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Isolation and Characterization of Endophytic Bacteria from the Vegetative Phase of Indonesian Garlic cv. 'Tawangmangu Baru'

(Pengasingan dan Pencirian Bakteria Endofit daripada Fasa Vegetatif Bawang Putih Indonesia cv. 'Tawangmangu Baru')

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ABSTRACT

The high demand for garlic (*Allium sativum* L.) has led to efforts to improve its productivity. Garlic endophytic bacteria have been found to boost the growth of garlic plants and protect them from diseases without any harmful effects on the host. The objective of the study was to isolate and characterise the endophytic bacteria present in the healthy local Indonesian garlic cv. 'Tawangmangu Baru' towards developing them as plant growth-promoting bacteria in improving garlic quality and productivity. The isolation of endophytic bacteria was performed from garlic leaves and roots during the vegetative phase. A total of 20 endophytic bacteria isolated from garlic leaves and roots were characterised by their morphology and tested for their ability to fix nitrogen (N), produce auxin (IAA), and solubilise phosphate. All the isolates were able to fix nitrogen, with 15 of the isolates found to be Gram-positive having bacilli cell shape. Furthermore, 8 isolates were shown to produce auxin, while 10 isolates were able to solubilise phosphate, with both groups originating from the root sample. Five potential isolates were identified as nitrogen-fixing, IAA-producing, and phosphate-solubilising, with the highest values of IAA concentration and the Phosphate Solubilisation Index (PSI) being 10.71 ppm and 2.00, respectively. These 5 potential endophytic bacterial isolates were genetically identified as similar to *Lysobacter tolerans*, *Enterobacter cloacae*, *Enterobacter huaxiensis*, *Klebsiella oxytoca*, and *Enterobacter hormaechei*. The potential endophytic bacterial isolates hold promise for developing garlic plant growth-promoting bacteria (PGPB) to enhance garlic production both qualitatively and quantitatively.

Keywords: *Allium sativum*; endophytic bacteria; IAA; nitrogen; phosphate

ABSTRAK

Permintaan yang tinggi untuk bawang putih (*Allium sativum* L.) membawa kepada usaha untuk meningkatkan produktivitinya. Bakteria endofit bawang putih didapati mempunyai keupayaan untuk meningkatkan pertumbuhan dan melindungi tumbuhan bawang putih daripada penyakit, tanpa kesan berbahaya pada perumah. Objektif kajian ini adalah untuk memencilkan dan mencirikan bakteria endofit yang terdapat dalam bawang putih tempatan Indonesia cv. 'Tawangmangu Baru' bagi membangunkannya sebagai bakteria penggalak pertumbuhan tumbuhan dalam meningkatkan kualiti dan produktiviti bawang putih. Pemencilan bakteria endofit dilakukan daripada daun dan akar bawang putih semasa fasa vegetatif. Sebanyak 20 bakteria endofit yang dipencilkan daripada daun dan akar bawang putih telah dicirikan morfologinya dan diuji kebolehannya untuk mengikat nitrogen (N), menghasilkan auksin (IAA) dan melarutkan fosfat. Semua pencilan dapat mengikat nitrogen dengan 15 pencilan didapati sebagai Gram-positif yang mempunyai bentuk rod. Tambahan pula, 8 pencilan telah menunjukkan keupayaan menghasilkan auksin, manakala 10 pencilan berupaya melarutkan fosfat, yang kesemuanya berasal daripada sampel akar. Sebanyak 5 pencilan berpotensi dikenal pasti sebagai pengikat nitrogen, penghasil IAA dan pelarut fosfat, dengan nilai kepekatan IAA dan IPF Indeks Pelarutan Fosfat (IPF) tertinggi, masing-masing ialah 10.71 ppm dan 2.00. Kesemua 5 pencilan bakteria endofit yang berpotensi ini dikenal pasti secara genetik menyamai *Lysobacter tolerans*, *Enterobacter cloacae*, *Enterobacter huaxiensis*, *Klebsiella oxytoca* dan *Enterobacter hormaechei*. Pencilan bakteria endofit yang berpotensi merupakan prospek dalam membangunkan bakteria penggalak pertumbuhan bawang putih untuk meningkatkan pengeluaran bawang putih secara kualitatif dan kuantitatif.

