Research Article

Effects of seaweed waste on the viability of three bacterial isolates in biological fertilizer liquid formulations to enhance soil aggregation and fertility

Novi Arfarita¹*, Takaya Higuchi², Cahyo Prayogo³

¹ Faculty of Agriculture, University of Islam Malang, Malang 65144, Indonesia.
² Division of Environmental Science and Engineering, Graduate School of Science and Engineering, Yamaguchi University, Yamaguchi 755-8611, Japan.
³ Faculty of Agriculture, University of Brawijaya, Malang 65145, Indonesia.
*corresponding author: arfarita@unsima.ac.id

Received 16 May 2019, Accepted 25 June 2019

Abstract: Biofertilizer production in Indonesia should fulfil the minimum requirement for being produced and released to the market. Problems occurred when those products are being absent on informing those expiration dates and the viability of microbial activity which then closely related to the quality of the product. Seaweed composted material are potential resources for producing Biofertilizer, but lacking on the optimization on their process as this material contain a various important component for soil and environment. The production of Biofertilizer from seaweed waste required an optimum condition, i.e.: pH and typical microbe which could germinate under specific formulation and temperature. This study aimed to determine the optimum pH in liquid fertilizer formulations made from seaweed waste in the form of composted material, to test the viability of three bacterial isolates and those pathogenicity properties, to examine the effect of metabolites release from bacterial isolates to green bean seed germination. The experimental design used was a completely randomized design with four treatments, which were as follow: P0 as a control (Peptone), RP1 (seaweed waste), RP2 (seaweed waste and glycerol), and RP3 (seaweed waste and PEG). The three bacterial isolates used were: (1) Bacillus licheniformis, (2) Psudomonas plecoglossicida and (3) Pantoea ananatis. This liquid fertilizer biological formulation was stored for 8 weeks at pH 5.5 and temperature 25°C. The results showed that the treatment of RP1 (seaweed waste) had high bacterial viability and could stimulate growth for green bean sprouts. The carrier material for seaweed waste with the addition of glycerol and PEG showed no effect of the disease and symptoms of a pathogenic bacterial consortium on germination of green beans.

Keywords: biofertilizer, microbe, compost, pathogenity, seaweed waste

To cite this article: Arfarita, N., Higuchi, T. and Prayogo, C. 2019. Effects of seaweed waste on the viability of three bacterial isolates in biological fertilizer liquid formulations and green bean sprout growth (Vigna radiata L.). J. Degrade. Min. Land Manage. 6(4): 1889-1895, DOI: 10.15243/jdmlm.2019.064.1889.

Introduction

The use of chemical fertilizers in the world continues to increase resulting in concern among environmental experts. This can increase the level of soil pollution which in turn can affect human health and soil microflora balance (Lingga and Marsono, 2000). At present farmers have started to use of various biological fertilizers in their farming system. Biofertilizers are inoculants made from active or latent organisms in a liquid or solid form that have the ability to mobilize, facilitate and increase the availability of non-available nutrients into available form through biological processes.

One of the problems on using biological fertilizers is the absence of expiration date information and those of microbial viability which then determine Biofertilizer quality (Husen, 2007). On the other hand, the application of seaweed
Effects of seaweed waste on the viability of three bacterial isolates in biological fertilizer formulations

waste for agricultural crops has long been carried out in several countries. The use of seaweed waste for plants such as various types or forms of seaweed fertilizer product such as : (1) liquid seaweed fertilizer (LSF), (2) seaweed liquid fertilizer (SLF), (3) liquid fertilizer (LF), and chopped powdered algae manure that are commonly distributed on market (Sedayu et al., 2013). Seaweed has long been used directly as a soil and fertilizer conditioner in various coastal regions (Haslam and Hopkins, 1996; Coccozza et al., 2011). Seaweed extract has also been widely marketed as an additive for the plant as a fertilizer in which those benefits and advantages of its use has been recently reported (Fornes et al., 2002; Padhiand Swain, 2006; Sivansankari et al., 2006). Chemically, seaweed consists of water (27.8%), protein (5.4%), carbohydrate (33.3%), fat (8.6%) crude fibre (3%) and ash (22.25%). Besides carbohydrates, proteins, fats and fibre, seaweed also contains enzymes, nucleic acids, amino acids, vitamins (A, B, C, D, E and K) and macro minerals such as nitrogen, oxygen, calcium and selenium as well as microminerals such as iron, magnesium and sodium.

