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Potency of Auxin Producing and Phosphate Solubilizing Bacteria from Dryland in Rice Paddy Field

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ABSTRACT

Rice (*Oryza sativa* L.) is one of important and suitable corps in East Indonesia including North Central Timor (NCT) Regency. However, farmer NCT Regency have not yet use microbe as biofertilizer. The research aimed to isolate Indole Acetic Acid (IAA) producing by soil bacteria from paddy field in NCT Regency and to apply selected microbe in rice cultivation. Eight isolates were obtained and analyzed their IAA ability based on a colorimetric method using Salkowski reagent. The result showed that the producing eight isolates was able to synthesize IAA with the highest concentration produced by EP.01 isolate. The ability of these isolate to solubilize phosphate was measured by using Pikovskaya media. The EP.02 isolate has the highest ability to solubilize phosphate. The growth curve and IAA synthesis were created using EP.01 and EP.02 isolates as a model. Production of IAA was in line with the cells growth. Both EP.01 and EP.02 isolates were closely related to *Bacillus* sp. with 97.7 and 98.1% maximum identity, respectively. The application of *Bacillus* sp. solubilizer as biofertilizer on paddy field using randomized block design with the type of fertilizer as a single factor. Application of fertilizer used compost enriched with 50% NPK, had the best result on the number of filled grains per clump, dry weight of 1000 grains which were 30.76, 29.37 g and 6.3 t ha^{-1}, respectively.

Key words: *Oryza sativa* L., production, North central timor, biofertilizer

INTRODUCTION

North Central Timor (NCT) Regency, Province of East Nusa Tenggara, East Indonesia, has an extreme climate with the intensity of rainfall about <500 mm $year^{-1}$ (Statistics NCT, 2012). It makes NCT become dry land area. However, many varieties of rice plant can grow and develop at that area. The efforts of NCT local farmer to achieve high productivity of rice plant by increasing the soil fertility is one of the important factor that has to be employed.

Soil from paddy in NCT regency has known that relatively mildly alkaline soil pH (7.8), the content of low soil C-organic (1:07%), low N nutrient N (0:11%), low P nutrient content (6.6 ppm), high K nutrient content of the soil (1.73 cM kg^{-1}) and Cation Exchange Capacity (CEC) middle were (24.36 cM kg^{-1}). Based on the chemical properties of these soil, the soil fertility status is low (Hardjowigeno and Widiatmaka, 2001). That fact triggered by the use of inorganic fertilizers for a long time. The use of inorganic fertilizers continuously will cause damage to the physical, chemical and biological properties of soil so that soil fertility will decrease (Havlin *et al*., 2005).

To increase yields, while maintaining high resource sustainability sustainable agriculture require alternative solution that refers to organic farming which more focus on local resources, such as compost, manure and rice straw. Organic fertilizer, such as compost is an important substance in the role of agriculture to improve soil physical and chemical properties, also producing energy source for microbial activity in the soil. The activity of soil microbes may play a role in improving soil fertility and producing phytohormones, fixing N_2 and solubilizing phosphate (P), so that is available to plants.

The IAA or auxin hormone active plays an important role in all plants for increasing of growth, such as initiating root elongation, cell expansion, vascular differentiation and initiating of flowering (Brandi *et al*., 1996). Plants naturally has produced the auxin hormone which called endogenous IAA, but the auxin hormone can also be produced by organisms other than plants which were called exogenous IAA. Exogenous IAA, which produced by microbes were affected the vegetative and reproductive growth of plants (Agustian *et al*., 2010). The other soil microbes also plays a role as solubilizer phosphate. Microbes release of phosphorus (P) from the bond of Fe, Al, Ca and Mg, so P in soil is available for plants (Rao, 1995). Yafizham (2003) stated that one cultured bacteria solubilizing phosphate can increase crop production 20-73% and directly able to increase the solubilization of P bound in the soil so that the available P in the soil was increased.

The high efficiency of chemical fertilizers and soil fertility problems also the availability of increasingly expensive fertilizer prices require new technology to overcome it. Beside the use of organic fertilizers, such as compost, biofertilizer was also an excellent alternative. Biofertilizer is a substance containing microbial life when applied to plants or soil can stimulate plant growth, improve soil fertility, increase crop production and did not cause side effects on farmers and the environment (Vessey, 2003). In addition, biofertilizer can also help control pathogenic organisms and can increase percentage of plant nutrients, such as nitrogen (N), phosphorus (P) and potassium (K) (Wu *et al*., 1995).

It is known that *Pseudomonas* sp. and *Bacillus* sp. are bacteria solubilizing phosphate and potassium compounds. *Bacillus* sp., *Pseudomonas* sp. and *Azotobacter* sp. can produce phytohormones such as IAA (Simanungkalit, 2001). However, these bacteria are still not used optimally in the agricultural sector, especially food crops such as rice plant in NCT regency. This research aimed to isolate bacteria from rice soil, identify the bacteria, characterize potential auxin-producing bacteria and solubilizer phosphate, also formulate selected bacteria as biological fertilizer and apply it to determine the effect of bacteria in enhancing the growth and rice productivity at NCT.