Kata kunci: *Allium sativum*; bakteria endofit; fosfat; IAA; nitrogen

INTRODUCTION

Garlic (*Allium sativum* L.) is a widely used spice that is also known for its medicinal properties in traditional and modern therapeutics (Singh, Khar & Verma 2021). In 2022, garlic production in Indonesia reached 30,194 tons, which was significantly lower than the national demand. To meet the high demand, 554,020 tons of garlic were imported in 2022 (BPS-Statistics Indonesia 2023). Additionally, garlic is a significant contributor to monthly inflation in Indonesia, accounting for 0.07% in the first semester of 2023, following cigarettes (Ministry of Agriculture 2023). However, efforts to address the food crisis include plans to improve garlic production capacity to reduce the need for imports. Garlic is targeted for production improvement in 2024, aiming for a total of 45.91 thousand tons (Ministry of Agriculture 2023).

The excessive use of inorganic fertilizers and pesticides to improve productivity has negative impacts on human health and agroecosystem balance (Gunstone et al. 2021). Advancements in agricultural technologies have been made to reduce the use of inorganic fertilizers and promote sustainable production. One such innovation is biofertilizer, which harnesses the role of beneficial living microorganisms for soil fertility and plant growth, with minimal negative impact. Biofertilizers, especially in the form of plant growth-promoting bacteria (PGPB), are widely used as an alternative to chemical fertilizers to reduce agricultural input costs and increase productivity (Costa Júnior et al. 2020; Srivastav et al. 2024).

The study of plant-microorganism association, known as the plant microbiome has shown mostly positive interactions. Research has shown that the plant microbiome can enhance plant growth and survival by improving physical and physiological functions (Bhattacharjee, Dubey & Sharma 2023; Koskey et al. 2021). Endophytic microbes, such as *Enterobacter cloacae* and *Burkholderia cepacian* have been found to promote the growth of garlic plants, making them more resistant to diseases and enhancing their physiological attributes (Costa Júnior et al. 2020). These endophytic microbes can solubilise macronutrients like phosphorous, potassium, and zinc, as well as fix atmospheric nitrogen, synthesise phytohormones, siderophores, hydrogen cyanide, and ammonia, and act as biocontrol agents against various plant pathogens (Rana et al. 2020; Ryan et al. 2008). This study aimed to isolate and characterise the endophytic bacteria present in the healthy local Indonesian garlic variety 'Tawangmangu Baru' to develop them as plant growth-promoting bacteria in improving garlic quality and productivity.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM GARLIC PLANTS

Endophytic bacteria were isolated from the leaves and roots of garlic plants (var. Tawangmangu Baru). The samples were thoroughly washed with running tap water to remove adhered soil particles and surface sterilised to remove any epiphytic microorganisms following standard protocol. This involved dipping the sample tissue in 70% ethanol for 3 min, followed by 0.5% NaOCl for 3 min, and then dipping it again in 70% ethanol for 30 s. Finally, the tissues were washed with double-distilled water. The samples were dried on sterile tissue for several minutes before being cut into small pieces and inoculated on Nutrient Agar (NA) media. The samples were then incubated for 2 - 14 days at 25 – 27 °C (room temperature). Direct colony counting was performed after the incubation period. The macroscopic characteristics of the colonies, including their edge, shape, elevation, and color, were observed. The isolates obtained were gram-tested using basic fuchsin stain according to gram-stain protocols (Smith & Hussey 2005).

EVALUATION AND SELECTION OF PLANT GROWTH-PROMOTING BACTERIA

AUXIN (IAA) PRODUCTION

The quantification of IAA production was carried out by inoculating endophytic bacterial isolates in Nutrient Broth (NB) media and incubating them at 28 °C for 48 h. Each isolate was then transferred to a new medium containing tryptophan (100 µg/mL) and incubated at 30 °C for 72 h in a dark room. Subsequently, the cultures were centrifuged at 15300 × g for 5 min to obtain the supernatant.

The determination of IAA production was performed by mixing 1 mL of supernatant with 1 mL of Salkowski Reagent (a solution made from 1.875 g $FeCl_3.6H_2O$, 100 mL H_2O, and 150 mL 35% H_2SO_4) (Gordon & Weber 1951). The mixtures were then incubated at 30 °C for 15 min in a dark room. The formation of a reddish-pink color indicated the presence of IAA, which was then observed for absorbance at 530 nm. The observed value was compared with a standard curve generated from a series of pure IAA concentrations to measure the IAA production.

BIOLOGICAL NITROGEN FIXATION

The nitrogen-fixing bacteria were identified by inoculating endophytic bacterial isolates on selective Yeast Extract

Mannitol (YEM) media. Each 1 L YEM media was made from 10.0 g mannitol, 0.5 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 1.0 g yeast extract, 20 g agar, and 10 mL of a stock solution of 250 mg bromothymol blue in 100 mL water, with the pH adjusted to 7. Bromothymol blue (BTB) was used as an indicator for nitrogen fixation activity. After 3 days of incubation, yellow circles around the blue colonies were observed. The yellow color formed indicated acid production from the nitrogenase activity of the nitrogen-fixing bacteria, which caused an increase in the pH of the medium (Ekowati et al. 2021; Wang et al. 2017).

