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# MOLECULAR APPROACH AND PRELIMINARY SCREENING OF CULTURABLE FREE-LIVING *RHIZOBIUM* AS PLANT GROWTH ENHANCER

Tri Ratna Sulistiyani[1a]*, Siti Meliah[2a], Masrukhin[3a], Mia Kusmiati[4a], Agus Budiyanto[5b]*, Dwi Agustiyani Muslichah[6c]

**Abstract:** The population increase and changes in consumption are the main challenges in agriculture production related to the increase in basic needs, especially food. The high demand for food leads to fertilizer application to effectively speed up and manage plant growth. The search for alternative biofertilizers from unique microorganisms is needed to tackle this issue. *Rhizobium* is known as a plant growth enhancer. In this study, they were isolated from Aek Natonang, famous as "the lake above the lake", located on Samosir Island, North Sumatra, at more than 1400 m. These geographical conditions offer various potential unique microorganisms. The collected free-living *Rhizobium* from the sample was identified by morphological characteristics and molecular approach through 16S rDNA sequence. They were assessed for their abilities as plant growth promoters. Based on the morphological characterization, they showed a creamy yellow colony, excessive mucus production, Gram-staining negative, and catalase positive. Through the molecular approach, they have 98-100% similarity with *Rhizobium* sp.. A combination of morphological and molecular approaches strengthens the identification results. Six *Rhizobium* sp. produced IAA, siderophore, ACC Deaminase, and ammonia. They could also solubilize phosphate tolerated to high pH and salt concentrations. All the collected *Rhizobium* were qualitatively potential to enhance the plant growth. They performed diverse abilities in vitro, and *Rhizobium radiobacter* strain DT 14.16 displayed the best features. Hence, they can be a candidate for biofertilizer. However, none of the collected *Rhizobium* could suppress the growth of *Ralstonia solanacearum*. Using quality indigenous bacteria such as *Rhizobium*, is an environmentally friendly technology to increase agricultural productivity and hopefully provide better yield.

***Keywords:*** *Agriculture, PGPR, Rhizobium, strain, Toba.*

## 1. Introduction

Indonesia's population reached 270 million, becoming a big problem in every sector, including agriculture. The increase in population and changes in consumption are the main challenges in agriculture production related to the increase in basic needs, especially food. This high demand for food leads to fertilizer application to accelerate plant growth and control plant diseases. However, an extreme application of chemical fertilizers could induce severe problems for human health and environmental pollution. Furthermore, chemical fertilizers can also change the diversity of soil microorganisms essential for soil fertility (Dinca et al., 2022). Therefore, in line with human understanding of healthy food, agricultural practices changed to nature-based through eco-friendly approaches. Gradually, chemical fertilizers are replaced with biofertilizers, and microbial application is one of the strategies to fulfil the need for healthy food.

**Authors information:**
[a]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (NRIA), Cibinong, INDONESIA. E-mail: trir003@brin.go.id[1]; sitimeliah@gmail.com[2]; masrukhin21@gmail.com[3]; miakusmiati.charis@gmail.com[4]
[b]Research Center for Agroindustry, National Research and Innovation Agency (NRIA), Cibinong, INDONESIA. E-mail: agusbdyanto@gmail.com[5]
[c]Research Center for Applied Microbiology, National Research and Innovation Agency (NRIA), Cibinong, INDONESIA. E-mail: titinagustin@yahoo.com[6]
*Corresponding Author: trilisty01@gmail.com; agusbdyanto@gmail.com

Soil microorganisms are involved in soil health and agricultural product sustainability. Besides promoting plant growth, microorganisms are considered inexpensive and environmentally safe (Dinca et al., 2022). They would reduce the need and reliance on chemical fertilizers. Studies have reported that microorganisms can increase plant growth due to their capability to generate active compounds such as growth hormones, antibiotics, and enzymes. Additionally, they are capable of performing nitrogen fixation from the environment. The genus of bacteria that are ubiquitous in the rhizosphere and widely applied in agricultural farmlands are *Rhizobium*, *Bacillus, Azotobacter, Azospirillum, Serratia, Pseudomonas, Burkholderia, Agrobacterium, Delftia* (Rajawat et al., 2019; Venieraki et al., 2021).

