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Exploration and Screening of Clove Plant Growth Promoting Rhizobacteria in Pucaksari Soil

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ABSTRACT: PGPR are soil bacteria in the plant root zone (rhizosphere) that provide nutrients for plant growth, plant development, and plant protection against certain pathogens. The use of PGPR in Indonesia, especially in Pucaksari Village, Buleleng Regency as a biostimulant and its application to clove plants to increase agricultural production is still very rarely studied. The purpose of this study was to find the presence of PGPR rhizosphere bacteria in clove plants in the root zone of the Pucaksari Buleleng plantation that have the ability to fix nitrogen, produce IAA and dissolve phosphate. The method of data analysis of characteristics and bioactivity tests of PGPR was compiled and analyzed descriptively. Isolation was carried out by dilution using clove plant rhizosphere soil samples. Phosphate solubilization ability was carried out using Pikovskaya media and calculating the Phosphate Solubility Index. Detection of nitrogen fixation was tested using Ashby's Mannitol Agar growth media. IAA production was qualitatively tested using tryptic soy broth enriched with tryptophan and Salkowsky's reagent. Three isolates (BRC01, BRC02, and BRC03) were isolated from the rhizosphere of clove plants. All isolates could solubilize phosphate and produce IAA. However, only one isolate successfully fixed nitrogen, as seen from its growth on Ashby's medium. Isolate BRC02 had the highest phosphate solubilization with an IKF of 1.86 and produced 14.71 ppm of IAA. This bacterium was identified as Klebsiella aerogenes, which has potential as a biostimulant agent.

KEYWORDS: PGPR, phosphate solubilization, nitrogen fixation, IAA

I. INTRODUCTION

Cloves are one of the most popular commodities in Buleleng Regency, Bali. Dried clove flowers produced in Buleleng as the main raw materials for kretek cigarettes, a signature Indonesian cigarette. However, clove production in Buleleng Regency has massively decreased over the past thirty years because farmers' interest in cultivating clove plants reduced due to unstable weather and limited water availability, which impact soil fertility.

One strategy for improving soil organic matter availability is to combine the use of PGPR (*Plant Growth Promoting Rhizobacteria*) with organic fertilizers. These biological agents have a direct effect by providing and mobilizing the absorption of various nutrients and altering the concentration of growth-promoting phytohormones. Indirectly, PGPR can increase the resistance of cultivated plants by producing metabolites with antibiotic properties.

Some of the roles of PGPR include synthesizing plant growth regulators such as IAA (Indole-3-acetic acid) and acting as nitrogen (N) and phosphorus (P) suppliers, thus enhancing resistance to certain diseases. The use of PGPR as biostimulants and bioprotectants has been widely developed in agriculture. This research will explore the existence of clove rhizosphere bacteria in Pucaksari Village, Buleleng Regency, and test the capability of the bacteria found to produce IAA, phosphate solubilization, and fix nitrogen.

II. METHODS

Rhizosphere Bacteria Isolation

Twenty-five grams of soil samples were suspended in 225 mL of 0.85% NaCl solution and shaken on a rotary shaker for 60 minutes at 27°C 120 rpm. The samples were diluted and transferred to Tryptic Soy Agar (TSA) media containing 200 mg L^{-1} L-tryptophan for the production of Indole-3 acetic acid (IAA) agar media, and Pikovskaya (PVK) for the phosphate solubilization test containing 10 g Glucose; 0.2 grams NaCl; 2.5 grams Ca_3PO_4; 0.2 grams KCl; 0.1 grams $MgSO_4$. $7H_2O$; 0.025 grams $MnSO_4$; 0.025 grams $FeSO_4$. $7H_2O$; 0.5 grams $(NH_4)_2SO$; 1 g Yeast Extract; and 15 g Bacto Agar in 1000 mL Aquades. Ashby's Mannitol Agar was used for testing

nitrogen-fixing bacteria containing K_2HPO_4 0,2 g; MgSO4. $7H_2O$ 0,2 gram; NaCl 0,2 g; K_2SO_4 0,01g; $CaCO_3$ 5g; *Agar* 15 g in 1000 mL Aquades. Total plate count (TPC) was performed on each growth medium to determine the number of cells of each bacterial isolate.

