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The Potential of Cabbage Rhizosphere Actinomycetes to Control Black Rot Disease Caused by *Xanthomonas campestris* pv. *campestris*

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ABSTRACT

Actinomycetes are Gram-positive soil bacteria known for decomposing organic matter and producing secondary metabolites, such as antibiotics and hydrolytic enzymes, which contribute to pathogen suppression and soil health. This study aimed to isolation of actinomycetes from the cabbage rhizospheric and evaluate their antagonistic activity against *X. campestris* pv. *campestris* (*Xcc*), the causative agent of black rot disease in cabbage. The study consisted of four main stages: isolation, selection, characterzation, and identification of potential isolates. A total 55 actinomycetes isolates were successfully isolated from cabbage rhizospheric in Garut, Cianjur and Bandung, West Java. Among them, 16 isolates were non-pathogenic to plant and mammals. *In vitro* assays showed inhibition of *Xcc* by 3.96-20.83%. Seed germination and growth tests on cabbage revealed a vigor index of 41.7-88.3% and germination rate of 50.83-93.33%. Based on the Analytical Hierarchy Process (AHP) method, three isolates CPT5, CPT8, and SNT10 were identified as the most potential biocontrol agenst *Xcc*, as they exhibited peroxidase enzyme activity. Moleculer identification through 16S rRNA sequencing confirmed that CPT5 was identified as *Streptomyces polychromogenes*, CPT8 as *Streptomyces vinaceus*, and SNT10 as *Streptomyces rochei.* These findings indicated that actinomycetes from the cabbage rhizospheric have potential to control *X. campestris* pv. *campestris*.

1. Introduction

Xanthomonas campestris pv. *campestris* is a phytopathogenic bacterium that causes black rot disease on cabbage, and causes significant losses for farmers. Nugroho (2012) reported that the incidence of this disease was up to 61% in Kopeng Village, Semarang Regency Central Java. Similarly, Pratama *et al.* (2016) reported the disease intensity of 15.1 to 21.8%, with an incidence rate of 32.5% and 44.3% in Cianjur, West Java. Singh *et al.* (2018) stated that this disease could result in yield losses up to 90%. Ramachandra *et al.* (2024) reported an average disease incidence of 52.7%, with yield losses ranging from 50 to 70% in Meghalaya, India. Lingaraj *et al.* (2023) stated that black rot can reduce cabbage yields by more than 50% globally and up to 100% under severe infection conditions, particularly in Tanzania. In Karnataka, India, the severity of the disease in certain cabbage varieties was reached up to 77.77%. The characteristics symptom include V-shaped chlorotic lesions at leaf margins, which progress to necrosis and eventually cause leaf abscission (Hakalová *et al.* 2022). Conventional control methods typically rely on chemical pesticides; however, their prolonged use can result in pathogen resistance and pose environmental hazards. Therefore, biological control using actinomycetes presents an eco-friendlier alternative. Actinomycetes are Gram-positive filamentous bacteria capable of producing antibiotics, degradative enzymes, and antifungal compounds, as well as inducing systemic resistance in plants (Fatimah *et al.* 2022).

Previous studies have demonstrated the effectiveness of actinomycetes in inhibiting plant pathogens.

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Nurmujahidin *et al.* (2023) found that isolates ST1, ST27, and BT23 could suppress *Burkholderia glumae*, the causal agent of bacterial grain rot in rice. Wijayanti *et al.* (2021) reported that actinomycetes could inhibit *Fusarium oxysporum* by up to 53.67% while also producing phosphate solubilizers and indole-3-acetic acid (IAA), which promote plant growth. Navitasari *et al.* (2022) demonstrated that actinomycetes were effective in controlling *Ralstonia solanacearum* in tomatoes. Based on these studies, further exploration is necessary to identify more effective actinomycete isolates for inhibiting *X. campestris* pv. *campestris*. Therefore, the present study aims to explore actinomycetes from the cabbage rhizospheric in West Java and to identify isolates with strong biocontrol potential against *X. campestris* pv. *campestris*.

2. Materials and Methods

2.1. *X. campestris* pv. *campestris* Isolate

The *Xcc* isolate was re-cultured from the Laboratory Plant Bacteriology collection, Department of Plant Protection, IPB University (Af'idzatuttama *et al.* 2023). The Virulence of was pathogen maintained by inoculating into cabbage plants.

2.2. Isolation and Selection of Actinomycete Bacteria from the Cabbage Rhizospheric

The Rhizospheric of healthy cabbage plant from Cianjur, Bandung, and Garut Districts, West Java, each was composited 1 kg sample was taken to the laboratory for the analysis. Ten grams soil sample from each location was suspended into 10 mL sterile distilled water. After serial dilution up to 10^{-6} in 0.85% NaCl, 100 µL of each dilution was plated onto WYE and SCA media. Plates were incubated at 28°C for 1-2 weeks for actinomycete growth. Actinomycete isolates were purified on ISP2 and incubated at 28°C, while their population and species diversity were determined based on colony morphology (Ni *et al.* 2021).

