

Application of a Single and Combination of Plant-growth Promoting Bacteria and Actinomycete to Increase Chili (*Capsicum annum L.*) Health and Growth

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Plant growth promoting bacteria (PGPB) are known for their ability to produce plant hormones and antimicrobial substances, to induce plant resistance, and to solubilize phosphate. In this study, two bacterial isolates *Bacillus* sp. ST02 and *Lactobacillus plantarum* ST03, one endophytic bacteria Sp 1E, and one rhizospheric actinomycetes Sp10 R were assayed for their ability in solubilizing phosphate and producing indole acetic acid (IAA), and in affecting chili growth and health. Bacterial ability in solubilizing phosphate was assayed in Pikovskaya's agar, while the ability in producing IAA was spectrophotometrically measured using Salkowski reagent. To know bacterial potential in promoting plant growth and in reducing fungal disease of *Fusarium oxysporum*, bacterial isolates were applied in chili seedling grown in small scale field. The results showed that only two isolates *Bacillus* sp. ST02 and *Lactobacillus plantarum* ST03 were capable of solubilizing phosphate, but all isolates produced IAA to some extent. Single and combination of ST02 and Sp 10R showed to affect more in increasing plant performance and reducing fungal disease caused by *F. oxysporum*. This result indicated that the PGPB isolates were considered as a promising microbial inoculant for plant health and growth.

Keywords: *Indole acetic acid, phosphate solubilizing bacteria, fungal control, plant performance.*

To control plant pathogenic fungi and to increase plant performance, ones mainly use chemical compounds such as pesticides and fertilizer. Recently, many efforts have been conducted to reduce chemical pesticide and fertilizer application by exploring a possibility of utilization of biological agent as biofertilizer and biocontrol agent. Among biocontrol and biofertilizer, bacteria are the most interesting group of organisms that have already been utilized as beneficial microorganism. A diverse group of

beneficial microorganisms have been found, but their roles in environments have not been fully understood. Many studies have been conducted to isolate and to utilize plant-benefit microbes such as plant growth promoting bacteria (PGPB). These include free-living, specific symbiotic, and cyanobacteria (Glick, 2012).

PGPB are group of bacteria enhancing and protecting plant growth through a wide variety of mechanisms such as by producing plant hormones, increasing the availability of soil nutrient, and controlling plant pathogen by inducing plant resistance or by producing antimicrobial compounds (Chakraborty *et al.*,

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2013; Przemienieck *et al.*, 2015; de Souza *et al.*, 2015). Furthermore, they may assist plants to cope with a variety of abiotic stresses (da Silva Sousa *et al.*, 2008; Thijs *et al.*, 2014; Gagné-Bourque *et al.*, 2015), and reduce environmental problems (Glick 2012; Thijs *et al.*, 2014). Among the bacteria *Bacillus*, *Pseudomonas*, and *Acinetobacter* (Chakraborty *et al.*, 2013; Dias *et al.*, 2013; Ngoma *et al.*, 2013; Thijs *et al.*, 2014), and actinomycetes (Dombou *et al.*, 2001; Franco-Correa *et al.*, 2010; Gopalakrishnan *et al.*, 2013; de Souza *et al.*, 2015) are known as PGPB.

Chili is one of the most important spices in our tradition. The consumption of fruit of this crop is very high everyday, and even higher in special holidays, with raised price tremendously. Since it is considered as important plant, chili is planted in almost all area in this region, from area close to the sea to highland area.

The chili fruit production depends on good culture practices such as to keep pest and disease controlled, to provide sufficient nutrient to the plant, and to grow the plant in proper season. To control pest and disease, ones utilize synthetic pesticides which may harm to environment and health, increase pest and disease resistant, and kill non target organisms. The need of free chemical on agricultural product together with the awareness of environmental issues increases the attempt to replace the chemicals with more environmentally friendly ones. In this study, PGPB of local isolates were assayed for their ability to solubilize phosphate, to produce indole acetic acid (IAA), and to assay the isolate in controlling fungal disease and enhancing chili productivity.

