

Consortia of endophytic bacteria for controlling *Colletotrichum gloeosporioides* causing anthracnose disease in chili plant

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Abstract. Nurbailis, Yanti Y, Resti Z, Djamaan A, Rahayu SD. 2023. Consortia of endophytic bacteria for controlling *Colletotrichum gloeosporioides* causing anthracnose disease in chili plant. *Biodiversitas* 24: 3503-3511. Anthracnose disease caused by *Colletotrichum gloeosporioides* is the main disease in chili plants, which reduces yields by up to 90%. Using a consortium of endophytic bacteria is an alternative way to control the disease at a low cost and is environmentally friendly. The aim of the study was to obtain the best consortium of endophytic bacteria to control anthracnose caused by *C. gloeosporioides* and improve the growth and yield of chili plants. This research was arranged in an experimental method consisting of two stages, namely (i) compatibility test between endophytic bacterial consisting of 28 species, and (ii) test of the endophytic bacterial consortium ability to control anthracnose in chili using a Completely Randomized Design (CRD) with 14 treatments and provided with 3 replications. All treatments of endophytic bacterial consortium showed the potential to suppress anthracnose disease caused by *C. gloeosporioides* and improve the growth and yield of chili plants. The best bacterial consortium treatment in suppressing the development of anthracnose disease by *C. gloeosporioides* and increasing the growth and yield of chili plants was the consortium *B. cereus* SNE 2.2 + *B. cereus* TLE 1.1 with dis-ease severity of 0.00%, plant height of 58.33 cm and fruit weight of 208.97 g.

Keywords: Anthracnose, chili, *Colletotrichum gloeosporioides*, consortia

INTRODUCTION

Anthracnose disease caused by *Colletotrichum* spp. is one of the most severe and important causes of yield losses in chili pepper ranging from 10 to 80% (Asare-Badiako et al. 2015). *Colletotrichum gloeosporioides* is one of the important pathogens causing anthracnose disease in chili, which can cause yield losses of up to 90% (Gautam et al. 2012). Anthracnose disease control commonly carried out in the field involves the intensive use of pesticides. Intensive and unwise use of chemicals (pesticides) may leave toxic effects in the environment, and the breeding resistant genes have no longlasting durability (Ji et al. 2008), leading to a risk for public health, natural waters, and non-target organisms (Gikas et al. 2022). Thus, other environmentally friendly alternatives are needed, one of which is the using biological agents from the group of endophytic bacteria (Yanti et al. 2020).

The mechanism of biological agents in controlling plant diseases is direct and indirect (Ludwig-Muller 2015). The direct mechanism is to produce antibiotic compounds, lytic enzymes, and siderophores and compete for nutrients and space (Beneduzi 2012). Meanwhile, the indirect mechanism is through the induction of plant resistance (Li et al. 2017). Endophytic bacteria induce plant resistance by producing metabolic compounds that play a role in plant resistance, including defense enzymes (phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase, β -1,3 glucanase, and phytoalexins) (Purwar et al. 2012).

Among them, the *Bacillus* genus is recognized as a promising group with a wide soil distribution. Due to their strong antagonistic activity, broad inhibitory spectrum, and high viability, members of the *Bacillus* species have effectively controlled soilborne, airborne, and postharvest diseases (Shafi et al. 2017). Suppression of plant pathogens from these bacteria might be due to microbial antagonisms due to the production of different varieties of secondary metabolites and enzymes like chitinase (Niazi et al. 2014)

Bacillus amyloliquefaciens significantly inhibited *C. gloeosporioides* with subsequent growth inhibition of 61.75% (Figueroa 2022). Meanwhile, combining endophytic bacteria of *B. cereus* TLE 1.1, *B. cereus* SNE 2.2, *B. toyonensis* EPL1.1.3, *B. anthracis* EPL 1.1.4, *Serratia nematodiphila* TLE 2.3, *Serratia marcescens* EIAB 2.1, *Enterobacter cloacae* EIAB 1.2, *Chryseobacterium rhizoplanae* KLE 3.3, and *Klebsiella michiganensis* TLE 2.2 from tomato plants have the potential to stimulate tomato plant growth and suppress the attack of *Ralstonia solanacearum* and *Fusarium oxysporum* fsp. *lycopersici* (Yanti et al. 2017).

Endophytic bacteria can be singly applied in a consortium; compatible endophytic bacteria can be used as a combination (a consortium). A consortium of endophytic bacteria control plant pathogens and improve plant growth (Kumar and Jagadeesh 2016). This method consists of designing mixtures of compatible strains that complement each other regarding the mechanism and ecological attributes. This strategy may increase the efficacy and

reliability of biocontrol in different environmental conditions, as well as provide a broader spectrum activity due to the synergistic effect of different mechanisms of the introduced biocontrol strains (Bonaterra et al. 2022)

Previous research results showed that the consortium of *Bacillus* sp. strain SJI, *Bacillus* sp. strain HI, *S. marcescens* strain ULG1E4, and *S. marcescens* strain JBIE3 from shallot plants was able to suppress purple spot disease (*Alternaria porri*) indirectly through the induction of plant resistance (Resti et al. 2021). The consortium of *B. cereus* AGBE 3.3 BB, *B. cereus* SLBE 1.1 SN, and *B. cereus* AGBE 1.2TL from chili plants were effective in suppressing the development of anthracnose disease with disease severity of 5% (Yanti et al. 2020). *B. subtilis* strain ZSH-1 displayed a strong antagonism towards *C. gloeosporioides* with inhibition rates ranging from 44.0 to 89.1%, and the greenhouse experiment also revealed that strain ZSH-1 had a 47.6% (12 days) efficacy in controlling poplar anthracnose than the control (Huang et al. 2020)

This study aimed to determine the best consortium of endophytic bacteria for controlling anthracnose caused by *C. gloeosporioides* and therefore improving the growth and production of chili.

