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Lactic Acid Bacteria in the Green Biocontrol against some Phytopathogenic Fungi: Treatment of Tomato Seeds

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ABSTRACT

Lactic acid bacteria (LAB) were evaluated against some plant-fungal pathogens with special reference to *Fusarium oxysporum* as *Fusarium* wilt is one of the major yield-limiting factors for growth of tomato plants. For an eco-friendly and sustainable management of such a disease, this study was conducted to evaluate seed treatment effects by culture broth of LAB for management of root-rot disease and plant-growth promoting activities in tomato plant. Initially, *in vitro* tests showed the potential effect and inhibition efficacy of LAB against growth of *Sclorotium rolfsii* and *Rhizoctonia solani*, whereas *Fusarium oxysporum* was the most resistant fungus. So, *F. oxysporum* was included in the *in vivo* experiments. Pot trial results verified a marked increase in root-, shoot-, and seedling lengths as well as vigor index, and plant weight and healthy. Protective effect of LAB for tomato plants significantly increased after challenging inoculation by *F. oxysporem*; the number of roots reached 57 and 64 (271 and 305 % compared to the control). Shoot lengths were 38.96 and 38.92 cm (221 and 216% of control), whereas the root lengths were 17.67 and 19.75 cm (151 and 169 % of control). Furthermore, height of the most vigor healthy plants reached 70 and 73 cm (292 and 304 % of control), whereas maximum total fresh weight were 33 and 37g (275 and 285 % of control). We have demonstrated the capability of LAB to act as plant-growth promoting bacteria and effective biocontrol agent against some phytopathogenic fungi under *in vitro* and *in vivo* tests.
KEY WORDS: Lactic Acid Bactria, Root, Shoot, Plant pathogens, Vigor index, Green biocontrol.

INTRODUCTION

Bacterial and fungal pathogens cause serious damage in agriculture, resulting in critical loss of crops yield and quality. Due to the serious effects of using chemical fungicides against phytopathogens as well as insecticides in plant treatment, biofertilizers are used as alternatives (Dhanya and Adeline 2014). Biofertilizers, used in biological control, are a natural and specific way to control pathogens and enhance crop yield by growth promoting advantages of environment friendly microorganisms. Based on the reduction of pathogenic activity, biological control has been developed successfully due to the natural presence of one or more organisms or their metabolites, through the management of plant-host interactions or antagonists (Dhanya and Adeline 2014). Currently, there is an ongoing rigorous research worldwide to explore a wide range of plant growth-promoting rhizobacteria (PGPR) possessing novel traits like heavy metal detoxifying potentials, pesticide degradation/tolerance, salinity tolerance, biological control of phytopathogens and insects, along with the normal plant growth promoting properties such as, phytohormone, siderophore, hydrogen cyanate (HCN), and ammonia production, as well as nitrogenase activity and phosphate solubilization (Sivasakthi *et al*. 2014). Mechanisms used by PGPR to promote plant growth can be classified in four groups (Noumavo *et al*. 2014, Munees and Kibret 2014): ***Biofertilizers*** (increasing the availability of nutrients to plant by solubilization of mineral phosphates, asymbiotic N2 fixation), ***Phytostimulators*** (promoting plant growth, generally through ability to produce phytohormones), ***Rhizoremediators*** (degrading organic pollutants) and ***Biopesticides*** (controlling diseases, mainly by production of siderophores, synthesis of antibiotics, enzymes, and antimicrobial metabolites).

Environmentally safe and ecofriendly methods of disease management are an urgent need. Biological control agents for sustainable agriculture are now used in many countries for combating diseases and increasing crop yield and seed quality. Soil application of biocontrol agent (PGPR) is not feasible, as large quantities are needed. Thus application of antagonist through seed treatment is a viable alternative for the introduction and establishment of a biocontrol agent (Negi *et al*. 2014). Biological seed treatment is usually very specialized and uses specific microorganisms that attack or interfere with specific pathogens or types of pathogens. The biological seed treatment involves the use of biological organisms to protect and control pathogens located in the seed or in soil. We