*Rhizobium* exists in the soil independently as a free-living bacteria or in symbiosis with roots in a legume nodule. *Rhizobium* can fix atmospheric nitrogen ($N_2$) in the air and convert it into ammonia ($NH_3$), which is further converted into amino acid used for plant growth. *Rhizobium* also produces biologically active compounds to promote plant development (Saghafi et al., 2018), directly by providing nutrients or indirectly by producing antibiotic compounds (Jaiswal et al., 2021). *Rhizobium*'s uniqueness is its symbiotic ability with Leguminosae/Fabaceae leguminoceae plants by forming nodules on the roots. The *Rhizobium* characteristic is that they infect the root hairs of legumes in temperate climates or topical areas, curl the hair

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roots, and, lately, produce root nodules. These processes make *Rhizobium* an intracellular symbiosis. The uniqueness and ability to generate active compounds beneficial for plants cause *Rhizobium* to be categorized as plant growth-promoting rhizobacteria (PGPR). Indonesia has the largest lake, also become the largest in the world, called Lake Toba. Aek Natonang, known as the "lake above the lake", which located on Samosir Island in the middle of Lake Toba. Being at an altitude of > 1400 m makes Aek Natonang unique regarding its geographical conditions, causing a relatively high microorganism diversity in that location. Therefore, the present study aimed to explore free-living *Rhizobium* diversity from unique conditions in the tropical rain forests around Lake Toba, screening the physiological activity of a plant-growth-promoting bacteria and their tolerance under abiotic stress conditions such as high salt levels and pH variations.

## 2. Materials and Methods

### *Rhizobium* isolation

As many as eleven rhizosphere soils were taken from the Lake Toba area, namely Aek Natonang and Eden Park 100 in North Sumatra, Indonesia, at a high altitude of >1400 m above sea level. Isolation was carried out using serial dilution and spread on Ashby Mannitol Agar media. It was followed by incubation at 28 °C for 3-7 days. The colony showing *Rhizobium* characteristics was purified and preserved using a freezing method at -80 °C for the following analysis.

### Morphology and Phenotypic Characterization

Somasegaran and Hoben (1994) refer to the morphological characterisation of bacterial colonies. The characteristics observed, i.e., size, shape, border, elevation, colour, mucousity, and transparency of colony after three days of incubation on Yeast Mannitol Agar (YMA). Gram reaction was conducted using a Gram staining reagent following the instructions provided by the manufacturer (Merck). *Rhizobium*'s ability to produce acids or bases was checked by growing on a YMA medium added with 1% Congo red or 0.025% Brom Thymol Blue (BTB). Isolate was cultured on YMA and incubated for seven days at 28 °C under dark conditions, and then the medium colour alteration was observed. As an acid-producing and fast-growing bacteria, the medium colour was changed from green to yellow. In contrast, base-producing and slow-growing bacteria were changed from green to blue.

### Molecular Identification

The DNA extract as a DNA template was collected by cell disruption at 98 °C for 5 min, as Packeiser et al. described. (2013). The bacterial DNA was amplified using primers: Forward, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and Reverse, 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991) with the PCR conditions: initial denaturation (95 °C, 90 sec), followed by 30 cycles of denaturation (95 °C, 30 sec); annealing (50 °C, 30 sec); elongation (72 °C, 90 sec), and final extension (72 °C, 5 min). Amplified DNA was loaded into wells on 1% agarose gel electrophoresis, followed by DNA visualization using a UV transilluminator.

The DNA sequencing was performed in Macrogen, South Korea. The chromatogram sequence was analyzed using Chromas Pro software, and the possible identity was searched through the EzBioCloud 16S database (Yoon et al., 2017). Construction and visualization of the phylogenetic tree were executed using the MEGA 11 programme (Hall, 2013). Contig and reference sequences were aligned using Clustal W and followed by construction using the Neighbor-Joining Method (Tamura et al., 2011), Kimura 2-Parameter as a suggested model, and 1000 bootstrap replication.

### Plant Growth Promoting Ability Assay

Isolate ability to fix nitrogen was tested using Jensen-free nitrogen media. Isolates were grown on Jensen-free nitrogen media and kept at 28 °C for seven days. The growth was observed daily. Vaccination was made in two replications.

Inorganic phosphate solubilization ability was determined using Pikovskaya Agar (Jasim et al., 2013). The isolate was streaked on the Pikovskaya Agar and placed in the incubator at 28 °C. A clear zone around the colony revealed a positive result. Vaccination was made in two replications.

