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Actinomycetes as Effective Biocontrol Agent Against Soft Rot Disease on Choy sum Caused by *Erwinia carotovora* pv. *carotovora*

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Abstract

Soft rot disease caused by *Erwinia carotovora* pv. *carotovora* severely affects Choy sum (*Brassica rapa* var. *parachinensis*) production, particularly in tropical regions such as Thailand. Conventional control methods rely heavily on chemical pesticides, raising concerns about environmental sustainability. This study aimed to explore indigenous Actinomycetes from rice field soil in Suphan Buri Province, Thailand, for their potential as biological control agents against soft rot disease and as plant growth promoters. Sixty actinomycete isolates were screened using the agar plug diffusion method, and four isolates (SPB1012, SPB1001, SPB1035, and SPB1022) exhibited significant antagonistic effects against *E. carotovora* pv. *carotovora*. Among them, SPB1012 demonstrated the highest inhibitory activity and effectively promoted growth parameters such as shoot height and stem diameter in greenhouse experiments with Choy sum. Molecular characterization revealed 100% sequence similarity between the isolates and *Streptomyces hygroscopicus* subsp. *hygroscopicus* NBRC 13472^T. These findings suggest that indigenous Actinomycetes from rice fields can serve as promising biocontrol agents for sustainable vegetable production, reducing reliance on chemical pesticides and contributing to environmentally friendly agricultural practices.

Keywords: Actinomycetes, *Erwinia carotovora* pv. *carotovora*, *Streptomyces hygroscopicus*, Soft rot disease, Biological control

1. Introduction

One of Chinese Mustard green; Chinese flowering cabbage; Choy sum (*Brassica rapa* var. *parachinensis*) is the vegetable within the family Brassicaceae. Soft rot disease, caused by *Erwinia carotovora* pv. *carotovora* (Ecc.), is among the most severe bacterial diseases affecting Brassica vegetables like Chinese Mustard greens, cauliflower, cabbage, Chinese cabbage, pak-choi, spinach, and carrot in tropical regions (Phokum et al., 2006). In Thailand soft rot disease was a major factor contributing to the destruction of economically important crops such as cruciferous and leafy vegetables. Each year, warnings were issued by the Ministry of Agriculture and Cooperatives (Thailand) to monitor the outbreak of soft rot during periods of continuous heavy rainfall, overcast skies, and limited sunlight. These conditions, combined with high humidity, created an environment conducive to the spread of the disease. This disease annually diminishes the quality and yield of vegetables, leading to significant crop losses of up to 55% in various nations. Symptoms typically manifest during the rainy season due to elevated humidity and temperature (Buensanteai et al., 2016).

The strategy focused on disease management and prevention to ensure crops were protected from soft rot disease. Biological control was identified as an effective method of reducing chemical usage, garnering significant interest due to its environmentally friendly approach. Current research emphasized the use of Actinomycetes, a group of bacteria known for their ability to produce various enzymes, such as β-1,3-glucanase, chitinase, cellulase, and protease (Araujo & Ramos, 2000). In addition to their effectiveness in suppressing pathogens, Actinomycetes

also promote plant growth by enhancing plant immunity. Actinomycetes are regarded as biocontrol agents because of their abilities in antifungal and antimicrobial synthesis, alongside their promotion of plant growth, known as plant growth-promoting bacteria (PGPB). PGPB can inhabit the soil surrounding the roots (rhizosphere), reside on the root surface, or within root cells (endophytes). Therefore, this group of bacteria can be referred to as Plant Growth-Promoting Rhizobacteria (PGPR) (Gratao et al., 2005).

According to the study by Rattanakavil (2020), Actinomycetes isolated from rice rhizosphere soils in Nakhon Sawan Province, located in central Thailand, exhibited considerable biodiversity. These isolates demonstrated antifungal activity against rice blast disease caused by *Pyricularia* sp. (CRI6000). Similarly, a study on endophytic actinobacteria from jasmine rice (*Oryza sativa* L. KDML 105) conducted in Roi-Et Province revealed that several *Streptomyces* isolates exhibited antimicrobial activity against rice pathogens such as *Xanthomonas oryzae* and *Pyricularia grisea*, despite the absence of typical biosynthetic genes like PKS-I, PKS-II, and NRPS, suggesting the presence of alternative mechanisms and their potential use as biocontrol agents (Kampapongsa & Kaewkla, 2016).

In this study, actinomycete strains were collected from rice field soil in Suphan Buri Province, and the evaluation for their ability to promote Choy sum (*Brassica rapa* var. *parachinensis*) growth and inhibit *E. carotovora* pv. *carotovora* in greenhouse conditions were performed. As rice cultivation in Thailand is predominantly practiced under wetland conditions, which have been shown to harbor unique and diverse Actinomycetes (Basik et al., 2020), the selected soil serves as a valuable source of strains with potential biocontrol and plant growth-promoting properties.

2. Materials and Methods

2.1 Isolation and preservation of Actinomycetes

2.1.1 Collection of soil samples

Soil samples were collected from a rice cultivation area in Suphan Buri. The organic rice field located at 78, Village No.2, Sa Kra Chom Subdistrict, Don Chedi District, Suphan Buri Province, Thailand (coordinates: 14º38'06.9"N, 99º53'57.3"E) after 60 days of crop growth on the 16th of October 2022. Sampling was conducted at seven designated points, each covering an area of 5 × 5 m. Approximately 500 g. of soil were taken from a depth of 1–2 cm. at each site and stored in clear plastic bags for subsequent analyses of soil acidity, soil temperature, and temperature. Each sampling location was thoroughly documented and processed at Department of Science, King Mongkut's Institute of Technology, Ladkrabang, in the laboratory within 24 hours after sampling. The samples were air-dried at room temperature for three days before analysis (Ogunmwonyi et al., 2008).

2.1.2 Isolation of Actinomycetes

The soil sample was divided into two parts. The first part was incubated (BINDER 1.2) at a temperature of 70°C, while the second part was baked at 100°C for 1 hour to compare the effects of the two temperatures on the isolation of microorganisms. The samples were finely ground using a mortar and pestle. Then, 1 gram of the sample was diluted in a 0.1% Tween 80 solution (Takizawa et al., 1993). The sample was further diluted to a level of 10^{-4} using a 0.85% NaCl solution. A 0.1 mL aliquot of the sample solution at dilutions of 10^{-2} to 10^{-4} was spread onto Zhang's Starch Soil Extract (ZSSE) agar (Zhang & Zhang, 2011), which was supplemented with 100 mg of the antibiotic Nystatin, and the pH was adjusted to 7.0–7.2. The agar plates were incubated (BINDER CONTROL E2) at 30°C for 7 days. The suspected Actinomycetes were subculture onto the International *Streptomyces* Project No. 2 (ISP2) medium using the streak plate technique. The Actinomycetes were preserved in 20% glycerol (w/v) of a cooler (Panasonic SF-PC1497) at -20°C for culture stock (Waksman, 1961).

2.1.3 Morphological characteristics of Actinomycetes

Actinomycetes were streaked onto International *Streptomyces* Project 2 (ISP2) media and incubated at 30°C for 7–14 days. Observations were made regarding growth characteristics, including the surface texture of the colonies, the color of aerial mycelium, the color of substrate mycelium, the color of spores, and the color of soluble pigments. These characteristics were compared to standard color charts from the ISCC-NBS System (Kelly, 1964). All actinomycete strains were examined for spore chain morphology under a light microscope (Leica DM750, Leica, Germany).

