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Identification and production of indole-3-acetic acid by bacteria isolated from eco-enzymes

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Abstract. *Meitiniarti VI, Kasmiyati S, Nugroho RA, Krave AS. 2025. Identification and production of indole-3-acetic acid by bacteria isolated from eco-enzymes. Biodiversitas 26: 111-117.* Phytohormone-producing microorganisms are an essential component of biofertilizers. One example of a phytohormone is Indole Acetic Acid (IAA). IAA-producing microorganisms can be originated from various habitats. In this study, IAA-producing bacteria will be isolated from eco-enzyme, a liquid-fermented organic material rich in benefits and contains numerous microorganisms and IAA. The research involves processes of isolation, detection of cell and IAA production, and molecular identification. Through the processes of isolation and purification, 14 bacterial isolates were obtained. After testing their ability to produce IAA using a medium containing L-tryptophan and Salkowski's reagent, only 11 isolates were found to produce IAA. The DNA of these 11 isolates was isolated, amplified, sequenced, and identified through molecular analysis. The nucleotide sequences of these 11 bacterial isolates have been registered in the gene bank and assigned accession numbers PQ095569 to PQ095579. Based on alignment and phylogenetic tree analysis, the 11 isolates were grouped into three categories: the *Bacillus* group, consisting of *Bacillus altitudinis*, *Bacillus subtilis*, *Bacillus licheniformis*, *Priestia megaterium*, and *Paenibacillus* sp.; lactic acid bacteria, including *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*; and vibrio-shaped bacteria, including *Vibrio* sp. and *Vibrio diazotrophicus*. The *Bacillus* group (including *Paenibacillus megaterium*) could produce high levels of IAA. However, among the members of this group, *P. megaterium* exhibited the highest cell production capability and IAA production, with values of 2982.208 $mg \cdot L^{-1}$ and 35.49 $mg \cdot L^{-1}$, respectively. This high growth ability and IAA production make *P. megaterium* a promising candidate as an inoculum for use as a PGPR (Plant Growth-Promoting Rhizobacterium).

Keywords: Biofertilizer, eco-enzyme, IAA-producing bacteria, molecular identification, *Priestia megaterium*

INTRODUCTION

Modern agricultural practices today demand a more sustainable and environmentally safe approach. Therefore, using chemical fertilizers that do not comply with regulations and have a negative impact on physical, chemical, and biological properties should be avoided. Biofertilizers are the most suitable option. Biofertilizer is an organic fertilizer product containing live microorganisms beneficial to plants (Seenivasagan and Babalola 2021).

Phytohormone-producing bacteria are a vital component of Plant Growth-Promoting Rhizobacteria (PGPR) (Zhang et al. 2021a) and are also commonly found among the microbes in biofertilizers (Mohite 2013; Anugrah et al. 2021). Microorganisms classified as PGPR have been proven to produce phytohormones such as indole-acetic acid, cytokinins, and gibberellins, which enhance plant growth and productivity (Kumar et al. 2018a). These bacteria are not only widely seen in the rhizosphere (Sukmawati et al. 2021; Giang et al. 2024). Still, they can also be found in various other sources, such as decomposed or fermented organic materials (Saputro and Kurniawati 2024). Phytohormone-producing bacteria may also be present in eco-enzymes. According to Farma et al. (2023), eco-enzyme is a fermentation product that produces Indole-3-Acetic Acid (IAA), a phytohormone essential for plant growth.

Eco-enzymes have been familiar to people in Indonesia for a long time. They were first developed in 2006 by a Thai researcher, Dr. Rosukon Poompanvong (Rasit and Chee Kuan 2018). The widespread activity of making eco-enzymes is motivated by the ability to process organic waste, typically discarded in trash cans, into hydrolytic enzymes with many uses (Gu et al. 2021). Additionally, processing waste into eco-enzymes reduces pollution due to the formation of methane gas from waste dumps (Krause et al. 2023). Making these eco-enzymes is also a waste management method that transforms kitchen scraps into useful ones (Vama and Cherekar 2020).

Eco-enzymes are solutions resulting from the fermentation of organic waste, such as fruits and vegetables, combined with a mixture of water and sugar (Hemalatha and Visantini 2020). Eco-enzyme fermentation lasts for 90 days. The essential basic components of this fermentation are molasses, organic waste, and water, which are in a ratio of 1:3:10 (Novianti and Muliarta 2021). According to Verma et al. (2019) and Rusdianasari et al. (2021), liquid eco-enzymes produced through the fermentation process display a dark brown color and emit a sour and sweet aroma, typical of the fermented substance.