Indole Acetic Acid (IAA) compounds analysis followed Rahman et al. (2010) protocol. Isolates were cultured on a liquid Yeast Mineral Extract (YEM) media enriched with 0.2% (v/v) L-tryptophan and kept in a shaker incubator at 28 °C for three days. On the third day, culture centrifugation at 10000 rpm for 10 min, then 200 µL of Salkowski's reagent was put into 100 µL of supernatant and placed in the darkroom for 30 min. Finally, absorbance measurement at 530 nm. Analysis was made in two replications.

Siderophore production was evaluated using Chrome Azurol S (CAS) agar (Alexander & Zuberrer, 1991). Isolate was streaked on the CAS agar, and then placed at 28 °C for 72 hours. A circular yellow-orange around the colony indicated isolate could generate a siderophore. Vaccination was made in two replications.

Inoculated isolates checked ACC deaminase activity on Tryptone Yeast (TY) media. It was incubated for 48 ± 2 hours at 28 ± 1 °C (Beringer, 1974). Isolates possessing ACC deaminase activity were indicated by growth ability in TY media. Vaccination was made in two replications.

Ammonium production was performed by inoculating the isolate in 5 mL of Peptone water media, then incubating at 28 ± 2 °C for 72 hours. Subsequently, 0,5 mL Nessler reagent was added to the inoculated media and observed brown colour formed in the media. Vaccination was made in two replications.

### Screening for Salt and pH Tolerance

The isolate was streaked in Yeast Mannitol Broth (YMB) media containing 0.5-5.0% (w/v) NaCl, with an interval of 0.5%. Acid or alkaline tolerance was evaluated using YMB media adjusted to pH 3-9, with a rise of 1 pH unit. Cell growth in the medium indicated their salt or potential hydrogen tolerance is given.


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**Biocontrol Assay Against *Ralstonia solanacearum***

The nineteen isolates were tested for antagonistic activity against *R. solanacearum*. The agar spot-on-the-lawn method carried out the antimicrobial assay with a slight modification of the agar diffusion method (van Reenen et al., 1998; Sabo et al., 2020). Prior to the assay, *R. solanacearum* was grown in the triphenyl tetrazolium chloride (TZC) agar, incubated for 48 hours, then inoculated into nutrient agar (NA) medium and incubated overnight. The concentration of *R. solanacearum* was adjusted according to the McFarland turbidity standard 0.5 and streaked to the Mueller Hinton Agar (MHA) densely. Isolates were grown in YMA medium, incubated for 72 hours, then spotted into MHA medium-containing *R. solanacearum* and incubated at 37 °C for 48-72 hours.

## 3. RESULTS

**Isolation and Morphological Characterization**

A total of 77 bacterial isolates were successfully collected from 11 rhizosphere samples. Phenotypically, 19 of 77 collected bacterial isolates showed the same characteristics as *Rhizobium*. The isolates formed circular, smooth, convex, raised, milky cream to yellow colour, opaque, and mucoid colonies. They were rod-shaped and pink in colour under Gram stains; therefore, they are considered Gram-negative bacteria. The morphological characteristics of bacterial isolates are presented in Table 1.

The notable characteristics of *Rhizobium* were that it produced catalase enzyme and mucoid texture. The mucus appeared after three days of incubation at 28°C due to exopolysaccharide production. The ability of the *Rhizobium* strain to generate acid or alkaline can be visualized by stain absorption on the YMA medium, and generally, rhizobia absorbs the dye weakly. On the other hand, 84% of the tested isolates changed medium colour from deep green to yellow. The alteration of medium colour indicates that the isolates mainly were acid-producing and fast-growing bacteria.

