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BACTERIA FROM ARBUSCULAR MYCORRHIZAL FUNGI SPORES *Gigaspora* sp. AND *Glomus* sp. : THEIR ANTAGONISTIC EFFECTS TOWARDS SOILBORNE FUNGAL PATHOGENS AND GROWTH STIMULATION OF *Gigaspora* sp. *IN VITRO*

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ABSTRACT

This research was aimed at obtaining bacterial isolate from arbuscular mycorrhizal fungi (AMF) spores showing antagonistic effects against fungal pathogens and yet compatible with AMF. Seven isolates of bacteria were isolated from surface-sterilized AMF spores of *Gigaspora* sp. (GG) and five isolates of bacteria isolated from *Glomus* sp. (GL). All bacterial isolates were identified based on its morphological methods and biochemical reaction and revealed that 9 isolates belong to genus *Bacillus*, while the other 3 isolates belong to genus *Pseudomonas*, *Proteous* and *Enterobacter* respectively. The tests to the antagonists against fungal pathogens and stimulation of AMF hyphal development of *Gigaspora* sp. *in vitro* showed that there were 3 isolates of bacteria (*Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5 and *Enterobacter hormaechei* GL3) had the ability to inhibit the growth of pathogens and to enhance the development of AMF hyphae *Gigaspora* sp *in vitro*. These three bacteria isolates potentially can be used to enhance the quality of AMF inoculants as biocontrol and biofertilizers. Enzymatic activity test showed that there were 7 isolates of bacteria that produced cellulase and protease activities, i.e. *Bacillus subtilis* GG1, *Bacillus cereus* GG3, *Bacillus laterosporus* GG6, *Bacillus pasteurii* GG7, *Proteus penneri* GL2, *Bacillus firmus* GL4 and *Bacillus cereus* GL5.

Key words: bacteria, Arbuscular Mycorrhizal Fungi Spores, antagonistic effects, stimulation effects, fungal pathogens

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INTRODUCTION

Many kind of microorganisms have been observed to be associated with mycorrhizosphere of different host plants. The functional significance of the microbial associate in the ecosystem has been well documented (Budi *et al.* 2012) including associative N_2-fixing bacteria (Cruz & Ishii 2011), plant growth-promoting rhizobacteria (Siddikee *et al.* 2010), phosphate solubilizing bacteria (Khan *et al.* 2009), and antagonists of plant pathogens (Artursson *et al.* 2006, Bakhtiar *et al.* 2010). In addition, the bacteria associated with spores of AMF *Glomus constrictum* and *Glomus geosporum* (Roesti *et al.* 2005), *Gigaspora margarita* (Cruz *et al.* 2008, Cruz & Ishii 2011, Horii & Ishii 2006, Budi *et al.* 2012), *Glomus intraradices* and *Glomus mosseae* (Bharadwaj *et al.* 2008) have been reported. These AMF spores associated bacteria has been reported significantly increased AMF hyphal growth and suppress fungal pathogens (Cruz & Ishii 2011).

Associated microorganisms may complement mycorrhizal activities, particularly in biological control and biofertilizer in agricultural systems. Nowadays, the awareness of international community to food safety has increased due to negative impacts of the use of chemical substances in crop production. The movement of "back to nature" of international community increases and organic food has become a new trend for their life style. Intensive agriculture in many countries including Indonesia have led to excessive inputs of agrochemicals (fertilizers, pesticides) but agricultural policies are now tending towards more organic for sustainable agricultural systems. Sustainability in agriculture has been defined as the successful management of resources for agriculture to satisfy enhancing the quality of the environment and conserving resources (Ladha 1992). In this respect, the Government of Indonesia c.q Ministry of Agriculture has declared "Go Organic in 2010". It implies better soil management to avoid modifications that have adverse effects on chemical and biological processes supporting plant growth. One approach is the decrease in chemical inputs to rational (economic but non-polluting) levels through the development of alternative strategies to ensure acceptable yield. An increasing demand for low-input of chemical substance in agriculture has resulted in greater interest in soil microorganisms that increase soil fertility or improve plant nutrition and health including the use of AMF as biofertilizer and biocontrol agents.