Eco-enzymes offer many benefits, including acting as antifungal, disinfecting agent, and being used as fertilizers (Ismail et al. 2024). The efficacy of eco-enzymes as liquid fertilizers is due to the presence of microorganisms in them.

Barman et al. (2022) identified secondary metabolites in eco-enzymes and found that they consist of enzymes such as amylase, trypsin, and lipase, along with phenols, alcohols, and organic acids. One of the organic acids in eco-enzymes is acetic acid, produced by the bacterial metabolism of fruit and vegetable residues. Several studies have shown that eco-enzymes contain many microorganisms, including lactic acid bacteria (Ibrahim et al. 2017) and fungi (Aulia and Handayani 2022).

Because eco-enzymes are also helpful as liquid fertilizers, some of them are likely capable of producing indole acetic acid (IAA). IAA is important role in plant growth and is commonly found in the plant rhizosphere. The utilization of IAA-producing microbes will be essential in biofertilizers. Eco-enzymes contain many microorganisms that play a role in fertilizing plants, but there is limited information on whether these microorganisms can produce IAA. No research has been conducted on isolating IAA-producing microbes from eco-enzyme. Therefore, it is necessary to study the microorganisms in eco-enzymes capable of producing phytohormones. The production of IAA by microbes depends on the species of microorganism and culture conditions (Mohite 2013). Several types of microorganisms are capable of producing IAA with varying concentrations. For example, *Fusarium* sp. and *Trichoderma* sp. (Wisdawati et al. 2020), a member of the Enterobacteriaceae family (Ramadhani et al. 2020), and several species of *Bacillus* (Hashem et al. 2019; de O. Nunes et al. 2023).

The lack of information on IAA-producing bacteria in eco-enzymes and the potential to harness these bacteria form the basis of this research. This research aimed to isolate bacteria from eco-enzymes that produce IAA, evaluate their IAA production capabilities, and determine their molecular identity.

MATERIALS AND METHODS

Eco-enzyme

The eco-enzyme solution used was produced by fermenting organic materials, water, and molasses in a ratio of 3:10:1. The organic materials used consist of a mixture of watermelon peel, orange, star fruit, lemon, and tomato in equal proportions (1:1:1:1:1). All organic materials were thoroughly washed and cut into pieces of about 1-2 cm. A clean plastic bottle (washed with soap and rinsed with water) was prepared. All ingredients were placed in a plastic bottle with a lid. The plastic bottle has a capacity of approximately 1.5 times the volume of the fermented material. The fermentation process is carried out for 90 days (Barman et al. 2022).

Isolation and purification of IAA-producing bacteria

The isolation procedures for IAA-producing bacteria were performed according to Giang et al. (2024), with the sample and isolation medium modifications. Five mL of the eco-enzyme sample was diluted into 45 mL of sterile 0.9% NaCl solution, and the sample was diluted until 10^{-6}. 0.1 mL was taken from each dilution series and then spread onto Nutrient Agar (NA) plate media. The plates were incubated at 37°C for 48 h. The single colonies on the NA medium plates were further purified on NA medium plates. The pure isolate was ready to be tested for its ability to produce IAA.

Growth of bacterial isolates and their ability to produce Indole-3-Acetic Acid (IAA)

One loop of each pure bacterial culture on slanted NA media was inoculated into a Nutrient Broth (NB) medium. Cultures were prepared with a volume of 50 mL, and incubation was done in a shaker incubator at 28°C and 120 rpm for 48 h, each with three replicates. Samples were taken every two hours. The optical density (OD) of the sample was measured using a Shimadzu UV/Visible Spectrophotometer at λ 600 nm (Kalsooma et al. 2021). The OD values obtained were converted into cell dry weight. The increase in cell dry weight over time was plotted on a graph with the Y-axis representing cell dry weight and the X-axis representing time to obtain a growth curve (Wang et al. 2015). The cell dry weight during the logarithmic phase was then used to calculate the specific growth rate.