**Table 1**. Morphological characters of selected isolates

| Isolate code | Gram stain | Cell shape | Form | Elevation | Colour | Mucosity | Transparency | CR absorption | BTB reaction (7th day) |
|---|---|---|---|---|---|---|---|---|---|
| TE 29.1 | - | Rod | circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| TE 29.10 | - | Rod | circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| DT 21.16 | - | Rod | circular | Convex | Creamy yellow | Soft mucoid | Opaque | NA | R |
| DT 20.2E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Translucent | NA | R |
| DT 14.7 | - | Rod | Small | Convex | Creamy yellow | Firm gummy | Opaque | NA | NR |
| DT 14.19 | - | Rod | Circular | Convex | Creamy yellow | Firm | Opaque | NA | NR |
| DT 14.12 | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Translucent | NA | R |
| DT 14.23 | - | Rod | Circular | Raised | Milky cream | Firm gummy | Opaque | NA | R |
| DT 14.1E | - | Rod | circular | Raised | Milky cream | Soft mucoid | Translucent | NA | R |
| DT 14.5 | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Translucent | NA | R |
| DT 14.16 | - | Rod | Circular | Raised | Milky cream | Soft mucoid | Translucent | NA | NR |
| DT 14.21 | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| MSP.3a.E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Translucent | NA | R |
| DT 14.2 | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| DT 21.20 | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| TE 09.2E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| TE 29.1E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| DT 20.1E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |
| TE 09.1E | - | Rod | Circular | Convex | Milky cream | Soft mucoid | Opaque | NA | R |

Note: CR: Congo Red; BTB: Brom Thymol Blue: NA: Not Absorb; R: Reacted; NR: Not reacted

**Molecular Identification**

In addition to morphological characterization, isolates were identified through the molecular approach 16S rDNA sequence. All identified bacteria belong to *Rhizobium*. The searches through the EzTaxon database generated 98.39-100% similarity value with type strain references on GenBank. This approach fully supports the morphological characteristics of selected isolates. The closest species identity and percentage similarity are given in Table 2. The evolutionary relationship of selected isolates is shown in Figure 1.


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**Table 2**. The closest identity of *Rhizobium* sp. based on the 16S rDNA.

| Isolate code | The closest species identity | Homonym/ correct name | Top hit strain | Similarity (%) |
|---|---|---|---|---|
| TE 29.1 | *Rhizobium leucaenae* | - | USDA 9039(T) | 99,85 |
| TE 29.10 | *Rhizobium calliandrae* | - | CCGE524(T) | 100 |
| DT 21.16 | *Rhizobium tropici* | - | CIAT 899(T) | 99,84 |
| DT 20.2E | *Rhizobium tropici* | - | CIAT 899(T) | 100 |
| DT 14.7 | *Rhizobium altiplani* | - | BR10423(T) | 98,87 |
| DT 14.19 | *Rhizobium altiplani* | - | BR10423(T) | 99,14 |
| DT 14.12 | *Rhizobium grahamii* | - | CCGE 502(T) | 99,54 |
| DT 14.23 | *Rhizobium smilacinae* | - | PTYR-5(T) | 98,42 |
| DT 14.1E | *Rhizobium radiobacter* | *Agrobacterium radiobacter* | ATCC 19358(T) | 99,62 |
| DT 14.5 | *Rhizobium radiobacter* | *Agrobacterium radiobacter* | ATCC 19358(T) | 99,69 |
| DT 14.16 | *Rhizobium radiobacter* | *Agrobacterium radiobacter* | ATCC 19358(T) | 99,92 |
| DT 14.21 | *Rhizobium radiobacter* | *Agrobacterium radiobacter* | ATCC 19358(T) | 99,92 |
| MSP.3a.E | *Rhizobium radiobacter* | *Agrobacterium radiobacter* | ATCC 19358(T) | 99,7 |
| DT 14.2 | *Rhizobium multihospitium* | | HAMBI 2975(T) | 100 |
| DT 21.20 | *Rhizobium multihospitium* | | HAMBI 2975(T) | 100 |
| TE 09.2E | *Rhizobium multihospitium* | | HAMBI 2975(T) | 100 |
| TE 29.1E | *Rhizobium multihospitium* | | HAMBI 2975(T) | 100 |
| DT 20.1E | *Rhizobium rhizogenes* | | NBRC 13257(T) | 99,89 |
| TE 09.1E | *Rhizobium miluonense* | | HAMBI 2971(T) | 100 |

**Physiological characterization**

*Rhizobium* strains possess several characteristics as plant-growth promoters, such as producing active compounds IAA, siderophore, ammonium, fixing nitrogen, solubilized inorganic phosphate, and also have ACC deaminase activity (Table 3). A qualitative assay for nitrogen fixation was detected in 89% of isolates, ammonium was produced in 95% of isolates, and inorganic phosphate solubilization was exhibited in 53% of isolates. As many as 74% of isolates could produce siderophore, 79 % have ACC deaminase activity, and all bacterial isolates can produce IAA.

