An Ability of Endophytic Bacteria from Nutgrass (*Cyperus rotundus*) from Lafau Beach of North Nias in Producing Indole Acetic Acid and in Solubilizing Phosphate

Atriani Zega, Dwi Suryanto*, Yurnaliza
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jln. Bioteknologi No. 1, Medan 20155, Indonesia

*Email: dwisuryanto@usu.ac.id

**Abstract**: Endophytic bacteria have taken much attention for their potency to promote plant growth. This study was aimed to isolate endophytic bacteria from nutgrass (*Cyperus rotundus*) and to examine their potency in producing indole acetic acid (IAA) and in solubilizing phosphate. Isolation of endophytic bacteria was done by slicing and sterilizing root, stem, and leaf sample surface with alcohol 70% and sodium hypochlorite 2%, followed by incubation of the sliced samples in nutrient agar medium. Morphological characterization and simple biochemical tests were performed on bacterial isolates. All bacterial isolates were examined for their ability to produce indole acetic acid and to solubilize phosphate. Three isolates (AZ5, AZ12 and AZ6) out of fifteen indicated the ability to produce indole acetic acid and to solubilize phosphate. IAA producing test using spectrophotometry method showed that AZ5, AZ12, and AZ6 produce more IAA with concentration of 49,91, 48,18, and 44,45 ppm, respectively. Phosphate solubilizing test using Pikovskaya agar medium showed that the three isolates were able to solubilize phosphate with index of 6.27, 3.31, and 3.41 respectively.

**Keywords**: *Cyperus rotundus*, plant growth promoting bacteria, IAA production, phosphate solubilizing

1. Introduction
Endophytes are a group of bacteria that live in the plant tissue as neutral or symbiotic to the host plant. Strobel and Daisy [8] explained that endophytic bacteria can live in plants during a certain period of their life cycle. The role of endophytic bacteria is allegedly supported by their role in supplying nutrients for the plant growth either by phosphate solubilization, nitrogen fixation [1], or phytohormones such as indole-3-acetic acid (IAA), abscisic acid, gibberellic acid, and jasmonic acid [2,3,4,5]. Another benefit of endophytic bacteria that support plant growth is by protecting plants from pathogen infection through competition for habitat and nutrients [6,7].

Indole acetic acid plays a role in cell enlargement, as well as rapid change of gene expression, which causes the cells in the area of extension to produce new proteins as a constituent of the cell walls that affect plant development. Patten and Glick [12] reported that the endophytic bacteria that produce IAA stimulate the growth of the host rooting system.
It was reported that endophytic bacteria from paddy rice are capable of producing hormones IAA [11]. Duangpaeng et al. [13] reported that thirty five endophytic bacteria on organic rice crops are capable of producing hormones IAA. Montañez et al. [14] isolated twenty two endophytic bacteria from Zea mays L. that produces IAA. He showed that the bacteria contributed to increase in root and stem biomass of corn.

Phosphate is the macronutrients for plant growth. Many endophytes were known for their ability to solubilize phosphate to help plant growth [10]. Chen et al. [15] reported that endophytic bacteria from Manihot esculenta Crantz root are potential to solubilize phosphate. Joe et al. [16] also reported that endophytic bacteria from plant Phyllanthus amarus Schum & Thonn was capable to solubilize phosphate.

In this study, isolation of endophytic bacteria of nutgrass (Cyperus rotundus) was done. Nutgrass is common grass species of tropical area and easily found in various regions of Indonesia. So far, the endophytic bacteria of nut grass with the ability both to produce IAA and solubilize phosphate have not been studied yet. The aims of this study were to know the potential of endophytic bacteria from nutgrass of Lafau Beach of North Nias to produce IAA and to solubilize phosphate.

2. Materials and Methods

2.1 Plant samples

Three nutgrass samples of C. rotundus were collected from three different locations in Lahewa, North Nias. The plant materials used in this study were root, stem and fresh leaf.

2.2 Isolation of endophytic bacteria

Samples of root, stem, and leaves were cut into 1-2 cm long. These pieces were rinsed with sterile water, surface-disinfected by soaking in 70% alcohol for 3 minutes, rinsed with sterile water, soaked in 1% NaOCl for 5 minutes, rinsed again with sterile water, soaked in 70% alcohol for another 1 minute, and put into sterile water. The sample was placed on a surface of filter paper aseptically, and put on the surface of the nutrient agar (NA) containing ketoconazole 0.3% (w/v). The samples were incubated at 37°C for three days. Colonies of bacteria that appeared were further purified using streak plate method and stored in a slant-butt in NA [17].