MATERIALS AND METHODS

Isolation of bacteria from rice paddy field soil: Soil sample that used for this study from NCT regency, Province East Nusa Tenggara, Indonesia. One kilogram soil was taken to a depth of 5-10 cm from 5 point in the rhizosphere. Then, the isolation of bacteria with serial dilution method to induce glucose lactose media (LGI) (Aquilantia *et al*., 2004) and Pikovskaya (Widawati, 2011). One gram soils were inserted into a tube containing 10 mL of 0.85% NaCl and then performed a serial dilution. The result of dilution 10^{-3}, 10^{-4} and 10^{-5} (0.1 mL) was spreaded to LGI selective medium and Pikovskaya, then incubated at room temperature for 5-7 days. pH medium was measured to 6.8 before sterilization. Morphological characteristic identified based on Holt *et al*. (1994) method.

Selection of IAA producing bacteria: The IAA-producing bacteria was measured by a colorimetric method using Salkowski reagent (Gordon and Weber, 1951). Isolates were cultured in 50 mL LGI liquid medium and Pikovskaya which added 1.0 mM L-Trp. The cultures were incubated for 3-7 days in shaker incubator at 120 rpm. Bacterial cultured (1 mL) was centrifuged at 10000 rpm for 10 min. Supernatant (1 mL) was taken and treated with 4 mL of reagent Salkowski. Then, suspensions were measured absorbance at 520 nm using a spectrophotometer (Genesys). The concentration of IAA in medium can be determined by using a standard curve with the range concentration of IAA from 0-100 ppm. IAA positive assay characterized by a color change to pink (Patten and Glick, 2002).

Selection of phosphate solubilizing bacteria: Phosphate solubilizing bacteria was selected with spotted on Petri dish containing sterile solid Pikovskaya medium. After incubation at room temperature for 3 days, clear zone around the colonies were observed and measured index of phosphate solubilizer (IP) (Nautiyal, 1999). Based on equation:

$$\text{Index of phosphate solubilizer} = \frac{\text{Diameter of clear zone (mm)-Diameter of colony (mm)}}{\text{Diameter of colony}(\text{mm})}$$

Antagonism and hypersensitivity assay: Antagonism assay was done using double layer method in Nutrient Agar (NA) medium. The bacteria isolates were plated on NA medium and spread with a solution of other isolates. Control using sterile distilled water and incubated for 3-7 days. Isolates of potential synergism was indicated by the formation of inhibition zone. Hypersensitivity assay was used tobacco leaves. One milliliter bacterial suspensions was cultured for 24 h and taken with a sterile hypodermic syringe (without needle) and injected at intervena of tobacco leaves.

Determination of growth curves and IAA synthesis: One loop of bacterial cultures (24 h) were grown in 10 mL of Nutrient Borth (NB) medium added 1.0 mM L-Trp as stock and shaken with 100 rpm at room temperature for 12 h. Then, 3 mL culture was taken and transferred into a threaded tube containing 3 mL of NB medium with dilutions of 1:1, 1:2, 1:4, 1:8 and 1:16. Each dilution (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) was measured absorbance at 620 nm. For the determination of IAA synthesis, every 3 h measured absorbance at 520 nm.

Identification of bacteria: Selected isolates bacteria which produce auxin and solubilize phosphate were identified using API 50 CHB kit. This kit is a standard system with 49 types of biochemical testing. Kit consists of 50 wells. Well number 0 did not contain the active ingredient and was used as a negative control, whereas the numbers 1-49 wells containing sugar and its derivatives. During incubation (24-48 h) sugars will be fermented into acid, which lowers the pH and it can be observe in color change. Confirmed positive test results when a change of color from red to yellow, but specifically for esculin test (well number 25) color changes from red to purple-black. These result as a biochemical profile which was used to identify microbial species using software APIWEB.

Preparation of biofertilizer and compost: Selected potential bacteria isolates rejuvenated first, and then inoculated into the liquid medium and incubated on shaker until the number of cells

reached 10^8 cells mL^{-1}. A total of 2000 mL culture medium containing the bacteria centrifuged at 2851x g for 15 min to produced paste cells, then 50 mL pasta cells mixed into 1 kg of sterile peat. The sterile peat that has been given a bacterial cell paste hereinafter referred as biofertilizer. Composting was done with the preparation of straw and cow manure (1:1 w/w). Straw and cow manure were arranged each in layers, then covered using a plastic sheet. Reversal was done every 10 days. After three weeks (half-baked), compost enriched by biofertilizer as much as 2.5% of the fresh weight of raw material of compost. Compost continued incubate until harvest. Biofertilizer was applied in the early of planting much as 150 g per plot (2 t ha G1).

