J Appl Biol Chem (2014) 57(2), 153–157
http://dx.doi.org/10.3839/jabc.2014.023

Online ISSN 2234-7941
Print ISSN 1976-0442

Original Article: Bioactive Materials

Effects of Inoculation of Rhizomicrobial Strains on Plant Growth at the Early Germination Stage

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Received: 21 November 2013 / Accepted: 27 November 2013 / Published Online: 30 June 2014

Abstract Plant-growth-promoting rhizobacteria can affect plant growth by various direct and indirect mechanisms. This study was conducted to determine the ability of some rhizobacterial strains to enhance the seed germination of *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish). Seeds were inoculated using a spore suspension (1×10^7 cfu mL^{-1}) and incubated in a growth chamber at 28°C under dark conditions and 65% RH. *Azotobacter chroococcum* and LAP mix inoculation increased the plumule length of *L. sativa* by 1.3, 0.8, and 0.7 cm, respectively, in comparison to the uninoculated control. *Pseudomonas putida* showed an increase of only 0.6 cm in plumule length when compared to the control. Inoculation of *A. chroococcum*, *P. putida*, and LAP mix enhanced the seed germination rate of *R. sativus*, by 10, 5, and 30%, respectively, in comparison with the uninoculated seeds. The results demonstrated that the inoculation of seeds by select rhizobacterial strains showed remarkable enhancement to the radicle length of lettuce and radish seedlings.

Keywords PGPR · Plumule length · Radicle length · Rhizobacteria

Introduction

The application of massive amounts of chemical fertilizers and fungicides has obstructed the development of sustainable agriculture. Free-living soil bacteria that are beneficial to plant growth, usually referred to as plant-growth-promoting rhizobacteria (PGPR), are capable of promoting plant growth by colonizing the roots. Wu et al. (2005) were among many researchers who have clearly demonstrated the effects of PGPR strains on different crops. An improvement in seed germination in a variety of plants by treatment with rhizobacteria has been reported. These plants include sorghum (Raju et al., 1999), pearl millet (Niranjan-Raj et al., 2004), as well as wheat and sunflower (Shaukat et al., 2006).

Rhizobacteria that possess some direct mechanism or capacity to promote plant growth are promising alternatives to chemical usage and helpful in maintaining a cleaner ecosystem. Most of the interest has been focused on non-symbiotic rhizobacterial strains, particularly *Pseudomonas* and *Bacillus* species. However, free-living bacteria that have unique growth-enhancing effects on host plants need to be studied further (Ping and Boland, 2004; Bais et al., 2006). Bacterial inoculants enhance plant growth, germination, and seedling emergence (Lugtenberg et al., 2002).

Increasing the synthesis of plant hormones such as indole acetic acid (IAA) and gibberellins is an important factor that triggers the activity of specific enzymes that promote seed germination and growth (Bharathi et al., 2004). The free-living, heterotropic *Azotobacter* helps in nitrogen fixation and thus enhances the seedling's progress. Genetic improvement of PGPR strains, in order to enhance their colonization and efficacy, may result in one or more factors related to plant growth promotion (Glick 1995; Lubeck et al., 2000; Bloemberg and Lugtenberg, 2001).

Biofertilizers are composed of effective microorganisms that colonize the rhizosphere and promote growth by increasing the availability of primary nutrients to the host plant when applied to seed, soil, or even plant surface (Vessey, 2003). Furthermore, the use of co-inoculation of PGPR, with a greater understanding of their functions, is of interest as these formulations may increase uniformity in the field (Jetiyanon and Kloepper, 2002; Siddiqui and Shauka, 2002). Rhizosphere microorganisms tend to produce large quantities of growth-promoting substances that affect, either directly or indirectly, the plant morphology and yield. Growth regulators control growth by influencing the physiological and morphological processes at trace concentrations (Arshad and Frankenberger, 1998).

