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Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet: Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4:187. www.molecularsystembiology.com

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Antagonist and plant growth promoting potential of indigenous bacteria isolated from oil palm empty fruit bunches

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Abstract. *Dermiyati, Suharjo R, Telaumbanua M, Yosita R, Sari AW, Andayani AP. 2023. Antagonist and plant growth promoting potential of indigenous bacteria isolated from oil palm empty fruit bunches. Biodiversitas 24: 1136-1142.* The present study aimed to identify of bacteria isolated from the suspension extract of oil palm empty fruit bunches (EFB) and evaluate their potential against *Ganoderma boninense* fungus and plant growth promoter. Bacterial isolates from the previous studies were tested for their ability through antagonistic tests. A plant growth promoter test was carried out on the selected isolates with the inhibition percentage criteria > 50%, a clear zone index > 3 in the phosphate solvent test and a clear zone index > 2 in the chitin-reducing test. The identification of bacterial isolates was carried out molecularly using a Polymerase Chain Reaction (PCR) analysis. A total of 220 bacterial isolates were tested, consisting of 84 isolates under aerobic, 68 isolates under anaerobic, and 68 isolates under facultative anaerobic conditions. It was found that the bacteria to antagonize 156 bacterial isolates (70.90%), dissolve phosphate 118 bacterial isolates (53.64%), and none of the bacterial isolates could reduce chitin. A significant increase occurred in root length, root wet weight and root dry weight so that bacterial isolates with isolate codes ASPB1, ANSP14, and SSPB2 had the potential to promote plant growth. These three isolates were identified into the genus *Bacillus*, namely species of *B. velezensis* (in aerobic condition), *B. paramycoides* (in anaerobic condition), and *B. tequilensis* (in facultative anaerobic condition).

Keywords: Antagonists, *Ganoderma boninense*, indigenous bacteria, oil palm empty fruit bunches

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the essential plantation crops in the agricultural sector which contributes in oil palm production and to the world economy (Nurfatriani et al. 2019). Oil palm is the main crop that can meet the global oil demand. The need for oil palm is estimated to reach 240 million tons by 2050, so the production potential must be increased by 11-18 tons per hectare (Barcelos et al. 2015). According to the Directorate General of estate crops (2018), oil palm production in Indonesia continued to increase annually until 2017, reaching 35.359.384 tons with an area of 12,307,677 hectares. Increasing palm oil production is a measure of success in managing oil palm plants. However, there are obstacles in the cultivation of oil palm plants, one of which is basal stem rot disease (Siddiqui 2021) which has an average oil palm infection rate of ca. 45% in Sumatera, Indonesia (Paterson 2019) and can cause losses of up to 50-80% per hectare (Rees et al. 2012).

Stem rot is a major disease in oil palm plantations caused by *Ganoderma boninense*, a pathogenic fungus that completes most of its life cycle in the soil and is very difficult to control. It is very hard to realize its presence in the soil and host plant. Once the symptoms appear, the plant is in the late state of infection. In this case, it is too late to carry out control of *G. boninense* on the infected plants. Preventive method using beneficial microbes is one of the promising eco-friendly and long-life control strategies.

The severity of *G. boninense* infection in oil palm plants is affected by the lack of the availability of bacteria that are competitive against pathogens in the soil (Siddiqui 2021). Once the population of beneficial microbes and pathogens is imbalanced, the beneficial microbes could not fully compete the plant pathogen (i.e. *G. boninense*) causing massive invasion and infection of the plant pathogens to the host plant. Here, introduction of indigenous beneficial microbes, including bacteria, is strongly recommended to create balance ecosystem in the oil palm rhizosphere.

Oil palm bunch is a waste of oil palm plantation that can be a source of indigenous beneficial microbes that can be applied in the field. Some studies suggest that

indigenous bacteria showed multi beneficial task. It has been reported that some of indigenous bacteria showed antagonistic capability against plant pathogens (Beneduzi et al. 2012). According to Hushiarian et al. (2013), some of antagonistic bacteria can be used as biocontrol agents in suppressing stem rot disease caused by *G. boninense*. Besides, indigenous bacteria also have the ability as a phosphate solvent (Satyaprakash et al. 2017; Dermiyati et al. 2019), reduce chitin (Suryanto et al. 2012), and improve the quality of plant growth (Vishwakarma et al. 2018). According to Lai et al. (2017), examples of bacteria that can dissolve phosphate are *Pseudomonas* sp. and *Bacillus* sp. Those two bacteria also have the potential to decompose Empty Fruit Bunches (EFB) and biocontrol agents (Gusmawartati et al. 2017). The results of identifying the types of bacteria in the local microorganism solution of banana weevil are *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. (Roeswitawati et al. 2018).