Experimental design: The experimental design used was a Randomized Block Design (RBD) with one factor treatment: (1) Without biofertilizer, as a negative control (P0), (2) NPK dosage 100% as a positive control (P1), (3) Compost (P2), (4) Compost enriched (P3), (5) Compost enriched +50% NPK (P4), (6) Compost +50% NPK (P5). Unit experiment was repeated 3 times, so totally 18 experimental units.

Land preparation and planting: Land preparation was done manually with plot 2×3 m. Randomization plot follow the design used. The planting distance was 20×20 cm. Each plot was selected 10 rice plant samples for the measurement of the rate of vegetative growth and its production. Rice plant used this research was IR64 varieties and the cultivation was done in NCT regency.

Observation: The parameters were observed in this research i.e., (a) The vegetative growth of rice plants include: Plant height, number of tillers, fresh and dry weight of plants. Observations plant vegetative growth was done every 2 weeks: 14 Days after Planting (DAP), 28 42 56 and 70 HST, (b) Production of rice plants include: Number of panicles per clump, total weight of grains per clump, 1000 grain weight of dry grain harvest, (c) The number of bacteria in compost-enriched using plate count method on Pikovskaya medium. Counting the number of bacteria were performed after harvesting (115 days after planting).

Statistical analysis: The data of vegetative growth and crop production were subjected to Analysis of Variance (ANOVA) using SPSS v.21 at $p \leq 0.05$.

RESULTS

Bacterial isolates: A total of eight bacterial isolates were obtained from rice soil samples originated from North Central Timor regency, Indonesia. Three isolates i.e., EAZO.01, EAZO.02 and EAZO.03 were obtained from LGI medium, while five isolates i.e., EP.01-EP.05 were obtained from Pikovskaya medium. Eighth isolates were dominated by circular and irregular shapes. Seven isolates showed white colonies while the other isolate showed a yellow with flat edges and a convex elevation. Based on Gram staining showed that the eight isolates has the shape of stem cells (Rod). Five isolates belonged to Gram-negative while three isolates (EP.01, EP.02 and EP.03) belonged to Gram-positive (Table 1).

Isolates of producing IAA and solubilizing phosphate: Eighth isolates were capable of producing IAA with various concentrations between 3.25-119.5 ppm. Four isolates produce IAA with concentrations less than 10 ppm; two isolates produce IAA between 30-55 ppm. While the two

Table 1: Morphological characteristic of bacterial isolates from NCT regency

	Type of colony			Type of cells		
Isolates codes	Shape	Colour	Edge	Elevation	Shape	Gram
EAZO.01	Circular	White	Flat	Convex	Rod	Negative
EAZO.02	Small circular	Less-white	Flat	Convex	Rod	Negative
EAZO.03	Small circular	Less-white	Flat	Convex	Rod	Negative
EP.01	Circular	White	Flat	Convex	Rod	Positive
EP.02	Circular	White	Flat	Convex	Rod	Positive
EP.03	Circular	White	Flat	Convex	Rod	Positive
EP.04	Circular	White	Flat	Convex	Rod	Negative
EP.05	Circular	Yellow	Flat	Convex	Rod	Negative

Table 2: Auxin producing bacteria in LGI medium with added 1.0 mM L-triptofan and solubilizing phosphate bacteria in Pikovskaya medium

Isolates codes	IAA production (ppm)	Index phosphate solubilizer
EAZO.01	4.53	1.0
EAZO.02	51.89	1.0
EAZO.03	3.25	1.2
EP.01	119.50	1.1
EP.02	4.90	3.5
EP.03	113.50	1.4
EP.04	32.49	1.0
EP.05	7.19	2.1

Table 3: Potential isolates produce highest IAA and phosphate solubilizer based on antagonism and hypersentivity assay

Assay isolates	Antagonism	Hypersensitivity
EP.01	-	-
EP.02	-	-
EAZO.02	-	+

+: Clear zone and phatogen -: Unclear zone and no phatogen

isolates were able to produce IAA more than 100 ppm. The EP.01 isolates have known able to produce IAA, with the highest concentration of 119.5 ppm. The solubilizing phosphate was marked by a clear zone surrounding the bacterial colonies. The data showed index phosphate solubilizer was ranges from 1 until 3.5. EAZO.01, EAZO.02 and EP.04 isolates have lowest clear zone index (1) while, EP.02 isolate known to have the highest index (3.5) (Table 2).

Antagonism and hypersensitivity assay: The result of antagonism between the selected isolates (EP.01, EP.02 and EAZO.02), showed no growth inhibition between isolates (Table 3). Their compatibility or synergism between isolates is a very important factor at the time of application in the field. Based on hypersensitivity assay with tobacco leaves after 48 h during incubation (injections), an isolate (EAZO.02) showed hypersensitive that causes tobacco leaves become necrotic and two isolates i.e., EP.01 and EP.02 showed negative hypersensitive.

