Study of cross inoculation of *Rhizobium tropici* with other potential soil microbes on their ability to support the growth of Soybean

S Lekatompessy, I Nurjanah, and H Sukiman

Research Center for Biotechnology, Indonesian Institute of Sciences Jl. Raya Bogor KM 46, Cibinong, Bogor Telp. (021) 8754587, Fax. (021) 8754588, Indonesia

Email: sylviajr1@yahoo.com

Abstract. *Rhizobia* bacteria have been well known as nitrogen-fixing bacteria which could promote the growth of soybean plants. The bacteria could establish a symbiosis living with crops and trees legume. The successful symbiosis living is normally identified by the specific compatibilities between the bacteria and host plants. However, there is a chance that the bacteria which was isolated from trees legumes could also infect and be compatible in developing the symbiosis living with crops plant such as soybean. This study aims to confirm that *Rhizobium* bacteria isolated from tree legume, *Acacia mangium*, could successfully develop the symbiosis living with soybean. In addition, this research also studies the possibility of *Rhizobium* bacteria in working with other potential soil microbes such as *mycorrhizae* and others potential microbes on supporting the growth of soybean. Green house experiment showed that *Rhizobium tropici* combined with other potential soil microbes could significantly support the growth of soybean. This is shown with the increase of upper plant biomass. A number of pods although the data of visible soybean seed did not make significant different. This research finally opens the possibility of using biodiversity of nitrogen-fixing microbes to be packed as an update quality biofertilizer for soybean.

1. Introduction

*Rhizobium* inoculation of cross *Acacia mangium* from the soybean crop is unique and interesting to be a focus of research as it can be further developed for up to date quality biofertilizer. *Rhizobium* combination of plant origin mangium with other potential microbial fertilizer can be expected to be utilized in accordance with the needs of the existing land. *Rhizobium* is a symbiotic bacteria groups under legumes. This group of bacteria is able to infect plant roots and form root nodules. Nodule functioning took atmospheric nitrogen and provided it as a necessary plant nutrient. *Rhizobium* is able to provide an element of N in the form of amino acids needed by soybean plants.

Nitrogen (N) is the most important element for the growth of soybean plants, but the availability of N in the tropics, including Indonesia is low. Artificial fertilizer N which used natural gas as a raw material has limitations due to the condition of natural gas that cannot be updated. Therefore, the necessary technologies are biological N fixation by utilizing microorganisms by means of inoculation of *Rhizobium* bacteria to make efficient the N fertilizer on soybean plants.

Endophytic bacteria are bacteria that live inside plant tissues during certain periods of their life cycle. Endophytic bacteria can colonize plant tissue without harming its host. In the plant tissue likely found several types of endophytic microbes [17]. Endophytic bacteria have the potential to be utilized as the production of secondary metabolites such as that contained in the host plant [14]. Some
endophytic bacteria can produce hormones that can stimulate plant growth. One of the hormones produced by endophytic microbes are Indole Acetic Acid (IAA) or better known as auxin. Auxin acts as a promoter of hormones growth in plants and is usually found in the meristem tissue [16]. IAA produced by bacteria in the plant increase the number of root hairs and lateral roots of plants [10]. Hormones produced by the bacteria will be absorbed by the plant so that the plant will grow faster or bigger. Hormones IAA is able to synthesize biological substances and increase seed germination, height and the growth of plant [4].

This study aims to confirm that *Rhizobium* bacteria isolated from tree legume, *Acacia mangium*, could successfully develop the symbiosis living with soybean. In addition, this research also studies the possibility of *Rhizobium* bacteria in working with other potential soil microbes such as *mycorrhizae* and others potential microbes on supporting the growth of soybean.

