ISOLATION OF NITROGEN FIXING AND PHOSPHATE SOLUBILIZING BACTERIA FROM NONI FRUIT WASTE

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Abstract - One method to reduce the excessive use of chemical fertilizer in the agricultural sector is to utilize organic fertilizer that supplies the soil nutrient for the plants. Organic fertilizer serves to improve soil structure since the organic matter is capable of fixating the soil particle to generate excellent aggregates, improving the soil pore size distribution to maximize the soil absorption capacity and soil aeration.Nitrogen and phosphate are two essential nutrients in any stage of plant's life cycle. However, the availability of these nutrients are frequently low in the soil. Phosphate solubilizing bacteria are able to solubilize inorganic phosphate by releasing organic acids such as formic acid, acetate, propionate, lactate, glycolate, fumarate, and succinate [16]. In addition to phosphate solubilizing bacteria, nitrogen-fixating bacteria also have the capability of improving the efficient use of available nitrogen in the soil. The bacteria use nitrogen for the synthesis of protein cell where the protein will be mineralized in the soil after the bacteria are decomposed. In such a method, the bacteria contributed positively to the nitrogen availability in plants. The use of biological nitrogen-fixating and phosphate solubilizing bacteria contained in the noni fruit waste could promote the plant's growth by increasing the N and P nutrient's availability. This study aims to collect nitrogen-fixating and phosphate solubilizing bacteria to improve the quality and growth of forages.

Keywords - Bacteria, Nitoren, Phospate, Noni

I. INTRODUCTION

The role of organic fertilizer in reducing chemical fertilizer use at the present day has been one of the most concerned issues among the researchers since excessive and long-term chemical fertilizer use could negatively affect the soil structure. This includes soil compaction. According to [2], chemical nitrogen fertilizer (N) may cause soil compaction due to the glueing effects of urea substances. Soil compaction due to the excessive urea fertilizer use will cause declining infiltration of soil [7]. In addition, according to [8], synthetic chemicals can negatively affect the environment, human and animal health.

In liquid organic fertilizer production, there have been various bio activators used. One of them is noni fruit waste. According to a report by Indonesian Horticulture Production Statistics, the harvest capacity of noni reached up to 739.906 trees with a total production of 5.577.347 kg (StatistikProduksiHortikultura, 2014) Noni is rich in minerals including 5.0% Nitrogen (N), 0.25% Phosphorus (P), 2.5% Potassium (K), 0.3% Magnesium (Mg), 125 ppm Zinc (Zn), 1722 ppm Iron, 3317 ppm copper (Cu), 46 ppm mangenese (Mn) and 3.0 ppm calcium (Ca) [6].

Noni fruit waste as bio activator contained Sphingomonas, Pseudomonas is a nitrogen fixating bacteria, Halomonas and Geobacillius [15]. [2] statedthat Pseudomonas aeruginosa bacteria are nitrogen-fixating bacteria capable of solubilizing phosphate in soil and improving the aggregate stability to resist water erosion.

II. DETAIL EXRIMENTAL

2.1 Noni Bacteria Isolation

1. The Isolation was performed by cutting the noni fruit waste and was stored in the laboratory. The fruit waste was cut with a sterile sharp knife with a size of 1 x 1 cm (10 grams). The cut pieces were washed with sterile water and sterilized with 70% ethanol solution for one minute. After that, they were washed with sterile water for twice and aerated.

The collected pieces were crushed in mortar and pestle and were added with 100 ml sterile aquades. Dilution was performed by preparing 1 ml stock. The pieces were diluted to the expected level 10^{-5}. The dilution was spread with a spatula onto nutrient agar and sealed with parafilm plastic and incubated for a period of 7 days with room temperature. The growing bacterial colony was separated from the other different colony to acquire pure culture.

2. Screening Of Isolates. A single colony was produced from the subcultured isolate through streaking method with ose needle on the petri dish (9 cm) containing nutrient agar medium. After this procedure, it was incubated at the temperature of 37ºC. The subculture media of nutrient agar was heated on the hot plate by using a magnetic stirrer to produce a homogenous solution. After that, The nutrient agar media were stored in the autoclave at the temperature of 101oC for 45 minutes.

Approximately 20 isolates from noni fruit were selected based on the growth rate and resistance to contaminants characterized physiochemically.

2.2 Phospate Solubilizing Capacity

The soluble phosphate concentration was detected by using liquid Phikovskaya media. As much as 30 ml isolate culture was cultured in the Phikovskaya media for 7 days and was incubated for 7 days with shaker. The suspension of bacteria was filtered by filter paper (Whatman No. 1) and was centrifuged for 15 minutes at 10.000 grams. As much as 5 ml supernatant was pipetted and added with 0.05 ml P-reactors (P-reactors: 12 g ammonium molybdate, 0.277 g potassium antimonyl tartrate). After that, it was shakened for several minutes and left for 30 minutes. Before performing abosrbance measurement of phosphate solubility, the pH of filtrate solution and its acidity were measured. The absorbance was measured at the spectrophotometer UV-VIS 693 nm.