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IAA can be biosynthesized by bacteria through a tryptophan-dependent pathway and the formation of indole pyruvic acid. PGPR can promote plant growth directly, through the synthesis of beneficial compounds, or indirectly, when controlling phytopathogens (Zhender et al., 1997; Medeiros et al., 2005). Many PGPR strains can act as inducers of ISR (Kloepper et al., 1992), and PGPR-mediated ISR may be promising alternatives to the use of chemicals or pathogens for inducing SAR. PGPR strains offer an attractive way to replace the use of chemical fertilizers, pesticides, and other supplements; therefore, they have gained worldwide importance and acceptance of their agricultural benefits.

The purpose of the present study was to evaluate the capability of select rhizobacterial strains to accelerate the early seed germination of *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish).

Materials and Methods

Microorganisms and inoculum preparation. Bacteria that were effective producers of IAA were previously isolated from *Panax schinsen* rhizospheres in a Korean ginseng farm in Chuncheon city, Gangwon-do, South Korea. These bacteria possess plant-growth-promoting (PGP) features, such as improving early seed germination (Bacilio et al., 2004). The strains were subcultured in tryptic soy agar of pH 7.2. One milliliter of the freshly grown spores (1×10^6 cfu mL^{-1}) was transferred to 250 mL of new TSB supplemented with 5% NaCl and incubated at 30°C for 48 h.

Inoculation of seeds. The bacterial spore suspension was adjusted to 1×10^7 cfu mL^{-1} for each strain by using a UV-VIS spectrophotometer (AvaSpec-ULS3648, Avantes, USA) at 600 nm. Seeds were agitated at 150 rpm with their corresponding spore suspensions for 2 h at room temperature. The seeds were then removed from the spore suspension and placed on plates with 10 seeds/plate. The seeds were placed on a sterile filter, Whatman No. 1, which was humidified by 5 mL of sterile distilled water. The experiment was conducted in triplicate. All petri dishes were incubated at 28°C, under dark conditions, in a plant growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Korea). The progress of germination was examined daily for four days. The number of germinated seeds was recorded every 24 h.

Statistical analysis. Analysis of variance (ANOVA) of the data was performed. The significance was tested at a 5% level by using least significant difference (LSD) with the SAS program version 9.1 (SAS, 2009).

Results

The highest rate of germination of *L. sativa* seeds after 24 h was shown by *Lactobacillus* sp. and LAP mix, which showed a 35% increase in seed germination after 24 h compared to the control.

Table 1 The effect of PGPR on the seed germination, root length, and plumule length of lettuce, *Lactuca sativa*

Inoculation	Germination (%)	Radicle length (cm)	Plumule length (cm)
	24 h	96 h	96 h
Uninoculated	50±1.41b	2.68±0.75de	1.71±0.5c
A. chroococcum	60±0ab	3.94±0.53a	2.57±0.19ab
B. subtilis	50±1.41b	2.29±0.8e	2.29±0.07b
Lactobacillus sp.	85±0.71a	3.33±0.47bc	3.04±0.59a
P. putida	50±0b	2.94±0.36cd	2.19±0.58bc
LAP mix	85±0.71a	3.59±0.37ab	2.41±0.27b

Table 2 The effect of PGPR on the seed germination, root length, and plumule length of radish, *Raphanus sativus*

Inoculation	Germination (%)	Radicle length (cm)	Plumule length (cm)
	24 h	96 h	96 h
Uninoculated	25±0.5ab	1.93±1.93c	2.36±1.69bc
A. chroococcum	35±0.5ab	5.05±5.05ab	4.30±0.97ab
B. subtilis	5±0.5b	1.50±1.5c	1.92±1.97c
Lactobacillus sp.	25±0.71a	3.22±3.23b	4.39±0.67ab
P. putida	30±0.5ab	2.37±2.38c	3.97±1.56ab
LAP mix	55±0.5ab	5.47±5.48a	4.61±0.82a

