TOMATO GROWTH PROMOTION INDUCED BY BACILLUS SPP

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Abstract - Biofertilizer containing plant growth promotingrhizobacteria offers great promise to promote agricultural sustainability. However, biofertilizer application is often restricted in the field application. To gain high-quality biofertilizer, seven bacteria producing spore, i.e., Bacilluslicheni form is F1-1, B.licheniformis F1-2, B. amyloliquefaciens ZH-3, B. altitudinis ZH-4, B. safensis ZH-5, B. licheniformis ZH-6, B.siamensis CM-2, were isolated from the rhizosphere soil. Two strains, i.e., F1-1, F1-2, showed cellulase activity. Strain CM-2 showed the production of IAA (79.58 mg L^{-1}). All strains except CM-2 showed antimicrobial activity against plant pathogens (i.e., Rhizoctoniasolani, Fusarium oxysporum, Botrytis cinereapers). Inoculation with isolates resulted in differential growth responses of tomato. B.licheniformis F1-1 inoculation showed the highest increase in tomato fresh and dry weight over control plants, while B.siamensis CM-2 gained the maximum length of tomato. Hence, strains F1-1 and CM-2 have greater potential to be used for the production of biofertilizer to reduce chemical fertilizer.

Keywords - Bacillus, Biofertilizer, Plant Pathogens, Cellulose Activity.

I. INTRODUCTION

Large amount of chemical fertilizers have been applied to gain higher agricultural production in the new agricultural practice [1]. However, frequent application of chemical fertilizers is not only expensive but also eco-unfriendly such as loss of crop genetic diversity, aquatic and terrestrial acidification, and eutrophication and contamination of ground-water resources [2]. Therefore, it is of great importance to take agronomic measures of reducing fertilizer inputs and new variety breeding to improve fertilizer-use efficiency, yield and disease resistance [3, 4]. Biofertilizer containing some plant growth promotingrhizobacteria (PGPR) is the most promising candidate for reducing the use of chemical fertilizer in sustainable agriculture practices[5]. Rhizobacteria, plant-associated bacteria, could colonize and persist inside and (or) outside seedlings, forming rhizospheric and (or) endophytic colonies in roots, stems and leave[6]. These colonized PGPR stimulate host plant growth and development directly or indirectly through multiple action mechanisms such as nitrogen fixation, potassium solubilization, phosphate solubilization, siderophore secretion and indole-3-acetic acid (IAA) production [7].Paenibacillus[8],Azospirillum[9], Bacillus[10], Pseudomonas[11] and Klebsiella[12]are genera which have already been widely reported for known benefits on various plants. Lots of researches of PGPR have been carried out on various plants including maize [8], potato [9], cucumber [10], barley and tomato [11]. However, biofertilizer application is restrictedin the field application [13]. First, some adverse environmental variables (soil pH and salinity,climatic conditions) could hinder PGPR application [14]. Second, some biofertilizershave many disadvantages, such as unstable activity and short retention period, which lead to great difficulties to practical application[8]. However, the ability of forming endospores can allow PGPR to survive in a wide range of environmental variables [15], thus helping to produce the high quality biofertilizer having long retention period. In this study, we focused only on isolating PGPR which could produce the spores.

II. DETAILS EXPERIMENTAL

A. Soil Sampling and Bacterial Isolations

Soil samples were collected from different plant rhizosphere. Serial dilutions (up to 10^{-5}) were made and isolated on Luria Bertani (LB) agar plate after heating at 85℃ for 10 min. The plates were incubated for 2 days at 30°C, and then colonies were picked and purified by three times on LB agar plates and then stored at 4°C. In order to long-term storage, these purified strains were stored in the medium with 30% glycerol (v/v) at −80°C.