After 48 h, 10 mL of each culture was sampled and tested for IAA production according to Sukmawati et al. (2021) by inoculating them in 40 mL NB medium supplemented with 100 mgL^{-1} tryptophan. The cultures were incubated at 28°C and 120 rpm for 24 h. After incubation, the culture was centrifuged at 9000 rpm. A supernatant of 0.5 mL was mixed with 2 mL of Salkowski reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) in a microtube. The microtubes were incubated at room temperature, in the dark, for 30 minutes. After that, the absorbance of colored tris-(indole-3-acetate) iron (III) complex was measured at λ 520 nm using a Shimadzu UV/Visible Spectrophotometer. Sample absorbance was converted into IAA concentration using the IAA standard curve.

The standard IAA curve was prepared by creating a series of IAA solutions with concentrations of 0, 10, 20, 30, 40, 50, and 60 $mg \cdot L^{-1}$. Each solution series was prepared in triplicate. The standard solutions were treated identically to the samples, and their absorbance was measured at λ 520 nm using a Shimadzu UV/Visible spectrophotometer. The absorbance data and corresponding concentrations were plotted on an XY graph, yielding the IAA standard curve equation ($y = 0.0094x + 0.0247$, $R^2 = 0.9914$).

Data analysis

The growth and IAA production data obtained in this study were statistically analyzed using a one-way analysis of variance with the SAS program version 9.1.3. Differences in population values were further evaluated using Duncan's test at a significance level of $\alpha = 5\%$.

Molecular identification

Bacterial isolates capable of producing IAA were cultured on slanted NA medium. Following the manufacturer's protocols after 72 h of incubation, bacterial DNA was isolated using the Quick-DNA Fungal/Bacterial Miniprep

Kit (Zymo Research, D6005). The DNA extraction results were analyzed using 1% (w/v) agarose gel electrophoresis containing 10 μL.L^{-1} ethidium bromide (10 mg.mL^{-1}). Electrophoresis was carried out at 60 V and 50 mA for 30 minutes. DNA visualization was performed using a GelDoc system at λ 254 nm.

PCR was performed to amplify near-full-length 16S rRNA gene sequences using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5′-TACCTTGTTACGACTT-3') (Nugroho et al. 2020) in a thermal cycler (Eppendorf Nexus GSX1). PCR was performed with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 54°C for 30 seconds, and elongation at 72°C for 30 seconds. The amplified DNA fragments were checked by gel electrophoresis using 1% agarose (w/v) (Lee et al. 2012).

PCR products were sent to the 1st BASE Laboratories for sequencing with primers 27F and 1492R. Sequencing was performed bidirectionally via the Sanger DNA Sequencing method with Capillary Electrophoresis. The nucleotide sequences were aligned and compared with the available standard sequences in the GenBank database (https://www.ncbi.nlm.nih.gov) using BLAST to identify all isolates. The nucleotide sequences of 16S rRNA from all bacterial isolates have been deposited in the GenBank database.

A phylogenetic tree was constructed using Mega software (Kumar et al. 2018b) with sequences from closely related strains retrieved from GenBank. ClustalW aligned the sequences to reconstruct a phylogenetic tree using the Neighbor-joining tree methods and Kimura 2-parameter model (Tamura et al. 2021). Bootstrap values (more than 75%) based on 1000 replications were listed at nodes.

Nucleotide sequence accession numbers

The nucleotide sequences had been deposited in GenBank under accession numbers PQ095569 to PQ095579.

RESULTS AND DISCUSSION

Isolation, growth, and IAA production capability

Fourteen bacterial isolates were obtained through the isolation process on NA media, followed by purification. These isolates were coded and subsequently cultured in liquid nutrient media to monitor their growth and determine their specific growth rates. The growth curves of the 14 bacterial isolates are presented in Figure 1, and their specific growth rates are summarized in Table 1.

Based on the growth curve (Figure 1) and specific growth rate calculations, isolate I-6 exhibited the fastest growth rate (0.134 h^{-1}) and the highest biomass production (2982.208 mg L^{-1}). The subsequent two isolates with slightly lower growth rates were I-13 (0.126 h^{-1}) and III-7 (0.122 h^{-1}), respectively. However, at 48 hours, isolates III-7 (2098.460 mg L^{-1}) and I-2 (1714.712 mg L^{-1}) produced the second and third highest biomass amounts, respectively, after isolates I-6. Biomass production by isolate I-13 was slightly lower than III-7 and I-2, at 1398.460 mg L^{-1}.