2.3. Biosafety Testing of Actinomycete Isolates

A hypersensitivity reaction test was conducted by growing actinomycete isolates in liquid ISP2 medium for seven days, after which 5 mL of the suspension was injected into a spot on tobacco leave. The appearance of necrosis within 3-5 days indicated potential pathogenicity, and such isolates were excluded from the further testing. A hemolysis test was performed by culturing the isolates on blood agar for three days. Isolates with a clear zone around the colony were considered hemolytic activity and were not used in subsequent experiments (Bernal *et al.* 2015).

2.4. Antagonistic Test of Actinomycetes Against *X. campestris* pv. *campestris In Vitro*

Antibiosis testing was conducted using a modified cross-streak method (Nurmujahidin *et al.* 2023). Actinomycete isolates were streaked onto one-third of the NA medium and incubated for 5-7 days. *X. campestris* pv. *campestris* was the streaked perpendicularly at a distance of approximately ±0.5 cm. The pathogen was streaked in three lines, each with a length of approximately 4 cm and a width of ±0.2 cm. The percentage of bacterial inhibition (PBI) was calculated using the following formula:

$$\text{PBI} = \frac{\text{Length of } Xcc \text{ in control - Length of in treatment}}{\text{Length of } Xcc \text{ in control}} \times 100\%$$

2.5. Effect of Actinomycetes on Cabbage Seed Germination and Vigor

The germination test of cabbage seeds was conducted by soaking the seeds in an actinomycete suspension (10^8 cfu/mL) for 12 hours after surface sterilization with 1% NaOCl. For the control treatment, the seeds were soaked in 0.5% CMC. The seeds were then sown in the planting medium consisting of sterilized soil and compost (3:1). The germinated seeds were counted on 5 days after sowing. The measured parameters included germination rate (%), vigor index (%), plant height (cm), and root length (cm). The vigor index (Vin) was calculated based on the percentage of normal seedlings (NS) at the first count.

$$\text{Germination Rate (GR) (\%)} = \frac{\sum \text{NS Count I} + \sum \text{NS Count II}}{\sum \text{Planted seeds}} \times 100\%$$

NS Count I: 5 days
NS Count II: 7 days

2.6. Analytical Hierarchy Process (AHP)

This study used the AHP method to determine actinomycete isolates with potential as biological control agents against *X. campestris* pv. *campestris*. Weighting was performed based on the prioritization of criteria and sub-criteria, following the method report by Saaty (2008), The isolate with the highest AHP score to be selected as the best candidate. Subsequently, the top three isolates were identified using molecularly.

2.7. Effect of Actinomycetes on the Activity of Peroxidase Enzyme (PO)

One gram of cabbage seedlings frozen in liquid nitrogen was ground and extracted with 10 mL of sodium phosphate buffer (pH 6.8–8.8) containing 1% PVP. The extract was centrifuged at 15,000 rpm for 15 minutes at 4°C, and the supernatant was used for the analysis of PO enzyme activity and protein content. PO activity was measured using guaiacol and H_2O_2 as substrates, and absorbance was recorded at 470 nm. One unit of PO activity was defined as the increase in absorbance per second. Protein content was determined by measuring absorbance at 260 and 280 nm, using BSA as the standard. Specific enzyme activity was expressed as units per kilogram of protein per second (Zhang *et al.* 2016).

2.8. Characterization and Molecular Identification of the Most Potential Actinomycete

Actinomycete isolates with strong inhibitory activity against *X. campestris* pv. *campestris* were molecularly identified based on the 16S rRNA gene sequence. DNA extraction was carried out following the method of Abd-Elsalam *et al.* (2003), and amplification was performed using the primers 27F and 16Sact1114R. PCR products were visualized by electrophoresis on a 1% agarose gel and observed under a UV transilluminator. The resulting sequences were analyzed using the BLASTN tool from NCBI.

3. Results

3.1. The Virulence of *X. campestris* pv. *campestris* Isolate

The *Xcc* isolate was cultured on YDCA medium and retained its virulence, as evidenced by the development of characteristic black, V-shaped lesions on cabbage leaves (Figure 1).

3.2. Abundance of Rhizospheric Actinomycetes on Cabbage Plants

The population of rhizospheric bacteria in cabbage plants varied among the sampling sites. Ciputri Village, located in Cianjur Regency, West Java recorded the highest actinomycete density at 5.1×10^6 CFU g^{-1} soil, followed by non-actinomycetes at 3.3×10^6 CFU g^{-1}. Meanwhile, Ciherang Village, Cianjur Regency, West Java recorded the lowest counts, with 6.3×10^4 CFU g^{-1} for actinomycetes and 1.7×10^4 CFU g^{-1} for non-actinomycetes (Table 1).