Isolate Bio-activity Test

IAA production test was conducted by testing bacteria using Tryptic Soy Broth (TSB) and Salkowski (1.05 g $FeCl_3.6H_2O$; 60 mL absolute H_2SO_4; 100 mL distilled water). The procedure for selecting auxin-producing bacterial isolates was by using TSB added with 0.1 g of L-tryptophan. Pure isolates were placed in Erlenmeyer flasks containing liquid TSB media. Then, the Erlenmeyer flasks were incubated on a shaker at 100 rpm for six days at room temperature until the bacterial isolates grew on the media. The color of the growing bacterial isolates would become cloudy, then 5 mL was taken with a micropipette and transferred into a microtube and centrifuged for 30 minutes at 7000 rpm. Next, one mL of supernatant was taken and added with 4 mL of Salkowski reagent. The supernatant was incubated for 24 hours and the color changes observed. If a color change occurs and the color of the sample isolate turns pink, this indicates that the isolate can produce IAA (Pattern & Glick 2002). The intensity of the pink color indicating positive IAA production was measured using a UV-VIS spectrophotometer at a wavelength of 515,5 nm. From a standard curve made with a known concentration of IAA, the quantity of IAA in the culture was determined and expressed as $mg.mL^{-1}$.

Phosphate solubilization test was performed by isolating bacterial colonies by serial dilution and spread plate method. Approximately 2 g of soil sample was dispersed in 9 mL of sterile distilled water and vortexed thoroughly. Subsequent serial dilutions of this stock were performed to obtain 10 8 dilutions and 100 µL of each dilution was poured into nutrient agar medium. To isolate phosphate solubilizing bacteria, each colony was taken and inoculated onto Pikovskaya agar (PVK) medium (Goenadi *et al.,* 2000) containing insoluble tricalcium phosphate and incubated at 28 °C for 7 days for bacterial growth. The presence of a clear zone around the bacterial colony after 2 days of incubation was used as an indicator of positive phosphate solubilization (Husen, 2003). Pure cultures of PSB were inoculated into PVK medium and incubated at 28°C for 48-72 hours to detect their phosphate solubilization efficacy (Sharma *et al.,* 2012). Phosphate solubility index (SI) was calculated for all potential phosphate solubilizers and pure cultures were analyzed for colony morphology and further evaluation was carried out.

Nitrogen fixation test was conducted by adding one gram of each soil sample into a 250 ml Erlenmeyer flask containing 100 ml of Ashby's medium (Mannitol, 20 g; K_2HPO_4, 0.2 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.2 g; K_2SO_4, 0.1 g and $CaCO_3$, 0.5 g. distilled water, 1000 ml (Subbarao, 2015) then incubated for 14 days at 27°C. One mL of liquid medium was taken aseptically then diluted to a dilution of 10-3 then inoculated on solid Ashby's with the spread plate method then incubated for 7 days at 27°C. Separate colonies were purified three times by the streak method. Pure colonies that grew with different morphological appearances were then characterized morphologically. Isolates that have been characterized then stored in agar slant for further analysis needs.