The use of AMF for sustainable agriculture has been widely used and reported significantly increased plant productivity (Wu & Zou 2009; Siddikee *et al.* 2011), but the avaibility of high quality inoculants is very limited due to the production of this fungi as biofertilizer. Up to now, biocontrol is carried out in open pot culture which is sensitive to contamination by other organism or soil borne plant pathogen. Therefore, the development of AMF inoculum production is needed in order to obtain high quality and purity of AMF inoculum. One approach to produce a high quality and purity of AMF inoculum is by using AMF spore associated with bacteria. The objective of this study was to acquire bacteria from surface-sterilized spores of AMF that showed antagonistic effects against fungal pathogens and yet compatible with AMF which potential to improve inoculum quality.

MATERIALS AND METHODS

Isolation and identification of bacteria from the AMF spores

The spores of AMF *Gigaspora* sp. and *Glomus* sp. originally from the collection of Silviculture Laboratory, Faculty of Forestry IPB were sieved by wet sieving according to method of Gardeman and Nicholson (1963). Hundred spores of each species were surface sterilized according to the method described by Budi *et al.* (1999a). The surface sterilized spores were crushed using sterile needles and transferred to Petri dishes containing sterile *Pseudomonas* agar base (Sigma-Aldrich) and Nutrient agar (Oxoid) and then incubated in the dark at 30°C for 24 h. The bacteria that grew were transferred to another Petri dishes containing the same media until single colonies were obtained. A single colony was then identified based on morphological methods using biochemical reaction developed by bioMerieux and carried out at Laboratory of Identification and Determination, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia.

Antagonistic effects of isolated bacteria towards soil borne plant pathogen *in vitro*

Isolated bacteria were tested toward soil borne plant pathogen *in vitro*. The common soil borne plant pathogens like *Slerotium* sp., *Rhizoctonia* sp. and *Ganoderma* sp. originally from the Laboratory of Pest and Diseases, Faculty of Forestry IPB, were used in this study. The test was carried out according to the method of Varese *et al.* (1996). The pathogens were grown on Petri dishes containing Potato Dextrose Agar (Difco) Media. Colony of bacteria were placed about 1.5 cm from the pathogen. The Petri dishes were then sealed with parafilm, incubated in the dark at 25°C for 7 days. The radial growth of mycelium were measured and compared to the control treatment. The experiment was carried out in a completely randomized design composed of 12 bacterial treatments and one control in triplicates for each pathogenic fungi. Data were analysed by one-way ANOVA.

Stimulation effect of bacteria on hyphal growth of AMF spores *in vitro*

The AMF *Gigaspora* sp. spores were collected and surface sterilized according to the method of Budi *et al.* (1999a). The spores were then placed in Petri dishes containing sterile zeolit medium supplemented with nutrient. Ten spores were put on sterile filter paper saturated with 50 l suspension of bacteria and were then placed on each Petri dish. The control sterile filter paper was saturated with sterile water. Petri dishes were then sealed with parafilm, incubated in the dark at 25°C for 21 days. The hyphal growth were recorded starting from germ tube and only the main hyphae was measured (Fig. 1) and compared to the control treatment. The experiment was performed in a completely randomized design and composed of 12 bacterial treatments and one control in triplicates. Data were analysed by one-way ANOVA.