A total of 55 actinomycete isolates were successfully obtained from rhizospheric samples collected in Garut, Cianjur, and Bandung, showing variation in isolate diversity among villages. Garut had the highest diversity in Cibodas Village (15 types) and the lowest in Giriawas (4 types). In Cianjur, Ciputri had 14 types, while Ciherang had only 5 types. Bandung exhibited lower diversity, with 2 types in Santosa and 8 types in Sukamanah. This variation was influenced

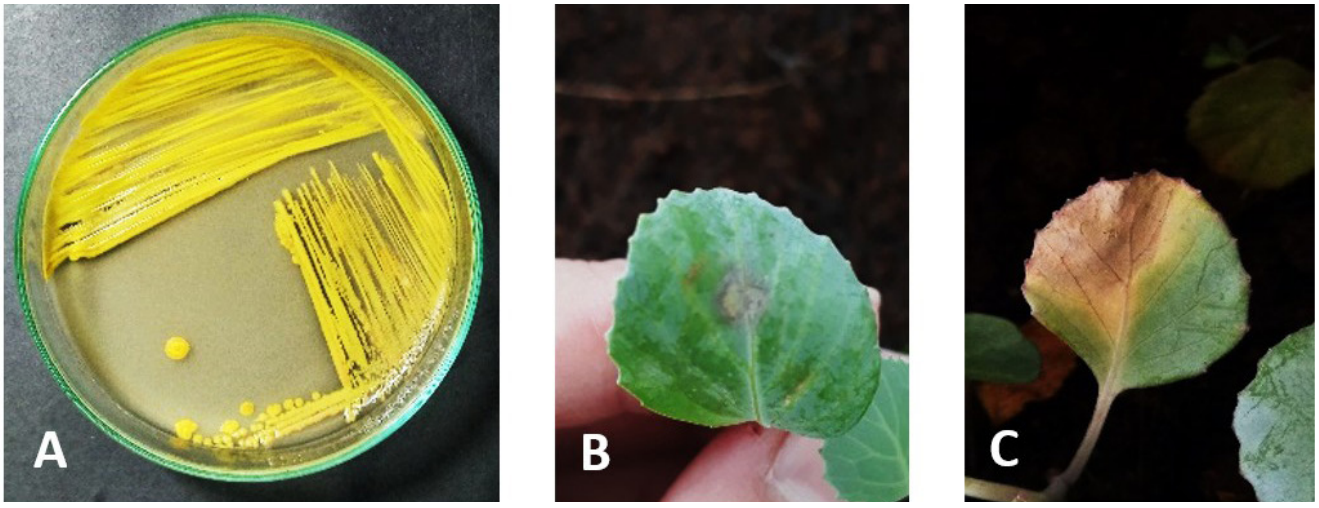

Figure 1. Rejuvenation and virulence testing of *Xcc* isolates on cabbage seedlings. (A) Xcc isolate on YDCA medium, (B) early symptoms, (C) advanced symptoms

by environmental factors such as soil conditions and organic matter availability (Table 1).

3.3. Biosafety of Actinomycetes Isolates

Based on the HR test on tabacco of 55 isolates actinomycetes, 20 isolates were exhibited a hypersensitive response in the form of necrosis, indicating pathogenicity toward plants. Based on Hemolysis testing of the remaining 35 isolates after HR test, 19 isolates were positive produced α and β hemolysis and were therefore not to be tested on the further tests. A total of 16 non-pathogenic isolates (HR negative), 8 isolates were isolated from Cibodas Village, Garut Regency, West Java. 2 isolates Panggilingan Garut Regency, West Java, 5 isolates Ciputri, Cianjur Regency, West Java, and 1 isolate Sukamanah Bandung Regency, West Java, and were used for further testing (Table 2).

3.4. Antagonistic Activity Against *X. campestris* pv. *campestris In Vitro*

Based on antagonistic testing, 16 rhizospheric actinomycetes were positively inhibit the growth of the *Xcc*. The results Isolates CPT5 and CPT8 demonsated the highest inhibition, with values of 20.83% and 19.58%, respectively, while CPT45 and CBS6 showed the lowest inhibition at 3.96% and 4.79%. (Figures 2 and 3).

3.5. Cabbage Seedling Growth and Germination

Isolate CPT5 significantly improved seedling performance, achieving a germination rate of 90.83% and a seedling height of 9.72 cm, highlighting its potential to promote early cabbage development. Isolates CPT8, PGN27, and CPT45 also had positive effects, particularly on germination and root length. In contrast, SNT10 showed a high germination rate (89.17%) but resulted in shorter plants (4.16 cm), suggesting a specific influence on plant morphology (Figure 4).