**Table 3.** Physiological characterization of *Rhizobium* spp.

| Isolate code | Nfix activity | Inorganic Phosphate solubilization | IAA production | Siderophore activity | ACC Deaminase activity | Ammonia production |
|---|---|---|---|---|---|---|
| TE 29.1 | + | + | + | - | + | + |
| TE 29.10 | + | + | + | + | + | + |
| DT 21.16 | + | - | + | - | + | + |
| DT 20.2E | + | + | + | + | + | + |
| DT 14.7 | - | + | + | - | - | + |
| DT 14.19 | - | + | + | + | + | + |
| DT 14.12 | + | - | + | + | + | + |
| DT 14.23 | + | - | + | + | - | - |
| DT 14.1E | + | - | + | + | + | + |
| DT 14.5 | + | - | + | + | + | + |
| DT 14.16 | + | - | + | + | + | + |
| DT 14.21 | + | - | + | + | + | + |
| MSP.3a.E | + | - | + | - | - | + |
| DT 14.2 | + | + | + | + | + | + |
| DT 21.20 | + | + | + | + | + | + |
| TE 09.2E | + | - | + | + | + | + |
| TE 29.1E | + | + | + | + | + | + |
| DT 20.1E | + | + | + | + | + | + |
| TE 09.1E | + | + | + | - | - | + |

Note: +: positive activity; -: No activity


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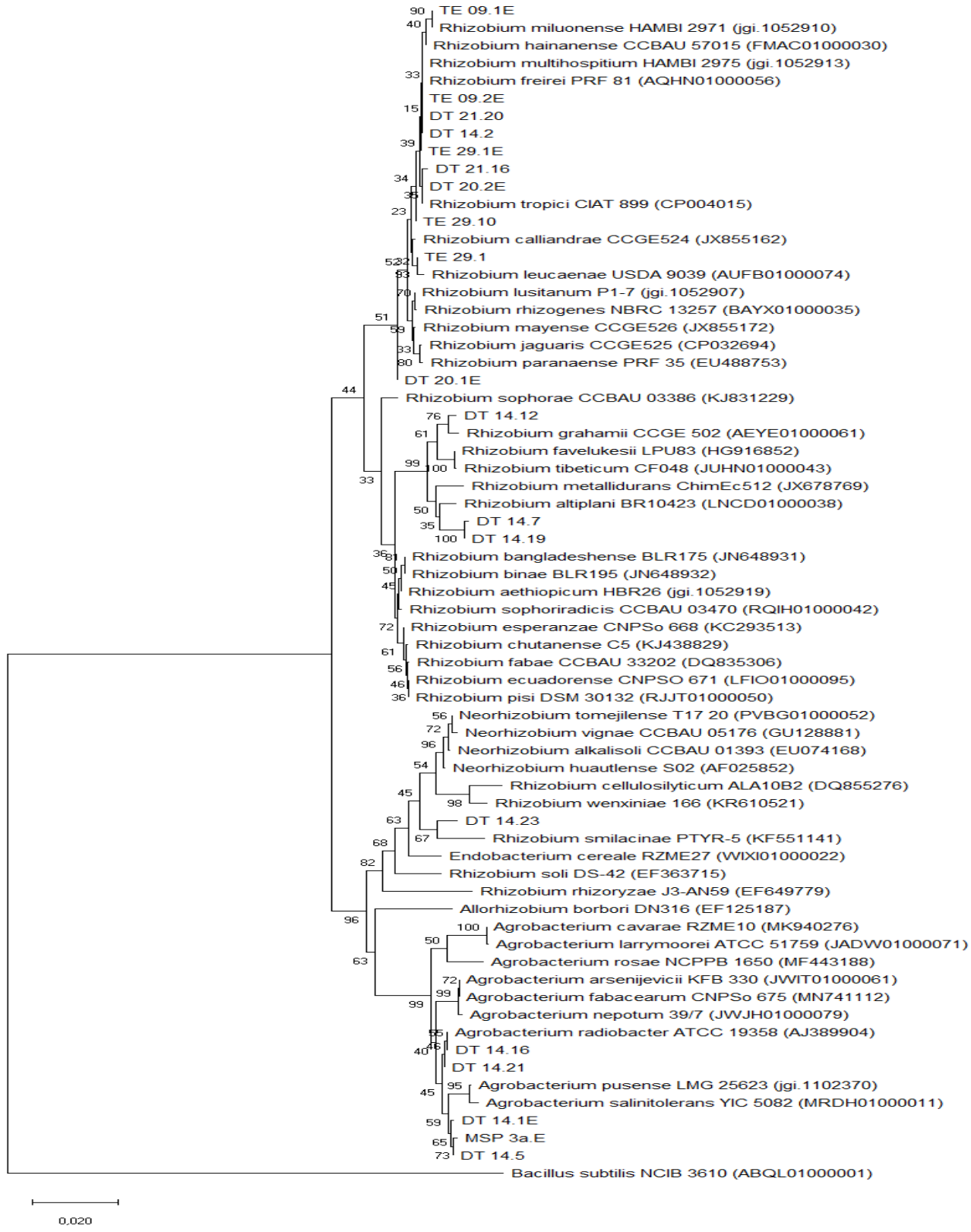