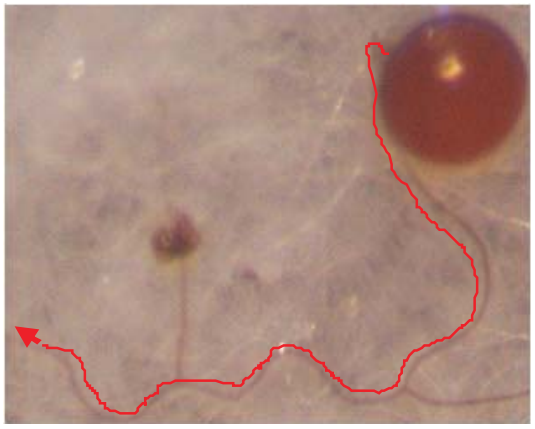

Figure 1. Hyphal growth measurement

Plate assay for enzymatic activities test

Bacterial isolates were grown in Erlenmeyer flasks for 24 h on a culture shaker at 30^0C in Nutrient Broth buffered medium. Ten micro liters of each bacterial suspension $\pm 10^8$ CFU/m/l was spotted onto plates containing the substrate of the enzyme to be tested and grown at 25^0C for 48 h. Cellulolytic activity was assessed as described by Teather and Wood (1982) using a solid medium, containing $MgSO_4 7H_2O$ (0.1 g/l), $CaCl_2 2H_2O$ (0.2 g/l), $FeSO_4 7H_2O$ (0.04 g/l), NaCl (0.2 g/l), KH_2PO4 (0.3 g/l), K_2HPO4 (0.5 g/l), CMC (carboxymethylcellulose) (Sigma) (5 g/l), yeast extract (0.1 g/l) and Bacto agar (15 g/l). For visualization of β-D-glucan hydrolysis, the agar medium containing CMC was flooded with an aqueous solution of congo red (1 mg/ml) for 15 min (Budi *et al.* 2000). The clear zones was measured after 48 h incubation. The proteolytic activity test was performed according to the method described by Dunne *et al.* (1997). The experiment was performed in a completely randomized design and composed of 12 bacterial treatments and one control with triplicates. Data were analysed by one-way ANOVA.

RESULTS AND DISCUSSION

Isolated bacteria from AMF spores

A total of 12 bacteria isolates were isolated and identified from surface sterilized spores of AM fungi. There were 7 isolates of bacteria isolated from *Gigaspora* sp. and 5 isolates isolated from *Glomus* sp. The morphological characteristics of each isolate of bacterial colony and its identification are shown in Table 1.

Table 1. Species identification and morphology characteristics of isolates of bacteria isolated from AMF spores of *Gigaspora* sp. and *Glomus* sp.

Source of isolate	Isolates Code	Morphological characteristics of bacterial colony (colour, colony surface and type)	Gram	Species name
Gigaspora sp.	GG1	Circular, entire, umbonate, cream, opaque	Positive	*Bacillus subtilis*
	GG2	Circular, undulated, umbonate, cream, opaque	Positive	*Bacillus licheniformis*
	GG3	Circular, serrate, raised, opaque	Positive	*Bacillus cereus*
	GG4	Circular, undulated, raised, surface dried, opaque	Positive	*Bacillus brevis*
	GG5	Circular, entire, convex, transparent, yellowish	Negative	*Pseudomonas diminuta*
	GG6	Circular, filamentous, raised, opaque	Positive	*Bacillus laterosporus*
	GG7	Circular, undulate, filamentous, raised, opaque	Positive	*Bacillus pasteurii*
Glomus sp.	GL1	Circular, entire, umbonate, translucent	Negative	*Bacillus circulans*
	GL2	Circular, entire, convex, translucent, yellow	Negative	*Proteus penneri*
	GL3	Circular, entire, convex, transparent	Negative	*Enterobacter hormaechei*
	GL4	Irregular, undulate, raised, transluent	Positive	*Bacillus firmus*
	GL5	Circular, serrate, raised, opaque,	Positive	*Bacillus cereus*

Antagonistic effects of isolated bacteria against fungal pathogen.