B. Determination Cellulose Activity, IAA Production Antimicrobial Activity

The determination of cellulose activity was performed according to the description of Li, et al [16]. The determination of IAA production was performed according the description of Li, et al [8]. Above bacterial isolates were evaluated for antimicrobial activity against plant pathogens including Rhizoctoniacerealis, Fusarium oxysporum and Botrytis cinereapers on potato dextrose agar (PDA) medium. After 3-7 days incubation at 30°C, the inhibition zones around the bacterial growth were visualized and measured in millimeters. All tests were performed in triplicate.

C. Morphological Characterization of Isolates

For observation of colony morphology, the bacterial strains were spread on LB agar. After 2 d incubation at 30℃, signal colony were observed. Cell morphology was viewed by optical microscopy (Olympus CX22LED, Japan).

D. Sequence Analysis of 16S rRNA

Strains were identified based on the 16S rRNA gene sequences. This assay was carried out according to the description of Li, et al [8]

E. Evaluation of Isolates for Growth Promotion

A pot experiment was employed in the greenhouse of China Agricultural University, Beijing, China to evaluate the effects of these isolates on tomato growth and development. The plant growth promoting activities were determined by the procedure described by Li et al [8].

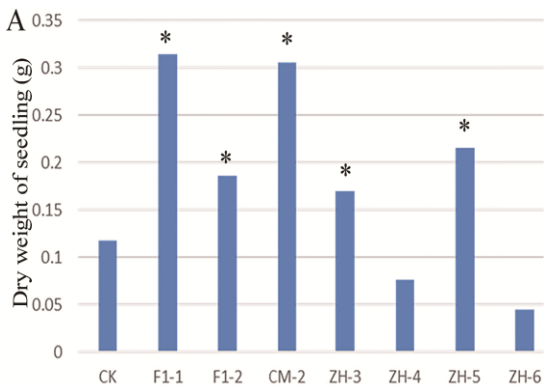
III. RESULTS

A. Isolation and Identification of Bacteria Isolation

In this study, 61 rhizosphere soil samples were collected from different plants in different areas. 308 bacterial strains (date not shown), which could produce the spores, were obtained after heating at 85°C for 10 min. Seven strains were chose based on plant growth promoting potential (Table 1). F1-1 and F1-2 showed cellulose activity, and CM-2 could produce IAA. F1-1 could inhibit 2 plant pathogens (i.e., R.solani and F.oxysporum), F1-2, ZH-5 and ZH-6 could inhibit R.solani, ZH-3 could inhibit 3 plant pathogens (i.e., R. solani, Botrytis cinereapers, F. oxysporum). Based on the analysis of 16S rRNA gene sequence, F1-1, F1-2 and ZH-6 were presumably identified as Bacillus licheniformis, ZH-3 as B.amyloliquefaciens, ZH-4 as B.altitudinis, ZH-5 as B.safensis, and CM-2 as B. siamensis. All strains were rods.

B. Growth promotion potential of isolated bacteria

To assess the impacts of above isolate inoculation on tomato growth, we analyzed the biomass (i.e., dry and fresh matter, plant length) in inoculated and un-inoculated plants (Figure 1). Inoculation with F1-1, F1-2, CM-2, ZH-3, ZH-5 significantly increased plant dry matter (Fig. 1A). Tomato inoculated with F1-1, F1-2, CM-2 and ZH-5 showed significantly higher fresh weight (Fig. 1B) and plant height (Fig. 1C), compared to un-inoculated tomato. Strain F1-1 showed maximum increase of tomato dry and fresh weight, while strain CM-2 showed maximum increase of tomato height.