Based on the previous research by Dermiyati et al. (2020), as many as 220 bacterial isolates were obtained due to isolation from the suspension of local microorganisms from EFB. However, each isolate is not yet known with certainty the ability and the type of the bacterium that has potential as antagonist and plant growth promoter. Therefore, this research aimed to study the ability and identification those bacterial isolates as antagonists, phosphate solvents, reducing chitin, plant growth promoters, or Plant Growth Promoting Bacteria (PGPB) as a pathogen.

MATERIALS AND METHODS

Source of bacterial isolates

The bacterial isolates were isolated from the extract suspension of EFB, which were established from the previous research by Dermiyati et al. (2020). The bacterial isolates were preserved in the Biotechnology Laboratory, Lampung University.

Antagonistic activity of *Ganoderma boninense*

The method used to evaluate the antagonistic activity of the bacterial isolates was adopted from Bivi et al. (2010). A five mm diameter disc was taken from the five-day-old PDA culture of *G. boninense* and plugged centrally in the nutrient so that the plate and the colonies of bacteria were streaked three cm away from the *G. boninense* plug.

The ability of the bacterial isolates to inhibit the growth of *G. boninense* was assessed after seven days of incubation by measuring the radius of the *G. boninense* colony in the direction of the antagonist colony (R2). The data were later transformed into percentage inhibition of radial growth (PIRG) with the radial growth of *G. boninense* in the control plate (R1) using the formula:

$$PIRG=\frac{R1-R2}{R1} \times 100\%$$

Based on the large percentage of inhibition, the ability of antagonistic bacteria is divided into two categories: the high category of percentage inhibition value of >50% and the low category of percentage inhibition value of <50% (Dewi 2015).

Phosphate solubilizing test

The ability of a phosphate solubilizing bacteria (PSB) was evaluated by scratching the bacteria onto sterile plastic Petri dishes containing Pikovskaya media (Himedia®; India) (Dermiyati et al. 2019). Three similar bacterial isolates was put on each petri dish. Observations the wide clear zone area formed around the bacterial colony were performed every day for 7 days. The clear zone area was measured using millimeter blocks that showed bacterial isolates' ability to dissolve phosphate. The wider clear zone area created by the colony indicateed a higher capability to solubilize phosphate.

In this study, the ability to dissolve phosphate is categorized as the phosphate dissolving index (PDI). PDI is divided into 5 levels based on the range of clear zone area; that is a very low capability (the range of 0.1-1.0), a low capability (the range of 1.01-2.0), a medium capability (the range of 2.01-3.0), a high capability (the range of 3.01 -4.0) and a very high capability (more than 4.0) (Matos et al. 2017).

Chitin reduction test

The culture media preparation for chitinolytic testing was carried out in two stages. Firstly, for the preparation of colloidal chitin, 5g of chitin powder from crab shells were added with 60 mL of concentrated HCl and homogenized using a hot magnetic stirrer for 1 hour. The mixtures were filtered through glass wool, and the filtrate was added to 200 mL of ethanol and re-homogenized. The solution was transferred to a glass funnel with filter paper and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). Colloidal chitin attached to filter paper was taken with a spatula, weighed and stored in the dark at 4 °C. Secondly, the preparation of chitin medium, which consists of 12 g of colloidal chitin, 1 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4, 1.6 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$, 0.02 g $CaCl_2.2H_2O$ and 20 g trunk agar (Souza et al. 2009).

The ability to produce bacterial chitin was done by scratching the bacteria into the petri dish and each petri dish consisted of 1 bacterial isolate with 3 replications. Observations the wide clear zone formed around the bacterial colony were performed every day for 7 days. Clear zone area is stated in the index standard in two categories: high category with a clear zone area index > 2 and low category with a clear zone area ≤ 2 (Setia and Suharjono 2015).

Plant Growth Promoting Bacteria (PGPB) test

PGPB test was performed on selected bacterial isolates with high antagonistic phosphate solvent and chitin-reducing abilities. This test used the cucumber as an indicator plant. Cucumber seeds were disinfected with 70% ethanol and 2% sodium hypochlorite, then sown and

incubated for 2 days. Furthermore, transplanting was carried out in polybags containing mixture of sand and compost (1:1) as much as 600 g .

