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Enhancement of Growth and Yield of Tomato by *Rhodopseudomonas* sp. under Greenhouse Conditions

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A greenhouse test was carried out to examine the effects on tomato growth of application of purple nonsulfur bacterium *Rhodopseudomonas* sp. which had enhanced germination and growth of tomato seed under axenic conditions. The shoot length of tomato plant inoculated by *Rhodopseudomonas* sp. KL9 increased by 34.6% compared to that of control in 8 weeks of cultivation. During the same period, this strain increased 120.6 and 78.6% of dry weight of shoot and root of tomato plants, respectively. The formation ratio of tomato fruit from flower was also raised by inoculation of KL9. In addition, *Rhodopseudomonas* sp. KL9 treatment enhanced the fresh weight and lycopene content in the harvested tomato fruits by 98.3 and 48.3%, respectively compared to those of the uninoculated control. When the effect on the indigenous bacterial community and fate of the inoculated *Rhodopseudomonas* sp. KL9 were monitored by denaturing gradient gel electrophoresis analysis, its application did not affect the native bacterial community in tomato rhizosphere soil, but should be repeated to maintain its population size. This bacterial capability may be applied as an environment-friendly biofertilizer to cultivation of high quality tomato and other crops including lycopene-containing vegetables and fruits.

***Keywords*:** lycopene, plant growth promotion, purple nonsulfur bacteria, *Rhodopseudomonas*, tomato

It has been well known that plant growth can be enhanced by some plant growth promoting bacteria (PGPB), and the most widely studied group of PGPB are plant growth promoting rhizobacteria (PGPR) colonizing the root surface and the closely adhering soil interface, the rhizosphere (Kloepper *et al*., 1999; Gray and Smith, 2005). Although the precise mechanisms of plant growth promotion by PGPB are not fully understood, but are thought to include the production of plant hormones, such as auxins, gibberellins, cytokinins (Karadeniz *et al*., 2006), nitrogen fixation (Mantelin and Touraine, 2004), and solubilization of mineral phosphates and other nutrients (Rodríguez and Fraga, 1999). Recently purple nonsulfur bacteria (PNSB) are being used in agriculture even though their precise mechanisms of plant growth promotion have not been elucidated (Elbadly *et al*., 1999). Unexpectedly, PNSB occur widely in aquatic and terrestrial environments, and most species of this bacterial group, such as *Rhodopseudomonas* spp. are able to grow anaerobically in the light or aerobically in the dark with many carbon sources and electron donors (Zhang *et al*., 2002). The pigments, metabolites, and nutrients made by PNSB could give some positive effects on plant growth. Although nitrogen fixation (Maudinas *et al*., 1981) and auxin production (Rajasekhar *et al*., 1999) by PNSB have been reported, a systematic field application of PNSB for growth promotion and quality improvement of agricultural crops has not been carried out many times. In our previous study, isolated PNSB strains *Rhodopseudomonas* sp. KL9 and BL6 showed the efficient growth enhancement of tomato seedlings under axenic conditions, and the production of indole-3-acetic acid (IAA) and 5-aminolevulinic acid (ALA) and solubilization of insoluble phosphate may be responsible for growth promotion of tomato seedling (Koh and Song, 2007).

The objective of this work is the examination of the effects of PNSB application on the growth of tomato plant and yield and lycopene content of tomato fruit in a greenhouse soil. The fate of the inoculated bacteria was also monitored by denaturing gradient gel electrophoresis (DGGE) analysis which has been widely used for the detection of certain bacteria and bacterial community analysis (Muyzer *et al*., 1993). Lycopene is a major carotenoid pigment in ripe tomato. Since tomato fruit has the antioxidizing, anticalcinogenic and antiatherogenic effect (Omoni and Aluko, 2005), its value as a well-being food is increasing nowadays. If PNSB can help to produce tomato with high lycopene content, it may be utilized as a biofertilizer especially for various lycopene-containing crops including tomato.

Materials and Methods

Preparation of purple nonsulfur bacteria

The *Rhodopseudomonas* strains KL9 and BL6 tested in this study had been isolated from river sediment and identified in the previous study (Koh and Song, 2007). They were grown on the modified Biebl and Pfennig's medium (Archana *et al*., 2004). The PNSB cultures were incubated under anaerobic conditions in the presence light (light intensity of 280~325 μE m^{-2} s^{-1} from fluorescent lamps) in 1 L screw

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cap bottle at 30°C for 7 days. When the culture showed reddish purple color, 50 ml inoculum was transferred into a fresh medium and incubated again under the same conditions. PNSB cells were removed by centrifugation (5,000×g, 50 min), resuspended with 0.1 M $MgSO_4 \cdot 7H_2O$ solution and adjusted to 10^7 cells/ml with a spectrophotometer (Shimadzu Co., Japan) at 660 nm.