PGPR Identification through DNA Screening

PGPR isolates were grown in Tryptic Soy Broth media (casein peptone: 17 g, soy peptone: 3 g, NaCl: 5 g, 2.5 g K_2HPO_4: 2.5 g, glucose: 2.5 g and sterile water to make 1 liter) and incubated in an orbital shaker in the dark at room temperature (28+2°C) for 24 hours. DNA extraction and purification were carried out using the Gene JET Genomic DNA Purification Kit procedure (Thermo Fisher Scientific Inc., USA). Two primers, namely 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3') were used to amplify the 16S rRNA gene. The reaction was carried out in a GeneAmp® PCR system 2700 (Applied Biosystem, California, USA) with the following conditions: pre-denaturation at 94°C (2 minutes 2 seconds) followed by 30 cycles of denaturation at 94°C (15 seconds), annealing at 50°C (30 seconds), extension at 72°C (1 minute 30 seconds), and final extension at 72°C for 10 minutes. Purified KtB3 DNA was sequenced using a Genetic Analyzer (Applied Biosystem ABI PRISM310, California, USA). The sequences were aligned to GenBank using the BLAST-N program from the National Center for Biotechnology Information (NCBI) to determine the similarity of the test isolates with other previously identified isolates. Phylogenetic analysis was performed using the neighbor-joining method MEGA version 6.0 with 1000x bootstrap. Phylogenetic trees were developed using TreeGraph 2.0.

III. RESULTS

A. Isolation of Clove Rhizosphere Bacteria in Pucaksari Village

Based on table 1, there were three isolates found in the clove rhizosphere of Pucaksari Village, Buleleng (BRC01, BRC02 and BRC03). The results of the Total Plate Count (TPC) calculation on the three isolates showed different numbers on each growth medium. Isolate BRC01 on Pikovskaya media showed $17x10^3$ CFU/mL, while on Ashby's Mannitol Agar media it did not grow successfully. Isolate BRC02 on both Pikovskaya and Ashby's Mannitol Agar media was able to grow with the highest colony count of $30x10^3$ on Pikovskaya media and $77x10^3$ on Ashby's Mannitol Agar media. Isolate BRC03 showed $15x10^3$ on Pikovskaya media

and did not grow on Ashby's Mannitol Agar media. Based on the data from the TPC test results of clove rhizosphere bacteria, it can be concluded that isolate BRC02 is the selected isolate for further research by identification through DNA screening.

Table 1. Total Plate Count (TPC) Value of Clove Rhizosphere Isolates in Pucaksari

No.	Isolate Code	PSB on Media Pikovskaya (CFU/mL)	NFB on Media Ashby's Mannitol Agar (CFU/mL)
1.	BRC01	$17x10^3$	-
2.	BRC02	$30x10^3$	$77x10^3$
3.	BRC02	$15x10^3$	-

B. IAA Production Results of Clove PGPR in Pucaksari Village, Buleleng

Qualitative measurement of IAA concentration is indicated by a color change in bacterial isolate cultures. Samples showing a positive reaction will turn pink. Figure 1 shows a faint pink color in the isolate, indicating its ability to produce IAA. Sukmawati *et al.,* 2021, stated that the color formed from a positive reaction indicates the presence of various indole compounds, products of tryptophan metabolism.

Figure 1. Qualitative Measurement of IAA Concentration of Clove Rhizosphere Bacterial Isolates

Absorbance measurements were performed using spectrophotometry at a wavelength of 515.5 nm. The regression equation is shown in Figure 2, where y = 0.0342x + 0.0466, and the regression value is 0.8944. The sample absorbance measurement result, 0.55, was used as the y value and was calculated into the regression equation to find the x value. The x value represents the IAA concentration produced by the sample. The isolate sample measured content of 14.71 ppm.

