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Screening and characterization of endophytic bacteria isolated from celery with the potential to promote plant growth

NURIYANAH[1,2], TIWIT WIDOWATI[1,♥], ANDI MASNANG[2], LISEU NURJANAH[1], DAINTY NOVILASARI[3], SYLVIA J. R. LEKATOMPESSY[1], RUMELLA SIMARMATA[1]

[1]Research Center of Applied Microbiology, National Research and Innovation Agency. Jl. Raya Bogor KM 46, Cibinong, Bogor 16911, West Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: tiwidowati@gmail.com

[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Nusa Bangsa. Jl. Sholeh Iskandar KM 4, Tanah Sareal, Bogor 16166, West Java, Indonesia

[3]Department of Technobiology, Faculty of Technobiology, Universitas Teknologi Sumbawa. Jl. Raya Olat Maras, Moyo Hulu, Sumbawa 84371, West Nusa Tenggara, Indonesia

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Abstract. *Nuriyanah, Widowati T, Masnang A, Nurjanah L, Novilasari D, Lekatompessy SJR, Simarmata R. 2023. Screening and characterization of endophytic bacteria isolated from celery with the potential to promote plant growth. Biodiversitas 24: 6897-6904.* Endophytic bacteria that inhabit plant tissue contribute to the enhancement of host plant growth by producing secondary metabolites. They can improve plant growth by phytohormone modulation and nutrient uptake. In addition, endophytic bacteria can increase plant health and tolerance by antibiotic and hydrolytic enzyme production. This study aims to screen and characterize endophytic bacteria of celery as plant growth promoter agents. Thirty endophytic bacteria were isolated successfully from part of the celery plant using the spread plate and plant piece method which grew at Nutrient Agar media. Based on the characterization assay, 30 isolates may produce IAA with various concentrations of 0.21-7.18 mg/L. In addition, 3 of 30 isolates had phosphate solubilizing activity, in different indexes ranging from 0.71-1.43. Twenty-one isolates were able to grow in an N-free medium, indicating the capability of isolates to fix nitrogen qualitatively. The hydrolytic enzymes assay resulted in 14 amylolytic, 17 proteolytic, and 12 cellulolytic activities. In addition, nine of 30 isolates show multi-activity in amylase, protease, and cellulase enzyme production. Based on the screening results, there are four potential isolates (SLBg 1.2; SLBg 1.5; SLAg 1.1, and SLAg 1.5) as promising plant-growth promoters and were molecularly identified as members of the *Bacillus* genus. These isolates can be developed as biofertilizers for supporting the cultivation of celery plants.

Keywords: Celery, characterization, endophytic bacteria, identification, plant growth agents

INTRODUCTION

Celery (*Apium graveolens* L.) is widely known as a vegetable and healthy food in the Middle East, Asia, Europe, and the United States (Yan et al. 2022). Celery is one of the annual or perennial plants that grow throughout tropical and subtropical countries (Gauri et al. 2015). Celery is one of the medicinal plants that is used in pharmaceutical, food, and ornamental plant industries. As medicinal plants, all parts of the celery plant such as leaves, roots, seeds, and stems can be used for medicinal purposes (Septiana et al. 2023). The leaves and stems of celery are also used for seasoning and garnishing dishes. There are many physiological functionalities of celery, such as lowering the cholesterol level, anti-inflammatory, antioxidant, antirheumatic, antihypertension, antidiabetic, antimicrobial, and anticancer activity (Zhu et al. 2014; Khairullah et al. 2021). Celery contains many bioactive compounds, such as flavonoids, saponins, tannins, apigenin, vitamins A, B, C, and volatile oil, which are used in the pharmaceutical and cosmetic industries (Kooti and Daraei 2017).