2.3 Morphological and biochemical characterization of endophytic bacteria

Bacterial isolates were morphologically characterized by observing their edges, color, elevation (appearance from the side), Gram staining, and motility test, as well as for their physiological characterization including starch and gelatin hydrolysis, triple sugar iron agar (TSIA) test and citrate utilization test [18].

2.4 Phosphate solubilization

The ability of endophytic bacteria to solubilize phosphate was determined on Pikovskaya agar using spot inoculation method [19]. Each isolates were grown in Pikovskaya’s medium and incubated at 35 ± 2°C for five days. Diameter of the colony as well as the clear zone was measured. Phosphate solubilization Index (PSI) was calculated as:

\[
\text{PSI} = \frac{\text{Diameter of clear zone}}{\text{Diameter of bacterial colony}}
\]  

(1)[20]
2.5 Measurement of IAA
IAA production was determined using modified Salkowski reagent as described by Gordon and Weber [21]. A 100 µL of 24 hours bacterial culture in Pikovskaya broth (OD$_{600}$ ≈ 0.5) was inoculated into 100 mL of nutrient broth medium in 250 mL flask containing 0.1 mg mL$^{-1}$ L-tryptophan. The flask was incubated at 35 ± 2°C for 48 hours in a rotary shaker (120 rpm). The culture was centrifuged for 30 minutes at 10,000 rpm. Two mL of the supernatant was mixed with equal volume of Salkowski reagent (0.1 M FeCl$_3$ + 400 mL of concentrated sulfuric acid (H$_2$SO$_4$) + 580 mL of aquadest). The solution were mixed by shaking and allowed to stay at ambient temperature for 30 minutes until pink color appeared. IAA concentration was measured spectrophotometrically of 530 nm. Un-inoculated broth was used as control.

3. Results and Discussion

3.1 Bacterial isolation
A total of 21 endophytic bacterial isolates were found in root, stem and leaves of nut grass (Table 1). Montañez et al. [14] isolated twenty two endophytic bacteria of IAA producer from corn.

| Isolate code | Form   | Colony Morphology | Cell morphology |
|--------------|--------|------------------|----------------|
| AZ$_1$       | Irregular | Undulate  | Flat  | white  | Diplo |
| AZ$_2$       | Irregular | Lobate  | Flat  | white  | Bacilli |
| AZ$_3$       | Circular | Entire   | Flat  | white  | Bacilli |
| AZ$_4$       | Circular | Entire   | Raised | White-yellowish | Diplobacilli |
| AZ$_5$       | Circular | Entire   | Raised | Off-white | Diplobacilli |
| AZ$_6$       | Circular | Undulate | Flat  | white  | Coccibacillus |
| AZ$_7$       | Irregular | Lobate  | Umbonate | White-yellowish | Bacilli |
| AZ$_8$       | Circular | Undulate | Flat  | Off-white | Bacilli |
| AZ$_9$       | Irregular | Undulate | Flat  | white  | Bacilli |
| AZ$_10$      | Circular | Entire   | Flat  | white  | Bacilli |
| AZ$_11$      | Irregular | Undulate | Raised | Off-white | Diplobacilli |
| AZ$_12$      | Circular | Undulate | Raised | White-yellowish | Bacilli |
| AZ$_13$      | Circular | Undulate | Raised | White-yellowish | Coccobacillus |
| AZ$_14$      | Circular | Entire   | Raised | White-yellowish | Bacilli |
| AZ$_15$      | Circular | Undulate | Raised | White-yellowish | Bacilli |
Table 3. Physiology and biochemistry characteristics of the endophytic bacterial isolates from nutgrass

| Isolate code | Starch utilization | Gelatin hydrolysis | Citrate utilization | Hydrogen sulphide producing | Motility test |
|--------------|--------------------|--------------------|--------------------|-----------------------------|--------------|
| AZ_1         | +                  | -                  | -                  | +                           | -            |
| AZ_2         | -                  | -                  | -                  | +                           | -            |
| AZ_3         | +                  | +                  | -                  | +                           | +            |
| AZ_4         | +                  | +                  | -                  | +                           | +            |
| AZ_5         | +                  | +                  | -                  | +                           | +            |
| AZ_6         | -                  | -                  | +                  | +                           | -            |
| AZ_7         | -                  | -                  | -                  | +                           | +            |
| AZ_8         | +                  | +                  | -                  | +                           | +            |
| AZ_9         | -                  | +                  | -                  | +                           | +            |
| AZ_10        | -                  | -                  | +                  | +                           | -            |
| AZ_11        | +                  | -                  | -                  | +                           | -            |
| AZ_12        | -                  | -                  | -                  | +                           | +            |
| AZ_13        | +                  | -                  | +                  | +                           | +            |
| AZ_14        | -                  | +                  | -                  | +                           | +            |
| AZ_15        | -                  | -                  | +                  | +                           | +            |

3.2 Assay of phosphate solubilization of endophytic bacteria

This test was performed to observe the ability of endophytic bacterial isolates in dissolving phosphate. Screening was done by growing the 24-hours pure culture of the isolates in Pikovskaya medium containing Ca$_3$(PO$_4$)$_2$ for seven days.

