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Research Article

Characterization of Plant Growth Promoting Rhizobacteria from Dominant Weeds Inhabited in Kaligarang River Delta, Indonesia

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Abstract: Delta is a bulk mass of river sediment that encompasses a variety of vegetation and soil microorganisms in beneficial relationships. The Kaligarang river delta in Semarang, Indonesia, has typical weed vegetation of *Ageratum conyzoides*, *Galinsoga parviflora*, *Eleusine indica*, and *Kyllinga brevifolia*, with impressive growth that may have been encouraged by rhizobacteria. Therefore, this study aimed to characterize rhizobacteria from the aforementioned deltaic weed vegetation, beginning with colony and cell morphology, biochemical and phytohormone analyses, and genotypic analysis of the notable performer. The result displayed distinctive colonies from *A. conyzoides* and *G. parviflora* rhizosphere samples with 3 of each. Meanwhile, *K. brevifolia* and *E. indica* rhizosphere unveiled 4 and 2 isolates, respectively. Most of the colonies were Gram-positive bacilli with circular or punctiform forms, entire edges, convex elevations, and the potential for catalase, starch hydrolysis, nitrogen fixation, phosphate solubilization, potassium solubilization, and auxin production. One of the *A. conyzoides* isolates achieved full biochemical competence and the highest auxin enhancement during 48 h of incubation without endospores. The sequence homology and phylogenetic tree analyses of the 16S rRNA gene suggested that the colony was *B. cereus*. The isolate characterization was in line with other research on *B. cereus* colony features and the tryptophan-dependent indole-3-acetic acid biosynthesis pathway.

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1. Introduction

Delta is a sedimentary soil on a riverbank that forms a bulky mass of various compounds and microorganisms transported by currents, with a wide range of particle sizes (Lyu et al., 2015; Li et al., 2019). The various sizes of soil particles affect soil texture and structure, which, in turn, influence soil performance (Olorunfemi et al., 2016). The distribution of soil particles is positively correlated with the storage capacity of water and organic matter. The larger the diameter of soil particles, the more groundwater flows down to lower soil bodies, and the more space is available for organic matter. Soil

particles, along with water and organic matter, form soil aggregates with cavities between them, which are colonized by plant roots (Han et al., 2021). Each aggregate is composed of several smaller aggregates with microorganisms in between. In this case, aggregates provide space for reciprocal relationships between vegetation and soil microorganisms, and thus, vegetation becomes highly dependent on the soil ecosystem (Costa et al., 2018).

Delta vegetation is either shrubs or grass. Shrubs occupy solid and annual delta communities, while grasses remain in fluctuating delta communities (Han et al., 2021). A study on vegetation restoration and ecological reconstruction of Shell Island in the Yellow River Delta, China, found that shrub and grass vegetation provide high soil water retention due to reduced gravel and coarse sand and elevated fine sand, silt, and clay. In other words, vegetation improved soil physical properties and water storage capacity (Chen et al., 2019; Xia et al., 2020).

The Kaligarang River delta in Semarang, Indonesia, has a typical type of weed vegetation. The delta shrubs were generally *Ageratum conyzoides* and *Galinsoga parviflora*. *A. conyzoides* has extraordinary growth potential, and *G. parviflora* withstands extreme environments (Kaur et al., 2023; Nyamwamu and Jeruto, 2023). Once *A. conyzoides* was grown adjacent to *Eupatorium catarium*, it gained a higher biomass. When both were co-planted with *V. cinerea*, none could outperform *A. conyzoides* in terms of biomass product (Huang et al., 2016). Meanwhile, *G. parviflora* demonstrated resistance to stressors, such as drought in sandy soil, and to biotic stressors, including insect, virus, and nematode attacks (Macharia et al., 2016; Santosa et al., 2020).

Further, the most common Kaligarang river delta grasses were *Eleusine indica* and *Kylinnga brevifolia*. *E. indica* is listed as one of the top five most problematic weeds in the world due to robust growth and extensive root system (Kalimashe, 2019). Likewise, *K. brevifolia* spreads widely through underground rhizomatous growth or seed germination. The plant was not easily uprooted or terminated with herbicides such as ALS (Westbury et al., 2022).

The mentioned grasses and shrubs are generally considered weeds, which exhibit better growth and survival than cultivated plants because of their greater tolerance to nutrients, water, sunlight, humidity, and limited growing space (Naeem et al., 2022). The growth and survival of weeds are also influenced by the association between beneficial rhizobacteria and the plant root (Coskun et al., 2017). Bacteria alleviate plant stress, enhance nutrient uptake, and support plant growth by modulating phytohormone levels, especially auxin (Gontia-Mishra et al., 2016; Afzal et al., 2019; Uçar et al., 2023). Recent research reinforced an important contribution of short-range auxin transport of exogenous auxin conceivably from rhizobacteria (Korver et al., 2018). The auxin then gives rise to root plasticity, development, and elongation; hinders lateral shoot and abscission; enlarges xylem-phloem tissue; and inhibits most abiotic stresses, such as salinity and water deficit (Zhang et al., 2022).