Celery has very good prospects, both in the domestic market and as an export commodity. However, celery cultivation in Indonesia has not been able to be managed commercially. Celery production in Indonesia is constrained by the limited area of productive land (Embarsari et al. 2015). In addition, celery is only used as a side crop cultivated with an intercropping system. Based on data from the Badan Pusat Statistik (BPS) (2014) about the survey results of vegetable crops in Indonesia, there are no data on harvest area and celery production nationally. In addition, as a subtropical plant, celery is usually cultivated in upland areas (Iman et al. 2023). The cultivation of celery in the lowlands has not yet gotten attention in both main commodities and research priorities. Because the celery plant has very good prospects in the future, production will be increased to supply consumer needs. An alternative to increase the yield of celery cultivation is through fertilization using biofertilizers.

Biofertilizers are one of the solutions to overcome the utilization of inorganic fertilizers in the long term, which have an impact on reducing soil fertility. The utilization of biofertilizers is one of the efforts to reduce negative impacts on the environment, both water and soil pollution, due to excessive use of inorganic fertilizers. Biofertilizers can help improve soil fertility and health, increase fertilizer efficiency, and provide nutrients for plants so that they can increase crop productivity (Daniel et al. 2022). The use of biological fertilizers that utilize microorganisms, such as nitrogen-fixing endophytic bacteria and phosphate

solubilizing bacteria can increase plant growth and production and reduce the excessive use of chemical fertilizers (Mazid and Khan 2015). In addition, biofertilizers can also improve soil properties (Daniel et al. 2022).

Endophytic bacteria are bacteria that inhabit internal plant tissue and mutually symbiosis with the host plant. They enter the plant tissue and spread throughout the tissue of the host plant (Tshikhudo et al. 2023). Endophytic bacteria derive nutrients from plant metabolism and protect plants from pathogen attacks, while plants obtain nutrients and bioactive compounds (Singh et al. 2017). Endophytic bacteria can be collected from diverse plants such as crops, medicinal, flowers, and wild and perennial plants (Afzal et al. 2019; Wu et al. 2021).

Endophytic bacteria can produce metabolites that are almost the same as active compounds derived from their host plants (Wu et al. 2021). They have several beneficial effects on their plant host directly, such as assisting plants to get nutrients to improve plant growth by regulating phytohormones (IAA) (Ma et al. 2016). Indirectly, endophytic bacteria produce antibiotic and hydrolytic enzymes to suppress and protect the plants from pest and disease attacks (Miliute et al. 2015). Endophytic bacteria in plant tissues can produce growth-promoting substances, carry out nitrogen fixation, solubilize phosphates, and act as biocontrol agents (Borah et al. 2019), increasing amounts of limiting plant nutrients such as nitrogen, phosphorus, and iron (Glick 2012). Information related to endophytic bacteria of celery that has potential as a plant growth promoter has not been studied. This research aims to obtain endophytic bacteria from the celery plant which have the potential to produce plant growth-promoting agents.

MATERIALS AND METHODS

Isolation of endophytic bacteria

The Celery plant was acquired from the vegetable garden, Research Center for Biotechnology, National Research and Innovation Agency (6.4919° South Latitude and 106.8453° East Longitude). After being cleaned under running water, fresh celery was divided into leaves, stems, and roots. Each piece was cut ± 2 cm in length then soaked with 75% alcohol for 2 min and washed thrice with sterile water (Duhan et al. 2020). The endophytic bacteria were isolated using 2 methods (spread plate and plant piece method). The leaf and stem samples were cut into 2 parts and aseptically placed over a Nutrient Agar (NA) plate containing Nystatin 100 $mg.L^{-1}$ (Ezeobiora et al. 2021). The spread method was carried out by samples mashed using a sterile mortar and adding 1 ml of sterile water (Sudewi et al. 2020). Fifty µL of sample water was spread over the NA plate containing Nystatin. The last rinse water is taken as much as 100 µL and spread on NA media to ensure that there is no contamination and that the surface sterilization process is carried out correctly. For 2-4 days, the culture media were incubated at room temperature. The bacteria were picked and streaked out of the fresh NA media to purify the colonies. One colony of bacteria was put into the NA medium of the test tube as stock and working culture.