PHOSPHATE SOLUBILISATION EFFICIENCY OF PHOSPHATE SOLUBILISING BACTERIA (PSB)

The qualitative assay of phosphate solubilisation was conducted using the spot test method on Pikovskaya agar. The composition of the Pikovskaya agar medium was as follows: 10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 25 mg MnSO4, 25 mg $FeSO_4$, and 20 g bacto agar in 1 L of water, with a pH of approximately 7. All isolates were spot inoculated using a sterile toothpick on Pikovskaya agar medium and then incubated for 72 h at room temperature (Azizah, Rahajeng & Jatmiko 2020). After the incubation period, the diameter of the phosphate solubilising zone around the bacterial colonies, indicated by a clear zone around the colonies, was measured. The clear zone formed is measured as the phosphate solubilisation index (SI), which is calculated as the ratio of the total diameter (colony + clear zone) to the colony diameter (Pande et al. 2017). Isolates exhibiting clear zones around their colonies could easily be identified as phosphate-solubilising bacteria (PSBs).

$$PSI = \frac{Colony\ diamter + Clear\ zone\ diameter}{Colony\ diameter}$$

GENETIC ASSESSMENT OF POTENTIAL ENDOPHYTIC BACTERIA

The genomic DNA was extracted from the isolates capable of producing IAA, nitrogen fixation, and phosphate solubilisation using the Tianamp Bacteria DNA Kit (Tiangen) following the manufacturer's instructions. The PCR reaction was carried out in a Wee32® Thermal Cycler (HiMedia). The gene fragment was amplified using general 16S ribosomal RNA gene PCR primers that were B341 primers (Forward primer 5'-CCTACGGGGGCWGCAG-3' and reverse primer 5'-GACTACHVGGGTATCTAATCC-3'). These primers showed higher internal diversity within certain bacterial taxonomic groups (Fadeev et al. 2021).

For the PCR amplification, a 50 µL PCR reaction mixture was prepared, which included 2 µL of template DNA (100 ng), 25 µL of MyTaq HS Red mix (bioline), and 1 µL of each forward and reverse primer. The PCR amplification cycle involved an initial denaturation step of 1 min at 95 °C, followed by 35 cycles of 15 s at 95 °C (denaturation), 15 s at 55 °C (annealing), and 10 s at 72 °C (extension), with a final extension step for 10 min at 72 °C. The PCR products were analyzed by electrophoresis using 2% agarose stained with GelRed® Nucleic Acid Gel Stain (biotium). The PCR products of the isolates were purified using a PCR purification kit (DNA Clean & Concentrator-5, Zymo Research) and sequenced on the Sanger sequencing platform at the genomic laboratory of the National Research and Innovation Agency for sequencing.

The endophytic bacterial isolates were identified by performing BLAST analysis of their respective gene partial sequences. The NCBI databases were used to determine the exact taxonomical classification of individual organisms. NCBI Blast was used for the determination of the closest type strains. The sequence data were aligned using the Clustal W software, and the distances were calculated according to Kimura's two-parameter method. Phylogenetic trees were produced by the neighbor-joining method, and bootstrap analysis was based on 1000 bootstraps. The MEGAXI package was used for all phylogenetic analyses.

RESULTS AND DISCUSSION

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM GARLIC VAR TAWANGMANGU BARU

A total of 20 endophytic bacterial colonies were isolated, with 9 originating from the roots and 11 from the leaves of the garlic variety 'Tawangmangu Baru'. The isolated bacteria were observed for their morphological characteristics, including shape, edges, elevation, surface, and color. The morphotypes exhibited distinct macroscopic features (Figure 1; Table 1). Eleven isolates were identified as having round shapes, while the rest displayed irregular and oval shapes. The round-shaped colonies had smooth edges, whereas the irregular ones had undulated edges. The elevation analysis showed that nearly all isolates obtained from the leaves had a flat elevation, while those from the roots displayed more diverse elevations.

Out of the total isolates, 15 were Gram-positive, consisting of 11 bacilli and 4 cocci forms. Additionally, 5 isolates were Gram-negative, with 4 being bacilli form and 1 being cocci form (Figure 2; Table 2). Previous research has shown that Gram-positive endophytic bacteria play crucial roles in bioremediation, biocontrol, plant growth, symbiotic mutualism, commensalism, trophobiotic interactions, control of soil-borne pathogens, and support of host plant defenses against environmental stress (Ryan et al. 2008).

