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RESEARCH

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Screening of indigenous actinobacteria as biological control agents of *Colletotrichum capsici* and increasing chili production

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Abstract

Background Anthracnose disease caused by *Colletotrichum capsici* is a major disease in chili plants that is difficult to control. Actinobacteria have potential biological control agents for *C. capsici* because of their antifungal properties and dominant presence in soil. The study aimed to obtain actinobacteria isolates that have the potential to control anthracnose in planta and increase the growth and production of chili plants. The study consisted of three stages: isolation, selection, and characterization of the best actinobacteria isolates. A total of 20 isolates of indigenous actinobacteria were isolated, and 16 isolates were successfully selected based on the results of the biosafety test.

Results The *in planta* test showed that eight isolates could control anthracnose with 100% effectiveness. Of the eight isolates, as many as five players increased the production of chili plants by 169.51–218.53 g. Actinobacterial isolates that have the potential to control anthracnose disease *in planta* and increase the growth and production of chili plants are ARAI 3221, ARAC 3221, ARAC 2211, ARAC 3321, and ARTI 1312. These isolates produced indole acetic acid (IAA) with concentrations of 25.82–88.87 ppm, and four isolates were able to dissolve phosphate. Five isolates produced chitinase enzyme with the chitinolytic index of 0.32–1.78.

Conclusion The introduction of actinobacteria in chili plants was also proven to extend the incubation period, reduce the incidence of disease, and reduce the severity of anthracnose disease compared to negative controls and mancozeb. Actinobacteria can suppress pathogenic microorganisms that can inhibit plant growth. Actinobacteria have the potential to increase the growth and production of chili plants. The results of 16S rRNA sequences showed that the five potential isolates were identified as *Streptomyces cellulose*, *S. fradiae*, *S. olivaceus*, *S. pseudogriseolus,* and *S. griseoflavus*.

Keywords Actinobacteria, Anthracnose, *Colletotrichum capsici*, Chitinase enzyme, IAA

Background

Anthracnose disease caused by (*Colletotrichum capsici* (Syd.) Butler & Bisby) and *C. gloeosporioides* (Penz) is an essential pathogen in chili plants (Sharma and Kulshrestha 2015). This pathogen can cause yield losses of 60–100% in Indonesia (Yanti et al. 2020). The part of the chili plant that is most often infected with *C. capsici* is the fruit. The initial symptoms on the fruit, namely the appearance of small black spots and slightly curved, then the further symptoms of the fruit will shrink, dry, rot, and

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eventually detach from the stem (Nurjasmi and Suryani 2020). Efforts to control anthracnose that have been carried out are the use of resistant varieties (Agastya et al. 2017), soaking seeds in hot water at 55 °C for 30 min. (Hasyim et al. 2014), and the use of fungicides (Selviani et al. 2021). Excessive use of fungicides for a long time can harm the environment and resistance to controlled pathogens (Sila and Sopialena 2016) and can damage the environmental balance by killing non-target microorganisms that can benefit plants (Ghanbarzadeh et al. 2016).

One group of microorganisms that can be used as biological agents is actinobacteria (Subramaniam et al. 2016). Using biological agents such as actinobacteria is one solution to reduce the dangers of using fungicides that the community or farmers intensively carry out. The principle of using biological agents is non-destructive and environmentally friendly (Sopialena 2018).

Actinobacteria are the dominant bacteria in the soil, can overgrow, and have an essential role in protecting plants from pathogen attacks. Most actinobacteria can also produce plant growth hormones (Loqman et al. 2009). Actinobacteria are Gram-positive, filamentous bacteria that produce bioactive compounds and act as antimicrobials (Retnowati et al. 2018). Goudjal et al. (2016) stated that actinobacteria can produce chitinase enzymes and -1.3-glucanase, which can degrade microbial cell walls. Actinobacteria have the potential to dissolve phosphate, produce siderophores, produce IAA (indole acetic acid), produce ammonia, and other lytic enzymes (Jog et al. 2012). Actinobacteria can suppress the development of plant pathogens and are also known as plant growth-promoting rhizobacteria (PGPR) (Rani et al. 2018). Actinobacteria can increase plant height, root length, dry weight, and photosynthetic pigments because they produce phytohormones (auxins, gibberellins, and cytokinins), siderophores, ammonia, dissolve phosphates and produce hydrogen cyanide (Chukwuneme et al. 2020). This study aimed to identify the isolates of Actinobacteria that have the potential to multiply anthracnose and have the potential to trigger plant growth and chili production.