MATERIALS AND METHODS

Microbial isolates and culture condition

Two potential phosphate solubilizing bacterial isolates *Bacillus* sp. ST02 and *Lactobacillus plantarum* ST03 isolated from guano of bat cave, one endophytic bacteria Sp 1E and one actinomycete Sp10 R of white lettuce rhizosphere of potential in inhibiting fungal growth, and a chili pathogenic fungi *Fusarium oxysporum* were used in this study. Microbial isolates were kept in refrigerator on either chitin contained medium or Pikovskaya's agar for bacteria and actinomycete,

and Sabaroud dextrose agar for fungal isolate. All isolates were grown in proper media at ambient temperature for further study.

Assay on Phosphate Solubilizing Potential of Bacterial Isolates

Assay on phosphate solubilizing potential of bacterial isolates was done by dropping a 10 μ l of bacterial suspension of OD₆₀₀ \approx 0.5 in the center of Pikovskaya's agar in petridish. Culture was incubated at ambient temperature for 7 days. Phosphate solubilizing index (PSI) was calculated as the ratio of diameter of clear zone divided by diameter of colony.

Assay on Potential of Indole Acetic Acid Production of Bacterial Isolates

IAA production was measured as described by Gordon and Webber (1951). A 10 ml of bacterial culture of OD₆₀₀ \approx 0.5 grown in broth medium was centrifuged at 10.000 rpm for 15 minutes. Supernatant was taken and mixed with Salkowski reagent in ratio of 1:1. Mixture was incubated at ambient temperature for 60 minutes. IAA was detected as pink color in the mixture. IAA concentration was measured as absorbance using spectrophotometer at 530 nm.

Assay on Effect of Bacterial and Actinomycetes Isolates in Controlling *Fusarium oxysporum* and Chili Performance in Field

All bacterial and actinomycete isolates were grown in nutrient broth. Chili seedling of 2 weeks were planted in soil mixed with compost in small scale field of Kebun Percobaan Tanaman Sayuran Berastagi, Balai Penelitian Tanaman Sayuran, Tongkoh, Deli Serdang, North Sumatra. No fertilizer and pesticide were applied, and no prior soil sterilization was carried out. Bacterial suspension of \approx 10⁸ cell/ml was applied next to chili seedling 2 weeks after planting, while fungal suspension of \approx 10⁸ conidia/ml was infested one week after bacterial infestation. Treatment was applied as described in Table 1.

Observation on control of *Fusarium oxysporum* and chili performance

Observation on plant health and performance included chlorophyll content, fresh-weight and dry-weight, and frequency of infected plant, disease coverage, and rotten fruit of chili fruit was conducted at the end of study of 16 weeks, while plant height and number of branch were

observed at 14 weeks after planting. Chlorophyll content was measured as described by Wintermans and de Mots (1965).

RESULTS

Phosphate Solubilizing Assay

A diverse group of bacteria and actinomycetes has been reported to solubilize phosphate to make it available to plants (da Silva Sousa *et al.*, 2008; Dias *et al.*, 2013; Ngoma *et al.*, 2013). By growing them in Pikovskaya's agar, these microorganisms show clearing zone around their colony measured as PSI. In this study, bacterial isolates *Bacillus* sp. ST02 and *Lactobacillus plantarum* ST03 showed to solubilize phosphate, whether the Sp1 E and Sp 10R did not showed the ability to solubilize phosphate (Figure 1). PSI of that two bacterial isolates was considerably higher compared to that of previous studies by Kasmita (2010) and Ruwandani *et al.* (2014). *Lactobacillus plantarum* ability in solubilizing phosphate has not been reported so far. However, the ability of *Lactobacillus plantarum* in solubilizing phosphate was believed due to its ability in producing organic acids. Previous studies have shown that *Lactobacillus* species was isolated as endophyte and had potential as biocontrol agent for plant disease pathogens. Pereira *et al.* (2012) reported that 6 out of 45 isolated endophytic bacteria from strawberry plants belong to the genera *Lactobacillus*. In addition, Limanska *et al.* (2015) demonstrated that *Lactobacillus plantarum* might reduce the percentage of tumor formation by

Agrobacterium tumefaciens on carrot plants up to 53%.