Curve of growth and IAA synthesis: The EP.01 and EP.02 isolates were produced of IAA in NB medium which added 1.0 mM L-tryptophan. EP.01 isolate initiate to synthesize IAA at the 9th h. IAA concentration continues to increase and reaches the highest concentration (8.53 ppm) at first stationary phase, i.e., at the 24th h. EP.02 isolate initiate to synthesize IAA at the 9th h and continues to increase at log phase. The highest IAA concentration was entered the stationary phase at the 21st h, i.e., 6.70 ppm (Fig. 1).

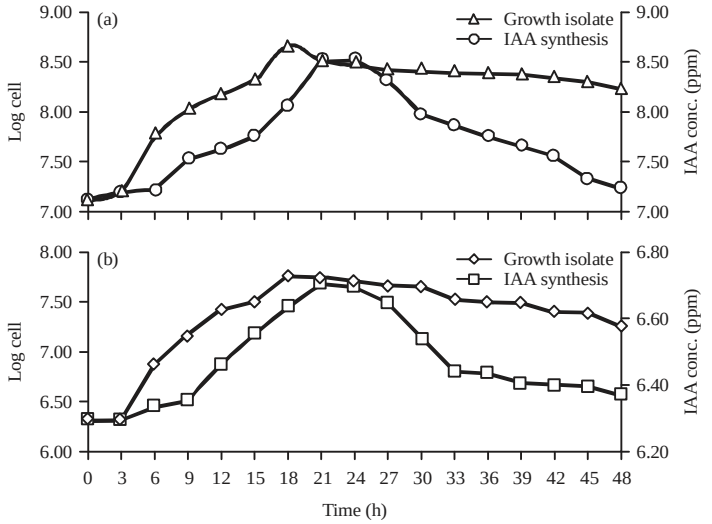

Fig. 1(a-b): Growth and IAA synthesis of (a) EP.01 isolate and (b) EP.02 isolate with added 1.0 mM L-trp in NB medium

Characteristic of bacteria: Identification of isolates were done using Analytical Profile Index (API) 50 CHB, EP.01 and EP.02 isolates showed a positive test results in the test: glycerol, L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, arbulin, esculin, maltose and trehalose. The test results lactose EP.01 isolate showed negative results whereas EP.02 isolate showed positive results. The results of species identification using software APIWEB, EP.01 and EP.02 isolates were closely related to *Bacillus* sp. with 97.7 and 98.1% maximum identity, respectively.

Biofertilizer treatment in paddy field: Biofertilizer containing *Bacillus* sp. that was IAA-producing bacteria and solubilizing phosphate. It can be used with natural materials such as compost as soil organic material. Application of biofertilizer combination with compost through the fertilization treatment was compost enriched +50% NPK (P4) significantly different ($p<0.05$), increased plant height and number of tillers (Fig. 2), also the biomass plant weight (Fig. 3).

Observations of plant height and number of tillers were done, when the rice was 14-70 DAP. An analysis at 14 and 28 DAP in all treatment effected was not significantly different ($p>0.05$) compared with control (P0), about plant height and number of tillers. At the age of 42-70 DAP, showed the highest plant height and number of tillers i.e., at compost enriched +50% NPK dosage (P4) treatment, compared with control (P0). While, they were not significantly different ($p>0.05$) with compost enriched fertilization treatment (P3) and 100% NPK treatment (P1). Fertilization treatment was used compost +50% NPK (P5) and compost treatment (P2) were generated plant height and number of tillers were not significantly different ($p>0.05$) compared with no fertilization (P0). The treatment of compost +50% NPK (P5) and compost (P2) also without fertilization treatment (P0) were generated more lower plant height and number of tillers.

Observation of plant biomass after harvest was done at 115 DAP (Fig. 3). The observed parameters include dry weight and fresh weight. The results showed that the dry and wet weight in compost enriched +50% NPK (P4) treatment was significantly different ($p<0.05$) with the treatment of without fertilization (P0) but not significantly ($p>0.05$) with 100% NPK (P1) treatment.