MATERIALS AND METHODS

Study area

The research was carried out from January to May 2022 in the Laboratory of Microbiology and Phytopathology, Department of Plant Protection and the experimental field of the Faculty of Agriculture (0.914249, 100.470091), Universitas Andalas, Padang, West Sumatra, Indonesia which is at an altitude of 350 meters above sea level (masl).

Design of experiment and procedures

This study was conducted in an experimental method of two stages and arranged in a completely randomized design. The first stage was compatibility test consist consisting of 28 treatments (Table 1), and the second stage was an ability test of endophytic consortia of 14 treatments with 6 replications (Table 2). Data were statistically analyzed using Analysis of Variance and tested with Scott Knott at 0.05 significance level.

Compatibility test between various endophytic bacterial species

The experiment was carried out using 8 species of endophytic bacteria obtained from the collection of the Laboratory of Microbiology, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. The species used for the study were, *Serratia marcescens* EIAB 2.1, *Bacillus cereus* SNE 2.2, *Bacillus anthracis* EPL 1.1.4, *Chryseobacterium rhizoplaneae* KLE 3.3, *Bacillus toyonensis* EPL 1.1.3, *Enterobacter cloacae* EIAB 1.2, *Serratia nematodiphila* TLE 2.3, and *Bacillus cereus* TLE 1.1. The treatments consisted of 28 compatibility tests presented in Table 1.

Testing the ability of endophytic bacterial consortium for suppression of anthracnose disease in chili

The fourteen consortiums identified by the experiment's first stage and untreated control were analyzed to test the ability to suppress the pathogen. The experiment was laid out in CRD design with 6 and 3 replications per treatment at the seedling and the planting stage, respectively.

Table 1. Compatibility test treatment of endophytic bacteria

Treatment
<i>S. marcescens</i> EIAB 2.1+ <i>B. cereus</i> SNE 2.2
<i>S. marcescens</i> EIAB 2.1+ <i>B. anthracis</i> EPL 1.1.4
<i>S. marcescens</i> EIAB 2.1+ <i>C. rhizoplaneae</i> KLE 3.3
<i>S. marcescens</i> EIAB 2.1+ <i>B. toyonensis</i> EPL 1.1.3
<i>S. marcescens</i> EIAB 2.1+ <i>E. cloacae</i> EIAB 1.2
<i>S. marcescens</i> EIAB 2.1+ <i>S. nematodiphila</i> TLE 2.3
<i>S. marcescens</i> EIAB 2.1+ <i>B. cereus</i> TLE 1.1
<i>B. cereus</i> SNE 2.2+ <i>B. anthracis</i> EPL 1.1.4
<i>B. cereus</i> SNE 2.2+ <i>C. rhizoplaneae</i> KLE 3.3
<i>B. cereus</i> SNE 2.2+ <i>B. toyonensis</i> EPL 1.1.3
<i>B. cereus</i> SNE 2.2+ <i>E. cloacae</i> EIAB 1.
<i>B. cereus</i> SNE 2.2+ <i>S. nematodiphila</i> TLE 2.3
<i>B. cereus</i> SNE 2.2 + <i>B. cereus</i> TLE 1.1
<i>B. anthracis</i> EPL 1.1.4 + <i>C. rhizoplaneae</i> KLE 3.3
<i>B. anthracis</i> EPL 1.1.4+ <i>B. toyonensis</i> EPL 1.1.3
<i>B. anthracis</i> EPL 1.1.4+ <i>E. cloacae</i> EIAB 1.2
<i>B. anthracis</i> EPL 1.1.4+ <i>S. nematodiphila</i> TLE 2.3
<i>B. anthracis</i> EPL 1.1.4+ <i>B. cereus</i> TLE 1.1
<i>C. rhizoplaneae</i> KLE 3.3+ <i>B. toyonensis</i> EPL 1.1.3
<i>C. rhizoplaneae</i> KLE 3.3+ <i>E. cloacae</i> EIAB 1.2
<i>C. rhizoplaneae</i> KLE 3.3+ <i>S.nematodiphila</i> TLE 2.3
<i>C. rhizoplaneae</i> KLE 3.3 + <i>B. cereus</i> TLE 1.1
<i>B. toyonensis</i> EPL 1.1.3+ <i>E. cloacae</i> EIAB 1.2
<i>B. toyonensis</i> EPL 1.1.3+ <i>S. nematodiphila</i> TLE 2.3
<i>B. toyonensis</i> EPL 1.1.3+ <i>B. cereus</i> TLE 1.1
<i>E. cloacae</i> EIAB 1.2+ <i>S. nematodiphila</i> TLE 2.3
<i>E. cloacae</i> EIAB 1.2+ <i>B. cereus</i> TLE 1.1
<i>S. nematodiphila</i> TLE 2.3+ TLE 1.1

Table 2. Compatible bacterial consortium results from testing of 28 bacterial consortia