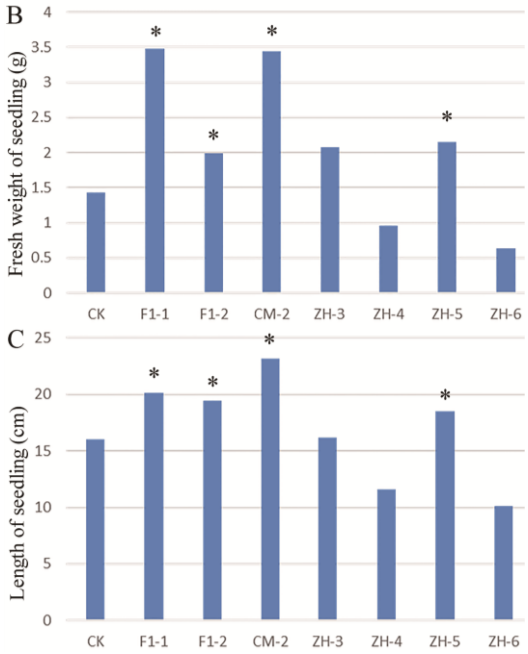


Fig.1. Effects of PGPR strains inoculation on tomato dry weight (A), fresh weight (B) and length (C). CK: un-inoculated seedlings. Single asterisk (*) indicates gnificant differences among different treatments determined by LSD at P<0.05.

IV. DISCUSSION

The rhizosphere, a narrow zone, is niches for various types of plant-associated microorganisms-beneficial, harmful, or neutral [8]. Some beneficial bacteria are called PGPR because they have some plant growth promoting mechanisms such as nitrogen fixation, potassium solubilization, phosphate solubilization, siderophore secretion and indole-3-acetic acid (IAA) production. In this study, identification based on 16S rRNA gene analyses showed that F1-1, F1-2, ZH-3, ZH-4, ZH-5, ZH-6 and CM-2 belong to know PGPR genera (Bacillus). Strains F1-1, F1-2 and ZH-6 were identified as affiliated with B. licheniformis, ZH-3 with B. amyloliquefaciens, ZH-4 with B.altitudinis, ZH-5 with B.safensis, CM-2 with B.siamensis. Some studies reported B. licheniformis[17], B. amyloliquefaciens[18, 19], B. altitudinis[20], B. safensis[21] and B. siamensis[22]were isolated form different plant rhizosphere soil.

In our study, B. licheniformis F1-1 and F1-2 showed cellulose activity. B. licheniformis 380, isolated from compost, showed carboxymethyl cellulose activity [23]. B. licheniformis 2D55, isolated form thermophilic environment, could produce celluloytic activity (CMCase 0.33 U/mL and FPase 0.09 U/mL) on microcrystalline cellulose [24]. All strains except CM-2 showed antimicrobial activity against plant pathogens. B. licheniformis UM88, isolated form the rhizosphere of Medicagotruncatula, owned antimicrobial activity against plant pathogens including Diaporthephaseolorum, Colletotrichumacutatum, R. solani, and F. oxysporum[25]. B. amyloliquefaciens SR1 showed antimicrobial activity against plant pathogens

ingcludingR. solani, Sclerotiumrolfsii, Alternariasolani, F. oxysporum under in vitro dual culture plate assay [26]. CM-2 showed the ability of producing IAA. It was also reported that B. siamensis BE-76 could produce IAA in medium supplemented with and without L-tryptophan [27]Inoculation with F1-1, F1-2, CM-2, ZH-3 and ZH-5 significantly increased tomato biomass, compared to un-inoculated tomato. B. licheniformis A2 treated groundnut significantly increased fresh biomass, total length and root length under non- saline-alkaline and saline-alkaline stress conditions [28]. Under saline-alkaline stress, B. licheniformis SA03 inoculation increased Chrysanthemum survival rates, photosynthesis and biomass [29].Kalanchoedaigremontiana inoculated with B. amyloliquefaciens IN937a increased the total number of leaves per plant and the root biomass compared un-inoculated plants [30].

REFERENCES

[1] Leita L, De Nobili M, Mondini C, Muhlbachova G, Marchiol L, Bragato G, Contin M: Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. Biology and Fertility of Soils1999, 28:371-376, 1999.

[2] Kaur T, Brar BS, Dhillon NS: Soil organic matter dynamics as affected by long-term use of organic and inorganic fertilizers under maize-wheat cropping system. Nutrient Cycling in Agroecosystems2008, 81:59-69, 2008.