In this study, a liquid formulation of Biological fertilizer made from seaweed waste was added by three indigenous bacterial inoculants obtained from previous studies (Arfarita et al., 2016; 2017; 2019), namely: *Pseudomonas plecoglossicida*, *Bacillus licheniformis*, *Pantoea ananatis* as consortium bacteria isolates. This consortium isolate was grown on a 100 ml Erlenmeyer tube containing 50 ml NB (Nutrient Broth) with 5 pH treatments (5.0; 5.5; 6.0; 6.5; 7.0). The treatment was repeated four times (using the formula t (n-1) ≥ 15). Determination of bacterial consortium growth was carried out under a completely randomized design (CRD), by testing all each pure isolates (*Pseudomonas plecoglossicida*, *Bacillus licheniformis*, *Pantoea ananatis*) as consortium bacteria isolates. This consortium isolate was grown on a 100 ml Erlenmeyer tube containing 50 ml NB (Nutrient Broth) with 5 pH treatments (5.0; 5.5; 6.0; 6.5; 7.0).

Manufacturing of liquid biofertilizer

The liquid Biofertilizer formulation was made using seaweed waste in the form of composted material, which then PEG was added into, followed by the addition of glycerol, and the three consortium bacterial isolates. The ingredients were mixed according to the treatment, namely : P0 (Pepton Water 0.1% + 3 bacterial isolates), RP1 (Seaweed waste + consortium of 3 bacterial isolates), RP2 (Seaweed waste + consortium of 3 bacterial isolates + 1% glycerol), and RP3 (seaweed waste + consortium of 3 bacterial isolates + 1% PEG). The carrier materials were sterilized first, then added additional ingredients and liquid cultures of bacteria, and stirred until homogeneous. Then put in a plastic bottle packaging, stored in a dry place, at room temperature and avoid direct sunlight.

Viability test for bacteria

The viability test was conducted using the spread plate method on PCA media. TPC (Total Plate Count) is carried out by taking 10 μl of liquid.
bacterial culture in certain dilutions using peptone water. Petri dishes were incubated for 24 hours at 30ºC (Astuti., 2014).

Pathogenicity test
Green bean sprouts are grown in a test tube containing Yoshida's media. As much as 200 µml of liquid biofertilizer formulation was included in each test tube aseptically. Each treatment was repeated 4 times using a completely randomized design. The parameters observed from the pathogenicity test were the presence or absence of malformations, necrosis and decay. The observation was conducted to examine green bean sprout growth and total root length.

Data analysis
The analysis used was the analysis of variance (ANOVA) with a 5% F test to determine the effect of each treatment, followed by a 5% LSD test to find out the differences between treatments.

Results and Discussion
Microbial purification
Three bacterial isolates were purified with the streak plate method on NA (Nutrient Agar) media before added on to liquid culture as a bacterial consortium. After a single colony was obtained, then staining was done to observe the purity of the isolate (Figure 1).

Observation of optimum pH
Observation of optimum pH for bacterial growth consortium in liquid media was observed using a spectrophotometer. The quantity of bacterial growth was measured with light absorbed by the bacterial suspension. This was expressed in OD (Optical Density) at the OD600 value to read the level of turbidity in the Bacterial medium. Based on Figure 2, it can be seen that the optimum pH required by the three bacteria to grow in a consortium was at pH 5.5 with the results of OD 600 of 1.542.