A. chroococcum showed only a 10% increase in the seed germination relative to the control, while *P. putida* did not show any enhancement in seed germination. *B. subtilis* showed the worst results during the trial (Table 1). The radicle length increased by 1.3 cm relative to the control, with the help of *A. chroococcum* inoculation. LAP mix enhanced the radicle length by 1.0 cm, while *Lactobacillus* sp. showed a lesser increase in the length, with an increase of 0.7 cm more than the uninoculated seeds. Similar results were demonstrated for the plumule length of *L. sativa* seedlings, in which *Lactobacillus* sp., *A. chroococcum*, and LAP mix inoculation increased the plumule length by 1.3, 0.8, and 0.7 cm, respectively. *P. putida* showed an increase of 0.6 cm in plumule length compared to the control (Table 1).

Inoculation of *A. chroococcum*, *P. putida*, and LAP mix enhanced the seed germination rate of *R. sativus* by 10, 5, and 30%, respectively, in comparison with the uninoculated seeds. However, *Lactobacillus* sp. did not enhance the seed germination. The radicle length of *R. sativus* seedlings was enhanced with the help of *A. chroococcum* and LAP mix inoculation by 3.0 and 3.5 cm, respectively. *Lactobacillus* sp. inoculation increased the radicle length by 1.3 cm compared to the uninoculated. However, *P. putida* showed a lower result (Table 2). *A. chroococcum* and LAP mix inoculation increased the plumule length of *R. sativus* by 2.0 and 2.3 cm, respectively. *Lactobacillus* sp. and *P. putida* inoculation increased the plumule length of *R. sativus* by 2.0 and 1.5 cm, respectively. However, *B. subtilis* decreased the plumule length by 0.4 cm compared to the uninoculated control (Table 2).

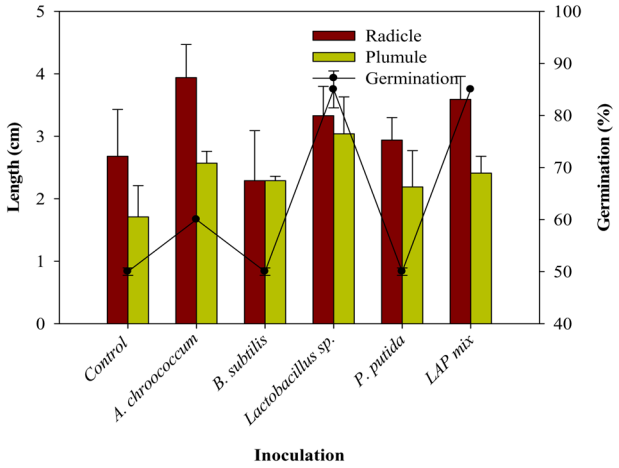

Fig. 1 The effect of different inoculations on the length of the radicle and plumule, as well as the seed germination percentage, of lettuce, *Lactuca sativa*, after 96 h.

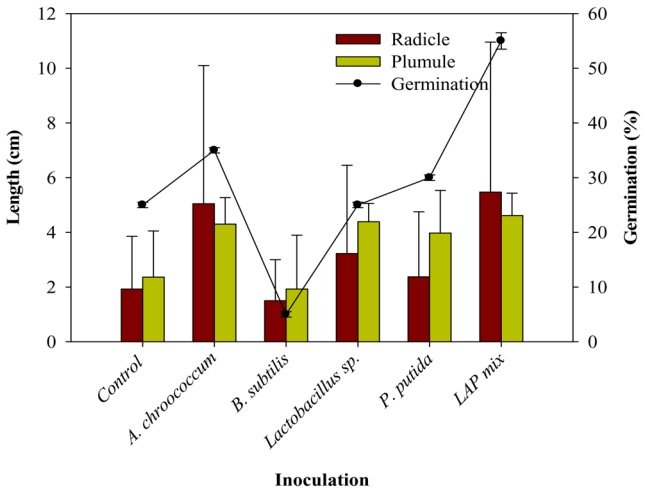

Fig. 2 The effect of different inoculations on the length of the radicle and plumule, as well as the seed germination percentage of radishes, *Raphanus sativus*, after 96 h.