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introduced LAB for the first time as an efficient biocontrol agent, through a study for protection of tomato plants against some phytogenic fungi, to improve seed germination, shoot- and root-length as well as improving plant fresh weight and healthy (Hamed *et al*. 2011). Studies on LAB as a biocontrol agent against phytopathogenic fungi are, however, very limited (Asma *et al*. 2013, Limanska *et al* 2013, kang *et al*. 2014, Nebia *et al*. 2014, Skaidre *et al*. 2014). The purpose of this study was to: 1) estimate the influence of LAB against some plant-pathogenic fungi by *in vitro* tests; 2) evaluate the efficacy and potential of LAB for protection of tomato plants, under *in vivo* conditions against *F. oxysporum* in pot trials, using culture broth of LAB applied as seed treatment.

MATERIALS AND METHODS

Lactic acid bacteria

The strains of LAB, used in the present study, were isolated from yoghurt and milk [Yomna 2000]. These strains included: *Lactobacillus* sp. (LAB-1), *Lactobacillus acidophilus* (LAB-2), *Lactobacillus plantarum* NRRL B-4524 (LAB-3), and *Lactobacillus* sp. (LAB-4), *Lactobacillus* sp. (LAB-5). *Lactobacillus plantarum* NRRL B-4524 was from the National Center for Agricultural Utilization Research (USA). All strains were kept on MRS agar (van den Berg *et al.,*1993). Fresh cultures are grown in MRS broth at 30^{o}C for 24hrs before use in experiments (de Man *et al*. 1960).

Plant-pathogenic fungal strains

High virulent strains of pure pathogenic fungi, previously isolated from diseased plants, were used in this study. *F. oxysporum* (isolated from tomato), *R. solani* (isolated from tomato), and *S. rolfsii* (isolated from onion). Fungal strains were maintained at 4^{o}C on potato dextrose agar (PDA containing: 4.0 g of potato extract, equivalent to 200 g of infusion from potatoes; dextrose 20 g; and agar 15 g at final pH 5.6 ± 0.2, Murray *et al*. 1995).For preparation of spore suspension, fungal strains were grown in PDB at 28^{o}C. After incubation for 7 days, fungal biomass was homogenized in a blender for one minute. Spore suspensions were prepared using sterile distilled water to a concentration of 5x10^{5} spores. The spore suspension was prepared just before each experiment.

Determination of inhibitory activity

In vitro assays for antifungal activity by LAB were determined using conical flasks (250-ml) containing PDB as the growth medium for all test fungi. Flasks supplemented with LAB were inoculated with the test fungi and incubated at 28^{o}C for each of all fungal strains. After incubation period, 7days, fungal growth were filtered, washed several times with distilled water, and dried at 55^{o}C to a constant weight. Percentage of growth inhibition (GI) was calculated using the formula: GI (%) = $C_0 - C_F$ / C_o x 100%, where C_0 is the dry weight of fungal mycelium (control), C_F is the dry weight of fungal mycelium after inhibition by LAB.

Seed treatment and Preparation of Tomato seedlings

Tomato seeds (UC 97, 30 seeds) were soaked in culture broth of LAB strains for 30 min (0.5-ml of 1x10^{6}/ml, for one seed). Seeds were washed 3-times with water and air dried. Peat moss soil was dispensed into plastic trays (160 eyes) for growth of tomato seedlings. Moisture content of peat moss was sustained at a proper level throughout seedlings growth. After 45 days, vigorous healthy seedlings were transplanted for pot trials. Experiments and controls were performed in triplicate and the mean of all treatments was calculated.

Pot trials

Vigorous healthy seedlings were placed in pots, 30 cm diameter, filled with unsterilized natural soil. The pots were un-inoculated or inoculated with *F. oxysporum*, at a rate of 10-ml homogenized culture per pot, prepared one day before planting, without supplementation of soil with LAB (seed treatment). Tomato seedlings were drenching at intervals and left to grow for another 30 days. *In vivo* tests were performed during 3-months, from April up to June, where the atmospheric temperature ranged from 25 - 38^{o}C. Pot trials were performed in the National Research Center, Giza, Egypt.