IAA Production Assay

All four isolates of bacteria and actinomycetes used in this study showed to vary in producing IAA (Figure 2). Sp10 R showed to have relatively higher IAA production. IAA is one of the most physiologically active auxins (Yasmi *et al.*, 2016). The higher production of IAA by the isolates is expected to promote plant growth. Many bacteria and actinomycetes have been reported to produce plant hormones such as auxine and gibberelic acid (Ponmurugan and Gopi 2006; da Silva Sousa *et al.*, 2008; Saharan and Nehra 2011; Dias *et al.*, 2013; Ngoma *et al.*, 2013; Przemienieck *et al.*, 2015).

Effect of Bacterial Treatment in Controlling of *Fusarium oxysporum* and Chili Performance

Application of bacterial isolates and actinomycete, both singly or in combination showed to have different effect in controlling chili disease caused by *F. oxysporum* compared to that of (+) control and (-) control. It seemed that combination of Sp 10R+ST02 showed to have lower frequency of infected plant, disease coverage, and rotten fruit. This was also shown with application of the single isolates of both (Table 2), in which lower infected plant and rotten fruit were observed. It was observed that chili with no microbial application still got fungal disease with mild disease coverage, which was susceptible to be infected by any fungal pathogen of unsterilized soil.

Application of phosphate solubilizing bacteria of isolate ST02 both singly and in

Table 1. PGPB treatments in chili

Treatments	Type of treatments
(+) control	Soil was applied with <i>F. oxysporum</i>
(-) control	No microbial infestation
Sp 1E	Soil was applied with <i>F. oxysporum</i> and Sp 1E
Sp 10R	Soil was applied with <i>F. oxysporum</i> and Sp 10R
ST02	Soil was applied with <i>F. oxysporum</i> and <i>Bacillus</i> sp. ST02
ST03	Soil was applied with <i>F. oxysporum</i> and <i>Lactobacillus plantarum</i> ST03
Sp 1E+Sp 10R	Soil was applied with <i>F. oxysporum</i> , Sp 1E, and Sp 10R
Sp 1E+ST02	Soil was applied with <i>F. oxysporum</i> , Sp 1E, and <i>Bacillus</i> sp. ST02
Sp 1E+ST03	Soil was applied with <i>F. oxysporum</i> , Sp 1E, and <i>Lactobacillus plantarum</i> ST03
Sp 10R+ST02	Soil was applied with <i>F. oxysporum</i> , Sp 10R, and <i>Bacillus</i> sp. ST02
Sp 10R+ST03	Soil was applied with <i>F. oxysporum</i> , Sp 10R, and <i>Lactobacillus plantarum</i> ST03
ST02+ST03	Soil was applied with <i>F. oxysporum</i> , <i>Bacillus</i> sp. ST02, and <i>Lactobacillus plantarum</i> ST03

combination to chili plant rhizosphere seemed to noticeably increase plant height, number of branch, and fresh-weight and dry-weight of chili fruit which was similar to that of single SP10 R of IAA producer (Figure 3, 4, and 5). Sufficient of nutrient such as phosphate, reduction of fungal disease, and adequate of plant hormones contributed to this result. IAA may directly influence of shoot growth and development (Yasmi *et al.*, 2016). However, no effect was shown in chlorophyll content of chili plant (Figure 6). Dias *et al.* (2013) isolated and screened of bacterial strains producing IAA and capable of phosphate solubilization. It was found that 59 out of 79 strains had both abilities, in which 7 strains promoted crop growth under greenhouse conditions in unsterilized soil. However, he observed that growth promoting capability was not consistently related *in vitro* to IAA production and

phosphate solubilization. In this study, it seemed that isolate both producing more IAA and more capable of solubilizing phosphate promoted more chili growth and health.