2.2 Antimicrobial activities

2.2.1 Actinomycetes with the ability to inhibit *E. carotovora* pv. *carotovora* using the agar plug diffusion method

Actinomycetes were prepared using the cross-streak method on ISP2 medium and incubated at 30°C for 7–14 days. The test strain *E. carotovora* pv. *carotovora* was obtained from a culture collection (Plant Protection Research and Development Office, Thailand) and then was prepared on Nutrient Agar (NA) and incubated at 30°C for 1 day. The test microorganism was mixed with a sterile 0.85% sodium chloride (NaCl) solution, and the turbidity of the test culture was adjusted to match the McFarland standard number 0.5 (approximately 1.0×10^8 CFU/mL). The NA medium was swabbed with the test culture using a sterile swab. A cork borer was used to punch out a sample of the Actinomycetes, which was then placed onto the swabbed NA medium. The plates were incubated at 30°C for 1 day, and the clear zone radius around the Actinomycetes was measured in duplicate (Balouiri et al., 2016).

2.3 Actinomycetes with inhibitory effects against *E. carotovora* pv. *carotovora* in Choy sum

2.3.1 Inhibitory effect on *E. carotovora* pv. *carotovora* in Choy sum (*Brassica rapa* var. *parachinensis*)

The most effective actinomycete isolate that could inhibit the pathogenic bacteria causing soft rot in Choy sum was selected. One isolate was tested for its disease control efficacy in Choy sum, with the experimental design divided into five treatments (T1–T5), each with 10 replications (Tsuda et al., 2016).

T1: Plants were watered with cell suspension of SPB1012.

T2: Plants were watered with cell suspension of SPB1012 and inoculated with *E.* pv. *carotovora*.

T3: Plants were watered with cell suspension of *E.* pv. *carotovora*.

T4: Plants were watered with cell suspension of *E. carotovora* pv. *carotovora* and treated with Funguran (CuOH) for plant disease protection.

T5: Plants were watered with distilled water (control group).

The experiment was conducted by planting Choy sum seeds in plastic pots for 7 days. The effectiveness of Actinomycetes in inhibiting the bacterial pathogen was tested. In T1 and T2, actinomycete broth was applied around the soil area at a volume of 20 mL + 20 mL of distilled water per plant, five times at 7-day intervals, specifically on days 7, 14, 21, 28, and 35. On day 36, wounds were made on the plant stems and treated with a culture of *E. carotovora* pv. *carotovora* in T2, T3, and T4 at a volume of 20 mL + 20 mL of distilled water per plant. Using a sterile toothpick, cutting wounds were made on the stems. In T4, plants were treated with chemical solutions five times at 7-day intervals, specifically on days 7, 14, 21, 28, and 35, with a volume of 20 mL + 20 mL of distilled water per plant. In T5 (the control group), cutting wounds on the stems were treated with 40 mL of distilled water per plant. The disease severity index (DSI) and protection value (PV) were assessed, and measurements were taken for plant height, root length, stem diameter, number of leaves, leaf width, leaf length, and fresh weight after inoculation with *E. carotovora* pv. *carotovora* for 3 days (Cui et al., 2019; Tsuda et al., 2016).

The severity level of the rot disease was assessed by dividing the symptoms into five levels (0–4) as follows:

Level 0: No symptoms were observed.

Level 1: Small water-soaked spots were present on the plant.

Level 2: Water-soaked spots were observed on parts of the plant.

Level 3: Widespread water-soaked and bruised spots were present throughout the plant.

Level 4: The plant was rotten, mushy, and exhibited a dark or brown color.

The severity levels of the disease were used to calculate the Disease Severity Index (DSI) and the Protection Value (PV).

$$\text{Disease Severity Index (DSI)} = \frac{\Sigma(number\ of\ plants\ showing\ symptoms\ X\ severity\ index) X\ 100}{4X\ total\ number\ of\ plants}$$

Protective Value (PV) = (DSI in control – DSI in treatment) × 100/DSI in control.

2.3.2 Preparation of Actinomycete culture medium

One actinomycete isolates with the best inhibitory effect against the bacteria pathogen was selected and cultured on ISP2 medium at 30°C for 7 days. A cork borer with a diameter of 5 mm was used to cut out agar pieces. The agar pieces were transferred into ISP2 broth and incubated on a shaker (Incubator shaker, OrbitTM 1900 High-Capacity Lab) at 110 rpm at 30°C for 7 days. Bacterial spore count was evaluated using a haemacytometer at 1×10^8 CFU/mL (Tsuda et al., 2016).

2.3.3 Preparation of *E. carotovora* pv. *carotovora*

E. carotovora pv. *carotovora* from Plant Protection Research and Development Office was cultured on Nutrient Agar (NA). cultured on NB broth and incubated at 30°C on a shaker at 150 rpm for 72 hours. The bacterial spore count was evaluated using a hemocytometer at 1×10^8 CFU/mL (Tsuda et al., 2016).

2.3.4 Preparation of Choy sum seedlings

Choy sum (*Brassica rapa* var. *parachinensis*) seeds were planted in plastic pots containing soil. The soil was sterilized using an autoclave (HV-25/50/85/110) twice, with a 24-hour interval between the first and second sterilization (Tsuda et al., 2016).

2.4 Genotypic characteristics of the selected Actinomycetes strain by 16S rRNA gene analysis

2.4.1 DNA extraction and the amplification of DNA using colony PCR

The colony was picked up with a sterilized toothpick and resuspended in 40 µL of TE buffer (pH 8.0) to serve as the DNA template. The 16S rRNA gene was amplified and sequenced using the primers 9F (5′GAGTTTGATCITIGCTCAG3′) and 1541R (5′AAGGAGGTGATCCAGCC3′). PCR amplification and sequencing reactions were performed following the protocol of Yukphan et al. (2005). Each 50 µL PCR reaction included 25 µL of AccuPower® Taq PCR Master Mix (Bioneer), 18 µL of ddH_2O, 2.5 µL of each primer at a final concentration of 10 pM, and 2 µL of DNA template. The cycling conditions for 16S rRNA gene amplification were as follows: initial denaturation at 94°C for 3 min, followed by 25 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 3 min. The resulting PCR product (3 µL) was assessed on a 0.8% agarose gel containing SYBR® Safe DNA stain (Invitrogen, USA). The gel was run in 1X TAE buffer at 100 volts for 30 min, and DNA bands were visualized using an UltraSlim® LED illuminator.

2.4.2 PCR purification

DNA products were purified by FavorPrep™ GEL/PCR Purification Mini Kit and DNA purity was determined using Nanodrop™ Lift Spectrophotometer, with the A_{260}/A_{280} absorbance ratio for pure DNA.

2.4.3 Analysis of 16S rRNA gene and phylogenetic tree construction

The purified PCR product was analyzed at U2Bio (Thailand) Co., Ltd. The analysis primers used were 800R (5′-TACCAGGGTATCTAATCC-3′), 1492R (5′-TACGGYTACCTTGTTACGACTT-3′), and 518F (5′-CCAGCAGCCGCGGTAATACG-3′). The 16S rRNA gene sequences were assembled into contigs using the BioEdit Sequence Alignment Editor (version 7.0.0). The actinomycete strains were compared with type strains from the EzTaxon server (Kim et al., 2014). Nucleotide alignment for the potential and type strains was performed using Clustal W in BioEdit, with gaps and unidentified nucleotides removed. A phylogenetic tree was constructed using the maximum likelihood method of Kumar et al. (2016) in MEGA7, and the distance matrices for aligned sequences were calculated using Kimura's two-parameter method (Kimura, 1980). Accession numbers for the nucleotide sequences of the potential strains were submitted to the DDBJ server.

2.5 *In vitro* screening of plant growth-promoting activities (PGP)

2.5.1 Phosphate solubilization

The qualitative phosphate solubilization ability of Actinomycetes isolates was determined following the protocol of Pikovskaya (1948). A 6 mm cork borer was used to cut samples of Actinomycetes grown on ISP2 medium, which were then inoculated onto Pikovskaya's agar plates and incubated at 30°C for 14–21 days. The formation of clear zones around the actinomycete colonies was observed, indicating phosphate solubilization.

