2 g $Na_2HPO_4 \cdot 2H_2O$ (Adhikari, Mew & Leach 1999). Potato infusion for MWM was prepared by boiling 30 g of diced potato in 100 mL distilled water for 15 min. A 100 µL suspension of KKU215 (~10^6 spores/plate on day 0) was spread onto each medium and incubated at 37 °C; spore numbers were counted on days 5, 10, 15, and 20 using a hemacytometer.

The PB design, based on Liu et al. (2020), evaluated seven variables: beef extract, peptone, potato, sucrose, $Ca(NO_3)_2$, $Na_2HPO_4 \cdot 2H_2O$ (selected from MWM and NA media), and incubation time. These variables were assessed for their effects on KKU215 spore production. The MWM and NA media, as well as incubation time, played a key role in achieving the highest spore production. Each variable was tested at three levels: low (-), central (0), and high (+), to ensure replication and accuracy. The levels were beef extract 1.0, 3.0, and 5.0 g/L; peptone 3.0, 5.0, and 7.0 g/L; potato 100.0, 300.0, and 500.0 g/L; sucrose 10.0, 20.0, and 30.0 g/L; $Ca(NO_3)_2$ 0.1, 0.5, and 0.9 g/L; $Na_2HPO_4 \cdot 2H_2O$ 1.0, 2.0, and 3.0 g/L; and incubation time 10, 15, and 20 days. A 15-run PB design was used (Table 1), with

each run conducted in triplicate, and statistical analysis (ρ-value < 0.05) with a Pareto chart determined key factors. Significant factors were further optimized using the Box-Behnken (BB) design. The starter culture and incubation temperature were fixed at 1% spore suspension and 37 °C, respectively.

BB design in RSM is a widely used technique for optimizing process parameters (Liu et al. 2025). In this study, the BB design was applied to determine optimal parameters for KKU215 spore production. Three significant variables (p-value < 0.05) determined from the PB design, peptone (X_1), potato (X_2), and incubation time (X_3), were further investigated at low (-), central (0), and high (+) levels corresponding to 3.0, 7.0, and 11.0 g/L for peptone; 300.0, 500.0, and 700 g/L potato infusion equivalent; and 10, 15, and 20 days for incubation time, respectively, for a total of 17 experimental runs. All runs are being performed in triplicate, including five center point replicates (Table 2). The potato infusion for each run was prepared by boiling the specified amount of fresh, peeled, and diced potato (corresponding to 300, 500, or 700 g) in 1 L of distilled water for 20 min. The resulting infusion was filtered through two layers of cheesecloth, and the volume of the filtrate was adjusted to exactly 1 L with distilled water before being used as the main component of the culture medium for each run. The experiment conditions followed those used in the screening experiment. A quadratic model was constructed from experimental data to describe the effects of these variables on spore production. The predicted optimal conditions were validated experimentally, confirming the model's accuracy.

STATISTICAL ANALYSIS

Data were analysed using SPSS version 29. An independent t-test was used to compare the means of seedling growth parameters, while one-way ANOVA with Tukey's HSD post-hoc test was applied to data from pot experiments and spore production assays. PB and BB design data were analysed using the trial version 23.1.8 (64-bit) of Stat-Ease® 360 (Stat-Ease, MN, USA). A one-sample t-test was conducted to evaluate the difference between the experimental mean spore count under optimized conditions and the corresponding predicted value obtained from the model. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

BIOCONTROL AND PLANT GROWTH–PROMOTING TRAITS

KKU215 exhibited multiple biocontrol and plant growth-promoting traits, including five extracellular lytic enzyme activities, siderophore production, biofilm formation (Figure 1), nitrogen fixation, ammonia production, and IAA production (Figure 2). Maximum IAA and ammonia levels reached 13.03 µg/mL (with 5 mg/mL tryptophan) and 1.43 µmol/mL (after 7 days), respectively. These traits support KKU215's potential as a biocontrol and growth-promoting agent. Similarly, Awla et al. (2017) reported ammonia, siderophore, amylase, protease, and lipase production by *Streptomyces* UPMRS4, while Kaari et al. (2022a) found biofilm formation in several *Streptomyces* strains. Kaari et al. (2022a) and Vurukonda, Giovanardi & Stefan (2018) further emphasized the role

TABLE 1. PB design and results

Std	Run	Be (g/L)	Pe (g/L)	Po (g/L)	Su (g/L)	Ca (g/L)	Di (g/L)	In (day)	Number of spore (spores/plate)
14	1	3.0	5.0	300.0	20.0	0.5	2.0	15	5.012×10^9
13	2	3.0	5.0	300.0	20.0	0.5	2.0	15	4.956×10^9
1	3	5.0	7.0	100.0	30.0	0.9	3.0	10	1.900×10^7
7	4	5.0	3.0	100.0	10.0	0.9	1.0	20	2.100×10^7
10	5	1.0	7.0	500.0	30.0	0.1	1.0	10	2.900×10^9
11	6	5.0	3.0	500.0	30.0	0.9	1.0	10	1.800×10^8
9	7	5.0	7.0	500.0	10.0	0.1	1.0	20	9.950×10^9
3	8	5.0	3.0	500.0	30.0	0.1	3.0	20	2.400×10^8
4	9	1.0	7.0	100.0	30.0	0.9	1.0	20	1.980×10^9
2	10	1.0	7.0	500.0	10.0	0.9	3.0	20	9.898×10^9
12	11	1.0	3.0	100.0	10.0	0.1	1.0	10	1.100×10^7
6	12	1.0	3.0	100.0	30.0	0.1	3.0	20	3.030×10^7
5	13	1.0	3.0	500.0	10.0	0.9	3.0	10	9.800×10^8
15	14	3.0	5.0	300.0	20.0	0.5	2.0	15	5.120×10^9
8	15	5.0	7.0	100.0	10.0	0.1	3.0	10	1.500×10^7