Many actinomycetes especially of the genus *Streptomyces* have been utilized as biocontrol agent of plant disease. Their ability to control plant disease is due to adequate production secondary metabolites, such as antibiotics, siderophores, and extracellular enzymes (Dias *et al.*, 2013; H Etesami *et al.*, 2014). They have also been reported to produce plant hormone and to solubilize phosphate, and play a role in decomposition and nutrient mineralization (Ngoma *et al.*, 2013; H Etesami *et al.*, 2014; Laid *et al.*, 2014). Moreover, actinomycetes has been reported to produce degrading enzymes such as pectinase, xylanase, cellulase, lipase, amylase that may help

Table 2. Effect of PGPB application on chili disease

Treatments	Frequency of infected plant	Percentage of Disease coverage	Rotten Fruit
(+) control	26.7	93.3	3.5
(-) control	13.3	40	0
Sp 1E	16.7	41.7	4.3
Sp 10R	6.7	53.3	3.3
ST02	0	73.3	3.7
ST03	33.3	100	4.1
Sp 1E+Sp 10R	20	53.3	19.2
Sp 1E+ST02	13.3	100	4.1
Sp 1E+ST03	13.3	40	1
Sp 10R+ST02	5.6	27.8	0.9
Sp 10R+ST03	20	66.7	4.9
ST02+ST03	26.7	66.7	1.4

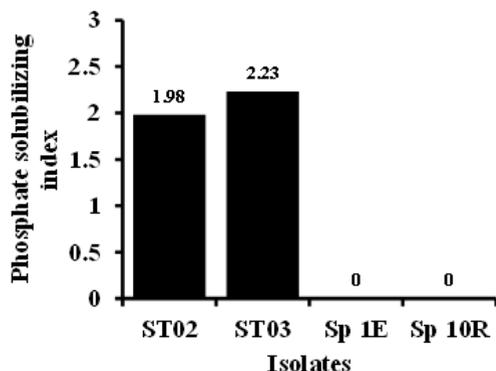


Fig. 1. PGPB isolate ability to solubilize phosphate

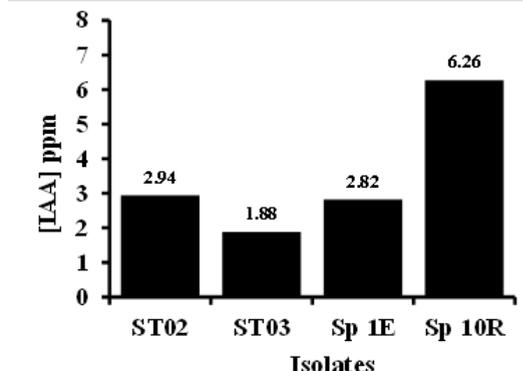


Fig. 2. PGPB isolate ability to produce IAA

it to decompose organic material in soil and in turn provide nutrient for plant (Ogbo and Okonkwo 2012).

DISCUSSION

Beneficial microorganisms like PGPB can be used as an alternative to substitute agrochemical

compounds in agriculture in controlling plant disease. In addition, these microorganisms might provide sufficient plant nutrient by solubilizing phosphate and fixating nitrogen (Dias *et al.*, 2013; Ngoma *et al.*, 2013; Yasmi *et al.*, 2016). Moreover, these microorganisms act as plant growth regulator by producing amino acids, vitamin, and plant hormones such as auxine and giberelic acid, as