2.5.2 Indole-3-acetic acid (IAA) production

IAA production was assessed according to the protocol of Patten and Glick (1996), with modifications from Gopalakrishnan et al. (2014) and Kaur et al. (2012). Actinomycetes were cultured in Yeast Extract–Malt Extract (YM or ISP2) broth supplemented with 150 µg/mL of the amino acid tryptophan. The cultures were incubated in a shaker incubator at 180 rpm and 30°C for 7–14 days. After incubation, the cultures were centrifuged (HERMEL Z383K) at 10,000 rpm for 10 minutes. The supernatant (1 mL) was collected and was mixed with 2 mL of Salkowski's reagent. The mixture was incubated at room temperature for 30 min and the development of a pink color was observed, indicating the presence of IAA.

2.5.3 Hydrogen cyanide (HCN) production

Actinomycetes were streaked onto ISP2 medium supplemented with 0.44% glycine. Whatman filter paper No. 1 was soaked in a solution of 2% sodium carbonate and 0.5% picric acid, placed over the plates, and sealed with parafilm. The plates were incubated at 30°C for 14 days. The production of HCN was determined by a color change in the filter paper from yellow to red, indicating a positive result, following the protocol of Lorck (1948).

2.5.4 Ammonia production

Ammonia production was tested in 10 mL of Peptone broth. Actinomycetes were inoculated into the medium and were incubated at 30°C for 14 days. After incubation, 0.5 mL of Nessler's reagent was added to the culture. The appearance of yellow to brown coloration was observed, indicating positive ammonia production (Dede et al., 2020).

2.5.5 Siderophore production

Actinomycetes were streaked onto Chrome Azurol S (CAS) agar plates and were incubated at 30°C for 7 days. Siderophore production was determined by the change in agar color from blue to yellow color around the growth spot of Actinomycetes (Krewulak & Vogel, 2008; Schwyn & Neilands, 1987).

2.6 Data analysis

This study employed a complete randomized design (CRD) for all treatments. The data collected were analyzed using the One Way ANOVA BY using IBM SPSS statistics program. Treatment means were compared through Duncan's multiple range test (Duncan, 1955), and percentage values were converted into appropriate formats prior to conducting the statistical analysis.

3. Results

3.1 Isolation of Actinomycetes

3.1.1 Collection of soil sample

The actinobacteria were isolated from organic rice fields in Don Chedi District, Suphan Buri, at coordinates 14º38'06.9" N 99º53'57.3" E, during the early growth stage of rice, which was 60 days old. Samples were collected from the surrounding area of the rice plants on October 16, 2022. Soil samples were randomly collected from seven points, and actinobacteria were isolated following the experimental methods described in sections 2.1.1 and 2.1.2 This process resulted in 60 pure isolates, as shown in Table 1.

Table 1. The isolated numbers of Actinomycetes obtained from organic rice field soil in Suphan buri.

Area	Isolation Number
1	SPB1001, SPB1003, SPB2048
2	SPB1006, SPB1007, SPB1011, SPB1012, SPB1013, SPB1016, SPB1018, SPB1019
3	SPB1022, SPB2049, SPB2050, SPB2051, SPB2052, SPB2053, SPB2054
4	SPB2057, SPB2058, SPB2059, SPB2060, SPB2063, SPB2064, SPB2065
5	SPB1025, SPB1026, SPB1027, SPB1028, SPB1031, SPB2069, SPB2070, SPB2072, SPB2073, SPB2080
6	SPB1035, SPB1036, SPB066, SPB068, SPB2083, SPB2084, SPB2085, SPB2086, SPB2087, SPB2088, SPB1092, SPB1093, SPB1096, SPB1097, SPB1098, SPB1100, SPB1101, SPB2104, SPB2105
7	SPB1042, SPB1043, SPB2107, SPB2108, SPB2109

3.2 The ability of Actinomycetes to inhibit the pathogen *E. carotovora* pv. *carotovora* by agar plug diffusion

From the test of the inhibitory effect on *E. carotovora* pv. *carotovora* at 7 days old, it was found that 20 actinomycete isolates inhibited the bacterial pathogen, with clear zones observed. The actinobacterial isolates SPB1012, SPB1001, SPB1035, and SPB1022 exhibited significant antagonistic effects against the pathogen, with clear zone diameters of 5.30 mm, 4.68 mm, 3.45 mm, and 3.03 mm, respectively, as shown in Table 2 and Figure 1. The *Actinomycete* isolate SPB1012 showed the highest effectiveness in inhibiting *E. carotovora* pv. *carotovora*. This isolation was selected for further testing to evaluate its ability to inhibit *E. carotovora* pv. *carotovora*, which causes soft root in Choy sum, and promotes plant growth.

Table 2. The ability of Actinomycetes to inhibit the pathogen *E. carotovora* pv. *carotovora* by agar plug diffusion.

Isolation of Actinomycetes	Clear zone (mm.)
SPB1001	4.68±0.03
SPB1012	5.30±0.02
SPB1018	1.66±0.02
SPB1022	3.03±0.07
SPB1031	0.84±0.30
SPB1035	3.45±0.01
SPB2049	0.56±0.0
SPB2050	0.95±0.50
SPB2053	1.86±0.15
SPB2060	1.10±0.28
SPB2064	0.41±0.11
SPB2065	2.22±0.21
SPB2069	0.94±0.25
SPB2073	1±0.15
SPB2086	1.21±0.85
SPB1092	1.73±0.02
SPB1098	2.23±0.45
SPB1100	0.43±0.03
SPB1104	0.47±0.07

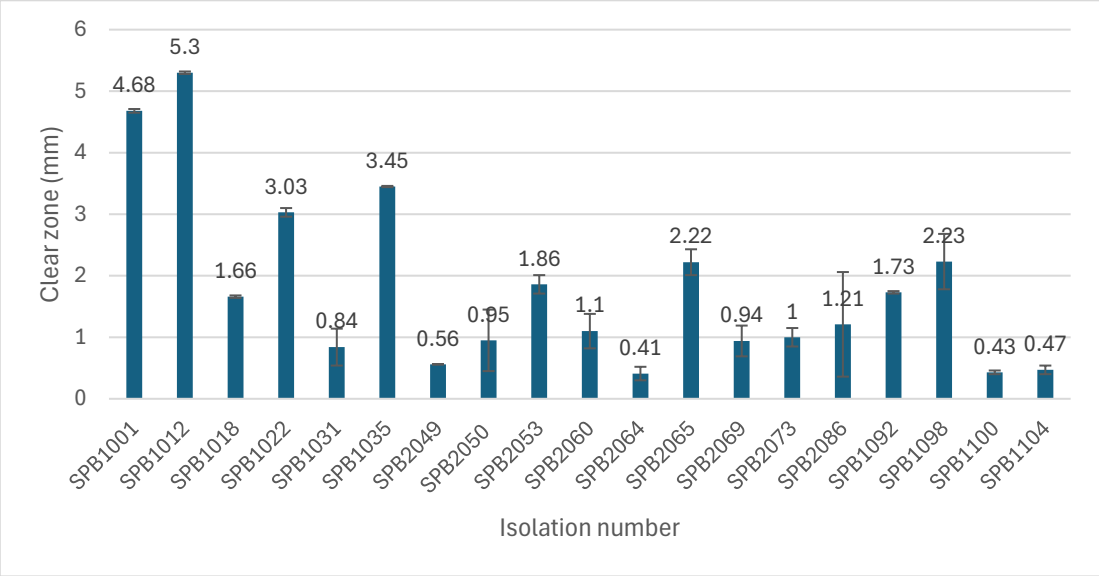


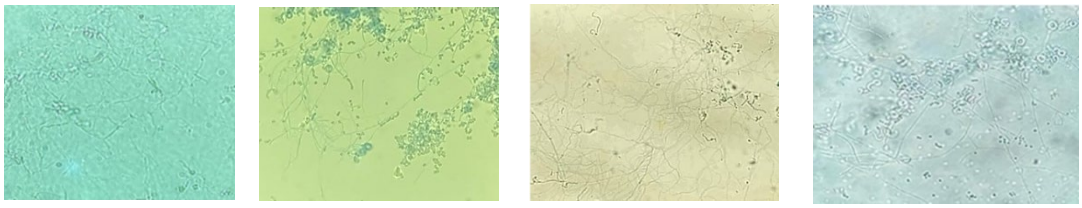
Figure 1. The ability of Actinomycetes to inhibit the pathogen *E. carotovora* pv. *carotovora* by agar plug diffusion (mm).