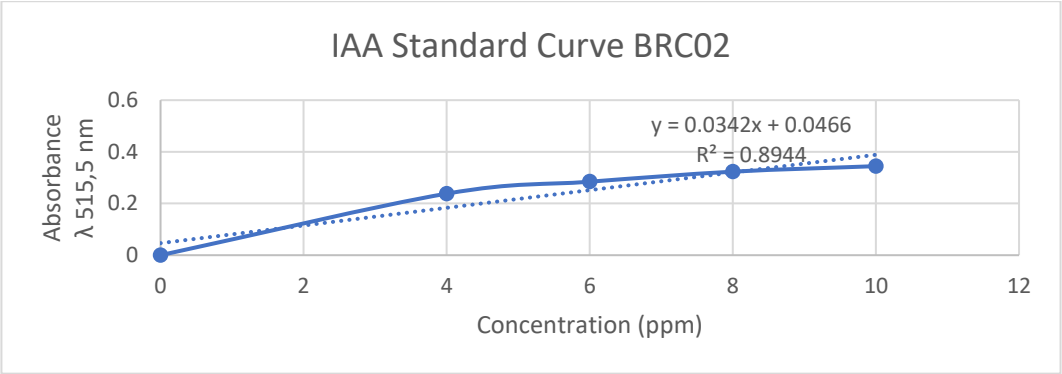

Figure 2. IAA Standard Curve of Clove Isolates from Pucaksari Village

C. Results of Phosphate Solubilization of Clove PGPR in Pucaksari Village, Buleleng

Ruwandani *et al.,* (2014) categorized the ability of bacteria to dissolve phosphate based on the phosphate solubility index (PSI) into four categories: low (PSI ≤ 1.59), moderate (PSI 1.6 - 2), high (PSI 2.15 - 2.59), and very high (PSI 2.6 - 3).

Based on Table 2, the phosphate solubility index of phosphate-solubilizing bacteria shows that isolate BRC02 has the highest phosphate solubility index, at 1.86, which falls into the moderate category. This is followed by isolate BRC01, which has a

phosphate solubility index of 1.84, with the largest colony diameter and clear zone of the three isolates, also categorized as moderate. Isolate BRC03 has the lowest phosphate solubility index, at 1.5, which falls into the low category.

Table 2. Phosphate Solubilitazion Index Isolate

Isolate Code	Colony Diameter (mm)	Clear Zone (mm)	Phosphate Solubilization Index
BRC01	19	16	1,84
BRC02	15	13	1,86
BRC03	10	5	1,5

D. Nitrogen Fixation Results of Clove PGPR in Pucaksari Village, Buleleng

Based on the qualitative nitrogen fixation test, on Ashby's Mannitol Agar media, 1 out of 3 isolates was able to grow on the media (Table 3). This proves that the BRC02 isolate has the potential to fix nitrogen. Islam *et al.,* (2019) reported that 18 isolates of non-symbiotic diazotrophic bacteria obtained from oil palm plantations were each able to grow on liquid NFB media and Ashby's Mannitol Broth media as an indicator, from these results, 3 isolates had high potential in fixing nitrogen.

Table 3. Uji Fiksasi Nitrogen pada Media *Ashby's Mannitol Agar*

Isolate Code	Nitrogen Fixing Bacteria Result
BRC01	Negative
BRC02	Positive
BRC03	Negative

E. Results of PGPR Isolate Identification through DNA Screening

Isolate BRC02 was selected as a potential isolate for molecular identification using the 16S rRNA gene because it can produce IAA, has the highest Phosphate Solubility Index (IKF), and can fix nitrogen. The BRC02 isolate DNA was then subjected to molecular identification using the universal primer 63F to amplify the 16S rRNA gene. The amplified DNA was then analyzed by 0.8% agarose gel electrophoresis to determine the DNA size.

BLAST analysis of the BRC02 isolate showed 99.86% homology with Klebsiella aerogenes, while the results of constructing a phylogenetic tree of the BRC02 isolate using the MEGA X program and the Neighbor Joining Tree method with 1,000 replications of the bootstrap test indicated that the bacterial isolate was aligned with Klebsiella aerogenes strain 18-2341 with the same bootstrap value of 91%.

The identification results based on BLAST homology and phylogenetic tree reconstruction stated that isolate BRC02 has similarities with Klebsiella aerogenes which has the characteristics of rod-shaped colonies, flat edges, convex elevations, white color and is a type of Gram-negative bacteria in the form of a bacillus.