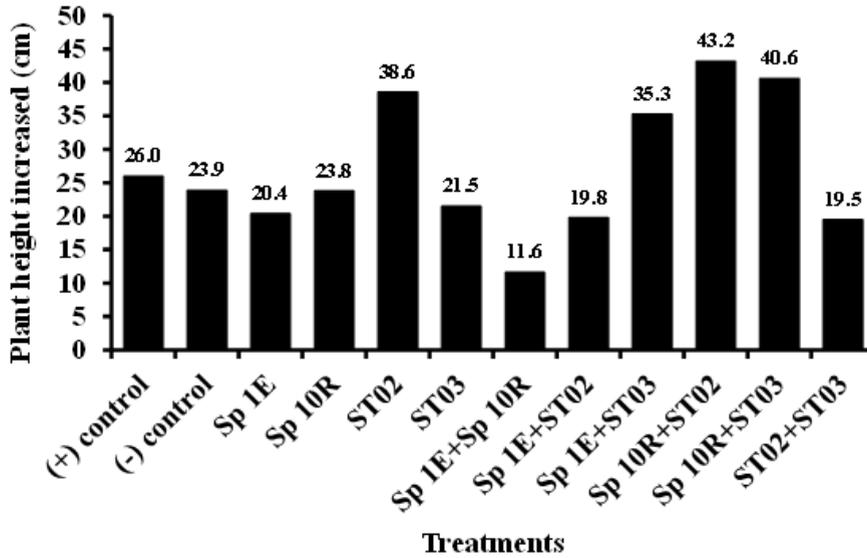


Fig. 3. Effect of PGPB isolate application in increasing plant height

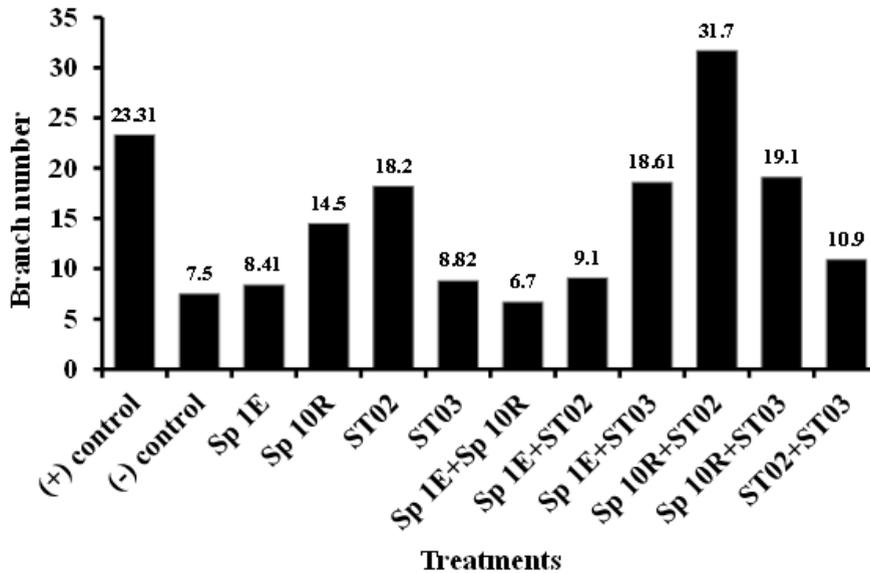


Fig. 4. Brach number of chili treated with PGPB

seen in the enhancement of seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes (Ponmurugan and Gopi 2006; Saharan and Nehra 2011).

Phosphor (P) is the second key important macronutrient after nitrogen for plant growth. Plants absorb phosphorus mainly in the form of phosphate ion, $H_2PO_4^-$ and HPO_4^{2-} . Phosphate

availability in soil is usually limited. In acidic soil, phosphate bond together with aluminum (Al) and iron (Fe), while in alkaline soil they tend to bond with calcium (Ca) and magnesium (Mg). An alternative in phosphate solubilization for improving the efficiency of natural phosphate utilization is through microbial metabolism. The main mechanism to solubilize phosphate is related to organic acids synthesized by microorganisms (da Silva Sousa *et al.*, 2008). The ability of PGPB to

