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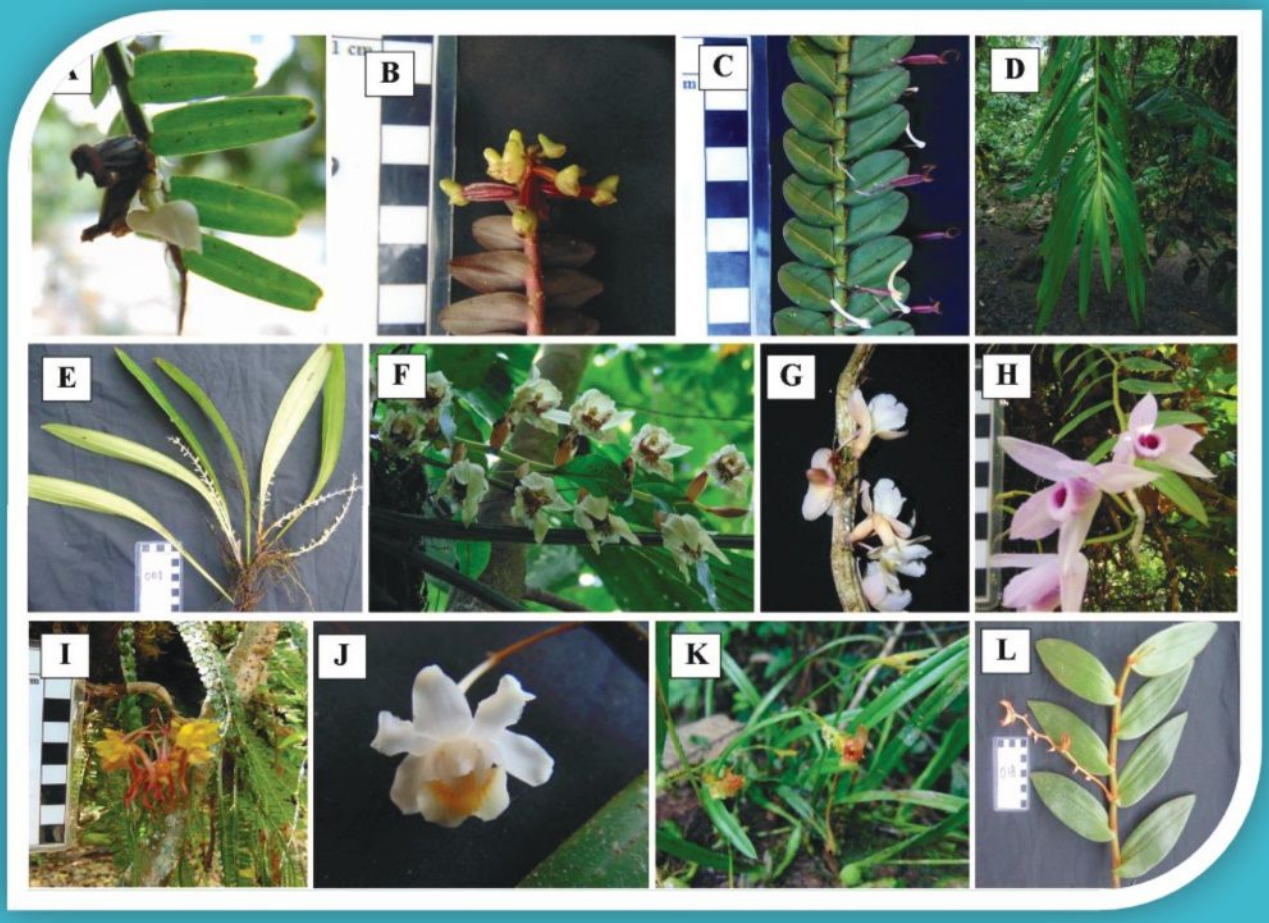

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Alamat (*Address*)
Pusat Penelitian Biologi-LIPI
Kompleks Cibinong Science Center (CSC-LIPI)
Jalan Raya Jakarta-Bogor KM 46,
Cibinong 16911, Bogor-Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (021) 8765059
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id
jurnalberitabiologi@gmail.com

Keterangan foto cover depan: Jenis anggrek epifit di kaki gunung Liangpran.
(*Notes of cover picture*): (*The epiphytic orchids in the foothill of Mount Liangpran)* sesuai dengan halaman 312 (*as in page 312*).