**Figure 1**. Phylogenetic tree of nineteen *Rhizobium* sp. using Neighbor-Joining method, Kimura-2 parameter model, and bootstrap value 1000


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Amongst the *Rhizobium* strain, ten 19 isolates have a phosphate solubilization index ranging from 1,06 to 1,67, with the highest index being *R. altiplani* strain DT 14.7 (Figure 2). As many as fourteen *Rhizobium* strains can produce siderophore with index values from 1 to 14,5; *R. multihospitium* strain TE 29.1E was the highest, while the lowest was *R. multihospitium* strain DT 14.2 and *R. grahamii* strain DT. 14.12. In addition, ACC deaminase activity was also detected in fifteen *Rhizobium* strains.

Quantitatively measurement of IAA production using a spectrophotometer revealed that IAA concentration ranged from 0,792 to 30,6 ppm, with the highest IAA producer being *R. altiplani* strain DT 14.7 and *R. grahamii* strain DT 14.12, while the lowest IAA producer being *R. multihospitium* strain TE 29.1E (Figure 3).

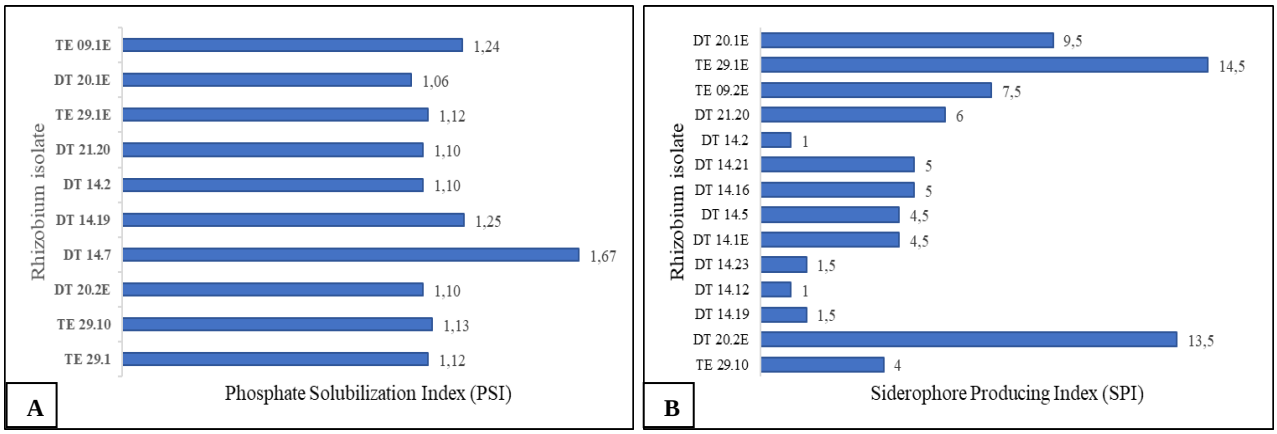


**Figure 2.** The capability of *Rhizobium* spp. in dissolving inorganic phosphate (A) and producing siderophore (B).

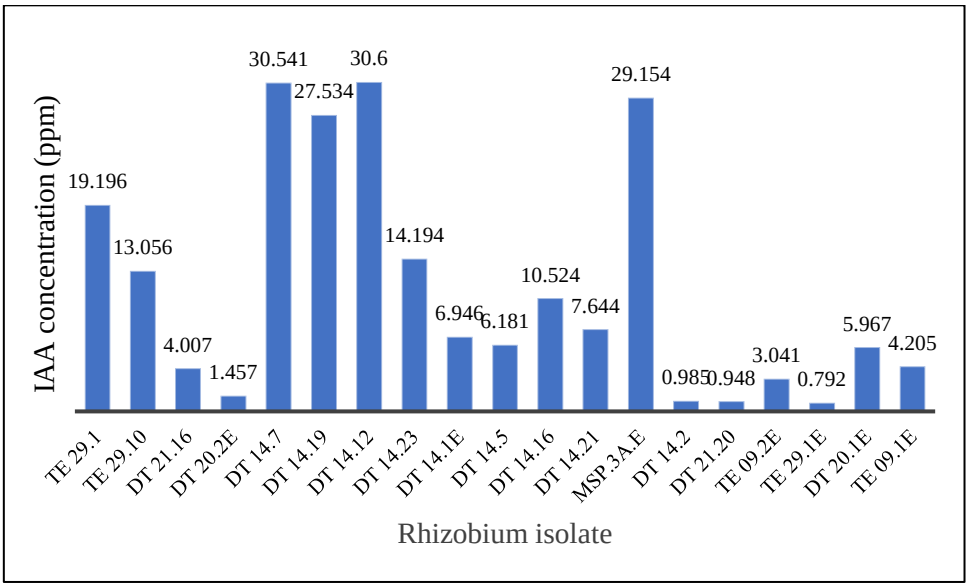


**Figure 3**. IAA concentration produced by *Rhizobium* spp.

**Salt and pH Tolerance**

The nineteen *Rhizobium* strains can grow in 0.5 and 1% NaCl. However, *R. radiobacter* strain DT 14.16 could grow in 0.5-5% NaCl. The optimum growth condition of *Rhizobium* was pH 7. The result of the present study showed that the nineteen *Rhizobium* spp. could grow in pH 3-9 (data not presented).

**Biological Control Against *Ralstonia solanacearum***

According to the antimicrobial assay, nineteen *Rhizobium* strains could not control *R. solanacearum* growth (data not shown). Chloramphenicol as positive control showed inhibition against *R. solanacearum*.


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## 4. Discussion