Bacterial isolates in PPGA media were homogenized in 300 mL of water and applied as much as 20 mL per plant. Observations were carried out every two days for 21 days, including plant height, leaf greenness, number of leaves, root length, root wet weight, root dry weight, canopy wet weight, and canopy dry weight.

Molecular identification

Bacteria identification was carried out by 16s rRNA sequencing method (Suharjo et al. 2014). The bacterial DNA from PPGA media was extracted with 20 µL instagene (Bio-rad). The DNA amplification stage using forward and reverse primers: fD1 (5 'CCGAATTCG TCGACAACAGAGTTTGATCCTGGCTCAG 3') and rP2 (5'CCCGGGATCCAAGCTTACGGCTACCTTTACGACTT 3') was done with Thermal Cycle Sensoquest PCR machine. PCR amplification was carried out at the initiation stage (95ºC; 5 minutes), denaturation (95ºC; 1 minute), annealing (58ºC; 1 minute), Extention (72ºC; 1 minute), and elongation (72ºC; 5 minutes).

DNA from PCR amplification was electrophoresed for 60 minutes in 0.5% agarose gel added with 1 µL Ethidium Bromide (EtBr 1 µg ML-1). Each agarose gel well was given 3 µL of DNA extraction mixed with 1 µL loading dye. The results of the electrophoresis were visualized with the digi doc imaging system. The PCR results were sent to PT. Genetica Science at Jakarta, Indonesia, for sequencing and results were analyzed by using the Mega 6 program (Tamura et al. 2013).

Statistical analysis

Data analysis was carried out to test bacteria's ability to boost plant growth using a completely randomized design. The difference in mean values compared by Least Significant Difference (LSD) test, at a significance level of 5%.

RESULTS AND DISCUSSION

Bacteria have antagonistic abilities to be used as biocontrol agents in suppressing stem rot caused by *G. boninense* (Hushiarian et al. 2013). The amount of inhibition percentage is an indication of antagonistic behavior in each bacterial isolate. The results of antagonistic tests showed that the ability to inhibit the percentage of bacterial isolates against *G. boninense* fungi varied from less than 1% up to 90% (Figure 1).

The percentage of inhibition of high-category bacterial isolates (> 50%) was obtained in 64 bacterial isolates (29.09%) consisting of 11.82% under aerobic conditions, 7.27% under anaerobic conditions, and 10.00% under facultative anaerobic conditions. Meanwhile, the percentage inhibition of bacteria with a low category (<50%) was obtained in 156 bacterial isolates (70.91%) consisting of 26.36% under aerobic conditions, 23.64% under anaerobic conditions, and 20.91% under conditions Facultative anaerobes (Figure 2).

The difference in the inhibitory ability of bacterial isolates against *G. boninense* is suspected because each bacterium has a different inhibition mechanism. According to Haidar et al. (2016), each bacterium issued a different antibiotic compound and the antagonistic bacterial inhibition mechanism was also different. Zain et al. (2019) reported a competition against growing media between antagonistic bacteria (isolates from sugarcane and cotton) with *Fusarium* pathogenic fungi, namely *F. oxysporum*, *F. solani*, and *F. moniliforme*.

According to Gofar et al. (2014), besides having the ability to be an antagonist, bacteria are also able to reduce chitin, dissolve phosphate and increase plant growth. Bacterial isolates that can dissolve phosphate in pikovskaya media are shown by the formation of clear zones around bacterial colonies as a sign of the activity of phosphate solvent bacteria and expressed in the index of phosphate solvents (Teng et al. 2019) (Figure 3).

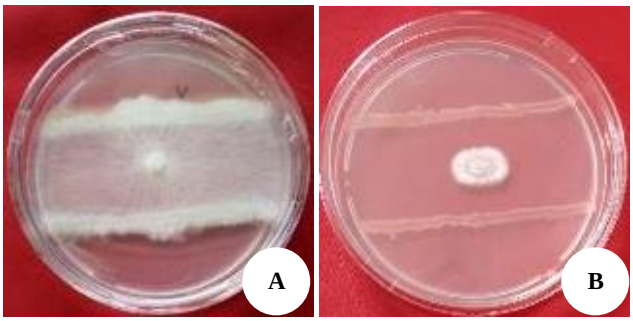

Figure 1. Inhibition of bacterial isolates against fungal growth *G.boninense*: A. inhibition <50% (low category) and B. inhibition > 50% (high category)