Morphological and physiological characterization

A single colony of endophytic bacteria was observed macroscopically for morphological characterization. Macroscopic observation includes the shape, size, color, edge, and elevation of the colony (Hikmawati et al. 2019). The selected bacteria were characterized microscopically by the Gram staining method.

Hypersensitivity test

All isolates were tested for hypersensitivity reactions in tobacco plants to determine bacterial isolates with potential pathogens (Sudewi et al. 2020). Nutrient Broth (NB) medium was inoculated with bacterial isolates and then subsequently stirred for 24 hours at 150 rpm. One mL of bacterial culture was injected into the tobacco leaves through the bottom side using a sterile syringe. *Escherichia coli* and *Pseudomonas solanacearum* were used as positive control, while the negative control used sterile aquadest. After inoculation, the reaction was observed at 3-5 days. The necrosis reaction on the tobacco leaves indicates a positive pathogen for plants.

Hemolytic test

Bacteria isolates were grown in Blood Agar media and incubated at room temperature for 24-48 hours. A clear zone was formed around the colony, indicating that the isolate can do lysis on media Blood Agar and is considered to be a pathogen for mammals. Blood agar is a culture media that differentiates bacteria based on their ability to lyse red blood cells (Sudewi et al. 2020).

Screening of plant growth-promoting activities

IAA production assay

Isolates were grown on NB media containing 0.2 mM L-tryptophan (Widowati et al. 2023). The presence of L-tryptophan can stimulate microorganisms to produce much greater quantities of auxins compared to the absence of L-tryptophan (Naveed et al. 2015). The bacterial culture was shaken and incubated at 28° C, 150 rpm for 24 h. The cultures were centrifuged at 150 rpm for 10 min. Two mL supernatant was mixed with 2 mL Salkowsky reagent and incubated in a dark room for 60 min then read absorbance at wavelength 530 nm. The appearance of pink color indicates IAA production.

Phosphate solubilization assay

Each isolate was grown on Pikovskaya media and cultured at 28° C for 72 hours. The ability of the isolates to solubilize phosphate is indicated by the formation of a clear zone around the isolate (Sharon et al. 2016). The colony that gave a clear zone is calculated for its phosphate solubilization index.

$$\text{Solubilization Index (SI)} = \frac{\text{Clear zone diameter (mm) - Colony diameter (mm)}}{\text{Colony diameter (mm)}}$$

Nitrogen fixation assay

Each endophyte bacteria was grown on Nutrient Broth (NB) media and then transferred on semi-solid Nitrogen-free Bromthymol Blue (NfB) media (Baldani et al. 2014). NfB media consist of 5 g malic acid; 0.5 g K_2HPO_4; 0.2 $MgSO_4.7H_2O$; 0.1 NaCl; 0.02 g $CaCl_2.2H_2O$; 2 mL microelement (0.04 g $CuSO_4.5H_2O$; 0.012 g $ZnSO_4.7H_2O$; 0.14 g H_3BO_3; 0.1 g $Na_2MoO_4.2H_2O$; 0.15 g $MnSO_4.H_2O$ and 100 mL water), 2 ml Bromthymol Blue solution (11.2 g KOH; 0.5 g BTB and 100 mL water), 4 mL Fe-EDTA 1.64%; 4 g KOH, 1 mL vitamin solution (0.01 g Biotin; 0.02 g pyrodoxol HCl and 100 mL water); 2.3 g agar and 1 L water. Culture isolates were incubated at a temperature of 28ºC for 72 h. The white pellicle formation below the surface of semisolid NfB media and the change of color of the media from green to blue indicated that the isolate could fix nitrogen.