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THE PHYSIOLOGICAL CHARACTERS OF BACTERIA ISOLATED FROM BANANA TREE'S RHIZOSPHERE FROM MALAKA, EAST NUSA TENGGARA, AND THEIR ROLES ON PLANT GROWTH PROMOTION ON MARGINAL LAND

[Karakter Fisiologi Bakteri yang Diisolasi dari Rizosfer Pisang asal Malaka, Nusa Tenggara Timur, dan Peranannya sebagai Pemacu Tumbuh Tanaman pada Lahan Marjinal]

Toga P. Napitupulu*✉, Atit Kanti, and I Made Sudiana

Microbiology Divison, Research Center for Biology, Indonesian Institute of Sciences
Jl. Raya Bogor Km 46, Cibinong 16911, Indonesia
email: toga.napitupulu@gmail.com

ABSTRACT

The objective of the study was to isolate microorganisms that can produce growth promoting hormone, fix atmospheric nitrogen, and solubilize inorganic phosphate. They may be used for enhancing the growth of banana tree in marginal land. A total of 25 bacteria associated with banana tree that belonged to six genera were isolated from Malaka, East Nusa Tenggara, Indonesia. The genera are *Acinetobacter*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Rhizobium*, and *Cupriavidus* genera. *Gammaproteobacteria* is the dominant class, followed by *Betaproteobacteria* and *Alphaproteobacteria.* All isolates were screened for multiple plant growth promoting traits which may play roles in banana tree growth, namely fixing nitrogen, solubilizing phosphate, and producing Indole Acetic Acid (IAA). Twenty-two isolates were capable to fix nitrogen, 21 isolates can solubilize insoluble phosphate, and 15 isolates produced IAA that is dependent on L-Tryptophan presence. Despite most of the bacteria isolates exhibited one plant growth-promoting activities, *Enterobacter* and *Klebsiella* genera showed three of the plant growth promoting bacteria traits.

Key words: Banana, marginal land, growth promoting bacteria

ABSTRAK

Tujuan penelitian ini adalah untuk mengisolasi mikroorganisme yang dapat menghasilkan hormon pertumbuhan, memfiksasi nitrogen atmosfer, dan melarutkan fosfat anorganik yang dapat digunakan untuk meningkatkan pertumbuhan pisang di lahan marjinal. Sebanyak 25 bakteri yang terkait dengan pisang telah diisolasi dari Malaka, Nusa Tenggara Timur, Indonesia yang terdiri dari enam genera dari genotipe *Acinetobacter*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Rhizobium*, dan *Cupriavidus*. *Gammaproteobacteria* adalah kelas dominan, diikuti oleh *Betaproteobacteria* dan *Alphaproteobacteria*. Semua isolat diuji untuk mengetahui beberapa karakteristik peningkat pertumbuhan tanaman khusunya pada pisang, yaitu memfiksasi nitrogen, melarutkan fosfat, dan memproduksi Indole Acetic Acid (IAA). Dua puluh dua isolat mampu memfiksasi nitrogen, 21 isolat mampu melarutkan fosfat yang terlarut, dan 15 isolat menghasilkan IAA yang tergantung pada ketersediaan prekursor L-Tryptophan. Meskipun sebagian besar isolat bakteri menunjukkan satu aktivitas pertumbuhan tanaman, *Enterobacter* dan *Klebsiella* genera menunjukkan tiga karakteristik promosi pertumbuhan tanaman.

Kata Kunci: pisang, tanah marjinal, bakteri pemacu tumbuh

INTRODUCTION

In general, marginal land has been defined as unsuitable land for food production due to its low quality land, thus economically inferior (Shortall, 2013). The limitations of marginal lands include low quality soil, very low rainfalls, extreme temperatures, steep land, or other problems that make it unsuitable for agriculture. In Indonesia itself, total amount of marginal land reach approximately 2% of total land (Milbrandt and Overend, 2009). However, marginal land has great potential and possibility to be converted into favorable land with more beneficial features to fulfill global needs, especially the availability of energy and food resources.

Microorganisms are key components of the soil environment as well as quality and play a very important part in order to enhance the suitability of land including marginal land. Their roles can include nitrogen fixation, solubilizing insoluble phosphate, or production of growth stimulants (Souza, Ambrosini, and Passaglia, 2015). In the sense of marginal lands, soil microorganisms can have ability to alleviate either abiotic or biotic stress toward plants. Furthermore, some plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas chlororaphis, Bacillus amyloliquefaciens,* and *Gluconacetobacter diazotrophicus* were reported to enhance drought stress adaptation in various plants

*Kontributor Utama
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(Lim and Kim, 2013). Some microorganisms have ability to suppress plant diseases by directly confront pathogens or induce system resistance of the plants (Beneduzi, Ambrosini, and Passaglia, 2012; Napitupulu T.P.; Ilyas M.; Kanti A.; and Sudiana IM., 2019).