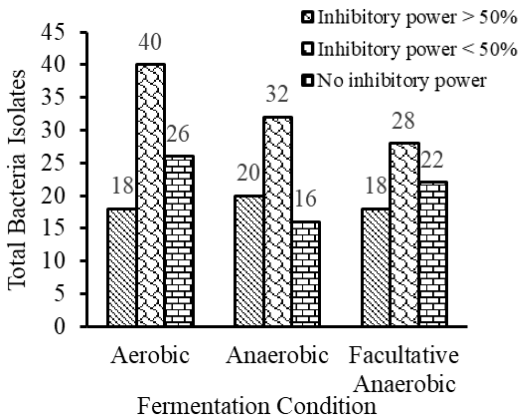

Figure 2. Antagonistic test on the bacterial isolates from extraction suspensions oil palm empty fruit bunches

The results showed that the phosphate solvent index in different bacterial isolates was in the range of 0-5.35. The ability of bacteria to dissolve phosphate is divided into four categories: high, medium, low, and very low. In addition to these 4 categories, several bacterial isolates cannot dissolve phosphate. Of the 220 bacterial isolates from aerobic, anaerobic and facultative anaerobic conditions, 118 bacterial isolates (53.64%) were able to dissolve phosphate and 102 bacterial isolates (46.36%) were unable to dissolve phosphate (TMMF). In aerobic conditions, the bacterial isolates with the ability to dissolve phosphate in the high category were 7.27%, the moderate category was 4.09%, the low category was 9.09%, the very low category was 2.27%, and the unable to dissolve phosphate was 15.45%. Moreover, in anaerobic conditions, the bacterial isolates with the ability to dissolve phosphate in the high category were 1.82%, the medium category was 5.45%, the low category was 3.64%, the very low category was 4.55%, and the unable to dissolve phosphate were 16.82%. While, in facultative anaerobic conditions, bacterial isolates with the ability to dissolve phosphate in the high category were 3.18%, the moderate category was 3.18%, the low category was 5.00%, the very low category was 5.45%, and the unable to dissolve phosphate was 14.09% (Figure 4).

The difference in the width of the clear zone is due to the ability of each bacterial isolate to produce different organic acids, where the organic acid influenced the dissolution of the P elements that are bound by Al, Fe, and Ca to become available to plants (Satyaprakash et al. 2017). Phosphate solubilizing bacteria can be used as an agent of plant growth booster because, in addition to providing the element, P can also produce IAA compounds (Saleemi et al. 2017). Examples of phosphate-solubilizing bacteria are *Pseudomonas* and *Bacillus* (Babu et al. 2017).

In the chitinolytic test, all bacterial isolates did not generate a clear zone around the chitin medium (Figure 5). This indicates that the bacterial isolates found could not reduce chitin. Due to the absence of a clear zone in the chitin media, it is suspected that the substrate concentration in the chitin media is so low that the bacteria cannot produce the chitinase enzyme. According to Veliz et al. (2017), the presence of chitin colloidal substrate in the production media influences bacteria in secreting chitinase enzymes out of cells, so the higher the substrate concentration, the increase in enzyme activity which is indicated by the greater clear zone.

Testing the ability of bacterial isolates as plant growth promoters or PGPB was conducted using selected bacterial isolates. Determination of selected bacterial isolates was based on the criteria of negative soft rot (not causing soft rot symptoms), negative hypersensitivity (not causing necrosis), hypovirulent (DSI value ≤2), antagonistic (percent inhibition >50%), having the ability to act as a phosphate solvent (phosphate solvent index >3 or high category) and chitin reducers (chitin index >2 or high category). Based on these criteria, 6 bacterial isolates were obtained with isolate codes, namely ASPB1, ASB10, ASPB2, ANSP14, ANSPB1, and SSPB2 (Table 1).

The analysis of variance (data not shown) in the PGPB test showed no significant differences in plant height, leaf greenness, leaf number, shoot wet and dry weight. However, there were significant differences in root length, root wet and dry weight.

Based on the LSD test at 5% level, the PGPB test on root length showed that SSPB2 bacterial isolate treatment had highest root length and was not different from the ANSPB1 bacterial isolate treatment, but it was different from other treatments and controls. Meanwhile, the root length in the ANSPB1 isolate treatment was also not different from the treatment of ANSP14, ASPB1, and ASB10 isolates, but it higher than that of the ASPB2 bacterial isolate treatment and controls (Table 1).