3.6. Selection of Potential Actinomycetes Using the AHP Method

The Analytical Hierarchy Process (AHP) method facilitated the prioritization of actinomycete isolates by evaluating their biocontrol potential through antagonistic activity and plant growth-promoting traits. The top three ranked isolates were CPT5 and CPT8 from Ciputri Village, Cianjur, and SNT10 from Santosa Village, Bandung.

3.7. Peroxidase Enzyme Activity (PO)

Peroxidase enzyme activity, as illustrated in Figure 5, was highest in cabbage seedlings treated with isolate SNT10 (0.00027541 UAE), followed by CPT8, while

Table 1. Abundance of rhizospheric actinomycete populations in cabbage plants

Regency	Village	Actinomycetes (CFU g^{-1} sample)	Non Actinomycetes (CFU g^{-1} sample)	Number of actinomycete types
Garut	Cibodas	1.25×10^5	7.7×10^4	15
	Giriawas	1.9×10^5	3.6×10^5	4
	Panggilingan	9.0×10^5	1.4×10^6	7
Cianjur	Ciherang	6.3×10^4	1.7×10^4	5
	Ciputri	5.1×10^6	3.3×10^6	14
Bandung	Santosa	8.0×10^5	1.3×10^6	15
	Sukamanah	1.2×10^5	3.6×10^5	4

Table 2. Hypersensitivity response (HR) test and hemolysis test of actinomycete isolates from cabbage rhizospheric

Regency	Village	Hypersensitivity test (n = 55)		Hemolysis test (n = 35)	
		Positive	Negative	Positive	Negative
Garut	Cibodas	4	11	3	8
	Giriawas	2	2	2	0
	Panggilingan	3	4	2	2
Cianjur	Ciherang	1	4	4	0
	Ciputri	6	8	3	5
Bandung	Santosa	4	4	3	1
	Sukamanah	0	2	2	0

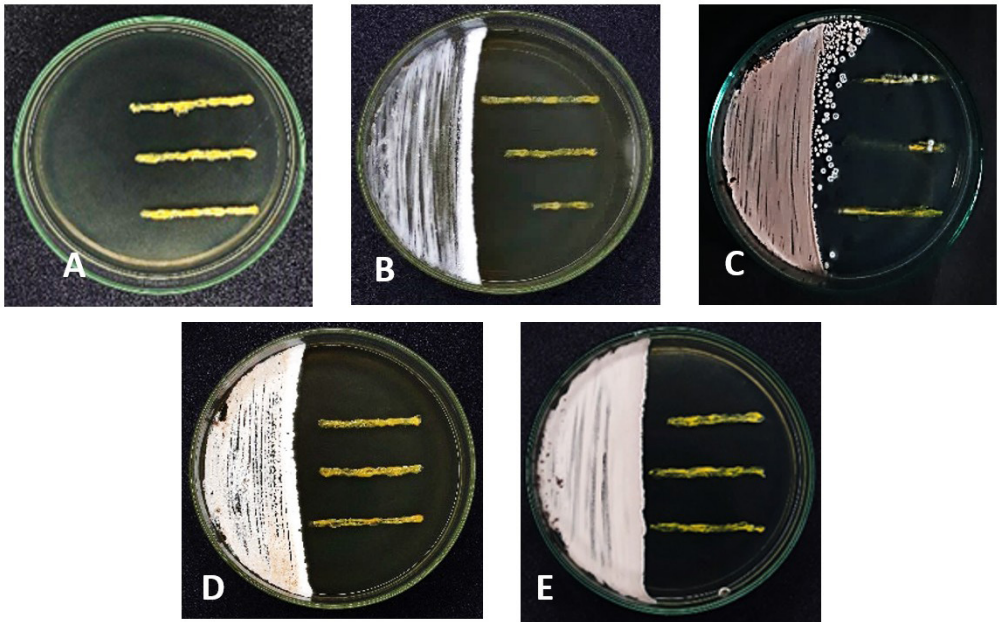

Figure 2. Results of the cross-streak antagonistic test. (A) Control, (B) CPT5, (C) CPT8, (D) CPT45, (E) CBS6. (Actinomycetes: scratch on the left side, pathogen: scratch on the right side)

