Open Access Research Journal of
Science and Technology
Journals home page: https://oarjst.com/
ISSN: 2782-9960 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Biofertilizer gel contains Phosphate Solubilizing Microbes (PSMs) plus and its effect on phosphate dynamics in Inceptisols Jatinangor-Indonesia

Mega Kartika Hermawan *, Betty Natalie Fitriatin A and Nenny Nurlaeny

Universitas Padjadjaran, Jatinangor-Indonesia.

Open Access Research Journal of Science and Technology, 2022, 04(01), 058–070

Publication history: Received on 20 December 2021; revised on 31 January 2022; accepted on 02 Februay 2022

Article DOI: https://doi.org/10.53022/oarjst.2022.4.1.0025

Abstract

Dependence on inorganic fertilizers is a major risk in today's food supply efforts, along with the increasing population. One of the efforts to reduce this risk is to apply biological fertilizers that are specially formulated on the suitable carrier material so that they are able to work effectively and efficiently in substituting the need for inorganic fertilizer inputs and increasing plant yield components. Inceptisol series Jatinangor has characteristic soil texture is dominated by clay fraction, neutral pH 7.01. Under neutral conditions, in general, nutrients in the soil tend to be more readily available, but the availability of P is actually very low at only 2.82 $mg.kg^{-1}$ of soil. In this neutral to alkaline pH, the solubility of metal cations Ca and Mg increased, indicated by a high Ca^{2+} content of 12.60 $cmol.kg^{-1}$ and Mg^{2+} which was also high at 4.91 $cmol.kg^{-1}$. The nutrient imbalance is also due to the low C-organic content of the soil, namely 1.83%, so that the soil's support capacity is low. Low organic C causes the presence of indigenous microbes which is quite high, especially PSMs, namely 1 x 10^4 $cfu.g^{-1}$, which is not able to increase the availability of soil nutrients, especially P due to lack of carbon sources. The experiment aimed to determine the compatibility and characteristics of inoculants in the biofertilizer gel contains PSMs plus (BG) and its effect on the dynamics of phosphate in Inceptisols Jatinangor. BG contains a consortium of phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) which are packaged in hydrogel carriers and additives. The PSB used were *Bacillus subtillis, Pseudomonas maleii,* and *Bulkholderia cepacea*, while the PSF used was *Trichoderma asperellum*. The experiment were carried out in vitro at the Soil Biology Laboratory of Universitas Padjadjaran in collaboration with PT Agritek Tani Indonesia and at the Experimental Land of the Department of Soil Science of Universitas Padjadjaran. The experimental design used in this study was a single randomized block design (RBD) with 9 treatments and 3 replications, consisting of treatments (control: 0 BG + 0 P); (1 P); (1 BG); (1 BG + ½ P); (1 BG + ¾ P); (1 BG + 1 P); (½ BG + ¾ P); (¾ BG + ¾ P); and (1 ½ BG + ¾ P). The dose of fertilizer used was BG 50 kg. ha^{-1}, SP-36 100 $kg.ha^{-1}$, Urea 350 $kg.ha^{-1}$, KCl 50 $kg.ha^{-1}$, and sheep manure as basic fertilizer 2 $t.ha^{-1}$.The results showed that each isolate had the ability to dissolved phosphate, produced IAA, and one of them was antagonistic against the fungal pathogen *Fusarium* sp.. The combination of fertilizers had an effect on regulate soil pH, and increasing phosphatase activity, soil available P, soil potential P, and PSMs population in the soil. The formulation of 1 BG + ¾ P consistently showed the best effect on soil phosphate dynamics in Inceptisols Jatinangor.

Keywords: Biofertilizer; Hydrogel; Phosphate; Inceptisols; *Fusarium* sp.

1. Introduction

Inorganic fertilizer residues caused soil compaction, reduced available nutrients and soil organic matter, loss of soil biodiversity which indirectly reduces soil fertility and makes plants more susceptible to disease [1]. This causes soil fatigue which in turn has an impact on leveling off productivity. In addition, the process of manufacturing inorganic fertilizers requires a lot of energy, costs, and produces large greenhouse gas emissions. Thus, there is a need for alternatives to meet plant P needs without causing soil fatigue, productivity sloping, and environmental impacts.

* Corresponding author: Mega Kartika Hermawan
Universitas Padjadjaran, Jatinangor-Indonesia.

The use of biofertilizers is currently reported to reduce the need for inorganic fertilizers while at the same time improving soil health and crop yields. The application of phosphate solubilizing microbes (PSMs) was able to reduce the need for inorganic P fertilizer for maize by up to 50% [2].

Almost 75–90% of P fertilizer added to the soil is deposited by metal cation complexes present in the soil so that it cannot be absorbed by plants and apart of them measured as P-potential [3]. Habi *et al.* (2018) stated that the P-potential in Inceptisols tends to be high. If the land continues to be exploited without a sustainable agricultural approach, it is certain that the land will experience soil fatigue [4].

The use of biofertilizer gel contains PSMs plus (BG) in this study aimed to improve P dynamics in Inceptisol soils e. The BG consists of several phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) which were packaged in a hydrogel carrier. The PSB used were *Bacillus subtillis, Pseudomonas maleii,* and *Bulkholderia cepacia*. The PSF used was *Trichoderma asperellum.* Hybrid maize plants with the Pioneer P-36 variety used in this study because they are quite adaptive to environmental stresses and are responsive to fertilization so that it will facilitate the observation of responses.

Compatibility testing and characterization of each isolate aimed to obtain superior isolates that will live well on hydrogel carrier that was specially formulated so that the BG will have good viability, good shelf life, effective and efficient in carrying out their functions when applied. The selection of the microbial consortium was based on the research results of Fitriatin *et al.* (2020) which showed that the application of a mixture of bacteria *B. subtillis, P. maleii, P. fluorescens, B. cepacia* and P solubilizing fungi had a better effect on maize yields than only P solubilizing bacteria or fungi as single [4]. *Pseudomonas mallei* is compatible with *B. substilis, Burkholderia sp.,* and *B. megatherium*.