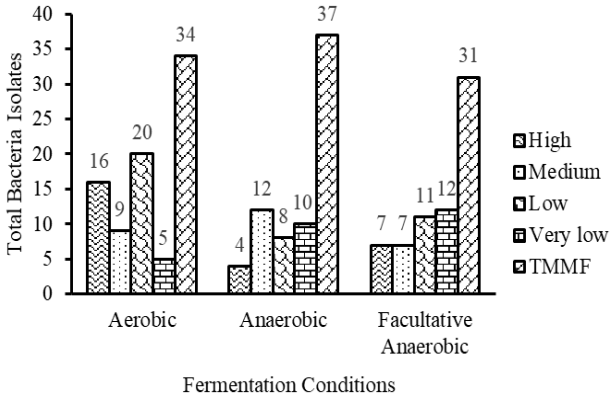

Figure 4. The ability of bacterial isolates from suspension extract of EFB to dissolve phosphate based on the clear zone index under various fermentation conditions

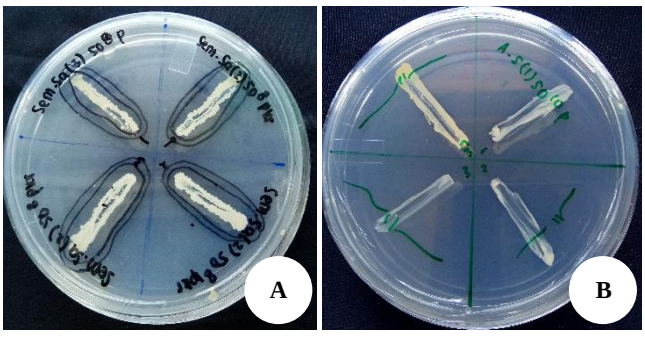

Figure 3. Phosphate solvent test in bacterial isolates: A. clear zone formed and B. no clear zone is formed

Figure 5. The chitinolytic test on the bacterial isolates from suspension extract of oil palm empty fruit bunches

In the root wet weight variable, the SSPB2 bacterial isolates treatment showed the highest root wet weight of 1.05 g and differed from all other isolate treatments and controls. Furthermore, the root wet weight in treating ASPB1, ANSP14, and ANSPB1 bacterial isolates did not differ from those of ASB10, ASPB2, and control treatments. Likewise, the root dry weight of the SSPB2 isolate treatment had the highest value, but it was not different from the ANPS14 isolate treatment but was different from the treatment of other isolates and controls (Table 1).

Based on its ability as a plant growth promoter shown in all observation variables, especially root length, root wet and dry weight, as well as testing for bacterial ability, 3 bacterial isolates were selected for molecular identification. The three isolates were ASPB1, ANSP14, and SSPB2.

Munif et al. (2012) reported in a study that 12 bacterial isolates isolated several rice varieties could boost the growth of rice plants as compared to other bacterial isolates and significantly different from controls. This study revealed the variations in root length in these 12 bacterial isolated inoculated plants. The difference in root length, root wet weight and root dry weight (Table 1) suggested that selected bacterial isolates have the ability as a phosphate solvent so that the bacteria can produce phytohormones such as auxin (IAA) or cytokinins that play a role in increasing plant growth (Poveda and González-Andrés 2021). According to Anggara et al. (2014), sufficient concentrations of phytohormones can act as promoters in root lengthening and accelerate cells in producing protein as a constituent of cell walls which affects the growth and development of plants.

Furthermore, molecular identification was carried out with bacterial isolates with antagonistic ability, phosphate solvents, chitin reduction and plant growth promoters. The results of molecular identification on bacterial isolates with isolate codes ASPB1 (aerobic condition), ANSP14 (anaerobic condition), and SSPB2 (facultative anaerobic condition) showed that the isolate was from the genus *Bacillus*. The bacterial isolates of ASPB1 (S3) belongs to group *B. velezensis*, of ANSP14 (S2) belongs to group *B. paramycoides* and of SSPB2 (S1) belongs to group *B. tequilensis* (Figure 6).