Methods

Isolation of actinobacteria from chili rhizosphere

Soil samples (root soil of healthy chili plants aged 6–10 weeks around chili plants with anthracnose symptoms) were taken using the purposive sampling method from chili production centers and *C. capsici* endemic areas in West Sumatra, namely Kab. Solok, Kab. Tanah Datar and Kab. Religion. One gram of the soil sample was suspended and diluted to 10^{-7}. Dilution levels 10^{-6} and 10^{-7} were placed into test tubes that already contained International Streptomyces Project-2 (ISP2) media and Simmons Citrate Agar (SCA) media (Saryono et al. 2019) 1 ml of liquid was homogenized using a vortex and poured into a Petri dish, incubated for 14 days. A single colony of actinobacteria was purified.

Biosafety test

Hypersensitivity reaction test

Hypersensitivity testing aimed to determine whether actinobacteria are pathogenic or not. Actinobacteria isolates were suspended and then diluted to a total spore density of 10^8 spores/ml, calculated using a hemocytometer (Kawuri 2012), then infiltrated on the lowest surface of the petals at four o'clock (*Mirabilis jalapa* L). The infiltrated leaves were covered with plastic and incubated for 2×24 h. The test results showed that the actinobacteria obtained were not pathogenic, characterized by no necrotic appearance on the infiltrated leaves.

Pathogenicity test

The pathogenicity test used healthy chilies that were surface sterilized first by rinsing with sterile distilled water and then soaking with 1% NaOCl for 2 min and then rinsing with sterile distilled water. The chilies were dried and then pierced with a sterile needle, and 10^8 spores/ml were applied to the center of the chilies and incubated for 6 days at room temperature. Isolates that cause necrotic in chili were not used for further testing because they are potential pathogens in chili (Yanti et al. 2020).

Hemolysis test

Actinobacteria isolates were cultured on blood agar medium (5% sheep blood agar) and incubated for 5 days. There are three types of hemolysis, namely α, β and γ. Isolates that produce α or β hemolysis reactions are not used for further testing because they can potentially be pathogenic to humans and mammals (Beutin 1991).

Propagation of actinobacteria

Pure isolates of actinobacteria from microtubes were rejuvenated by the scratch method in ISP2 and SCA medium and then incubated for 2×24 h. One single colony of actinobacteria was placed into 25 ml of ISP2 and SCA medium in a culture bottle and then incubated on a rotary shaker at 70 rpm for 7×24 h (Kawuri 2012). The population with 10^8 spores/ml density was used for the introduction.

Chili seed planting

Chili seedlings were transferred to the field as many as six with six replicates 21 days after sowing. The roots of the chili seeds were cleaned with water and then immersed in a suspension of actinobacteria with a density of 10^8 spores/ml. The subsequent treatment was soaked with a

fungicide with the active ingredient mancozeb, for control, soaked with sterile distilled water. Each treatment was soaked for 15 min. Furthermore, chili seeds are planted as much as one seed per polybag.

Rejuvenation and propagation of *C. capsici*

Rejuvenation and propagation of *C. capsici* isolates using isolates collected by Dr. Yumira Yanti, SSi. MP at the Microbiology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University. Rejuvenation and propagation were carried out by cutting the fungal mat on PDA media and incubating it for 2 weeks (Yanti et al. 2020).