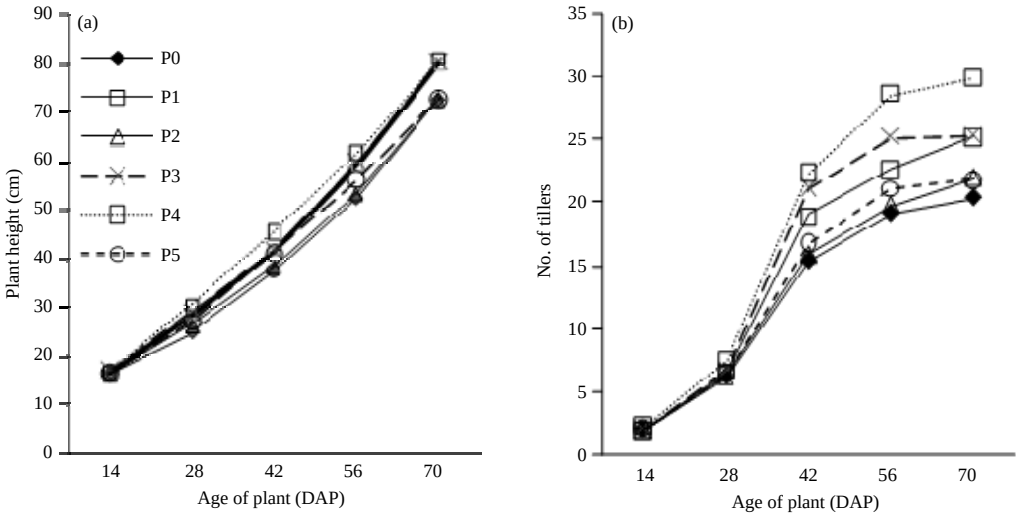

Fig. 2(a-b): Rice vegetative growth at 14-70 days after planting (HST). (P0: Without biofertilizer, P1: 100% NPK, P2: Compost, P3: Compost enriched, P4: Compost enriched +50% NPK, P5: Compost +NPK 50%)

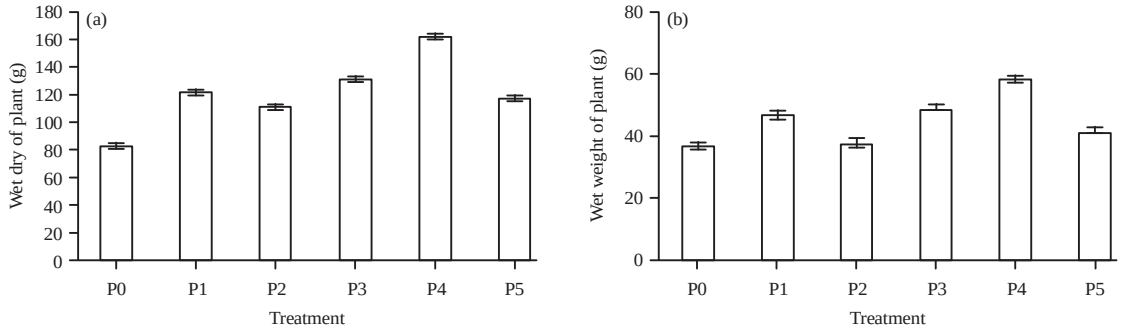

Fig. 3(a-b): Effect biofertilizer to dry weight and fresh weight after 115 DAP. (P0: Without fertilizer, P1: 100% NPK, P2: Compost, P3: Enriched compost, P4: Enriched compost +50% NPK, P5: Compost +50% NPK)

Applications of compost enriched treatment (P3) was given more weight biomass plant which was comparable to NPK 100% treatment (P1). Whereas, the fertilization were used compost +50% NPK treatment (P5) and compost treatment (P2) were given the dry and fresh weight were not significantly different ($p>0.05$) with the treatment without fertilization (P0). Rice production with biofertilizer containing *Bacillus* sp. and compost through composting treatment enriched +50% NPK (P4) was produced the number of panicles per clump were significantly different ($p<0.05$) with the treatment of 100% NPK (P1), but grain per panicle and weight of 1000 grains were not significantly different ($p>0.05$). The treatment of the compost enriched (P3) was produced the number of panicles per clump, grains per clump and the weight of 1000 grains were not significantly different ($p>0.05$) with 100% NPK treatment (P1). The used of compost (P2) and compost +50% NPK (P5) in this research were not significantly different ($p>0.05$) with the treatment of without fertilization (Po) but significantly different $p<0.05$) with 100% NPK

Table 4: Rice production after 115 DAP

Fertilization treatment	No. of panicles per clump	Total of grains per clump (g)	Weight 1000 grains (g)	Dry grain weight (GKP) (t ha^{-1})
P0 (without fertilizer)	17.73^{d}	24.87^{c}	25.83^{c}	4.7^{c}
P1 (100% NPK)	22.63^{ab}	28.15^{ab}	28.67^{ab}	7.4^{a}
P2 (compost)	19.56^{cd}	24.75^{c}	26.03^{c}	5.6^{b}
P3 (compost enriched)	24.16^{b}	29.40^{a}	27.93^{b}	6.0^{a}
P4 (compost enriched +50% NPK)	27.90^{a}	30.76^{a}	29.37^{a}	6.3^{a}
P5 (compost +50% NPK)	20.80^{cd}	25.72^{ab}	26.40^{c}	5.7^{b}

Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05)

Table 5: Number of cells EP.01 dan EP.02 isolates in biofertilizer and 115 DAP at compost enriched plot (P3)

Type of microbes	Isolates in biofertilizer (before planting) ($\times10^{8}$) cell mL^{-1}	Isolates 115 HST ($\times10^{9}$) cell mL^{-1}
Bacillus strain EP.01	2.0	4.7
Bacillus strain EP.02	3.0	5.6

Table 6: Analysis of soil fertility at plots: without fertilization (P0), compost (P2) and compost enriched (P3) after 115 DAP