The results indicated that there are 2 bacterial isolates originated from *Gigaspora* sp. spores (*Bacillus subtilis* GG1 and *Pseudomonas diminuta* GG5) and 2 bacterial isolates from *Glomus* sp. spores (*Proteus penneri* GL2 and *Enterobacter hormaechei* GL3) have the ability to inhibit the growth of three pathogens tested. The percentages of inhibition

Table 2. Radial growth of pathogenic fungal mycelium *in vitro*

No	Isolates	Radial growth of mycelium (cm)* and % Inhibition					
		Sclerotium sp.	%	*Rhizoctonia* sp.	%	*Ganoderma* sp.	%
1	Control	9.00 a	0	9.00 a	0	9.00 a	0
2	GG1	1.70 d	81.11	4.17 d	53.66	1.04 c	88.44
3	GG2	9.00 a	0	9.00 a	0	9.00 a	0
4	GG3	9.00 a	0	9.00 a	0	9.00 a	0
5	GG4	9.00 a	0	9.00 a	0	9.00 a	0
6	GG5	1.88 d	79.11	4.35 d	51.66	1.12 c	87.55
7	GG6	9.00 a	0	9.00 a	0	9.00 a	0
8	GG7	9.00 a	0	9.00 a	0	9.00 a	0
9	GL1	9.00 a	0	4.65 b	48.33	9.00 a	0
10	GL2	2.99 c	66.77	4.16 d	53.77	0.92 d	89.77
11	GL3	3.86 b	57.11	4.86 c	46	3.68 b	59.11
12	GL4	9.00 a	0	9.00 a	0	9.00 a	0
13	GL5	9.00 a	0	9.00 a	0	9.00 a	0

*Values in a column followed by the same letter do not differ significantly from each other at *P* value of 0.05.

varied among pathogens tested. The bacterial isolates of *Bacillus subtilis* GG1 and *Pseudomonas diminuta* GG5 inhibited the growth of mycelium of *Sclerotium* sp. by 79-80%. While four isolates of bacteria (*Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5, *Proteus penneri* GL2, and *Enterobacter hormaechei* GL3) showed inhibitory effects to *Rhizoctonia* by 46-54%. Testing against *Ganoderma* sp. showed that bacterial isolates *Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5, *Proteus penneri* GL2 and *Enterobacter hormaechei* GL3 gave inhibitory effects of 59-90 %. (Table 2).

Stimulation effect of isolated bacteria on hyphal growth of AMF spores *Gigaspora* sp. *in vitro*

Among the 12 isolates of bacteria tested, one isolate (*Pseudomonas diminuta* GG5) had a significantly higher effect than the control, while 6 other isolates (*Bacillus subtilis* GG1, *Bacillus licheniformis* GG2, *Bacillus cereus* GG3, *Bacillus brevis* GG4, *Bacillus laterosporus* GG6 and *Enterobacter hormaechei* GL3) increased AMF hyphal development higher than the control treatment but were statistically not different. Of the remaining 5 isolates, 2 isolates significantly inhibited the AMF hyphal growth, i.e. *Bacillus pasteurii* GG7 and *Bacillus cereus* GL5 while the other 3 isolates inhibited the AMF hyphal growth but were not significally different from the control treatment (Table 3).

Enzymatic activity characterization of Bacteria

Ten out of the 12 isolates of bacteria produced cellulase activities, i.e. *Bacillus subtilis* GG1, *Bacillus licheniformis* GG2, *Bacillus cereus* GG3, *Pseudomonas diminuta* GG5, *Bacillus laterosporus* GG6, *Bacillus pasteurii* GG7, *Proteus penneri* GL2, *Enterobacter*

Table 3. Stimulation of AMF hyphae of *Gigaspora* sp. growth after 21 days incubation by bacteria

No	Isolates	Hyphal length (μm)*	% Increased/ decreased (-)
1	Control	202.44 bc	-
2	*Bacillus subtilis* GG1	286.31 ba	41.43
3	*Bacillus licheniformis* GG2	369.84 ba	82.69
4	*Bacillus cereus* GG3	231.79 bc	14.50
5	*Bacillus brevis* GG4	287.37 ba	41.95
6	*Pseudomonas diminuta* GG5	499.19 a	146.59
7	*Bacillus laterosporus* GG6	363.89 ba	79.75
8	*Bacillus pasteurii* GG7	19.62 e	-90.31
9	*Bacillus circulans* GL1	139.46 bcd	-130
10	*Proteus penneri* GL2	160.68 bcd	-20.62
11	*Enterobacter hormaechei* GL3	324.47 ba	60.28
12	*Bacillus firmus* GL4	181.46 bc	-11.56
13	*Bacillus cereus* GL5	156.38 bcd	-22.75