Treatment code	Description
A	<i>B. cereus</i> TLE 1.1 + <i>B. anthracis</i> EPL 1.1.4
B	<i>B. cereus</i> TLE 1.1 + <i>B. toyonensis</i> EPL 1.1.3
C	<i>B. cereus</i> SNE 2.2 + <i>C. rhizoplaneae</i> KLE 3.3
D	<i>B. cereus</i> SNE 2.2 + <i>B. cereus</i> TLE 1.1
E	<i>B. anthracis</i> EPL 1.1.4 + <i>B. toyonensis</i> EPL 1.1.3
F	<i>C. rhizoplaneae</i> KLE 3.3 + <i>B. toyonensis</i> EPL 1.1.3
G	<i>E. cloacae</i> EIAB 1.2 + <i>C. rhizoplaneae</i> KLE 3.3
H	<i>C. rhizoplaneae</i> KLE 3.3 + <i>B. cereus</i> TLE 1.1
I	<i>C. rhizoplaneae</i> KLE 3.3 + <i>B. anthracis</i> EPL 1.1.4
J	<i>E. cloacae</i> EIAB 1.2 + <i>B. cereus</i> TLE 1.1
K	<i>E. cloacae</i> EIAB 1.2 + <i>B. cereus</i> SNE 2.2
L	<i>S. marcescens</i> EIAB 2.1 + <i>S. nematodiphila</i> TLE 2.3
M	<i>B. cereus</i> SNE 2.2 + <i>B. anthracis</i> EPL 1.1.4
N	<i>B. cereus</i> SNE 2.2 + <i>B. toyonensis</i> EPL 1.1.3
O	Control (treatment without consortium and <i>C. gloeosporioides</i> inoculation)

Rejuvenation and confirmation of endophytic bacteria.

The isolate stock stored in microtubes was rejuvenated in Tryptic Soy Agar (TSA) medium for *B. cereus* SNE 2.2, *B. cereus* TLE 1.1, *B. toyonensis* EPL 1.1.3 and *B. anthracis* EPL 1.1.4. Meanwhile, Nutrient Agar (NA) medium was used for *S. marcescens* EIAB 2.1, *S. nematodiphila* TLE 2.3, *E. cloacae* EIAB 1.2 and *C. rhizophanae* KLE 3.3) using the scratch method and incubated for 48 hours.

KOH test. Gram test was carried out by dripping 3% KOH solution on the slide surface, then a single colony of endophytic bacteria was placed. If there is mucus when the needle is removed, the bacteria are Gram-negative, while if there is no mucus, the bacteria are Gram-positive (Schaad et al. 2001).

Hypersensitivity reaction. The endophytic bacteria were suspended using sterile distilled water with a population density of 10^8 cells mL⁻¹, homogenized with a vortex for a minute, and infiltrated intercellularly into the lower surface tissue of the four o'clock flower plant (*Mirabilis jalapa* L.) with a syringe until saturated. The infiltrated leaves were covered with clear plastic and incubated for 2x24 hours. If there are no necrotic symptoms on the leaves, it means that it is not a plant pathogen or a negative reaction (Klement et al. 1990)

Compatibility test between endophytic bacteria species.

Endophytic bacteria compatibility test was performed using the cross streak method. Two different endophytic bacteria were scratched by crossing (vertical and horizontal) on a petri dish containing TSA medium for the *Bacillus* bacteria group and NA for the *Serratia*, *Chryseobacterium* and *Enterobacter* bacteria groups were then incubated for 48 hours at room temperature. Inhibition zones formed between isolates were observed (James et al. 2003).

Propagation of the consortium of endophytic bacteria.

Confirmed non-pathogenic endophytic bacteria were rejuvenated on TSA or NA media and propagated in liquid culture consisting of two stages. In the first stage (preculture), one colony of endophytic bacteria was put into 10 mL of Nutrient Broth (NB) and incubated on a rotary shaker at 150 rpm for 24 hours at room temperature. In the second stage (main culture), a consortium of endophytic bacteria was created by combining two compatible species of endophytic bacteria (the results of the first stage of the experiment). Furthermore, one mL of liquid culture of each endophytic bacterial species (preculture results) was transferred to 23 mL of sterile coconut water in a culture bottle (50 mL) and incubated for 2x24 hours on a rotary shaker at 150 rpm at room temperature (Yanti et al. 2020). The density of the bacterial population was determined by comparing the turbidity of the bacterial suspension with a McFarland scale 8 solutions (population density of 10^8 cells/mL⁻¹) (Yanti et al. 2018a).

Ability test of the endophytic bacterial consortium for anthracnose disease control in chili

Preparation of planting media. The soil used was from the experimental field of the faculty of agriculture, Andalas University, Padang with ultisol soil type. The soil was mixed with manure (cow dung) in a ratio of 2:1 (v/v), put into a 5 kg clear plastic bag, and sterilized for 1 hour

using a boiler at 100°C and cooled. Growing media was put as much as 20 g per hole in the seed tray for the nursery and 10 kg into polybags measuring 35×35 cm for planting (Yanti et al. 2020).

Endophytic bacterial consortium application

The endophytic bacterial consortium was applied in two stages, namely on seeds and seedlings. The seeds were sterilized in 1% NaOCl solution for 1 minute, which were then rinsed with sterile distilled water and dried. The seeds were then soaked in each suspension of the endophytic bacterial consortium (population density of 10^8 cells/mL) for 15 minutes. For the control treatment, the seeds were soaked in sterile distilled water. The seeds were planted on a tray (three seeds per hole) and treated for 21 days. The seedlings at age of 21 days were removed from the seed tray, and the roots were cleaned from the soil with water. Subsequently, the roots were immersed in a consortium suspension of endophytic bacteria (population density of 10^8 cells/mL) for 15 minutes. For the control treatment, the roots were immersed in sterile distilled water. Afterward, the seedlings were planted in polybags containing a mixture of soil and manure, and 2 chili seedlings were planted in each polybag.