3.3 Morphological characteristics of Actinomycetes

The Actinomycetes were cultured on International *Streptomyces* Project **2 (**ISP**2)** medium using the Cross Streak method and incubated at 30°C for 7-14 days. The morphological characteristics were studied as shown in Figures 2, 3, and Table 3.

(a) (b) (c) (d)

Figure 2. The characteristics of Actinomycetes colony grown on International *Streptomyces* Project (ISP2) medium for 14 days with the isolate SPB1001 (a), SPB1012 (b), SPB1022 (c), SPB1035 (d).



(a) (b) (c) (d)

Figure 3. The characteristics of Actinomycetes grown on International *Streptomyces* Project (ISP2) medium and spore arrangements for 14 days with the isolate SPB1001 (a), SPB1012 (b), SPB1022 (c), SPB1035 (d).

Table 3. Morphological characterization of the selected Actinomycetes strains.

Isolation number	Aerial mycelium	Substrate mycelium	Soluble pigment	Spore arrangement
SPB1001	White to Medium gray	Moderate orange yellow	-	Verticillati Chains
SPB1012	White to Dark grayish yellowish brown	Dark orange yellow	-	Retinaculiaperti chains
SPB1035	White to Dark grayish olive green	Moderate orange yellow	-	Retinaculiaperti chains
SPB1022	White to Light greenish gray	Deep orange	Dark orange yellow	Retinaculiaperti chains

Note: The symbol, - means the non-producing soluble pigment

3.4 The ability of Actinomycetes to promote growth in Choy sum

From the selection of Actinomycetes with inhibitory effects against *E. carotovora* pv. *carotovora* using the agar plug diffusion method found that the isolated actinomycete SPB1012 exhibited the highest inhibitory efficiency. When tested with Choy sum (*Brassica rapa* var. *parachinensis*), measurements were taken for plant height, root length, number of leaves, leaf width, leaf length, stem width, and fresh weight at 39 days of plant age. It was found that T1 (Actinomycetes SPB1012) had an average stem height of 131.14 mm, an average root length of 108.59 mm, 7 leaves, an average leaf width of 36.64 mm, an average leaf length of 60.56 mm, an average stem width of 3.99 mm, and a fresh weight of 3.92 g. Meanwhile, T5 (the control group) had an average stem height of 129.63 mm, an average root length of 88.57 mm, 6 leaves, an average leaf width of 31.11 mm, an average leaf length of 55.64 mm, an average stem width of 3.36 mm, and a fresh weight of 3.49 g (Table 4 and Figures 4-7.).

The experiment demonstrated that Actinomycetes had a positive influence on stem dimensions compared to the control group, T5.

Calculation of the disease incidence index and disease protective value revealed that T2 (SPB1012 + *E. carotovora* pv. *carotovora*) has a disease severity index of 37.5% and a protective value of 62.5%. T3 (*E. carotovora* pv. *carotovora*) has a disease severity index of 100% and a protective value of 0%. T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH) has a disease severity index of 20% and a protective value of 80%, as shown in Table 5-6 and Figures 8-10. The experimental results showed that T2 (SPB1012) treated with Actinomycetes was effective in inhibition comparable to Plant pathological protection by chemical substances.

(a) (b) (a) (b) (a) (b) (a) (b)

Figure 4. The characteristics of the Choy sum (*Brassica rapa* var. *parachinensis*) including the stem, leaves and roots in promoting the growth at 39 days old with the isolate SPB1012 (a), T5 control group (b).

Table 4. Results of One-Way ANOVA statistical analysis at a 95% confidence level of Actinomycetes isolate SPB1012 on the promoting of growth in Choy sum (*Brassica rapa* var. *parachinensis*) after 39 days of cultivation.

Treatment	Shoot length (mm)	Roots length (mm)	Leaves count (No.)	Leaf width (mm.)	Leaf length (mm)	Stem width (mm)	Fresh weight (g)
T1	131.14±0.46^{a}	108.59±34.34^{a}	7±2.97^{a}	36.64±**8.47**^{a}	60.56±**14.53**^{a}	3.99±**0.65**^{a}	3.92±**0.83**^{a}
T5	129.63±0.68^{b}	88.57±**25**.75^{b}	7±2.01^{a}	31.11±**3.78**^{b}	55.64±**11.10**^{b}	3.36±**0.50**^{b}	3.49±**1.45**^{b}

Note: T1 means isolation SPB1012, T5 means Control

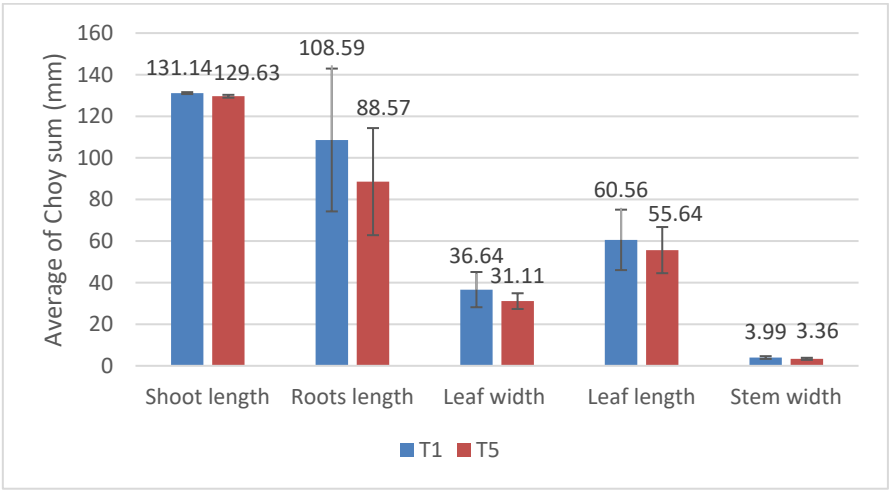


Figure 5. The average of Choy sum (*Brassica rapa* var. *parachinensis*) of Actinomycetes isolates SPB1012 in promoting growth after 39 days of cultivation. T1 means isolation SPB1012 and T5 means Control group (error bars (n=10))

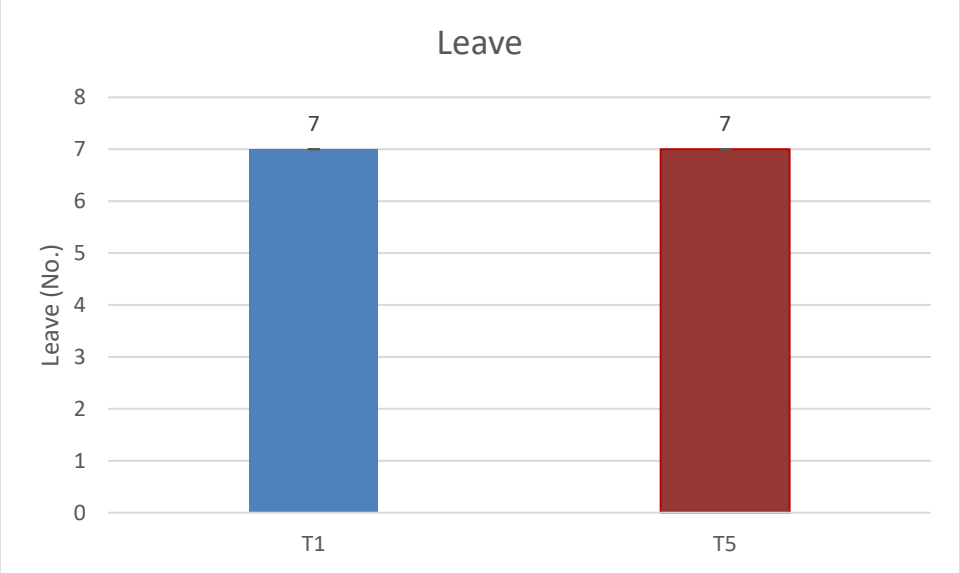


Figure 6. The average Leave (No.) of Actinomycetes isolates SPB1012 in promoting the growth after 39 days of cultivation. T1 means isolation SPB1012 and T5 means Control group (error bars (n=10))