A study of the rhizobacteria in river delta weed vegetation has never been done before. Thus, this study aimed to characterize rhizobacteria from the aforementioned deltaic weed vegetation, beginning with colony and cell morphology, biochemical and phytohormone analyses, and genotypic analysis of the notable performer. This study provides new evidence that optimal growth of delta vegetation was supported not only by the accumulation of organic matter but also by the presence of plant growth-promoting rhizobacteria.

2. Material and Methods

2.1. Soil sampling

Sampling of the delta vegetation rhizosphere was carried out downstream of the Kali Garang River, in Banjir Kanal Barat of Semarang City, Central Java, Indonesia, at coordinates 6°59'31.7148"S and 110°24'9.3672"E during April 2023. The location is the river's estuary mouth, with significant sedimentation. The temperature, moisture, and light intensity were measured on average. There are 5 rhizosphere plot samplings for each vegetation, determined by purposive sampling due to the occurrence of *Ageratum conyzoides*, *Galinsoga parviflora*, *Eleusine indica*, and *Kylinnga brevifolia*.

50 grams of soil was collected from the soil adjacent to each vegetation root of each plot and kept in a Falcon tube. Under aseptic conditions, samples from each vegetation type across all five plots were mixed and used for subsequent analyses. Hence, 4 soil samples were collected for each vegetation, with prior pH and nutrient analysis.

2.2. Isolation of Rhizobacteria

1 gram of soil sample mixture from each sample was put into a test tube containing 9 ml of 0.1% NaCl and vortexed. This solution was then diluted to 10⁻¹⁰ and inoculated into NA media containing nystatin using the spread plate method. The inoculants were incubated for 48 hours at 37 °C. Distinctive colonies were then regrown in NA medium by using the streak plate method and incubated at 37 °C for 24 hours to obtain refined isolates (Komaresofla et al., 2019).

2.3. Characterization of Rhizobacteria

Bacterial identification was performed using colony and cell characterization. The observed colonies' morphological features included margins, elevations, and shapes (Doh et al., 2019). The cell was described based on morphology and biochemical features. Cell morphology was Gram-negative, and endospore analysis included endospore formation, endospore morphology, and endospore germination (Chaiya et al., 2021). Biochemical assays included catalase, starch hydrolysis, plant nutrient provision, and plant growth hormone analysis (Chaiya et al., 2021). Finally, the colony, which produced abundant plant nutrients and plant growth hormone, was genotypically analyzed (AlAli et al., 2021).

2.3.1. Gram staining

Each isolate was fixed onto a sterile glass slide. The slides were successively soaked in crystal violet for 1 min, iodine solution for 1 min, decolorized acetone for 30 s, and safranin for 1 min. Each slide was observed under a microscope at 100x magnification by using immersion oil (Li et al., 2020).

2.3.2. Endospore staining

Each isolate was smeared onto a sterile glass slide. Each slide was alternately dipped in 0.5% Malachite green, heated until a little steam forms, and counterstained with safranin. Each sample was observed under a microscope at 100x magnification with immersion oil handling (Shen and Zhang, 2023).

2.3.3. Catalase test

The catalase test was performed by dropping 3% hydrogen peroxide onto bacterial colonies. A positive reaction was indicated by the presence of air bubbles. Aerobic bacteria tend to be catalase positive (Fedoruk et al., 2023).

2.3.4. Starch hydrolysis analysis

Starch hydrolysis was assessed on NA plates containing 10% starch, incubated at 37 °C for 24h. Thenceforth, iodine-KI was dropped onto the plate. A positive test result was indicated by an intense gap surrounding the colony, which showed starch-hydrolysis ability.

2.3.5. Plant nutrient provision

The most common plant nutrient requirements are nitrogen, phosphorus, and potassium. Thus, the analysis focused on these. Rhizobacteria's ability in atmospheric nitrogen fixation was determined by cultivating each colony in separated plates containing non-nitrogenous Jensen's medium at 37 °C for 72h. Rhizobacteria were confirmed to be positive for nitrogen fixation when emergence occurred (Denaya et al., 2021).

Rhizobacteria capable of mobilizing phosphate were categorized as phosphorus-solubilizing bacteria (PBS). The PBS test was performed by cultivating bacteria in Pikovskaya medium supplemented with tricalcium phosphate. The incubation was done at 37 °C for up to 7 days by inverting the agar medium. A positive test was recorded if a clear halo zone around the bacteria appeared (Pande et al., 2017).

