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Jatinangor, Indonesia
8–9 August 2017

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I Made Joni and Camellia Panatarani

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Table of Contents

Preface: The 1st International Conference and Exhibition on Powder Technology Indonesia (ICePTi 2017)

The 1st International Conference and Exhibition on Powder Technology Indonesia (ICePTi 2017) was held in Universitas Padjadjaran, Jatinangor Campus, Bandung, Indonesia during 8th – 9th August 2017. Jatinangor, the house for various Universities amidst the pleasant environment is an ideal venue for the conference.

ICePTi 2017, organized by Nanotechnology and Graphene Research Center (Print - G), Science & Technology Park Unpad (KST-Unpad), Material Science & Engineering (MSE) Study Center Unpad, Indonesian Powder Association and managed by JP Global Transtech. In connection with this event, The 4th Japan Powder Technology Forum was held in Indonesia. The objectives of this forum is to acquaint with the field of powder technology and technologies developed by Japanese companies.

This conference is aimed at elevating the scientific communities, explore new ideas, research progress, technological developments and light up collaboration between researchers and industries, in powder technology and its applications. Exhibition was also organized to promote the new products or technologies developed under these four categories: Manufacturing & Processing Equipment, Instrumentation, Measuring & Laboratory Equipment, Materials Engineering, Science & Technology Park Unpad.

There were 14 invited speakers, 49 abstracts and 63 scientific participants from countries like Indonesia, Japan, India and United States, contributing to the overall success of the Conference. The abstracts were considered under two categories, powder science & technology and powder applications. Among the total number of presented abstracts, 52 articles were published in AIP Conference Proceedings.

Generous support for the conference was provided by Rector, Directorate of Research and Community Service and Innovation, Universitas Padjadjaran.

On behalf of the organizing committee, we would like to register our thanks to the advisory committee, participants and all who have supported to the success of this scientific meeting. We would also like to thank all the members of the organizing committee, it is our privilege and honour to be the corresponding Editors.

Corresponding Editors

I Made Joni

Camellia Panatarani

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Cell Viability of Mycorrhiza Helper Bacteria Solid Inoculant in Different Carrier Material

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Abstract. Roots of food crops are colonized by nonpathogenic mycorrhizal fungi which show natural ability to control plant pathogen. Mycorrhizal establishment in plant roots is affected by rhizobacteria, known as mycorrhiza helper bacteria (MHB), which has synergetic effects on mycorrhizal associations. Laboratory experiment has been conducted to assess the best carrier material to develop well-qualified MHB of *Pseudomonas diminuta* and *Bacillus subtilis* solid inoculant. Carrier materials were 100 mesh organic matter of agricultural waste. Different spore concentration of both bacterial liquid inoculants were grown on three kinds of 100-mesh organic matter and stored at room temperature up to 90 days. Cell viability of both MHB were counted by serial dilution plate method by using specific medium. The results showed that sugar cane baggase ash was the best carrier material to maintain cell viability for both MHB. However, the population of *Pseudomonas diminuta* and *Bacillus subtilis* in sugar cane baggase ash were slightly decreased after 90 days. The use of sugarcane baggase ash for solid MHB inoculant development could be suggested.

INTRODUCTION

Micorrhyza Helper Bacteria (MHB) behaves as helpers for mycorrhiza to perform its function or role. These endophytic bacteria, residing within the body of mycorrhiza, have pivotal role in mycorrhizal development [1-2]. Previous studies have discovered that bacteria isolated from mycorrhizal fungi can stimulate mycorrhizal infection, spore production and also resistance to plant pathogens [1; 3-5]. MHB has four mechanisms in assisting the effectiveness of mycorrhizal infection in plants i.e., MHB effects on root acceptability, MHB effects on root recognition with fungi, MHB effect on fungal growth and modification of rhizosphere by MHB [1] respectively.

MHB from Genus Bacillus and Pseudomonas are able to function as biological control agents and also to increase the plant growth known as Plant Growth Promoting Rhizobacteria (PGPR) for its ability to increase nutrition, to produce growth hormone and to induce plant resistance known as induced systemic resistance (ISR) [6].

Among various types of MHB discovered in previous studies, such as *Pseudomonas diminuta, Bacillus licheniformis, B. laterosporus, Enterobacterhormaechei, B. brevis, B. subtilis, B. cereus (GG)* and *B. firmus* [7], *Pseudomonas diminuta* and *B. subtilis* are two potential MHB acting as a controlling agent of parasitic nematodes. Inoculation of *P. diminuta*and *B. subtilis* exerts significant effect in suppressing *Pratyenchuscoffeae* nematode population. Treatment of *B. subtilis* with density of 10^8cfu can suppress nematode population up to 71.3%. It means it is not significantly different from carbofuran synthetic nematicide which can suppress population up to 89.7%. Similarly, *P. diminuta* bacteria with density of 2.10^8 are able to suppress *P. coffeae* population up to 64.2% [8]. Other studies administered through the addition of *B. subtillis* and *P. diminuta* may increase the mycorrhizal ability to decrease *P. coffeae* population, crown damage score and root damage score and to increase plant height, stem diameter and genuine leaf coffee seedlings [9].