Sampling and analysis

After 75 days of growth, tomato plants were harvested. To reveal the effect of LAB on the growth characteristics, each plant was measured for shoot- and root-length, number of secondary roots, and total fresh weight of plants. The germination percent and seedling Vigor Index were calculated: Germination % = Number of germinated seeds / Number of total seeds x 100 %.

Normal seedlings were evaluated for seedling vigor index. The root- and shoot-length of normal seedlings were measured and seedling vigor index was calculated by using the following formula: Seedling vigor index = [Mean root length (cm) + mean shoot length (cm)] × germination percentage (Ashwini and Giri 2014)

Statistical analysis

Data were analyzed using SPSS for windows (SPSS Inc.) by means of a one-way ANOVA and subsequently differences between treatments were determined using least significant differences (LSD) at $P \leq 0.05$ level.

RESULTS

***In vitro* tests**

Inhibition effects of *in vitro* tests by LAB against the pathogenic fungi differed widely. Low inhibition effects were observed by all the strains against *F. oxysporum* (Fig. 1). The inhibition effects against *F. oxysporum* were 17, 16, 13, 19, and 15 % by LAB-1 to LAB-5, respectively. On other hand, highly inhibition effects were observed against *R. solani* and *S. rolfsii* by LAB-2, LAB-3, and LAB-4. Inhibited effects against *R. solani* were 81, 75, 72%, whereas *S. rolfsii* was inhibited by 91, 88, and 84%, respectively (Fig. 1). Thus, *in vitro* tests revealed that LAB-1 and LAB-5 showed the lowest inhibition effects against the tested pathogens, while the fungal strains, *R. solani* and *S. rolfsii* were the most inhibited pathogens by LAB-2, LAB-3 and LAB-4. In addition, *F. oxysporum* was the most resistant fungus to all the strains of LAB under *in vitro* tests, therefore, it was chosen for all *in vivo* pot trials.

***In vivo* Tests**

Plant growth-promoting properties by LAB indicated vigorous seedlings growth of tomato seeds, pre-treated with LAB. Enhanced growth of tomato seedlings was observed in early stages of growth when compared with control (Fig. 2). Moreover, in absence of *F. oxysporum*, results of pot trials with LAB, applied as seed treatment, revealed their ability to enhance plant growth compared with controls (Fig. 3). However, plant growth-promoting properties and antifungal activities by LAB in presence of *F. oxysporum* were more effective. Results of pot trials with LAB revealed their ability to enhance plant growth, especially after challenging with *F. oxysporum*, compared with controls (Fig. 4 and Fig. 5).

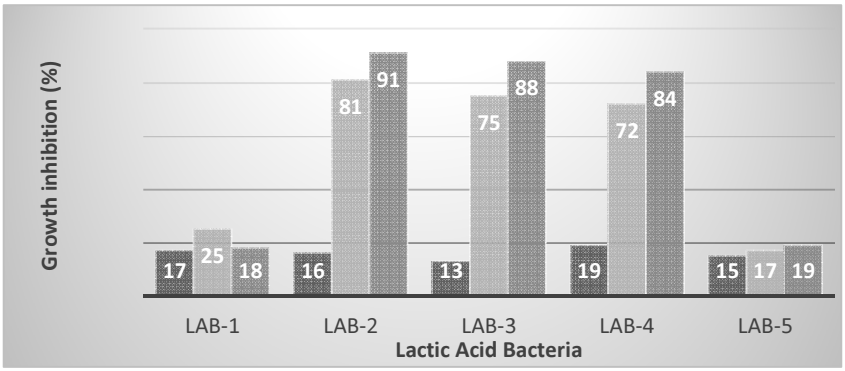

Fig. 1. The percentage of growth inhibition by lactic acid bacterial strains against phytopathogenic fungi: (■) *Fusarium oxysporum,* (■) *Rhizoctonia solani,* and (■) *Sclorotium rolfsii*. (■)

Fig. 2. Growth-promoting properties by lactic acid bacteria from seeds pre-soaked with LAB, in soil infested with *Fusarium oxysporum*. Growth of tomato seedlings, in early stages (*left image*) and, fruiting formation(*right image*) of tomato plant after 75 days.