Note : * Values in a column followed by the same letter do not differ significantly from each other at *P* value of

Figure 2. Celullase activity (A) and Protease activity (B) as indicated by clear zones (halo)

hormaechei GL3, *Bacillus firmus* GL4, and *Bacillus cereus* GL5, while 8 isolates produced protease activities, i.e. *Bacillus subtilis* GG1, *Bacillus cereus* GG3, *Bacillus brevis* GG4, *Bacillus laterosporus* GG6, *Bacillus pasteurii* GG7, *Proteus penneri* GL2, *Bacillus firmus* GL4, and *Bacillus cereus* GL5. There were 7 isolates that produced both cellulase and protease activities, i.e. *Bacillus subtilis* GG1, *Bacillus cereus* GG3, *Bacillus laterosporus* GG6, *Bacillus pasteurii* GG7, *Proteus penneri* GL2, *Bacillus firmus* GL4 and *Bacillus cereus* GL5, as indicated by clear zone (halo) around the colony (Table 3, Fig. 2).

Bacteria have been observed to live in close association with AMF (Gopal *et al.* 2012), and have been isolated from different AMF spores (Xavier & Germida 2003; Roesti *et al.* 2005; Horii & Ishii 2006; Cruz *et al.* 2008; Bharadwaj *et al.* 2008;,

Table 4. Enzymatic activities of bacteria isolated from *Gigaspora* sp. and *Glomus* sp. as indicated by clear zones diameter after 48 h incubation

No	Bacteria Isolates	Enzymatic activities (Ø clear zones, cm)	
		Celluase	Protease
1	*Bacillus subtilis* GG1	4.80 cd	4.80 ba
2	*Bacillus licheniformis* GG2	2.83 e	0.00 c
3	*Bacillus cereus* GG3	5.70 b	6.50 a
4	*Bacillus brevis* GG4	0.00 f	5.77 a
5	*Pseudomonas diminuta* GG5	5.10 cb	0.00 c
6	*Bacillus laterosporus* GG6	5.87 b	6.20 a
7	*Bacillus pasteurii* GG7	7.17 a	5.83 a
8	*Bacillus circulans* GL1	0.00 f	0.00 c
9	*Proteus penneri* GL2	5.07 cb	4.73 ba
10	*Enterobacter hormaechei* GL3	2.97 e	0.00 c
11	*Bacillus firmus* GL4	3.23 e	3.73 b
12	*Bacillus cereus* GL5	4.10 d	4.73 ba

* Values in a column followed by the same letter do not differ significantly from each other at *P* value of ,0.05

Cruz & Ishii 2011). Cruz and Ishii (2011) found 2 bacteria *Bacillus* sp. and *B. thuringiensis* isolated from liquid present inside AMF spore of *Gigaspora margarita*, while Budi *et al.* (2012) found four bacteria *Bacillus* sp. *Bacillus megaterium, Bacillus subtilis* and *Bacillus flexus strain* KSC_SF9c isolated from surface sterilized AMF spores *G. margarita*. Xavier and Germida (2003) found 5 bacteria, *Alcaligenes*, *Bacillus* sp. *Burkholderia*, *Flavobacterium* and *Pseudomonas* sp. from surface sterilized AMF spores of *Glomus clarum*. This study demonstrated different bacteria species isolated from surface sterilized AMF spores of *Gigaspora* sp. and *Glomus* sp. We found 7 bacteria from *Gigaspora* sp. and 5 bacteria from *Glomus* sp. as shown in Table 1. As reported by Roesti *et al.* (2005), the bacterial community associated with the AMF spores was more influenced by the AMF identity (AMF species) than by the host plant. The difference in composition of the spore walls or exudates of AMF species may have played a major role in the selection of bacterial populations living on the spore (Roesti *et al.* 2005).