2.3 Nitrogen-Fixation Ability of the Isolate

Testing procedure employed burk's n-free media. The composition of burk's n-free media consisted of sucrose 20 g, K_2HPO_4 0.64 g, KH_2PO_4 0.16 g, Mg2SO4.7H2O 0.20 g, NaCl 0.20 g, CaSO4.2H_2O 0.05 g, Na2MoO42H_2O (0.05%) 5 ml, FeSo4.7H_2O (0.3%) 5.0 ml and 15 g bacto agar. The media were set to pH 7.3 before stored at autoclave. Mg2SO4.7H2O dan FeSo4.7H_2O were filtered based on the method performed by (Kesaulya, et al, 2017) referring to Stellahdan Suhaimi (2010).

The bacterial isolates that were capable of growing in the nutrient agar N-Free media were categorized as fixating nitrogen bacteria. The measurement of nitrogen content was performed by using K-jedall method.

III. RESULT AND DISCUSSION

3.1 Phospate Solubilizing Capacity

The bacterial isolation of noni fruit was performed by using NA media. A produced single colony of bacteria from the isolation growing in NA media was moved to other NA media to acquire some bacterial isolates for selection.

Each of the isolates resulted from the isolation had various colony morphology although some colonies appeared to be similar (Figure. 1). About 20 bacterial Isolates from the waste of noni fruit that have been selected based on the growing speed and resistance to contaminants (figure 2).

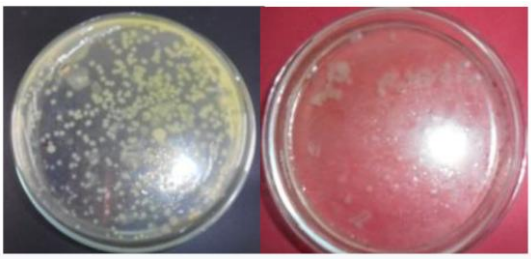


Fig 1. Results of Bacterial Isolation

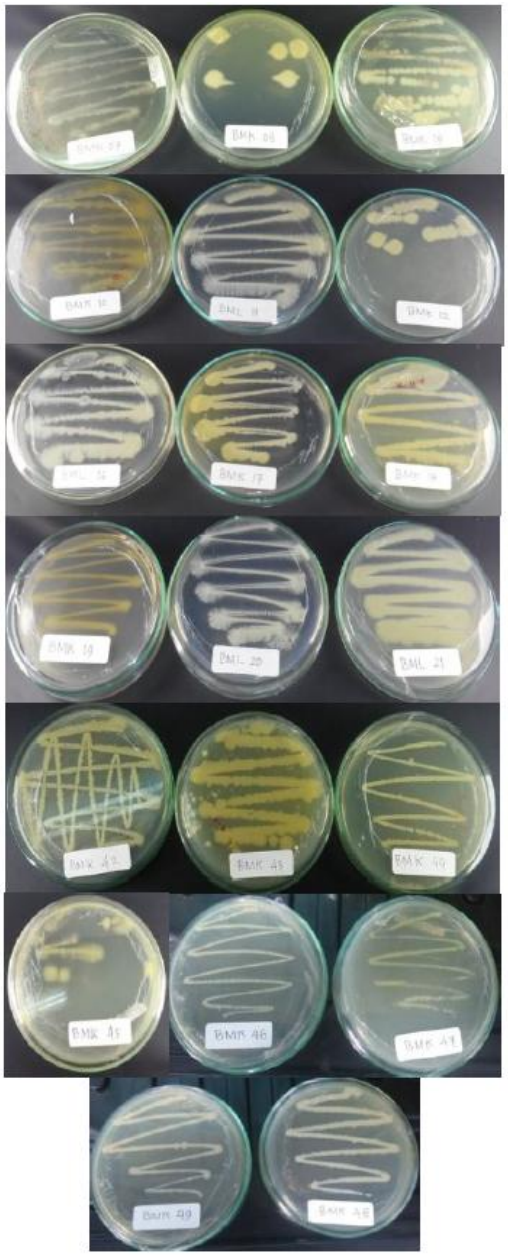
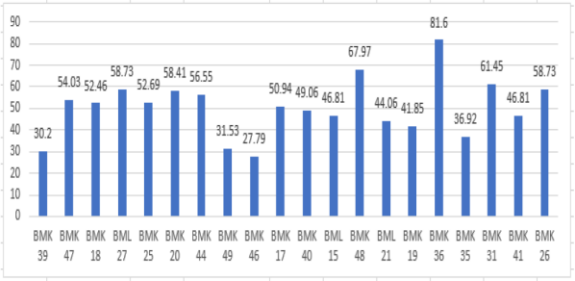