Screening of enzymatic activities

Amylolytic activity assay was carried out by grown isolates on Starch Agar (SA) media consisting of 20 g NA, 1 g soluble starch, and 1 L water. Cultures were incubated at 37° C for 24-48 h and then added to an iodine solution. The ability of the isolate to degrade amylase was characterized by the formation of a clear zone around the colony (Duhan et al. 2020). The activity of proteolytic was tested on Skim Milk Agar (SMA) media consisting of 1 g peptone, 5 g NaCl, and 500 mL water. One hundred g of skim milk was dissolved in 500 mL water and sterilized at 100°C for 10 min. Skim milk media was mixed with agar media and poured into a Petri dish. Isolates were grown at Petri dish media and incubated at 28°C for 72 h. A clear zone was formed around the colony, indicating that the isolate was able to degrade protease (Alkahtani et al. 2020). The isolates were grown on 1% carboxymethyl cellulose (CMC) media. CMC media consisted of 10 g CMC; 0.2 g $MgSO_4.7H_2O$; 0.75 g KNO_3; 0.02 g K_2HPO_4; 0.04 g $CaCl_2.H_2O$; 15 g agar and 1 L phosphate buffer. Cultures were incubated at 28° C for 48 h, then washed with 0.1% congo red for 10 min and rinsed with 1% NaCl. The formation of clear zones around the colony indicates positive results (Dogan and Taskin 2021). The amylolytic, proteolytic, and cellulolytic index was measured with the formula:

$$\text{AI/PI/CI} = \frac{\text{Clear zone diameter (mm) - Colony diameter (mm)}}{\text{Colony diameter (mm)}}$$

AI/PI/CI = Amylolytic, Proteolytic, Cellulolytic Index

Identification of selected isolates based on 16S rRNA gene sequencing

The 16S rRNA gene amplification was done for bacterial identification using the colony PCR method (Packeiser et al. 2013). Isolates were grown on a nutrient agar plate. A single colony of isolates was inserted into a microtube, and the PCR mix was added. The PCR reaction mixtures were carried out in a volume of 50 µL containing 10 µL MyTagTM HS Red Mix, 1 µL primer forward 27F, 1 µL primer reverse 1492R, and 38 µL ddH_2O. The universal primer forward (5' - AGA GTT TGA TCC TGG CTC AG - 3') and reverse (5' - GTT TAC CTT GTT ACG ACT T - 3') were used for amplification of the 16S rRNA gene. PCR was initiated with pre-denaturation at 95°C for 1 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 5°C for 1 min, elongation at 72°C for 1 min, and ended final elongation at 72°C for 5 min. The PCR product was checked by electrophoresis with 1.2% agarose at 100 V for 35 min (BIO-RAD mupid-exU-75577). The PCR products were processed at PT Genetica Science. The sequences were further aligned to the other closely related bacterial species in the National Centre for Biotechnology Information (NCBI) database using the BLASTn program from the NCBI website and BioEdit.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria

Thirty isolates have been isolated from part of the celery plant. Based on the isolation method, the spread plate method resulted in endophyte bacteria more than the plant piece method (Table 1). This is probably due to the difference in sample size and isolation process (Sulistiyani and Lisdiyanti 2016). The crushed samples allowed the cell bacteria to be distributed into the water so that more endophyte bacteria were obtained. The sample size of the plant piece method is larger, limiting the opportunity for endophytic bacteria to be distributed in the growing media (Sulistiyani et al. 2014).

Morphological and physiological characterization

The endophyte bacteria of celery showed different morphological characteristics. Most isolates were circular in shape, small in size, flat elevation, and undulated edge (Table 2). The colony's color varied from yellow, white, cream, orange, and pink, where the yellow color was dominant in as many as 8 isolates followed by white color. The difference in colony color is caused by the capability of bacteria to release the diffusible pigment into the agar and produce specific metabolites during the colony growth (Fiscarelli 2019). The Gram staining of selected bacteria resulted from a rod-shaped bacterium that was blue and classified as Gram-positive bacteria.

Out of 30 isolates injected into tobacco leaves, 6 isolates (SLB 1.1, SLD 1.1, SLDg 1.1, SLDg 1.6, SLDg 1.8 and SLAg 1.2) showed symptoms of necrosis. Twenty-four isolates were not pathogenic to plants and could potentially be used as biostimulant agents. The hemolysis activity test showed that 4 isolates (SLB 1.2, SLD 1.4, SLDg 1.1, and SLDg 1.7) form a clear zone in the Blood Agar. Isolates with negative activity (Table 2) indicate that these bacteria are not pathogens for humans and animals.