Bacteria associated with AMF spores have been reported to play an important role in antagonistic effect of soil borne plant pathogens, AMF spore germination and hyphal growth (Horii & Ishii 2006; Cruz & Ishii 2011). The interesting thing from this study was that among the 12 isolate bacteria tested, 3 bacterial isolates (*Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5 and *Enterobacter hormaechei* GL3) have the ability to inhibit the growth of three pathogens and stimulate hyphal growth of *Gigaspora* sp. (Table 2 and 3). This finding was in line with Cruz and Ishii (2011), who found that *Bacillus* sp., *B. thuringiensis* and *Paenibacillus rhizosphaerae* isolated from surface sterilized

AMF spores *Gigaspora margarita* had antagonistic effect to the pathogenic fungi *Rosellimia necatrix, Phytium ultimum, Fusarium oxysporum* and *Rhizoctonia solani* and stimulate hyphal growth of *G. margarita*. Many studies have reported that *B. subtilis* has the potential to be used as biocontrol agent against soil borne plant pathogens (Nalisha *et al.* 2006; Velmuragan *et al.* 2009). Phae *et al.* (1990) found that *B subtilis* produced *iturin*, a bioactive compound that are active as antifungal agents against several plants pathogenic fungi as well as antibacterial. The production of hydrolytic enzyme protease by *Paenibacillus* sp. isolated from mycorrhizosphere that caused cell wall degradation of pathogenic fungi have also been reported (Budi *et al.* 2000). In this experiment, *B. subtilis* also produced hydrolitic protease enzyme. We assume that combination of protease enzyme with the other active compounds produced by *B. subtilis* could inhibit pathogenic fungal growth.

Other bacteria such as *Pseudomonas* and *Enterobacter* have been reported effectively control diseases caused by soil borne plant pathogens (Thomashow *et al.* 1990; Chermin *et al.* 1995, Barea *et al.* 1998). These bacteria produced bioactive compound Phenazine-1-Carboxylic Acid responsible for suppression of soil borne plant pathogenic fungi (Thomashow *et al.* 1990). In this study only *Pseudomonas diminuta* GG5 produced hydrolytic protease enzyme but not *Enterobacter hormaechei* GL3, indicating that the role of these enzymes in controlling the growth of soil borne plant fungal pathogens is not clear and it seems that the inhibitory effect of these bacteria to soil borne plant fungal pathogen development is probably derived from more than one mechanism (Budi *et al.* 2000). Further investigation is necessary to isolate bioactive compound and its mode of action from these 3 bacteria isolates.

The stimulation of AMF hyphal growth by AMF spores associated with bacteria have been reported by several researchers (Budi *et al.* 1999b; Cruz & Ishii, 2011). In this study *Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5 and *Enterobacter hormaechei* GL3 have the ability to stimulate hyphal growth of *Gigaspora* sp. and supressed several soil borne fungal pathogens *in vitro*. Four others isolates only have the ability to stimulate hyphal growth of *Gigaspora* sp. *in vitro* (Table 3). The exact mechanism of hyphal growth stimulation by spores associoated with bacteria is not known, but Cruz and Ishii (2011) reported that bacteria released volatile compounds and exudates involved in stimulating hyphal growth. Futher study is needed to investigate the possible isolate volatile compounds and exudate released by these 3 bacteria isolates.