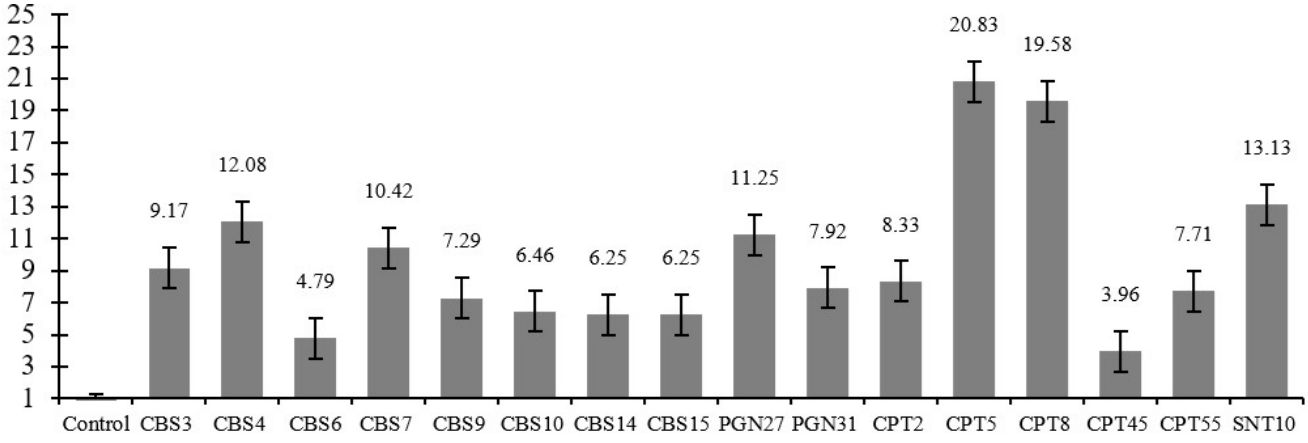

Figure 3. Antagonistic activity of actinomycete isolates against *X. campestris* pv. *campestris*

CPT5 showed the lowest activity. This suggests that SNT10 is more effective in stimulating peroxidase production than the other isolates.

3.8. Characterization and Identification of Potential Rhizospheric Actinomycetes

Morphological characterization was carried out for the selected isolates CPT5, CPT8, and SNT10, which were obtained from the cabbage rhizospheric (Table 3). Morphological observations and 16S rRNA sequence analysis identified CPT5 as *Streptomyces polychromogenes*, CPT8 as *Streptomyces vinaceus*, and SNT10 as *Streptomyces rochei*, all of which are known for their antimicrobial properties (Table 4).

Growth curve analysis showed that CPT8 grew the fastest, reaching the highest OD value (0.5007 at 24 hours), followed by CPT5 and SNT10, which had final OD values of 0.2257 and 0.2224, respectively (Figure 6).

4. Discussion

This study commenced with the isolation of rhizospheric actinomycetes from cabbage cultivated in three regions of West Java Garut, Cianjur, and Bandung which revealed variability in abundance and species diversity. The isolation process resulted in 55 actinomycete isolates with varying abundance and diversity across different