In order to convert marginal land for agricultural purpose, some betterments of soil conditions are needed. The limited nutrients followed by other extreme conditions in marginal land need microbes with plant growth promoting features. The utilization of microbial approach and technology is considered more environmental friendly for agricultural practice (Glick, 2012). Therefore, searching and screening of suitable microorganisms that have high adaptability in non-favorable condition is desired. The objective of this study was to isolate microorganisms that have plant growth promoting characteristics for enhancing the growth of banana tree in marginal land. Banana as a staple food is one of the most important and cultivable crops that shows the increase of demand annually. Consequently, cultivation optimization of banana tree through application of plant growth promoting bacteria is expected to gain banana production yields.

MATERIALS AND METHODS

Isolation of Bacteria

Soil sample was collected from rhizosphere of banana plantation in Malaka, East Nusa Tenggara, Indonesia. The sample was suspended in minimal salt broth and incubated for 7 days without shaking at room temperature. Further, it was transferred to different media in dilution from 10^{-2} to 10^{-3}, poured to Pikovskaya agar, Yeast Mannitol Agar (Yema), Sucrose-Ashby agar, and Caceres agar, then incubated at 30 ^{o}C for 2 weeks. For each medium, colonies were selected and picked up according to their different color and size, then transferred to new plates containing initial kind of agar media until obtaining single colony as pure culture. Isolates were preserved and stored in 10% glycerol at -80 ^{o}C for long term storage.

PCR amplification of 16S rRNA gene

All isolates were grown on selected medium for overnight then picked up by using sterile toothpicks, suspended in 15–20 µl of sterile nuclease free water (NFW), boiled at 80 ^{o}C for 10 min, then spin down for a while. One µl of genomic DNA from bacteria was used as a DNA template for colony PCR. The PCR mixture, containing 12.5 µl GoTaq Green Master Mix (Promega), 1 µl primer 27f (5'-AGA GTT TGA TCC TGG CTC AG-3'), 1 µl 1492r (5'-GGT TAC CTT GTT ACG ACT T-3'), 1 µl DNA template and 0.5 µl DMSO was made up to 25 µl with nuclease free water (Schumann, 1991). The PCR conditions were consisted of an initial denaturation at 96 ^{o}C for 5 min, followed by 30 cycles of denaturation at 96 ^{o}C for 30 sec, annealing at 55 ^{o}C for 30 sec and extension at 72 ^{o}C for 1 min, followed by final extension at 72 ^{o}C for 7 min in a thermocycler Arktik Thermo Cycler (Thermo Scientific). All PCR products (1.5 µl volumes) were analyzed on 1% agarose gel (1st BASE) by electrophoresis Mipid-exu (Advance) for 23 min at 100 V then soaked in EtBr for 30 min and washed in 1x TAE buffer. The gel was viewed under UV light in Gel DocTM EZ Imaginer (Bio-Rad). These PCR products were sent to 1st BASE (Singapore) for sequencing. The sequencing results were analyzed using BioEdit software (Hall, 1999), then uploaded to EzTaxon (Chun *et al.*, 2007; Kim *et al.*, 2012; Yoon *et al.*, 2017) to align and found out the similarity with other sequences in database. The phylogenetic trees were performed by MEGA7 program (Kumar, Stecher, and Tamura, 2016).

Screening for plant growth promoting traits

Nitrogen fixation. All isolates were transferred to semi solid NFb medium for the analysis of their ability to fix nitrogen. The composition of semi solid NFb medium is as follows (l^{-1}): Malic acid (5 g); K_2HPO_4 (0.5 g); $MgSO_4.7H_2O$ (0.2 g); NaCl (0.1 g); $CaCl_2.2H_2O$ (0.02 g); KOH (4 g); bromothymol blue 0.5% in 0.2 N KOH (2 ml); 1.64% Fe EDTA (4 ml); filtered vitamin solution (1 ml); filtered micronutrient solution (2 ml) and agar (2 g). The pH was adjusted to 6.8. The vitamin solution contains, in 100 ml, biotin (0.01 g) and pyridoxol HCl (0.02 g), dissolved at 100 ^{o}C in a water bath stirer. The micronutrients solution consist of the foloowing (l^{-1}): $CuSO_4.5H_2O$ (0.4 g); $ZnSO_4.7H_2O$ (0.12 g); H_2BO_3 (1.4 g); $Na_2MoO_4.2H_2O$ (1.5 g); $MnSO_4.H_2O$ (1.5 g) (Eckert *et al.*, 2001).