Fig. 3. Growth-promoting properties by LAB-5, after 75 days. *Left image*: growth of tomato seedlings by seeds non-soaked with LAB in soil non-infested with *Fusarium oxysporum*, and *Right image*: seedlings of pre-soaked seeds with LAB in soil non-infested with *Fusarium oxysporum*.

Fig. 4. Growth-promoting properties and antifungal activity by LAB-1 against *F. oxysporum*, after 75 days. *Left image*: growth of tomato seedlings from seeds non-soaked with LAB in soil infested with *F. oxysporum*, and *Right image*: seedlings of pre-soaked seeds with LAB, after challenging with *F. oxysporum*.

Fig. 5. Growth-promoting properties and antifungal activity by LAB-5 against *F. oxysporum*, after 75 days. *Left image*: growth of tomato seedlings from seeds non-soaked with LAB in soil infested with *Fusarium oxysporum*, and *Right image*: seedlings of pre-soaked seeds with LAB, after challenging with *Fusarium oxysporum*.

Plant Measurements and Vigor index

Plant-growth characteristics significantly differed with LAB strains in soil infested with *F. oxysporum.* Shoot length, root length, number of roots as well as germination rate and vigorus index significantly differed among LAB. Number of roots (57 and 64) and seed germination (100%) were obtained with LAB-1 and LAB-5 (Table 1). Furthermore, maximum values of shoot length were 37.96and 38.92 cm with LAB-1 and LAB-5, respectively, whereas values of root length reached 17.67 and 19.75 cm withLAB-1 and LAB-5, respectively in comparison with the less values of LAB-2, LAB-3, LAB-4, and controls (Table 1). On other hand, values of seedling vigor index reached 5563 and 5867 with LAB-1 and LAB-5 followed by 3177, 3320, and 3780 for LAB-2, LAB-3, and LAB-4, respectively (Fig. 6).

Table 1. Number of secondary roots per root system, and mean shoot- and root-length (cm)of tomato plants treated with lactic acid bacteria, in soil infested with *Fusarium oxysporum*

LAB	Number of roots	Shoot length (cm)	Root length (cm)	Germenation (%)
Control*	21	17.60[b]	11.67[ab]	43
LAB-1	**57**	**37.96[a]**	**17.67[b]**	**100**
LAB-2	41	26.42[c]	15.00[c]	73
LAB-3	38	24.32[d]	15.29[c]	67
LAB-4	35	26.79[c]	14.67[c]	83
LAB-5	**64**	**38.92[a]**	**19.75[a]**	**100**

***Control for seedlings from seeds non-soaked in LAB and infested with *Fusarium oxysporum.*
Germination %: >90 = very good, 80-90% = good, 70-80% = moderate, 70% = poor

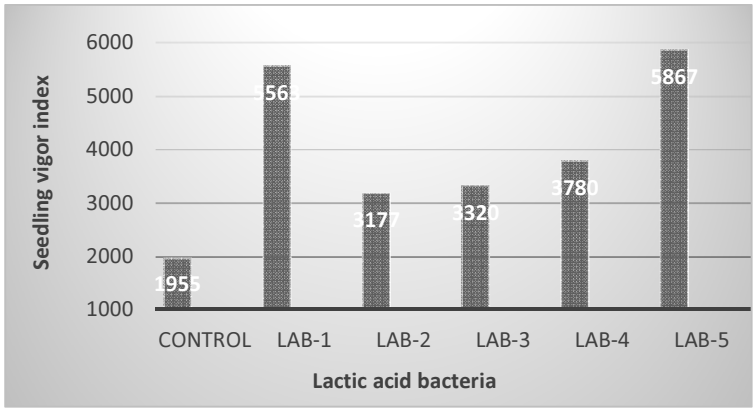

Fig. 6. *In vivo* efficacy of LAB for protection of tomato plants against *F. oxysporum* through the vigor index.