Table 1. The ability test of selected bacterial isolates as a plant growth promoter

Treatment	Root length (cm)	Root wet weight (g)	Root dry weight (g)
Control	13.90^{c}	0.45^{c}	0.03^{d}
ASPB1	16.20^{bc}	0.78^{b}	0.05^{bc}
ASB10	16.76^{bc}	0.44^{c}	0.04^{cd}
ASPB2	14.44^{c}	0.45^{c}	0.05^{bc}
ANSP14	16.10^{bc}	0.75^{b}	0.06^{ab}
ANSPB1	19.16^{ab}	0.67^{b}	0.05^{bc}
SSPB2	21.28^{a}	1.05^{a}	0.07^{a}
LSD 5%	4.48	0.17	0.01
CV (%)	2.90	6.30	4.90

Note: The values followed by the same letter in the same column are not different based on the LSD Test at $\alpha = 0.05$.

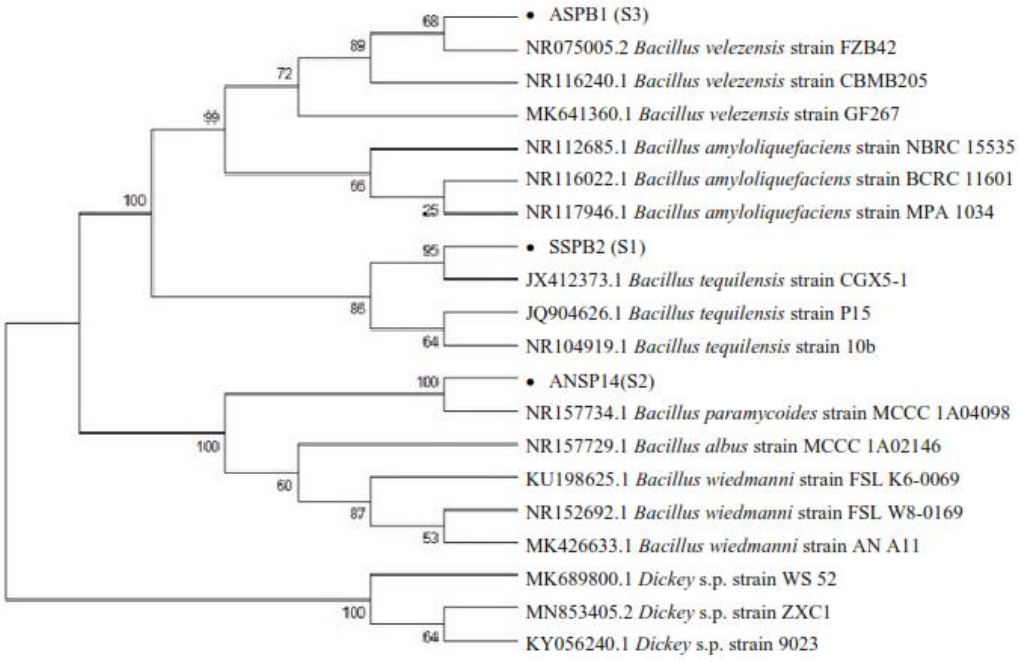

Figure 6. The phylogenic tree from 16S rDNA sequence analysis using the program MEGA6 with the Neighbor-Joining Tree method. (●: tested bacterial isolates)

Bacillus bacteria can be found in soil, water or decomposed plant residues and have white to yellowish or cloudy white colony with edges that are generally uneven, round, gram-positive, aerobic or facultative anaerobic (Emmyrafedziawati 2013). According to Fan et al. (2018), *B. velezensis* is included in gram-positive bacteria that can boost plant growth. Sondang et al. (2019) stated that *B. paramycoides* is a gram-positive bacterium that has the ability as a phosphate solubilizer. Meanwhile, *B. tequilensis* has the ability as a biocontrol agent against rice blast disease caused by the pathogen *Magnoporthe oryzae* (Li et al. 2018), as co-composting because it helps the composting process in accelerating biodegradation of lignocellulosic biomass contained in oil pam waste and as a biocontrol agent for fungi *G. boninense* (Chin et al. 2017).

The results showed that bacterial isolates from EFB can be used as a starter or a decomposer and have the ability to be antagonistic phosphate solubilizers and growth promoters which can later be used as biological agents and biofertilizers. Of the 220 bacterial isolates tested, the ability of bacteria to inhibit the growth of *G. boninense* fungi varied from ≥1% to 90%, the phosphate solvent index was from 0 to 5.35 and none of the isolates were able to reduce chitin. Three selected bacterial isolates have potential as a plant growth promoter and are identified into the genus *Bacillus* namely *B. velezensis*, *B. paramycoides* and *B. tequilensis* species with isolate codes ASPB1, ANSP14 and SSPB2, respectively. From these results, the further research on the ability of the three selected bacterial isolates (indigenous bacteria) in planta is needed to be done.

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