Phosphate Solubilizing. The isolates were cultured on Pikovskaya's plates supplemented with $CaCO_3$ (HiMedia), then incubated for 3–7 days at 30 ^oC. A clear zones formation around colony indicates positif result of phosphate solubilizer bacteria.

Indole Acetic Acid (IAA) production. All isolates were cultured on half-strength Tryptic Soy Agar (TSA) plates, then transferred to half-strenght TSA supplemented with L-Tryptophan (200 ppm) as the precursor. Further, the colonies were poured with Salkowsky's reagent, stored at dark room for 30 min. A pink color indicates positive result of IAA production. The composition of the half-strenght TSA is as follows (1^{-1}): Peptone (10 g), NaCl (2.5 g) and agar (20 g). The pH was adjusted to 7.0 and 200 µl/ml L-Tryptophan was filtered and added. The Salkowsky's reagent contains 250 ml of 35% HClO4 (70–72%) and 250 ml water, added with 1 ml of 0.5 M $FeCl_3.6H_2O$ (Gordon and Weber, 1951).

RESULTS

Isolation and Identification of Bacteria

Twenty five bacteria species were isolated from the rhizosphere soil of banana using various kind of agar (Table 1). MSA was used for isolation to obtain several bacteria that were capable to growth in extreme conditions with very limited nutrition. The soil samples was initially suspended in minimal salt broth in order to resemble condition in marginal lands that have limited resources, particularly carbon source. The culture broth was then poured to different medium for specific selection.

Table 1. Bacterial isolates of banana tree's rhizosphere soil from Malaka, East Nusa Tenggara **(***Isolat Bakteri dari Rizosfer Pisang asal Malaka, Nusa Tenggara Timur*)

No.	Sample code (*Kode Sample)*	Species Name (*Nama Spesies)*	Bacterial Genera (*Genera Bakteri)*	Classes (*Kelas)*
1	B1	*Acinetobacter baumannii*	*Acinetobacter*	*Gammaproteobacteria*
2	B2	*Enterobacter cloacae subsp. dissolvens*	*Enterobacter*	*Gammaproteobacteria*
3	B3	*Pantoea stewartii subsp. indologenes*	*Pantoea*	*Gammaproteobacteria*
4	B4	*Enterobacter aerogenes*	*Enterobacter*	*Gammaproteobacteria*
5	B5	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
6	B6	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
7	B7	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
8	B8	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
9	B9	*Enterobacter aerogenes*	*Enterobacter*	*Gammaproteobacteria*
10	B10	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
11	B11	*Enterobacter xiangfangensis*	*Enterobacter*	*Gammaproteobacteria*
12	B12	*Pantoea stewartii subsp. indologenes*	*Pantoea*	*Gammaproteobacteria*
13	B13	*Klebsiella pneumoniae subsp. pneumoniae*	*Klebsiella*	*Gammaproteobacteria*
14	B14	*Enterobacter xiangfangensis*	*Enterobacter*	*Gammaproteobacteria*
15	B15	*Acinetobacter baumannii*	*Acinetobacter*	*Gammaproteobacteria*
16	B16	*Rhizobium radiobacter*	*Rhizobium*	*Alphaproteobacteria*
17	B17	*Acinetobacter baumannii*	*Acinetobacter*	*Gammaproteobacteria*
18	B18	*Acinetobacter baumannii*	*Acinetobacter*	*Gammaproteobacteria*
19	B19	*Acinetobacter baumannii*	*Acinetobacter*	*Gammaproteobacteria*
20	B20	*Acinetobacter pittii*	*Acinetobacter*	*Gammaproteobacteria*
21	B21	*Cupriavidus oxalaticus*	*Cupriavidus*	*Betaproteobacteria*
22	B22	*Cupriavidus oxalaticus*	*Cupriavidus*	*Betaproteobacteria*
23	B23	*Enterobacter bugandensis*	*Enterobacter*	*Gammaproteobacteria*
24	B24	*Rhizobium pusense*	*Rhizobium*	*Alphaproteobacteria*
25	B25	*Cupriavidus alkaliphilus*	*Cupriavidus*	*Betaproteobacteria*

Nitrogen fixation ability

Due to a very important role of nitrogen toward plant growth, 25 of the isolates were screened for the ability to fixate nitrogen, while 22 isolates were capable to fix nitrogen (Table 2). The nitrogen-fixing abilities of these strains were confirmed using qualitative in vitro analysis growth in NFb medium. These strains formed white ring on NFb medium indicating a positive result for their ability to fix atmospheric nitrogen.