B. substilis is able to produce organic acids that can release orthophosphate from metal cations that bind it through chelation reactions. The organic acids produced include glucionic acid, acetic acid, succinic acid, propionic acid, and lactic acid [5]. Another ability is to convert organic P into inorganic P through the secretion of phosphatase enzymes or what is known as the mineralization process. The PSB also produces IAA phytohormones which can increase corn root elongation up to 67.7-74.5%.

According to Montesano *et al.* (2015), *Trichoderma* sp. able to increase available P and P uptake of cucumber plants by producing extracellular hydrolytic enzymes and increasing soil organic matter content [6]. Alori *et al.* (2018) also explained that *Trichoderma* sp. has the ability to become a bio-stimulant, biocontrol, and is able to dissolve several minerals including phosphate in vitro through the mechanism of regulating the pH of the medium, the production of metal chelating metabolites, and the activity of the phosphatase enzyme [7]. The reaction between organic acids secreted by PSMs can form stable organic chelates with Al, Fe, and Ca, or Mg to liberate bound phosphate ions [8].

The interweaving of functional soil fungal hyphae in strengthening soil aggregation, and initiating soil biodiversity, especially the formation of microbial communities around roots. A group of cellulolytic microbes that gather will excrete cellulase enzymes that can degrade cellulose into simpler forms. The results of this cellulose degradation can then glue the soil aggregates around these microbes [9].

Apart from the superior potential of active ingredients (inoculants), phosphate solvent gel biological fertilizers also have the advantage that they are not voluminous due to the nature of the hydrogel in binding water which can reach 400 times the mass of the water [10]. In this study, a consortium of superior PSMs was inoculated in liquid form into a hydrogel so that the PSMs biomass could spread according to the size of the gel formed. This is in line with the research results of Suman *et al.* (2016) who reported that hydrogels were known to increase the microbial population higher than formulations of liquid carriers and lignite up to $>10^8$ CFU.mL^{-1} after 90 days incubation at room temperature with a potential shelf life of up to 2 years [11].

According to Yan *et al.* (2020) hydrogels are able to maintain microbial activity with a long shelf life [12]. This long shelf life is a distinct advantage of hydrogel carriers which are not commonly used as biofertilizers. This is because some commonly used carrier materials such as peat, wood charcoal, and lignite are not able to maintain the shelf life of biological fertilizers as indicated by a decrease in the PSMs population from 4×10^7 CFU.g^{-1} to 3×10^7 CFU.g^{-1} within 1 months of extended shelf life [13].

Simanungkalit *et al.* (2013) stated that quality biological fertilizers must have good viability of active ingredients, active ingredients in the form of microbial inoculants must be superior and effective in increasing the quantity and quality of plants [14]. Biological fertilizer carrier materials must be able to support microbial viability during the production,

transportation, and storage processes before inoculants are applied to plants, and must have good shelf life to show the guarantee period of inoculant effectiveness. These criteria are actualized in Ministry of Agriculture Decree 261/2019.

The BG in this study was in the form of a semi-solid gel preparation, which was derived from acrylic acid powder which was 499 times its initial mass. Based on the measurement results of the swelling ratio, it is known that each available cavity is able to accommodate the PSMs consortium which was inoculated stably and in large quantities and in a viable condition. These characteristics are expected to answer the weakness of biological fertilizers in liquid form which are often less stable in accommodating inoculants due to competition between the indigenous microflora of the soil, soil physicochemical conditions, and adverse fluctuations in pH and temperature [15].

Hydrogel is known to be very soluble in water because it is polar, so that when applied it does not clog the sprayer nozzles. When dissolved in water, the gel undergoes syneresis then the inoculant particles attached to the gel move and spread into a uniform biofertilizer solution. This is expected to be able to answer the shortage of solid biofertilizers which are often clogged in the sprayer nozzles.

Biofertilizers containing superior inoculants, not voluminous, and practical when used are expected to be able to reduce production costs so that the selling price can become more economical, and can increase farmers' interest in using biological fertilizers to support sustainable agriculture. In this case, the constraints in the production and socialization of the use of biological fertilizers according to Basu *et al.* (2021) which include technical, biosafety, financial, regulatory, quality control, field level, carrier material, and marketing constraints will be overcome [16].

It is also hoped that the sustainable production of phosphate-solvent gel biofertilizers will not be constrained by the availability and price of raw materials, or security reasons for both living things and the environment. According to the description of Ahmed (2015), synthetic gel raw materials such as acrylic acid are very easy to obtain, use sparingly, are able to bind large quantities of the dispersing phase, and maintain their quality for a long time, and are environmentally friendly [17]. BG has the potential to increase soil P dynamics which in turn can reduce the need for inorganic P fertilizers, especially SP-36.

2. Methods

The experiment were carried out in vitro at the Soil Biology Laboratory of Universitas Padjadjaran in collaboration with PT Agritek Tani Indonesia and at the Experimental Land of the Department of Soil Science of Universitas Padjadjaran. The experimental design used in this study was a single randomized block design (RAK) with 9 treatments and 3 replications, consisting of treatments (Control: 0 BG + 0 P); (1 P); (1 BG + P); (1 BG + P); (1 BG + 1 P); (½ BG + P); (¾ BG + P); and (1 BG + P). The dose of fertilizer used was BG 50 kg.ha^{-1}, SP-36 100 kg.ha^{-1}, Urea 350 kg.ha^{-1}, KCl 50 kg.ha^{-1}, and sheep manure as basic fertilizer 2 t.ha^{-1}.

The experiment was carried out in three stages, namely:

2.1. Stage I: Compatibility Test and Characterization of Various Microbes Compounding BG

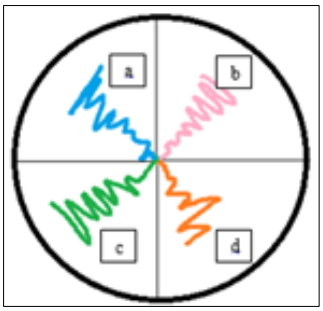

Figure 1 Isolate streak pattern on NA: a.) *B. cepacia*, b.) *B. subtilis,* c.) *P. maleii*, and d.) *T. asperellum*

Compatibility test was carried out in vitro using the streak method on universal media which could be grown by all isolates that make up the phosphate-plus solvent gel biofertilizer [18]. The media used is Nutrient Agar which is a universal medium for bacteria and can still be grown by the fungus *Trichoderma* sp. on a petri dish. Each isolate was scratched on the agar media with a pattern as shown in Figure 1, then incubated for 24 hours, and the growth was observed. Isolates that are mutually compatible form colonies that meet and intersect. Observations were continued at

three, five, and seven days after inoculation to see the zone of inhibition around the surface of the isolate. If there is one isolate whose growth is stunted, then the isolate is declared incompatible.