Table 1. Number of endophyte bacteria from celery using spread plate and plant piece method

Part of plant	Plant piece		Spread plate	
	No. of isolates	Isolate code	No. of isolates	Isolate code
Leaf	4	SLD 1.1, SLD 1.2, SLD 1.3, SLD 1.4	8	SLDg 1.1, SLDg 1.2, SLDg 1.3, SLDg 1.4, SLDg 1.5, SLDg 1.6, SLDg 1.7, SLDg 1.8
Stem	3	SLB 1.1, SLB 1.2, SLB 1.3	8	SLBg 1.1, SLBg 1.2, SLBg 1.3, SLBg 1.4, SLBg 1.5, SLBg, 1.6, SLBg 1.7, SLBg 1.8
Root	-		7	SLAg 1.1, SLAg 1.2, SLAg 1.3, SLAg 1.4, SLAg 1.5, SLAg 1.6, SLAg 1.7

Table 2. The morphological and physiological characterization of endophytic bacteria from celery

Isolate code	Colony morphology					Hypersensitive reaction	Hemolysis
	Color	Size	Shape	Elevation	Edge		
SLB 1.1	Yellow	Small	Circular	Flat	Undulate	+	-
SLB 1.2	Broken white	Small	Circular	Flat	Undulate	-	+
SLB 1.3	Cream	Small	Circular	Flat	Undulate	-	-
SLBg 1.1	Yellow	Small	Circular	Flat	Undulate	-	-
SLBg 1.2	Milky white	Medium	Circular	Flat	Undulate	-	-
SLBg 1.3	Transparent	Small	Circular	Flat	Undulate	-	-
SLBg 1.4	*Orange*	Small	Circular	Flat	Undulate	-	-
SLBg 1.5	Yellow	Small	Circular	Umbonate	Undulate	-	-
SLBg 1.6	Transparent	Small	Circular	Flat	Entire	-	-
SLBg 1.7	White	Small	Circular	Umbonate	Undulate	-	-
SLBg 1.8	Yellow	Small	Circular	Flat	Entire	-	-
SLD 1.1	Transparent	Small	Circular	Flat	Entire	+	-
SLD 1.2	Cream	Small	Circular	Flat	Undulate	-	-
SLD 1.3	White	Small	Circular	Flat	Undulate	-	-
SLD 1.4	Cream	Small	Circular	Flat	Undulate	-	+
SLDg 1.1	Cream	Medium	Circular	Flat	Entire	+	+
SLDg 1.2	Pink	Small	Circular	Flat	Entire	-	-
SLDg 1.3	*Orange*	Small	Circular	Flat	Undulate	-	-
SLDg 1.4	Transparent	Small	Circular	Flat	Entire	-	-
SLDg 1.5	Yellow	Small	Circular	Flat	Entire	-	-
SLDg 1.6	Transparent	Small	Circular	Flat	Undulate	+	-
SLDg 1.7	Cream	Small	Circular	Umbonate	Undulate	-	+
SLDg 1.8	Milky white	Small	Circular	Umbonate	Undulate	+	-
SLAg 1.1	Cream	Small	Circular	Flat	Entire	-	-
SLAg 1.2	Yellow	Small	Circular	Flat	Entire	+	-
SLAg 1.3	White	Small	Circular	Flat	Entire	-	-
SLAg 1.4	Yellow	Small	Circular	Flat	Entire	-	-
SLAg 1.5	Yellow	Small	Circular	Flat	Entire	-	-
SLAg 1.6	White	Large	Irregular	Flat	Lobate	-	-
SLAg 1.7	White	Medium	Circular	Flat	Undulate	-	-