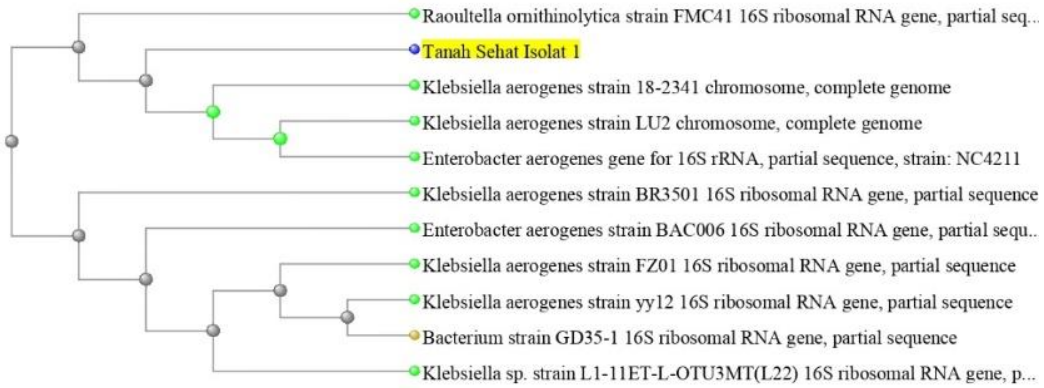

Figure 3. Phylogenetic Tree of Isolate BRC02

IV. DISCUSSION

Based on its classification, *Klebsiella aerogenes* belongs to the genus Klebsiella, formerly known as Enterobacter aerogenes. This bacterium has undergone various modifications. The term *Klebsiella pneumoniae* is used for the entire species, which is further divided into four subspecies: *K. pneumoniae* subsp. *aerogenes*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *pneumoniae*, and *K. pneumoniae* subsp. *rhinoscleromatis*. Indole-producing strains that resemble *K. pneumoniae* subsp. *aerogenes* are biochemically classified as a separate species, *K. oxytoca* (Kumar, 2022).

Morphologically, Klebsiella bacteria are straight rod-shaped with rounded or slightly pointed ends, encapsulated, and can be found singly, in pairs, or in short chains. The diplobacillary form is commonly found in vivo. These bacteria measure 1–2 µm in length and 0.5–0.8 µm in width, with parallel or protruding sides. *K. aerogenes* is a gram-negative bacterium and a facultative anaerobe species that can produce citrate and glucose as absorbable carbon sources and ammonia as a nitrogen source (Cassan *et al.,* 2020). Klebsiella can grow well on standard media between 12°C and 43°C, but optimal growth is achieved when cultured at 37°C for an incubation period of 18–24 hours. Colonies on MacConkey's agar typically appear large, slimy, and red. The slimy nature of the colonies is due to the capsule produced by the organism. This capsule is a protective layer that covers bacterial cells and is composed of polysaccharides or proteins, giving the colonies a wet and slippery appearance. The capsule functions to develop the immune system and enhance the bacteria's ability to survive in extreme environmental conditions (Sydow *et al.,* 2022).

Several researchers have reported the activity of Klebsiella found in the root zone of plants. Chaudhary *et al.,* (2021) in their research revealed that many species of bacteria and fungi were found to have the ability to dissolve inorganic phosphate, known as phosphate-solubilizing bacteria, such as: Arthrobacter, Bacillus, Delftia, Gordonia, Phyllobacterium, Pseudomonas, Rhodococcus and Serratia sp., Xanthomonas, Chryseobacterium, Xanthobacter agilis, Vibrio proteolyticus, Azotobacter, Enterobacter, Pantoea, Klebsiella, *Pseodomonas* sp., and *Rhizobium leguminosarum*. Phosphorus is the second essential element required by plants after nitrogen. Phosphate is needed in various processes such as photosynthesis, carbon absorption, membrane formation, energy transfer, signal transduction, disease resistance capacity, and seed formation (Lebrazi *et al.,* 2020). The P content available in the soil cannot be utilized by plants because its fixation is very complex and insoluble.

