Phytotoxicity Test of Probiotic Bacteria in Jack Bean Seed (*Canavalia ensiformis* L) Through Seed Viability Test

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Abstract. Phytotoxicity test of probiotic bacteria was carried out to determine its effect on the germination of jack bean seeds (*Canavalia ensiformis* L). The study was conducted in February to March 2017, at the Seed Health Laboratory and Seed Center of the Bogor Institute of Agriculture. The study was designed in a Randomized Block Design with seven treatments and three replications. The test results showed that three pairs of probiotics were toxic and three other pairs of probiotics that were not toxic to the growth of *Canavalia ensiformis*. The three pairs of probiotics that are not toxic are AcCKB20-P24 (D1), AcCKB4-B28 (D3), and AcCKW5-P24 (D4). The three pairs of probiotics that are not toxic are recommended for use in making bio-organic fertilizers for *Canavalia ensiformis*.

Introduction

One seed is worthless, but from one seed it will produce millions of new seeds. If you grow one seed in the middle of the ocean, a large island will form.

Seed viability is the viability of seeds which is indicated by growth activity or metabolic activity. Seed viability can be measured through vigor testing for growing strength. The forms of growth strength vigor test include maximum growth potential (MGP), growth speed (GS), simultaneous growth (SG), vigor index (VI) and normal sprouts dry weight (NSDW).

The research aims to obtain a combination of probiotic bacteria that are not toxic to the jack bean seed. Four types of probiotic bacteria used are *Actinomycetes* sp and *Azotobacter* sp as nitrogen-fixing bacteria from the rhizosphere while *Pseudomonas* sp and *Bacillus* sp are saprotrophic soil bacteria that act as phosphate solvents or as decomposers. The research is a form of a screening of six bacterial combinations that will be used in making bio-organic fertilizers

Material and Method

The research was conducted in February to March 2017, at the Seed Health Laboratory of the Department of Agronomy and Horticulture and the Seed Center of the Bogor Institute of Agriculture. Materials used in the form of jack bean seeds, agar media (jelly), Nutrient Broth, rice flour, aqua dest, probiotic solution, bio-organic, while the tools used in the form of laboratory equipment: laminar
airflow, autoclave, Sacher, digital scales, electric ovens, Petri dishes, measuring cups, goblets cups, hand sprayers, scales, germination boxes, camera, writing instruments and data processing tools.

The experiment was carried out through the stages of making a liquid suspension of eight types of bacteria (six types of phosphate solubilizing bacteria and two types of nitrogen-fixing bacteria) based on compatibility tests so that six pairs of probiotic bacteria were obtained. Furthermore, the toxicity test is carried out through seed viability testing to obtain a couple of probiotic bacteria that are not toxic to jack bean seeds. Liquid suspension preparation refers to the Method of Manikandan et al [1]. The microbial incubation process of Pseudomonas sp and Bacillus sp was carried out for 24 hours while Azotobacter sp for 48 hours [2] until the number of cells reached at least $10^7$ cells / mL. Propagation of Pseudomonas sp, Bacillus sp and Azotobacter sp using agar media, whereas the multiplication of Actinomycetes sp microbes using rice flour media was then incubated for 14 days [3]. The volume of liquid suspension each made as much as 500 mL for soaking 75 seeds. The probiotic bacterial toxicity test for the jack bean seed consisted of seven treatments of probiotic pairs with three replications and analyzed in a randomized block design. 75 seeds were soaked in 500 mL probiotic solution for 17 hours, then the seeds were air-dried for one hour then every 25 seeds were planted in three germination boxes (35 cm x 27 cm) with sterile sand media and kept in moisture during the test. Toxicity test using seven treatments consisting of: D0 = without probiotics (control using sand media and water), D1 = probiotic pair AcCKB20-P24 (probiotic solution), D2 = probiotic pair AcCKB9-P24 (probiotic solution), D3 = probiotic pair AcCKB4-B28, (probiotic solution), D4 = probiotic pair AcCKW5-P24 (probiotic solution), D5 = probiotic pair Azl9-P24 (probiotic solution), D6 = probiotic pair Azl7-P24 (probiotic solution). Treatment that has the same or higher viability compared to control treatments means that probiotics are not toxic to seeds so that they can be used to make bio-organics for the cultivation of jack beans. Observation of seed viability includes germination percentage (GP), maximum growth potential (MGP), germination speed (GS), germination uniformity (GU), vigor index (VI), and normal sprouts dry weight (NSDW).

Result and Discussion

Observation of the number of bacterial colonies in six pairs of probiotic solutions is shown in Table 1. Bacillus sp (B28 = 2.85 x 10^6) is the highest solvent phosphate bacteria. Actinomycetes sp Cikabayan4 isolates (AcCKB4 = 2.64 x 10^4) are nitrogen-fixing bacteria with the highest number of colonies. Azotobacter sp (Azl9 = 1.26 x 10^3) is the nitrogen-fixing bacteria with the lowest number of colonies while Azotobacter sp (Azl7 = 1.22 x 10^6) as the phosphate solvent bacteria with the lowest number of colonies.