Note: + : pathogen, - : non pathogen

Screening of plant growth-promoting activities

IAA production assay

The ability of thirty endophytic bacteria to produce IAA has been tested and resulted from varying concentrations of IAA (Table 3). The result showed that 3 isolates were classified as moderate IAA producers (2-10 µg/mL) and 27 isolates were as low IAA producers (<2 µg/mL) (Fatmawati et al. 2019). Isolate SLAg 1.5 produced the highest IAA concentration (7.18 µg/mL), followed by SLBg 1.5 (4.35 µg/mL) and SLAg 1.1 (2.96 µg/mL). The lowest IAA concentration resulted in SLBg 1.3 and SLAg 1.6 isolates (0.21 µg/mL).

Phosphate solubilization assay

Three isolates solubilized tricalcium phosphate more effectively than others (Table 3). The highest score of phosphate solubilizing index (PSI) resulted from SLD 1.1 isolate (1.43), followed by SLAg 1.2 (1) and SLB 1.1 (0.71). Several isolates, SLB 1.2, SLB 1.3, SLBg 1.1, SLD 1.3, SLD 1.4 and SLAg 1.5 showed relatively inconspicuous halo areas. Twenty-one isolates showed an absence of phosphate solubilizing activity. Several factors influenced the phosphate solubilizing index, including the concentration of insoluble phosphate source perfectly, the thickness of agar media, microbial growth rate, type of microbes, phosphate sources, environmental conditions, and the microbial capability to release organic acid compounds (Sudewi et al. 2020).

Nitrogen fixation assay

A total of 23 isolates showed the capability to fix nitrogen after growing onto NFb media (Table 3). They showed the formation of white pellicles and the change of color of the medium from green to blue after 4 days of incubation. The nitrogen fixation activity was determined by a color change from green to blue and the development of a pellicle on the media surface demonstrated that bacteria can adapt to their environment (Arsita et al. 2020). The color change occurs because the bromothymol blue indicator on the media turns blue at alkaline pH. The formation of a pellicle is an indicator that bacteria can reduce the source of N from the media as a nitrogenase activity (Aryantha and Hidiyah 2018).

Table 3. Plant growth-promoting and enzymatic activities of endophytic bacteria from celery

Isolate code	IAA concentration (µg/mL)	Growth in N-free medium	Phosphate solubilization index	Amylolytic index	Proteolytic index	Cellulolytic index
SLB 1.1	0.38	+	+	-	-	-
SLB 1.2	0.35	+	+	1.62	1.53	2.67
SLB 1.3	0.77	+	+	1.45	0.45	4.57
SLBg 1.1	0.55	+	+	-	-	-
SLBg 1.2	0.93	+	-	1.27	2.08	5.40
SLBg 1.3	0.21	+	-	-	-	-
SLBg 1.4	0.56	+	-	-	-	-
SLBg 1.5	4.35	+	-	0.31	0.92	-
SLBg 1.6	0.31	-	-	-	-	-
SLBg 1.7	0.41	+	-	0.47	2.11	3.12
SLBg 1.8	0.24	-	-	-	-	-
SLD 1.1	0.83	+	+	-	-	-
SLD 1.2	0.37	+	-	0.87	1.43	2.87
SLD 1.3	0.46	+	-	1.45	1.65	4.57
SLD 1.4	0.54	+	-	0.59	1.41	6.55
SLDg 1.1	0.52	+	-	-	1.02	2.16
SLDg 1.2	0.77	-	-	1.79	-	-
SLDg 1.3	0.59	+	+	0.73	-	-
SLDg 1.4	0.41	-	+	0.73	-	-
SLDg 1.5	0.35	+	-	-	-	-
SLDg 1.6	0.38	-	-	2.00	-	2.16
SLDg 1.7	0.8	+	-	0.63	0.80	7.31
SLDg 1.8	0.59	+	-	2.32	0.19	3.69
SLAg 1.1	2.96	+	-	-	0.08	-
SLAg 1.2	0.27	+	+	-	-	-
SLAg 1.3	0.61	+	-	-	1.45	-
SLAg 1.4	0.7	-	-	-	1.92	-
SLAg 1.5	7.18	+	+	-	3.47	-
SLAg 1.6	0.21	-	-	-	0.23	-
SLAg 1.7	0.28	+	-	-	0.95	-