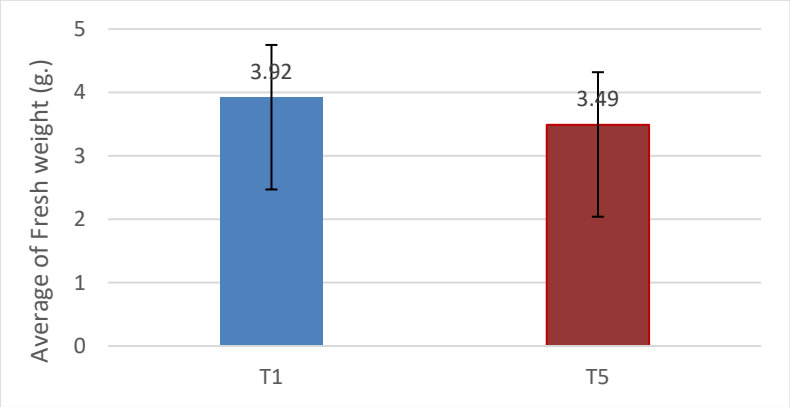


Figure 7. The average of Fresh weight of Actinomycetes isolates SPB1012 in promoting the growth after 39 days of cultivation. T1 means isolation SPB1012 and T5 means Control group (error bars (n=10))

(a) (b) (c)

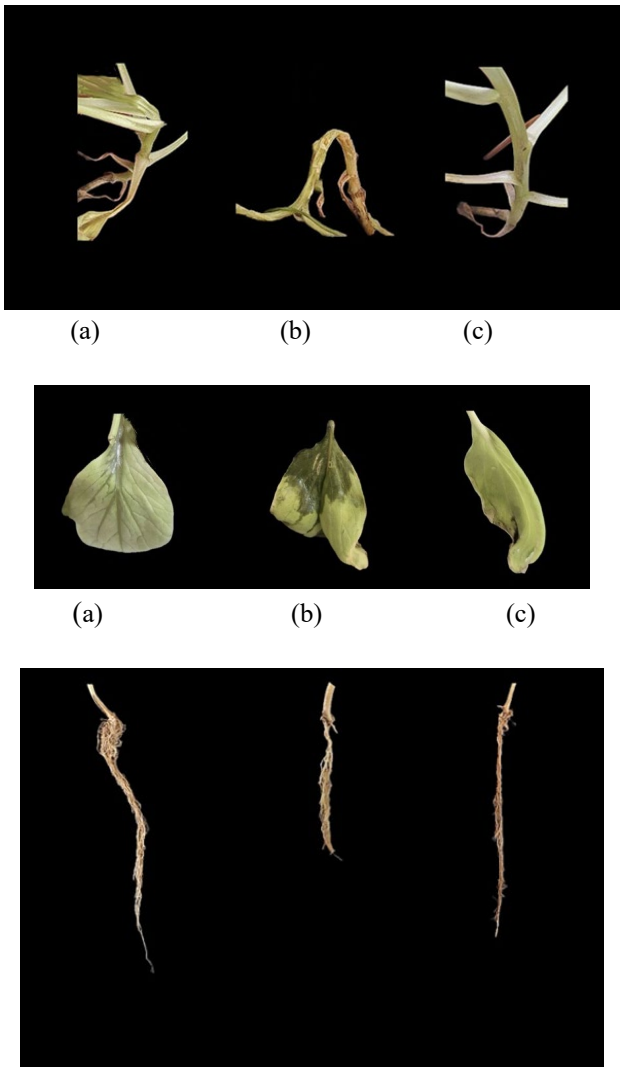


Figure 8. The characteristics of the Choy sum (*Brassica rapa* var. *parachinensis*), including the stem, leaves, and roots, that have been tested for the inhibition of *E. carotovora* pv. *carotovor*a at 39 days old.

a) T2 (SPB1012 + *E. carotovora* pv. *carotovora*)
b) T3 (*E. carotovora* pv. *carotovora*)
c) T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH))

Table 5. The results of the One-Way ANOVA statistical analysis at a level of confidence 95% for the severity index of soft rot disease on 39-day old Choy sum (*Brassica rapa* var. *parachinensis*).

Treatment	Severity index
T2	1.5 ± 0.52^{b}
T3	4.0 ± 0^{c}
T4	0.8 ± 0.42^{a}

Note: T2 (SPB1012 + *E. carotovora* pv. *carotovora)*, T3 (*E. carotovora* pv. *carotovora*), T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH))

Table 6. Actinomycetes isolate SPB1012 in inhibiting *E. carotovora* pv. *carotovora* on 39-day old Choy sum (*Brassica rapa* var. *parachinensis*).

Treatment	Disease severity index (DSI)	Protective value (PV)
T2	37.5% [b]	62.5% [b]
T3	100% [c]	0% [c]
T4	20% [a]	80% [a]

Note: T2 (SPB1012 + *E. carotovora* pv. *carotovora)*, T3 (*E. carotovora* pv. *carotovora*), T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH))

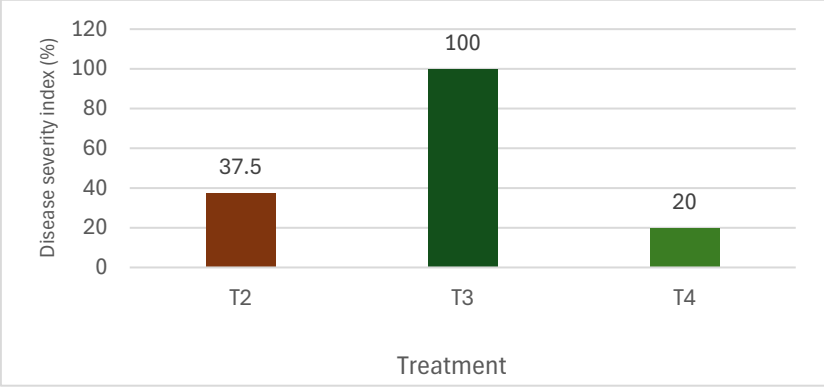


Figure 9. Disease senverity index of *E. carotovora* pv. *carotovora* on Choy sum T2 (SPB1012 + *E. carotovora* pv. *carotovora*), T3 (*E. carotovora* pv. *carotovora*) and T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH)).

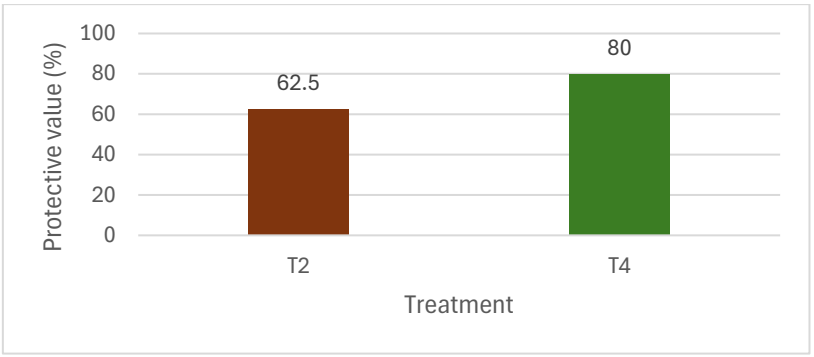


Figure 10. Protective value of *E. carotovora* pv. *carotovora* on Choy sum T2 (SPB1012 + *E. carotovora* pv. *carotovora*) and T4 (*E. carotovora* pv. *carotovora* + Plant pathological protection (CuOH)).