Treatments	pH	C-organic (%)	N-total (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (cM kg^{-1})	CEC
Without fertilization (P0)	7.7	1.03	0.12	3.9	0.22	24.40	9.08	15.06
Compost (P2)	7.0	1.79	0.18	7.6	0.19	45.22	20.01	33.05
Compost enriched (P3)	7.0	3.11	0.27	18.2	0.27	42.18	19.51	39.70

fertilization treatment (P1) as controls positive. In addition, based on analysis, compost fertilizer treatment (P4) and compost enriched +50% NPK treatment (P5) were given dry grain harvest (GKP) were not significantly different ($p>0.05$) with 100% NPK fertilizer application (P1) but significantly different ($p<0.05$) compared with no fertilization (P0). The GKP yield was produced from the compost enriched treatment (P3) was produced a yield of 6 t ha^{-1}; compost enriched plus inorganic fertilizer 50% dosage (P4) of 6.3 t ha^{-1}, 100% NPK (P1) of 7.4 t ha^{-1} while, without fertilization (P0) was produced a yield of 4.7 t ha^{-1} (Table 4).

Total bacteria and soil fertility: The number of bacterial cells before using biofertilizer that containing *Bacillus* sp., ranges from 2.0-3.0×10^{8} cells mL^{-1}. The number of bacterial cells in soil until harvest time (115 days after planting has increased by 200-500% (Table 5). After the application of biofertilizer, the soil fertility in plots of compost enriched treatment (P3) neutral pH values (7), the organic content of C-3.11, N-total of 0.27, 18.2 ppm P-soluble, K nutrient content 0.27 and the CEC has risen 39.70 (Table 6).

DISCUSSION

Eight isolates were successfully isolated from rice soil of NCT regency. Samples were taken from rhizosphere of rice plants. The bacterial rhizosphere get energy intake of metabolites released by plants through the roots (Lebuhn *et al*., 1997). The metabolites can form compounds sugars, amino acids, organic acids, glycosides, compounds of nucleotides, vitamins, enzymes and indole. Metabolite which was released by plants into soil through the roots can determine microbes condition in rhizosphere. In addition, the source of isolation of bacteria was taken from rice soil that have low fertility that caused by using inorganic fertilizer. Least a population of bacteria in rice plant rhizosphere were affected by pH and nutrients in rhizosphere where the higher the nutrient, so bacterial population will be increased (Agustian *et al*., 2010; Acuna *et al*., 2011).

Selection of IAA producing bacteria was added 1.0 mM L-tryptophan in the culture medium for growth. Saharan and Verma (2014) stated that IAA production was strongly influenced by growth level and availability of L-tryptophan substrate in medium. The IAA synthesis on bacterial

strain can use more than one pathway. Among these pathways i.e., Indole Acetamide (IAM), indole-3-pyruvate acid (IPyA), triptamin (TAM) and indole-3-acetonitrile (IAN) (Patten and Glick, 1996). The IpyA pathway has known to be inducible by tryptophan compounds. An addition of tryptophan was known to increase the IAA biosynthesis through IpyA pathway. On this pathway, conversion of tryptophan to IPyA by aminotransferase, then IPyA was decarboxylated into indole-3-acetaldehyde (IAAId) by indole-3-pyruvate decarboxylase (IPDC). In the last step, IAAId was oxidized to IAA (Spaepen *et al*., 2007). The IAA production can be known from supernatant, which was reacted with a reagent Salkowski and incubated in dark room. Salkowski is a dye reagents that can be used to test the indole compounds including IAA concentration, so giving the concentration with a pink color (Glickmann and Dessaux, 1995).

Eighth isolates were capable of solubilizing the phosphate in Pikovskaya medium. Insoluble P used in this research that was tricalcium diphosphate ($Ca_3(P0_4)_2$), because it is based on the results of soil analysis showed that soil was alkaline. This was due to the high content of alkaline elements. At alkaline pH insoluble P was binded with Ca formed a complex Ca-phosphate, one of them ($Ca_3(P0_4)_2$). The ability of isolates solubilizing phosphate were characterized by the formation of a clear zone surrounding the bacterial colonies (Hefdiyah and Shovitri, 2014). Clear zone in around the colony was indicated of bacterial activity in solubilizing P-bound. The isolates were solubilized ($Ca_3(P0_4)_2$) which contained in Pikovskaya medium. In this study, eight isolates have differences to solubilizing a phosphate. The IP qualitatively (Table 3) showed the level of isolates phosphate solubilizer in their ability to solubilize the P-insoluble phosphate into a P-soluble form. The EP.02 isolate has known to capable of solubilizing of phosphate with the highest index was 3.5. Premono (1998) stated that the bacteria solubilize phosphate was closely related to the organic acids. These organic acids were include citric acid, glutamic acid, succinic acid, lactic acid, oxalic acid, malic acid, fumaric acid and α-ketobutyric acid. The organic acids will chelate cations in the form of a stable complex with Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}. In addition, beside with organic acids, P can be removed because of the enzyme phosphatase and phytase (Idriss *et al*., 2002). Acid phosphatase and phytase are the most common activities because of their substrates were dominant in the soil.