Note: + : produce, - : unproduced

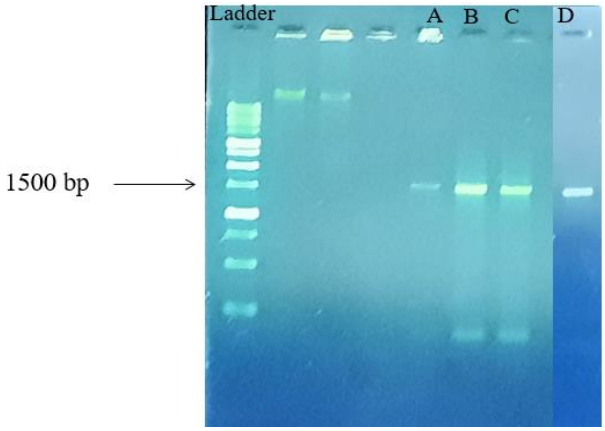

Figure 1. Gel electrophoresis of 3 PCR products of selected endophytic bacteria of celery. A. SLAg 1.5, B. SLBg 1.2, C. SLBg 1.5, D. SLAg 1.1

Screening of enzymatic activities

The endophytic bacteria of celery were evaluated for hydrolytic enzyme activities. A clear zone formed around the colony indicated the enzymatic activity of bacterial isolates in degrading the media. The assay result showed 14 amylolytic, 17 proteolytic, and 11 cellulolytic isolates (Table 3). The amylolytic activity test showed that the largest amylolytic index resulted from the SLDg 1.8 isolate (2.32), while the smallest amylolytic index showed by SLBg 1.5 (0.31). In addition, the highest proteolytic index resulted from SLAg 1.5 isolate (3.47), followed by SLBg 1.7 (2.11) and the last is SLAg 1.1 (0.08). Isolate SLDg 1.7 showed the largest cellulolytic index (7.31) and the smallest result by SLB 1.2 isolate (0.36). Several isolates had triple enzyme activities, such as SLB 1.2, SLB 1.3, SLBg 1.2, SLBg 1.7, SLD 1.2, SLD 1.3, SLD 1.4, SLDg 1.7 and SLDg 1.8.

Table 4. Identity of 4 selected isolates from endophytic bacteria of celery

Isolate code	Name of species	Similarity (%)	Accession number
SLBg 1.2	*Bacillus* sp.	99.44%	KY283147.1
SLBg 1.5	*Bacillus megaterium*	99.52%	KF963621.1
SLAg 1.1	*Bacillus cereus*	100%	HQ670530.1
SLAg 1.5	*Bacillus aryabhattai*	99.66%	MK517594.1

Identification of selected isolates based on 16S rRNA gene sequencing

Four isolates were selected for molecular identity based on their capability to produce IAA, fix nitrogen, solubilize phosphate, and be non-pathogenic for humans and plants. The DNA fragments of selected isolates showed 1500 bp in length after amplification of the 16S rRNA. The result of BLASTn analysis showed that the selected isolates belonged to *the Bacillus* genus (Table 4). The isolates were identified based on UV visualization on product PCR (Figure 1). BLAST analysis of all isolate sequences on the NCBI database showed homology with genus *Bacillus* with similarity 99-100% (Table 4).

Discussion

In this study, we obtained that the abundance of the culturable endophyte bacteria varied between leaves, stems, and roots. A total of 30 isolates of bacteria were isolated from the surface-sterilized leaves, stems, and roots of celery. Endophyte bacteria can be found in all parts of the plant host, including roots, stems, leaves, fruits, and tubers. The population and types of bacteria are influenced not only by species, variety, age, health, and developmental stage of the plant but also by environmental factors such as climate and seasons (Elmagzob et al. 2019).