Pathogenicity test

The pathogenicity test used a suspension of *C. capsici* (10^6 conidia/ml), prepared by adding 10 ml of sterile distilled water to the culture, then the conidia were removed using a soft brush. The suspension was transferred into a test tube and then homogenized with a vortex. One milliliter of the suspension was taken with a dropper, and the number of conidia was calculated using a hemocytometer. Healthy chili fruit is surface sterilized and then injured by pricking it with a sterile needle. Next, the suspension of *C. capsici* fungus (10^6 conidia/ml) was inoculated into the chili fruit using a syringe (a syringe without a needle) until the fruit was wet. The fruit is placed in a plastic box lined with a moist tissue and incubated until symptoms appear (Yanti et al. 2020).

Inoculation of *C. capsici*

Inoculation of *C. capsici* was carried out on chilies 1 month after flowering. *C. capsici* suspension (10^6 conidia/ml) was inoculated on healthy chilies by wounding the chilies with sterile needles at the base, middle, and bottom, then sprayed with *C. capsici* suspension (10^6 conidia/ml) all over the fruit surface until wet. The fruit was placed in a plastic box containing moist tissue and observed for symptoms to appear four days after being inoculated (Yanti et al. 2020). Chili fruit with symptoms of anthracnose disease was inoculated when chili using the spread method of as many as three pieces per stem.

Observation variable

Incubation mass (days after inoculation)

The incubation period was observed every day after *C. capsici* was inoculated until the plants showed the first symptoms of anthracnose disease on chili peppers, namely the surface of the fruit skin marked by blackish brown spots. The spots formed will expand and form indentations with various concentric shapes that are dark in color and dark red-brown around them.

Disease incidence

The disease incidence is the proportion of the fruits that are attacked by pathogens in a plant population. Disease incidence was observed every day after inoculation and when one of the plants showed the first symptoms.

Disease severity

Disease severity was observed and calculated on the same day as the disease incidence.

First flower emergence (day)

Observations of the first flower emergence were carried out on the first day after the appearance of flowers on each plant.

Number of fruits

The number of fruits was observed by counting the fruit in each treatment after harvest.

Fruit weight (g)

The chilies that have been harvested are weighed and added up each time they are harvested.

Test ability to produce chitinase enzyme, dissolve phosphate, produce IAA and fix nitrogen

The selected actinobacteria were tested for their ability to produce chitinase enzymes using the method of Hsu and Locwood (1975); dissolving phosphate using the Pikovskaya method and medium; produce IAA using the Gordon and Weber method (1951), and fixing Nitrogen (N) using the Dobereiner and Day method (1976).

Molecular identification

DNA extract was amplified with primers ActF (5'-CGCGGCCTATCAGCTTGTTG-3′) and ActR (CCG TACTCCCCAGGCGGGG-3′). GreenTaq PCR Master Mix (Thermo Scientific) and its protocol were used in the PCR process. The reaction mixture was prepared according to the protocol for each reaction volume of 25 l with the mixture for each reaction, namely Master Mix 12.5 l, Primer Forward 1 l, Primer Reverse 1 l, and Nuclease Free Water 7.5 l. A 25 l of the PCR reaction mixture was put into 100 l microtubes, and 3 l of DNA template samples were added to each tube. The initial conditions of the PCR were set at 94^0C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 3 min, annealing at 55^0C for 1 min and extension at 72 °C for 2 min, and final extension at 72 °C for 10 min.

Amplificon was electrophoresed using 1% agarose gel in 1 × TAE buffer and visualized with gel imaging, and obtained a baseband measuring ± 1500 bp.