The abbreviations Be, Pe, Po, Su, Ca, Di, and In represent Beef extract, Peptone, Potato, Sucrose, $Ca(NO_3)_2$, $Na_2HPO_4 \cdot 2H_2O$ and Incubation time, respectively

TABLE 2. BB design and results

Std	Run	Peptone (g/L)	Potato (g/L)	Incubation time (day)	Number of spore (spores/plate)	
					Experiment	Predicted
5	1	3.0	500.0	10	1.257×10^9	1.489×10^9
13	2	7.0	500.0	15	2.263×10^9	2.143×10^9
14	3	7.0	500.0	15	2.160×10^9	2.143×10^9
17	4	7.0	500.0	15	1.912×10^9	2.143×10^9
15	5	7.0	500.0	15	2.065×10^9	2.143×10^9
7	6	3.0	500.0	20	9.987×10^9	1.006×10^{10}
16	7	7.0	500.0	15	2.317×10^9	2.143×10^9
3	8	3.0	700.0	15	4.780×10^9	4.447×10^9
1	9	3.0	300.0	15	2.560×10^9	2.579×10^9
10	10	7.0	700.0	10	1.440×10^9	1.541×10^9
2	11	11.0	300.0	15	5.130×10^9	5.463×10^9
11	12	7.0	300.0	20	1.130×10^{10}	1.120×10^{10}
12	13	7.0	700.0	20	1.300×10^{10}	1.325×10^{10}
8	14	11.0	500.0	20	1.510×10^{10}	1.487×10^{10}
6	15	11.0	500.0	10	1.510×10^9	1.428×10^9
4	16	11.0	700.0	15	6.320×10^9	6.300×10^9
9	17	7.0	300.0	10	1.142×10^9	8.901×10^8

of *Streptomyces* spp. in biocontrol and plant growth promotion via siderophores, lytic enzymes, IAA, ammonia, and nitrogen fixation.

SEEDLING GROWTH

On day 30, tomato seedlings from seeds treated with KKU215 spores showed significantly greater shoot and root lengths (15.21 cm and 16.65 cm), as well as higher fresh and dry weights (29.38 mg and 2.12 mg), compared to the control (Figure 3(A) and 3(B)). The leaf size was also larger (Figure 3(C)). Similarly, *Streptomyces rochei* BT02 has also significantly stimulated shoot and root lengths, and fresh and dry weights of tomato seedlings (Devi, Sharma & Manhas 2022).

POT EXPERIMENT

At 56 days after planting, treatment with KKU215 spores (T2) significantly reduced the severity of bacterial wilt caused by RsDOA-BCC1954, resulting in a disease index of 12.48%, compared to the pathogen control (T4), which exhibited a disease index of 95.75% (Figure 4(A)). In T4, disease symptoms appeared within 7 days, starting with shoot tip wilting and progressing without leaf yellowing (Figure 4(D)). This study suggests that the ability of KKU215 to control BW disease may be attributed to its production of a variety of enzymes, including lipase, amylase, protease, cellulase, and chitinase, as well as siderophores and biofilms. Kaari et al. (2022a), Lee et al. (2023), and Tan et al. (2006), found that these compounds effectively inhibit the growth of Rs, thereby controlling bacterial wilt disease. These findings align with other studies on *Streptomyces* species, which have shown a role in disease prevention. Enzymes produced by *Streptomyces* species help break down complex molecules and inhibit pathogens, and siderophores sequester iron, depriving pathogens of this essential nutrient (Khan et al. 2023), while biofilms produced by *Streptomyces* species inhibit pathogens by forming protective barriers around plant roots and surfaces, preventing pathogen colonization (Kaari et al. 2022b). Kaari et al. (2022a) found that *Streptomyces* sp. UT4A49 produces amylase, protease, cellulase, and siderophores, which inhibit the pathogen Rs.

KKU215 also significantly promoted tomato growth, as indicated by increased shoot and root lengths, as well as greater fresh and dry biomass in treatments T1 and T2, compared to the controls (T3 and T4) (Figures 4(B), 4(C)). This growth enhancement may be attributed to the abilities of KKU215 in nitrogen fixation, ammonia production, and IAA synthesis, i.e., factors that potentially stimulate plant growth. Similarly, Devi, Sharma and Manhas (2022) found that *Streptomyces* sp. SP5 significantly enhances shoot and root length, as well as the fresh and dry weight of plants through IAA production, while Du et al. (2022) demonstrated that *Streptomyces albidoflavus* St-220 possesses nitrogen fixation, which significantly increases

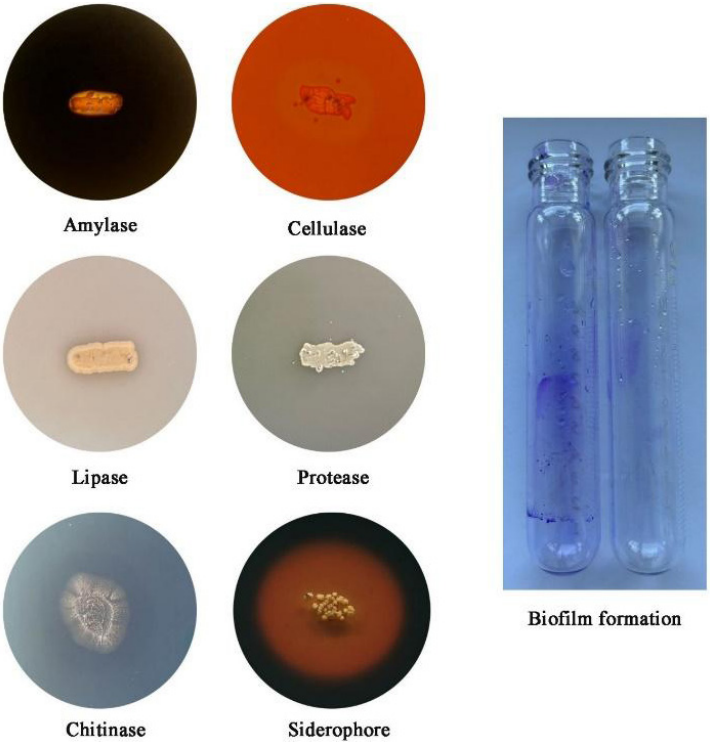

FIGURE 1. Characterization of extracellular lytic enzymes, siderophores, and biofilm production by KKU215