Plant Healthy and Fresh Weight

Maximum of plant injury severity was observed with seeds non-soaked with LAB in soil infested with *F. oxysporum* (Table 2). Height of the most vigor healthy plants were 70 and 73 cm (292 and 304 % compared to the control) with LAB-1 and LAB-5, respectively, whereas maximum total fresh weight were 33 and 37g with LAB-1 and LAB-5, respectively. LAB-2, LAB-3, LAB-4, showed 44, 42, and 53 cm as plant height and 15, 24, 28g as total fresh weight, respectively, in comparison with LAB-1 and LAB-5 (Table 2).

Table 2. Plant growth-promoting and antifungal activity by lactic acid bacteria strains

Treatment	Plant injury severity[c]	Plant height (cm) [d]	Height (% of control)	Total Fresh Weight (g) [d]
Plant[a]	++	26	-	19
Plant + F[b] (control)	+++++	24	-	12
Plant + F + LAB-1	-	**70**	**292**	**33**
Plant[a]	++	27	-	10
Plant + F[b](control)	+++++	20	-	7
Plant + F + LAB-2	+	44	220	15
Plant[a]	++	24	-	11
Plant + F[b] (control)	+++++	19	-	7
Plant + F + LAB-3	+	42	221	24
Plant[a]	++	31	-	8
Plant + F[b] (control)	++++	20	-	6
Plant + F + LAB-4	-	**53**	**265**	**28**
Plant[a]	++	28	-	22
Plant + F[b](control)	+++++	24	-	13
Plant + F + LAB-5	-	**73**	**304**	**37**

[a]Tomato seedlings from seeds treated with LAB in soil non-infested with *Fusarium oxysporum.*
[b]Tomato seedlings from seeds not treated with LAB in soil infested with *Fusarium oxysporum.*
[c] Severity: +, slight; ++, moderate; ++++, high; +++++, strong; -, no severity.
[d]From three plant replicates, the best was chosen.

Thus, in absence of *F. oxysporum*, results of pot trials with LAB revealed their ability to enhance plant growth when compared with controls (Fig. 2 and Fig. 3). On other hand, however, plant growth characteristics were significantly increased over control after challenging with *F. oxysporum*. Obvious increment is occurred in shoot and root lengths, number of roots, plant height as well as seedling vigorous index and total fresh weight (Table 1, Table 2, and Figs. 4, 5, and 6).

DISCUSSION

Plant rhizosphere is known to be preferred ecological niche for various types of soil microorganisms due to the rich nutrient availability. Some groups of beneficial bacteria can aggressively colonize the roots and protect the plants against soil-borne diseases whose most of which are caused by fungi. These beneficial bacteria are identified as plant growth promoting rhizobacteria (PGPR). Microbial activities and beneficial effects in the rhizosphere enriched the root area and supply available nutrients to plants, thereby modifying the quality and quantity of root exudates and plants as well. Organic molecules around the roots are further metabolized by PGPR and other microorganisms as carbon and nitrogen sources, and some microbe-oriented molecules are subsequently re-taken up by plants for healthy growth and development. Root exudates promote the plant-beneficial symbiotic interactions with PGPR and inhibit growth of the competing plant-pathogenic species **(**Rana *et al*. 2014**)**. Being one of the most industrially important strains, LAB was thought to be used as a biocontrol agent. We introduced LAB for the first time in biocontrol and verified its potential and efficacy as a biocontrol agent for protection of tomato plants (Hamed *et al*. 2011). Efficacy of LAB as PGPR on growth of tomato greatly enhanced overall plant growth over control. In the present study, culture broth of LAB was used as seeds pretreatment for protection of tomato plants against some phytopathogic fungi.