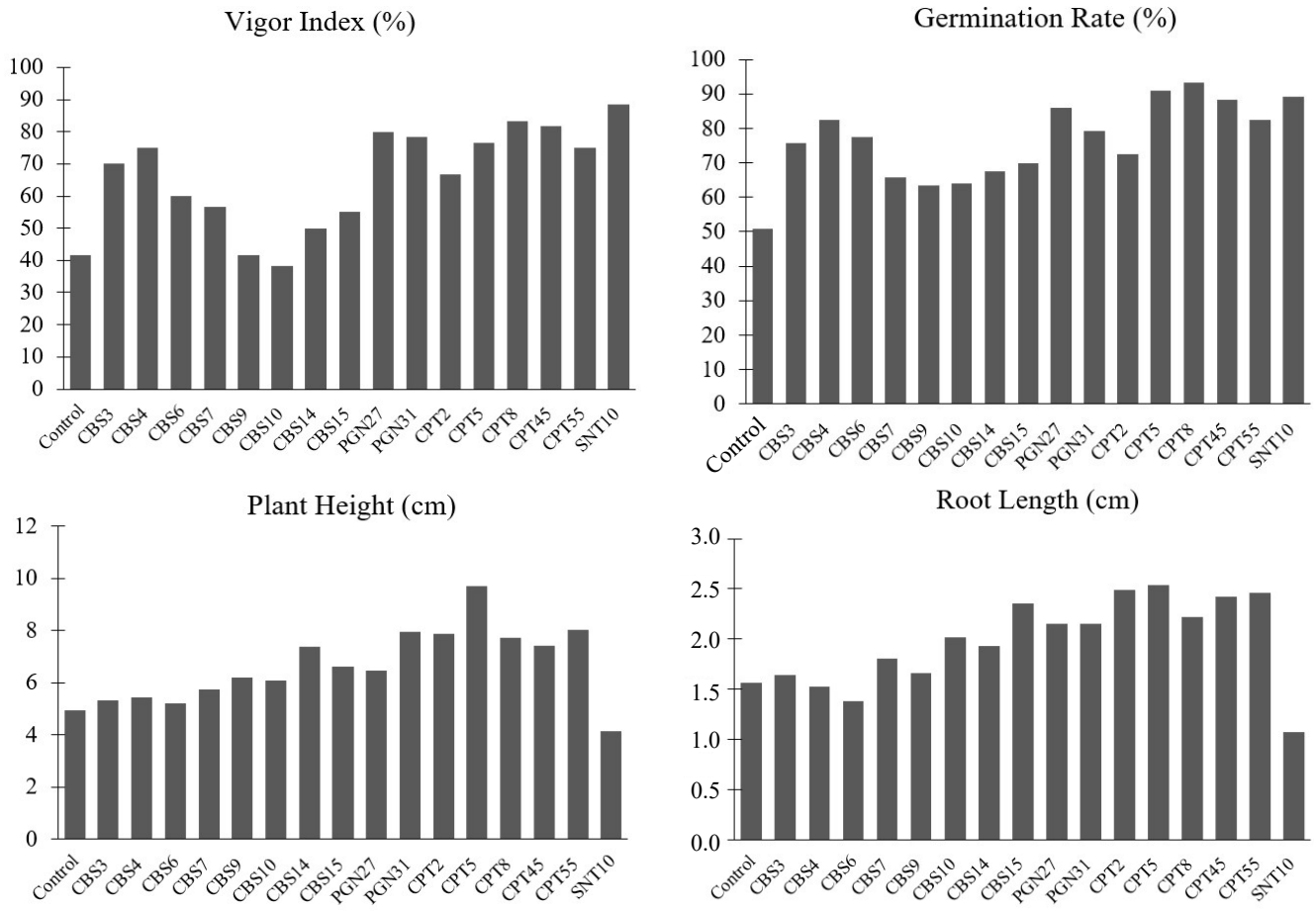

Figure 4. Vigor index, germination rate, plant height, and root length of cabbage seedlings in each actinomycete treatment

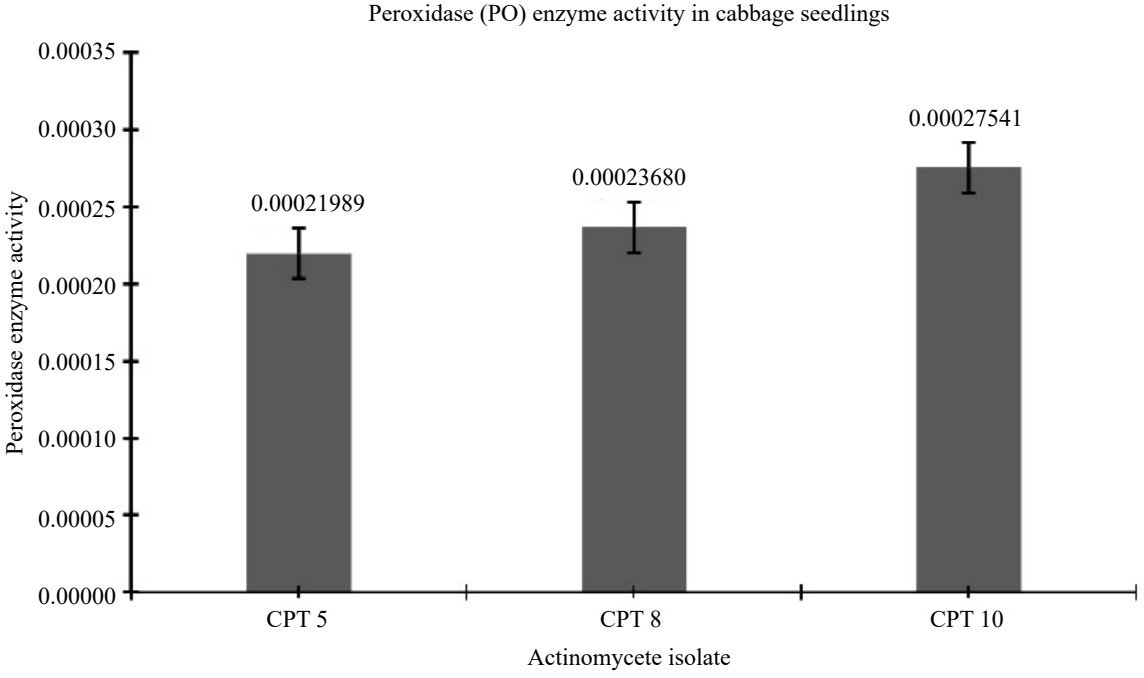

Figure 5. Effect of actinomycetes on peroxidase (PO) enzyme activity

Table 3. Morphological characteristics of selected actinomycete isolates

Colony characteristics	CPT5	CPT8	CPT8
Isolate			
Color	Pinkish white	Orange red	Gray
Shape	Circular	Irregular	Irregular
Elevation	Convex	Convex	Flat
Margin	Irregular	Undulate	Irregular