Experimental design and statistical analysis

This research is an experimental study that consists of 16 treatments and three replications, conducted using a Completely Randomized Design (CRD). The treatments included: (1) Actinobacteria isolates as many as 16 isolates. (2) Positive control (without inoculating *C. capsici* compared with plant growth parameters). (3) Negative control (treatment of *C. capsici* compared with biocontrol test). (4) Fungicide treatment with active ingredient mancozeb (*C. capsici* inoculation compared to biocontrol test). The data were analyzed using analysis of variance (ANOVA) and continued with the LSD test with a level of 5%. Software used SAS version 9.0

Results

Actinobacteria isolates that have been isolated from the roots of chili plants obtained as many as 20 isolates. The mycelium color in the lowest view is generally yellow and cream, while the upper mycelium color is generally white and green (Table 1). The results of the biosafety test showed that four isolates showed hypersensitivity reactions to the *Mirabilis jalapa* plant, four isolates caused hemolysis reactions on blood agar medium, and three isolates showed positive reactions in the pathogenicity test. Actinobacterial isolates that showed negative reactions in the biosafety test were used for further testing.

Disease progress

Chili plants introduced with actinobacteria showed that 13 isolates could slow down the incubation period compared to control; 8 isolates, until the end of the observation, did not show any symptoms of being attacked by *C. capsici*. (Table 2).

The initial symptoms of *C. capsici* are blackish-brown spots, and then later symptoms will expand and form indentations with various concentric shapes that are dark in color and dark red-brown around them. The comparison of chilies with anthracnose symptoms and healthy chilies can be seen in Fig. 1.

Plant growth (generative phase)

Chili plants introduced by actinobacteria showed significant differences in the first flower emergence, number of fruits, and fruit weight of chili compared to mancozeb and control (Table 3). The best four actinobacteria isolates were found in the first flower, which showed significantly different results from the control treatment and not significantly different from other

Table 1 Morphological diversity of actinobacteria isolates and their biosafety test

No	Isolate	Mycelium color		Elevation	Margin	Dimensions (mm)	Hypersensitivity reaction test	Pathogenicity test	Hemolysis test
		Lower view	Upper view						
1	ARSC 2311	Cream	White	Undulate	Convex	2	–	–	–
2	ARSI 2112	Yellow	Green	Undulate	Convex	2	–	–	–
3	ARTI 3221	Brown	Green	Undulate	Convex	2	–	–	–
4	ARTI 1312	Pink	Pink	Undulate	Convex	2	–	–	–
5	ARTI 3121	Yellow	Green	Entire	Convex	2	–	–	–
6	ARTI 3311	Brown	White	Undulate	Convex	2	–	–	–
7	ARAI 3121	Yellow	White	Undulate	Flat	2	–	–	–
8	ARAI 3312	Yellow	Grey	Undulate	Flat	2	–	–	–
9	ARSI 1121	Yellow	Cream	Entire	Flat	2	–	–	–
10	ARAI 3211	Yellow	Green	Undulate	Convex	2	–	–	–
11	ARAI 1211	Brown	White	Entire	Convex	2	–	–	–
12	ARAC 3221	Cream	Yellow	Undulate	Convex	2	–	–	–
13	ARAC 2211	Yellow	White	Undulate	Convex	2	–	–	–
14	ARTI 3112	Cream	Grey	Entire	Convex	2	–	–	–
15	ARAC 3321	Yellow	Cream	Undulate	Convex	2	–	–	–
16	ARAI 3221	Brown	Cream	Undulate	Convex	2	–	–	–
17	ARAE3224	Brownish-yellow	Brown	Undulate	Convex	2	+	–	+
18	ARAC2422	Brown	Cream	Undulate	Convex	2	+	+	+
19	ARAC1252	Brown	Cream	Undulate	Convex	2	+	+	+
20	ARAMR 4252	Yellow	Cream	Undulate	Convex	2	+	+	+

Table 2 The incubation period, disease incidence, and severity of anthracnose disease in chili plants that have been introduced to actinobacteria