Approximately 90–95% of phosphate is present in insoluble, immobile, and sedimentary forms (Cabugao *et al.,* 2017). Phosphorus is an essential element for plant development and growth. Plants utilize orthophosphate, a form of phosphorus, by synthesizing siderophores, hydroxyl ions, organic acids, carbon dioxide, and protons. Microbes play a crucial role in converting insoluble phosphorus into plant-accessible orthophosphate. The limited availability of soluble or inorganic phosphorus, particularly orthophosphate, in the soil significantly impacts crop yields, leading to reduced agricultural productivity (Wang *et al.,* 2017).

Oksana *et al.,* (2020) in their research identified bacteria of the genus Klebsiella in Ultisol soil isolated from Rumbai District, Pekanbaru. In this study, Klebsiella obtained the highest phosphate solubility index value among the 4 isolates found. Biochemically, Klebsiella tested positive for catalase, negative for oxidase, positive for fermentation, TSIA showed a yellow color, negative for motility, positive for glucose, positive for lactose, positive for mannitol, positive for maltose and positive for sucrose. In the study by Chuong *et al.,* (2024) found bacteria of the genus Klebsiella isolated from the mangrove rhizosphere that could affect the growth of corn plants by 27.7 cm but did not significantly affect root length, number of leaves, fresh weight or dry weight of roots.

Walpola *et al.,* (2014) revealed that Klebsiella oxycota, the closest relative of *K. aerogenes*, was proven to be able to dissolve phosphate in a wide pH range (4-10), temperature (20 to 40 °C) and salt concentration of 0 - 7.5% NaCl). As reviewed from the results of plant growth tests on the activity of 1-aminocyclo-propane-1 carboxylic acid (ACC) deaminase and the production of ammonia, hydrogen cyanide (HCN) and acetic acid (IAA), the strain was proven to be a good plant growth promoter, which was further confirmed by the increase in growth of mung bean seedlings inoculated with the strain (31.88 and 45.53%) higher shoot and root length compared to the uninoculated control. Based on these results, the *K. oxycota* strain can be identified as an ideal candidate for inclusion in the development of microbial inoculants suitable for stressful environments. *Gupta et al.,* (2024) examined the three best isolates from the rhizosphere of various drought-exposed plants and identified three strains: *Enterobacter cloacae, Serratia marcescens*, and *Klebsiella aerogenes*, selected based on their ability to produce EPS, IAA, ammonia, and good siderophores, as well as nutrient solubilizers (K, Zn, P). These isolates were used for biopriming Avena sativa seeds, which were then exposed to water stress (5 days) in a completely randomized design to examine the effect of bacteria on plant growth.

Water stress caused significant changes in growth, protein, and chlorophyll content of *Avena sativa*. However, biopriming improved the physiological condition of oat plants by increasing osmolytes (proline, phenols, and sugars) under water-stressed conditions. Furthermore, *K. aerogenes* exhibited substantial phosphate-solubilizing activity, as evidenced by significant clear zones in in vitro screening. This activity was directly related to increased growth. It can be concluded that *K. aerogenes* is the best isolate as an efficient plant growth promoter in water-deficient soil.

Coutinho *et al.,* (2024) explored the growth-promoting potential of rhizosphere bacteria in wheat under high salinity and drought conditions. Pot tests showed that coating wheat seeds with *K. aerogenes* successfully had a positive impact on plant parameters under salt stress, with increases of 2.4-2.6-fold, 1.3-1.6-fold, and 1.4-1.8-fold observed in root length, shoot length, and fresh weight of inoculated plants. In the drought-tolerant isolate producing IAA, there was no increase in shoot length or root length. However, a 1-2-fold increase was observed in the fresh weight of inoculated plants compared to the control under drought conditions.

Nitrogen is essential for plant growth. Mineral fertilizer and nitrogen fixation occur with the help of several microorganisms and are essential for plant growth in agricultural soils (Grzyb *et al.,* 2021). The role of microbes in the N cycle is crucial. Bacteria possess a key enzyme, nitrogenase, which is responsible for exclusively fixing atmospheric N, both symbiotically and asymbiotically (Mahmud *et al.,* 2020). Soil N availability and supply can be improved in sustainable agriculture by using several microbial inoculants such as PGPR, rhizobia, and arbuscular mycorrhizal fungi (Tyagi *et al.,* 2022).