2. Materials and Methods

2.1. Experimental Design

The study was conducted at the Laboratory of Plant Symbiotic Microbes, Research Center for Biotechnology, LIPI, Cibinong. Three replications were made from each treatment. The treatments were: control without chemical fertilizer and potential bacteria inoculation (KO), Control with chemical fertilizers (KN), *Rhizobium* from plant of *Acacia mangium* (DCM), *Mycorrhizae* (Miko), Endophytic bacteria from the plant of *Shorea selanica* (Endo), Phosphate solubilizing bacteria from plant of Gamal (*Gliricidia sepium*) (BPF), and *Rhizobium* from plant of *Glycine max* (RH). All treatments were then combined so in total there were 18 combined treatments.

2.2. Biological Material

Soybean seed varieties used are Anjasmoro. Soybean seeds that will be used are selected then tested germination. Preparation of microbes for the production of biomass to be used as an inoculant. The medium used for microbial bacteria *Rhizobium is* Yeast Extract Mannitol Broth (YEMB) media [15], endophytic bacteria were grown on nutrient broth media, phosphate bacteria biomass production using liquid Pikovskaya media, biomass production Mycorrhiza fungus using soil media and corn as a host plant. Soybean seeds were germinated to normal growth and good germination. Soybean sprouts then inoculated with free variation. The volume of the suspension inoculation was ± 30 ml, and soaking sprouts was done for 30 minutes. For the treatment of fungal *mycorrhiza*, every polybag with *mycorrhiza* treatment was given 2 grams of powder *mycorrhiza*. Then the soybean sprouts were grown in a polybag with a volume capacity of 10 kg of soil and compost media using 1: 1. Maintenance was done with regular watering and NPK fertilizer 50% as a recommended standard. Fertilization was done 14 days after planting and 45 days after planting. Plants were harvested after ± three months old.

2.3. Measurements and Analyses

The parameters measured were plant heights (measurements was performed when the plants were one and two months old), wet and dry weight of biomass crops from the top and bottom, number of plant pods, pod weight from wet biomass and seed production. Then the results of measurements of these parameters are analyzed based on the average value. The next measurement analysed are symbiotic capacity (SC) and symbiotic effectiveness (SE) of the soybean crop.

3. Result and Discussion

Based on the results, it showed that the ability of *Rhizobium tropici* from *A. mangium* (DCM) could be crossed on other host plants, namely soybean plants. In addition, *Rhizobium* bacteria (DCM) can be inoculated using a 1 to 5 crossed microbes. Generally, the results confirmed that the incorporation of potential microbes influence could support plant growth. The incorporation of these microbes can also be combined according to the needs of the existing land.

As we know, most of the degraded land in Indonesia are complex and expensive to be dealt with. One of the extreme land damage comes from coal mining activities, petroleum and others. As a result
of these activities, the soil has lost their topsoil and also experienced dryness, compaction of the soil, low water holding ability of soil, nutrient-poor soil, accumulation of toxic elements in the soil, as well as pH of acid soils. For example, utilization of soil microbes such as fungi mycorrhiza is potential to be a solution in the rehabilitation of mined land and is expected to be one alternative to improve the quality of degraded lands [9].

Utilization continues to grow microbes; microbial incorporation studies are expected to further improvement of the quality of biological fertilizers. For example, cooperation between mycorrhiza with bacterial N fixing will yield favorable conditions for the surrounding soil microbes so it will positively affect plant growth. The contribution of elements obtained by these plants can not be ascertained because more research is needed on this subject [5]. Plants can also obtain IAA hormone body of endophytic microbes that have the ability to produce IAA producing growth hormone which helps the growth of plant and plant roots at the same time so that the N fixing is more powerful and healthy.

![Figure 1. The height at age 1 month and 2 months on soybean plants.](image)

Figure 1 shows crossed Rhizobium inoculation on soybean plants DCM combined with another microbial effect on soybean plant height. This proves the potential use of microbial inoculation of cross manner to support plant growth. Soil microbes serve as a change agent biochemical complex organic compounds into inorganic compounds. The form of inorganic compounds is the element carbon, nitrogen, sulfur, and phosphorus. This process is called mineralization in which there occurs a large number of chemical changes and the role of various species of microbes in the soil. The level of the balance amount in the soil nutrients will affect the amount and activity of soil microbes. This will affect the level of soil fertility [18].

