In vitro test for compatibility of biofertilizers containing phosphate solubilizers and nitrogen-fixing bacteria

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Abstract. The quality of biofertilizers is determined by the content of isolates and synergism between isolates. The experiment was aimed to investigate the compatibility of biofertilizers containing P-solubilizers and N-fixing bacteria. In vitro test using isolates of PSB and N-fixing bacteria was done to study the synergistic character by using streak method on nutrient agar for the bacteria and potato dextrose agar for the fungi. Species of P-solubilizers were Pseudomonas mallei, P. cepaceae, Aspergillus niger, and Penicillium sp., and species of N-fixing bacteria were Azotobacter chroococum and Azospirillum sp. The results revealed that the P-solubilizers microbes and N-fixing bacteria were synergistic and did not antagonistic to each other. Biofertilizers which contain P-solubilizers and N-fixing bacteria can be used for agricultural purposes.

1. Introduction

Several microbes in the soil show the ability to dissolve P in order to enhance P available in soil. This microbes are known as phosphate solubilizing microbes or P-solubilizers which has the ability to extract P from bonds with Al, Fe, Ca, and Mg, so that it can dissolve inavaiable P to become available for plant uptake. P-solubilizers microbes produced organic acids [12] which can form stable complexes with P-binding cations in the soil. The microbes can also produce enzyme phosphatase and fitohormones [9]. P-solubilizers application on soil showed a good influence on improving nutrient status and increasing crop yield.

Some bacteria can convert atmospheric nitrogen into ammonia which grows freely in plant rhizosphere (non-symbiotic) or which symbiotes with plants. Non-symbiotic N-fixing bacteria include Clostridium, Azotobacter, Azomonas, Azospirillum, Klebsiella, Blue green algae as well as some Bacillus and Pseudomonas [6]. While the symbiotic N-fixer, among others, has been widely known, namely root nodules or Rhizobium. The nitrogen fixation process in all species involves the nitrogenase enzyme.

The importance of N-fixing bacteria and P-solubilizers as well as their interactions with mineral P fertilization in improving crop productivity and fertilizers efficiency [1]. The potential of N donated by N-fixing bacteria in plant tissue (endophytic) is greater than that of non-endophytic (in rhizosphere Azotobacter) is N-fixing bacteria that is capable of producing substances that stimulate growth of gibberellins, cytokines and indole acetic acid [10], so that Azotobacter inoculation can stimulate root growth [3]. Inoculation of Azospirillum brasilense and Pseudomonas fluorescens improved crop production [8]. The application of phosphate solubilizing microbes (Pseudomonas mallei, P.cepaceae, A.niger, and Penicillium sp.) and N-fixing bacteria (Azotobacter chroococum and Azospirillum sp.) combined with humic acid increased yield of paddy rice and anorganic fertilizers efficiency [4].
Interactions between microbes can also be seen from the growth or population of microbes in an ecosystem. Density of microbes can be heavily influenced by interspecies interactions [11]. The objective of the research was to determine the compatibility or synergistic interaction between isolates of biofertilizers containing P-solubilizers and N-fixing bacteria.

2. Materials and Methods
In Vitro test were carried out in the Soil Biology laboratory of Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. to study the compatibility of several P-solubilizers isolates (Pseudomonas mallei, P. cepaceae, Aspergillus niger, and Penicillium sp.) and N-fixing bacteria (Azotobacter chroococum and Azospirillum sp.). In vitro on nutrient agar using the streak method [5]. Pure cultures of each isolate are prepared on a slant. To verify the synergic interaction between bacterial isolates, each isolate was scratched to each other on plate nutrient agar. Growth observations were carried out at three, five and seven days after inoculation by investigating clear zones which characterizes as inhibition zones around the isolate intersection. The interaction of isolate was synergistic when there was no inhibition zone in the intersection area of the two isolates, and was antagonistic when there is an inhibition zone in the area where the two isolates intersected.