Nitrogen assimilation by plants is the most limiting factor in plant growth (Courty *et al.,* 2015). Nitrogen (78%) present in the atmosphere remains unavailable to plants. No plants are known to be able to fix atmospheric nitrogen into a usable form. However, biological nitrogen fixation is known to convert nitrogen to ammonia by the nitrogen-fixing enzyme nitrogenase. This enzyme complex consists of two components, dinitrogenase reductase, an iron protein, and dinitrogenase, a metal cofactor. The first component provides electrons, and the second utilizes electrons to reduce nitrogen to ammonia. Mo-nitrogenase, V-nitrogenase, and Fe-nitrogenase have been identified as key metal cofactors.

Symbiotic and non-symbiotic associations lead to nitrogen fixation with the help of nitrogen-fixing organisms. Azospirillum, Gluconacetobacter diazotrophicus, and cyanobacteria are some non-symbiotic nitrogen-fixing bacteria. Members of the Rhizobiaceae family are nitrogen-fixing bacteria. Very small amounts of fixed nitrogen are supplied to plants by non-symbiotic bacteria. Symbiotic associations by bacteria lead to colonization within the roots of legume plants. Nodule formation is observed in the intracellular compartments of plant cells where rhizobia cells colonize (Böhme *et al.,* 2018). Biological nitrogen-fixing organisms have proven to be a promising alternative for balancing nitrogen status in plants. Worldwide, significant consideration has been given to nitrogen limitation in agricultural soils, and new tools and techniques are being developed to prevent the application of chemical fertilizers for crop improvement. The combination of free-living diazotrophic nitrogen-fixing bacteria such as Anabaena and Azolla in flooded rice fields can compensate for nitrogen deficiency. Therefore, Anabaena is considered a "biofertilizer" (Fosu *et al.,* 2015).

Wei *et al.,* (2013) in their research revealed that *Klebsiella variicola* isolates were found in the rhizosphere of sugarcane and demonstrated that the *K. variicola* strain was able to colonize sugarcane plants, fix N2, and enhance plant growth. *K. variicola* was able to survive in soil and colonize root epidermal cells, intercellular spaces in the root cortex, and leaf mesophyll and vascular tissue. *K. variicola* was able to enhance sugarcane growth by fixing N2 and solubilizing phosphate and potassium under greenhouse conditions. Chen *et al.,* (2024) investigated the nitrogen metabolism of the aerobic heterotrophic nitrifying and denitrifying bacterium, *Klebsiella aerogenes*. Overall, *K. aerogenes* was found to assimilate 82.47% of the available nitrate into cellular nitrogen.

Yoshidome *et al.,* (2024) developed a strategy for glutamate production from atmospheric nitrogen using the nitrogen-fixing bacterium *K. oxytoca*. We demonstrated that simultaneous application of glucose and citrate as carbon sources increased nitrogenase activity in *K. oxytoca*. In the presence of glucose and citrate, a *K. oxytoca* strain genetically engineered to increase the availability of 2-oxoglutarate, a precursor of glutamate synthesis, produced more than 1 g L−1 of extracellular glutamate from atmospheric nitrogen.

Shukla *et al.* (2022) studied two endophytic bacterial isolates, *Bacillus subtilis* and *K. aerogenes*, co-cultured on *Bacopa monnieri* cuttings. Plants inoculated with the consortium significantly increased plant biomass and Bacoside A content compared to single inoculation without treatment. Inoculation with the *B. subtilis* and *K. aerogenes* consortium increased the fresh and dry weight of *B. monnieri* plants by 117.08% and 57.63%, respectively, compared to control plants. A significant increase of 46.63% was observed in N content in the microbial combination treatment; However, an insignificant effect of the endophyte combination on K content was observed. Similarly, a significant increase in Bacoside A content of 46.99% was observed in plants treated with the microbial consortium compared to plants in the control group.