Along with the PBS test, the potassium solubilized bacteria (KSB) test was performed using Aleksandrow medium containing insoluble mica powder as the potassium source. The incubation was performed at 37 °C for up to 15 days. A positive test was recorded if a clear halo zone around the bacteria appeared (Yaghoubi Khangahi, 2018).

2.3.6. Plant growth hormone analysis

Auxin analysis was done as follows. 5 mL of isolate with a cell count of 6×10^8 CFU/mL was added to a flask containing 50 mL of NB medium with 0.005 g tryptophan. The culture was then cultivated in a shaker incubator at 120 rpm, 300 °C for 96 hours. Auxin content was measured every 24 hours by seizing 2 ml of inoculum. This 2 ml inoculum was centrifuged at 10 000 rpm for 10 minutes to separate supernatant from bacterial cell pellets. 1 ml of supernatant was combined with 3 ml of Salkowski reagent and incubated in the dark for 30 minutes. A positive reaction was marked by red color development, indicating the presence of auxin. The auxin concentration was then determined based on the spectrophotometer absorbance at a wavelength of 530 nm. A standard curve was generated using indole-3-acetic acid from Merck over the range of 10 to 200 ppm (Gilbert et al., 2022). Alongside hormone analysis, the cell growth was also monitored at OD 600.

2.3.7. Genotype analysis

The genome of the determined colony was extracted by using the TaKaRa genomic DNA extraction kit as instructed in the user manual. The PCR was performed using universal primers 27F and 1492R to amplify 16S rRNA, with 30 cycles of amplification consisting of 94 °C denaturation for 45 s, 55 °C annealing for 60 s, and 72 °C extension for 60 s. The amplified DNA was then run on a 1% agarose gel prior to sequencing by Applied Biosystems. The sequence was compared to other 16S rRNAs in the GenBank database and phylogenetically analyzed using BLAST at NCBI.

3. Results

Table 1. pH and nutrient of each vegetation rhizosphere

Rhizosphere sample	pH	Nutrient		
		Nitrogen (ppm)	Phosphorus (ppm)	Potassium (ppm)
<i>A. conyzoides</i>	7.2 ± 0.36	165.33 ± 28.75 ^a	161.33 ± 19.55 ^a	162.33 ± 15.69 ^a
<i>G. parviflora</i>	7.5 ± 0.55	85.33 ± 5.51 ^b	90 ± 2.65 ^c	84.33 ± 10.97 ^c
<i>K. brevifolia</i>	7.0 ± 0.43	71.67 ± 4.73 ^b	60.67 ± 6.03 ^d	69.67 ± 10.79 ^d
<i>E. indica</i>	7.1 ± 0.35	162.00 ± 28.93 ^a	129.67 ± 16.19 ^b	127.33 ± 11.37 ^b

Distinct superscript letters adjacent to values indicate a statistically significant difference of mean at $p < 0.05$ levels.

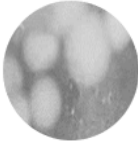
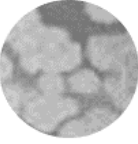
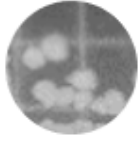
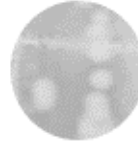
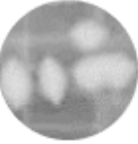
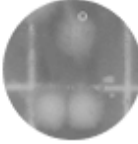
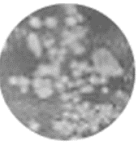
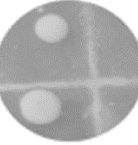
Bacteria live within river sediment. They are crucial for pollutant degradation, organic material mineralization, and biogeochemical processes of macronutrients such as nitrogen, phosphorus, and potassium (Ouyang et al., 2020). These communities tend to vary in response to chemical and physical changes, depending on environmental conditions (Obi et al., 2016). In view of that, the current experiment highlights the environment factor as an explanatory variable. The average temperature, moisture, and light intensity during sampling were 34 °C, 63.33%, and 72%, respectively. The chemical factors of the sediments are detailed in Table 1 above.