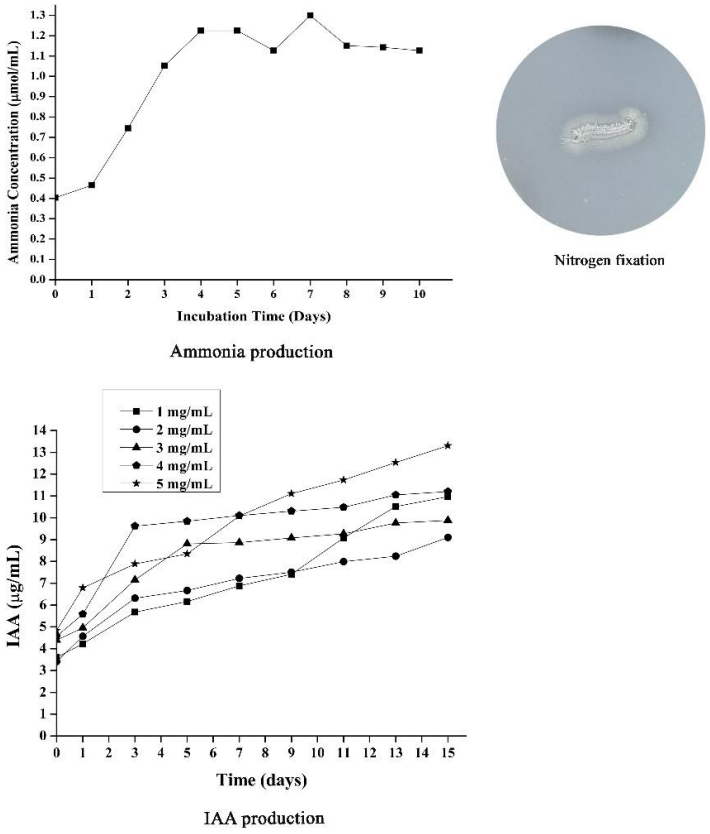

FIGURE 2. Characterization of nitrogen fixation, ammonia, and IAA production by KKU215

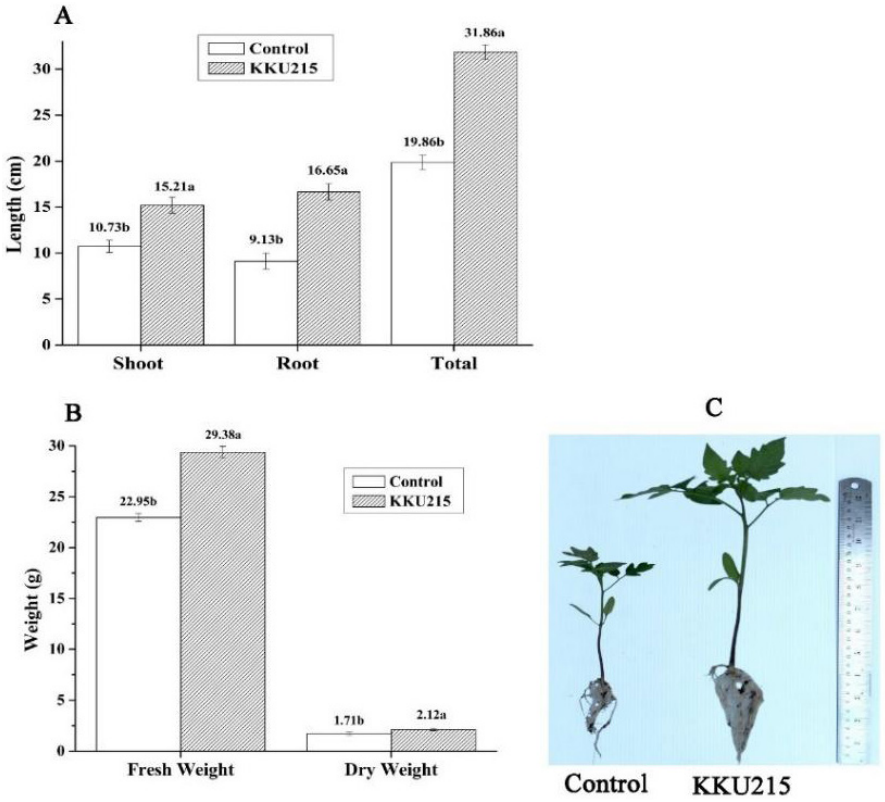

FIGURE 3. Effect of KKU215 on tomato seedling growth 30 days after planting in tray experiment. (A) shoot and root lengths, (B) fresh and dry weights, and (C) photographs of seedlings