According to Gonzalez and Aranda (2023), the specific growth rate represents the increase in cell population biomass per unit of biomass concentration. It is typically determined when the bacterial population enters the logarithmic phase (Fernández-Martínez et al. 2024), when bacterial cells grow at their maximum rate without being constrained by limiting factors. Notably, bacterial isolates with high specific growth rates may produce lower biomass, if biomass production is measured during the stationary phase. Therefore, the relationship between specific growth rate and biomass production is most accurately assessed during the logarithmic phase. Based on the growth curve (Figure 1), the logarithmic phase of the 14 bacterial isolates occurred between 6 and either 18 or 24 hours, depending on the isolate.

Table 1. Specific growth rate and biomass production over 48 hours for the 14 bacterial isolates

Bacterial isolate code	Specific growth rate (h^{-1})	Biomass production (mg.L^{-1})
I-2	0.104 ± 0.012^{C}	1714.712 ± 75.125^{C}
I-6	0.134 ± 0.002^{A}	2982.208 ± 40.576^{A}
I-13	0.126 ± 0.004^{B}	1398.460 ± 112.466^{D}
I-18	0.008 ± 0.001^{E}	226.633 ± 7.382^{F}
II-1	0.079 ± 0.013^{C}	1202.654 ± 131.457^{D}
II-4	0.010 ± 0.001^{E}	213.163 ± 8.782^{F}
II-9	0.017 ± 0.003^{D}	431.436 ± 60.151^{E}
II-10	0.007 ± 0.001^{E}	225.133 ± 10.581^{F}
II-12	0.022 ± 0.003^{D}	535.620 ± 65.151^{E}
III-7	0.122 ± 0.005^{B}	2098.460 ± 50.453^{B}
III-8	0.089 ± 0.012^{C}	1181.750 ± 112.466^{D}
III-15	0.108 ± 0.013^{C}	1117.775 ± 121.756^{D}
III-16	0.022 ± 0.003^{D}	431.436 ± 60.351^{E}
III-17	0.102 ± 0.012^{C}	1134.060 ± 109.356^{D}

Note: Values in the same column followed by the different letters indicate significant differences of at least P<0.05

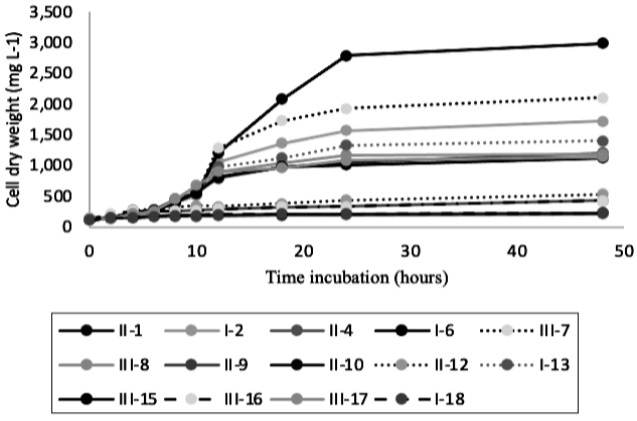

Figure 1. The growth of 14 bacterial isolates from eco-enzymes in nutrient broth media during a 48-hour incubation

In general, rhizosphere microorganisms associated with various plants commonly produce indole acetic acid as a secondary metabolite, facilitated by the abundant supply/ availability (Spaepen et al. 2007). Secondary metabolite synthesis typically occurs toward the end of or during the stationary phase of bacterial growth. In this study, samples for measuring IAA production were taken at the 48th hour, based on the assumption that the bacteria had entered the stationary phase. The stationary phase in this study generally began after the 24th hour.

Khianngam et al. (2023) reported that two strains of endophytic bacteria, VG2 and MG9, produced the highest IAA levels in NB medium supplemented with 100 $mg \cdot L^{-1}$ L-tryptophan after 48 hours of incubation. Sampling at the 72nd hour showed lower IAA production. However, out of the 14 successfully isolated strains, only 11 bacterial isolates produced IAA (Table 2). The isolates that were unable to produce IAA were isolates I-18, II-4, and II-10. These three isolates likely failed to produce IAA either because they inherently lack the capability or because they were unable to grow well in the liquid nutrient medium, thus inhibiting their ability to produce IAA.