Table 1. The average number of colony (CFU/ml) across different of the liquid biofertilizer treatments over a period of 8 weeks incubation.

| Treatments | Average of number of bacterial colony (CFU/ml), in weeks |
|------------|--------------------------------------------------------|
|            | W0  | W1  | W2  | W3  | W4  | W5  | W6  | W7  | W8  |
| P0         | 6.32ab | 10.61a | 11.68a | 13.52 | 12.87a | 12.87a | 12.33 | 12.32 | 11.84b |
| RP1        | 6.36b | 10.98bc | 12.36b | 13.06 | 12.94a | 12.94a | 12.13 | 12.00 | 12.75c |
| RP2        | 6.66b | 10.80ab | 11.99a | 12.83 | 14.32b | 14.32b | 12.47 | 12.30 | 12.00b |
| RP3        | 6.00a | 11.17c | 11.73a | 13.5 | 13.50a | 12.77a | 12.22 | 12.05 | 11.07a |
| LSD 5%     | 0.34 | 0.20 | 0.32 | Ns  | 0.53 | 1.39 | Ns  | Ns  | 0.35 |

Note: Number with a similar letter within a column showed not significantly different at a significant test of LSD 5%. Ns= Not significantly different.
Graphs of viability and growth of the consortium of three bacterial isolates from all treatments during the 8 weeks of observation showed significantly different (P<0.05) are presented in Figure 3. Treatment of P0, RP1 and RP3 reach the peaks of growth at the 3rd week, however, RP2 treatment obtained the best growth for the following week. After the eighth week of cultivation, all treatments meet declining population phase except for the treatment of RP1. By the end week of observation, the highest viability value was obtained at the treatment of RP1 (Table 1).

Pathogenicity test

Pathogenicity tests were observed until green bean sprouts were 6 days old by observing symptoms of necrosis, decay and sprout malformations compared to controls showed negative results (Figure 4). Whereas sprout growth is presented in Table 2. From Table 2, it can be seen that in term of the parameters of sprout length, the treatment of control and P0 was not significantly different in sprout length compare to RP1 and RP3 treatments, but not for RP2. In addition, this treatment was not significantly different compared to RP1 and RP3. Furthermore, in term of root length measurement, there was not significantly different across the treatments.

Table 2. Growth of green bean sprout across various liquid biofertilizer treatments.

| Treatment | Sprout length (cm) | Root length (cm) |
|-----------|--------------------|------------------|
| Control   | 4.74a              | 2.64             |
| P0        | 4.71a              | 2.46             |
| RP1       | 7.5ab              | 2.25             |
| RP2       | 9.3b               | 2.35             |
| RP3       | 6.9ab              | 2.04             |
| LSD 5%    |                    |                  |
| Sig       |                    | N-Sig            |

Note: Numbers with similar letters within a column showed not significantly different at a significant test of LSD 5%.

Discussion

Observation of optimum pH for bacterial consortium growth

The main factors that are affecting the growth of microorganisms include the supply of nutrients, time, temperature, water, pH and the availability of oxygen. Each organism has a range of pH values where growth is still possible, and each usually has an optimum pH. Most microorganisms can grow within the range of pH 6.0-8.0, and the pH value outside the range 2.0 and 10.0 is usually destructive (Winarwi, 2006). From observations of bacterial growth by observing the absorbance value of OD600 (Figure 2), it was found that at the 72nd hour of observation all treatments showed differences and the best results were shown at pH 5.5. Therefore, from this result, we can conclude that soil aggregate-fixing bacteria (*Pseudomonas plecoglossicida*), phosphate solvents (*Pantoea ananatis*) and N fixing bacteria (*Bacillus licheniformis*) can grow as a consortium with optimum pH 5.5. From the previous studies, it was known that *Pseudomonas plecoglossicida* grew optimally at pH 7.3 and optimum temperature 28°C (Borica, 2009). Deepa (2009) shows that *Pantoea ananatis* can grow at pH 7.0 (pH minimum 4.0 and maximum 10.0) with optimum temperature 28°C - 30°C (minimum temperature 15°C and maximum 37°C). *Bacillus licheniformis* forms spores in the soil and produces various extracellular enzymes that are related to the cycle of nutrients in nature. The optimal growth temperature is at 50° C. The optimal temperature for enzyme secretion is at 37° C. Because it can grow in alkaline conditions, these bacteria are able to produce proteases that can survive at high pH. Protease *Bacillus licheniformis* has an optimal pH of about 9 and 10 (Pepe et al., 2003).
Viability test of bacteria in liquid biofertilizer

The liquid biofertilizer formulation used in this study is composted material from factory waste for seaweed. The addition of the carrier materials used is to add food content to prolong the life span of the bacteria in liquid biofertilizer formulations.