Members of the bacilli class are primarily known for producing bioactive secondary metabolites and important antimicrobial enzymes (Ek-Ramos et al. 2019). Another study focusing on the isolation of endophytic bacteria from garlic roots found that out of 48 isolates, 31 were

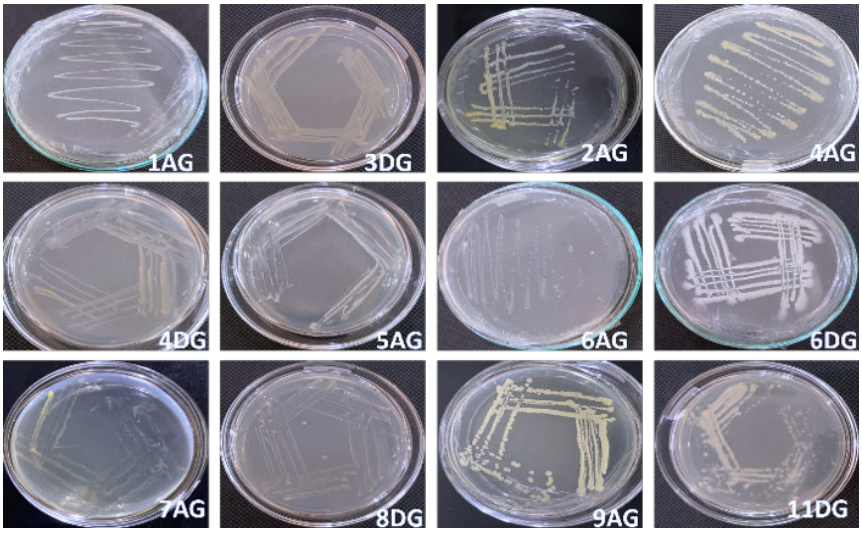


FIGURE 1. Macroscopic performance of garlic endophytic bacteria isolates

TABLE 1. Macroscopic characteristics of garlic endophytic bacteria isolates

No	Isolate Code	Shape	Edge	Elevation	Color
1	1AG	round	entire	flat	white
2	2AG	round	entire	convex	clear
3	3AG	irregular	undulate	umbonate	white
4	4AG	oval	undulate	convex	white yellowish
5	5AG	round	entire	raised	white
6	6AG	round	entire	flat	clear
7	7AG	round	entire	convex	clear yellowish
8	8AG	round	entire	flat	clear
9	9AG	oval	undulate	flat	white
10	1DG	round	entire	raised	white
11	2DG	irregular	undulate	flat	clear
12	3DG	irregular	undulate	flat	white
13	4DG	round	entire	flat	clear
14	5DG	irregular	undulate	flat	yellow
15	6DG	oval	rhizoid	flat	white
16	7DG	round	entire	flat	clear
17	8DG	round	entire	flat	clear
18	9DG	irregular	undulate	raised	white
19	10DG	round	entire	flat	clear
20	11DG	irregular	undulate	flat	white