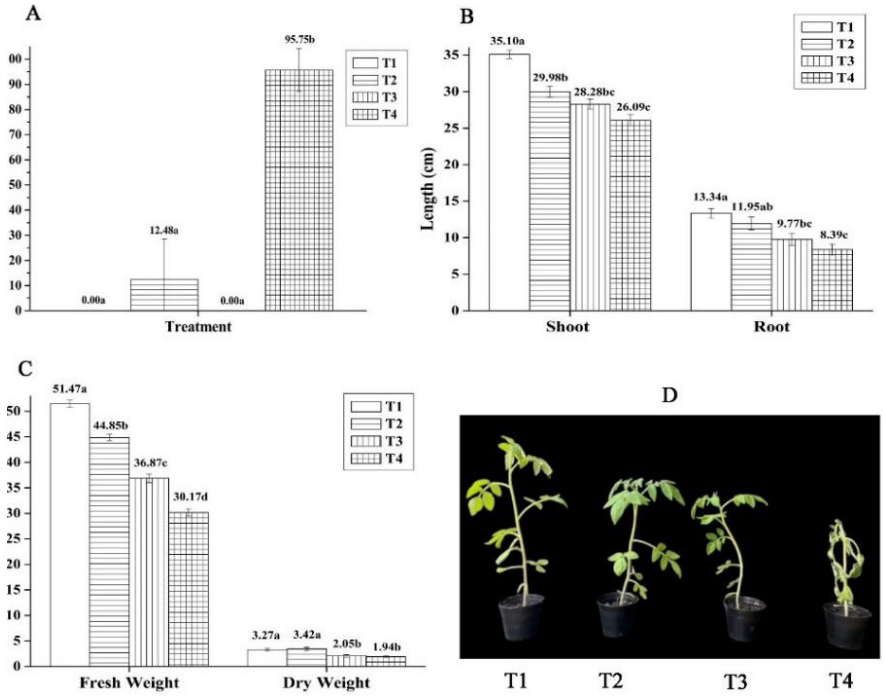

FIGURE 4. Efficacy of KKU215 in controlling BW disease and promoting tomato growth 56 days after planting in a pot experiment. (A) disease index, (B) shoot and root lengths, (C) fresh and dry weights, and (D) photographs of seedlings

the total fresh and dry weight of plant growth. These findings align with other studies that highlight the role of *Streptomyces* species in promoting plant growth. Nitrogen fixation and ammonia production increase the availability of nitrogen, an essential nutrient for plant growth, while IAA promotes plant development by enhancing cell elongation and root growth (Vurukonda, Giovanardi & Stefan 2018).

OPTIMIZATION OF PARAMETERS FOR ENHANCING SPORE PRODUCTION

Spore production by KKU215 was significantly affected by the type of medium and incubation time. Spore production increased rapidly within the first 10 days in most media, except for ISS and ½ PDA, which exhibited minimal increases. This was likely due to the lower nutrient content in these media, which are insufficient to support robust sporulation compared to nutrient-rich media. From day 10 to day 20, spore production continued to rise steadily. After 20 days of incubation, MWM and NA media supported the highest spore yields ($9.51 \pm 0.18 \times 10^9$ and $9.28 \pm 0.21 \times 10^9$ spores/plate, respectively), significantly higher than other media ($p < 0.05$) (Table 3). The study showed that MWM and NA medium, and incubation time all influenced spore production. The six media components (beef extract, peptone, potato, sucrose, $Ca(NO_3)_2$, and $Na_2HPO_4 \cdot 2H_2O$), along with incubation time, were selected for screening using the PB design to identify key factors affecting spore production.

The PB design was used to screen seven factors affecting the culture conditions of KKU215. In Table 4, Analysis of Variance (ANOVA) indicated that peptone (p-value = 0.0066), potato (p-value = 0.0085), sucrose (p-value = 0.0352), and incubation time (p-value = 0.0200) are significant factors (p-value < 0.05). The Pareto chart of standardized effects (Figure 5) showed that peptone, potato, and incubation time significantly exceeded the reference line, confirming their statistical significance ($p < 0.05$) in enhancing spore production. These factors were therefore selected for further optimization. In contrast, sucrose, which had a negative effect, was excluded from the optimization process. Consequently, peptone, potato, and incubation time were selected for further optimization using the RSM with BB design.

Peptone, a rich nitrogen source, plays a crucial role in promoting bacterial growth and spore formation (Hsieh & Labbe 2007; Liggins, Ramírez & Abel-Santos 2023). Potato serves as a source of both carbohydrates and minerals such as Ca, Fe, and Mg (Beals 2019), which are present in adequate amounts to support the transition to sporulation in *Streptomyces* (Yague et al. 2013). Incubation time increases the possibility of sporulation by allowing bacteria to undergo the transition from vegetative growth to the sporulation phase (Aouadhi et al. 2016). Meanwhile, sucrose was excluded from further optimization due to its negative effect on spore production. This may be attributed to the fact that sucrose acts as a single carbon source, whereas potato provides not only carbohydrates but also essential minerals, and peptone acts as a complex nitrogen source, thereby supporting spore formation more effectively.

Following the PB design results, a BB design was carried out with 5 replicates at the central point. Peptone, potato, and incubation time were selected as independent variables in a three-factor, three-level test, with spore count as the response variable. A total of 17 experimental trials were performed, and the results are presented in Table 2. The regression analysis with quadratic terms, used to describe the relationship between the independent variables and the response variable, resulted in the following equation:

$$Y = 3.20475 \times 10^{10} - 1.67806 \times 10^9 X_1 - 2.85128 \times 10^7 X_2 - 3.605551 \times 10^9 X_3 - 3.21875 \times 10^5 X_1X_2 + 6.07531 \times 10^7 X_1X_3 + 3.50475 \times 10^5 X_2X_3 + 8.74152 \times 10^7 X_1^2 + 28888.90625 X_2^2 + 1.36865 \times 10^8 X_3^2$$

where Y represents the predicted number of spores, and X_1, X_2, and X_3 represent peptone, potato, and incubation time, respectively.

Table 5 shows that the ANOVA results indicate the model is highly significant ($p < 0.0001$), with no significant lack of fit ($p = 0.0540$), indicating a good fit between the model and the experimental data. The model's high R^2 (0.9982), Adjusted R^2 (0.9959), and Predicted R^2 (0.9759) values confirm its high accuracy and predictive power. Among the tested variables, peptone, potato, and incubation time had significant linear and quadratic effects on spore production ($p < 0.05$). Interaction between peptone and incubation time, as well as between potato and incubation time, also showed significant influence. In contrast, the interaction between peptone and potato was not significant ($p = 0.1209$), meaning that they were statistically insignificant in affecting the response. This suggests that changes in both factors may not have a significant statistical impact on spore production when assessed together.