Based on antagonism assay for three isolates (EP.01, EP.02 and EAZO.02) were indicated a relationship of synergism. None of the bacteria that inhibit the growth of other bacteria. This was indicated by the formation of inhibition zone around bacterial colonies. Their compatibility or synergism of the two inoculated bacteria is a factor that is very important in order to the three of these bacteria can be function properly. At the hypersensitivity assay was used tobacco leaves because this plant is a model plant. Based on hypersensitivity assay showed that EP.01 and EP.02 isolates did not triggered a hypersensitivity reaction in tobacco leaves. According to Lindsay *et al*. (1993) the hypersensitive response was defined as a rapid defense reactions of plants to uncompatible pathogens also rapid cell death in the tissue on area injected bacterial suspension, so its presence did not affected the growth of the host plant.

The data about growth curve of EP.01 and EP.02 isolates and IAA concentrations obtained in this study were required for the formulation of biofertilizers. The growth curve of EP.02 and EP.01 isolates showed that IAA produced pattern comparable with the growth of bacteria cells. The two isolates of bacteria IAA was produced significantly at 3-21 h incubation. Wahyudi *et al*. (2011) stated that the abundant IAA hormone levels produced by the bacteria at the stationary phase. The IAA was synthesized as a secondary metabolite which produced in conditions of bacterial growth when available precursor amino acid tryptophan. This condition indicated that IAA is secondary metabolite compound of these bacteria.

Based on eighth morphological identification (Table 1), especially isolates that produce IAA with the highest concentration of EP.01 isolate, have morphological characters colonize round with flat edges, white and elevation of convex. The EP.02 isolate that produce index phosphate solubilizer (IP) has the highest character circular colonies with flat edges, creamy white and elevation of convex. Based on Gram staining EP.01 and EP.02 isolates belonged to Gram-positive. Based on physiological identification as confirmation of EP.01 and EP.02 isolates using the tool kit Analytical Profile Index (API) 50 CHB showed that EP.01 and EP.02 isolates has similarities with *Bacillus* sp. According to Agustian *et al. (*2010), color and character of isolates with the same morphology was not necessarily derived from the same species. *Bacillus* sp. is a group of soil bacteria, have cell walls containing peptidoglycan with 90% polysaccharides form tekoat acids which are embedded in the cell wall. *Bacillus* sp. can form endospores in critical environmental conditions including nutrient limitation for example carbon and nitrogen deficiency. *Bacillus* sp. including aerobic or facultative aerobic bacteria that use oxygen as a final electron acceptor in cell respiration phase. *Bacillus* sp. has a lot of potential i.e produce IAA, solubilize phosphate, secrete siderophores and biocontrol agents (Compant *et al*., 2005). Thus, the *Bacillus* sp. EP.01 strains and *Bacillus* sp. EP.02 strains of rice plant soil originated from NCT was potential as a biofertilizer.

Applications of biofertilizer containing *Bacillus* sp. with compost enriched through fertilization +50% NPK treatment (P4) significantly ($p<0.05$) increased of plant height and number of tillers (Fig. 2), the weight of the biomass plant (Fig. 3) compared to treatment without fertilization (P0), but not significantly different ($p>0.05$) with 100% NPK fertilizer treatment (P1). It was assumed because of *Bacillus* sp. as a producer of auxin and phosphate solubilizer that plays an important role in rice plant growth. In addition, the compost can also provide nutrients for bacteria contained in the biofertilizer for metabolic processes so that, before the bacteria applied to plants has increased the number of its population. According to Triadiati *et al*. (2013) compost was provided the elements C and N for metabolic processes of bacteria. This fact showed that the addition of organic fertilizers, such as compost as an additional source of nutrients other than as a provider of nutrients needed by plants, also as a source of energy and nutrients for the bacterial growth that plays as a biofertilizer to increase plant growth, among others, stimulated by the growth hormone that produced by the bacteria.

Increased of plant growth could not be separated from their mutual interaction between the bacteria with plants. The bacteria will be colonized of plant roots because it requires the metabolites produced by plants in the form of exudate which contains many amino acids, carbohydrates and indole as nutrients for bacterial growth (Rodriguez and Fraga, 1999). The accumulated of bacteria in rhizosphere will synthesis of auxin hormone to promote plant growth. According to Vessey (2003), an increase of plant growth was caused by cleavage and cell elongation were driven by the auxin hormone produced by the bacteria.

Furthermore, *Bacillus* sp. in particular of biofertilizer especially as phosphate solubilizer clearly plays a role in releasing of phosphate bonded to phosphate solubilized. The condition was indicated that the bacteria contained in biofertilizer can increase the availability of P. Elements of P has function to promote growth of the number of tillers, root development, flowering and ripening (Ivanova *et al*., 2006). According to Rao (1995) phosphate solubilizing bacteria was secreted of organic acids. In addition, P may be removed from organic compounds by non-specific phosphatase enzymes which performed phosphorylation of the bound phosphoester organic matter and phytase acid that causes P regardless from phytic acid, specifically (Idriss *et al*., 2002). The role of biofertilizers containing *Bacillus* sp. have been effected on phosphate solubilization, so that P

becomes available and can be absorbed by plants. Increased nutrient absorption was effected on the number of tillers formation, root development, flowering and ripening which can affect to plant biomass (Ivanova *et al*., 2006).