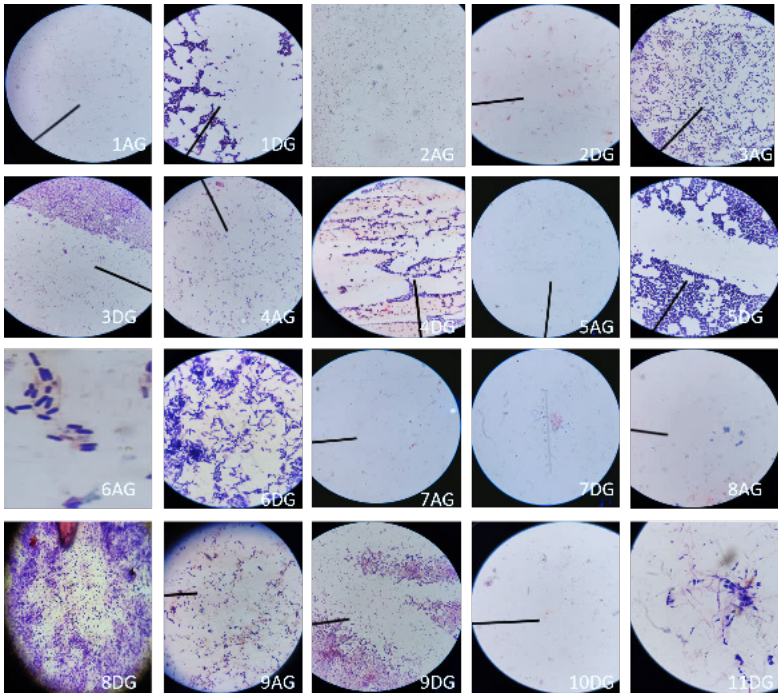


FIGURE 2. Microscopic performance of garlic endophytic bacteria gram stain

TABLE 2. Shape and gram stain test result of garlic endophytic bacteria

No	Isolate code	Cell shape	Gram stain
1	1AG	bacilli	negative
2	2AG	bacilli	positive
3	3AG	bacilli	positive
4	4AG	bacilli	positive
5	5AG	cocci	negative
6	6AG	bacilli	positive
7	7AG	bacilli	negative
8	8AG	bacilli	positive
9	9AG	bacilli	positive
10	1DG	cocci	positive
11	2DG	bacilli	negative
12	3DG	bacilli	positive
13	4DG	cocci	positive
14	5DG	cocci	positive
15	6DG	bacilli	positive
16	7DG	cocci	positive
17	8DG	bacilli	positive
18	9DG	bacilli	positive
19	10DG	bacilli	negative
20	11DG	bacilli	positive

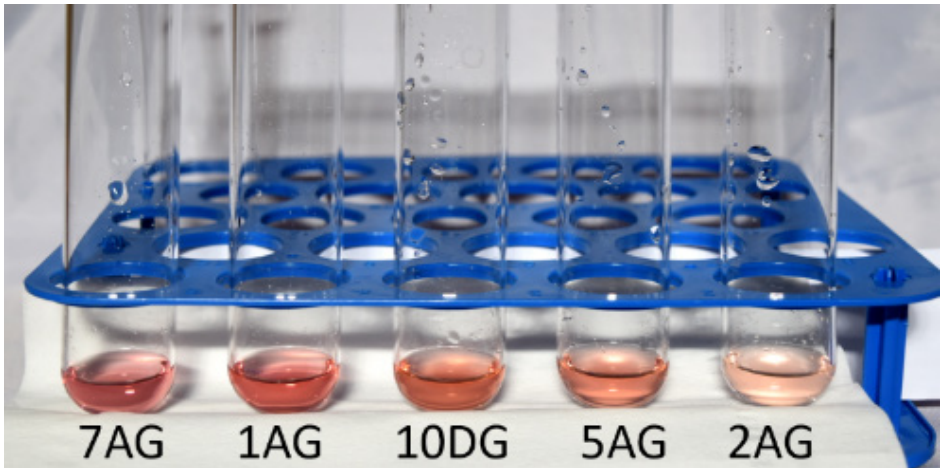


FIGURE 3. Qualitative IAA production of garlic endophytic bacteria

TABLE 3. IAA production, nitrogen fixation, and P-solubilizing test of garlic endophytic bacteria isolates

No	Isolate code	IAA contents		Nitrogen fixation	P-Solubilize (clear zone)	
		Color	Concentration (ppm)		PSI	Consistency
1	1 AG	+++	10.71	+	1,28	+++
2	2 AG	+	4.45	+	1,39	+
3	3 AG	-	-	+	1,55	+++
4	4 AG	-	-	+	1,21	+
5	5 AG	+++	9.63	+	2,00	+++
6	6 AG	++	6.11	+	-	-
7	7 AG	+++	8.84	+	1,38	+++
8	8 AG	-	-	+	-	-
9	9 AG	-	-	+	1,44	+++
10	1 DG	-	-	+	1,17	+
11	2 DG	+	4.40	+	-	-
12	3 DG	-	-	+	-	-
13	4 DG	-	-	+	-	-
14	5 DG	-	-	+	1,26	+
15	6 DG	++	5.53	+	1,19	+
16	7 DG	-	-	+	-	-
17	8 DG	-	-	+	-	-
18	9 DG	-	-	+	1,14	+
19	10 DG	+++	10.19	+	-	-
20	11 DG	-	-	+	-	-