Table 2. Colonies morphology

Strain	Colony morphology		
	Shape	Margin	Elevation
AC1	Circular	Entire	Convex
AC2	Irregular	Undulate	Flat
AC3	Circular	Entire	Convex
GP1	Punctiform	Entire	Raised
GP2	Rhizoid	Undulate	Flat
GP3	Circular	Entire	Convex
KB1	Circular	Entire	Convex
KB2	Circular	Entire	Convex
KB3	Irregular	Undulate	Flat
KB4	Circular	Entire	Convex
EI1	Punctiform	Entire	Raised
EI2	Punctiform	Entire	Raised

Colony morphology determines the type of bacteria. Thus, isolates of each vegetation type were typified on the basis of colony morphology, including shape, edge, elevation, and texture, as shown in Tables 2 and 3. 12 distinct colonies were found in each vegetation rhizosphere, randomly distributed. In the *A. conyzoides* rhizosphere, 3 colonies were found: AC1, AC2, and AC3. *G. parviflora* rhizosphere is liable to have 3 colonies coded as GP1, GP2, and GP3. The results also detected 4 colonies in the *K. brevifolia* rhizosphere: KB1, KB2, KB3, and KB4. Meanwhile, only 2 isolates of *E. indica* rhizosphere were identified: EI1 and EI2.

Table 3. The appearance of the colonies

Strain	Appearance	Strain	Appearance	Strain	Appearance
AC1		GP2		KB3	
AC2		GP3		KB4	
AC3		KB1		EI1	
GP1		KB2		EI2	

Most of the colonies were circular or punctiform, with entire edges and convex elevations. A comparable rhizobial colony shape was identified in the soil of East Java, Indonesia, with potential for phosphate solubilization and auxin production (Astriani et al., 2020). Thus, the dominant colonies provide an estimate of the potency of the newly acquired colonies. Moreover, fewer colonies exhibited irregular rhizoid morphology with undulate edges, which were identified as fertilizer elicitors, such as those obtained with *Tephrosia candida* and *Oryza sativa* leaf litter (Ghosh and Tripathi, 2021) and with xenobiotic-contaminated soil (Maity et al., 2022). For that reason, these kinds of colonies conceivably support the breakdown of complex compounds as a source of plant nutrients, as well as bioremediation of the delta soil.

However, some of the circular colonies formed a slightly oval shape. Colony morphology is highly diverse, with shape a species characteristic that persists across generations. However, periodic variations can occur alongside cell division and life cycles, which are generally influenced by environmental conditions and serve self-defense, nutrient acquisition, distribution, interaction, and even stress resistance (van Teeseling et al., 2017). Thus, it can be an indicator of phenotypic variation as a bacterial adjustment to overcome environmental stress. One form of stress is the density of colonies on media plates. The denser the colony, the more fluctuating its morphogenesis becomes. Some changes observed include a reduction in size or a more irregular margin (Sousa et al., 2013). The colonies in the current experiment likely encountered a density problem, leading to shape adjustments.

Further identification was based on cell characteristics, including Gram stain, cell shape, and endospores, as summarized in Tables 4 and 5. Most of the bacteria in this experiment were Gram-positive bacilli. The cell wall is a peptidoglycan polymer with a complex scaffold glycan backbone, a loose, irregular sacculus, and large pores with a diameter of 1–10 nm (Turner et al., 2013; Kim et al.,

2015). Therefore, Gram-positive bacteria have thicker cell walls than Gram-negative bacteria. In contrast, Gram-negative bacteria impede the permeation of the crystal violet dye, resulting in the absence of a bluish color (Schneewind and Missiakas, 2014).

Gram-positive bacteria exhibit a thick exoskeleton, which determines cell shape and protects the cytoplasmic membrane while resisting intracellular turgor pressure. The cell wall forms a matrix containing polysaccharides and proteins, such as teichoic acid, which functions in metal cation chelation, substrate adhesion, and protection against harmful substances (Poxton, 2015). Apart from these original benefits, Gram-positive bacteria also have the potential to support plant growth, namely by protecting plants from pathogens and promoting plant growth, specifically by enhancing nutrient and hormone uptake (Etesami and Alikhani, 2017; Fan et al., 2018; Ek-Ramos et al., 2019).

Only 2 colonies found in *K. brevifolia* and *E. indica* rhizospheres, i.e., KB2 and EI2, were Gram-negative bacilli, which were obtained at the vegetative cell stage, so contained no endospore. The other cells encountered in the vegetative stage were Gram-positive, including colonies of *A. conyzoides* (EI1 of *E. indica*), GP1 and GP2 of *G. parviflora*, and KB3 of *K. brevifolia*. Despite this, some Gram-positive bacteria had oval endospores at their centers (Priyani et al., 2023). Endospores are resistant bodies formed by bacterial cells to enable survival under conditions of competition or extreme environmental changes, for example, when nutrients are scarce (Soni and Keharia, 2021). Endospores are inanimate objects that are very resistant to heat, ultraviolet radiation, and even toxic chemicals (Lv et al., 2021). Gram-positive genera were eminent as endospore formers, such as members of the *Bacillus* and *Clostridium*. Only a few Gram-negative bacteria generate an endospore, such as *Sporomusa*, or an exospore, such as *Myxobacteria* (Abel-Santos, 2015).