Based on the observation and statistical analysis showed that the productivity of rice plants in compost enriched +50% NPK treatment (P4) was given the results were not significantly different ($p>0.05$) with 100% NPK fertilization treatment (P1). The treatment of compost enriched +50% NPK (P4) was given the number of filled grain per clump and the weight of 1000 grains of 30.76 and 29.37 g, respectively. The estimated harvest of dry grain yield (GKP) on rice plant cultivation, at the treatment of compost enriched (P3) was given a yield of 6 t ha^{-1}; compost enriched plus inorganic fertilizer 50% dosage (P4) of 6.3 t ha^{-1}, 100% NPK (P1) of 7.4 t ha^{-1} while without fertilization (P0) was given a yield of 4.7 t ha^{-1}. These results were supported by research of Gofar and Marsi (2013) that the treatment of compost enriched with biofertilizer (N fixing, phosphate solubilizer and potassium, plant growth) were capable of producing a harvest of dry grain weight, number of grains per panicle and rice production was better than inorganic fertilizers treatment on soil ultisol. Hartanto and Melati (2013) suggested that a sustainable compost application can increases of yield. Compost treatment in the same field during two season of planting was produced two rice production of 2 t ha^{-1} increased to 5.04 t ha^{-1} (Hartanto and Melati, 2013). Based on this results of this research, the application biofertilizer, compost and 50% dosage of inorganic fertilizer in ricefield, the use of as an additional source of nutrients are needed to provide the best productivity results.

The number of bacterial cells in rice soil until harvest time 115 days after planting has increased by 200-500% (Table 5). This fact was comparable with research conducted (Rao, 1995) that in order to increase the number of soil microbes were required the addition of biofertilizers containing microbes. Another fact research was conducted (Danapriatna *et al*., 2012) that the application of biofertilizer able to increase the population of *Azotobacter* sp. and *Azospirillum* sp. In addition, rice straw compost application also can support directly increase rice plant soil 5% C-organic (Yan *et al*., 2007). The increase of cells bacterial number during planting was due to the presence of organic materials contained in compost. Compost provides an element of C and N were used for bacterial metabolic processes that support its growth (Triadiati *et al*., 2013). Soil conditions after planting in compost enriched treatment better than without fertilization (Table 6). The increasing of N, P and C nutrient were affect from biofertilizer contained bacteria. Bacterial phosphate solubilizer, fixing N_2 and lignocellulosic degrader add to content of their element in soil. According to Mezuan *et al*. (2002), the used of biofertilizers contained microbes can improve the physical, chemical and biological properties of soil, also able to increase the crop yields. The higher of P nutrient content was found at the end of this research (18.2 ppm). This was assumed due to an increase of microbial population in soil with a biological fertilizer. Giving a high compost (5 t ha^{-1}) and biofertilizer were assumed very effective to react with Fe and Al, so that the fixation of phosphorus in soil will decreased and increasing their availability. Organic materials such as compost was able to bind colloidal and cations which capable of fixing P in soil becomes mineralized, also the presence of organic acids decomposition of organic material which capable of solubilizing P element from it binder (Hanafiah *et al*., 2007). An increase C-organic content in soil of 1.25% (initial soil analysis) to above 3.11% (analysis of compost enriched plots) was indicated that its treatment has already restoration of soil health. This condition was caused by C-organic of soil is a key indicator of soil health indirectly. The higher of C-organic content of soil was indicated the soil was healthier.

CONCLUSION

The eight bacterial isolates from North Central Timor (NCT) capable of producing IAA and solubilizing phosphate. The EP.01 isolate was produced the highest IAA concentration of 119.5 ppm in medium with addition 1.0 mM tryptophan, while EP.02 isolate has highest index of phosphate solubilizer (IP) was 3.5. Selected isolates (EP.01 and EP.02) were belongs to Gram-positive bacteria. These isolates were closely related to *Bacillus* sp. with 97.7 and 98.1% maximum identity, respectively. The application of biofertilizers containing *Bacillus* sp. and compost in NCT rice fields, clearly showed that it capable of increasing the vegetative growth and production of rice plants, also increasing the number of bacterial cells. The treatment of compost enriched +50% NPK, showed the best results on the vegetative growth of rice plants and the number of grains per clump, weight of 1000 grains and dry grain harvest (GKP) which were 30.76, 29.37 g and 6.3 t ha^{-1}, respectively. The number of bacterial cells in compost-enriched plots (P3) has increased by 200-500% and increasing the fertility of rice soil, especially after treatment in P3 plot an increase C-organic nutrients, P-soluble and N-total.

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