Isolation and identification of C. gloeosporioides

The isolation was carried out by the moist chamber method. First surface sterilization is done on symptomatic fruit by washing it with sterile aquadest. The fruit is soaked in a plastic box containing Natrium hypochlorite 1% (NaOCl 1%) for 3 minutes and then rinsed with sterile aquadest. The fruit is cut into 0.5 cm size by inserting symptomatic and healthy parts. Five pieces of fruit were placed in a plastic petri dish covered with moistened filter paper and incubated for 48 hours. The fungus that grows on the fruit was isolated in a petri dish containing a PDA medium, and the fungal colony that has the characteristic of *C. gloeosporioides* was isolated again until its pure culture was obtained (Nurbailis et al. 2019). Furthermore, the fungi were identified macroscopically by observing the color and growth of the colony. Meanwhile, the hyphae structure and conidia shape were microscopically observed. Identification refers to (Barnet and Hunter 1972; Alexopoulos et al. 1996; Than et al. 2008).

Preparation of the suspension of C. gloeosporioides

Suspension of conidia of *C. gloeosporioides* was prepared by dilution using a pure culture of *C. gloeosporioides* aged 14 days in a petri dish, which was added with 10 mL of sterile distilled water. Then the dilution was made up to 10^{-3} . Conidia density was calculated at a dilution level of 10^{-3} using an improved Neubauer-type hemocytometer. The conidia density required was 10^6 conidia/ml (Suparman et al. 2018).

Pathogenicity Test of *C. gloeosporioides*. The pathogenicity test was performed using healthy chilies. Suspension of *C. gloeosporioides* (10^6 conidia/mL) was inoculated by smearing with a brush on the injured part. The fruit was placed in a plastic box filled with a moist paper tissue and observed until symptoms appeared.

Symptoms are shown by lesions on the infected fruit (Ibrahim et al. 2017).

Inoculation of *C. gloeosporioides*. *C. gloeosporioides* inoculation was carried out on chili plants using the scatter method (Suparman et al. 2018). Healthy chilies were surface sterilized using 1% NaOCl and sterile distilled water. Then the chilies were air-dried and wounded using sterile needles at the base, middle, and bottom. Furthermore, the suspension of *C. gloeosporioides* (10^6 conidia/mL) was inoculated by applying a brush to the wounded area. The chilies were placed in a mica plastic box containing a moist tissue, and a plastic straw was used as a support inside the box. The box was tightly closed and incubated for four days. The criteria for infected fruit were the presence of dry rot on the part of the fruit that is concave to the inside. In the middle of the rot is orange or brown and then will turn black (Than et al. 2008). Chili fruits showing symptoms of anthracnose disease (*C. gloeosporioides*) were inoculated into 72-day-old chili plants. The symptomatic chili fruits were placed around the stems of chili plants (three fruits in a polybag of chili plants).

Maintenance. Maintenance was conducted by watering every morning and evening (according to plant conditions), weeding, installing stakes, and mechanical pest control. Weeds that grow directly weeded. The stakes are installed when the chili plants are 3 weeks old. Plant-disturbing organisms are controlled mechanically, by taking them directly from the plant or the surrounding environment and then killing them.

Fertilizer. Fertilizer was given a third of the dose on chili plants 3, 6, and 9 weeks after planting. Fertilizers consist of Urea 1 g/polybag equivalent to (Urea 100 kg/ha), SP-36 1.5 g/polybag (SP-36 150 kg/ha), ZA 3 g/polybag (ZA 300 kg/ha) and KCl 1.5 g/poly bag (KCl 150 kg/ha). Fertilizer is given by making holes around the roots as deep as 5 cm and with a distance of 3 cm from the base of the stem (Sumarni and Muharam 2005).

Harvesting. The chili fruits were harvested when they were physiologically ripe, with the criteria of fruit being 75% red. Harvesting was done nine times by picking the fruit and stems by hand. The harvested fruits were separated between the damaged and the whole ones.

Parameters observed

Compatibility test between different endophytic bacteria

A compatibility test between different endophytic bacteria was performed by observing inhibition zones that appeared at the intersection of vertical and horizontal streaks (James et al. 2003). The absence of a clear zone between the vertical and horizontal streak junctions characterized the consortium of compatible endophytic bacteria.

Ability test of the endophytic bacterial consortium to control anthracnose disease in chili

Disease development

Incubation period (days after inoculation). The incubation period was observed every day after the inoculation of *C. gloeosporioides* until the plants showed early anthracnose symptoms in chili. The initial symptoms

of *C. gloeosporioides* are characterized by the presence of round, grooved brown spots. The effectiveness of suppression of the incubation period was calculated using the formula (Sivan and Chet 1986).

$$E = \frac{T-nc}{nc} \times 100\% \dots\dots\dots [1]$$

Where :
 E : effectiveness
 T : the value of treatment
 nc: the value of Negative control

Disease incidence (%). The disease incidence was observed after the first anthracnose symptoms appeared until the fruit was harvested at an interval of a week. Disease incidence was calculated by formula 2, and effectiveness was calculated using formula 1.

$$DI = \frac{n}{N} \times 100\% \dots\dots\dots [2]$$

Where:
 DI : disease incidence (%)
 n : number of infested fruits
 N : number of observed fruits

Disease severity (%). The disease severity was observed starting after the first symptoms appeared until harvesting at an interval of a week. The disease severity was calculated using formula 3 (Suprapta 2022) and effectiveness was calculated using formula 1.

$$S = \frac{\sum ni \times vi}{N \times V} \times 100\% \dots\dots\dots [3]$$

Where:
 S: disease severity
 ni: number of fruits in each attack category
 vi : scale value of each attack category
 N : number of fruits observed
 V : highest numerical score in the attack category

Next, to calculate the severity of anthracnose disease in chili plants, the scale presented in Table 3 was used.