The characterization of each isolate was carried out by testing the ability of the isolates against the following parameters:

2.1.1. Determination of the Growth Curve of Each Isolate

In vitro tests were made using the Total Plate Count method. The isolate sample was taken as much as 1 ose and then cultured in 9 mL of Nutrient Broth (NB) liquid media. The number of samples made was adjusted to the number of petri dishes containing selective media according to the number of observations. Observations were made for 1 day with 2 hour intervals. The sample suspension was put into a petri dish containing 1 mL of selective media each, then incubated at room temperature, and observed for growth [19].

2.1.2. Phosphate Solubility Index (IP) on Pikovskaya

IP testing on pikovskaya media aimed to determine the ability of PSMs in dissolving P qualitatively [20]. Phosphate solubilization index is the ratio between the total diameter of the clear zone (hallo zone) and the diameter of the colony, then divided by the diameter of the colony to measure the PSMs' ability.

2.1.3. Phosphatase Activity

Measurement of phosphatase enzyme activity from soil was carried out using p-nitrophenol phosphate (pNP) as a substrate [21].

2.1.4. Trichoderma sp. Antagonism Test to Fusarium spp.

Tests carried out in vitro were made using the modified dual culture method [22].

2.2. Stage II: Shelf Life of BG.

The BG was firstly identified for its physical, chemical, and biological characteristics, which included shape, water content, swelling ratio, pH, and contaminant content of *Salmonella* sp. and *Escherichia coli.*

Tests of water content and swelling ratio were carried out using the thermogravimetric method [23]. The swelling ratio test was carried out with several modifications. Dry powder acrylic acid (W_o) was weighed as much as ± 10 mg and then put into 100 mL of solvent mixture, and stirred at 400 rpm at room temperature. The gel that has been formed is filtered using a tea filter (± 200 mesh). The entire supernatant was allowed to drip into the beaker (± 1 hour). The volume of the supernatant accommodated in the beaker was weighed as (W_1). The swelling ratio of the hydrogel was calculated by Equation (1).

$$\text{Swelling ratio} = (W_1\text{-Wo})/\ \text{Wo} \quad \text{.....(1)}$$

Where:

W_o= Weight of initial dry hydrogel

W_1= Weight of hydrogel in a state of swelling at a given time.

In general, solid biofertilizers only have a shelf life of 6-12 months [29]. Therefore, the viability test was carried out for 4.5 months which represents half the shelf life of solid biofertilizers in general. Sampling and viability testing of Phosphate plus solvent gel biofertilizer were carried out continuously at 2-week intervals, namely T_{14}, T_{28}, T_{42}, T_{56}, T_{70}, T_{84}, T_{98}, T_{112}, and T_{126}. Samples were stored on a storage rack in a closed room with a temperature range of 26^0C-29^0C and relative humidity (RH) 54%-82%. BG packaged in glass vial which sealed by using aluminum foil.

The viability test was carried out to determine the shelf life of BG. The test was carried out using the TPC method with serial dilutions at the dilution rank required by Ministry of Agriculture Decree No. 261/2019.

2.3. Stage III: Application of BG to Maize (*Zea mays L.*) in Inceptisols Jatinangor

The application of various dosage combinations of BG and inorganic P fertilizer was carried out to determine the best formulation that could improve soil P dynamics in Inceptisols Jatinangor. The experimental design used in this study

was a single randomized block design (RBD) with 9 treatments and 3 replications, consisting of treatments (control: 0 BG + 0 P); (1 P); (1 BG); (1 BG + ½ P); (1 BG + ¾ P); (1 BG + 1 P); (½ BG + ¾ P); (¾ BG + ¾ P); and (1 ½ BG + ¾ P). The dose of fertilizer used was BG 50 kg.ha^{-1}, SP-36 100 kg.ha^{-1}, Urea 350 kg.ha^{-1}, KCl 50 kg.ha^{-1}, and sheep manure as basic fertilizer 2 t.ha^{-1}.

2.3.1. Study Performed

Compatibility and Characteristics of Various Microbes Compounding BG

Figure 2 shows that the four isolates were compatible because they could grow together in the same agar medium. The four isolates were synergistic with each other because there was no zone of inhibition between the growing colonies. A microbial consortium has complementary metabolic functions in an ecosystem so that its performance can increase (Rifai *et al.,* 2020)[24]. PSMs consortium can increase the availability of phosphate more effective [6].

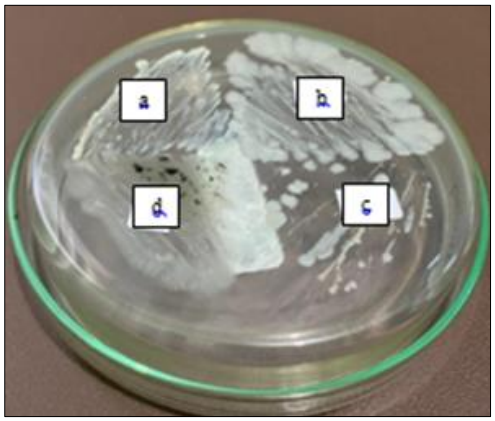

Figure 2 Isolate compatibility using streak method on NA media. a.) *B. cepacia*, b.) *B. subtilis,* c.) *P. mallei*, d.) *T. asperellum*

Characterization was carried out on each isolate that made up the consortium to assess the ability to dissolve phosphate, perform dual roles as PGPR, especially in producing IAA and controlling *Fusarium* sp.