3.5 Genotype characteristics by 16S rRNA gene of the Actinomycetes

The study of the morphological characteristics and inhibition of *E. carotovora* pv. *carotovora* using the Agar plug diffusion method found that the actinomycete isolate SPB1012 SPB1001 SPB1035 and SPB1022 exhibited the highest inhibitory efficiency. Subsequently, isolates were analyzed for nucleotide sequences in the 16S rRNA gene for further details.

3.5.1 DNA extraction and DNA amplification by colony PCR method

The actinomycete isolates, SPB1001, SPB1012, SPB1035 and SPB1022 were analyzed for nucleotide sequences on the DNA strand in the 16s rRNA gene region using the colony PCR method. Two primers were used: 9F (5'- GAGTTTGATCCTGGCTCAG -3') and 1541R (5'- AAGGAGGTGATCCAGCCGCA -3') as the reverse primer. PCR reactions were then performed using the T100TM Thermal Cycler (Bio-Rad, USA)

3.5.2 Purification of PCR product

The PCR product was assessed for DNA quality using gel electrophoresis with the OwlTM EasyCastTM B2 Mini Gel Electrophoresis System at 100 volts for 30 minutes, and the DNA bands were observed using the UltraSlim® LED Illuminator. The DNA was then purified using the FavorPrepTM GEL/PCR Purification Mini Kit. The purity (A_{260}/A_{280}) and concentration of the DNA (nanograms per microliter) were measured using the NanoDropTM Lite Spectrophotometer (Thermo Scientific, USA).

3.5.3 Analysis of 16S rRNA and phylogenetic tree construction

The nucleotide sequence of the 16s rRNA gene was determined by U2Bio (Thailand) Co., Ltd. using primers 9F and 1541R. The sequences SPB1012, SPB1001, SPB1035 and SPB1022 were analyzed with the BioEdit Sequence Alignment Editor and compared to species similarity values from the EzBioCloud database. The actinomycete fourth of isolate have exhibited 100 percent similarity with *Streptomyces hygroscopicus* subsp. *hygroscopic* NBRC 13472^T, the phylogenetic tree was constructed utilizing Maximum-likelihood methods via MEGA7, applying Kimura (1980) two-parameter method with a bootstrap value of 1,000, as illustrated in Figure 11 and Table 7.

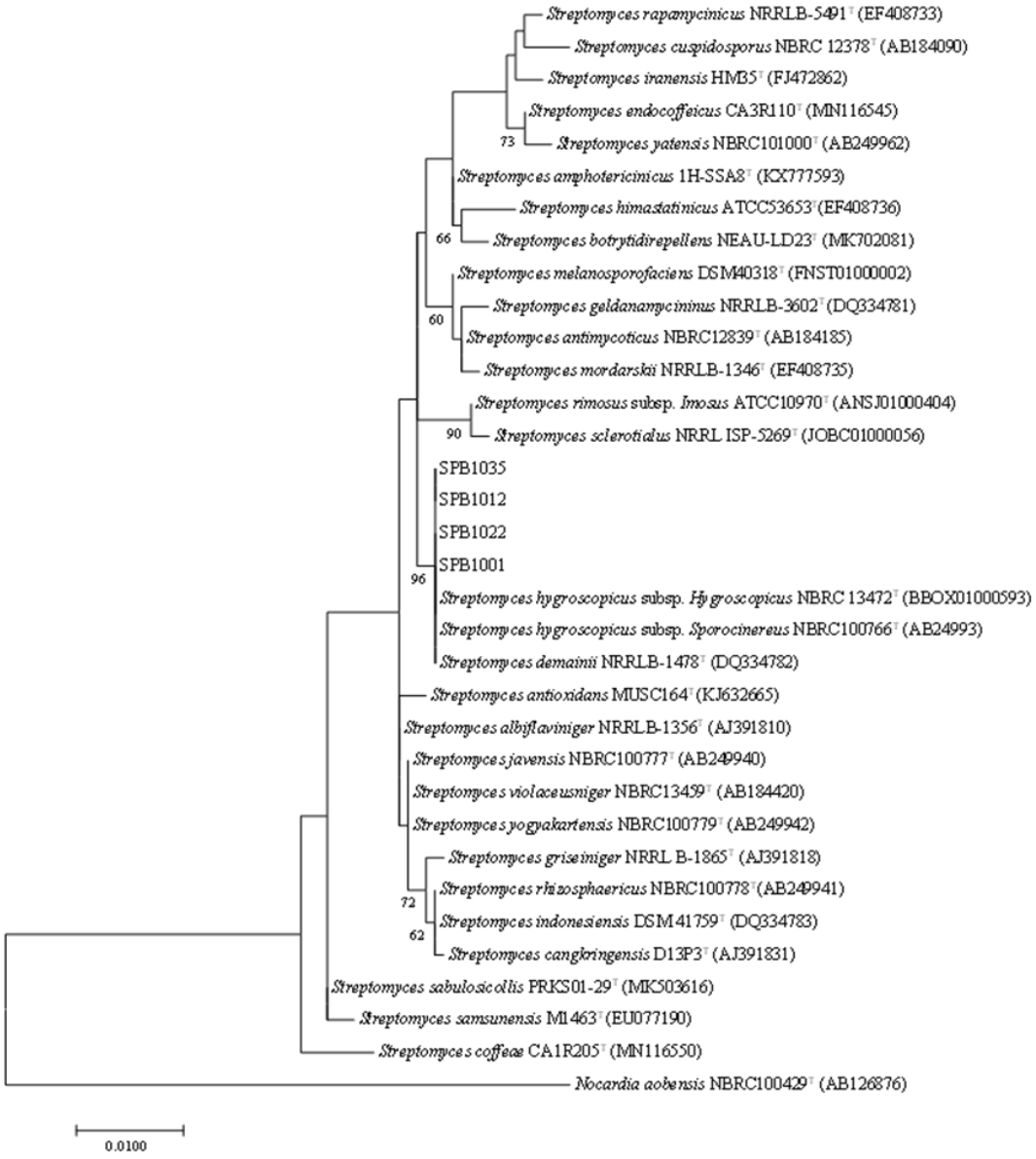


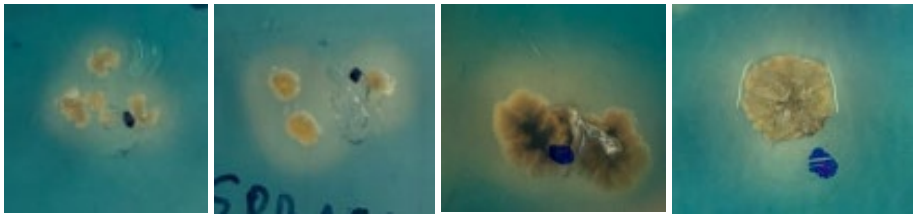
Figure 11. Maximum likelihood phylogenetic tree based on the 16S rRNA gene sequences of selected Actinomycetes 4 strains and their closely related type strains.

Table 7. Nucleotide sequence analysis on the DNA strand in the 16s rRNA gene of Actinomycetes isolates, SPB1001, SPB1012, SPB1035 and SPB1022.