Table 4. 16S rRNA gene sequence alignment of CPT5, CPT8, and SNT10 based on universal actinomycete primers

Isolate code	Species name	Query cover (100%)	Homology (100%)	Country of origin	Accession number
CPT5	*Streptomyces polychromogenes*	100	99.99	India	KF032545.1
CPT8	*Streptomyces vinaceus*	99	98.93	South Korea	CP023692.1
SNT10	*Streptomyces rochei*	98	98.67	Thailand	KP797913.1

villages. Variations in actinomycete populations among the locations were primarily attributed to environmental conditions, including soil structure, pH, organic matter content, and nutrient availability. Villages with higher diversity, such as Cibodas and Ciputri, had better soil conditions for actinomycete development. Conversely, the low number of species in Santosa Village may indicate limitations in soil microbial life-supporting factors, such as a lack of organic matter or suboptimal environmental conditions.

Variations in the abundance and diversity of actinomycetes across different villages are influenced by several environmental factors. Soil conditions are the primary determinant of the abundance of these microorganisms. Soils rich in organic matter and with good structure tend to support larger and more diverse populations of actinomycetes. Additionally, soil pH and nutrient content play crucial roles in influencing actinomycete abundance and diversity. Soils with a neutral to slightly alkaline pH are more favorable for actinomycete growth compared to those that are highly acidic or strongly alkaline (Pakaya *et al.* 2025). In soils with extreme pH values, even if colony numbers are high, diversity may decrease because only a few species can survive.

Biosafety assessments revealed that several isolates elicited a hypersensitive response or exhibited hemolytic activity, indicating potential pathogenicity. These isolates were excluded from further testing to ensure environmental and biological safety. However, isolates CPT5, CPT8, and SNT10 were proven to be safe and have potential as biocontrol agents, as they did not show pathogenic characteristics toward plants or animals.

In vitro antagonistic assays demonstrated that isolates CPT5 and CPT8 exhibited the strongest inhibitory effects against *X. campestris* pv. *campestris* (*Xcc*), likely due to their ability to produce antimicrobial secondary metabolites and effectively compete for nutrients and ecological niches. Antagonistic actinomycetes are characterized by the formation of an inhibition zone around the main actinomycete streak, indicating suppression of pathogen growth through the production of antimicrobial compounds. This inhibitory effect may result from antibiotic production that disrupts pathogen protein or cell wall synthesis, competition for space and nutrients that prevents pathogen colonization, and the secretion of lytic enzymes such as chitinase and cellulase, which degrade the pathogen's cell wall. The larger the inhibition zone, the greater the actinomycete's capacity to suppress pathogen growth (Nurjasmi and Suryani 2020). Previous studies have also demonstrated that actinomycetes, particularly those belonging to the genus *Streptomyces*, produce natural antibiotics effective against various plant pathogens (Palaniyandi *et al.* 2019).

A previous study by Promnuan *et al.* (2021) demonstrated that three actinomycete isolates ACZ2-27, ASC3-2, and