Effects on growth promotion of tomato plant by *Rhodopseudomonas* sp. in a greenhouse

Fifty seedlings (15-day grown, average length 18.5±2.7 cm) of tomato (*Lycopersicon esculentum* Mill cv. Zeus) in pots were purchased from a nursery company in Chuncheon, Korea. They were cultivated additional 7 days and each seedling was transplanted into a 5,000 cm^3 pot containing 4 kg mixture of coarse sand and sandy loam soil (1:4, v/v), and cultivated in a greenhouse in Kangwon National University. For the application of PNSB, either 400 ml of live bacterial suspension (10^7 cells/ml) or autoclaved bacterial suspension was applied on the surface soil of each pot for transplanting. The tomato plants were irrigated every day with 400 ml of water per pot, and the control plant received 400 ml of sterile distilled water. Tomato plants were grown under the typical growing conditions in the greenhouse [temperatures of 18°C (night) and 24°C (day)] during 8 weeks (Siddiqui *et al*., 2001). Each tomato plant was a single replication in a pot and there were five replications per treatment (n=5).

The length of shoot, dry weight of tomato plant, and production (yield) and lycopene content of harvested tomatoes were determined during or after 8 weeks of cultivation. The length of shoot of tomato plant was measured from a bottom of first leaf stem of plant to a top of new leaf once a week followed by the inoculation of 400 ml suspension of *Rhodopseudomonas* sp. KL9 and BL6 (10^7 cells/ml). After 8 weeks cultivation, dry weights of the root and the stem were measured after cutting into small pieces and complete drying at 80°C for 7 days. At this time, the weight of the roots detached from the main root or remaining in the pot soil were measured after recovery by filtration of pot soil using a sieve (dia. 8 mm), washing several times with sterile water, and then drying at 80°C for 2 days. After 5 weeks of cultivation the tomato fruits began ripening, and they were harvested three times a week until the end of cultivation period. During the cultivation of tomato plant, the numbers of tomato flower and tomato fruit were enumerated and the formation ratio of fruit from flower was calculated. Yield of tomato fruit was determined by measuring the average fresh weight of tomato fruits per plant.

Analysis of lycopene content in tomato fruits

All tomato samples from each plant were homogenized in a domestic blender to obtain a representative sample for lycopene analysis. Five gram of the homogenized sample was placed in a flask, protected from light, and mixed with 100 ml of lycopene extraction solution (hexane 50:acetone 25: ethanol 25, v/v/v). The mixture was mixed on a magnetic stirrer until the tomato pulp became completely colorless. This extract was added 15 ml distilled water and centrifuged (5,000×g, 50 min). The supernatant was separated and evaporated to dryness by a rotary vacuum evaporator. The dry extract was dissolved with 10 ml of the lycopene dissolution solution (tetrahydrofuran 15:acetonitrile 30:methanol 55, v/v/v), and evaporated to dryness. This washing process was repeated twice. The final residue was dissolved to 10 ml hexane, filtered through membrane filter (0.45 μm pore), and 15 μl of final extract was injected to HPLC for the analysis of lycopene. The analytical column was Luna 5 μ C18 (300 mm×4.6 mm, 5±0.3 μm of pore size, Phenomenex, USA). Samples were analyzed under isocratic condition (methanol 8:tetrahydrofuran 2, v/v), and lycopene was detected by a UV detector at 350 nm. The total run time was approximately 20 min at a flow rate of 1.2 ml/min. Lycopene was quantified by comparison to the peak area of the lycopene standard obtained from Sigma Chemical Co. (Fish *et al*., 2002; Olives Barba *et al*., 2006).

All experiments were carried out in five or ten-replicate, and the data were analyzed by Student's 'T'-test.