Treatments	Incubation period	Disease incidence (%)	Disease severity (%)
ARAI 3221	35.00 a*—	0.00 a*	0.00 a
ARAC 3221	35.00 a*—	0.00 a*	0.00 a
ARAC 2211	35.00 a*—	0.00 a*	0.00 a
ARAI 3121	35.00 a*—	0.00 a*	0.00 a
ARAI 3312	35.00 a*—	0.00 a*	0.00 a
ARTI 3121	35.00 a*—	0.00 a*	0.00 a
ARTI 3311	35.00 a*—	0.00 a*	0.00 a
ARSI 2112	35.00 a*—	0.00 a*	0.00 a
ARSI 1121	27.33 ab	1.70 a	1.42 ab
ARAI 1211	27.00 ab	1.52 a	1.52 ab
ARTI 1312	25.66 ab	2.83 a	1.95 ab
ARAI 3211	18.33 bc	4.23 a	2.93 ab
ARSC 2311	17.33 bc	3.53 a	2.64 ab
ARTI 3221	12.00 cd	3.80 a	2.38 ab
ARTI 3112	9.00 cd	4.67 a	3.38 ab
MANKOZEB	9.00 cd	12.37 b	13.07 c
ARAC 3321	8.66 cd	2.09 a	5.04 b
CONTROL—	4.33 d	86.15 c	71.05 d
Variety coefficient	5.91	19.13	16.76

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level

*Plant show no symptomps until the end of observation

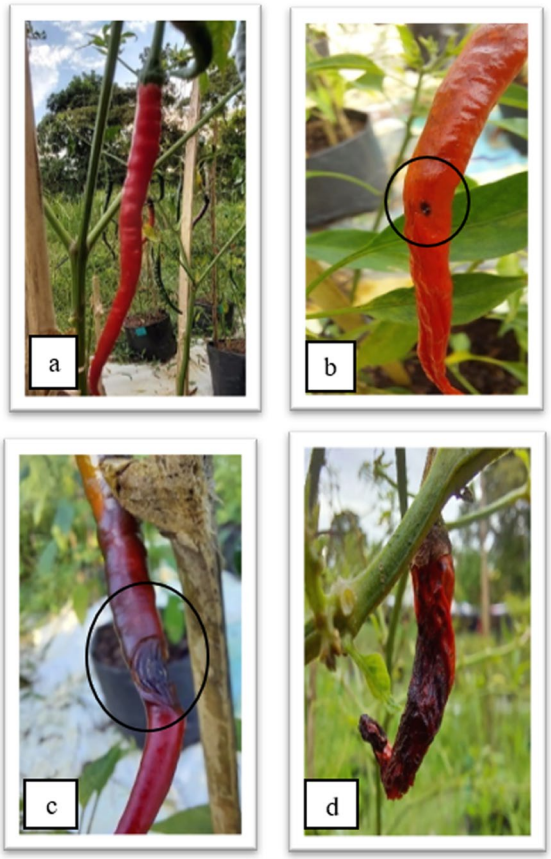

Fig. 1 The severity of anthracnose symptoms on chili fruits. **a** Chili fruit without anthracnose symptoms. **b** Initial symptoms of blackish brown spots on chili fruits. **c** The spots extend to form concentric indentations. **d** The spots spread to all parts of the chili fruits

actinobacterial treatments. The ARAC 2211 isolate was the best isolate against the number of chili plants, and the ARAC 2211 was the best isolate against the fruit weight of chili. The comparison of the appearance of the first flowers of chili plants introduced by actinobacteria isolates with the control can be seen in Fig. 2, and the comparison of the number of chilies can be seen in Fig. 3.

Ability of actinobacteria to produce chitinase enzymes, dissolve phosphate, produce IAA, and fixing nitrogen

Selected actinobacteria capable of producing chitinase enzymes with indexes ranging from 0.32 to 1.78, dissolving the highest phosphate in isolate ARAI 3221, producing IAA with concentrations of 25.82–85.87 ppm, and one isolate unable to fix nitrogen (Table 4).

Molecular identification

After sequencing DNA (Table 5), 4 actinobacteria isolates are from Streptomyces genera, but in different species were get.