Fig 2. Selection Results of 20 Bacterial Isolates

3.2 Microbial Assay of Nitrogen Fixation Ability

The assay results from 20 isolates of nitrogen fixation bacteria suggested that the highest bacterial nitrogen fixation ability appeared at BMK 36 (81.6 ppm) while the lowest was at the 46 BMK bacterial isolate (27.79 ppm). Nitrogen plays an important role in the formation of cell, protein, cytoplasm, chlorophyll, and other cell components [4] to produce carbohydrate from the photosynthesis process. This would play a role in the development of the root system [9[. Plants lack of N will indicate poor plant's growth. The leaf turns yellow and died [3]. The bacteria would also require nitrogen to support their metabolism. Nitrogen fixating bacteria are capable of supplying nitrogen needs by fixating atmospheric nitrogen through the aid of the nitrogenase enzyme and convert it to protein. The effectiveness of

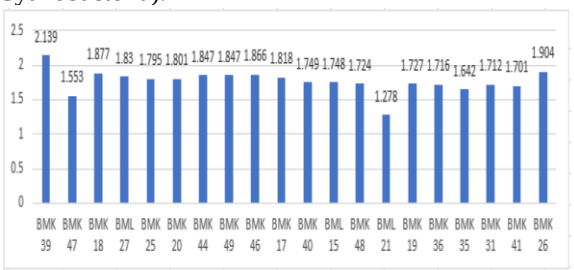
nitrogenase enzyme activity in fixating nitrogen may differ from one isolate to other isolates [7].



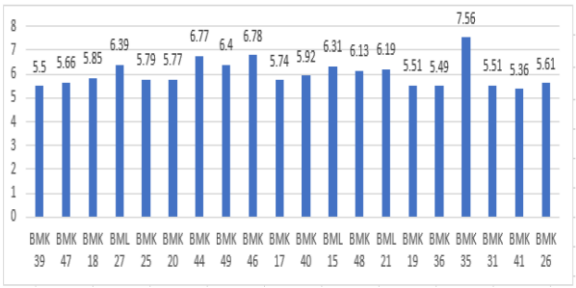
Graphic 1. Microbial Assay of Nitrogen Fixation Ability

3.3 The Microbial Assay of PH and Phosphate Solubilizing Ability.

Based on the microbial assay of PH and phosphate solubilizing bacteria, it was identified that all 20 isolates were able to solubilize phosphate. The BMK 39 isolate appeared to be the highest phosphate solubilizing index (2.139 ppm) with decreasing pH (5.5) while BML 21 appeared to be the lowest (1.278 ppm) with increasing pH (6.19). The results of a study performed by [17] indicated that FPF 4 isolate of soil microbes appeared to be the highest index of phosphate solubility (1.065). This indicated that the phosphate solubilizing bacteria from noni fruit waste seemed to have the higher solubilizing ability. The dissolution of P was also indicated by organic acid production. According to a study performed by [4], the primary mechanism of P distribution was caused by the production of organic acids by phosphate solubilizing bacteria. The production of organic acid resulted in the decreasing pH and P will be released by proton substitution. The produced organic acids will react to the Ca2+, Fe3+ and Al3+ ion that fixates phosphate to a stable degree and it is released to provide nutrient for plants. [13] in his study also reported that there are several microbial mechanisms in solubilizing phosphate. These include the competition of anion orthophosphate, pH change due to organic acids production, metal fixation forming organic metal, and chelation of organic ligands. The pH value identified was also in line with a study performed by [15] that the pH reaching 7 indicating high microbial activity. Soil pH below 5.5 caused low microbial activities. The results of the research by [10] indicating that the supply of biofertilizerCyanobacteriahad the higher soil pH compared to the control (without biological fertilizer Cyanobacteria).



Graphic 2.The Microbial Assay of Phosphate Solubilizing Ability.



Graphic 3. Bacterial pH

IV. CONCLUSIONS

All 20 isolates were observed to be able to fixate nitrogen and solubilize phosphate. The highest nitrogen fixation index was at the BMK 36 (81.6 ppm) bacterial isolate while the lowest was at BMK 46 (27,79 ppm). The highest phosphates solubility index was the BMK 39 (2.139 ppm) bacterial isolates with a pH decreasing to 5.5 while the lowest was at BML 21 (1.278) with a pH increasing to 6.19.