A comparison between efficiency of LAB with some other beneficial PGPR is represented in Table 3. Hameeda *et al*. (2008) reported that, seed germination by *E. asburaie* PS13 and *S. marcescens* EB reached 90 and 94% with a vigor index of 5021 and 5787, respectively, while *Azospirillum lipoferum* was reported to enhance seed germination by 98 and a vigor index of 2365 (Noumavo *et al*, 2013). Damodaran *et al*. (2014) reported that, germination of seeds by *B. pumilus* CRS-B-2 was 95 % with a vigor index of 4566. Seed germination by *Trichoderma viride* reached 71% with a vigor index of 1466 (Ashawini and Giri 2014), whereas a combination of *Trichodermaharzianum* and *P. fluorescens* increased the seed germination to 94% and vigor index to 5707 (Negi *et al*. 2014). Seed germination by LABI and LABII were 78 and 80 % with a vigor index of 1113 and 1130, respectively (Murthy *et al*. 2012).In the present study, however, LAB-1 and LAB-5 showed maximum values of 100% seed germination and a vigor index of 5563 and 5867, respectively. Moreover, the plant height reached 70 and 73 cm with total fresh weight 33 and 37 g by LAB-1 and LAB-5, respectively. Our results revealed capability of LAB to be considered as PGPB.

Table 3. A comparison between the efficiency of LAB with different bacteria in plant-growth promotion

Strain	Shoot length (cm)	Root length (cm)	GP*	Fresh weight (g)	Vigor Index	Plant Height	Reference
***E. asburaie* PS13**	22	33	**90**	1.96	**5021**	-	Hameeda *et al*. 2008
***S. marcescens* EB**	27	34	**94**	2.16	**5787**		Hameeda *et al*. 2008
Strain UPMR48	5.66	5.66	81	-	951	-	Mia and Shamsuddin 2009
LABI**	5.76	8.47	78	-	1113	-	Murthy *et al*. 2012
LAB II	8.83	5.80	80	-	1130	-	Murthy *et al*. 2012
Azospirillum lipoferum	7.34	16.79	**98**	-	**2365**	-	Noumavo *et al*. 2013
***B. pumilus* CRS-B-2**	35.13	7.63	**95**	-	**4566**	-	Damodaran *et al*. 2014
Trichoderma harzianum* + *P. Fluorescens	28.50	16.40	**94**	-	**5707**	45	Negi *et al*. 2014
Strain AMET9345+BG	9.50	3.80	100	0.2	1330	-	Jayaprakashvel *et al*. 2014
***B. subtilis* BCA-6**	9.25	3.94	95	-	1258		Bellishree *et al*. 2014
***B. pumilus* BCA-19**	7.23	3.15	82	-	855	-	Bellishree *et al*. 2014
***B.megaterium* BCA-5**	8.31	3.45	87	-	1025	-	Bellishree *et al*. 2014
***C. oceanosedimentum* CEG**	11	20	-	0.45	2666	-	Audipudi *et al*. 2014
Trichoderma viride	11.5	9.15	71	-	1466	-	Ashwini and Giri 2014
LAB-1	**37.96**	**17.67**	**100**	**33**	**5563**	**70**	The present study
LAB-4	**26.79**	**14.67**	**83**	**28**	**3780**	**53**	The present study
LAB-5	**38.92**	**19.75**	**100**	**37**	**5867**	**73**	The present study

*Germination Percentage. Note: germinating duration differed from hours to a maximum of 75 days.
**LAB = Lactic acid bacteria

Plant growth promoting rhizobacteria are characterized by specific properties; they should: 1) be proficient to colonize the root surface; and 2) survive, multiply and compete with other microbiota, at least for the time needed to express their plant growth promotion/protection activities; 3) maintain viability for long periods; 4) existing and competing around or inside the root against pathogens; and 5) promote plant growth, vigorous, and healthy (Stephane *et al.,* 2005). In addition, a single PGPR will often reveal multiple modes of action (Munees and Kibret 2014). In our studies, LAB may exerted beneficial effects upon plant growth in terms of increased root and shoot growth, plant height, germination percentage as well as increased plant total fresh weight and vigor index (Table 1 and Table 2). In comparison with other potent bacteria such as *Trichoderma*, *Pseudomonas*, *Bacillus*, and *Serratia*, LAB in our studies are considered a potent PGPR (Table 3). Worthy mention is that, LB-1 and LB-5 showed higher antifungal activity under *in vivo* tests, which is contrary to their results of *in vitro* tests. Actually, *in vivo* tests ensure the efficacy of LAB as a biocontrol agent against phytopathogenic fungi, indicating that *in vitro* assays are not fully predictive for the inhibitory action confirmed under *in vivo* tests against a pathogen (Faina *et al.,* 2007). The antifungal effect of LAB under *in vivo* conditions may lead us for thinking about the efficacy of LAB as root colonizer. We can't exclude the possibility that on roots, LAB could attack, colonize, and reduce fungal growth much faster than under *in vitro* tests. On other hand, LAB are used for preservation of food and milk products from centuries and acquired the GRAS status. Thus, LAB and their metabolic products can be safely used in biocontrol of plant pathogenic fungi.