Discussion

The actinobacteria isolated from the roots of chili plants were 20 isolates from 3 districts in West Sumatra. Actinobacteria isolates were found to be the least in Solok Regency, then Tanah Datar Regency, and most commonly found in Agam Regency. Rhizosphere actinobacteria are potential actinobacteria as biological agents of *C. capsici*. A total of 5 actinobacteria isolates were able to inhibit the growth of *C. capsicum* and increase the growth of chili plants. Five potential isolates, ARSI 2112, ARAI 3312, ARAC 3221, ARAC 2211, and ARAI 3221, were selected based on the results of the antagonist test and the ability to produce chitinase enzymes. The five isolates were able to dissolve phosphate based on the physiological characterization test. The highest phosphate solubilization

Table 3 Appearance of the first flower, number of fruits, and fruit weight of chili introduced by actinobacteria isolates

Treatments	First flowers emergence	Number of fruit	Fruit weight (g)
ARTI 3221	63.00 a	39.00 ef	142.17 d
ARAC 2211	63.00 a	62.00 a	210.53 a
ARAC 3221	63.00 a	43.33 cde	151.75 cd
ARAI 3221	63.00 a	49.66 bc	169.85 bc
ARTI 1312	65.33 ab	50.33 bc	173.42 bc
ARSI 1121	65.33 ab	38.00 ef	139.00 de
ARAI 3312	65.33 ab	39.00 ef	139.96 d
ARTI 3112	65.33 ab	42.66 cdef	149.23 cd
ARAC 3321	65.33 ab	49.00 bcd	171.55 bc
ARSI 2112	67.66 abc	43.00 cdef	151.78 cd
ARTI 3311	67.66 abc	50.00 bc	172.92 bc
ARTI 3121	67.66 abc	40.66 def	153.33 cd
MANCOZEB	70.00 abc	34.33 fg	113.44 e
ARSC 2311	70.00 abc	54.33 ab	187.25 ab
ARAI 3211	70.00 abc	48.66 bcd	169.51 bc
ARAI 3121	70.00 abc	44.33 cde	154.24 cd
ARAI 1211	70.00 abc	43.33 cde	153.26 cd
Negative control	72.33 bc	25.66 g	83.45 f
Positive control	74.66 c	25.66 g	83.57 f
Variety coefficient	6.89	12.18	10.30

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level

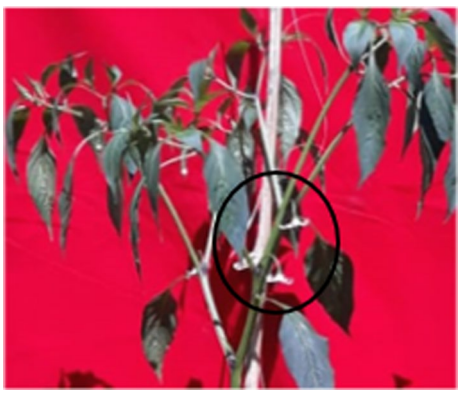

Fig. 2 The first flower appears on chili plants introduced by isolates of actinobacteria code ARAI 3221 (42 DAP)

index was shown by isolate ARAI 3221. The five isolates produced IAA with a concentration of 25.82–85.87 ppm. The highest IAA production was shown by isolates ARAI 3221. Isolates ARSI 2112, ARAC 3221, ARAC 2211, and ARAI 3221 were able to fix nitrogen.

Morphologically characterize actinobacteria by looking at aerial mycelium color, substrate mycelium color, Gram test, and hypersensitivity test. The color diversity

Fig. 3 Comparison of the number of chilies introduced by actinobacteria isolates with mancozeb and control at the sixth harvest. **a** Actinobacteria isolates ARAC code 2211, **b** mancozeb, **c** control

of the mycelium isolates of actinobacteria is caused by differences in the environmental conditions of the actinobacteria origin. Sapkota et al. (2020) stated that different environmental conditions affect the growth and diversity of actinobacteria. Fitri (2018) proved that actinobacteria have different colony colors due to differences in the pigment content of each actinobacterial constituent cell.