Generally, healthy soil around the roots will be inhabited by beneficial microorganisms that utilize organic substrates of organic matter as a source of energy and nutrients. A number of microbes play an important role in normal and healthy soil. The soil microbes is an indicator in determining the quality of the soil. Soil microbes also play a role in the decomposition of organic matter, releasing nutrients into forms available to plants, and degrade toxic residue. In addition, microbes also act as an agent to enhance plant growth which produces growth hormones (IAA), vitamins and various organic acids that play an important role in stimulating the growth of plant root hairs [6].

Figure 2 shows that the cross-inoculation of soybean plants with a combination of five microbial biomass significantly affect the top of soybean plants. Generally, the effect of cross-inoculation is well visible using 1 to 5 of potential microbes as it helps increase the top plant biomass compared to
(KO) control without fertilizer, without inoculation and (KN) controls the use of chemical fertilizers alone. Lower biomass plant DCM + Endo, RH DCM, DCM + Miko + BPF looks higher than the others. This is because microbes potentially help root growth. Endophytic hormone-producing bacteria and also the presence of IAA mycorrhiza soil fungi, besides bacteria phosphate solubilizing also helps in getting the needed P. Some bacteria are nutrient providers around the plant roots known as promoter Rhizobacteria otherwise known as PGPR [3].

Figure 2. The dry weight of the upper plant and root plant on soybean plant

IAA is not only a source of hormones produced by plants alone but can be generated by rhizobacteria or what we call microbe living around the plant roots. The use of microbial culture supernatant containing IAA is able to provide a physiological effect on the plant. The growth of hormone produced by the microbes around the roots are able to form root hairs and increased ion transport so that the transport of water by the roots increased [12].

Microbial activity around the plant roots is influenced by the exuded roots [1]. The content of this root exuded, which is one factor for the growth of plant microorganisms [19]. Organic acids such as citric acid released from plant roots also suspected to trigger the growth of bacteria so that the N fixing will occur between bacterial N fixing association with plant roots [11]. Figure 3 shows the results of the number of pods and pod weight at the highest inoculation of the cross using five potential microbes. In general, the cross-inoculation using two to five potential microbes very significantly affect the number of pods produced. Microbial population in the soil is influenced by several factors: the amount and kinds of nutrients, level of moisture, aeration rate, temperature, pH, and soil treatment such as the addition of fertilizer or natural disasters such as flooding which can cause an increase in the number of microbes [2,7,8]. This supports the results that more soil microbial population will help the plant so that the plant increases production of pods.
Microbial inoculation potential cross gives real advantages. One of the advantages by giving microbes is the ability to increase the uptake of phosphate solvent $p$ and plant height and weight of maize [13]. In addition, microbial phosphate solubilizing can also increase the production of peanuts by up to 73% and increase the solubility of P in the soil [20].

**Figure 3.** Number of pods, fresh weight of pods of soybean plant

| Note | 1 CO  | 7 DCM. Endo | 13 DCM. RH. BPF |
|------|-------|--------------|-----------------|
|      | 2 CN  | 8 DCM. Myco. Endo | 14 DCM. RH. Myco. Endo |
|      | 3 DCM | 9 DCM. Myco. BPF | 15 DCM. RH. Myco. BPF |
|      | 4 DCM. Myco | 0 DCM. Endo. BPF | 16 DCM. RH. Endo. BPF |
|      | 5 DCM. Endo | 1 DCM. RH. Myco | 17 DCM. Myco. Endo. BPF |
|      | 6 DCM. BPF | 2 DCM. RH. Endo | 18 DCM. Myco. Endo. BPF. RH |