[3] Hirel B, Le Gouis J, Ney B, Gallais A: The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot2007, 58:2369-2387, 2007.

[4] Tester M, Langridge P: Breeding technologies to increase crop production in a changing world. Science2010, 327:818-822, 2010.

[5] Choudhury ATMA, Kecskés ML, Kennedy IR: Utilization of BNF technology supplementing urea N for sustainable rice production. J Plant Nutr2014, 37:1627-1647, 2014.

[6] Ku YL, Xu GY, Tian XH, Xie HQ, Yang XN, Cao CL: Root colonization and growth promotion of soybean, wheat and Chinese cabbage by Bacillus cereus YL6. Plos One2018, 13, 2018.

[7] Gontia-Mishra I, Sapre S, Sharma A, Tiwari S: Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. Plant Biol2016, 18:992-1000, 2016.

[8] Li YB, Liu XM, Hao TY, Chen SF: Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates. International Journal of Molecular Sciences2017, 18:16, 2017.

[9] Naqqash T, Hameed S, Imran A, Hanif M, Majeed A, van Elsas J: Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. Front Plant Sci2016, 7:144, 2016.

[10] Li Y, Shr H, Zhang H, Chen S: Amelioration of drought effects in wheat and cucumber by the combined application of super absorbent polymer and potential biofertilizer. Peerj2019, 7, 2019.

[11] Scagliola M, Pii Y, Mimmo T, Cesco S, Ricciuti P, Crecchio C: Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (Hordeum vulgare L.) and tomato (Solanum lycopersicon L.) grown under Fe sufficiency and deficiency. Plant Physiology and Biochemistry2016, 107:187-196, 2016.

[12] Kuan KB, Othman R, Rahim KA, Shamsuddin ZH: Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. Plos One2016, 11, 2016.

[13] Zhang J, Liu J, Meng L, Ma Z, Tang X, Cao Y, Sun L: Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with fluorescein isothiocyanate. J Microbiol2012, 50:191-198, 2012.

[14] Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA: Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus2013, 2:31, 2013.

[15] Pérez-García A, Romero D, de Vicente A: Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. Current Opinion in Biotechnology2011, 22:187-193, 2011.

[16] Li Z, Chen X, Li Z, Li D, Wang Y, Gao H, Cao L, Hou Y, Li S, Liang J: Strain improvement of Trichoderma viride for increased cellulase production by irradiation of electron and $^{12}C^{6+}$ ion beams. Biotechnol Lett2016, 38:983-989, 2016.

[17] Jeong MH, Lee YS, Cho JY, Ahn YS, Moon JH, Hyun HN, Cha GS, Kim KY: Isolation and characterization of metabolites from Bacillus licheniformis MH48 with antifungal activity against plant pathogens. Microb Pathog2017, 110:645-653, 2017.

[18] Karimi E, Safaie N, Shams-Baksh M, Mahmoudi B: Bacillus amyloliquefaciens SB14 from rhizosphere alleviates Rhizoctonia damping-off disease on sugar beet. Microbiol Res2016, 192:221-230, 2016.

[19] Zhang QX, Zhang Y, Shan HH, Tong YH, Chen XJ, Liu FQ: Isolation and identification of antifungal peptides from Bacillus amyloliquefaciens W10. Environ Sci Pollut Res Int2017, 24:25000-25009, 2017.

[20] Sunar K, Dey P, Chakraborty U, Chakraborty B: Biocontrol efficacy and plant growth promoting activity of Bacillus altitudinis isolated from Darjeeling hills, India. J Basic Microbiol2015, 55:91-104, 2015.

[21] Kothari VV, Kothari RK, Kothari CR, Bhatt VD, Nathani NM, Koringa PG, Joshi CG, Vyas BR: Genome sequence of salt-tolerant Bacillus safensis strain VK, Isolated from Saline Desert Area of Gujarat, India. Genome Announc2013, 1, 2013.