Discussion

The rhizosphere is the part of the soil surrounding the root in which essential and intensive interactions take place between the plant, soil, and microfauna (Nihorimbere et al., 2011). The rhizosphere is a dynamic system controlled by the combined effects of soil properties, plants characteristics, and the interactions of plant roots with microorganisms and the surrounding soil (Bowen and Rovira, 1999). Rhizobacteria are those bacteria that, when introduced into a soil containing competitive microflora by plant inoculation, show a beneficial effect on plant growth and are termed PGPR which have representatives from very broad range of bacterial taxa (Vessey, 2003; Lucy et al., 2004).

Rhizobacteria can increase nutrient supply by the production of siderophores and enzymatic nutrient mobilization from organic matter (Anderson et al., 1993; Whiting et al., 2001; Jing et al., 2007). Most living species possess complicated mechanisms to control their need of iron, since iron is an essential element for almost all organisms; however, an overload of this element results in toxicity due to the formation of oxidant species.

IAA produced by bacteria enhances plant growth by increasing the number of root hairs and lateral roots (Okon and Kapulnik, 1986). IAA is biosynthesized by rhizosphere microbes due to tryptophan from root exudates or decaying cells (Benizri et al., 1998). The osmoprotectants exuded from and accumulated by the plant roots are likely to be utilized by *Azospirillum* spp. (Tripathi et al., 1998). *Azospirillum* is known to contribute to improving plant-microbe relationships. Therefore, understanding the mechanism of osmoadaptation of such rhizomicrobes is beneficial in order to increase crop productivity (Tripathi et al., 1998).

Our results demonstrated that inoculation with *A. chroococcum* showed remarkable enhancement in the radicle length of lettuce and radish seedlings (Figs. 1 and 2). Similarly, Brown (1982) revealed that use of *Azotobacter* spp. as biofertilizers promotes growth by a mechanism other than biological N fixation. PGPR stimulate plant growth via production of phytohormones, activation of phosphate solubilization, suppression of deleterious organisms, and promotion of the uptake of minerals and nutrients (Lalande et al., 1989). Direct stimulation of plant growth occurs when PGPR provide compounds such as phytohormones that affect plant metabolism or when they facilitate a plant's acquisition of an otherwise unavailable nutrient from the soil.

The highest rate of germination of lettuce seeds after 24 h was shown by *Lactobacillus* sp. and LAP mix, which showed a 35% increase in seed germination compared to the control after 24 h, respectively. *A. chroococcum* showed only a 10% increase in the seed germination rate compared to the control, while *P. putida* did not show any enhancement in seed germination. *B. subtilis* showed the worst results during the trial.

Many studies have shown that several soil bacteria, particularly those that are identified as PGPR, can produce cytokinins and gibberellins (De Salamone et al., 2001). Plant growth promotion by some cytokinin- or gibberellin-producing bacteria (e.g., *Azotobacter* spp., *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*) has been reported (Joo et al., 2005; Kang et al., 2009). Selection of PGP bacteria (PGPB) from various habitats can widen the PGPB spectrum. The strains used were isolated from *P. schinsen* rhizosphere and selected according to their strong potential production for IAA and siderophores that were shown in previous studies.

According to our results, the radicle length was 1.3 cm greater with the help of *A. chroococcum* inoculation. LAP mix enhanced the radicle length by 1.0 cm, while *Lactobacillus* sp. showed a lesser increase of 0.7 cm. PGPR improve the growth and health of plants via one or more mechanism(s) at different stages of plant growth. Among these, enhancing the plant's nutrient uptake and phytohormone production can influence plant growth (Zaidi et al., 2009).