**Figure 4.** Production of seeds produced from soybean plants for 3 months in a greenhouse conditions

| Note | 1 CO  | 7 DCM. Endo | 13 DCM. RH. BPF |
|------|-------|--------------|-----------------|
|      | 2 CN  | 8 DCM. Myco. Endo | 14 DCM. RH. Myco. Endo |
|      | 3 DCM | 9 DCM. Myco. BPF | 15 DCM. RH. Myco. BPF |
|      | 4 DCM. Myco | 10 DCM. Endo. BPF | 16 DCM. RH. Endo. BPF |
|      | 5 DCM. Endo | 11 DCM. RH. Myco | 17 DCM. Myco. Endo. BPF |
|      | 6 DCM. BPF | 12 DCM. RH. Endo | 18 DCM. Myco. Endo. BPF. RH |
Figure 4 shows that the role of microbes assists in plant growth compared with controls. The highest seed production can be seen in cross-inoculation potential of using microbes 5. In general, this microbial utilization of plant growth looks very helpful. Hopefully, by cross inoculating and combining these microbes, microbial potential can be utilized in accordance with the land needs to be used.

Table 1 shows that the cross-inoculation with five microbes have a potential symbiotic capacity and maximum effectiveness compared with others. However, the potential microbial capabilities are included in the effective category. These results are expected to up-date the use of microbial inoculation of cross 1 to 5 microbes to improve the quality of biofertilizer.

Cross-inoculation between microbes and plants on SC (symbiosis capacity) and SE (symbiosis effectiveness) is high. If it is not compatible with the microbe and plants, the required nutrient plants will not be achieved. Cross-inoculation is very favorable, so we can adjust according to the soil conditions prior to use. It is expected that cross-inoculation with potential microbe variation will show the quality of biofertilizers.

| Treatment               | Symbiosis Capacity (SC) | Category | Symbiosis Effectiveness (SE) |
|-------------------------|-------------------------|----------|-----------------------------|
| DCM                     | 4.55                    | e        | 117.2061                    |
| DCM+Miko                | 7.45                    | e        | 144.9744                    |
| DCM+Endo                | 3.88                    | e        | 136.6269                    |
| DCM+BPF                 | 3.33                    | e        | 117.8876                    |
| DCM+RH                  | 5.94                    | e        | 149.9148                    |
| DCM+Miko+Endo           | 1.30                    | e        | 102.385                     |
| DCM+Miko+BPF            | 5.52                    | e        | 147.8705                    |
| DCM+Endo+BPF            | 6.09                    | e        | 133.3901                    |
| DCM+RH+Miko             | 7.73                    | e        | 151.448                     |
| DCM+RH+Endo             | 3.58                    | e        | 126.7462                    |
| DCM+RH+BPF              | 2.91                    | e        | 105.6218                    |
| DCM+RH+Miko+Endo        | 6.09                    | e        | 133.3901                    |
| DCM+RH+Miko+BPF         | 7.73                    | e        | 151.448                     |
| DCM+RH+Endo+BPF         | 3.58                    | e        | 126.7462                    |
| DCM+Miko+Endo+BPF       | 3.73                    | e        | 134.5826                    |
| DCM+RH+Miko+Endo+BPF    | **11.52**               | e        | **170.1874**                |

4. Conclusion

*Rhizobium* cross-inoculation (DCM) on soybean plants in combination with other potential microbes on soybean plants is capable of supporting plant growth while improving the quality of biofertilizers. Updated biofertilizer can improve the quality of cross inoculated either with a host of other plants and other potential combinations of microbes in accordance with the needs of the land. This research opens the opportunity in using the biodiversity of potential microbes such as nitrogen fixing, phosphate solubilizing and plant growth hormone to be packed as an updated quality of biofertilizer for soybean plant.
5. References

[1] Bais H P, Weir T L, Perry L G, Gilroy S and Vivanco J M 2006 The role of root exudates in rhizosphere interactions with plants and other organisms *Annu. Rev. Plant Biol.* 57 233-266

[2] Barnesa R J, Baxtera S J, Lark R M 2007 Spatial covariation of Azotobacter abundance and soil properties: A case study using the wavelet transform *Soil Biol. Biochem.* 39 pp 295-310