Among these 11 bacterial isolates, isolate I-6 had the best ability to produce an IAA of 35.49 $mg.L^{-1}$. Three bacterial isolates, namely isolate II-12, III-16, and III-17 showed a low ability to produce IAA between 2.09 - 3.11 $mg.L^{-1}$. Based on the calculation of IAA production per cell mass (Table 2), the results showed that higher cell density does not always lead to higher IAA production. This finding contradicts the view of Kochar et al. (2013) and Ramadhani et al. (2020), who suggested that cell density is one of the factors influencing IAA production. Other influencing factors include bacterial species, growth phase, pH, and temperature. In this case, it is possible that the bacterial isolate's growth had not yet reached the phase suitable for IAA production.

Interestingly, isolates I-2, I-13, and III-7 produced relatively high levels of IAA but showed lower efficiency when production was calculated per cell weight. According to Elsoud et al. (2023), microbial IAA production varies significantly not only between species but also among strains within the same species. This variability is strongly influenced by nutritional and environmental conditions. Even within the same bacterial species, IAA can be produced in varying concentrations depending on production conditions. It is likely that the growth conditions and medium used in this study to assess IAA production capacity were not optimal, leading to submaximal IAA production. Therefore, further investigation is necessary to determine the optimum conditions for these isolates to produce IAA.

Molecular identification and evolutionary relations between IAA-producing bacterial strains

Based on the amplification results, a DNA amplicon of approximately 1400bp was obtained (Table 3). The Basic Local Alignment Search Tool (BLAST) analysis results to match with the NCBI GenBank database showed that most of the isolates (nine out of eleven) belong to the Bacillus group. The nine isolates are I-2, III-7, and III-8, which are similar to *B. altitudinis*; isolate I-13, which is comparable to *B. subtilis*; isolate III-15, which is identical to *B. licheniformis*; isolate I-6, which is identical to *Priestia megaterium*; and isolate I-2, which is identical to *Paenibacillus* sp. *P. megaterium* is the new name given to *Bacillus megaterium* due to evidence of phylogenetic solid and molecular differences (Gupta et al. 2020). Originally, *Paenibacillus* was included in the genus *Bacillus*; however, it was reclassified into its genus in 1993 (Grady et al. 2016).

Table 2. IAA production by the 14 bacterial isolates

Bacterial isolate code	IAA-production concentration	
	($mg.L^{-1}$)	(mg.mg cell dry $weight^{-1}$)
I-2	7.12 ± 0.38^{BC}	0.0042
I-6	35.49 ± 1.98^{A}	0.0119
I-13	6.45 ± 0.34^{CD}	0.0046
I-18	0	0
II-1	5.42 ± 1.23^{DE}	0.0045
II-4	0	0
II-9	2.47 ± 0.57^{GH}	0.0057
II-10	0	0
II-12	2.09 ± 0.24^{H}	0.0039
III-7	8.40 ± 0.65^{B}	0.0035
III-8	4.54 ± 0.69^{EF}	0.0038
III-15	3.90 ± 0.22^{FG}	0.0035
III-16	3.04 ± 0.16^{GH}	0.0070
III-17	3.11 ± 0.37^{FGH}	0.0027

Note: Values in the same column followed by the different letters indicate significant differences of at least $P<0.05$

Table 3. Isolation code, molecular characteristics, and the value of the closeness of the species

Isolation code	Accession number	Sequence length (bp)	Related species	Related score
I-2	PQ095569	1416	*Bacillus altitudinis*	99.93%
I-6	PQ095570	1412	*Priestia megaterium*	100.00%
I-13	PQ095571	1396	*Bacillus subtilis*	99.86%
II-1	PQ095572	1431	*Paenibacillus* sp.	99.44%
II-9	PQ095573	1442	*Lacticaseibacillus paracasei*	99.86%
II-12	PQ095574	1454	*Lactiplantibacillus plantarum*	100.00%
III-7	PQ095575	1414	*Bacillus altitudinis*	100.00%
III-8	PQ095576	1415	*Bacillus altitudinis*	99.79%
III-15	PQ095577	1417	*Bacillus licheniformis*	99.79%
III-16	PQ095578	1444	*Vibrio* sp.	100.00%
III-17	PQ095579	1448	*Vibrio diazotrophicus*	100.00%