Chili seed growth. Observations of chili seed growth traits included field emergence, seedling height, Number of leaves, and seed dry weight. Field Emergence Power (%) Emergence was observed when new chili seeds emerged on the soil surface and the Number that emerged was calculated compared to the total seeds planted. Field emergence observations were carried out every day for up to 21 days.

Table 3. Anthracnose disease severity score on chili fruit (Suprapta 2022) is as follows

Score	The extent of fruit attack symptoms
0	Without attack
1	Symptoms occur on the fruit 1-10%
2	Symptoms occur on the fruit 10-30%
3	Symptoms occur on the fruit 30-50%
4	Symptoms occur on the fruit 50-75%
5	Symptoms occur on the fruit 75-100%

Notes: The effectiveness of each treatment was calculated using formula 1

The formula calculates the percentage of field emerging power:

$$P = \frac{b}{B} \times 100\% \dots\dots\dots [4]$$

Where:

- P : seed percentage appears
- b : number of seeds that appear
- B : number of seeds sown

The effectiveness of each treatment was calculated using the formula (Sivan and Chet 1986) as follows:

$$E = \frac{P - Kp}{Kp} \times 100\% \dots\dots\dots [5]$$

Where:

- E : effectiveness
- P : treatment
- kp : positive control

Seedling height is measured starting from the base of the stem to the highest growing point. Seedling height was observed once a week for 3 weeks. The Number of leaves was observed and counted every 1 week for 3 weeks since the first leaves appeared. Therefore, the seeds were wrapped in stencil paper to measure dry weight, dried in an oven at 60°C for 5 hours and weighed (until constant weight). The effectiveness of each treatment is calculated using formula 5.

Vegetative and generative growth

Observations of the vegetative and generative growth included plant height, Number of leaves, the appearance of the first flower, and fruit weight. Observation of plant height begins after the plants are 14 days old after planting until the plants flower with an interval of 1 week. Chili leaves starts from the first leaf until the first flower grows and appears once every 1 week until the first flower appears. The newly counted leaves are added to the previously counted leaves and each growing leaf is marked. Observations of the appearance of the first flowers were carried out every day until the first flowers appeared on each plant. The fruit weight was observed by harvesting healthy chilies, then weighing and adding up each time you

harvest. The effectiveness of each treatment is calculated using formula 5.

Data analysis

The data were statistically analyzed and tested with Skott-Knott at a significant level of 5%.

RESULTS AND DISCUSSION

Compatibility test between endophytic bacterial species

Endophytic bacteria tested for compatibility showed variations. Not all endophytic bacteria were compatible with each other to grow on the same medium. The results of the endophytic bacterial compatibility test are presented in Table 4.

The group of endophytic bacteria from *Bacillus* spp. was compatible with all bacterial strains of the genus *Bacillus* but not compatible with the genus *Serratia*. Based on Figure 1.A , the endophytic bacteria of *B. cereus* TLE 1.1 and *B. anthracis* EPL 1.1.4 were compatible because there was no clear zone between the vertical and horizontal strokes. Endophytic bacteria of *B. cereus* SNE 2.2 and *S. nematodiphila* TLE 2.3 were not compatible because of the clear zone between the vertical and horizontal strokes.

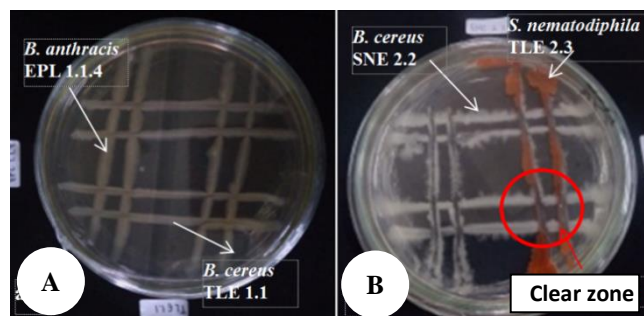


Figure 1. Compatibility between endophytic bacteria incubated for 2x24 hours, A. *Bacillus anthracis* EPL 1.1.4 and *Bacillus cereus* TLE 1.1 show compatibility; B. *Bacillus cereus* SNE 2.2 and *Serratia nematodiphila* TLE 2.3 show incompatible

Table 4. Compatibility results between different species of endophytic bacteria

	TLE 1.1	EPL 1.14	EPL 1.1.3	SNE 2.2	KLE 3.3	EIAB1.2	EIAB 2.1	TLE 2.3
TLE 1.1	█	✓	✓	✓	✓	✓	-	-
EPL 1.14	✓	█	✓	✓	✓	-	-	-
EPL 1.1.3	✓	✓	█	✓	✓	-	-	-
SNE 2.2	✓	✓	✓	█	✓	✓	-	-
KLE 3.3	✓	✓	✓	✓	█	✓	-	-
EIAB 1.2	✓	-	-	✓	✓	█	-	-
EIAB 2.1	-	-	-	-	-	-	█	✓
TLE 2.3	-	-	-	-	-	-	✓	█