Phytohormones are known to be produced by plants to promote growth, immunity, stress tolerance, and maturity. PGPR also produce phytohormones under stress conditions. They activate the plant's cellular machinery through the activation of transcription factors. Auxins, gibberellins, cytokinins, jasmonic acid, and brassinosteroids modulate plant growth and response to stress (Maheshwari *et al.,* 2015).

Auxin/Indole Acetic Acid (IAA) is responsible for promoting plant health and development through cell expansion, division, and differentiation, increased root and shoot elongation, apical dominance, and more. IAA is produced by various rhizobacteria, such

as Azospirillum, Agrobacterium, Bacillus, Paenibacillus, Pseudomonas, and Erwinia (Chandra *et al.,* 2018). IAA production can be regulated by environmental factors such as pH and the presence of tryptophan secreted by plant roots (Jaiswal *et al.,* 2018). L-tryptophan is an IAA precursor used by PGPR for IAA synthesis through deamination, decarboxylation, and/or hydrolysis reactions. IAA helps plants resist adverse conditions and promotes good plant growth (Lebrazi *et al.,* 2020).

In their study, Gang *et al.,* (2018) isolated the rhizosphere of the flowering plant *Dianthus caryophyllus* and found bacteria of the genus Klebsiella. These bacteria were shown to produce the hormone IAA, indicated by a pink color change in the Salkowski reagent. Subsequently, *D. caryophyllus* seedlings were soaked in a Klebsiella bacterial suspension, indicating root elongation.

Klebsiella sp. was also found in banana roots, which were then isolated and shown to produce IAA in the range of 20 to 302 µg ml−1. In addition to IAA production, the study also included phosphate solubilization and nitrogen fixation tests, which yielded positive results. The greenhouse study found significant increases ($P < 0.05$) in plant growth, chlorophyll, total phenolics, proline, catalase, and ascorbic acid oxidase in banana plants treated with PGP bacteria compared to the control. This study suggests that bacteria exhibiting diverse PGPR activities can be used as biostimulants to increase banana production (Patel *et al.,* 2017).

In their research, Khalifa *et al.* (2022) found *K. oxytoca* in the rhizosphere of *Lotus corniculatus*, then isolated it to test its biostimulant properties. *K. oxytoca* was shown to produce IAA (16.34 µg mL-1), solubilize phosphate, and fix nitrogen. Furthermore, in vitro inoculation of barley plants significantly increased root and shoot dry weight.

Ou *et al.* (2022) in their research explained that *K. aerogenes* possesses superior plant growth promoting (PGP) properties in vitro and in vivo against mulberry plants and is non-invasive and harmful to silkworm populations. *K. aerogenes* can actively colonize mulberry roots and subsequently move to stems and leaves. Furthermore, *K. aerogenes i*ncreases the content of indole acetic acid (IAA) in mulberry roots, indicating that this strain induces mulberry plant resistance to biotic and abiotic stress. In particular, cinnamic acid, isocoumarin, coumarin, and their derivatives, such as phenylpropanoid and polyketide compounds, were significantly upregulated in mulberry after *K. aerogenes* inoculation. Coumarins are known to play a key role in iron transport and mobilization in plants and are known to act as signaling agents that shape the host microbiome.

V. CONCLUSION

Three isolates were successfully isolated from the rhizosphere of clove plantations in Pucaksari Village, Busungbiu District, Buleleng Regency, Bali, isolates BRC01, BRC02, and BRC03. All three isolates potentially solubilizing phosphate and produce IAA (Indole Acetic Acid), but only 1 isolate can fix nitrogen, namely isolate BRC02 which was then selected for further research. This bacterial colony has an IAA content of 14.71 ppm, with the highest phosphate solubility index (PSI) of 1.86 and tested positive for growth on Ashby's Mannitol Agar media which indicates it can fix nitrogen. Furthermore, isolate BRC02 was identified through DNA screening which showed that BRC02 is a *Klebsiella pneumoniae* subsp. *aerogenes*.

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