Isolate	Nearest stain	Type stain	% Similarity	Length	Accesion Number
SPB1012	*Streptomyces hygroscopicus* subsp. *hygroscopicus*	NBRC13472^T	100	1,488 bp	BBOX01000593
SPB1001	*Streptomyces hygroscopicus* subsp. *hygroscopicus*	NBRC13472^T	100	1,513 bp	BBOX01000593
SPB1035	*Streptomyces hygroscopicus* subsp. *hygroscopicus*	NBRC13472^T	100	1,489 bp	BBOX01000593
SPB1022	*Streptomyces hygroscopicus* subsp. *hygroscopicus*	NBRC13472^T	100	1,479 bp	BBOX01000593

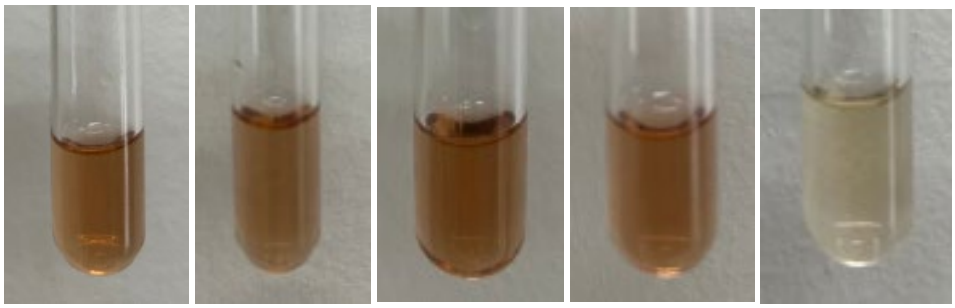
3.6 *In vitro* screening of plant growth-promoting activities (PGP)

Actinomycetes isolate SPB1012, SPB1001, SPB1035 and SPB1022 were studied of phosphate solubilization, indole-3-acetic acid (IAA) production, hydrogen cyanide (HCN) production, ammonia production and siderophore as presented in Table 8. Siderophore production was determined by yellow colour around the growth spot of Actinomycetes as presented in Figure 12 Isolate SPB1012, SPB1001, SPB1035 and SPB1022 showed positive result. Indole-3-acetic acid (IAA) production was determined pink color as presented in Figure 13. Isolate SPB1012, SPB1001, SPB1035 and SPB1022 showed a positive result.



(a) (b) (c) (d)

Figure 12. Screening of Plant Growth-Promoting Activities (PGP) siderophore production with the isolate SPB1022 (a), SPB1012 (b), SPB1001 (c) and SPB1035 (d).



(a) (b) (c) (d) (e)

Figure 13. Screening of Plant Growth-Promoting Activities (PGP) Indole-3-acetic acid (IAA) production with the isolate SPB1012 (a), SPB1001 (b), SPB1022 (c), SPB1035 (d) and Control (e).

Table 8. *In vitro* screening of plant growth-promoting activities (PGP).

Isolate	IAA production	Phosphate solubilization	HCN production	Ammonia production	Siderophore production
SPB1012	+	-	-	-	+
SPB1001	+	-	-	-	+
SPB1035	+	-	-	-	+
SPB1022	+	-	-	-	+

Note: The symbol, + means the positive reaction while symbol, - means the negative reaction

4. Discussion

In this study, sixty actinobacterial isolates from organic rice fields in Suphan Buri Province, Thailand, were evaluated for their antagonistic activity against *E. carotovora* pv. *carotovora* uses the agar plug diffusion method. Among these, twenty-two isolates produced visible inhibition zones, with isolates SPB1012, SPB1001, SPB1035, and SPB1022 exhibiting the most significant antagonistic effects. SPB1012, in particular, showed the highest inhibitory activity and was selected for greenhouse evaluation, where it also significantly enhanced plant growth, increasing shoot height and stem width in Choy sum (*Brassica rapa* var. *parachinensis*).

Molecular identification based on 16S rRNA gene sequencing revealed that SPB1012, SPB1001, SPB1035, and SPB1022 shared 100% sequence similarity with *Streptomyces hygroscopicus* subsp. *hygroscopicus* NBRC 13472^{T}. Although high sequence similarity provides strong preliminary identification, it does not necessarily confirm that the isolates belong to the same strain or even the same species. It has long been proposed that two bacterial strains should be classified as different species if their 16S rRNA gene sequence similarity falls below 97%, and as different genera if the similarity is below 95% (Stackebrandt & Goebel, 1994). This threshold was later revised, suggesting a 98.7% cut-off for species delineation (Stackebrandt & Ebers, 2006).

However, several studies have shown that these thresholds are not universally applicable across all bacterial genera. For example, strains in the genus *Edwardsiella* exhibit interspecies similarities ranging from 99.3% to 99.8% (Janda & Abbott, 2007), while in genera such as *Streptomyces* and *Chlorobium*, the lowest observed interspecies similarities are as low as 78% and 86.1%, respectively (Alexander et al., 2002). Moreover, unique phenotypic or chemotypic characteristics can justify the classification of strains into different species or genera despite high 16S rRNA gene sequence similarity (Huse et al., 2008). Therefore, although the isolates showed 100% similarity to *Streptomyces hygroscopicus* subsp. *hygroscopicus*, further phenotypic, chemotaxonomic, and whole-genome analyses are necessary for definitive species identification.

Salem and Abd El-Shafea (2018) studied the control of potato rot disease caused by *Erwinia carotovora* subsp. *carotovora* using four biological agents, namely *Streptomyces* spp., *Bacillus subtilis*, *Pseudomonas flurescence* and *P. aeruginosa*. It was found that *Streptomyces* spp. had the best efficiency in inhibiting pathogenic bacteria. This is in line with our research, which found that the most potent strains were those in the genus *Streptomyces.* In addition, Cao et al. (2021) studied *Streptomyces hygroscopicus* OsiSh-2 and found that it has the ability to produce Fe for plants with high efficiency by conducting Fe deficiency tests in rice plants. The test results showed that OsiSh-2 addition effectively increased the biomass of rice plants under Fe limitation. Which is consistent with our research that found the creation of Siderophore and IAA production.

Furthermore, Tanespimycin and rapamycin, metabolites obtained from *Streptomyces hygroscopicus*, were found to have antifungal activity against *Colletotrichum gloeosporioides*, the causative agent of anthracnose. Tanespimycin and rapamycin at concentrations of 0.01-0.1 μM were found to reduce the growth of the fungus in vitro by 60.4% and 40.4%, respectively. And the experimental results of tanespimycin and rapamycin were tested for inhibition and prevention of the growth of pathogenic fungi in mangoes after infection for 5 days, which could inhibit up to 73.7% and 78.4% respectively. It was also found that tanespimycin and rapamycin could maintain the quality and extend the shelf life of the fruits for 6 days when stored at 20 degrees Celsius (Cheng et al., 2023).

Additionally, Xu et al. (2019) studied the rice endophyte exhibiting antifungal activity of *Streptomyces hygroscopicus* OshiSH-2 as a potential biocontrol agent against rice leaf blast pathogen. Based on previous studies, screening and preliminary morphological characterization. *Streptomyces hygroscopicus* OshiSH-2 was found to have antagonistic activity against *Magnaportthe oryzae* in vitro, showing a strong inhibition of *M. oryzae* mycelial growth, and when tested on rice seedlings in the greenhouse and field, it showed a 23.5% and 28.3% reduction in disease incidence, respectively. In addition, *S. hygroscopicus* OshiSH-2 was found to induce chitin accumulation in cell wall and decrease Esgosterol content in cell membrane of *M. oryzae* and alter mitochondrial function in the pathogen, indicating that *S. hygroscopicus* OshSH-2 is antagonistic to *M. oryzae*.

Ultimately, the dual ability of SPB1012 to inhibit plant pathogens and promote plant growth highlights its potential as a biocontrol agent for sustainable agriculture. Development of products such as biofertilizers incorporating SPB1012 could provide an eco-friendly alternative to chemical pesticides, contributing to enhanced crop protection and productivity under field conditions.

5. Conclusions

This study demonstrated that actinobacterial isolates obtained from organic rice field soil in Suphan Buri Province, Thailand, exhibited significant antagonistic activity against *E. carotovora* pv. *carotovora*, the causative agent of soft rot disease in Choy sum (*Brassica rapa* var. *parachinensis*). Among the isolates, SPB1012 showed the highest inhibitory effect and promoted plant growth by increasing shoot height and stem diameter under greenhouse conditions.

Molecular identification revealed that SPB1012 and related isolates shared 100% sequence similarity with *Streptomyces hygroscopicus* subsp. *hygroscopicus*. These findings support the potential application of SPB1012 as an effective biocontrol agent and plant growth promoter. The integration of SPB1012 into agricultural practices, such as the development of biofertilizers or biopesticides, could offer an environmentally friendly alternative to chemical pesticides and enhance the sustainability of vegetable production systems.