[22] Shin DJ, Yoo SJ, Hong JK, Weon HY, Song J, Sang MK: Effect of Bacillus aryabhattai H26-2 and B. siamensis H30-3 on growth promotion and alleviation of heat and drought stresses in Chinese cabbage. Plant Pathol J2019, 35:178-187, 2019.

[23] Marco EG, Heck K, Martos ET, ST VDS: Purification and characterization of a thermostable alkaline cellulase produced by Bacillus licheniformis 380 isolated from compost. An Acad Bras Cienc2017, 89:2359-2370, 2017.

[24] Kazeem MO, Shah UKM, Baharuddin AS, AbdulRahman NA: Prospecting agro-waste cocktail: Supplementation for cellulase production by a newly isolated thermophilic B. licheniformis 2D55. Appl Biochem Biotechnol2017, 182:1318-1340, 2017.

[25] Hernandez-Salmeron JE, Prieto-Barajas CM, Valencia-Cantero E, Moreno-Hagelsieb G, Santoyo G: Isolation and characterization of genetic variability in bacteria with beta-hemolytic and antifungal activity isolated from the rhizosphere of Medicago truncatula plants. Genet Mol Res2014, 13:4967-4975, 2014.

[26] Nanjundan J, Ramasamy R, Uthandi S, Ponnusamy M: Antimicrobial activity and spectroscopic characterization of surfactin class of lipopeptides from Bacillus amyloliquefaciens SR1. Microb Pathog2019, 128:374-380, 2019.

[27] Ambawade MS, Pathade GR: Indole acetic acid (IAA) PRODUCTION by Bacillus siamensis BE 76 isolated from Musa balbisiana (banana). Journal of Chemical and Pharmaceutical Research, 10:6-10,

[28] Goswami D, Dhandhukia P, Patel P, Thakker JN: Screening of PGPR from saline desert of Kutch: growth promotion in Arachis hypogea by Bacillus licheniformis A2. Microbiol Res2014, 169:66-75, 2014.

[29] Zhou C, Zhu L, Xie Y, Li F, Xiao X, Ma Z, Wang J: Bacillus licheniformis SA03 confers increased saline-alkaline tolerance in Chrysanthemum plants by induction of abscisic acid accumulation. Front Plant Sci2017, 8:1143, 2017.

[30] Park YS, Park K, Kloepper JW, Ryu CM: Plant growth-promoting rhizobacteria stimulate vegetative growth and asexual reproduction of Kalanchoe daigremontiana. Plant Pathol J2015, 31:310-315, 2015.

Strain Code	Cellulase activity (D/d)	IAA production (mg L^{-1})	Antimicrobial activity
F1-1	4.20±0.15	---	*Rhizoctoniasolani, Fusarium oxysporum*
F1-2	4.58±0.73	---	*R. solani*
ZH-3	---	---	*R. solani, Botrytis cinerea Pers., F. oxysporum*
ZH-4	---	---	*B. cinerea Pers.*
ZH-5	---	---	*R. solani*
ZH-6	---	---	*R. solani*
CM-2	---	79.58	---

Table 1. Cellulase activity, IAA production and antimicrobial activity of isolates.

Strain Code	Cell Morphology	Colony Morphology	Maximum Similarity of 16S rRNA
F1-1	Rods	Creamy, Round	*Bacillus licheniformis*
F1-2	Rods	Mucoid, Convex	*Bacillus licheniformis*
ZH-3	Rods	Creamy, Convex	*Bacillus amyloliquefaciens*
ZH-4	Rods	Milky, Convex	*Bacillus altitudinis*
ZH-5	Rods	Vitelline, Round	*Bacillus safensis*
ZH-6	Rods	Creamy, Irregular	*Bacillus licheniformis*
CM-2	Rods	Vitelline, Round	*Bacillus siamensis*

Table 2. Characterization and identification of all strains

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