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REFERENCES

[1] Danapriatna N, 2010. Biokimiapenambatan nitrogen olehbakteri non simbiotik [biochemical nitrogen fixation by non-symbiotic bacteria] JurnalAgribisnisdanPengembangan Wilayah. Vol.1, pp1-10.

[2] Goenadi, D. H, 2006. Pupukdanteknologipemupukanberbasishayati [fertilizer and biotic-based fertilizing technology]. Yayasan John Hi-Tech Idetama, Jakarta.

[3] Hardjowigeno S. 1992. Ilmu Tanah [Soil Science]. Jakarta (ID): MediyatamaSarana Perkasa.

[4] Khan, M.S., A. Zaidi and P.A. Wani. 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture. Sustainable Agriculture. Springer Science Business Media, New York. pp551-570.

[5] Kraiser T, Gras DE, Gutierrez AG, Gonzalez B, Gutierrez RA. 2011. A holistic view of nitrogen acquisition in lants. Journal of Experimental Botany, Vol.62, no. 4,pp1455–1466.

[6] Mathivanan and G. Surendiran, 2007. Chemical and biological properties of Morinda spp.Inter.Journal of Noni Research, Vol. 2 no. 1-2, pp 62-74.

[7] Nugraha R, Asdyati A, Suharjono. 2014. Eksplorasibakteriselulolitik yang berpotensisebagaiagenbiofertilizerdaritanahperkebunanapelkotabatu, jawatimur [Cellulolytic bacterial exploration as a potential biofertilizer agent from apple farm in Kota Batu, East Java]. JurnalBiotropika, Vol. 2, no. 3, pp 159-163.

[8] Olness, A., & Archer, D, 2005. Effect of organic carbon on available water in soil, Vol. 170, no.2,pp 90–101.

[9] Padmini OS, Suwardi. 1998. Pengaruhdosispupuk N danpemindahanumurbibitterhadappertumbuhandanhasilpadi [The Effect of N fertilizer dosage and seed transfer age to the rice yields and growth]. Agrivet, Vol. 2, no 1, pp52-59.

[10] Pamungkas, R, Y, Budi Prasetya. 2017. The utilization of N fastening bacteria as biological fertilizers and nitrogen uptake

of soybean plants in the alfisol, Journal of Soil And Land Resources, Vol.4, no.2, pp 533-541.

[11] Paul,D, and S.N.Sinha. 2016. Isolation and characterization of phosphate solubilizing bacterium seudomonasaeruginosa KUPSB12 with antibacterial potential from river Ganga, India, Annals of Agrarian Science, Vol.15, no. 1, pp130-136

[12] Rahman, R, Anshar, M, Bahrudin. 2015. Applications solvents phosphate bacteria, nitrogen-fixing bacteria and mycorrhizae plants against pace of chili (Capsicum annum L.). e-J. Agrotekbis, Vol. 3, no.3, pp316 – 328.

[13] Setiawati, T.C. dan A. Mihardja. 2008. identifikasidanbakteripelarutfosfatdanpengaruhnyaterhadapaktivitasrhizoctoniasolanipadatanamankedelai[The identification and the effect of phosphate solubilizing bacteria to rhizoctoniasolani in soybean]. J. Tanah Trop., Vol 13, pp 233-240.

[14] Simanungkalit, R. D. M danSuriadikarta, D. A. 2006. Pupukorganikdanpupukhayati [Organic and biofertilizer]. BalaiBesarLitbangSumberdayaLahanPertanian. Bogor

[15] Syekhfani, 2009. Hubunganharatanah air dantanaman[The relationship of soil water nutrient and plants]. 2nd Ed. Malang. Indonesia pp 21 – 28.

[16] Suliasih, S.Widawatidan A. Muharam, 2010. The application of organic fertilizer and phosphate solubilizing bacteria to improve tomato plant's growth and soil microbial activity J. Hort, Vol. 20, no. 3, pp241-246.

[17] Tristiana, D, K, Sukmadewi, IswandiAnas, RahayuWidyastutidanAniaCitraresmini. 2017. Test of phytopathogenicity, hemolysis and microbial ability in solubilizing phosphate and potassium. J. Il. Tan.Lingk.,Vol. 19, no. 2 pp2549-2853.

[18] Yang L, Yinhu Li, Su Yao, Hui Wang, Yanhua Cao, Jie Li, FeirongBai, ChuangzhaoQiu, XinFeng, Wenkui Dai dan Chi Cheng, 2015. Diversity and distribution of endophytic bacterial community in the Noni (Morindacitrifolia L.) plant. African Journal of Microbiology Research. Vol. 9, no.25, pp. 1649-1657.

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