Table 1 Characterization of Various Microbes Compounding BG

Isolate	IP	Phosphatase (µL.g^{-1}.hour^{-1})	IAA
B. subtilis	1.82	0.30	positive
P. mallei	1.71	0.48	positive
B. cepacia	1.74	0.12	positive
T. asperellum	1.44	0.23	positive

Table 1 shows that the four isolates had the ability to dissolve the two forms of phosphate. The IP values ranged from 1.44 to 1.82, with the highest value was *B. subtilis* and the lowest was *T. asperellum.* However, *P. mallei* and *B. subtilis* had better ability to dissolve inorganic phosphate than other isolates. IP is a qualitative value that represents the number of $H_2PO_4^-$ ions released from the inorganic phosphate precipitated complex. Qualitatively, $H_2PO_4^-$ will produce a hallo zone around the microbial colonies on Pikovskaya agar whose main content is $Ca_3(PO_4)_2$ as shown by Figure 3. The four types of isolates were known to be able to produce phytohormones, namely IAA which can stimulate plant growth, especially root growth so that they can reach more water and nutrients so that plants can grow optimally.

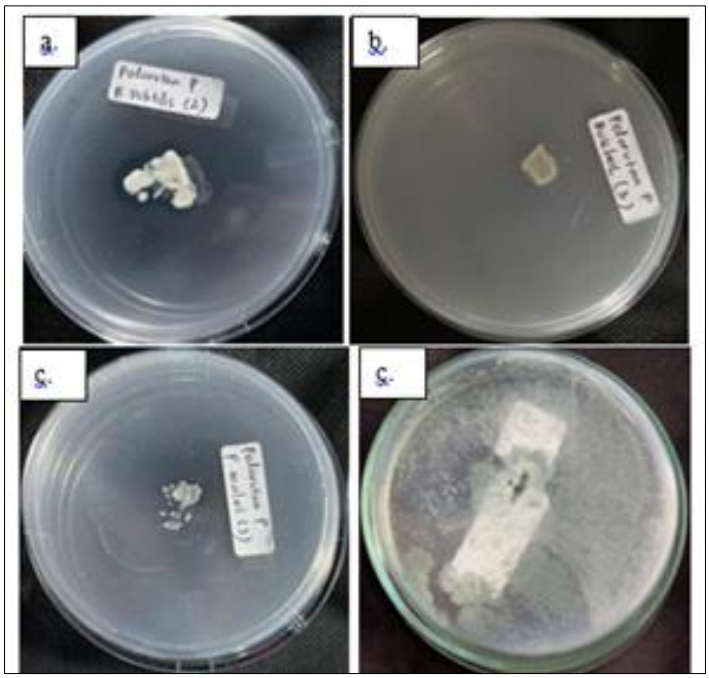

Figure 3 P Solubilizing on Pikovskaya. a.) *B. subtilis*, b.) *B. cepacia*, c.) *P.* mallei, d.) *T. asperellum*

T. asperellum in this study was known to be able to dissolve phosphate in the soil, and had the ability as a biocontrol, especially against the pathogenic fungus *Fusarium* sp. with an antagonism value of 100% as shown in Figure 4. These antagonist characters are indispensable in maize cultivation so that the harvest has good quality and is safe for consumption by humans or livestock. Suriani *et al.* (2016)[12] stated that *Fusarium* sp. able to reduce maize productivity up to 1.8 $t.ha^{-1}$.

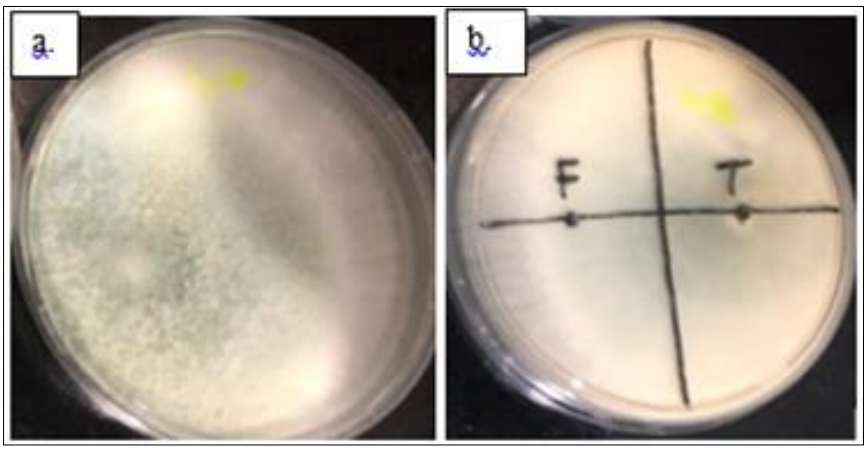

Figure 4 Antagonism test of *T. asperellum* with Pathogenic Fungi *Fusarium* sp. a.) The front view of the *T. asperellum* hyphae filled the entire media, b.) The rear view of the T*. asperellum* hyphae filled the entire media

Fusarium sp. is quite difficult to control because it is able to form chlamydiospores that can survive in the soil for years even under environmental stress and without host plants [25]. In addition, maize varieties that are moderately resistant to *Fusarium* sp. has not been widely reported circulating in Indonesia [26].

The selection of the four types of microbes aimed to produce biological fertilizers that are effective and efficient in dealing with various practical agricultural problems, especially in creating a balance of nutrients and protecting plants from various environmental stresses including pests and plant diseases.