Actinobacteria isolates, namely ARSI 2112, ARAI 3312, ARAC 3221, ARAC 221, and ARAI 3221, were able to produce chitinase enzymes, dissolve phosphate and produce IAA. Of the five isolates, only ARAI 3312 was unable to fix nitrogen. Indole-3-acetic acid (IAA) is the main auxin in the most plants. IAA is synthesized from tryptophan or indole in primordial leaves, young leaves, and at the time of seed formation. IAA also increases the rate of photosynthesis, stomatal conductance, and transpiration rate and reduces intercellular concentrations (Li et al. 2020). Nitrogen and phosphate are macro elements needed by plants for metabolism and are constituents of nucleotides that play a role in synthesizing amino acids and proteins. Nitrogen is also the main component of chlorophyll in plants (Grusak 2002).

The results of molecular identification using specific primers for actinobacteria 27F and 16Sact1114R identified five potential actinobacteria, namely: *Streptomyces cellulosae* (ARSI 2112), *S. fradiae* (ARAI 3312), *S. olivaceus* (ARAC 3221), *S. pseudogriseolus* (ARAC 2211), and *S. griseoflavus* (ARAI 3221). The homology value obtained was more than 99%. The rhizosphere actinobacteria were dominated by the genus *Streptomyces* by 95.3% (Williams and Wellington 1982). Previous research stated that *Streptomyces* spp. could increase the growth of tomato plants (Phankhajon et al. 2016), produce IAA, siderophores and dissolve phosphate (Wang et al. 2020), and inhibit the growth of several pathogens such as *F.*

Table 4 Ability of actinobacteria to produce chitinase enzymes, dissolve phosphate, produce IAA, and fix nitrogen

No	Isolate	Produce chitinase enzyme with chitinolytic index	Phosphate Dissolving Index	IAA concentration (ppm)	Fixing nitrogen
1	ARSI 2112	1.38	2.08	78.56	+
2	ARAI 3312	0.32	0.03	25.82	−
3	ARAC 3221	0.48	0.38	31.69	+
4	ARAC 2211	0.79	0.95	37.34	+
5	ARAI 3221	1.78	2.89	85.87	+

+ Positive reaction, − Negative reaction

Table 5 Sequence homology of 16sRNA 4 Selected isolates by the accession of GenBank

Isolate code	GenBank isolate	Query Cover (%)	Homology (%)	Number of accession
ARSI 2112	*Streptomyces cellulosae* LEM 24	100	99	KU180346.1
ARAI 3312	*S. fradiae* SMS SU 23	100	99	NJ777676. 1
ARAC 3221	*S. olivaceus* TB2210	100	99	MH411235. 1
ARAC 2211	*S. pseudogriseolus* 158	100	99	MN199546. 1
ARAI 3221	*S. griseoflavus* CB16	100	99	MK929476.1

oxysporum, *Botrytis cinerea*, and *Monilinia laxa* (Lu et al. 2008).

Actinobacteria produce hydrolytic enzymes such as proteases, cellulases, and chitinases (Song et al. 2020). Chitinase enzymes produced by Actinobacteria can control fungi, nematodes, and insects by breaking down chitin in fungal cell walls, nematodes, and insect exoskeletons into N-acetyl glucosamine (Parwati et al. 2018). Anggraini et al. (2018) reported that the production of IAA by actinobacteria responded to the length of sprouts and roots of red chili soaked with actinobacteria inoculum. Plants need nitrogen and phosphate in the metabolism and synthesis of amino acids and proteins, and nitrogen is also the main component of chlorophyll in plants (Wijayanti et al. 2022).