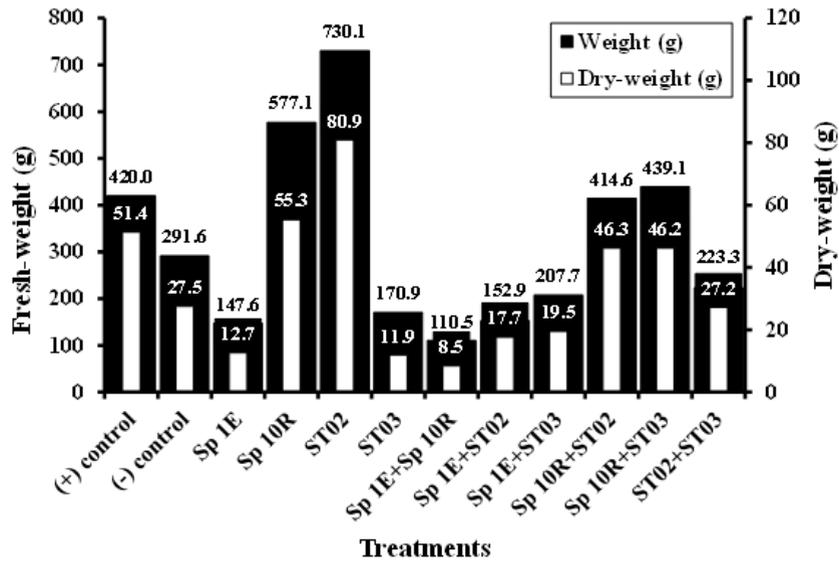


Fig. 5. Effect of PGPB isolate application in fresh-weight and dry-weight of chili fruit

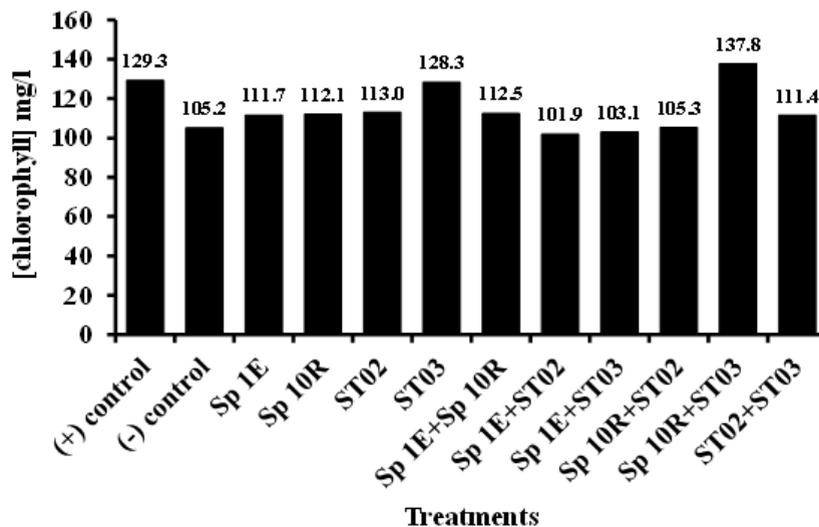


Fig. 6. Effect of PGPB isolate application in chlorophyll content

solubilize phosphate is also due to production of alkaline or acid phosphatase and other enzymes, and makes it available to the roots (Franco-Correa *et al.*, 2010; Przemieniecki *et al.*, 2015).

It is very important to protect plant growth in the early stage to yield good agricultural product (Przemieniecki *et al.*, 2015). Up until now, many agrochemical compounds are applied for that purpose. One alternative to decrease the agrochemical application in controlling plant disease is to use biological control agent. The ability of biological control agent to control plant disease is due to their ability to produce antimicrobial substances such as antibiotics, siderophore, and hydrogen cyanide (Ngoma *et al.*, 2013; H Etesami *et al.*, 2014; Laid *et al.*, 2016), lytic enzymes such as chitinase, glucanase, and other antifungal protein (Ogbo and Okonkwo 2012), and to induce plant resistance (Chakraborty *et al.*, 2013; Przemieniecki *et al.*, 2015).

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