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Given that *Pseudomonas diminuta* and *B. subtilis* have been proved to control *P. coffeae* and help the growth of mycorrhiza, an easy application of both MHB in the field is significantly required. Microbial formulations can be either liquid or dry, liquid formulations contain biomass suspensions in water, oil or a combination of both (emulsions). Dry formulas contain active or inactive biomass in the form of powder or granules [10]. The efficacy of the bioformula is affected by the carrier compound used. The use of organics (peat, rice flour) and inorganics (talk and bentonite) as carrier compounds enhances the stability and effectiveness of bioformulation [11-13]. Therefore, in order to find the best carrier material that can maintain bacterial cell viability, the study on the formulation of MHB in solid form is important.

MATERIALS AND METHODS

Materials

The MHB isolates used were *Pseudomonas diminuta* (the collection of Microbiology Lab of Jember University) and *Bacillus subtilis* (the collection of Soil Biology Laboratory of Universitas Padjadjaran) maintained in NA medium. Both isolates are Plant Growth Promoting Rhizobacteria (PGPR) and also capable of dissolving phosphate. Mass culture medium of MHB isolates was 2% molasses originating from Sugar Factory of Probolinggo, while the carrier materials of the bio formula are sugarcane bagasse ash (SBA), cow manure and goat manure with 100 mesh powder size.

MHB Propagule Preparation

B. subtilis and *P. diminuta* were cultured on tilted nutrient agar in test tubes by scratch method prepared for each. To form a suspension, after the incubation period at 30 ± 2ºC for 24 h, the MHB culture was removed by using 5 ml of sterile distilled water and then mixed with vortex in order to be homogeneous. A total of 1 ml of isolate suspension was inserted into 100 ml of Nutrient Broth (NB) and shake for 24 h at 100 rpm in room temperature.

Mass Cultivation of MHB Propagules

A total of 1 ml of MHB propagules was inserted into 99 ml of 2% molasses and then homogenized using a shaker for 3 x 24 h at100 rpm in room temperature. After 72 h, the liquid inoculants of *B. subtilis* and *P. diminuta* bacteria were mixed with a ratio of 2: 3 (*B. subtillis*: *P. diminuta*, v: v). The bacterial consortium was incubated for 3x24 h. After 3 days, the consortium is ready for formulation.

Formulation

This study consists of two stages, 1) determining the best carrier, selected from there types of carrier material and 2) determining the quality of selected carrier based on cell density for both the species of bacteria. The preparation of the formulation was initiated by adding a consortium of MHB cultures with cell densities of 10^8 and 10^9 cfu to the three types of carrier material up to 20% water content. Then, the formula was stored in a sealed container. To obtain the best carrier material to meet the Minimal Organic Minimum Technical Requirement of Indonesian Ministry of Agriculture, cell viability, measured in cell density (cfu) and pH were observed on day 3. To get well-qualified carrier materials, cell density (cfu) and pH observations were performed up to day 90 and nutrient content analysis was also conducted. The data obtained were scored to establish the quality of selected carriers based on cell density of both bacterial species.

RESULTS AND DISCUSSION

Characteristics of Mass Cultivation Medium

Mass cultivation medium should be a low cost organic material. Based on the test results in Soil Biology Laboratory of Universitas Padjadjaran the best mass cultivation medium for bacteria is molasses. Molasses is a by-

product of sugarcane commonly sold at a low price. Therefore, this study used 2% molasses as mass cultivation medium. Analysis of nutrient content was carried out to figure out the nutrient content of molasses (Table 1).

TABLE 1. Results of nutrient content analysis on molasses

Nutrients	Contents
Total-N	0.29 %
Total-P	15.20 mg/100 mg
P_2O_5	0.18 %
K_2O	0.39 %
Organic-C	56.79 %
C/N Ratio	19.58

Based on Table 1, the molasses used in this study contained very high Organic-C which is very appropriate for the use as bacterial mass culture medium. Similar results were also shown by [14-15], molasses contains 48-56% sugar, with 30-40% sucrose content and 4-9% glucose.

Quality of the Three Types of Solid Bioformula Carrier

Three types of carrier materials, namely sugarcane bagasse ash, cow manure and goat manure with a powder size of 100 mesh and an initial cell density of 10^8 and 10^9 cfu, were tested to decide the best solid bioformula carrier that can maintain the viability of the MHB isolates (Table 2).