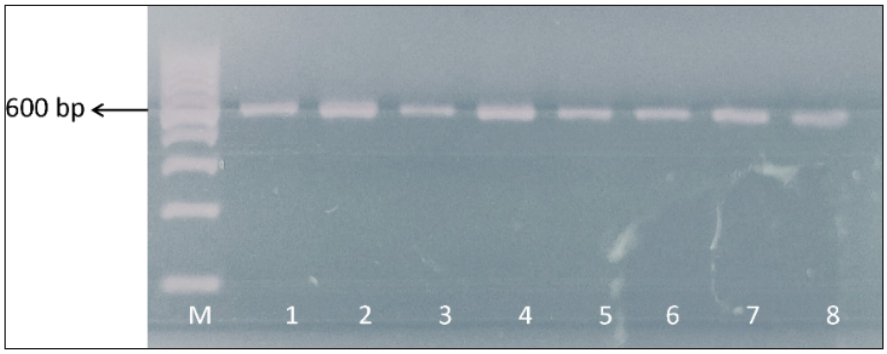
Gram-positive (Costa Júnior et al. 2020). Moreover, research conducted on enzyme-producing endophytes in the Thailand mangrove ecosystem by Khianngam et al. (2013) showed that Gram-positive bacteria exhibited higher hydrolytic activity compared to Gram-negative ones. These findings suggested that further tests should be carried out on the isolates, including assessments of bioactive compound production to promote plant growth.

EVALUATION AND SELECTION OF PLANT GROWTH-PROMOTING BACTERIA

Out of the 20 endophytic bacterial isolates that were tested for their ability to produce IAA (auxin), fix nitrogen, and solubilise phosphate, only 8 isolates produced the characteristic pink color when mixed with Salkowski's reagent in the auxin test. Among these 8 isolates, four showed a stronger pink color, indicating a higher presence of auxin in the mixture. These isolates were labeled as 7AG, 1AG, 10DG, and 5AG (Figure 3). The quantitative assay of auxin production showed that the amount of auxin produced by the isolates ranged from 4.40 to 10.71 µg/mL. The top three isolates in terms of auxin production were found to produce 10.71 µg/mL, 10.19 µg/mL, and 9.63 µg/mL, and were labeled as 1AG, 10DG, and 5AG, respectively (Table 3). The results indicated that 5 isolates that produced higher levels of auxin were isolated from the roots, while three were from the leaves.

These findings suggested that the producers of auxin were more prevalent in the roots, which are one of the meristematic tissues and are in a continuous state of division. Auxin plays a crucial role in root development, inhibiting the growth of side shoots, stimulating abscission, and in the formation of xylem and phloem tissue (Silitonga, Priyani & Nurwahyuni 2008).

The nitrogen fixation test showed that all of the isolates were able to fix nitrogen, as indicated in Table 3, by the yellow color changes in the medium. Nitrogen fixation is a biological process through which bacteria convert nitrogen in the air into a form that can be used by plants. The fact that all endophytic bacteria tested showed the capacity to carry out nitrogen fixation indicated that these bacteria have the potential to increase nitrogen availability for host plants. This is significant because nitrogen is a crucial element in plant growth.



Left to Right: 100 bp marker (M); 7AG (1); 1AG (2); 2AG (5); 5AG (7); dan 6DG (8)

FIGURE 4. Electrophoregram of garlic endophytic bacteria isolates

TABLE 4. The comparison of the percentage similarity of the rRNA gene of endophytic bacteria with several DNA sequences in Genbank using the BLAST program

Isolate code	Species	Strain	Accession Number	Similarity (%)
7 AG	*Klebsiella oxytoca*	KKP 3088	MT549686.1	97,82
1 AG	*Lysobacter tolerance*	CEMTC 2278	OP703672.1	91,49
2 AG	*Enterobacter cloacae*	P5-5	MN181079.1	93,97
5 AG	*Enterobacter huaxiensis*	gall3055	MW435508.1	98,25
6 DG	*Enterobacter hormaechei*	JRBHU-8	MK500939.1	96,26