Future studies should focus on formulation development, field trials, and the characterization of secondary metabolites responsible for antimicrobial and growth-promoting activities to fully harness the potential of SPB1012 for commercial applications.

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Conflict of Interest

No conflicts of interest.

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References

Alexander, B., Andersen, J. H., Cox, R. P., & Imhoff, J. F. (2002). Phylogeny of green sulfur bacteria on the basis of gene sequences of 16S rRNA and of the Fenna-Matthews-Olson protein. *Archives of Microbiology*, *178*, 131–140. https://doi.org/10.1007/s00203-002-0432-4

Araujo, R., & Ramos, M. A. (2000). *Action plan for Margaritifera margaritifera in Europe*. Council of Europe.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Basik, A. A., Juboi, H., Shamsul, S. S. G., Sanglier, J.-J., & Yeo, T. C. (2020). Actinomycetes isolated from wetland and hill paddy during the warm and cool seasons in Sarawak, East Malaysia. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(4), 774–780. https://doi.org/10.15414/jmbfs.2020.9.4.774-780

Buensanteai, N., Sompong, M., Saengchan, C., Phansak, P., & Thumanu, K. (2016). PGPR induces resistance against soft rot on Chinese green mustard. *Biological and Integrated Control of Plant Pathogens IOBC-WPRS Bulletin*, *116*, 131–135.

Cao, L., Gao, Y., Yu, J., Niu, S., Zeng, J., Yao, Q., Wang, X., Bu, Z., Xu, T., Liu, X., & Zhu, Y. (2021). *Streptomyces hygroscopicus* OsiSh-2-induced mitigation of Fe deficiency in rice plants. *Plant Physiology and Biochemistry*, *158*, 275–283.

Cheng, Y.-J., Lee, F.-W., Tsai, Y.-S., & Kuan, Y.-C. (2023). Tanespimycin and rapamycin exhibit antifungal activity against *Colletotrichum gloeosporioides* and enhance mango resistance to anthracnose via differentially modulating heat shock response. *Postharvest Biology and Technology*, *204*, 112474. https://doi.org/10.1016/j.postharvbio.2023.112474

Cui, W., He, P., Munir, S., He, P., He, Y., Li, X., Yang, L., Wang, B., Wu, Y., & He, P. (2019). Biocontrol of soft rot of Chinese cabbage using an endophytic bacterial strain. *Frontiers in Microbiology*, *10*, 1471. https://doi.org/10.3389/fmicb.2019.01471

Dede, A., Güven, K., & Şahin, N. (2020). Isolation, plant growth-promoting traits, antagonistic effects on clinical and plant pathogenic organisms and identification of actinomycetes from olive rhizosphere. *Microbial Pathogenesis*, *143*, 104134. https://doi.org/10.1016/j.micpath.2020.104134

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*, 1-42. https://doi.org/10.2307/3001478

Gopalakrishnan, S., Vadlamudi, S., Bandikinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., Kudapa, H., Katta, K., & Varshney, R. K. (2014). Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. *Microbiological Research*, *169*, 40–48. https://doi.org/10.1016/j.micres.2013.09.008

Gratao, P. L., Prasad, M. N. V., Cardoso, P. F., Lea, P. J., & Azevedo, R. A. (2005). Phytoremediation: Green technology for the cleanup of toxic metals in the environment. *Brazilian Journal of Plant Physiology*, *17*(1), 53–64. https://doi.org/10.1590/S1677-04202005000100005

Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A., & Sogin, M. L. (2008). Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, *4*(11), e1000255.

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/jcm.01228-07

Kampapongsa, D., & Kaewkla, O. (2016). Biodiversity of endophytic actinobacteria from jasmine rice (*Oryza sativa* L. KDML 105) grown in Roi-Et Province, Thailand and their antimicrobial activity against rice pathogens. *Annals of Microbiology*, *66*, 587–595. https://doi.org/10.1007/s13213-015-1140-z

Kaur, S., Pembleton, L. W., Cogan, N. O., Savin, K. W., Leonforte, T., Paull, J., Materne, M., & Forster, J. W. (2012). Transcriptome sequencing of field pea and faba bean for discovery and validation of SSR genetic markers. *BMC Genomics*, *13*, 104.

Kelley, K. L. (1964). *Inter-Society Colour Council-National Bureau of Standard Color name charts illustrated with centroid colours*. US Government Printing Office.

Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J.-S. (2014). Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Research*, *24*(6), 1012–1019.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120.

Krewulak, K. D., & Vogel, H. J. (2008). Structural biology of bacterial iron uptake. *Biochimica et Biophysica Acta*, *1778*, 1781–1804. https://doi.org/10.1016/j.bbamem.2007.07.026

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Physiologia Plantarum, 1*, 142–146.

Ogunmwonyi, I. N., Igbinosa, O. E., Aiyegoro, O. A., & Odjadjare, E. E. (2008). Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University, Nigeria. *Scientific Research and Essay*, *3*(3), 120–124.

Patten, C. L., & Glick, B. R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, *42*, 207-220.

Phokum, C., Jitareerat, P., Photchanachai, S., & Cheevadhanarak, S. (2006). Detection and classification of soft rot *Erwinia* of vegetables in Thailand by DNA polymerase chain reaction. *Acta Horticulturae*, *712*, 917-926. https://doi.org/10.17660/ActaHortic.2006.712.121

Pikovskaya, R. I., (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*, *17*, 362–370.

Rattanakavil, T., Kumlung, T., & Klanbut, K. (2020). Isolation and molecular characterization of antifungal production from rice fields rhizosphere soil, Thailand. *International Journal of Science and Innovative Technology*, *3*(1), 41–52.

Salem, E. A., & Abd El-Shafea, Y. M. (2018). Biological control of potato soft rot caused by *Erwinia carotovora* subsp. *carotovora*. *Egyptian Journal of Biological Pest Control*, *28*, 94. https://doi.org/10.1186/s41938-018-0100-x

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

Stackebrandt, E., & Ebers, J. (2006). Taxonomic parameters revisited: Tarnished gold standards. *Microbiology Today*, *33*, 152–155.

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, *44*(4), 846–849.

Takizawa, M., Colwell, R. R., & Hill, R. T. (1993). Isolation and diversity of actinomycetes in the Chesapeake Bay. *Applied and Environmental Microbiology*, *59*(4), 997–1002. https://doi.org/10.1128/aem.59.4.997-1002.1993

Tsuda, K., Tsuji, G., Higashiyama, M., Ogiyama, H., Umemura, K., Mitomi, M., Kubo, Y., & Kosaka, Y. (2016). Biological control of bacterial soft rot in Chinese cabbage by *Lactobacillus plantarum* strain BY under field conditions. *Biological Control*, *100*, 63–69. https://doi.org/10.1016/j.biocontrol.2016.05.010

Waksman, S. A. (1961). *The Actinomycetes: Vol. II. Classification, identification and description of genera and species*. The Williams and Wilkins.

Xu, T., Cao, L., Zeng, J., Franco, C. M. M., Yang, Y., Hu, X., Liu, Y., Wang, X., Gao, Y., Bu, Z., Shi, L., Zhou, G., Zhou, Q., Liu, X., & Zhu, Y. (2019). The antifungal action mode of the rice endophyte *Streptomyces hygroscopicus* OsiSh-2 as a potential biocontrol agent against the rice blast pathogen. *Pesticide Biochemistry and Physiology*, *160*, 58–69. https://doi.org/10.1016/j.pestbp.2019.06.015

Yukphan, P., Malimas, T., Potacharoen, W., Tanasupawat, S., Tanticharoen, M., & Yamada, Y. (2005). *Neoasaia chiangmaiensis* gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the α-*Proteobacteria*. *The Journal of General and Applied Microbiology*, *51*, 301–311.

Zhang, J., & Zhang, L. (2011). Improvement of an isolation medium for actinomycetes. *Modern Applied Science*, *5*(2), 124–127.