Certain Arbuscular Mycorrhizal Fungi hyphae produce hydrolytic enzymes which hydrolyses the biopolymers such as protein, chitin and cellulose that will help the AMF to degrade and infect the plant cell walls (Gopal *et al.* 2012). Furthermore, the hyphae and spores of AMF also harbour associated bacteria that produce hydrolytic enzyme (Budi *et al.* 1999b). In this experiment, 7 isolates produced both cellulase and protease activities, i.e. *Bacillus subtilis* GG1, *Bacillus cereus* GG3, *Bacillus laterosporus* GG6, *Bacillus pasteurii* GG7, *Proteus penneri* GL2, *Bacillus firmus* GL4 and *Bacillus cereus* GL5, as indicated by clear zone (halo) around the colony (Table 3, Fig. 2). As reported by Budi *et al.* (2012) *Bacillus subtilis* isolated from surface sterilized AMF spores *G. margarita* have shown to increase mycorrhizal root colonization of neem seedling in the nursery and also produced cellulase and protease activities.

Production of volatile compounds that can positively influence germination of AMF spores, provision of nitrogen through nitrogen fixation, solubilization of soil phosphate sources, detoxification of the fungal microhabitat, change of pH level of siderophores are also some of the proposed mechanisms underlying stimulation of mycorrhization (Bharadwaj *et al.* 2012). Further study is needed to test the consistency effects in the field, and to investigate the mechanisms involved in the antagonism towards fungal pathogens as well as stimulation effect to the hyphal growth of AM fungi.

CONCLUSIONS

There is a possibility that 3 isolate bacteria (*Bacillus subtilis* GG1, *Pseudomonas diminuta* GG5 and *Enterobacter hormaechei* GL3) isolated from surface sterilized spores of AM fungi *Gigaspora* sp. and *Glomus* sp. have the ability to inhibit the growth of 3 pathogens and stimulate hyphal growth of *Gigaspora* sp. *in vitro*. These bacteria have a great opportunity to be used as biocontrol agents for soil borne plant pathogens as well as for improving the AMF inoculum quality, since up to now the production of these fungi for biofertilizers and biocontrol is carried out in open pot culture sensitive to contamination by other organisms or pathogens.

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REFERENCES

Artursson V, Finlay RD, Jansson JK. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ Microbiol* 8: 1-10.

Bakhtiar Y, Yahya S, Sumaryono W, Sinaga MS, Budi SW, Tajudin T. 2010. Isolation and identification of Mycorrhizosphere bacteria and their antagonistic effects towards *Ganoderma boniense in vitro*. J Microbiol Indonesia 4 (2): 96-102.

Barea JM, Andrade G, Biancioto V, Dowling D, Lohkre S, Bonfante P, O'Gara F, Azcon-Aguilar C. 1998. Impact on arbuscular mycorrhiza formation on Pseudomonands strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Appl Environ Microbiol 64: 6.

Bharadwaj DP, Lundquist PO, Alstrom S. 2008. Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. Soil Biol Biochem 40: 2494-501.

Bharadwaj DP, Alstrom S, Lundquist PO. 2012. Interactions among *Glomus irregulare*, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro condition. Mycorrhiza 22: 437-47.

Budi SW, Blal B, Gianinazzi S. 1999a. Surface sterilization of *Glomus mosseae* sporocarpes for studying endomycorrhization *in vitro*. Mycorrhizae. 8: 15-18.

Budi SW, van Tuinen D, Negrel J, Martinotti G, Gianinazzi S. 1999b. Isolation from the mycorrhizosphere of *Sorghum bicolor* a bacterioum compatible with arbuscular mycorrhizal development and antagonistic towards soilborne fungal pathogens. Appl Environ Microbiol 65 (11): 5148 - 50.

Budi, SW, van Tuinen D, Arnould C, Dumas-Gaudot E, Gianinazzi-Pearson V, Gianinazzi S. 2000. Hydrolitic enzyme activity of *Paenibacillus sp.* strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Appl Soil Ecol 15: 191-9.

Budi SW, Bakhtiar Y, Nunang LM. 2012. Bacteria associated with Arbuscula Mycorrhizal Spores *Gigaspora margarita* and their potential for stimulating root Mycorrhizal colonization and Neem (*Melia azedarach* Linn) seedling growth. Microbiol Indonesia, 6(4): 157-65.