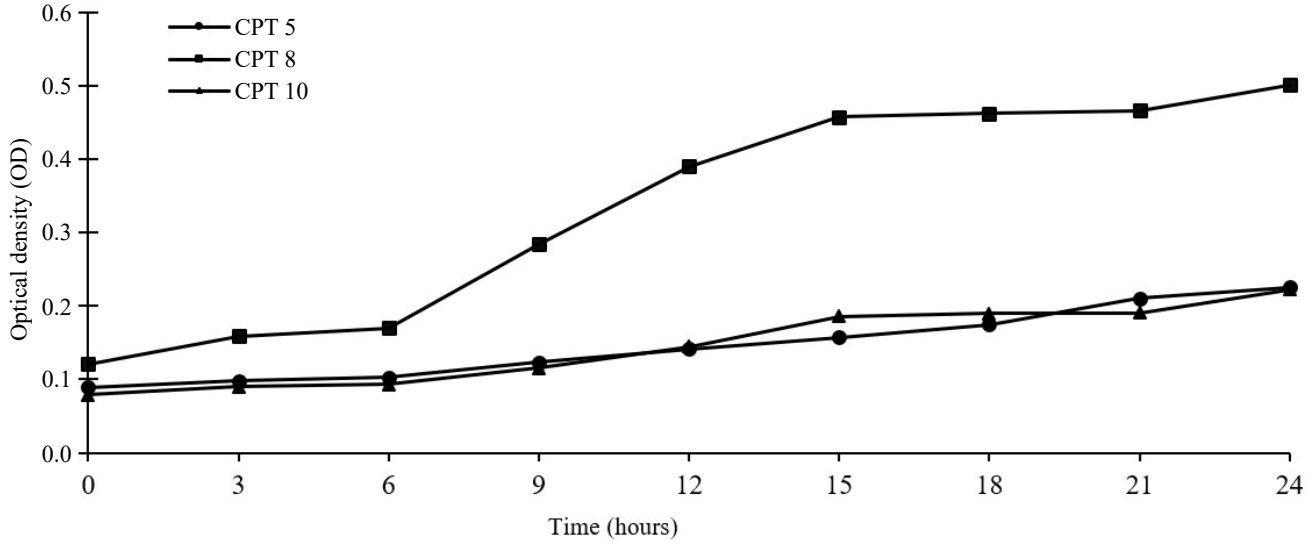

Figure 6.Growth of actinomycetes based on optical density (OD) values

ASC5-7P exhibited antagonistic activity against Xcc. The inhibition zones varied, with isolate ACZ2-27 showing the largest inhibition zone of 10.75 mm, followed by ASC5-7P with 9.00 mm, and ASC3-2 with 6.00 mm. These results indicate that actinomycetes have the potential as biocontrol agents in inhibiting *Xcc* growth.

The effect of actinomycete isolates on peroxidase enzyme activity and cabbage seedling growth was studied as part of a biological control effort against black rot disease caused by *Xcc*. The results showed that each isolate differed in effectiveness, both in enhancing peroxidase enzyme activity and suppressing pathogen growth. Among all isolates, SNT10 induced the highest peroxidase activity in cabbage seedlings, suggesting its involvement in triggering Induced Systemic Resistance (ISR) mechanisms, including reactive oxygen species (ROS) regulation and cell wall fortification (Torres *et al.* 2022).

Testing actinomycetes on cabbage seed growth and germination revealed significant variations in observed parameters, including vigor index, germination rate, plant height, and root length. Sriwahyuni *et al.* (2023) stated that actinomycetes function as biostimulants that support plant growth by producing secondary metabolites, organic compound-degrading enzymes, and enhancing nutrient availability in the rhizospheric. Additionally, actinomycetes contribute to plant resistance against abiotic and biotic stress, benefiting cabbage germination and early growth. Thus, the application of actinomycetes has the potential to naturally enhance cabbage productivity, reduce dependency on synthetic fertilizers, and promote sustainable agriculture. Actinomycetes benefit plant development by producing various growth hormones, such as Indole Acetic Acid (IAA), gibberellins, cytokinins, and ethylene. IAA, a natural auxin, plays a role in cell enlargement, inhibiting lateral shoot growth, stimulating abscission, and contributing to vascular tissue formation, such as xylem and phloem. Moreover, IAA influences root development and elongation, making it essential for overall plant growth (Baid *et al.* 2022).

Actinomycetes, particularly those belonging to the genus Streptomyces, are well-recognized for their capacity to produce antibiotics (e.g., streptomycin, rifamycin), lytic enzymes, and growth regulators, making them potent candidates for integrated disease management and sustainable agriculture. Their primary mechanism involves the production of antibiotics such as streptomycin and rifamycin, which inhibit the growth of pathogenic bacteria by disrupting protein synthesis and cell wall formation. In addition, actinomycetes are known to enhance soil health and nutrient availability, indirectly increasing plant resistance to pathogen attacks (Dow *et al.* 2023). The success of isolates CPT5, CPT8, and SNT10 in controlling disease in cabbage plants highlights the strong potential of actinomycetes as environmentally friendly alternatives to synthetic pesticides.

Molecular identification using 16S rRNA gene sequencing confirmed the identity of CPT5 as *Streptomyces polychromogenes*, CPT8 as *Streptomyces vinaceus*, and SNT10 as *Streptomyces rochei*, all of which have been reported to possess significant antimicrobial and plant-beneficial properties. These three species are known for

their ability to produce antimicrobial secondary metabolites and support plant growth. Thus, this study reinforces the role of actinomycetes as natural biocontrol agents that enhance plant resistance to pathogens and promote sustainable agriculture.

Streptomyces polychromogenes was originally isolated from soil in Venezuela and is known to produce O-carbamylserine, a bioactive compound. Meanwhile, *Streptomyces vinaceus* produces various bioactive compounds, including vitamin B12, viomycin, amicetin, and citreamycin delta, which play significant roles in medicine and biotechnology (Suárez *et al.* 2019). *Streptomyces rochei*, isolated from soil in Russia, is capable of producing bioactive compounds such as borrelidin, butyrolactones A and B, uricase, and streptotricin. In addition to its antifungal activity against *Fusarium oxysporum* f.sp. *lycopersici* and *Aspergillus fumigatus, S. rochei* also produces moenomycin and bambermycin, which are essential for controlling plant pathogens (Nonthakaew *et al.* 2022). With these capabilities, these three *Streptomyces* species hold great potential for applications in both healthcare and agriculture, particularly in the biological control of plant diseases.

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