Table 4. Cell morphology

Strain	Cell morphology		
	Gram	Cell shape	Endospore
AC1	Positive	Bacil	Vegetative cell only
AC2	Positive	Bacil	Vegetative cell only
AC3	Positive	Bacil	Vegetative cell only
GP1	Positive	Bacil	Vegetative cell only
GP2	Positive	Bacil	Vegetative cell only
GP3	Positive	Bacil	Oval, central
KB1	Positive	Bacil	Oval, central
KB2	Negative	Bacil	Vegetative cell only
KB3	Positive	Bacil	Vegetative cell only
KB4	Positive	Bacil	Oval, central
EI1	Positive	Bacil	Vegetative cell only
EI2	Negative	Bacil	Vegetative cell only

In addition to the colonies and cell morphology, a biochemical assay was performed using catalase, starch hydrolysis, and plant nutrient provision; the results are summarized in Table 6. In Table 6, it was noted that all colonies exhibit catalase activity and are therefore likely aerobic or facultatively aerobic microbes. Hydrogen peroxide is toxic due to enzyme inactivation and cell lysis (Li and Imlay, 2018). Microbes produce catalase to decompose hydrogen peroxide into water and oxygen, thereby reducing its bactericidal effect and protecting against oxidative damage (Kaushal et al., 2018). Although the oxygen product of catalase is a growth-limiting factor for some organisms, it also serves as a beneficial respiration substrate. Therefore, the enzyme serves as the indicator of aerobic or facultative aerobic microorganisms (Kaushal et al., 2018).

As shown in Tables 2-6, Gram-negative and punctiform bacteria tend to inhibit starch hydrolysis. Starch assay is commonly executed to distinguish *Bacillus* and *Clostridium*, in which α -amylase or oligo-1,6-glucosidase are activated (Janeček et al., 2014). Both genera are typical rhizobacteria with plant growth-promoting characteristics. Some *Clostridium* species lack starch-hydrolyzing enzymes but possess β -glucosidase (Yu et al., 2015). Aside from *Bacillus*, amylase is mainly utilized (Simair et al., 2017; Güney et al., 2025).

Table 5. The appearance of the cell

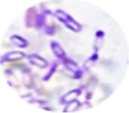
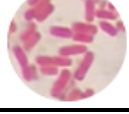
Strain	Appearance	Strain	Appearance	Strain	Appearance
AC1		GP2		KB3	
AC2		GP3		KB4	
AC3		KB1		EI1	
GP1		KB2		EI2	

Table 6. Biochemical characteristics of rhizobacteria colonies

Strain	Catalase	Starch hydrolysis	Plant Nutrient Provision		
			N fixation	P solubilization	K solubilization
AC1	+	-	+	-	-
AC2	+	+	+	+	+
AC3	+	+	+	+	+
GP1	+	-	+	-	-
GP2	+	-	+	-	+
GP3	+	-	+	-	-
KB1	+	+	+	+	+
KB2	+	-	+	-	+
KB3	+	+	+	+	+
KB4	+	+	+	+	+
EI1	+	-	+	-	-
EI2	+	-	+	+	+

(+) denotes positive and (-) denotes negative analysis.

Eventually, plant nutrient provision was variable enough that only bacteria capable of nitrogen fixation remained, leaving out phosphorus and/or potassium solubilization. The types of rhizobacteria were bacilli. *Bacillus* has been well known as one of the nitrogen-fixing bacteria with potential for application as a biofertilizer in agricultural management (Ezrari et al., 2021). Glutamine, the main amino acid produced by plants, plays an important role in nitrogen utilization by *Bacillus*. Therefore, there is a mutualistic relationship between the two, involving specific regulation via transcription factor engineering (He et al., 2023). Meanwhile, some *Bacillus* species were incapable of phosphorus solubilization, such as *B. megaterium*, due to the absence of propionic acid during the metabolism (Saeid et al., 2018). Similarly, potassium solubilization was possessed only by certain *Bacillus* species. Bacteria with such hormones are preserved around the plant, dissolving the surrounding minerals, such as mica and feldspar, and converting them into ionic potassium (Bagyalakshmi et al., 2017).

Afterward, bacterial growth and the emitted hormone were assessed to obtain the primary benefits. Figure 1 depicts the growth curves of each colony. All isolates entered the lag phase at incubation times of 0-12 hours. During this phase, the bacterial population density was low because of environmental adaptation. A logarithmic phase occurred at incubation times of 24 to 36 or 48 hours. At this phase, bacterial cells undergo rapid, constant growth, doubling the population (Maier et al., 2015). Finally, bacteria entered an initial stationary phase, indicated by persistent growth despite many stress factors, such as nutrient depletion, pH alteration, solute content, or by-product accumulation (Jaishankar

and Srivastava, 2017). One of these by-products is a phytohormone, which is part of a defense mechanism (Gilbert et al., 2022). During the 84-96 hour incubation period, bacterial population density decreases, indicating a bacterial collapse phase due to stress accumulation (Maier et al., 2015).