The ability of phosphate solubilization of the isolates was indicated by clear zone around the bacterial colonies (Figure 1). One out of fifteen bacterial isolates did not form a clear zone around the colony indicating unable to solubilizing phosphate (Table 4). It was observed that AZ_5 showed to have relatively high ability to solubilize phosphate with PSI of 6.27, while AZ_2, AZ_10, and AZ_14 showed low ability with PSI 1-1.15. Interestingly, the isolates showed to form clear zones in different days, indicated that each endophytic bacterial isolate had different capability in solubilizing phosphate. The ability to solubilize phosphate could be due to the medium complexity with the presence of other minerals as a complement, which makes the solubilization process more complicated [23].

![Figure 1](image-url)

Figure 1. (a). Clear zone around bacterial colony indicating endophytic bacterial isolates in solubilizing phosphate, and (b). bacterial colony
Table 4. Phosphate solubilization index of endophytic bacterial isolates

| Isolate code | PSI  |
|--------------|------|
| AZ1          | 1.33 |
| AZ2          | 1.15 |
| AZ3          | 1.83 |
| AZ4          | 3.75 |
| AZ5          | 6.27 |
| AZ6          | 3.41 |
| AZ7          | 1.36 |
| AZ8          | 1.16 |
| AZ9          | 1.71 |
| AZ10         | 1    |
| AZ11         | 2.35 |
| AZ12         | 3.31 |
| AZ13         | 2.75 |
| AZ14         | 1.15 |
| AZ15         | 3.13 |

3.3 Assay of indole acetic acid (IAA) production

All isolates showed to produce IAA. The production of IAA of the isolates seemed varied to some extent (Table 5). AZ5 showed to have relatively high IAA production, while AZ1 seemed to produce low IAA by 49.91 and 0.13 ppm, respectively (Table 5).

IAA is generally a type of plant hormone that is secreted by many bacterial symbionts regulating plant growth. Endophytic bacteria have a role in activating genes to colonize and to perform adaptation in plant tissues [24]. In this study, L-tryptophan was added in the culture as a precursor for IAA, although the production of IAA is also influenced by other precursor such as indol glycero phosphate [25].

Table 5. IAA production by endophytic bacteria isolates

| Isolate code | Concentration (ppm) |
|--------------|---------------------|
| AZ1          | 7.18                |
| AZ2          | 8.09                |
| AZ3          | 14.45               |
| AZ4          | 18.09               |
| AZ5          | 49.91               |
| AZ6          | 44.45               |
| AZ7          | 19.00               |
| AZ8          | 12.64               |
| AZ9          | 31.73               |
| AZ10         | 19.91               |
| AZ11         | 18.09               |
| AZ12         | 48.18               |
| AZ13         | 24.45               |
| AZ14         | 8.09                |
| AZ15         | 13.54               |

4. Conclusion

Fifteen endophytic bacteria were isolated from nutgrass (Cyperus rotundus). Eleven isolates were Gram-negative, and the rest were Gram-positive. Out of the fifteen isolates, AZ5
isolate showed to have relatively high IAA production and phosphate solubilization. This isolates might be potential to be used as biofertilizer.

Acknowledgements
The authors would like to express our greatest gratitude to North Nias District Government for funding this study.