The phylogenetic tree of the eleven IAA-producing isolates is presented in Figure 2. Nine isolates with rod-shaped cells were grouped into four different groups: five isolates (I-2, III-8, III-7, I-13, and III-15) belonging to the genus *Bacillus*, which were closely related to the isolate I-6, identified as *P. megaterium*. Additionally, two lactic acid bacteria isolates (II-9 and II-12) were closely related to the isolate II-1, identified as *Paenibacillus*. *Paenibacillus* is a distinct group, quite distant from other rod-shaped bacteria, because phenotypically, this group reacts weakly to Gram staining, and even young cultures appear Gram-negative (Chauhan et al. 2015). Based on the phylogenetic tree analysis, *Paenibacillus* sp. is more closely related to the lactic acid bacteria (II-9 and II-12) than members of the *Bacillus* and *Priestia* groups. One species of *Paenibacillus*, namely *P. polymyxa*, can produce a bacteriocin called polymyxin, and, along with lactic acid bacteria, can be used as a probiotic (Wang et al. 2021).

The remaining two isolates (III-16 and III-17) were classified into a slightly different group, namely vibrio-shaped bacteria. This group of *Vibrio* bacteria is closely related to *E. coli*. Both *Vibrio* bacteria and *E. coli* belong to the class *Gamma-proteobacteria*, a large group characterized by diverse metabolic and ecological traits. Additionally, both are Gram-negative bacteria (Kaberdin and Arana 2021). *Vibrio* spp. has received particular attention because it is often used to study the impact of climate change on the dynamics, distribution, and pathogenicity of microbial species inhabiting aquatic systems. Although *Vibrio* species are often studied for their pathogenicity, most *Vibrio* spp. are not pathogenic. For example, *V. diazotrophicus* can be isolated from marine habitats (Kerkar et al. 2012).

Regarding IAA production capabilities, most *Bacillus* species exhibit moderate levels, ranging from 3.9 to 8.4 $mg \cdot L^{-1}$. Members of the genus *Bacillus* are well-known as rhizobacteria commonly found on plant roots (Shi et al. 2022; Liu et al. 2022) and are recognized for their biofertilizer properties (Budiharjo et al. 2017). According to de O. Nunes et al. (2023), *B. licheniformis*, *B. subtilis*, and their mixture of both can enhance tomato growth and produce IAA. *B. altitudinis* is also known for its ability to produce IAA and promote plant growth (Elfira et al. 2020; Zhang et al. 2021b).