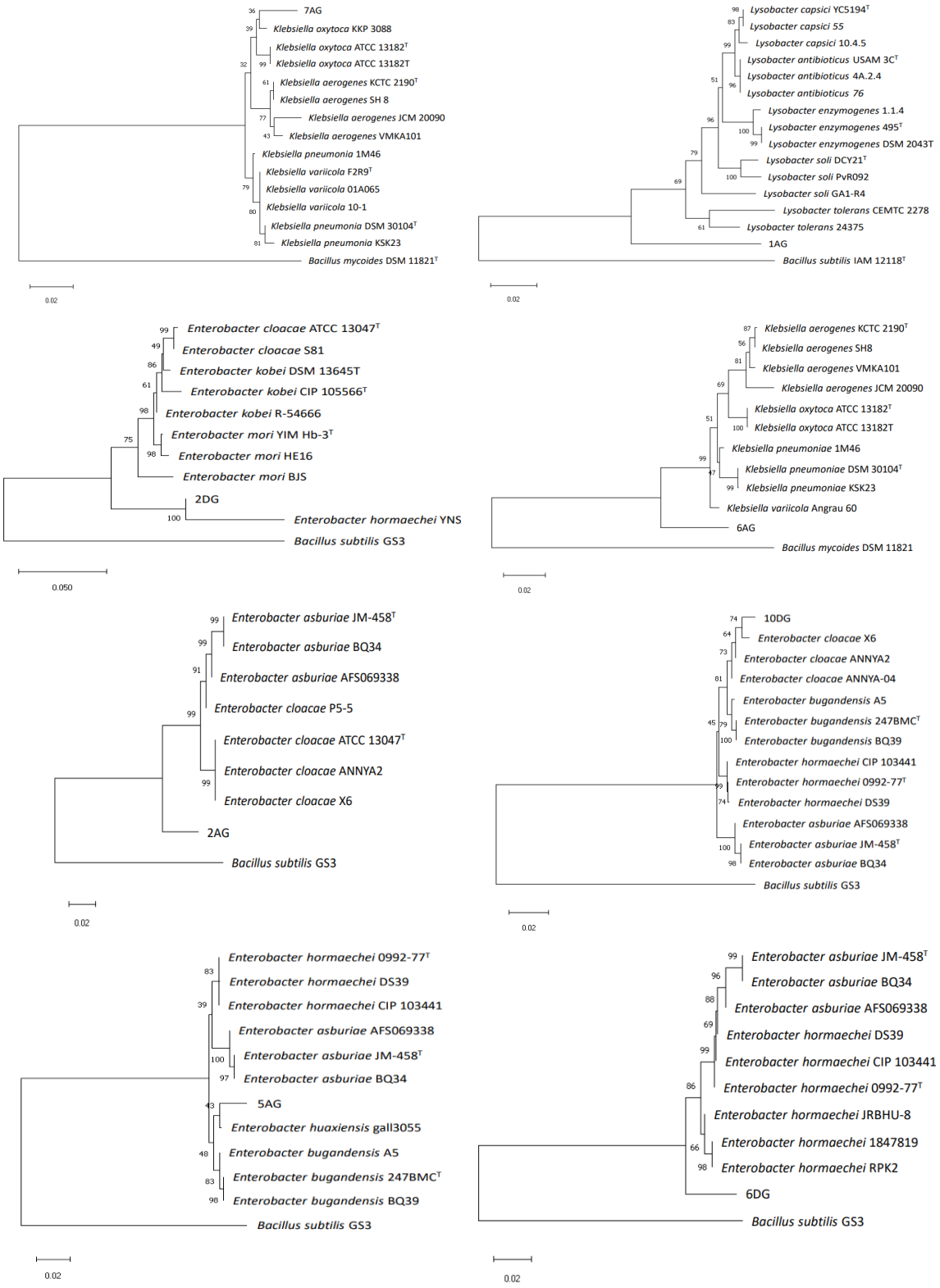


FIGURE 5. Phylogeny tree of garlic endophytic bacteria with reference species based on rDNA sequence similarity using the Neighbor-Joining algorithm and the Tamura-Nei method (1000x bootstrap)

An interesting finding of the research was that the isolates can perform auxin production, nitrogen fixation, and phosphate solubilisation. The phosphate solubilising tests were conducted by growing the isolates in a selective medium. The Pikovskaya medium was used for the tests and the results showed that 11 isolates were able to solubilise phosphate, indicated by the clear zone formed around the colony. The solubilisation zone occurs due to the presence of some substances, such as organic acids, released by microorganisms, which solubilise the phosphate in the medium. The presence of these substances generates a clear zone around the colonies, which indicates the solubilising capacity of the complex insoluble $Ca_3(PO_4)_2$ on Pikovskaya media (Matos et al. 2017). The colony and the clear zone diameter were measured as the Phosphate Solubilisation Index (PSI). The thicker the shown zone which is marked with (+++) consistency in the table, indicated the stronger ability to solubilise phosphate, but indeed needed to be quantitatively tested.

The higher consistency and PSI value displayed by the root endophytic bacterial isolates indicated that the phosphate solubilisers were more prevalent in the roots. Previous research showed that phosphate-solubilising bacteria (PSB) were found in the roots and around the rhizosphere of healthy rice plants in acid sulfate soil, and showed a higher population around the rhizosphere and roots compared to non-rhizosphere due to the presence of high levels of nutrients exuded from the roots (Purwaningsih, Dewi & Pujiasmanto 2019; Walia et al. 2017), which might enhance the possibility of the presence of PSB inside the roots.