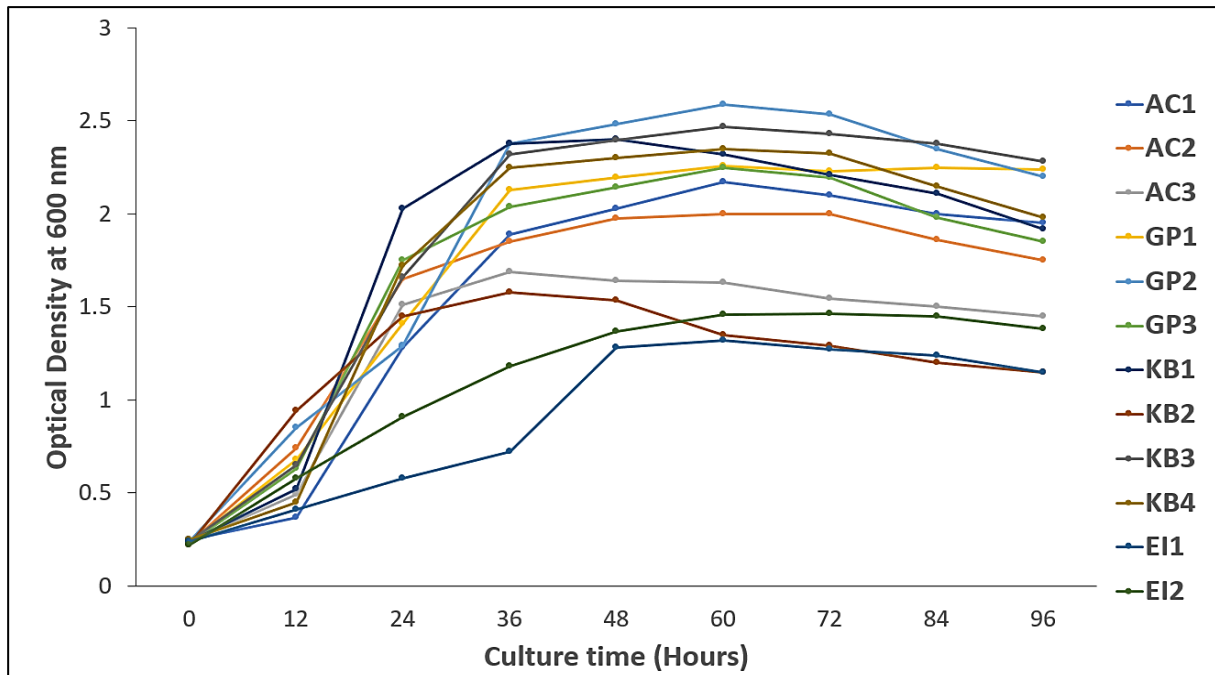


Figure 1. The growth curve of each vegetation isolates.

Every 24 hours, a growth evaluation was performed, followed by auxin analysis. The detection of each was positively indicated. Thus, a further quantification was performed, and the results were summarized in Figure 2.