Hadioetomo (1990) revealed that a medium is a material used to grow microorganisms above or in it. The manufacture of liquid biofertilizer formulation is performed by utilizing seaweed waste added with bacterial isolates. This seaweed waste is wasted material; its existence is abundant and has a low selling price. The use of seaweed waste as fertilizer or fertilizer additives is expected to be an alternative solution to environmental problems because it is safe for soil and plant microbes and also increases the economic value of seaweed in Indonesia.

Chemically seaweed consists of water (27.8%), protein (5.4%), carbohydrate (33.3%), fat (8.6%) crude fibre (3%) and ash (22.25%). Besides carbohydrates, proteins, fats and fiber, seaweed also contains enzymes, nucleic acids, amino acids, vitamins (A, B, C, D, E and K) and macro minerals such as nitrogen, oxygen, calcium and selenium as well as microminerals such as iron, magnesium and sodium (McHugh, 2003).

The viability test is carried out to determine bacterial growth. The growth of microorganisms is defined as the increase in cell weight. Cell weight is relatively the same in each cell cycle so that growth can be defined as an increase in the number of cells (Purwoko 2009). This phase shows the condition of the cell when it runs out of food, and there is a buildup of toxic metabolic products resulting in a decrease in the number of living cells (Fujikawa et al., 2004).

In general, bacterial media must contain sources of carbon, nitrogen, sulfur, phosphate, vitamins or ingredients that can promote bacterial growth such as meat extract or yeast. Meat extracts contain peptone and amino acid acids. Peptone is used in culture media as a source of nitrogen. Many simple nitrogen compounds are contained in peptones so that the nitrogen element is easily removed. Besides that, there are also bacteria that need fertilizers such as blood, serum and metals from inorganic salts as "trace elements" / microelements such as Ca, Mn, Na, Mg, Zn, Co, Fe, Cu (Collin and Lyne 1987). Peptone is an important component in microbial growth media which acts as a nitrogen source. Peptone by bacteria is broken down into amino acids, then absorbed to be used as an energy source and build cytoplasm (Wiranti, 2014).

Addition of peptone (treatment P0) with all three bacterial isolates able to add nutrients and to supply food to bacteria. The most important feature of peptone is its function as a source of nutrition for microorganisms so that it requires a high amount of nitrogen and amino acids that can support the growth of microorganisms (Astuti, 2014). However, peptone media is very expensive for the sake of commercialization and mass production for field applications. Its use here is as a control or comparison. The concentration used is based on the use of culture in the laboratory, and the appropriateness of costs for field applications. The treatment of RP2 consisting of seaweed waste, a consortium of three bacterial isolates and glycerol is known to be able to extend the viability of bacteria. Because the content of seaweed waste in the form of N, P and K is even rich in protein, flour, sugar, and vitamins A, C and D (McHugh, 2003). The protein contained in seaweed waste is able to become a food source for bacteria to maintain its survival. Glycerol is the simplest compound, with hydroxyl which is hydropholic and hygroscopic. Glycerol is a component that makes up various types of lipids, including triglycerides. Glycerol also functions as a stabilizer (stabilizer) from the carrier material and can extend the shelf life of the formulation (Advance et al., 2015). Glycerol (I, 2,3-propanetriol) or also called glycerin is a trihydric alcohol compound with the formula of building CH₂OHCHOHCH₂OH. Glycerol is a clear, hygroscopic, thick liquid, and tastes sweet. Glycerol is found in vegetable and animal oils and fats but is rarely found in its own form. Glycerol prepares oil and fat after mixing with fatty acids such as stearic acid, oleic acid, palmitic acid, and lauric acid (Kern, 1966).