Nineteen selected isolates were confirmed to be a group of rhizobia through morphology and physiology characterization as well as molecular identification, such as the creamy yellow colony, excessive mucus production, Gram-staining negative, catalase positive, and more than 98% similarity with *Rhizobium* species. Furthermore, the low absorption of Congo Red and BTB in the medium strengthens the assumption that the selected isolates belong to the rhizobia group. Kneen & Larue (1983) reported that rhizobia has white colonies or poorly absorbed dye indicators. The absorption of an indicator chemical is affected by medium composition and strain character. The colour alteration in the YMA medium containing BTB revealed that the selected bacteria was fast-growing and acid-producing, which is one of the characteristics of *Rhizobium* (Koskey et al., 2018).

Identification based on 16S rRNA gene sequence, the selected isolates showed a high similarity percentage to the reference strains. The 19 selected isolates were challenging to be identified solely based on their morphological appearance because all isolates were visually the same. However, the molecular technique was able to separate them into different species. Therefore, the combination of phenotypic and molecular approaches guaranteed that the selected isolates belonged to the genus *Rhizobium*. A point to be highlighted is *Rhizobium smilacinae* strain DT 14.23 has a slightly low percentage of similarity, 98.42%, compared to the reference species provided. Based on this value, the strain is potentially a candidate for new species. Unique locations such as Aek Natonang provide a great chance for new species discovery.

Rhizospheric microorganisms supply most of the nutrition plants require (Mendes et al., 2013). These bacteria contribute to the promotion of plant growth through both direct and indirect mechanisms. Indole acetic acid, popular as auxin, is a plant product utilized directly by plants. The IAA production highly depends on the type of bacteria and tryptophan availability in the environment (Duca et al., 2014). IAA is involved in cellular generation (Bhardwaj et al., 2014), controlling metabolite biosynthesis, and resisting environmental conditions (Rohini et al., 2018). Ul Hasan & Bano (2015) stated that L-tryptophan addition into the soil could be done to manage plant growth. Therefore, rhizospheric bacteria that could produce IAA, even in small concentrations, are necessary. The ability to utilize and produce IAA varies among bacterial strains, regardless of the presence or absence of tryptophan.