The hemolysis reaction test was used to observe the safety of the bacterial experiment on the health of mammals. The principle of hemolysis assay is to observe the change of media color when the isolates are grown in fresh blood agar media. After 48 h of incubation, the formation of a clear zone surrounding the colony demonstrated that bacteria could lyse red blood cells (Hikmawati et al. 2019). Bacteria which lyse red blood cells are pathogenic to mammals. Based on the results of the hemolysis and hypersensitivity assay, 5 isolates indicate necrotic formation in tobacco leaves and 4 isolates show hemolyzing reactions in the blood agar medium.

Indole-acetic acid is the most important of auxins synthesized by endophytic bacteria. IAA-producing endophytic bacteria can accelerate plant growth by playing a role in cell enlargement, formation of xylem and phloem tissues, development and elongation of roots, and also stimulate germination of seed and affect the biosynthesis of metabolites (Kabir et al. 2023). In this study, endophytic bacteria produce IAA ranging from 0.21 to 7.18 µg/mL using tryptophan as a precursor. The isolates SLAg 1.5, SLAg 1.1, and SLBg 1.5 were able to produce higher IAA than *Bacillus subtilis* Cb 11, endophytic of *Capsicum annuum* L., which produces IAA 1.37 µg/mL (Widowati et al. 2023).

Phosphorus is one of plants' essential macronutrients, which plays an important role in biological development. Phosphorus compounds are available in agricultural soils in the form of insoluble forms and cannot be utilized by plants. The use of bacteria possessing phosphate solubilizing activity can adapt insoluble phosphate into available forms for plants (Karthik et al. 2017). Other studies have mentioned that endophytic bacteria can be used to dissolve phosphate, which can produce organic and phosphatase acids that provide uptake of phosphorus resources for the growth and development of plants (Kandel et al. 2017).

A positive reaction in nitrogen-fixing is indicated by a color change from green to blue color and a ring/pellicle is formed on semi-solid NfB media. The color change of media is caused by organic acid, which is secreted by bacteria capable of living on nitrogen-free media (Aryantha and Hidiyah 2018). The color change indicated the nitrogenase activity of nitrogen-fixing bacteria. Endophytic bacteria can carry out nitrogen fixation through assimilation by taking nitrogen in the air and converting it into ammonia used by plants (Qin et al. 2022).

The hydrolytic enzymes produced by endophytic bacteria are essential for plant root colonization and endophytic migration through the degradation of cell walls (Walitang et al. 2017). The activity of hydrolytic enzymes is another mechanism used by endophytic bacteria to enhance plant growth and suppress the pathogen. Through the activity of these enzymes, plant growth-promoting bacteria play a role in protecting plants from biotic and abiotic stresses (Pacifico et al. 2019).

Bacillus is recognized as one of the most dominating genera as a cultivable endophytic of crops that have the potential to promote plant growth and produce enzymes. *Bacillus* species are known to produce a variety of secondary metabolites that can induce plant growth. *B. aryabhatai* improves rice productivity through increases in the production of nitrogen fixation, phosphate solubilization, and indole acetic acid (Sultana et al. 2020). In addition, *B. subtilis* was reported to be capable of producing indole acetic acid, growing in a nitrogen-free medium, and having proteolytic, amylolytic, and siderophore activity (Bolivar-Anilo et al. 2021). Kulkova et al. (2023) reported that *B. cereus* has many traits, including IAA and ACC deaminase production, phosphate solubilization, extracellular enzymes, and antibiotic lipopeptides production. In addition, the Bacillus genus has the potential as a biocontrol agent to reduce celery powdery mildew disease in vivo and in vitro (Ahmed et al. 2021).

Based on the activities assay, four isolates selected were molecularly identified. The 16S rRNA gene analysis revealed that all isolates belong to the genus *Bacillus*. Four isolates (SLBg 1.2, SLBg 1.5, SLAg 1.1, and SLAg 1.5) were prospected to be developed further as biofertilizer agents.

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