The three-dimensional response surface (3D) plots and contour plots are demonstrated in Figure 6. Figure 6(A) presents the 3D and contour plots illustrating the interaction between peptone and potato. The plots show that increasing both components to their highest levels results in optimal spore production. High concentrations of peptone provide the necessary nitrogen, which supports rapid cell division and facilitates the transition into the stationary phase, where sporulation is triggered (Hsieh & Labbe 2007; Liggins, Ramírez & Abel-Santos 2023). Likewise, high levels of potato enhance bacterial proliferation by supplying carbohydrates and nutrients. As the population increases, the nutrients become limited, triggering sporulation. The 3D and contour plots in Figure 6(B) (peptone and incubation time) and Figure 6(C) (potato and incubation time) show that higher levels of either peptone or potato, when combined with longer incubation time, significantly enhance spore production. This prolonged incubation time increases the possibility of sporulation by allowing bacteria to undergo the transition from vegetative growth to the sporulation phase (Aouadhi et al. 2016).

TABLE 3. Spore production of KKU215 under various media and incubation times

Media	Number of spore (spores/plate)				
	0 Day	5 Day	10 Day	15 Day	20 Day
MWM	1×10^6	$(9.13 \pm 0.19) \times 10^8$ a	$(1.89 \pm 0.03) \times 10^9$ a	$(6.23 \pm 0.14) \times 10^9$ a	$(9.51 \pm 0.18) \times 10^9$ a
NA	1×10^6	$(2.61 \pm 0.12) \times 10^8$ b	$(1.66 \pm 0.09) \times 10^9$ b	$(6.02 \pm 0.09) \times 10^9$ b	$(9.28 \pm 0.21) \times 10^9$ a
GYM	1×10^6	$(1.04 \pm 0.14) \times 10^8$ c	$(3.48 \pm 0.35) \times 10^8$ c	$(5.34 \pm 0.36) \times 10^8$ c	$(6.17 \pm 0.07) \times 10^8$ b
ATCC	1×10^6	$(1.69 \pm 0.12) \times 10^7$ d	$(4.21 \pm 0.46) \times 10^7$ d	$(3.06 \pm 0.22) \times 10^8$ d	$(5.16 \pm 0.10) \times 10^8$ bc
ISS	1×10^6	$(3.38 \pm 0.23) \times 10^6$ d	$(7.84 \pm 0.79) \times 10^6$ d	$(1.25 \pm 0.10) \times 10^8$ de	$(2.30 \pm 0.09) \times 10^8$ cd
½ PDA	1×10^6	$(1.77 \pm 0.15) \times 10^6$ d	$(5.18 \pm 0.13) \times 10^6$ d	$(1.65 \pm 0.16) \times 10^7$ e	$(2.83 \pm 0.13) \times 10^7$ d

Different letters within each column indicate statistically significant differences between means ($p < 0.05$)

TABLE 4. ANOVA for PB design results

Source	Sum of squares	Df.	Mean square	F-value	ρ-value
Beef extract	2.407×10^{18}	1	2.407×10^{18}	0.8787	0.3847
Peptone	4.523×10^{19}	1	4.523×10^{19}	16.51	0.0066*
Potato	4.060×10^{19}	1	4.060×10^{19}	14.82	0.0085*
Sucrose	2.009×10^{19}	1	2.009×10^{19}	7.33	0.0352*
$Ca(NO_3)_2$	3.831×10^{14}	1	3.831×10^{14}	0.0001	0.9909
$Na_2HPO_4 \cdot 2H_2O$	1.241×10^{18}	1	1.241×10^{18}	0.4530	0.5260
Incubation time	2.703×10^{19}	1	2.703×10^{19}	9.87	0.0200*
Residual	1.644×10^{19}	6			
Corrected total	1.724×10^{20}	14			