RP3 is a liquid formulation added by PEG in addition to compost wash seaweed as its basic ingredient. PEG is a polymer consisting of several monomer bonds. Polyethylene glycol (PEG) belongs to the synthesis polymer group. Polyethylene glycol (PEG) is a synthetic compound that is widely used in the food, pharmaceutical, and, cosmetics and agricultural industries. PEG has soluble properties in warm water, non-toxic, non-corrosive, odorless, colourless, has a very high melting point (580 ° F), is spread evenly, hygroscopic (volatile) and can also bind pigments, so PEG can be used as an additional ingredient for bacterial viability.

But if seen at week eight, there is a difference in the treatment of RP1. When all other treatments have experienced a decline in the bacterial growth population, the treatment of RP1 is still experiencing a log phase. This shows that there is a need for additional periods to test the viability of RP1 so that it is known when the phase of population decline occurs. Then if viewed from the...
Effects of seaweed waste on the viability of three bacterial isolates in biological fertilizer formulations

Based on the literature, the optimum pH for growth of each isolate in the bacteria of *Pseudomonas plecglossicida, Pantoea ananatis* and *Bacillus licheniformis* were different. However, when they were cultured under bacterial consortium for formulating liquid biofertilizer, the optimum pH was 5.5. Within the storage period up to 8 weeks, the formulation of bio-treatment RP1 (peptone with the addition of three bacterial isolates and glycerol) was giving the most optimal results for a long-term bacterial stationary period of 3 weeks, and the population of bacteria produced at 8 weeks was $12.75 \times 10^9$ CFU/ml. This was due to the formulation of the addition of glycerol can prevent bacteria cell wall integrity. The consortium of three isolates of bacteria did not produce any exudates in liquid form, which could result in diseases, for the germination of a green bean sprout. The significant difference of green bean sprout length, between the control and treatments, were likely due to the growth of hormones produced by the metabolism of the bacteria, or the effects of the additional material contained in that liquid fertilizer.

**Conclusion**

Based on the literature, the optimum pH for growth of each isolate in the bacteria of *Pseudomonas plecglossicida, Pantoea ananatis* and *Bacillus licheniformis* were different. However, when they were cultured under bacterial consortium for formulating liquid biofertilizer, the optimum pH was 5.5. Within the storage period up to 8 weeks, the formulation of bio-treatment RP1 (peptone with the addition of three bacterial isolates and glycerol) was giving the most optimal results for a long-term bacterial stationary period of 3 weeks, and the population of bacteria produced at 8 weeks was $12.75 \times 10^9$ CFU/ml. This was due to the formulation of the addition of glycerol can prevent bacteria cell wall integrity. The consortium of three isolates of bacteria did not produce any exudates in liquid form, which could result in diseases, for the germination of a green bean sprout. The significant difference of green bean sprout length, between the control and treatments, were likely due to the growth of hormones produced by the metabolism of the bacteria, or the effects of the additional material contained in that liquid fertilizer.

**Acknowledgement**

The authors wish to thank Oniek Zaqiyyah for her contribution to assist in laboratory work.

**References**

Advinda, L., Fifendy, M. and Rahmadeni, Y. 2014. The potency of *Pseudomonas fluorescent* isolates CAS3 on various formulation with the addition of glycerol stabilizer for controlling blood disease bacteria (BBB) under in-vitro condition. *Jurnal Sains Tek (*6(2): 102-109 (in Indonesian).

Arfarita, N., Hidayati, N., Rosyidah, A., Machfudz, M. and Higuchi, T. 2016. Exploration of indigenous soil bacteria producing-exopolysaccharides for stabilizing of aggregates land potential as biofertilizer. *Journal of Degraded and Mining Lands Management* 4(1): 697-702.

Arfarita, N., Lestari, M.W., Murwani, I. and Higuchi, T. 2017. Isolation of indigenous phosphate solubilizing bacteria from green bean rhizospheres. *Journal of Degraded and Mining Lands Management* 4(3): 845-851.

Arfarita, N., Muhibuddin, M. and Imai, T. 2019. Exploration of indigenous free nitrogen-fixing bacteria from rhizosphere of *Vigna radiate*. *Journal of Degraded and Mining Lands Management* 6(2): 1617-1623.