Indole-3-acetic acid producting bacteria

Indole-3-acetic acid or commonly abbreviated as IAA is the most common and naturally occurring of plant hormone of the auxin class that has function to modulate plant growth (Zhao, 2010). Biosynthesis of IAA by bacteria is mostly L-tryptophan dependent and use it as precursor (Mohite, 2013). A pink color solution indicated ability of bacteria to produce IAA after poured with Salkowski's reagent. Fifteen isolates, out of 25, had ability to produce IAA in various degree (Figure 1).

Phosphate solubilizer

This study presented that most bacteria species were solubilized inorganic phosphate indicated by a clear zone around colonies and shown in Figure 2. Twenty one out of 25 isolates showed phosphate solubilizing activity in various degree of solubilization. These phosphate solubilizing bacteria were belonged to genera of *Acinetobacter*, *Enterobacter*, *Klebsiella*, and *Pantoea*. *Rhizobium radiobacter* also shows phosphate solubilizing activity, but *Rhizobium pusense* has negative result according to this bioassay.

Table 2. Nitrogen fixation ability of bacterial isolates from banana rhizosphere soil from Malaka, East Nusa Tenggara **(***Kemampuan pengfiksasi nitrogen isolat-isolat bakteri dari rizosfir pisang asal Malaka, Nusa Tenggara Timur*)

No.	Code (*Kode*)	Nitrogen fixation* (*Fiksasi Nitrogen*)	Species name (*Nama Spesies*)
1	B1	+	*Acinetobacter baumannii*
2	B2	+	*Enterobacter cloacae* subsp. *dissolvens*
3	B3	-	*Pantoea stewartii* subsp. *indologenes*
4	B4	+	*Enterobacter aerogenes*
5	B5	+	*Enterobacter bugandensis*
6	B6	+	*Enterobacter bugandensis*
7	B7	+	*Enterobacter bugandensis*
8	B8	+	*Enterobacter bugandensis*
9	B9	+	*Enterobacter aerogenes*
10	B10	+	*Enterobacter bugandensis*
11	B11	+	*Enterobacter xiangfangensis*
12	B12	-	*Pantoea stewartii* subsp. *indologenes*
13	B13	+	*Klebsiella pneumoniae* subsp. *pneumoniae*
14	B14	+	*Enterobacter xiangfangensis*
15	B15	+	*Acinetobacter baumannii*
16	B16	+	*Rhizobium radiobacter*
17	B17	+	*Acinetobacter baumannii*
18	B18	+	*Acinetobacter baumannii*
19	B19	+	*Acinetobacter baumannii*
20	B20	+	*Acinetobacter pittii*
21	B21	+	*Cupriavidus oxalaticus*
22	B22	+	*Cupriavidus oxalaticus*
23	B23	+	*Enterobacter bugandensis*
24	B24	-	*Rhizobium pusense*
25	B25	+	*Cupriavidus alkaliphilus*