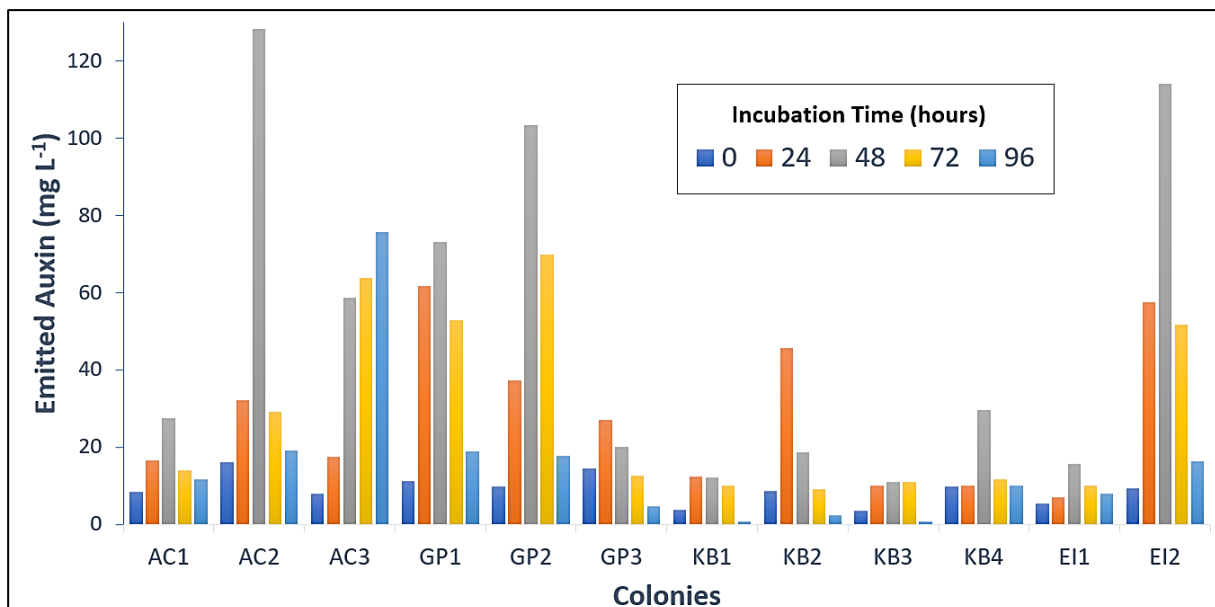


Figure 2. The amount of emitted auxin in every 24 hours of each colony.

Some isolates achieve optimal auxin production during the logarithmic phase at 24 hours of incubation, such as GP3, KB1, and KB2. GP3 and KB1 were Gram-positive bacteria, presumably growing rapidly due to endospore formation. KB2 was Gram-negative, lacked endospores, and had almost identical growth patterns. However, most of the others showed an increase in auxin production

at 48 hours of incubation, namely AC1, AC2, GP1, GP2, KB4, EI1, and EI, which were dominated by irregular, punctiform, or rhizoid-shaped Gram-positive bacteria with no endospores. Meanwhile, only AC3 produced optimal auxin after 96 hours of incubation.

Each bacterium exhibits growth variation due to several factors, specifically the wide range of genomes among species (Glick, 2018). Endospores imply dormant bacteria; likewise, low metabolism. Endospores form in response to prolonged stress. On the other hand, some bacteria survive stressful conditions without forming endospores but instead enhance secondary metabolite production, as in the case of auxin. Therefore, when the medium was cell-saturated, equivalent to the stationary phase, the cells encountered stress and then initiated auxin production (Zhang et al., 2022). After the bacteria could no longer endure the stress, the endospore formed. The earlier endospore formation, the sooner auxin production begins.

The L-tryptophan supplement suggested that the obtained bacteria synthesized auxin via a tryptophan-dependent biosynthetic pathway (P. Zhang et al., 2019). Tryptophan-dependent biosynthesis pathways are mostly established as indole-3-acetamide (IAM), indole-3-pyruvic acid (IPyA), and indole-3-acetonitrile (IAN) pathways (Keswani et al., 2020). The IAM pathway is catalyzed by tryptophan monooxygenase enzyme (Di et al., 2016). The IAN pathway is catalyzed by nitrile hydratase and indole-3-acetamide hydrolase. The IPyA pathway is catalyzed by tryptophan aminotransferase enzyme (Sun et al., 2022).

Based on Table 6 and Figure 2, the AC2 isolate achieved full biochemical competence and the highest auxin enhancement during a 48 h incubation without endospores. The result suggests that the isolate tends to regulate growth in a regular rhythm, so when stress occurs, the bacteria can slowly release auxin to the highest level without forming an endospore. Therefore, the isolate was subjected to genotype analysis. The PCR amplification of the AC2 genome yielded a 1500 bp fragment of 16S rRNA.

The 16S rRNA segment was sequenced to construct a phylogenetic tree using neighbor-joining. The sequence homology revealed 99.12% identity of the AC2 isolate to some *Bacillus* sp., especially *B. cereus*. Comparative analysis discovered that AC2 was closely related to other members of the Firmicutes phylum. The results suggest relationships between the isolated strain and some *B. cereus* strains, 2.3AL8, CUMB NS 07, and LSB24, and, typically, strain JUB1, as shown in Figure 3. Therefore, it is assumed that AC2 is *B. cereus*.