References
[1] Marra LM, Soares CRFS, de Oliveira SM, Ferreira PAA, Soares BL, de Fráguas Carvalho R, de Lima JM, de Souza Moreira F M. 2012. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant and Soil*, 57, 289-307.
[2] Cohen AC, Bottini R, Piccoli P. 2008. *Azospirillum brasilense* sp. 245 produces ABA in chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. *Plant Growth Regulation*, 54, 97-103.
[3] Gabriela F, Oscar M, Marí a JI, Sergio A, Daniel A, Guillermi A. 2010. Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. *Current Microbiology*, 61, 485-493.
[4] Malfanova N, Kamilova F, Validov S, Shcherbakov A, Chebotar V, Tikhonovich I, Lugtenberg B. 2011. Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. *Microbial Biotechnology*, 4, 523-532.
[5] Patricia P, Claudia T, Ana C, Laura S, Paula C, Ricardo M, Rubén B. 2011. An endophytic bacterium isolated from roots of the halophyte *Prosopis strombulifera* produces ABA, IAA, gibberellins A1 and A3 and jasmonic acid in chemically-defined culture medium. *Plant Growth Regulation*, 64, 207-210.
[6] Brooks D S, Gonzalez C F, Appel D N, Filer T H. 1994. Evaluation of endophytic bacteria as potential biocontrol agents for oak wilt. *Biological Control*, 4, 373-381.
[7] Kuklinsky SJ, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environmental Microbiology*, 6, 1244-1251.
[8] Strobel dan Daisy (2003) Bioprospecting for Microbial Endophytes an Their Natural Products. Microbiol. and Mol. Biology, 67(4): 63-68.
[9] Anzuay MS, Frola O, Angelini JG, Luduena LM, Fabra A, Taurian T (2013) Genetic diversity of phosphate-solubilizing peanut (*Arachis hypogaea* L.) associated bacteria and mechanisms involved in this ability. *Symbiosis*, 60, 143-154.
[10] Yurnaliza MW, Siregar dan Priyani N (2011) Peran Bakteri Endofit Penghasil IAA (Indole Acetic Acid) Terseleksi Terhadap Pertumbuhan Tanaman Padi (*Oryza sativa* L.). Prosiding Seminar Nasional Biologi, 219-228.
[11] Patten CL and BR Glick (2002) Role of Pseudomonas putida Indole Acetic Acid in Development of The Host Plant Root System. *Appli Environ Microbiol*. 68:3795-3801.
[12] Duangpaeng P, Phetcharat S, Chanthapho N, Boonkantong N, Okuda (2012) The Study *Porcedia Engineering*, 32,172-176.
[14] Montañez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro. Applied Soil Ecology, 58, 21-28.

[15] Chen Y, Fan JB, Du L, Xu H, Zhang QH, He QY (2014) The application of phosphate solubilizing endophyte Pantoea dispersa triggers the microbial community in red acidic soil. Applied Soil Ecology, 84, 235-244.

[16] Joe MM, Devaraja S, Benson A, Tongmin S (2016) Isolation of phosphate solubilizing endophytic bacteria from Phyllanthus amarus Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. Journal of Applied Research on Medicinal and Aromatic Plants, ISSN: 2214-7861.

[17] Hallmann JG Berg (2006) Spectrum and population dynamics of bacteri root endophytes. Berlin, Heidelberg, Germany, Springer-Verlag. 15-31.

[18] Mac Faddin JF (1979) Biochemical test for identification of medical bacteria. Baltimore (US). The William and Wilkins Company, 1(5), 2365-2370.

[19] Harley and LM Prescott (2003) Microbiology. 5th Edition. Mc Graw Hill, New York.

[20] Hidayati U 2014. Potensi bakteri endofit asal tanaman karet sebagai pemacu pertumbuhan bibit batang bawah tanaman karet (Hevea brasiliensis Müll. Arg.) [Disertation]. Bogor (ID): Bogor Institute of Agriculture.

[21] Gordon SA dan Weber RP (1951) Colorimetric Estimation of Indoleacetic Acid. Plant Physiol, 26(1):192–195.

[22] Yan Chen, Jian-Bo Fan, Lian Du, Huan Xu, Qi-Hai Zhang, Yuan-Qiu He (2014) The application of phosphate solubilizing endophyte Pantoea dispersa triggers the microbial Applied Soil Ecology, 84, 235-244.

[23] Narsian V, Ahmed Abu, Samaha SM, Patel HH (2010) Rock phosphate dissolution by specific yeast. Indian J. Microbiol. 50 (1), 57-62.

[24] Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances, 29, 248-258.

[25] Pollmann S, Düchting P, Weiler EW (2009) Tryptophan-dependent indole-3-acetic acid biosynthesis by ‘IAA-synthase’ proceeds via indole-3-acetamide. Phytochemistry, 70, 523-531.

[26] Aryanth, INP, DP Lestari and NPD. Pangesti (2005) Potensi Isolat Bakteri Penghasil IAA dalam Peningkatan Pertumbuhan Kecambah Kacang Hijau pada Kondisi Hidroponik. Jurnal Mikrobiologi Indonesia. 9(2):43-46.

[27] Doty SL (2011) Nitrogen-fixing endophytic bacteria for improved plant growth. Maheswari DK, editor. In Bacteria in Agrobiology: Plant Growth Responses. Springer Berlin Heidelberg. 183-199.

[28] Feng K, Lu HM, Sheng HJ, Wang Xl and Mao J (2006) Effect of organic ligands on biological availability of inorganic phosphorus in soils. Pedosphere. 14:85-92.

[29] Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital capacity of source microbial species. Microbiologiya,17, 362-370.