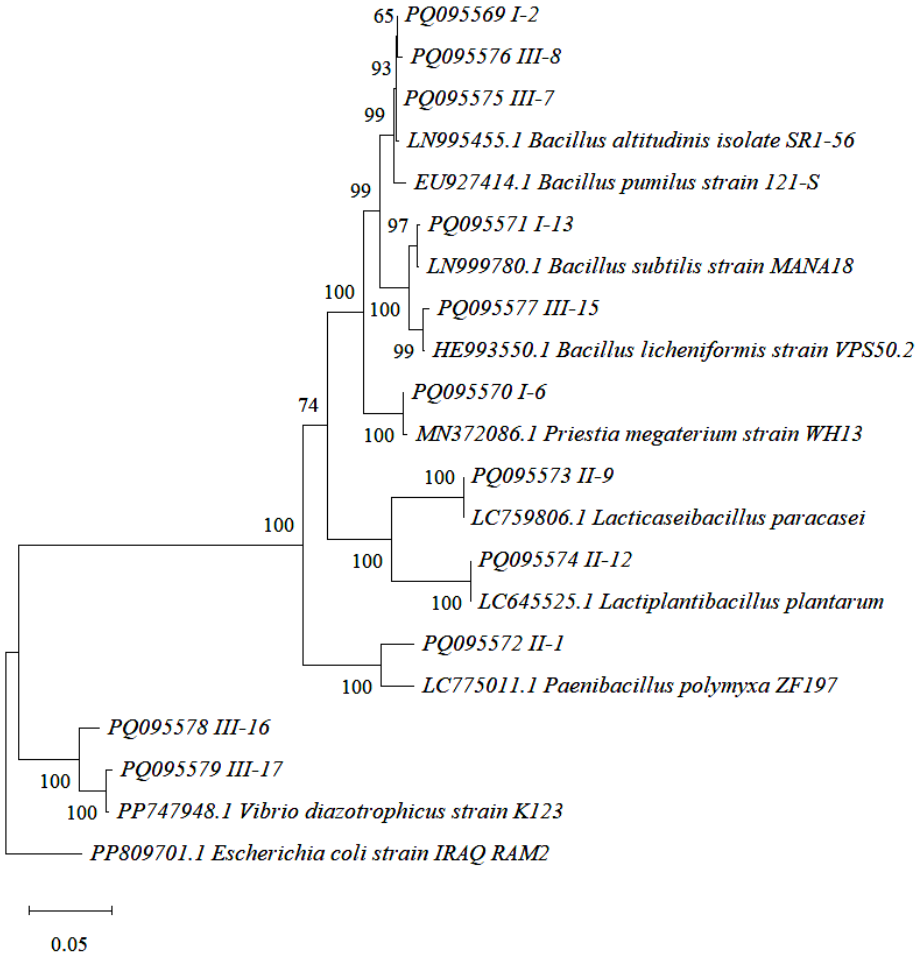

Figure 2. The phylogenetic tree constructed using MEGA software illustrates the close relationship between the11 IAA-producing isolates from eco-enzymes and several other IAA-producing isolates

In this study, isolate I-6, identified as *P. megaterium*, produced the highest amount of IAA at 35.49 mg.L^{-1}. Although *P. megaterium* produced the highest IAA in this study, its IAA production is lower compared to isolate RK (Sukmawati et al. 2021), isolated from mung bean roots, which produced 50.9 ppm of IAA, and isolate P37 (Putra et al. 2023), isolated from the roots of *Plumeria acuminata*, which produced 113.588 ppm of IAA. However, the identities of isolates RK and P37 remain unknown. *P. megaterium* is known to inhabit roots (as a rhizobacterium) and has the ability to produce IAA (Mohite 2013). According to Liu et al. (2022) and de O.Nunes et al. (2023), this bacterial species also produces antimicrobials, which protect plants against pathogens. Another rod-shaped bacterial isolate, *Paenibacillus* (isolate II-1), produced a relatively high amount of IAA (5.42 mg.L^{-1}). According to Grady et al. (2016), *Paenibacillus* has the ability to produce IAA; however, there has been no in-depth research on the extent of its production or the conditions that influence its IAA production.

Two isolates of lactic acid bacteria, namely isolates II-9 and II-12, are closely related to *L. paracasei* (II-9) and *L. plantarum* (II-12), respectively. Lactic acid bacteria, such as *L. casei* and *L. acidophilus* (Mohite 2013; Panetto et al. 2023), are known for their ability to produce IAA. In their research, Nunes et al. (2022) successfully identified lactic acid bacteria capable of controlling phytopathogens, stimulating plant growth, and producing IAA. These include *L. plantarum*, *L. paracasei*, *Lacticaseibacillus rhamnosus*, *Lactococcus lactis*, *Levilactobacillus brevis*, *Lactilactobacillus curvatus*, *Leuconostoc mesenteroides*, and *Leuconostoc citreum*.

IAA-producing isolates in the form of vibrios, namely isolates III-16 and III-17, belong to a group distinct from the others (Figure 2). This group is closely related to *E. coli*, also known to produce IAA (Li and Young 2013). These isolates exhibit relatively low IAA production. *Vibrio* species have been successfully isolated from biofilm mats and the grass rhizosphere in estuarine environments (Gutierrez et al. 2009; Kerkar et al. 2012). *Vibrio diazotrophicus* can produce 9.67 µg.mL^{-1} of IAA with the addition of tryptophan (Kerkar et al. 2012). Similarly, Shin et al. (2023) reported that *Vibrio* sp. can produce 0.25 mg.L^{-1} of IAA with tryptophan supplementation.

Based on the results of this research, it can be concluded that eco-enzymes contain various bacteria capable of producing IAA. Among the eleven IAA-producing isolates, the members of the genus *Bacillus*, i.e., *B. altitudinis*, *B. subtilis*, *B. licheniformis*, *Priestia megaterium*, and *Paenibacillus* sp. were the most frequently isolated and exhibited significant IAA production. The isolate with the highest IAA production (35.49 mg.L^{-1}) was *Priestia megaterium*. The other two groups with lower IAA production capabilities are lactic acid bacteria (*Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*) and vibrio-shaped bacteria (*Vibrio* sp. and *Vibrio diazotrophicus*). These bacterial isolates, particularly *Priestia megaterium*, show great promise as plant growth promoters. However, further research is required to evaluate their effectiveness in stimulating plant growth in field conditions.

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