*Represents significant, Df: degree of freedom

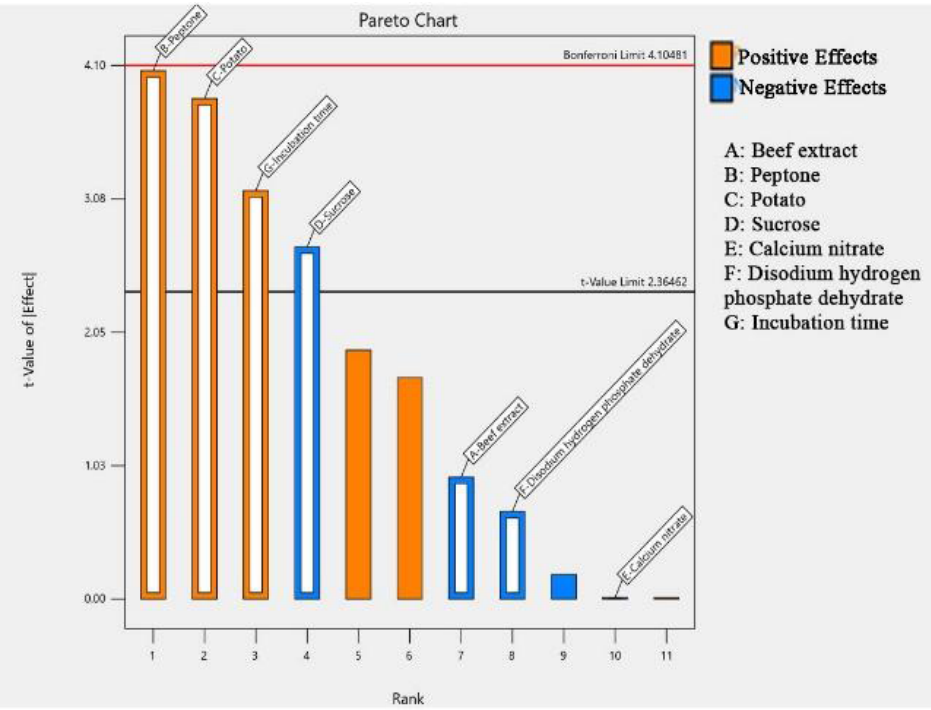

FIGURE 5. Pareto chart of standardized effects on KKU215 spore production

TABLE 5. ANOVA for BB design results

Source	Sum of squares	Df.	Mean square	F-value	ρ-value
Model	3.324×10^{20}	9	3.693×10^{19}	433.85	< 0.0001*
Peptone	1.122×10^{19}	1	1.122×10^{19}	131.86	< 0.0001*
Potato	3.656×10^{18}	1	3.656×10^{18}	42.95	0.0003*
Incubation time	2.424×10^{20}	1	2.424×10^{20}	2847.97	< 0.0001*
Peptone*Potato	2.652×10^{17}	1	2.652×10^{17}	3.12	0.1209
Peptone*Incubation time	5.906×10^{18}	1	5.906×10^{18}	69.38	< 0.0001*
Potato*Incubation time	4.913×10^{17}	1	4.913×10^{17}	5.77	0.0473*
Peptone*Peptone	8.237×10^{18}	1	8.237×10^{18}	96.77	< 0.0001*
Potato*Potato	5.622×10^{18}	1	5.622×10^{18}	66.05	< 0.0001*
Incubation time* Incubation time	4.929×10^{19}	1	4.929×10^{19}	579.13	< 0.0001*
Residual	5.958×10^{17}	7	8.512×10^{16}		
Lack of Fit	4.915×10^{17}	3	1.638×10^{17}	6.28	0.0540
Pure Error	1.043×10^{17}	4	2.607×10^{16}		
Cor Total	3.330×10^{20}	16			
$R^2 = 0.9982$ Adjusted $R^2 = 0.9959$ Predicted $R^2 = 0.9759$					

*Represents significant, Df: degree of freedom

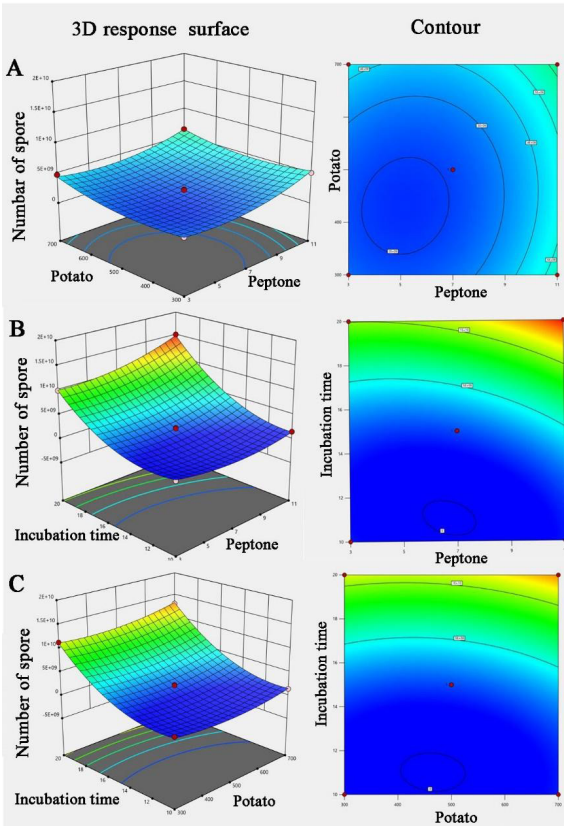

FIGURE 6. The 3D plots and contour plots showing the effect of (A) potato and peptone, (B) incubation time and peptone, and (C) incubation time and peptone

CONCLUSIONS

This study highlights the biocontrol and plant growth-promoting potential of KKU215. The strain produced extracellular lytic enzymes, siderophores, biofilms, IAA, and ammonia, and fixed nitrogen, supporting its biocontrol and plant growth-promoting roles. KKU215 reduced BW disease index and improved tomato growth, as shown by increased shoot and root lengths, as well as fresh and dry weights in tray and pot experiments. MWM and NA media yielded the highest spore production of KKU215 ($p < 0.05$), and their components were screened using PB design, showing peptone, potato, and incubation time as key factors. RSM optimization showed the optimal conditions: 7 g/L peptone, 700 g/L potato infusion equivalent, and 20 days of incubation, resulting in a 46% increase in spore yield, closely matching predicted values. This study demonstrates the potential of KKU215 as a sustainable biocontrol agent and plant growth promoter by effectively reducing the BW disease incidence and enhancing tomato seedling growth. Additionally, it showed high spore production under optimized culture conditions. These findings provide a strong foundation for developing KKU215-based bioproducts for agricultural applications. Future research should focus on field trials and commercial formulation development to promote sustainable disease management and reduce chemical pesticide use in crop production.

ACKNOWLEDGEMENTS

This study was funded by Thailand Science Research and Innovation (TSRI) and Sakon Nakhon Rajabhat University, Thailand.

REFERENCES

Adhikari, T.B., Mew, T.W. & Leach, J.E. 1999. Genotypic and pathotypic diversity in *Xanthomonas oryzae* pv. *oryzae* in Nepal. *Phytopathology* 89(8): 687-694.

Ajijah, N., Fiodor, A., Pandey, A.K., Rana, A. & Pranaw, K. 2023. Plant growth-promoting bacteria (PGPB) with biofilm-forming ability: A multifaceted agent for sustainable agriculture. *Diversity* 15(1): 112.

Akarapisan, A., Kumvinit, A., Nontaswatsri, C., Puangkrit, T. & Kositratana, W. 2021. Phylotype, sequevar and pathogenicity of *Ralstonia solanacearum* species complex from Northern Thailand. *Journal of Phytopathology* 170(3): 176-184.