Population of isolates were calculated by Total Plate Count method to determine growth of isolates in carrier based biofertilizers (mixed of peat and compost 1:1). Pikovskaya media was used to calculate the population of P-solubilizers, Ashbys media for Azotobacter while the Okon media to calculate Azospirillum. Population of isolates were measured at 7 days after incubation with a dilution series.

3. Results And Discussion
The results of synergistic effect from in vitro test between P-solublizers and N-fixing bacteria isolates showed that all isolates were synergistic each other and showed no antagonistic signs. Table 1 showed that all isolates was compatible, it’s mean that all isolates can growth in the same media or culture. The result of experiment showed that bacteria and fungi were compatible with each other. Futhermore, it can be make biofertilizers consortia between P-solubilizers and N-fixing bacteria. Inoculation of the phosphate solubilizing microbes and nitrogen fixing bacteria consortium has the potential to increase the availability of phosphate and nitrogen up take needed by plants compared to one type/singular bacteria.

| Isolates     | Compatibility |
|--------------|---------------|
| P. mallei    | ++            |
| P. cepaceae  | ++            |
| A. chroococum| ++            |
| Azospirillum sp. | ++  |
| A. niger     | ++            |
| Penicillium sp. | ++  |

Note : ++ (compatible)

The result showed that isolates were synergistic since no inhibition zone in the intersection area of the two isolates (Figure 1). Positive compatibility among N-fixing bacteria Azospirillum, Azotobacter, and phosphate solubilizing bacteria Pseudomonas fluorescens and Bacillus subtilis [7].
Figure 1. In vitro test for Compatibility of P-solubilizers and N-fixing bacteria: (a) *Apergillus niger* vs *P. cepaea*; (b) *Aspergillus niger* vs *Azospirillum*; (c) *Penicillium* sp. vs *P. mallei*

The microbial analysis of P-solubilizers and N-fixing bacteria in carrier based biofertilizers revealed that the growth of each isolate was very fast with bacterial density up to $10^8$ CFU g$^{-1}$ while fungal density up to $10^7$ CFU g$^{-1}$ (Table 2). This study indicated that each isolate in carrier based biofertilizers can grow optimally. In vitro test for compatibility of P-solubilizers isolates and N-fixing bacteria were supported by the microbial analysis in carrier based biofertilizer. The other research revealed that population of phosphate solubilizing bacteria was $4.7 \times 10^7$ CFU g$^{-1}$ and N-fixing bacteria *Rhizobium* was $5.4 \times 10^9$ CFU g$^{-1}$ in carrier based biofertilizers [2].

Table 2. Population of P-solubilizers and N-fixing bacteria in carrier based biofertilizers

| No | isolates                        | Method | CFU g$^{-1}$ |
|----|---------------------------------|--------|--------------|
| 1  | *Azotobacter chroococcus*        | TPC    | $12 \times 10^8$ |
| 2  | *Azospirillum* sp.               | TPC    | $16 \times 10^8$ |
| 3  | *Pseudomonas cepacea*            | TPC    | $26 \times 10^8$ |
| 4  | *Pseudomonas mallei*             | TPC    | $16 \times 10^8$ |
| 5  | *Penicillium* sp.                | TPC    | $46 \times 10^7$ |
| 6  | *Apergillus niger*               | TPC    | $16 \times 10^7$ |

4. Conclusions

The interaction between P-solubilizers *Pseudomonas mallei*, *P. cepacea*, *Aspergillus niger*, and *Penicillium* sp., and N-fixing bacteria *Azotobacter chroococcus* and *Azospirillum* sp. bacteria were synergistic and did not antagonistic to each other. The population of P-solubilizers and N-fixing bacteria in carrier based biofertilizers grow optimally and maintain the bacterial and fungal density up to $10^8$ CFU g$^{-1}$ and $10^7$ CFU g$^{-1}$ respectively.

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