Table 3 indicated that 5 isolates (1AG, 2AG, 5AG, 7AG, and 6DG) demonstrated the ability to produce auxin, fix nitrogen, and solubilise phosphate. The isolates with the highest values were identified as endophytic bacteria isolated from garlic roots. This study suggested that potential endophytic bacteria were more abundant in below-ground tissues than in above-ground tissues of the plant. These findings aligned with previous research showing that the population of endophytic bacteria is more diverse and more dense in the roots (Kaga et al. 2009; Lin et al. 2022; Shofiyah et al. 2023).

GENETIC ASSESSMENT OF POTENTIAL ENDOPHYTIC BACTERIA

Genetic assessment was conducted on the five potential isolates identified for their ability to produce IAA, fix nitrogen, and solubilize phosphate. The results of DNA electrophoresis indicated that the B341 primers successfully amplified the target DNA of endophytic bacteria. A clear visualization of one DNA band and the production of a 600 bp DNA fragment confirmed this (Figure 4). The resulting DNA fragments were purified and sequenced to determine the bacterial species present. The identification process was based on the similarity of the species to other identified bacterial species.

The BLAST analysis showed that three endophytic bacterial isolates belong to the genus *Enterobacter*, while one isolate was identified as belonging to the genus *Klebsiella* and another to the genus *Lysobacter*. The results of the phylogenetic tree construction using UPGMA with 1000 repetitions are available in Figure 5. Additionally, the comparison of the percentage similarity of the rRNA sequences of endophytic bacteria with several DNA sequences in Genbank using the BLAST program is available in Table 4.

Further analysis showed that the potential isolates found were more to the family *Enterobacteriaceae*, with four isolates identified as genetically similar to *Enterobacter cloacae* (2AG), *Enterobacter huaxiensis* (5AG), *Klebsiella oxytoca* (7AG), and *Enterobacter hormaechei* (6DG). Furthermore, one isolate (1AG) showed similarity (91.49%) with *Lysobacter tolerans* strain CEMTC 2278, and displayed rod-shaped and negative Gram-stained characteristics.

Even though the most abundant metabolite-producing Gram-positive bacteria endophytes found within diverse environments were *Bacillus* and *Streptomyces* species, there were Gram-negative bacteria including those in the genus *Enterobacter, Klebsiella, Pseudomonas, Enterococcus*, and *Staphylococcus*, which have anti-bacterial activity and resistance (Ek-Ramos et al. 2019). Genus *Lysobacter* includes several species that produce a range of extracellular enzymes and other metabolites with activity against bacteria, fungi, oomycetes, and nematodes (Gómez Expósito et al. 2015).

In Indonesia, *K. oxytoca*-C939A32 has been found in coffee plants and can dissolve phosphate and fix nitrogen (Halimah, Munif & Giyanto 2016). Additionally, the bacteria can control root wound nematode disease in coffee plants. Another study reported that the endophytic bacteria *K. oxytoca*-GR3 can dissolve phosphate and fix nitrogen, making it beneficial for stimulating plant growth, and identified its potential to promote the growth of rice (Jha & Kumar 2007). The sequencing results indicated that all five isolates of garlic endophytic bacteria belong to the *Enterobacter* genus, which is a member of the *Enterobacteriaceae* family. Two bacterial isolates were identified as *E. hormaechei* strain YNS (2 DG) and *E. hormaechei* strain JRBHU-8 (6 DG), respectively. The other two bacterial isolates were identified as *E. cloacae* strain P5-5 (2 AG) and *E. cloacae* strain ANNYA-04 (10 DG). One isolate was *E. huaxiensis* strain gall3055 (5AG). *E. cloacae* has been documented as capable of producing auxin, dissolving phosphate, and fixing nitrogen, as well as having potential as an antimicrobial against *Fusarium* pathogens and for increasing the availability of soil nutrients to promote the growth of cowpea (Ji et al. 2020).

CONCLUSIONS

Twenty endophytic bacteria were isolated from garlic leaves and roots. Fifteen of them were Gram-positive with a bacilli cell shape. Eight of the isolates produced auxin, and ten were able to solubilize phosphate. Five of the isolates were found to have the potential to fix nitrogen, produce auxin, and solubilize phosphate. These isolates had the highest concentrations of IAA and PSI. They were genetically identified as similar to *L. tolerans*, *E. cloacae*, *E. huaxiensis*, *K. oxytoca*, and *E. hormaechei*. These bacteria have the potential to be developed as garlic plant growth-promoting bacteria (PGPB) to improve garlic production.

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