3. Shelf Life of BG

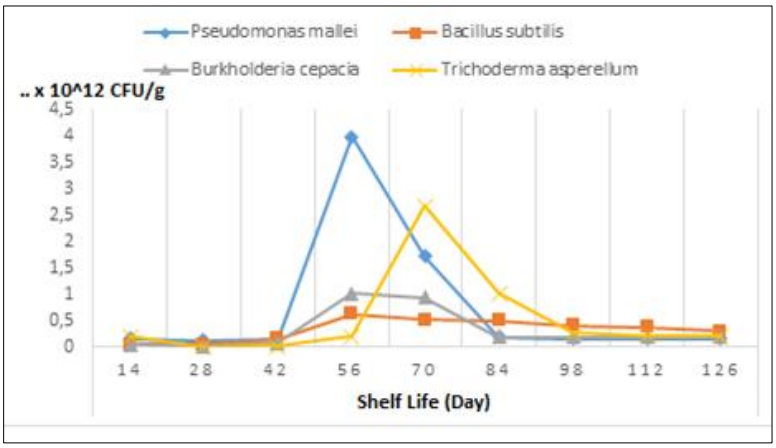

Figure 5 PSMs viability curve over 126 days shelf life

Figure 5 shows that the number of viable populations of each isolate that inoculated in hydrogel carrier (BG) was in the high population range, 1 x 10^{12} cfu.g^{-1}. This population range showed that the sample of BG still met the minimum number of viable microbes required in Ministry of Agriculture Decree No. 261/2019. The shelf life of 126 days or 4.5 months represents half of the shelf life of solid biological fertilizers which are generally only able to maintain viability according to quality standards for 6-12 months. Therefore, it can be concluded that the BG has the potential to have a longer shelf life of 6-12 months.

Figure 6 Color Change of Hydrogel Carrier During the Sterilization Process, Formulation, and Shelf Life of 126 Days. a.) non-sterile gel, b.) sterile gel, c.) biofertilizer with a shelf life of 0 days, d.) biofertilizer with a shelf life of 126 days

Figure 6 shows a color change in the hydrogel carrier material before sterilization (a) due to the sterilization process using the wet heat method using an autoclave (b), where the hydrogel preparation which was originally a colorless semi-solid (clear) turned yellow after being sterilized. This proves that the hydrogel formed from poly acrylate undergoes physical changes at temperatures above 50^0C, but does not change its other characteristics. Sterilization is carried out to minimize the presence of contaminating microorganisms, especially *E. coli* and *Salmonella* sp.

After formulation and addition of additives, the color of the hydrogel carrier material changed from yellow (b) to dark brown (c), then the growth of fungal hyphae was seen due to the presence of *T. asperellum* inoculants. The biofertilizer formulation process has the ultimate goal of producing live and effective inoculants when applied, and able to last a long time during storage in a suitable carrier. The success of a biofertilizer formula depends on the targeted plant species, cost, market accessibility, ecological limitations, and user-friendliness [27]. To extend the shelf life, various additives can be added that are able to support the needs of longer microbial life.

Additives added in this study include molasses, bean sprouts, and glycerol. Molasses serves as a carbon source for inoculants, while bean sprouts are a source of auxin and glycerol as an antifreeze to prevent dehydration. These additives are in accordance with the research results of Vassilev *et al.* (2017) which states that glycerol, trihydroxyalcohol are additives in biological fertilizers that are expected to function as cell viability protectors, but

further studies are needed regarding technical formulations [28]. Other additives that can be used are methyl cellulose, starch, and silica gel [29].

4. Effect of BG Application on Phosphate Dynamics in Inceptisols Jatinangor-Indonesia

Phosphate dynamics can be represented by the processes that occur in the soil solution during the growing period of maize. Soil solution is a medium where the surface of the root hairs meet with nutrients in the form of ions. In this study, the content of pH, available P, and potential P were analyzed from soil samples that were closest and even still attached to plant roots, thus representing the conditions that occurred in the soil solution.

The application of inorganic P fertilizer is the main source of phosphate for maize to meet their daily needs. Inorganic P fertilizers are generally easily available to plants, but most of these fertilizers quickly turn into unstable P fractions, namely P bound by metal cations in this study Ca^{2+} and Mg^{2+}. The other part turns into a non-labile P fraction, namely P which returns to minerals, but can be released back into a labile form very slowly [30].

Inceptisol series Jatinangor has characteristic soil texture is dominated by clay fraction, neutral pH 7.01. Under neutral conditions, in general, nutrients in the soil tend to be more readily available, but the availability of P is actually very low at only 2.82 $mg.kg^{-1}$ of soil. In this neutral to alkaline pH, the solubility of metal cations Ca and Mg increased, indicated by a high Ca^{2+} content of 12.60 $cmol.kg^{-1}$ and Mg^{2+} which was also high at 4.91 $cmol.kg^{-1}$. The nutrient imbalance is also due to the low C-organic content of the soil, namely 1.83%, so that the soil's support capacity is low. Low organic C causes the presence of indigenous microbes which is quite high, especially PSMs, namely 1 x 10^4 $cfu.g^{-1}$, which is not able to increase the availability of soil nutrients, especially P due to lack of carbon sources.

Table 2 Phosphatase activity and pH of Inceptisols influenced by application of BG and P fertilizer

Treatment	Phosphatase ($\mu L.g^{-1}.hour^{-1}$)	pH of soil
A : Control	18.75 ab	6.45 a
B : 1 P	18.20 ab	6.45a
C : 1 BG	17.71 ab	7.37 c
D : 1 BG + ½ P	20.29 ab	6.85 b
E : 1 BG + ¾ P	23.38 b	6.83 b
F : 1 BG + 1 P	17.33 a	7.43 c
G : ½ BG + ¾ P	17.63 ab	6.91 b
H : ¾ BG + ¾ P	17.43 a	7.21 c
I : 1 ½ BG + ¾ P	15.20 a	7.18 c

Note: Numbers followed by the same letter are not significantly different according to Duncan's multiple-distance test at 5% level.

Table 2 shows that the application of a combination of BG and P fertilizer had a significant effect on changes in soil pH, especially those around the roots. The initial soil had a high content of metal cations, namely Ca^{2+} 12.60 $cmol.kg^{-1}$ and Mg^{2+} 4.91 $cmol.kg^{-1}$. These cations were one of the causes of very low available P, which was only 2.82 $mg.kg^{-1}$. The control treatment was not significantly different from the 1 P treatment, but was significantly different from the 1 BG treatment or the combination treatment. This condition indicated that the application of BG was able to stimulate the soil buffering mechanism, namely the ability of the soil to maintain its optimum condition. The pH was adjusted so that the solubility of Ca^{2+} and Mg^{2+} decreased so that the bond with orthophosphate could be released.

The superior PSMs in BG was able to secreted organic acids, OH^- ions, and CO_2^{2-} ions which function to regulate soil pH so that it could released P from metal cations bonds [31]. PSMs also secreted COO^- (carboxyl group) which acted as a chelator of Ca^{2+} and Mg^{2+} cations by competing for P adsorption groups in the soil [32]. PSMs will only secrete these anions when there is a balanced exchange with root exudates.