Notes: ✓ (compatible), - (incompatible). TLE 1.1: *B. cereus* TLE 1.1; EPL 1.1.4: *B. anthracis* EPL 1.1.4; EPL 1.1.3: *B. toyonensis* EPL 1.1.3; SNE 2.2: *B. cereus* SNE 2.2; KLE 3.3: *C. rhizophanae* KLE 3.3; EIAB 2.1: *S. marcescens* EIAB 2.1; TLE 2.3: *S.nematodiphila* TLE 2.3

Table 5. The incubation period, disease incidence and disease severity of anthracnose on chili plants treated with the endophytic bacterial consortium (35 days)

Treatment	Incubation period (dai)	Effectiveness Incubation period (%)	Disease incidence (%)	Effectiveness Disease incidence (%)	Disease Severity (%)	Effectiveness Severity (%)
O=control	6.33 a	0.00	61.29 b	0.00	49.65 b	0.00
A	26.00 b	310.74	3.97 a	93.52	3.66 a	92.63
B	26.00 b	310.74	5.10 a	91.67	4.34 a	91.26
J	27.67 b	337.12	6.67 a	89.11	6.69 a	86.53
L	27.67 b	326.54	11.35 a	81.48	8.96 a	81.95
H	28.00 b	342.34	5.86 a	90.43	5.86 a	87.84
N	28.00 b	342.34	4.05 a	93.39	3.42 a	93.11
F	28.33 b	347.55	1.90 a	96.27	1.27 a	97.46
M	28.33 b	347.55	4.90 a	92.00	3.18 a	93.60
K	31.00 b	389.73	4.62 a	92.46	4.16 a	91.62
G	32.67 b	416.11	0.92 a	98.50	0.92 a	98.15
C	35.00 b	452.92	0.00 a	100.00	0.00 a	100.00
D	35.00 b	452.92	0.00 a	100.00	0.00 a	100.00
E	35.00 b	452.92	0.00 a	100.00	0.00 a	100.00
I	35.00 b	452.92	0.00 a	100.00	0.00 a	100.00

Notes: The mean value followed by the same letter was not significantly different according to the Scott-Knott Follow-up Test at the 0.05 significance level

Ability test of the endophytic bacteria consortium to control anthracnose disease in chili

Disease development

The consortium of endophytic bacteria significantly affected the control treatment on the incubation period, disease incidence, and anthracnose disease severity in chili peppers (Table 5). All endophytic bacteria consortia were able to inhibit the development of anthracnose disease in chili plants. There were four treatments with high effectiveness in suppressing the incubation period, disease incidence and disease severity as indicated by the absence of symptoms on chili plants at the end of the observation (35 days after inoculation), namely the C consortium treatment (*B. cereus* SNE 2.2 + *C. rhizophanae* KLE 3.3), consortium D (*B. cereus* SNE 2.2 + *B. cereus* TLE 1.1), consortium E (*B. anthracis* EPL 1.1.4 + *B. toyonensis* EPL 1.1.3), and consortium I (*C. rhizophanae* KLE 3.3 + *B. anthracis* EPL 1.1.4).

Chili seed growth

The growth of chili seeds treated with a consortium of endophytic bacteria showed significantly different responses. Further test results can be seen in Table 6.

Chili seeds introduced with a consortium of endophytic bacteria increased seed field emergence compared to the control treatment. Seven endophytic bacterial consortia have 100% field strength, namely E (*B. anthracis* EPL 1.1.4 + *B. toyonensis* EPL 1.1.3), B (*B. cereus* TLE 1.1 + *B. toyonensis* EPL 1.1.3), C (*B. cereus* SNE 2.2 + *C. rhizophanae* KLE 3.3), A (*B. cereus* TLE 1.1 + *B. anthracis* EPL 1.1.4), H (*C. rhizophanae* KLE 3.3 + *B. cereus* TLE 1.1), K (*E. cloacae* EIAB 1.2 + *B. cereus* SNE 2.2), and L

(*S. marcescens* EIAB 2.1 + *S. nematodiphila* TLE 2.3). All the endophytic bacteria consortium treatments were able to increase the growth of the seedling height compared to the control. The best treatment in increasing the height growth of chili seedlings was consortium D (*B. cereus* SNE 2.2 + *B. cereus* TLE 1.1) with a height of 8.90 cm and an effectiveness of 82.75%,

The Number of chili seed leaves introduced with a consortium of endophytic bacteria showed a significant difference compared with the control. Consortium K (*E. cloacae* EIAB 1.2 + *B. cereus* SNE 2.2) was the best treatment in increasing the number of seedling leaves, with an effectiveness of 136.60%.

The dry weight of chili seedlings introduced to a consortium of endophytic bacteria showed a significantly different effect between treatments. Several treatments showed the same abilities as controls. The best treatment for increasing the dry weight of chili seeds was consortium J (*E. cloacae* EIAB 1.2 + *B. cereus* TLE 1.1) with a weight of 0.10 g.

Chili plant growth

The endophytic bacterial consortia significantly affected plant height, the Number of leaves, first flower appearance, and fruit weight applied. Further test results can be seen in Table 7.

The treatments of various consortia of endophytic bacteria showed no significant effect on increasing plant height. Several treatments of the consortium of endophytic bacteria showed significant differences in the Number of leaves, appearance of flowers and fruit weight.