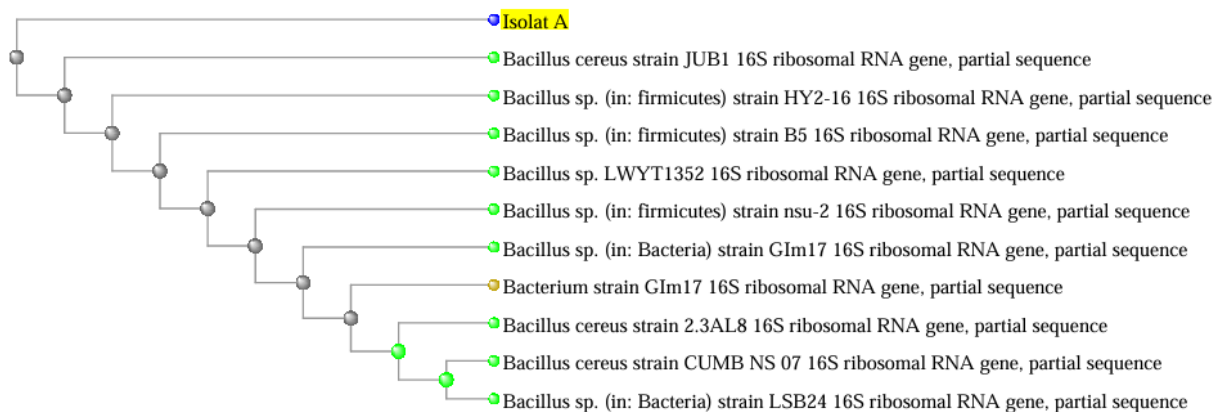


Figure 3. Phylogenetic Tree by Blast NCBI of isolate AC2 (Abbreviated isolat A).

The phylogenetic analysis was confirmed by the morphological and biochemical analysis of AC2. The isolate identification was consistent with another study on *B. cereus* colony characteristics, which described Gram-positive, bacil/rod-shaped, time-consuming sporulation bacteria with fitness for starch hydrolysis and catalase (Das et al., 2021; Elshikh, 2022). The nitrogen fixation analysis also confirmed findings from research on some tropical estuary bacillus nitrogenase activities, in which both *B. cereus* strains showed moderate *nifH* gene expression, higher than in *B. aerophilus*, *B. subterraneus*, and *B. licheniformis* (Yousuf et al., 2017). Comparable research on phosphorus solubilization reported that *B. cereus*, *B. subtilis*, and *B. megaterium* are proficient in utilizing bound phosphorus pentoxide (Saeid et al., 2018). *B. cereus* is also prominent as a potassium-solubilizing bacterium, which co-cultivated to overcome plant nutrient deficiency, insufficient metabolism, water deficit, lack of phloem

transport, low crop quality, and high stress of pests and disease (Verma et al., 2017). Lastly, the *B. cereus* genome holds an indole-3-pyruvate decarboxylase gene (*ipdC*). In the challenge of indole-3-pyruvic acid in tryptophan catabolism, the enzyme catalyzes the conversion of indole-3-pyruvate (IPyA) to indole-3-acetaldehyde (IAAId), which is subsequently transformed to IAA (Duca and Glick, 2020). This confirmed that AC2 auxin metabolism in *B. cereus* exploits the tryptophan-dependent indole-3-acetic acid biosynthesis pathway.

Conclusion

The study identified 12 distinct colonies: 3 from the *A. conyzoides* rhizosphere sample, 3 from the *G. parviflora* rhizosphere sample, 4 from the *K. brevifolia* rhizosphere sample, and 2 from the *E. indica* rhizosphere sample. The colonies were mostly Gram-positive bacil bacteria. The variation of edge and elevation was in the range of circular or punctiform form, entire edge, and convex elevation. The bacteria have the potential for catalase, starch hydrolysis, nitrogen fixation, phosphate solubilization, potassium solubilization, and auxin production. AC2, as one of the *A. conyzoides* isolates, demonstrated full biochemical competence and the highest auxin enhancement during 48 h of incubation without endospores. The sequence homology and phylogenetic tree analyses of the 16S rRNA gene suggested that the colony was *B. cereus*. The isolate characterization was in line with other research on *B. cereus* colony features and the tryptophan-dependent indole-3-acetic acid biosynthesis pathway. The result showed that the immense growth of delta vegetation was not only maintained by the abundance of organic substances but also by the presence of rhizobacteria with plant growth-promoting traits.

Ethical Statement

Ethical approval is not required for this study because it did not involve human or animal subjects, nor the collection of plants or bacteria from protected or restricted areas.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Artificial Intelligence Declaration

The authors declare that no generative artificial intelligence tools were used at any stage of the preparation of this manuscript, including the writing, editing, or refinement of the text, or the creation of any images, figures, graphics, tables, or related titles.

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Author Contributions

Talitha Widiatningrum contributed to the conceptualization of the study, supervised the research process, and designed the data analysis methodology. Lina Herlina contributed to the interpretation and validation of the research results. Kiki Rahma Aprilia contributed to data acquisition and data analysis. Fadhlika Nurhidayah contributed to data acquisition and data analysis. Fita Fatimah Hartanti contributed to data acquisition and data analysis. Silvia Dewi Ula Fatma contributed to data acquisition and data analysis. All authors read and approved the final manuscript.

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