According to Nurlaeny (2015) plant roots secrete exudate in the form of low molecular weight organic compounds that diffuse into the rhizosphere [33]. The exudate released by plant roots is specific and generally corresponds to the target

organ harvested in the plant. Exudates released by the roots of maize include free sugars such as glucose and sucrose, amino acids such as glycine and glutamate, and organic acids such as citric, malic, and oxalic [34].

The application of the combination of BG with P fertilizer had no significant effect on phosphatase activity, but in general the reduction of P fertilizer dose accompanied by the application of BG in the right amount could to produced higher phosphatase so potentially better in increasing soil P availability. Treatment of 1 BG + ¾ P produced more phosphatase 2.18 $\mu L.g^{-1}.hour^{-1}$ than treatment of 1 P. Treatment of 1 BG + ½ P produced more phosphatase of 2.09 $\mu L.g^{-1}.hour^{-1}$more compared with a 1 P treatment. This was because PSMs will only secrete its phosphatase when it is deficient in P to metabolize. According to the research of Magralef *et al.* (2017) phosphatase production depends on a combination of the P requirements of plants and microbes, the available organic P substrates and soil P limitations [35].

Table 3 shows the P content of maize rhizosphere, namely the zone affected by root exudate and microbial secretion. P is available in the soil in the form of orthophosphate ions that can be directly absorbed by plants, while the potential P in the form of P_2O_5 compounds and is a reserve of P in the soil that has the potential to be converted into P is available.

Table 3 Phosphatase activity and pH of Inceptisols influenced by application of BG and P fertilizer

Treatment	Available-P ($mg.kg^{-1}$)	Potential-P ($mg.100g^{-1}$)
A : Control	1.44 a	33.79 b
B : 1 P	2.50 a	25.90 a
C : 1 BG	12.40 b	27.22 a
D : 1 BG + ½ P	3.09 a	25.70 a
E : 1 BG + ¾ P	25.47 d	35.20 b
F : 1 BG + 1 P	18.80 c	35.06 b
G : ½ BG + ¾ P	10.98 b	51.47 d
H : ¾ BG + ¾ P	11.19 b	44.61 c
I : 1 ½ BG + ¾ P	17.37 c	33.67 b

Note: Numbers followed by the same letter are not significantly different according to Duncan's multiple-distance test at 5% level.

The available P content ranged from 1.44 -25.47 $mg.kg^{-1}$ of soil. Combination treatment of BG and P in general had a significant effect on the increase of available soil P. Treatment 1 BG + ¾ P showed the highest increase in available P among all treatments. The treatment caused 22.97 $mg.kg^{-1}$ P more available than the 1 P treatment, and produced the smallest difference in P potential. Treatment 1 BG also still produced 9.90 $mg.kg^{-1}$ P available more than treatment 1 P. Therefore, BG could be considered to have a good performance in optimizing P reserves in the soil for plant growth, so it had great potential to substitute. some of the fertilizer needs of P.

The potential P ranged from 25.70-51.47 $mg.100g^{-1}$ soil. The ½ BG + ¾ P treatment produced the highest potential P among all treatments so that there were more labile P reserves that had the potential to become P available. The performance of P provision could be judged from the difference between potential P and available P, where the smaller the difference indicated the greater the amount of labile P that was successfully converted into available P.

Table 4 shows the population of each PSMs that managed to remain in the rhizosphere zone after competing with indigenous microbes, both pathogenic and non-pathogenic. The post-application population was generally lower than the pre-application population.

The control treatment was not significantly different from the 1 P treatment or the 1 BG treatment. This happened because inoculants need to maintain their survival from indigenous microbes and contaminants as well as from environmental stresses, form similar communities, colonize plant roots, and then interact with plants, especially as PSMs and as PGPR. In the control and 1 P treatment, there were populations of *B. subtilis, B. cepacia*, and *P. mallei* in the ranged of 1×10^5 $CFU.g^{-1}$ soil and *Trichoderma* sp. in the ranged of 1×10^4 $CFU.g^{-1}$ soil, where the population range corresponded to the population of indigenous bacteria and fungi in the initial soil analysis.

Table 4 Population of PSMs in rhizosphere of Inceptisols influenced by application of BG and P fertilizer

	Population of PSMs of BG in rhizosphere			
	... x 109CFU.g-1			.... x 107 CFU.g-1
	B. subtilis	**B. cepacia**	**P. mallei**	**T. asperellum**
A	0.02 a	0.02 a	0.03 a	0.01 a
B	0.09 a	0.01 a	0.06 a	0.01 a
C	0.01 a	0.03 a	0.08 a	0.01 a
D	9.33 a	3.83 bc	6.67 a	1.33 b
E	27.67 a	1.63 ab	7.17 a	0.20 ab
G	14.00 a	0.90 ab	5.83 a	0.67 ab
H	115.33 b	1.50 ab	4.70 a	2.67 c
I	27.33 a	6.50 c	3.50 a	0.27 ab

Note: Numbers followed by the same letter are not significantly different according to Duncan's multiple-distance test at 5% level.

In combination treatment, PSMs in BG was able to adapt well to the soil as a new growth medium. The highest viable population of *B. subtilis* was found in 1½ BG + ¾ P treatment i.e. 1.33 x 10^{11} CFU.g^{-1} soil, and the lowest population in 1 BG treatment i.e. 7 x 10^{5} CFU.g^{-1} soil. The highest population of *B. cepacia* was found in the treatment of ¾ BG + ¾ P which was 6.50 x 10^{11} CFU.g^{-1} soil, and the lowest population was found in the treatment of 1 P which is 4.70 x 10^{5} CFU.g^{-1} soil.