*+ means positive result; - means negative results

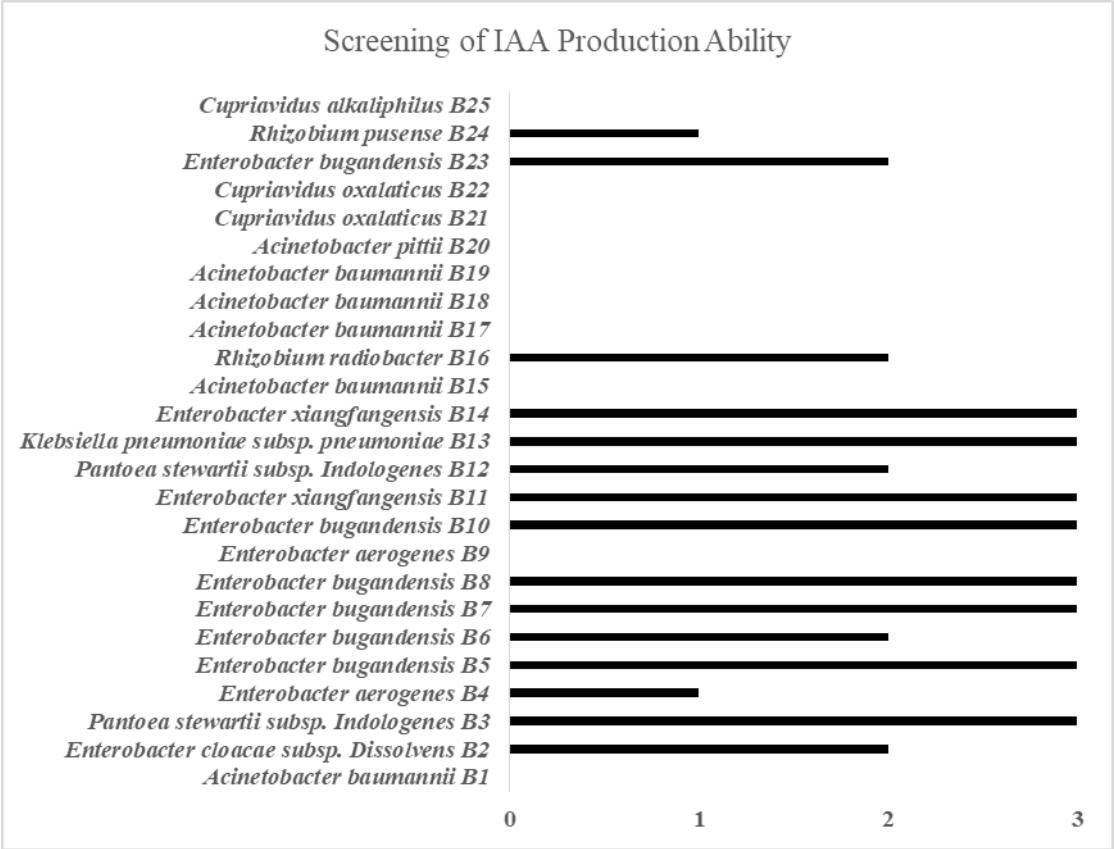

Figure 1. IAA production ability of bacterial isolates from banana tree rhizosphere soil from Malaka, East Nusa Tenggara. 0 means negative activity. The degree of activity to produce IAA increases with increasing number 1<2<3 **(***Kemampuan produksi IAA isolat-isolat bakteri dari rizosfer pisang asal Malaka, Nusa Tenggara Timur. 0 menandakan aktifitas yang negatif. Derajat aktifitas dalam memproduksi IAA meningkat seiring dengan peningkatan angka 1<2<3*)

DISCUSSION

According to molecular analysis based on 16S rRNA gene, 25 isolates of bacteria were belonged to *Gammaproteobacteria,* with the dominant genera was *Enterobacteriaceae*. The rest are *Alphaproteobacteria* and *Betaproteobacteria*, 2 and 3 strains respectively. Similarly, *Enterobacteriaceae*, among other high diversity bacteria, is found as dominant family of bacteria in soil of three different traditional banana farms in Uganda. Köberl, Dita, Martinuz, Staver, and Berg (2017) reported that diversity of *Gammaproteobacteria* is indicator of healthy banana plants in *Fusarium oxysporum* f.sp. *cubense* infected area in Central America. Hence, the *Gammaproteobacteria* class were associated with banana-associated areas in tropical countries. The presence of *Cupriavidus* sp. and *Acinetobacter* sp. is linked to environmental adaptability of heavy metal resistant bacteria in soil (Jiang, Pan, Xiao, Yang, and Zhang, 2017).

Typically less than 1% of microorganisms from an environmental sample can be cultured in the laboratory leaving the immense amount of the uncultured diversity unexplored (Amann, Ludwig, and Schleifer, 1995). Although study of diversity of bacteria through traditional culture-dependent technique can provide an overview regarding the soil condition, it is necessary to extend the observation by applying more cutting-edge approach in order to get more comprehensive information. Combination of culture-based methods with metagenomics analysis is able to improve