Similar results were demonstrated for the plumule length of *L.*

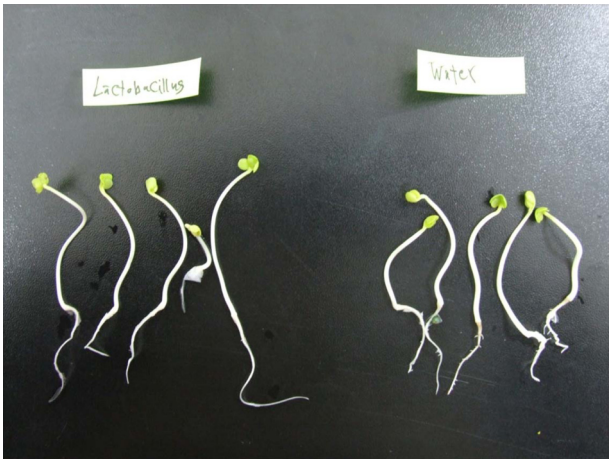

Fig. 3 The difference in radicle and plumule lengths of radish seeds inoculated by *Lactobacillus* sp. (left) and the uninoculated seeds (right).

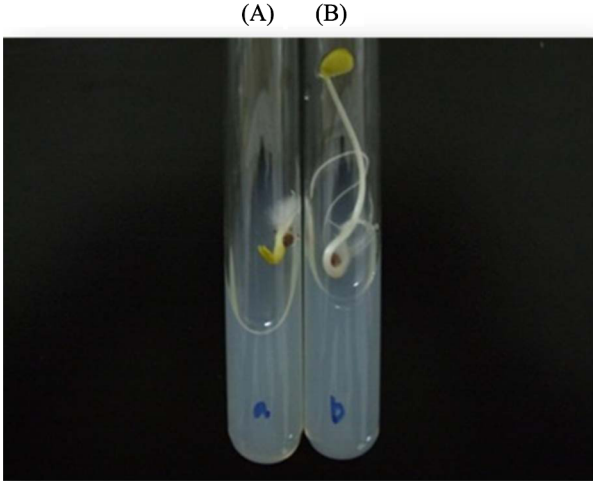

Fig. 4 The difference in growth between (A) the uninoculated radish seed and; (B) the LAP mix inoculated seeds.

sativa seedlings, in which *Lactobacillus* sp., *A. chroococcum*, and LAP mix inoculation increased the plumule length by 1.3, 0.8, and 0.7 cm, respectively, relative to the uninoculated control. *P. putida* showed an increase of only 0.6 cm in plumule length (Fig. 3). Promotion of root growth is a major marker that estimates the efficacy of PGPB (Glick, 1995). Elongation of the primary root and proliferation of lateral and adventitious roots are beneficial for seedlings as these increase the seedling's ability to establish themselves in the soil and take up water and nutrients from the environment, thus improving their survival (Pattern and Glick, 2002).

Inoculation with *A. chroococcum*, *P. putida*, and LAP mix enhanced the seed germination rate of *R. sativus* by 10, 5, and 30%, respectively, in comparison with the uninoculated seeds. *Lactobacillus* sp., however, did not enhance the seed germination. The radicle length of radish seedlings was enhanced with the help of *A. chroococcum* and LAP mix inoculation by 3.0 and 3.5 cm, respectively. *Lactobacillus* sp. inoculation increased the radicle length by 1.3 cm compared to the uninoculated control. Conversely, *P. putida* showed a lower result (Fig. 4).

Radish plumule length increased by *A. chroococcum* and LAP mix inoculation by 2.0 and 2.3 cm, respectively compared to the uninoculated control. *Lactobacillus* sp. and *P. putida* inoculation increased the radish plumule length compared to the uninoculated control by 2.0 and 1.5 cm, respectively. However, *B. subtilis* decreased the plumule length by 0.4 cm compared to uninoculated control (Table 2). Recently, many bacterial strains belonging to *Pseudomonas*, *Azotobacter*, *Enterobacter*, *Arthrobacter*, *Burkholderia*, *Bacillus,* and *Serratia* have been reported to enhance plant growth (Okon and Labandera-Gonzalez, 1994; Glick, 1995).

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