Chermin L, Ismailov Z, Haran S, Chet I. 1995. Chitinolytic Enterobacter agglomerans antagonistic to fungal plant pathogens. Appl Environ Microbiol 61:1720-6.

Cruz AF, Horii S, Ochiai S, Yasuda A, Ishii T. 2008. Isolation and analysis of bacteria associated with spores of *Gigaspora margarita*. J Appl Microbiol 104 :1711-7.

Cruz AF, Ishii T. 2011. Arbuscular mycorrhizal fungal spores host bacteria that affect nutrient biodynamics and biocontrol of soilborne plant pathogens. Biology Open 000 1-6.

Dunne C, Crowly JJ, Moenne-Loccoz Y, Dowling DN, de Bruijn FJ, O'Gara F. 1997. Biological control of *Pythium ultinum* by *Stenotromonas maltophilia* W18 is mediated by extra cellular proteolytic activity. Microbiology 143: 3921-39.

Gardemann JW, Nicholson TH. 1963. Spores of mycorrhizal Endogones species extracted from soil by wet-sieving and decanting. Trans Br Mycol Soc 46: 235-44.

Gopal S, Chandrasekaran M, Shagol C, Kim K, Sa T. 2012. Spore associated bacteria (SAB) of arbuscular mycorrhizal fungi (AMF) and Plant Growth Promothing Rhizobacteria (PGPR) increase nutrient uptake and plant growth under stress conditions. Korean J Soil Sci Fert 45(4): 582-92.

Horii S, Ishii T. 2006. Identification and function of *Gigaspora margarita* growth-promoting microorganisms. Symbiosis 41: 135-41.

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1(1): 48-58.

Ladha JK. 1992. Editorial comments. Plant and Soil, 141 pp.

Nalisha I, Muskhazali M, Nor Farizan T. 2006. Production of bioactive compounds by *Bacillus subtilis* against *Sclerotium rolfsii*. Malay Microbiol 2: 19-23.

Phae CG, Shoda M, Kubota H. 1990. Suppressive effect of *Bacillus subtilis* and its products on phytopathogenic microorganisms. J Ferment Bioeng 69 (1): 1-7.

Roesti D, Ineichen K, Braissant O, Redecker D, Wiemken A, Aragno M. 2005. Bacteria associated with spores of arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. Appl Environ Microbiol 71: 6673-9.

Siddikee MA, Chauhan PS, Anandham R, Gwang-Hyun Han, Sa TM. 2010. Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20(11): 1577-84.

Siddikee MA, Glick BR, Chauhan PS, Yim WY, Sa TM. 2011. Enhancement of growth and salt tolerance of red pepper seedling (*Capsicum annum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol Biochem 49: 427-34.

Theather RM, Wood JP. 1982. Use of Congo red polysacharide interaction in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43: 777-80.

Thomashow LS, Weller DM, Bonsall RF, Pierson S. 1990. Production of the antibiotic Phenazine-1-carboxylic acid by *Fluoresecent Pseudomonas* species in the rhizosphere of wheat. Appl Environ Microbiol 56(4): 908-12.

Varese GC, Portinaro S, Trotta A, Scannerini S, Luppi-Mosca AM, Martinotti MA. 1996. Bacteria associated with *Suillus grevillei* Sporocarps and Ectomycorrhizae and their effects on in vitro growth of the mycobion. Symbiosis 21: 129-47.

Velmurugan N, Choi MS, Han SS, Lee YS. 2009. Evaluation of antagonistic activities of *Bacillus subtilis* and *Bacillus licheniformis* against wood-staining fungi: *in vitro* and *in vivo* experiments. J Microbiol 47: 385-92.

Wu QS, Zou YN. 2009. Arbuscular mycorrhizal symbiosis improves growth and root nutrient status of citrus subjected to salt stress. *Science Asia* 35: 388-91.

Xavier LJC, Germida JJ. 2003. Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. Soil Biol Biochem 35: 471-8.