The highest population of *P. mallei* was found in the treatment of 1½ BG + ¾ P which is 1.68 x 10^{10} CFU.g^{-1} soil, and the lowest was found in the control treatment which is 2.67 x 10^{6} CFU.g^{-1} soil. The highest population of *T. asperellum* was found in the treatment of ½ BG + ¾ P which is 2.67 x 10^{7} CFU.g^{-1} soil and the lowest in the treatment of 1 organic fertilizer which was 2.30 x 10^{4} CFU.g^{-1} soil. In general, the overall combination of organic fertilizer and P fertilizer resulted in a higher PSMs population than controls, but tended not to differ significantly among fellow combination treatments. Therefore, the treatment of 1 BG + ¾ P could be considered to be effective enough to increase the availability of soil P optimally. This condition happened because before it could perform its function as a biological fertilizer that is to increase nutrient availability and plant health, inoculants must first be able to adapt to the new environment.

Figure 7 Colonization of plant roots by fungal hyphae of *T. asperellum* after application of BG

An important aspect of the association between plant roots, rhizosphere, and rhizobacteria is the increase in root growth and proliferation which is important in the transfer of nutrients and water to the upper part of the plant [3]. The total exudate released by plant roots is referred to as rhizodeposit which is mostly stored in the form of root biomass. The formation of plant roots is also influenced by the stimulation of hormonal transcription changes due to the auxin secreted by PSMs [36]. Auxin also works in increasing root biomass, as well as reducing the size and density of stomata [37].

The above explanation was supported by Figure 7 which showed the occurrence of root colonization by fungal hyphae after application of BG. If the fungal hyphae were visible, microscopically, the bacteria had to colonized the roots. The advantage of root colonization was that it expanded the rhizosphere area, which was the zone for exchanging P nutrients with root exudates in the form of dissolved organic matter [36]. In the zone of root elongation, precisely around the root hairs, more exudate is produced as a result of the ruptured cells during root elongation. The wider the nutrient exchange zone, the higher the absorption of nutrients and water by plants. The meeting place for fungal hyphae that enter the soil pores is called the porosphere which is one of the main locations for the exchange of root exudates and the secretion of microbial organic substances. The interwoven hyphae then form an aggregatusphere that strengthens soil aggregation so that nutrients are not easily leached and prevent erosion [38].

5. Conclusion

The conclusions that can be drawn based on the results of research that have been carried out are:

- Each of the PSMs isolates inoculated in the BG was mutually compatible so that they can be used as a synergistic consortium.
- The isolates had the ability to dissolve phosphate, produce phosphatase enzyme, and produce IAA. *Trichoderma asperellum* had a plus role, namely being able to act as a biocontrol against the pathogenic fungus *Fusarium* sp.
- Each of the PSMs isolates of BG namely *Bacillus subtilis, Bulkholderia cepacia, Pseudomonas mallei,* and *Trichoderma asperellum* had good viability, which was around 1 x 10^{12} cfu.g^{-1} at a shelf life of 126 days before being applied to the soil, and ranged from 10^{9} cfu.g^{-1} after application to soil, and had the potential to maintain this viability for a longer shelf life.
- Treatment 1 BG + ¾ P was the best formulation because it consistently significantly affected in increasing the dynamics of soil phosphate in Inceptisols Jatinangor-Indonesia.

Compliance with ethical standards

Acknowledgments

This research was supported by grants received from the Directorate General of Higher Education Ministry of Research and Technology Indonesia (applied research: 1207/UN6.3.1/PT.00/2021). We thank to staff Laboratory of Soil Biology Faculty of Agriculture, Universitas Padjadjaran and the Research and Development Laboratory of PT. Agritek Tani Indonesia for their cooperation.

Disclosure of conflict of interest

No conflict of interest between the authors.

References

[1] Sahu Y, Kumar A, Kumar N, Kumawat. Role of Pupuk hayatis in Agriculture. India. Popular Kheti Volume -5, Issue-4 (October-December). 2017.

[2] Fitriatin BN, M Agustina dan, R Hindersah. Populasi Bakteri Pelarut Fosfat, P-Potensial Dan Hasil Jagung Yang Dipengaruhi Oleh Aplikasi MPF Pada Ultisols Jatinangor. Agrologia. 2017; 6(2): 75-83.

[3] Khan N, Ali S, Shahid MA, Mustafa A, Sayyed RZ, Curá JA. Insights into the Interactions among Roots, Rhizosphere, and Rhizobacteria for Improving Plant Growth and Tolerance to Abiotic Stresses. 2021.

[4] Habi LM, Nendissa, Marasabessy, Kalay. Ketersediaan Fosfat, Serapan Fosfat, Dan Hasil Tanaman Jagung (Zea mays L.) Akibat Pemberian Kompos Granul Ela Sagu Dengan Pupuk Fosfat Pada Inceptisols. Agrologia. 2018; 7(1).

[5] Fitriatin BN, DF Manurung ET, Sofyan MR, Setiawati. Compatibility, Phosphate Solubility and Phosphatase Activity by Phosphate Solubilizing Bacteria. Universitas Padjadjaran – Jatinangor. United Arab Emirates Journal. 2020.

[6] Saeid A, E Prochownik, JD Iwanek. Phosphorus Solubilization by Bacillus Species. Published: 6 November 2018. Molecules. 2018; 23: 2897.

[7] Montesano F, A Parente, P Santamaria, A sannio, F Serio. Biodegradable Superabsorbent Hydrogel Increase water Retention Properities of Growing Media and Plant Growth. Agriculture and Agricultural Science Procedia. 2015; 4: 451-458.

[8] Gupta M, Kiran Gulati, Singh Tewari. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of Aloe barbadensis Miller. Microbiological Research. 2012; 167: 358– 363.

[9] Wilpiszeski RL, Jayde A Aufrecht, Scott T Retterer, Matthew B Sullivan, David E. Graham, Eric M Pierce, Olivier D. Zablocki, Anthony V. Palumbo, & Dwayne A. Elias. Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales. 2020.

[10] Ahmed, E. M. Hydrogel: Preparation, characterization, and applications: A review. In *Journal of Advanced Research* (Vol. 6, Issue 2, pp. 105–121). Elsevier B.V. https://doi.org/10.1016/j.jare.2013.07.006. 2015

[11] Suman A, Verma, Yadav A, Srinivasamurthy Singh, Prasanna. Development of Hydrogel Based Bio-Inoculant Formulations and their Impact on Plant Biometric Parameters of Wheat (Triticum aestivum L.). International Journal of Current Microbiology and Applied Sciences. 2016; 5(3): 890–901.