Table 1 The number of microbial colonies in a probiotic solution (CFU/g)

| Bacterial Pair Code | Pseudomonas sp | Bacillus sp | Actinomicetes sp | Azotobacter sp |
|---------------------|----------------|-------------|------------------|---------------|
| AcCKB20 vs P24      | $1.68 \times 10^6$ | X           | $2.24 \times 10^4$ | X             |
| AcCKB9 vs P24       | $1.42 \times 10^6$ | X           | $1.30 \times 10^4$ | X             |
| AcCKB4 vs B28       | X               | $2.85 \times 10^6$ | $2.64 \times 10^4$ | X             |
| AcCKW5 vs P24       | $1.58 \times 10^6$ | X           | $2.08 \times 10^4$ | X             |
| Azl9 vs P24         | $1.34 \times 10^6$ | X           | X                | $1.26 \times 10^3$ |
| Azl7 vs P24         | $1.22 \times 10^6$ | X           | X                | $1.31 \times 10^3$ |

Source: Primary data, laboratory test results

The six pairs of probiotic solutions were then used for phytotoxicity testing by soaking the seeds of jack beans for 17 hours and then planted in germination boxes. The results of phytotoxicity testing are presented in Table 2.
There are three combinations of probiotics with seed viability values that are not different compared to control treatments, namely AcCKB4-B28, AcCKB20-P24, and AcCKW5-P24 while the other three treatments are different and lower than controls which show probiotic treatment decreases seed viability. The high seed viability test results in the treatment of AcCKB4-B28, AcCKB20-P24, and AcCKW5-P24 are the strong reasons that the three pairs of probiotics are not toxic to seeds and are recommended for selection in bio-organic manufacturing. Actinomycetes sp isolates Cikabayan4 (AcCKB4) and Bacillus sp (B28) were the bacteria with the highest number of colonies (Table 1). The pair of these two types of bacteria also turned out to produce the best vigor of seed growth strength although it was not different from some other treatments (Table 2), even the dry weight of normal sprouts produced was higher and different than other treatments. The high dry weight of the sprouts shows that the sprout's metabolic activity is running well so that the accumulation of dry matter is more optimal.

According to Sutariati and Wahab (2012) [4], application of rhizobacteria to chili seeds can increase germination (DB), maximum growth potential (PTM), growth simultaneity (KST), vigor index (IV), growth index (KCT), dry weight of normal sprouts (BKKN), rate of sprouting (LPK) as well as a reduction in the time needed to germinate 50% (T50) where Bacillus sp gives the best results. The research results of Sutariati and Wahab (2012) [4] also support that Bacillus sp has a better ability to dissolve phosphate and produce IAA compared to Pseudomonas fluorescence and Serratia sp.

A germination is an event of an embryo growing in seed into a new plant. The seeds will germinate if the environmental conditions are appropriate, suitable temperature, adequate water supply, sufficient oxygen, humidity, light, and adequate planting media. In addition to environmental conditions, internal seed (seed maturity, weight and size, seed dormancy, inhibitors, and physical seed) are factors that influence seed germination. In the seed, there is an embryo as a candidate plant consisting of radicles (root candidates) and plumules (sprouts candidates). In the embryo, there are also food reserves (carbohydrates, proteins, fats, and phytin) stored in cotyledons while cotyledons are protected by testa as a coating to prevent damage to the embryo by bacteria and fungi. In the testa, there is a small hole (micropyle) and near the micropyle, there is a hilum (navel seed) and the umbilical cord that connects the seed to the placenta and functions as a food supplier during the process of filling and forming seeds.

The process of seed germination physiology begins with the imbibition event. Water plays a role in enzymatic reactions, increasing respiration through several pathways to produce adenosine triphosphate (ATP). ATP plays a role in cell organelle synthesis, synthesis of Ribonucleic Acid (RNA) and protein so that embryo initiation occurs. The advanced process of overhauling food reserves in seeds (carbohydrates, proteins, fats, and phytin) to produce energy through enzymatic reactions that

| Treatment          | MGP (%) | GP (%) | GU (%) | GS (%) | VI (%) | NSDW (g) |
|--------------------|---------|--------|--------|--------|--------|----------|
| D0 Without Probiotics | 89.3 a  | 80.0 ab| 50.7 a | 19.6 bc| 20.0 ab| 23.2 b   |
| D1 AcCKB20-P24     | 93.3 a  | 84.0 ab| 53.3 a | 21.4 ab| 25.3 a | 24.1 b   |
| D2 AcCKB9-P24      | 68.0 b  | 41.3 c | 22.7 b | 8.1 d  | 9.3 b  | 17.2 c   |
| D3 AcCKB4-B28      | 98.7 a  | 92.0 a | 56.0 a | 24.6 a | 25.3 a | 24.1 b   |
| D4 AcCKW5-P24      | 88.0 a  | 77.3 b | 42.7 a | 18.1 c | 22.7 ab| 20.6 bc  |
| D5 Azl9-P24        | 56.0 b  | 17.3 d | 8.0 b  | 3.1 e  | 2.7 b  | 16.2 d   |
| D6 Azl7P24         | 58.7 b  | 32.0 c | 8.0 b  | 5.3 de | 5.3 b  | 17.2 cd  |
| Mean               | 78.9    | 60.6   | 34.5   | 14.3   | 16.0   | 20.9     |
| Coefficient of variation | 6.5    | 9.4    | 19.9   | 10.7   | 0.4    | 7.7      |

Note: numbers followed by the same letter in the same column do not differ in the DMRT test level of 5%, *) Asarcin transformation
are regulated by phytohormones and the results are mobilized to the point of growth [5]. Thus the two important things in the initial process of seed germination are water and phytohormone. Water comes from outside the seed while phytohormone is naturally present in the seed (endogenous). Fito hormone can be exogenous, which is derived from microbes in the atmosphere. Phytohormone-producing bacteria can be isolated and modified in various forms (solid, liquid, paste, powder) to be applied in the form of biological fertilizers. Research result [6] shows the microbes in the form of liquid formulations tend to produce maximum growth potential, germination power and a higher vigor index of corn seeds than in paste form.

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