Astiti, W.R.E. 2014. Application of peptone from the rotten side fish catching raw material (HTS) as a component of bacterial and yeast media. Fisheries and marine science. Bogor Agricultural Institute (IPB). Bogor (in Indonesian).

Borica, H. and Fulekar, M.H. 2009. *Pseudomonas plecglossicida* as a novel organism for the bioremediation of cypermethrin. Department of Life Sciences, University of Mumbai.

Cocozza, C.A., Parente, C. Zaccoone, C. Mininni, P. Santamaria, T. and Miano. 2011. Comparative management of offshore Posidonia residues: composting vs. energy recovery. *Waste Management (Oxford) 31: 78-84.*

Collin, C.H. and Lyne, P.M. 1987. *Microbiological Methods, 5th Edition.* Butterworths, London. 419-431p.

Deepa. 2009. Isolation and characterization of plant growth-promoting strain Pantoce NII-186. From Western Ghat Forest soil, India.

Fornes, F., Sanchez, P.M. and Guadiola, J.L. 2002. Effect of a seaweed extract on the productivity of `de Nules’ Clementin endanarimna velina orange. *Botanica Marina* 45:486-489.

Fujikawa, H., Kai, A. and Morozumi, S. 2004. A new logistic model for *Escherichia coli* growth at
Effects of seaweed waste on the viability of three bacterial isolates in biological fertilizer formulations

constant and dynamic temperatures. Food Microbiology 21:501-509.
Hadioetomo, R.S. 1993. The basic of Microbiology in Practices. Gramedia. Jakarta (in Indonesian).
Haslam, S.F.I. and Hopkins, D.W. 1996. Physical and biological effects of kelp (seaweed) added to soil. Applied Soil Ecology 3:257-261.
Husen, E., Simanungkalit, R.D.M. dan Irawan. 2007. Characterization and quality assessment of Indonesian commercial biofertilizers. Indonesian Journal of Agricultural Science 8: 31-38.
Kern, J. 1966. Glycerol. Encyclopedia of Chemical Technology, Vol. 10. Interscience Publishers, New York.
Lingga, P. and Marsono. 2000. Guidelines for Fertilizer Application. Penebar Swadaya, Jakarta. Pp. 80 (in Indonesian).
McHugh, D.J. 2003. A Guide to Seaweed Industry. Food and Agriculture Oganization of the UN, Rome. 88p.
Padhi, S.B. and Swain, P.K. 2006. Effective Role of Microorganism dan Seaweed as Biofertilizer in Organic Farming for a Sustainable Environment. http://wgbis.ces.iisc .rne t /energy/lake 2006 /programme/programme. procee/fullpaper _pdfs/Sailabala%20Padhi.pdf. Akses on 13 October 2016.
Pepe O., Blaiotta, G. Moschetti, G. Greco, T. and Villani, F. 2003. Rope-producing strains of Bacillus spp. from wheat bread and strategy for their control by lactic acid bacteria. Applied Environmental Microbiology 69(4):2321.
Purwoko, T. 2009. Fisiology of Microbes. Jakarta (ID): Bumi Aksara. pp. 199-201 (in Indonesian).
Sedayu, B.B., Basmal, J. and Utomo. B.S.B. 2013. Identification of growth hormone from urine extract (cow) Eucheuma cottonii. Journal of Postharvest dan Marine Biotechnology and Fisheries 8(1): 1–8 (in Indonesian).
Sivasankari, S., Venkatesalu, V. Anantharaj, M. and Chdanrasekaran M. 2006. Effect of seaweed extract on the growth and biochemical constituents of Vigna sinensis. Bioresource Technology 97: 1745–1751.
Winariwi, 2006. Viability test of Bacteria dan Activity of Proteolytic bacterial enzyme on carrier media Carrier of bekatul. Thesis. Faculty of teacher training and educational sciences. The University of Sebelas Maret, Surakarta (in Indonesian).
Wiranti, N.G. 2014. Manufacturing of peanut Pepton by rough Papain enzyme for bacterial growth media. Bogor Agricultural Institut Bogor (in Indonesian).