[12] Yan M, Fotidis IA, Jéglot A, Treu L, Tian H, Palomo A, Zhu X, Angelidaki I. Long-term preserved and rapidly revived methanogenic cultures: Microbial dynamics and preservation mechanisms. Journal of Cleaner Production. 2020; 263.

[13] Shravani K, Triveni S, Latha PC, KDC. Evaluation of Shelf Life and Quality of Carrier and Liquid Based Evaluation of Shelf Life and Quality of Carrier and Liquid Based Pupuk hayatis. International Journal of Microbiology Research. 2019; 11(6): 1598–1601.

[14] Simanungkalit E. Husen, R Saraswati. Baku Mutu Pupuk Hayati dan Sistem Pengawasannya. 2009.

[15] Sahu Y. Kumar, A Kumar, N Kumawat. Role of Pupuk hayatis in Agriculture. India. Popular Kheti Volume -5, Issue-4 (October-December), 2017.

[16] Basu A, Priyanka P, Subha N, Sadaf K, Sayyed, Reddy, Hesham E. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. Basel. Switzerland. 2021.

[17] Ahmed, E. M. Hydrogel: Preparation, characterization, and applications: A review. In *Journal of Advanced Research* (Vol. 6, Issue 2, pp. 105–121). Elsevier B.V. https://doi.org/10.1016/j.jare.2013.07.006. 2015

[18] Widyawati I, Sugiyanta, Junaedi A, Widyastuti R. Peran bakteri penambat nitrogen untuk mengurangi dosis pupuk nitrogen anorganik pada padi sawah. J Agon Indones. 2014; 42(2): 96-102.

[19] Maier, Raina M. Environmental Microbilogy. Chapter 3 Bacterial Growth. Academic Press. Inc. 2009.

[20] Tabatabai MA. Soil Enzymes. Dalam: Methods of Soil Analysis,Part 2. Microbiological and Biochemical Properties. J.M. Bigham (Editor). American Society of Agronomy, Soil Science Society of America. Madison, Wisconsin. 1982; 775-834.

[21] Singh PK, Vijay K. 'Biological control of Fusarium wilt of Chrysanthemum with Trichoderma and botanicals', J. Agric Tech. 2011; 7(6): 1603-13.

[22] Erizal Dian P. Perkasa, Sulistioso G, Sudirman, Z Juniarti, Hariyanti. sintesis dan karakterisasi biodegradabel hidrogel superabsorben poli(kalium akrilat)-gglukomanan dengan teknik iradiasi gamma. Jurnal Sains Materi Indonesia. 2017; 19(1): 32-38.

[23] Simanungkalit RDM, Didi Ardi Suriadikarta, Rasti Saraswati, Diah Setyorini, Wiwik Hartatik. organic fertilizer and Pupuk hayati. Balai Besar Litbang Sumberdaya Lahan Pertanian Badan Penelitian dan Pengembangan Pertanian. 2006.

[24] Rifai, Hening Widowati, Agus Sutanto. sinergisme dan antagonisme beberapa jenis isolat bakteri yang dikonsursiumkan. Universitas Muhammadiyah Metro. 2020.

[25] Sudantha. Pengujian beberapa jenis jamur endofit dan saprofit Trichoderma spp. terhadap penyakit layu fusarium pada tanaman kedelai. Jurnal Agroteksos. 2010; 20(2): 90-102.

[26] Pakki S. Cemaran mikotoksin, bioekologi patogen Fusarium verticilloides dan upaya pengendaliannya pada jagung. Junal Penelitian dan Pengembangan Pertanian. 2016; 35(1): 11-16.

[27] Herrmann L, Lesueur D. Challenges of formulation and quality of Pupuk hayatis for successful inoculation. Applied Microbiology and Biotechnology. 2013; 97(20): 8859–8873.

[28] Vassilev N, Eichler-Löbermann B, Flor-Peregrin E, Martos V, Reyes A, Vassileva M. Production of a potential liquid plant bio-stimulant by immobilized Piriformospora indica in repeated-batch fermentation process. AMB Express. 2017; 7: 106.

[29] Macik M, Gryta A, Frac M. Pupuk hayatis in agriculture: an overview on concepts, strategies and effects on soil microorganisms. Adv. Agron. 2020; 162: 31–87.

[30] Mengel K, Kirkby EA. Principles of Plant Nutrition. International Potash Institute, Bern. 1982.

[31] Wei Y, Zhao Y, Shi M, Cao Z, Lu Q, Yang T. Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. Bioresour. Technol. 2018; 247: 190–199.

[32] Billah M, Khan M, Bano A, Hassan TU, Munir A, Gurmani AR. Phosphorus and phosphate solubilizing bacteria: Keys for sustainable agriculture. Geomicrobiol. J. 2019.

[33] Nurlaeny, Nenny. Bahan Organik Tanah dan Dinamika Ketersediaan Unsur Hara Tanaman. Bandung. Penerbit Unpad Press, Bandung. 2015.

[34] Lima, Olivares, Oliveira, Vega, Aguiar, Canellas. Root exudate profiling of maize seedlings inoculated with Herbaspirillum seropedicae and humic acids. Chemical and Biological Technologies in Agriculture. 2014; 1: 23.

[35] Magralef, Sardanz, Martinez, Horas, Jansens, Ciais, GollRichter, Obserteiner, Asensio, & Penuelas. Global patterns of phosphatase activity in natural soils. Scientific Reports. 2017; 7: 1337.

[36] Spaepen S, Bossuyt S, Engelen K, Marchal K, Vanderleyden J. Phenotypical and molecular responses of A rabidopsis thaliana roots as a result of inoculation with the auxin-producing bacterium A zospirillumbrasilense. New Phytol. 2014; 201: 850–861.

[37] Ruzzi M, Aroca R. Plant growth-promoting rhizobacteria act as biostimulants in horticulture. Sci. Hortic. 2015; 196: 124–134.

[38] Winger, Marlon. Unlock The Secrets in The Soil. Idaho. NRSC USDA. 2020.