Aoki, Y., Yoshida, M., Kawaide, H., Abe, H. & Natsume, M. 2007. Isolation and characterization of a spore germination inhibitor from *Streptomyces* sp. CB-1-1, a phytopathogen causing root tumor of melon. *Bioscience, Biotechnology, and Biochemistry* 71(4): 986-992.

Aouadhi, C., Rouissi, Z., Kmiha, S., Mejri, S. & Maarouf, A. 2016. Effect of sporulation conditions on the resistance of *Bacillus sporothermodurans* spores to nisin and heat. *Food Microbiology* 54: 6-10.

Awla, H.K., Kadir, J., Othman, R., Rashid, T.S., Hamid, S. & Wong, M.Y. 2017. Plant growth-promoting abilities and biocontrol efficacy of *Streptomyces* sp. UPMRS4 against *Pyricularia oryzae. Biological Control* 112: 55-63.

Beals, K.A. 2019. Potatoes, nutrition and health. *American Journal of Potato Research* 96: 102-110.

Boukaew, S., Chuenchit, S. & Petcharat, V. 2011. Evaluation of *Streptomyces* spp. for biological control of *Sclerotium* root and stem rot and *Ralstonia* wilt of chili pepper. *BioControl* 56: 365-374.

Boukaew, S., Yossan, S., Cheirsilp, B. & Prasertsan, P. 2022. Impact of environmental factors on *Streptomyces* spp. metabolites against *Botrytis cinerea. Journal of Basic Microbiology* 62(5): 611-622.

Chouychai, W., Sangdee, A., Phunee, A., Senarit, P. & Somtrakoon, K. 2022. Using *Streptomyces* spp. as plant growth-promoting inoculants for growth of napier grass under low water system. *Pertanika Journal of Tropical Agricultural Science* 45(2): 491-504.

Devi, S., Sharma, M. & Manhas, R.K. 2022. Investigating the plant growth promoting and biocontrol potentiality of endophytic *Streptomyces* sp. SP5 against early blight in *Solanum lycopersicum* seedlings. *BMC Microbiology* 22: 285.

Dionigi, C.P., Millie, D.F., Spanier, A.M. & Johnsen, P.B. 1992. Spore and geosmin production by *Streptomyces tendae* on several media. *Journal of Agricultural and Food Chemistry* 40: 122-125.

Du, Y., Wang, T., Jiang, J., Wang, Y., Lv, C., Sun, K., Sun, J., Yan, B., Kang, C., Guo, L. & Huang, L. 2022. Biological control and plant growth promotion properties of *Streptomyces albidoflavus* St-220 isolated from *Salvia miltiorrhiza* rhizosphere. *Frontiers in Plant Science* 13: 976813.

Elsharkawy, M.M., Nakatani, M., Nishimura, M., Arakawa, T., Shimizu, M. & Hyakumachi, M. 2015. Control of tomato bacterial wilt and root-knot diseases by *Bacillus thuringiensis* CR-371 and *Streptomyces avermectinius* NBRC14893. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science* 65(6): 575-580.

Fahsi, N., Mahdi, I., Mesfioui, A., Biskri, L. & Allaoui, A. 2021. Plant growth-promoting rhizobacteria isolated from the Jujube (*Ziziphus lotus*) plant enhance wheat growth, Zn uptake, and heavy metal tolerance. *Agriculture* 11(4): 316.

Fonseca, J.S., Sousa, T.F., Almeida, S.V.R., Silva, C.N., Castro, G.S., Yamagishi, M.E.B., Koolen, H.H.F., Hanada, R.E. & Silva, G.F. 2024. Amazonian bacteria from river sediments as a biocontrol solution against *Ralstonia solanacearum. Microorganisms* 12: 1364.

Hasan, M.M., Marzan, L.W., Hosna, A., Hakim, A. & Azad, A.K. 2017. Optimization of some fermentation conditions for the production of extracellular amylases by using *Chryseobacterium* and *Bacillus* isolates from organic kitchen wastes. *Journal of Genetic Engineering and Biotechnology* 15: 59-68.

He, D., Gao, C., Zhao, S., Chen, H., Li, P., Yang, X., Li, D., Zhao, T., Jiang, H. & Liu, C. 2024. Antibacterial, herbicidal, and plant growth-promoting properties of *Streptomyces* sp. STD57 from the rhizosphere of *Adenophora stricta. Microorganisms* 12(11): 2245.

Hernandez-Herrera, R.M., Santacruz-Ruvalcaba, F., Zanudo-Hernandez, J. & Hernandez-Carmona, G. 2016. Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. *Journal of Applied Phycology* 28: 2549-2560.

Hsieh, P.Y. & Labbe, R. 2007. Influence of peptone source on sporulation of *Clostridium perfringens* type A. *Journal of Food Protection* 70(7): 1730-1734.

Ilesanmi, O.I., Abiodun, A.E., Omolaiye, J.A., Olorode, E.M. & Ogunkanmi, A.L. 2020. Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. *Scientific African* 8: e00279.

Jain, K., Parida, S., Mangwani, N., Dash, H.R. & Das, S. 2013. Isolation and characterization of biofilm-forming bacteria and associated extracellular polymeric substances from oral cavity. *Annals of Microbiology* 63: 1553-1562.

Kaari, M., Joseph, J., Manikkam, R., Sreenivasan, A. & Venugopal, G. 2022a. Biological control of *Streptomyces* sp. UT4A49 to suppress tomato bacterial wilt disease and its metabolite profiling. *Journal of King Saud University - Science* 34: 101688.

Kaari, M., Joseph, J., Manikkam, R., Sreenivasan, A., Venugopal, G., Alexander, B. & Krishnan, S. 2022b. Anti-biofilm activity and biocontrol potential of *Streptomyces* cultures against *Ralstonia solanacearum* on tomato plants. *Indian Journal of Microbiology* 62(1): 32-39.