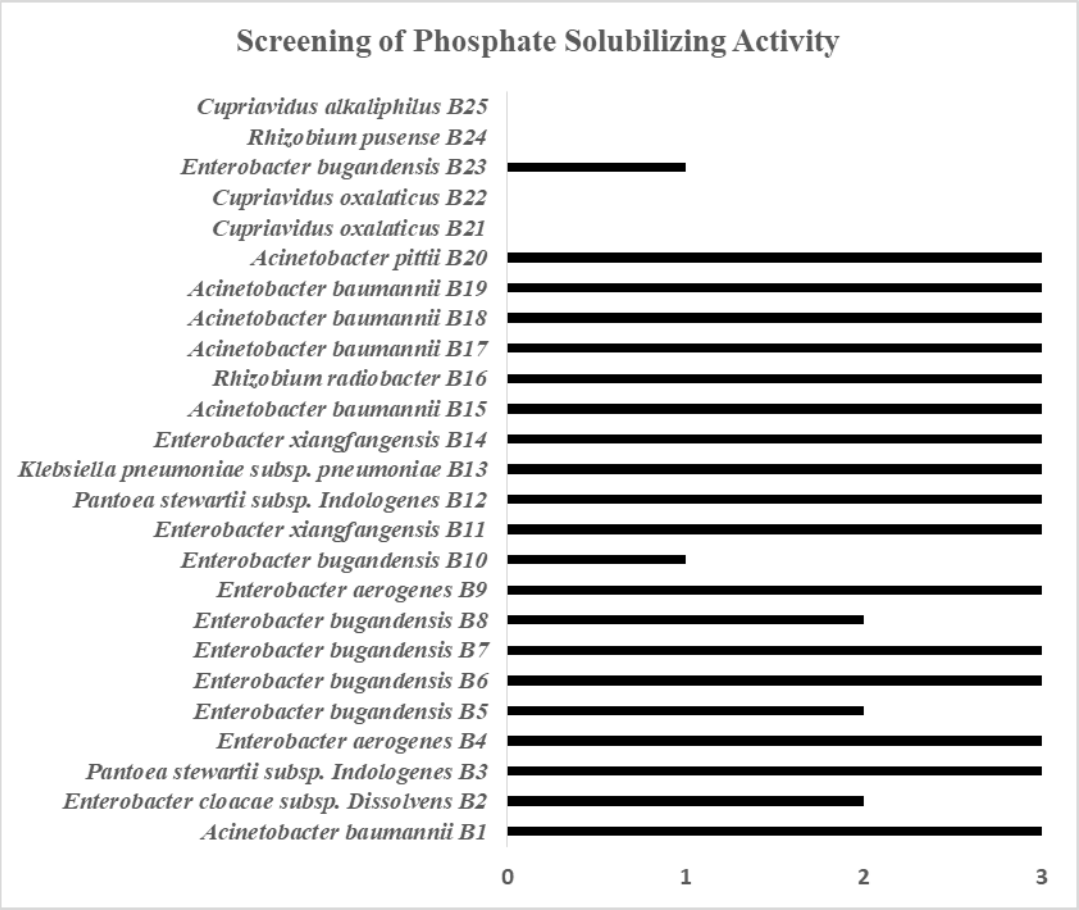

Figure 2. Phosphate solubilizing ability of bacterial isolates from banana tree rhizosphere soil from Malaka, East Nusa Tenggara. 0 means negative activity. The degree of activity to produce IAA increses with increasing number 1<2<3 **(***Kemampuan pelarutan fosfat isolat-isolat bakteri dari rizosfer pisang asal Malaka, Nusa Tenggara Timur. 0 menandakan aktifitas yang negatif. Derajat aktifitas dalam memproduksi IAA meningkat seiring dengan peningkatan angka 1<2<3*)

bioprospecting particularly in harsh and difficult environment (Vester, Glaring, and Stougaard, 2015).

Except *Pantoea* sp. and *Rhizobium pusense*, the rest of bacterial isolate show positive result as nitrogen-fixing bacteria. Instead of beneficial as plant growth promoting agent, *Pantoea stewartii* is an opportunistic bacteria to human which also a plant pathogen that causing stewartii wilt. Although *Rhizobium* genera are well-known as nitrogen fixing bacteria even in harsh condition and in arid climate (Zahran, 1999), our result shows that one of member of genera, *Rhizobium pusense*, did not show nitrogen fixation activity. Meanwhile, all *Enterobacter* genera isolates have ability to fix nitrogen. Likewise, *Enterobacter* associated with some crops such as sugarcane (Lin *et al*., 2012) and wild rice (Peng *et al*., 2009) also possess the ability to fixate nitrogen from the atmosphere.