Table 6. Field emergence, seedling height, Number of leaves and dry weight of chili seedlings introduced with the endophytic bacterial consortium (21 days)

Treatment	Field emergence (%)	Effectiveness field emergence (%)	Seedling height (cm)	Effectiveness seedling height (%)	Number of leaves	Effectiveness number of leaves	Dry weight (g)	Effectiveness dry weight (%)
D	94.44 b	21.43	8.90 d	82.75	4.66 b	21.67	0.09 c	350.00
J	91.66 b	17.86	8.73 d	79.26	4.83 b	30.54	0.10 c	400.00
F	94.44 b	21.43	8.63 d	77.20	5.00 b	30.54	0.06 b	200.00
E	100 b	28.58	8.48 d	74.13	4.83 b	30.54	0.09 c	350.00
B	100 b	28.58	8.45 d	73.51	4.66 b	21.67	0.07 b	250.00
C	100 b	28.58	8.43 d	73.10	4.50 b	17.49	0.07 b	250.00
M	94.44 b	21.43	8.37 d	71.87	4.83 b	30.54	0.06 b	200.00
A	100 b	28.58	8.00 c	64.27	4.50 b	17.49	0.06 b	200.00
H	100 b	28.58	7.70 c	58.11	4.16 b	8.62	0.05 a	150.00
N	94.44 b	21.43	7.58 c	55.65	4.50 b	17.49	0.03 a	50.00
G	91.66 b	17.86	7.55 c	55.03	4.33 b	13.05	0.06 a	200.00
K	100 b	28.58	7.55 c	55.03	5.33 b	39.16	0.05 a	150.00
I	83.00 b	7.15	7.48 c	53.59	4.50 b	17.49	0.06 a	200.00
L	100 b	28.58	6.87 b	41.07	4.66 b	21.67	0.04 a	40.46
O=control	77.77 a	0.00	4.87 a	0.00	3.83a	0.00	0.02 a	0.00

Notes: The mean value followed by the same letter was not significantly different according to the Scott-Knott Follow-up Test at the 0.05 significance level

Table 7. Plant height and Number of leaves of chili plants introduced with the endophytic bacterial consortium (56 Day After Plant (DAP))

Treatment	Plant height (cm)	Effectiveness Plant height (%)	Number of leaves	Effectiveness Number of leaves	First flowers appear (DAP)	Effectiveness First flowers appear (%)	Fruit weight (g) /plant	Effectiveness Fruit weight (%)
D	58.33 a	62.00	161.33 b	68.05	45.00 a	27.41	208.97 d	207.53
N	57.67 a	60.19	149.67 b	55.90	46.33 a	25.27	207.44 d	205.28
E	55.83 a	55.08	147.67 b	53.82	45.67 a	26.34	198.05 d	191.46
G	54.00 a	50.00	155.00 b	61.45	47.33 a	23.66	190.26 d	180.00
I	53.17 a	47.69	129.67 b	35.07	47.00 a	24.19	166.09 c	144.42
F	53.00 a	47.22	122.00 b	27.08	47.33 a	23.66	173.41 c	152.20
B	52.33 a	45.36	138.33 b	44.09	46.00 a	25.80	172.67 c	154.11
A	50.67 a	40.75	142.67 b	47.57	48.00 a	22.58	176.84 c	160.25
L	51.00 a	41.66	121.67 a	26.73	48.00 a	22.58	158.29 c	132.95
H	51.00 a	41.66	141.67 b	47.57	45.33 a	26.88	169.95 c	150.11
K	50.00 a	38.88	133.33 b	38.88	48.33 a	22.04	147.22 c	116.65
M	48.33 a	34.25	121.67 a	26.73	47.67 a	23.11	172.96 c	154.54
C	46.50 a	29.16	146.00 b	52.08	48.00 a	22.58	166.09 c	144.42
J	45.33 a	25.91	109.33 a	13.88	49.33 a	20.43	162.20 c	138.70
O=control	36.00 a	0.00	96.00 a	0.00	62.00 b	0.00	67.95 a	0.00

Notes: The mean value followed by the same letter was not significantly different according to the Scott-Knott Follow-up Test at the 0.05 significance level

Discussion

Endophytic bacteria that were used as a consortium were compatible with each other. A total of 14 combinations of endophytic bacteria were compatible with each other (Table 4). Compatible bacteria are characterized by the absence of a clear zone between the meeting point of the two endophytic bacteria (vertical and horizontal lines). Compatible bacteria are synergistic with each other or do not suppress each other in the same habitat. Tabacchioni et al. (2021) stated that compatible bacteria could live together without suppressing the growth of one another in controlling disease and plant growth.

The consortium of endophytic bacteria introduced into chili plants was able to prolong the incubation period and reduce disease incidence and severity compared to the

controls (Table 5). The treatments with four potential endophytic bacterial consortia showed no symptoms to appear until the end of the observation (35 days after inoculation). The bacterial consortia were *B. cereus* SNE 2.2 + *C. rhizoplanae* KLE 3.3, *B. cereus* SNE 2.2 + *B. cereus* TLE 1.1, *B. anthracis* EPL 1.1.4 + *B. toyonensis* EPL 1.1.3, and *C. rhizoplanae* KLE 3.3 + *B. anthracis* EPL 1.1.4. The endophytic bacterial consortium could increase plant resistance through a plant resistance induction mechanism in inhibiting the growth of *C. gloeosporioides*, thereby reducing the percentage of fruit attacks and the intensity of disease attacks. (Table 5). According to (Purwar et al. 2012), Endophytic bacteria induce plant resistance by producing metabolic compounds that play a role in plant resistance, including defense enzymes

(phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase, β -1,3 glucanase, and phytoalexins). Yanti et al. (2019) reported endophytic bacteria from the *Bacillus* spp. could increase the activity of plant defense enzymes, namely phenylalanine ammonia-lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO), in inducing systemic resistance in tomato plants in suppressing bacterial wilt disease (*Ralstonia syzygii* subsp. *indonesiensis*). Yanti et al. (2020) also reported that *B. cereus* TLE 1.1 and *B. toyonensis* EPL 1.1.3 had higher PAL activity than other bacteria, *B. cereus* SNE 2.2 produced the highest PO (59.46%), and *B. cereus* TLE 1.1 produced the highest PPO (342.86%). Besides, a consortium of *B. pseudomycooides* SLBE 3.1 AP, *B. thuringiensis* SLBE 2.3, and *B. toyonensis* AGBE2.1TL from chili plants was reported to suppress anthracnose disease caused by *C. capsici* in chili plants with disease severity of 5%. That proves a mutually supportive mechanism exists between endophytic bacteria to inhibit the development of *C. capsici*. Mukherjee et al. (2018) also mention that the endophytic bacterial consortium was more effective because the physiological activity of endophytic bacteria in a consortium gave better results, producing more diverse secondary metabolites.

Chili seeds introduced with a consortium of endophytic bacteria experienced increased field emergence, seedling height, Number of leaves, fresh weight, and dry weight (Table 6). The best endophytic bacterial consortium was the consortium of *E. cloacae* EIAB 1.2 + *B. cereus* TLE 1.1. This consortium significantly increased the field emergence, seed height, Number of leaves, root length, fresh weight, and dry weight to 91.66%, 8.73 cm, 4.83, 7.58 cm, 0.41 g, and 0.10 g, respectively, with an average effectiveness of 144.11% (Table 6 and 7). This result shows that the endophytic bacterial can produce growth hormones namely Indole Acetic Acid (IAA) and gibberelins (Prihatiningsih et al. 2023). Yanti et al. (2017) mention that these endophytic bacteria can produce IAA hormones of 26.8-42.5 ppm, associated with tomato plant roots. The *B. cereus* TLE 1.1 endophytic bacteria produced the highest IAA at 42.5 ppm. Various *Bacillus* spp. strains can elicit ISR in different plants and confer an enhanced defense mechanism against a various pathogens. Several studies have shown that Volatile Organic Compounds (VOCs) and Contract Laboratory Programs (CLPs), such as surfactin and fengycin, are involved in the immune response of plants elicitation (Cawoy et al. 2015). Srinivasan and Mathivanan (2011) reported that the endophytic bacterial consortium produced a higher IAA concentration than the single endophytic bacteria application. Furthermore, Rajer et al. (2022) also reported that the *Bacillus* spp. FA12 and FA26, applied as seed inoculants, significantly enhanced the vigor index of rice seedlings by 78.89% and 108.70%, respectively.

Compared to the controls, the endophytic bacterial consortium applied to chili plants increased plant height growth and the number of leaves. The best consortium of endophytic bacteria in increasing the growth of chili plants was the consortium of *B. cereus* SNE 2.2 and *B. cereus* TLE 1.1, with a chili plant height of 58.33 cm and 161.33 leaves. These endophytes can fix N_2 that live in the

intracellular spaces and the plant's vascular system and also take dissolved N_2 gas from the sap flow and convert it into amines and ammonium nitrogen for plant use (Hongrittipun et al. 2014). Yanti et al. (2017) reported that endophytic bacteria *B. cereus* TLE 1.1 produced the highest IAA at 42.5 ppm, and *B. cereus* SNE 2.2 produced IAA at 28.7 ppm. According to Cueva-Yesquén et al. (2021) The contribution of these genera to the promotion of plant-growth and germination is highlighted by their potential to produce IAA, solubilize phosphate, and synthesize siderophores.

The consortium of endophytic bacteria introduced into chili plants significantly accelerated the emergence of first flowers and increased the Number of fruits and fruit weight compared to the control. The best consortium of endophytic bacteria in increasing chili production was the consortium of *B. cereus* SNE 2.2 + *B. cereus* TLE 1.1. This consortium could accelerate the emergence of the first flower (45 days after inoculation), increase the Number of fruits, and increase fruit weight. This is because the endophytic bacterial consortium can dissolve phosphate in increasing production. This is supported by previous research from Yanti et al. (2017), reporting that endophytic bacteria *B. cereus* TLE 1.1 could dissolve phosphate, while *B. cereus* SNE 2.2 was not able to dissolve phosphate. Yanti et al. (2018b) stated that a consortium of *B. cereus* strain CCM 2010 and *B. toyonensis* strain BCT-7112 could accelerate flowering and increase tomato yield. Furthermore, Abedinzadeh et al. (2018) stated Endophytic bacteria could produce IAA, ACC deaminase, siderophore and phosphate solubilization that can promote plant growth and plant tolerance to environmental stresses.

In conclusion, all treatments of the endophytic bacteria consortium can potentially suppress anthracnose disease caused by *C. gloeosporioides*, increasing the growth and yield of chili plants. The best bacterial consortium treatment in suppressing the development of anthracnose disease by *C. gloeosporioides*, increasing the growth and yield of chili plants, namely the *B. cereus* SNE 2.2 + *B. cereus* TLE 1.1 consortium with disease severity of 0.00%, plant height 58.33 cm and weight fruit 208.97 g.

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