Khan, S., Srivastava, S., Karnwal, A. & Malik, T. 2023. *Streptomyces* as a promising biological control agents for plant pathogens. *Frontiers in Microbiology* 14: 1285543.

Kifle, M.H. & Laing, M.D. 2016. Isolation and screening of bacteria for their diazotrophic potential and their influence on growth promotion of maize seedlings in greenhouses. *Frontiers in Plant Science* 6: 1225.

Le, K.D., Kim, J., Nguyen, H.T., Yu, N.H., Park, A.R., Lee, C.W. & Kim, J.C. 2021. *Streptomyces* sp. JCK-6131 protects plants against bacterial and fungal diseases via two mechanisms. *Frontiers in Plant Science* 12: 726266.

Lee, J., Kim, S., Jung, H., Koo, B.K., Han, J.A. & Lee, S.H. 2023. Exploiting bacterial genera as biocontrol agents: Mechanisms, interactions and applications in sustainable agriculture. *Journal of Plant Biology* 66: 485-498.

Li, F., Xie, Y., Gao, X., Shan, M., Sun, C., Niu, Y.D. & Shan, A. 2020. Screening of cellulose degradation bacteria from min pigs and optimization of its cellulase production. *Electronic Journal of Biotechnology* 48: 29-35.

Li, Y., Narayanan, M., Shi, X., Chen, X., Li, Z. & Ma, Y. 2024. Biofilms formation in plant growth-promoting bacteria for alleviating agro-environmental stress. *Science of the Total Environment* 907: 167774.

Liggins, M., Ramírez, N.R. & Abel-Santos, E. 2023. Comparison of sporulation and germination conditions for *Clostridium perfringens* type A and G strains. *Frontiers in Microbiology* 14: 1143399.

Ling, L., Han, X., Li, X., Zhang, X., Wang, H., Zhang, L., Cao, P., Wu, Y., Wang, X., Zhao, J. & Xiang, W. 2020. A *Streptomyces* sp. NEAU-HV9: Isolation, identification, and potential as a biocontrol agent against *Ralstonia solanacearum* of tomato plants. *Microorganisms* 8(3): 351.

Liu, H., Zhang, D., Zhang, X., Zhou, C., Zhou, P. & Zhi, Y. 2020. Medium optimization for spore production of a straw-cellulose degrading actinomyces strain under solid-state fermentation using response surface method. *Sustainability* 12: 8893.

Liu, X., Martin, J.J.J., Li, X., Zhou, L., Li, R., Li, Q., Zhang, J., Fu, D. & Cao, H. 2025. Optimization of the fermentation culture conditions of *Bacillus amyloliquefaciens* ck-05 using response surface methodology. *Frontiers in Microbiology* 16: 1552645.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P. & Hens, L. 2016. Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Frontiers in Public Health* 4: 148.

Schwyn, B. & Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160(1): 47-56.

Shaikh, I.A., Turakani, B., Malpani, J., Goudar, S.V., Mahnashi, M.H., Al-Serwi, R.H., Ghoneim, M.M., El-Sherbiny, M., Mannasaheb, B.A., Alsaikhan, F., Sindagimath, V., Khan, A.A., Muddapur, U.M., Azzouz, S., Mohammed, T. & Shakeel Iqubal, S.M. 2023. Extracellular protease production, optimization, and partial purification from *Bacillus nakamurai* PL4 and its applications. *Journal of King Saud University - Science* 35(1): 102429.

Tan, H.M., Cao, L.X., He, Z.F., Su, G.J., Lin, B. & Zhou, S.N. 2006. Isolation of endophytic actinomycetes from different cultivars of tomato and their activities against *Ralstonia solanacearum in vitro. World Journal of Microbiology & Biotechnology* 22: 1275-1280.

Vailleau, F. & Genin, S. 2023. *Ralstonia solanacearum*: An arsenal of virulence strategies and prospects for resistance. *Annual Review of Phytopathology* 61: 25-47.

Vurukonda, S.S.K.P., Giovanardi, D. & Stefan, E. 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *International Journal of Molecular Sciences* 19(4): 952.

Wu, K., Fang, Z., Wang, L., Yuan, S., Guo, R. & Shen, Q. 2016. Biological potential of bioorganic fertilizer fortified with bacterial antagonist for the control of tomato BW and the promotion of crop yields. *Journal of Microbiology & Biotechnology* 26(10): 1755-1764.

Xia, J.L., Xiong, J., Zhang, R.Y., Liu, K.K., Huang, B. & Nie, Z.Y. 2011. Production of chitinase and its optimization from a novel isolate *Serratia marcescens* XJ-01. *Indian Journal of Microbiology* 51(3): 301-306.

Xue, Y., Yang, M., Li, S., Li, Z., Liu, H., Guo, Q. & Wang, C. 2019. The antibiotic activity and mechanisms of active metabolites (*Streptomyces alboflavus* TD-1) against *Ralstonia solanacearum. Biotechnology Letters* 41(10): 1213-1222.

Yague, P., Lopez-Garcia, M.T., Rioseras, B., Sanchez, J. & Manteca, A. 2013. Pre-sporulation stages of *Streptomyces* differentiation: State-of-the-art and future perspectives. *FEMS Microbiology Letters* 342(2): 79-88.

Yottakot, S. & Leelavatcharamas, V. 2019. Isolation and optimisation of polylactic acid (PLA)-packaging degrading actinomycete for PLA-packaging degradation. *Pertanika Journal of. Tropical Agricultural Sciences* 42(3): 1111-1130.

Zhuang, X., Gao, C., Peng, C., Wang, Z., Zhao, J., Shen, Y. & Liu, C. 2020. Characterization of a novel endophytic actinomycete, *Streptomyces physcomitrii* sp. nov., and its biocontrol potential against *Ralstonia solanacearum* on tomato. *Microorganisms* 8(12): 2025.

*Corresponding author; email: ssuvapa@hotmail.com