Enterobacter sp., *Pantoea stewartii*, and *Klebsiella pneumoniae* were the higher IAA producing bacteria in this study based on the color intensity. Most *Enterobacter* produced IAA but in various level of concentration. This is indication of the strain-dependent ability of *Enterobacter* species to biosynthesize growth hormone IAA. Moreover, production of IAA by *Enterobacter* sp was reported with their association with various plants like in *Abrus precatorius* (Ghosh, Sen, and Maiti, 2015) and sugarcane (Rodrigues, Forzani, Soares, Sibov,

and Vieira, 2016). All *Acinetobacter* in this assay did not produce IAA. In a study conducted by Huddedar *et al*. (2002), only approximately 20% of *Acinetobacter* isolated from rhizosphere of wheat have ability to produce IAA. *Klebsiella pneumoniae*, a human pathogen, also showed a plant growth promoting characteristic, in a high level producing IAA and solubilizing phosphate. Similar result reported by Bhardwaj, Shah, Joshi, and Patel (2017) that *Klebsiella pneumoniae* VRE36 associated with sugarcane exhibit important plant growth promoting traits.

In soil, most phosphate occur as insoluble form that cannot be absorbed by plants (Rengel and Marschner, 2005). In order to provide nutrient requirement of crops, chemical phosphate fertilizer is usually added to soil, but long term impacts on the environment in terms of eutrophication, soil fertilility depletion, and carbon footprint are major obstacles for the application (Sharma, Sayyed, Trivedi, and Gobi, 2013). Some microorganisms have ability to solubilize insoluble phosphate through these proposed mechanisms: acidification of the medium, production of chelating metabolites, and redox activity (Altomare, Norvell, Björkman, and Harman, 1999). Therefore, the utilizitation of this beneficial trait of microorganisms particularly bacteria to solubilize insoluble phosphate is important for plant phosphate nutrition.

All *Acinobacter* genera shows high phosphate solubilization. *Acinobacter* strains are reported to solubilize tri calcium phosphate through acidification of medium (the pH dropped to below 4.7 from 7.8) by production of gluconic acid (Ogut, Er, and Kandemir, 2010). This significant shifting of acidity explains the high level solubilizing phosphate activity. The capability of *Enterobacter* to solubilize insoluble phosphate shows variability even among same species. Similar with its capability to produce IAA, the phosphate solubilizing ability is also indicated to have strain-dependent tendency. A recent study showed that *Enterobacter* sp. are potential as phosphate solubizing bacteria and has significant impact on crop growth in field experiment (Borham, Belal, Metwaly, and El-gremy, 2017). *Cupriavidus* sp, member of *Betaproteobacteria*, have negative result both on IAA producing and phosphate solubilizing bioassay. However, some *Cupriavidus* species possess plant growth promoting traits, such as secretion of IAA, siderophores, and solubilization of phosphate (Yu, Liu, Zhu, Liu, and Mao, 2011).

CONCLUSION

Using culture-dependent technique, isolation towards banana tree's rhizosphere soil from Malaka, East Nusa Tenggara, shows diversity in which *Gammaproteobacteria* is dominant class, followed by *Betaproteobacteria* and *Alphaproteobacteria*. Total six genera were isolated, namely *Acinetobacter*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Rhizobium*, and *Cupriavidus.* All genera show at least one plant growth promoting traits, namely fixing nitrogen, solubilizing phosphate, and producing IAA, while *Enterobacter* and *Klebsiella* have all of them.

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.

8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al*. Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Jika sitasi beruntun maka dimulai dari tahun yang paling tua, jika tahun sama maka dari nama penulis sesuai urutan abjad. Contoh: (Anderson, 2000; Agusta *et al*., 2005; Danar, 2005). Penulisan daftar pustaka, sebagai berikut:

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      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565–1569.
   b. **Buku**
      Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Development and Tramsmission*. 2nd ed. CABI Publishing. New York. pp. 650.
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   d. **Makalah sebagai bagian dari buku**
      Sausan, D., 2014. Keanekaragaman Jamur di Hutan Kabungolor, Tau Lumbis Kabupaten Nunukan, Kalimanan Utara. Dalam: Irham, M. & Dewi, K. eds. *Keanekaraman Hayati di Beranda Negeri*. pp. 47–58. PT. Eaststar Adhi Citra. Jakarta.
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      Sundari, S., 2012. Soil Respiration and Dissolved Organic Carbon Efflux in Tropical Peatlands. *Dissertation*. Graduate School of Agriculture. Hokkaido University. Sapporo. Japan.
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      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

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