[3] Bashan Y and Holguin G 1998 Proposal for division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (Plant Growth Promoting Bacteria) and PGPB *Soil Biol. Biochem.* 30 1225-1228

[4] Berkum V P and Bohlool B B 1980 Evaluation of nitrogen fixation by bacteria in association with root s of tropical grasses *Microbiol Rev.* 44(3) 491-517

[5] Curl E and Bryan T 1985 The Rhizosphere (Springer-Verlag: Berlin Heidelberg New York, Tokyo) 290

[6] Hindersah R and Simarmata T 2004 Artikel Ulas Balik: Potensi rhizobakteri azotobacter dalam meningkatkan kesehatan tanah. *Jurnal Natur Indonesia* 5(2) 127-133

[7] Kusnadi P, Syulasmi A, Purwianingsih W, and Diana 2003 Mikrobiologi (Common Teksbook) Jurusan Pendidikan Biologi FPMIPA-UPI IMSTEP

[8] Ladha J K, George T and Bohlool B B 1992 Biological nitrogen fixation for sustainable agriculture (Kluwer Academic : London) 209

[9] Margaretha 2011 Eksplorasi dan identifikasi mikorisa indigen asal tanah bekas tambang batubara *Berita Biologi* 10(5) 641-647

[10] Okan Y and Kapulnik Y 1986 Development and function of *Azospirillum* inoculated root *Plant Soil* 90 3–16

[11] Olivares E, Peña E, Aguiar G 2002 Metals and oxalate in Tithonia diversifolia (Asteraceae): concentrations in plants growing in contrasting soils, and Al induction of oxalate exudation by roots *J. of Plant Physiol.* 159(7) 743-749

[12] Pamungkas F T, Darmanti S, and Rahardjo B 2009 Pengaruh konsentrasi dan lama perendaman dalam supernatant kultur *Bacillus sp.* 2 DUCC-BR-K13 terhadap pertumbuhan stek horizontal batang jarak pagar (*Jatropha curcasL.*) *J. Sains & Mat.* 17 131-140

[13] Prihartini T and Anas 1991 Peran jasad mikro pelarut phosphat terhadap tanaman jagung di tanah utisol Rangkas Bitung Risalah Seminar Latihan Magang Penelitian Pertanian dan Bioteknologi Sukamandi, Bogor

[14] Simanjuntak P, Parwati T, Bustanussalam, Prana T K and Shibuya H 2002 Produksi Alkaloid Kuninga oleh beberapa mikroba endofit dengan penambahan zat induser (Studi Mikroba Endofit Tanaman Cinchona sp. (2) *Majalah Farmasi Indonesia* 13(1) 1-6

[15] Somasegaran P and Hoben H J 1984 Methods in legume rhizobium technology University of Hawaii. NIFTAL: Hawaii *Project and Mircen*

[16] Spaepen S, Vanderleyden J and Remans R 2007 Reviews: Indole-3-acetic acid in microbial and microorganism-plant signaling *FEMS Microbiology* 10 1-24

[17] Strobel G A and Daisy B 2003 Bioprospecting for microbial endophytes and their natural products *Microbiology and Molecular Biology Review* 67(4) 419-502

[18] Ristia N, Sanusi M and Frieda N 2008 Isolasi dan identifikasi bakteri penambat nitrogen non simbiosis dari dalam tanah *Jurnal Penelitian dan Pengembangan Sains & Humanio* 2(1) 8-80

[19] Vlastimil V and Kunc F 1998 *Soil microbia associations* (Czechoslovakia: Prague Institute of Microbiology of the Czechoslovakia Academy of Sciences) 84-130

[20] Young C C, Chen C L and Chao C C 1990 *Effect of rhizobium, VAM and solubilizing bacteria on yield and mineral phosphorus uptake of crops in subtropical-tropical soils* (Taiwan: Dept of Soil Science)

Acknowledgements

We would like to thank Rumella Simarmata, Tiwit Widowati, Mr. Adang, Mr. Muplih and Nuriyanah that has helped us so this research can be done well.