Application of PGPR has been, however, hampered by inconsistent performance in field tests; this is usually attributed to their poor rhizosphere competence. Rhizosphere competence of biological control agents comprises effective root colonization combined with the ability to survive and proliferate along growing plant roots over a considerable time period in presence of the indigenous microflora (Stephane *et al.,* 2005).Currently, there is an ongoing rigorous research worldwide to explore a wide range of rhizobacteria possessing novel traits like heavy metal detoxifying potentials, pesticide degradation/tolerance, salinity tolerance, biological control of phytopathogens and insects. Rhizobacteria should also exhibit normal plant growth-promoting properties such as phytohormone, siderophore, hydrogen cyanate (HCN), and ammonia production, as well as nitrogenase activity and phosphate solubilization (Nebia *et al*. 2014, Sivasakthi *et al*. 2014). Use of LAB as biocontrol agent against phytopathogenic fungi presents both challenges and opportunities for management of plant diseases. The success of biological control in our research is surprising compare with the control. LAB may produce a variety of antifungal substances under *in vitro* tests, but under *in vivo* tests the mechanism of antifungal action is difficult to elucidate due to the complex and commonly synergistic interactions between different compounds and different soil microbiota (Naseby *et al.,* 2000). It could also be suggested that, a synergistic effect of LAB with other beneficial microorganisms in soil may provide an almost constant nutrient source for the plants. In the present study, *in vivo* tests were performed during 3-months, from April up to June, where the atmospheric temperature ranged from 25 - 38^{o}C. LAB thus, compete agent against *F.oxysporum* despite of stress conditions such as fluctuation in temperature, relative humidity, and a greater variety of competitive microorganisms. Moreover, bioprotection of tomato seeds with LAB support plant growth without plant severity despite of long duration (75 days of seed treatment). LAB may enriched the rhizosphere area with nutrient availability and subsequently encourage growth of other beneficial microorganisms. Furthermore, obvious elongation in both shoot- and root-length and the increasing number of secondary roots as well as the increment in total fresh weight of tomato plants may confirm capability of LAB to trigger the induced systematic resistance of tomato plants. Triggering the induced systematic resistance is well known to enhance production of growth regulators, stimulants, or plant hormones, by which elongation of plant, increment of total fresh weight and vigor index are occurred.

Conclusion

Several PGPR and their metabolites have been reported to give a good biocontrol effect against phytopathogens in various crops, and their use is increasingly applied worldwide. Such PGPR and their metabolites promote plant vigor and productivity. In the present research, however, LAB are proved to be a potent plant growth-promoting agent in comparison with other PGPR. We demonstrated for the first time that LAB are potent biocontrol agents; soil infested with *Fusarium* reduced root-, shoot-, and plant lengths as well as total fresh weight and vigor index. Bioprotection of tomato plants by LAB isolated from yoghurt and milk is a finding. Under *in vitro* tests, even in a medium suitable for *Fusarium*, LAB-2, 3, and 4 persist, compete, and inhibit the fungal growth. In *in vivo* experiments, LAB increased seedlings germination, plant healthy, fresh weight, and vigor index despite of one application as seed treatment; that is, no additional requirements for soil drench with culture broth of LAB was needed although a long period of cultivation (75 days). In addition, LAB-1, 4, and 5 were verified to persist in soil under hard conditions; LAB seem to be more resistant to stress conditions such as fluctuation in temperature and

relative humidity. Future research should confirm assays for lytic enzymes, antibiotics, production of plant hormones, and other plant-growth activities by LAB for management of plant diseases.

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