Bacteria capacity to generate IAA is also reported to have ACC deaminase with high activity (Glick, 2014). Through ACC deaminase expression, *Rhizobium* can convert 1-aminocyclopropane-1-carboxylic acid (ACC) into -ketobutyrate and NH3, then decrease plant ethylene level. Therefore, inoculating *Rhizobium* with ACC deaminase activity in plants with low ethylene content will help plants produce longer roots and avoid stress (Gopalakrishnan et al., 2015). Several *Rhizobium* species known to have ACC deaminase activity are *Rhizobium leguminosorum* Viciae., *R. hedysari, R. japonicum, R. gallicum* (Ma et al., 2003; Khan et al., 2022). In addition, Nascimento et al., (2016) stated in their study that rhizobia with the ability to produce ACC deaminase are also effective as a nitrogen fixer.

In agriculture, diazotrophs are a possible choice for reducing the excessive use of artificial nitrogen-based fertilizers (Santi et al., 2013). Seventeen of 19 isolates (89%) could fix nitrogen, as seen from their ability to grow on Jensen's media, proving their potential as nitrogen fertilizers. Not all *Rhizobium* can fix nitrogen, such as *R. altiplani* strain DT 14.19 and *R. altiplani* strain DT 14.7. Another critical parameter in plant growth promoters is the ability to dissolve inorganic phosphate and produce siderophores. Eight *Rhizobium* strains have phosphate solubilizing activities. Most of the phosphate solubilizing obtained were *R. radiobacter,* with various strengths activity seen from the index value of the phosphate solubilization. Fifteen isolates produced siderophore, noticed by developing an orange colour surrounding the bacterial colony on blue selective media (CAS medium). *R. radiobacter* was reported to produce siderophores (Ferreira et al., 2019). This report aligns with this study that *R. radiobacter* collected from the rhizosphere can produce a siderophore. Verma et al. (2020) study revealed that *R. radiobacter* LB2 is a promising biofertilizer candidate due to its capability. It could solubilize phosphate, produce active compounds, fix nitrogen, and grow in high NaCl concentrations.

To live in symbiosis, *Rhizobium* also lives as a saprophyte by competing with others to obtain infection sites on the legume roots. Therefore, *Rhizobium* must survive in extreme conditions such as varied pH and NaCl concentrations. Abiotic factors such as acidity and salinity affect plant development. A slight change in pH affects bacterial growth in the soil. All nineteen selected isolates could grow in the pH range from 3 to 9. Seven isolates were tolerant to salt concentrations above 2.5%, including *R. smilacinae* strain DT 14.23, *R. radiobacter* strain DT 14.1E, *R. radiobacter* strain MSP.3a.E, *R. radiobacter* strain DT. 14.16, *R. radiobacter* strain DT 14.21, and *R. radiobacter* strain DT 14.5. The best isolate was *R. radiobacter* strain DT 14.16 which survived at 5% salt concentration. Berrada et al. (2012) stated that the limiting aspect of the nitrogen fixation activity of *Rhizobium* is salinity. Bacteria with fast growth rates demonstrate higher tolerance to high salt concentrations and are suitable to be applied as biofertilizers in soils with high salt content. Tewari and Arora (2016) reported that exopolysaccharides contained in mucous play a role in tolerance of pH and high salinity.

## 5. Conclusion

The rhizosphere soil from tropical rain forests around Lake Toba in Sumatra, Indonesia, contains *Rhizobium.* As many as 19 bacterial isolates of *Rhizobium* collected from the unique geographical conditions are promising as plant growth enhancers and suitable for biofertilizer candidates. *R. radiobacter* strain DT 14.16 is the best candidate as a PGPR. However, none of the collected *Rhizobium* could control the *R. solanacearum* growth. Using *Rhizobium* in the consortium will provide better results, as each species or strain has different abilities. In addition, using


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indigenous bacteria with such quality is an environmentally friendly technology to increase agricultural productivity.

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