Actinobacteria have the potential to increase the growth and production of chili plants. The introduction of actinobacteria in chili plants increased flower emergence, fruit number, and fruit weight higher than positive controls. It is because actinobacteria can produce plant growth hormone, namely IAA. Kamal et al. (2017) stated that actinobacteria could produce phytohormones such as IAA, the primary plant hormone that can stimulate plant growth by promoting cell division and elongation. Sathya et al. (2017) stated that actinobacteria derived from the plant rhizosphere were able to produce growth hormones such as (IAA), cytokinins, and gibberellins. Dochhil et al. (2013) stated that the production of IAA, as much as 71 g/ml and 197 g/ml, increased seed germination and plant growth. The ability of actinobacteria to increase the growth of chili plants is because Actinobacteria can colonize the roots of chili plants. When actinobacteria colonize the roots of seedlings, actinobacteria perform essential roles such as breaking down organic matter and recycling complex nutrients in the plant rhizosphere into simple nutrients that can make it easier for the plants to absorb nutrients for growth. Following the statement of Vurukonda et al. (2018), which states that actinobacteria are well known for their productive activity in nutrient recycling by the degradation of chitin, cellulose, starch, lipids, and complex carbohydrates, which are converted into simple sugars by the secretion of various types of hydrolytic enzymes in the rhizosphere.

The introduction of actinobacteria in chili plants was also proven to extend the incubation period, reduce the incidence of disease, and reduce the severity of anthracnose disease than negative controls and mancozeb. Actinobacteria can suppress the development of pathogens by indirect mechanisms such as increasing the production of salicylic acid and jasmonic acid in plants. Aditi and Anupama (2015) stated that actinobacteria promote plant growth by two mechanisms: direct and indirect. According to Subramaniam et al. (2016), the indirect mechanism is the induction of resistance in plants by the presence of ISR (Induce Systemic Resistance) and PGP (Plant Growth Promoting). Indirect mechanisms include nitrogen fixation, phosphate dissolution, production of phytohormones such as indole acetic acid (IAA), utilization of 1-aminocyclopropane-1carboxylate (ACC), production of siderophores, cyanide (HCN), lytic enzymes, and antibiotics.

Actinobacteria can suppress pathogenic microorganisms that can inhibit plant growth. According to Omran and Kadhem (2016), actinobacteria generally produce various secondary metabolites, often referred to as antibiotics. These antibiotics include antitumor (doxorubicin and bleomycin), antifungal (amphotericin B and nystatin), immunosuppressive (FK-506 and rapamycin) (Grasso et al. 2016). Actinobacteria act as plant growth promoters (PGPR); to the statement of El-Tarabily and Sivasithamparam (2006) that actinobacteria have the potential as Plant Growth Promoting Rhizobacteria (PGPR). Some of the roles of actinobacteria involved in PGPR activity are producing the hormone Indole Acetic Acid (IAA) or auxin, producing siderophore compounds and plant growth hormones (Plant Growth Regulators), increasing the growth of mycorrhizal mycelium, helping the process of dissolving organic and inorganic phosphates, as well as bacteria. Nitrogen fixation without the need for symbiosis with the host plant (non-symbiotic nitrogen fixation). The results of this study showed that actinobacteria that have the potential to control anthracnose disease *in planta* and increase the growth and production of chili plants were isolated with the codes ARAI 3221, ARAC 3221, ARAC 2211, ARAC 3321, and ARTI 1312 and the molecular test results obtained the genus *Streptomyces* spp.

Conclusion

The introduction of actinobacteria in chili plants was proven to extend the incubation period, reduce the incidence of disease and the severity of anthracnose disease than negative controls and mancozeb. Actinobacteria can suppress pathogenic microorganisms that can inhibit plant growth. Actinobacteria had the potential to increase the growth and production of chili plants. The results of 16S rRNA sequences showed that the five potential isolates were identified as *Streptomyces cellulose*, *S. fradiae*, *S. olivaceus*, *S. pseudogriseolus,* and *S. griseoflavus*.

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Author contributions
MS and HH made drafts or revised the articles. R and N analyzed and interpreted data, while Y and NLS made conception and design. In addition to making conception and design, MSR and YY also performed analysis and interpretation of the data, revised articles. YY was a major contributor to writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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