TABLE 2. Cell density and pH of the MHB inoculants carrier and cell density differences in 3 days of storage time

Carrier Type	Initial Cell Density (cfu)	Average Cell Density (CFU) x 10^7		pH
		P. diminuta	*B. subtilis*	
Sugarcane Bagasse Ash (SBA)	10^8	85.50	17.50	7.57
	10^9	23.67	44.17	7.60
Cow Manure	10^8	23.33	20.33	9.73
	10^9	30.00	42.83	9.80
Goat Manure	10^8	34.67	28.17	8.53
	10^9	25.00	11.67	8.57

The solid bio-fertilizer must comply with the Technical Requirement of Minimum Solid Organic Fertilizer of Ministry of Agriculture of Republic of Indonesia (Permentan No 70 / Permentan /SR.140 / 10 / 2011), which requires a cell density of $\geq 10^7$ cfu/g dry weight sample with pH 5-8. Table 2 reveals that the cell density of those carriers meet the dry weight requirement, but the mean pH of cow and goat manure is above 8, which exceeds the maximum technical requirement of compound biomass 5-8. Therefore, sugarcane bagasse ash (SBA) was selected as the bioformula carrier in this study. The analysis of sugarcane bagasse ash nutrients is shown in Table 3.

TABLE 3. Nutrient content of sugarcane bagasse ash (SBA)

Nutrients	Contents
Total-N	1.89 %
Total-P	24.70 mg/100 mg
K_2O	1.58 %
Organic-C	29.09 %
C/N Ratio	15.39

Sugarcane bagasse ash (SBA) is a solid fibrous residue derived from the process of refining sap in sugar mills. In 2008, fifty-seven sugar mills in Indonesia produced more than one million tons of SBA and ash kettle over thirty-four thousand tons. This large amount of SBA is a potential bioformula carrier. The nutrient composition of SBA depends on the pattern of production and origin of sugarcane. The results of nutrient analysis on SBA (Table 3) unfold the nutrient contents needed for bacterial growth, among which are the elements of nitrogen, phosphate, potassium and carbon.

Quality of the Selected Solid Bioformula Carrier Material

To test the quality of the selected bioformula carrier material, sugarcane bagasse ash (SBA) (Table 2), the cell densities and pH were observed up to 90 days (Table 4).

TABLE 4. Inoculant cell density and pH of MHB on bagasse within 3, 7, 30, 60 and 90 days of storage time

Initial Cell Density	Storage Period	Cell Density (cfu) x 10^7		pH	Score Value
		P. diminuta	*B. subtilis*		
10^8	3 days	85.50	17.50	7.57	1.67
10^9		23.67	44.17	7.60	1.33
10^8	7 days	78.17	27.50	7.57	1.67
10^9		58.67	51.00	7.63	1.33
10^8	30 days	76.67	66.83	7.47	1.67
10^9		63.75	30.33	7.40	1.33
10^8	60 days	19.22	12.25	7.70	1.33
10^9		13.9	12.52	7.60	1.67
10^8	90 days	6.4	5.4	7.367	1.67
10^9		3.783	6.3	7.40	1.33

Based on the observations of cell density and pH on 3, 7, 30, 60 and 90 days of storage time, the solid bioformula with the initial cell density of 10^8 and 10^9 still met the minimum technical requirements of solid compound biochemical fertilizer ≥ 10^7 cfu /g dry weight sample with a pH of 5-8, but a sharp decline of the cell density occurred after 30 days.

The viability of MHB isolates was influenced by bacterial characteristics, initial cell density and storage time. *Bacillus subtilis*, a gram-positive, rod-shaped bacterium, can grow in aerobic and anaerobic conditions and has endospores as resistant structures when environmental conditions are not favorable [16]. *P. diminuta* is a gram-negative, straight-shaped bacterium with a length of 1.5-5 μm, which also does not form the body regeneration and also has no sheath around his body. The movement of *P. diminuta* bacterium was carried out by using one flagellum body [17].

On day 3 the cell viability of *B subtilis* was still low compared to *P. diminuta* possibly due to the adaptation to the new environmental conditions. *B. subtilis* is a bacterium capable of forming spores. In the early storage, the young spores were unable to survive at new environmental conditions, but their viability increased after 7 and 30 days of storage time. While those mature ones could adapt well to the new environment. However, the viability of *B. subtilis* decreased sharply on day 60 and 90. Different responses were shown by *P. diminuta* which show very high cell density on day 3. It did not form spores, so it could grow easily in the new environment, but its viability decreased along with the increasing storage time.

The decrease in the number of bacterial colonies was attributed to the reduced nutrients in the medium during the storage. In fact, well-qualified carriers would be able to meet the nutritional needs of bacteria and support endophytic bacteria survival during storage. In this study, the only source of nutrients was from the sugarcane bagasse ash (SBA) without any additional ones. The result of nutrient contents analysis on the SBA show low N and P element contents which were insufficient for bacteria to survive for long. Hence, to increase the bacterial lifetime optimization effort is required through the addition of various nutrient compositions on the SBA. In addition, the population decline was caused by the competition between bacteria in obtaining nutrients for growth.

CONCLUSION

The results showed that sugarcane bagasse ash (SBA) was the best carrier material to maintain cell viability of both MHB. Although the population of *Pseudomonas diminuta* and *Bacillus subtilis* in sugarcane bagasse ash were slightly decreased within 90 days, the use of sugarcane bagasse ash for solid MHB inoculant development is highly recommended.

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