Analysis of rhizosphere bacterial community by PCR-DGGE

PCR-DGGE analysis was performed to monitor the bacterial community in rhizosphere and the inoculated bacteria as described by Muyzer *et al*. (1993). Bacterial DNA was directly extracted from 0.25 g of soil sample using the Power Soil DNA kit (MOBIO Lab, USA). The final volume of extracted DNA solution was 20 μl, and PCR ampli-

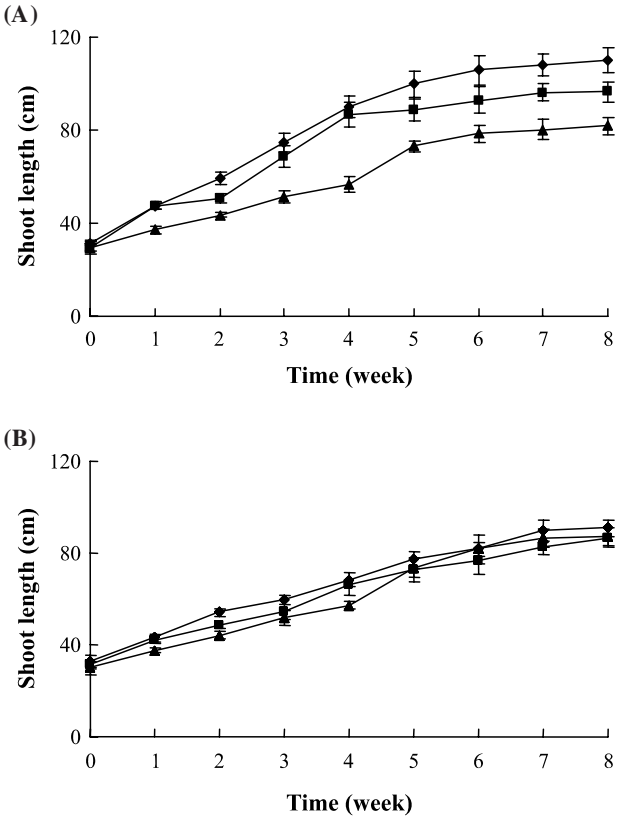

Fig. 1. Change of shoot length of the tomato plant treated with *Rhodopseudomonas* sp. [(A) live bacteria, (B) autoclaved bacteria] during the cultivation in a greenhouse. Symbols: control (▲), KL9 (◆), BL6 (■)

Table 1. Comparison of dry weight of the shoot, root, and fruit formation ratio from flower in tomato plant under different inoculation conditions

Inoculation	Dry weight (g)[a]		Fruit/Flower (%)[a]
	Root	Shoot	
Uninoculated control	4.2±0.3	10.7±1.3	24.5±4.3
Killed BL6	4.1±0.3	11.0±1.2	22.0±3.5
BL6	4.7±0.5	15.7±1.2	33.3±4.9
Killed KL9	5.3±0.2	14.3±0.9	27.8±5.5
KL9	7.5±0.3	23.6±1.3	46.2±2.9

Data were analyzed by Student 'T'-test (n=5).
[a] Dry weight of tomato plant and ratio of fruit formation were measured after 8 weeks of cultivation.

fication was performed with 20 μl reaction volume in a gradient thermal cycler (PTC DNA Engine System, USA). The 341F primer; 5'-CCTACGGGAGGCAGCAG-3' and 518R primer; 5'-ATTACCGCGGCTGCTGG-3' to conserved regions of the 16S rRNA gene were used. A 341F primer has an additional 40 GC-rich sequence; 5'-CGCCCGCCGCGCG CGGCGGGCGGGGCGGGGGCACGGGGGG-3' at 5' end. DGGE was performed with DCode system Model 2001 (BioRad, USA). PCR products were applied directly onto polyacrylamide gels in 1× TAE (40 mM Tris acetate; pH 7.4, 20 mM sodium acetate, 1 mM Na-EDTA) with gradient which contained 50~70% denaturant [7 M urea and 40% deionized formamide (v/v)]. Electrophoresis was performed at a constant voltage of 50 V and a temperature of 60°C. After electrophoresis, the gels were incubated for 15 min in distilled water containing ethidium bromide (0.5 mg/ml), rinsed for 10 min with distilled water, and photographed with Gel Logic imaging system (Kodak, USA).

Results

Effects on tomato plant growth by application of *Rhodopseudomonas* sp. in a greenhouse

The growth of shoot of tomato plant was enhanced by application of live *Rhodopseudomonas* sp. BL6 and KL9 strain during entire cultivation period. Strain BL6 and KL9 increased the shoot lengths by 17.6 and 34.6%, respectively, compared to that of the uninoculated control (Fig. 1A). The killed bacteria could not affect the growth of tomato plants (Fig. 1B). The application of *Rhodopseudomonas* spp. could also increase the fruit/flower ratio and biomass measured by dry weight of root and shoot harvested after 8 weeks of cultivation (Table 1). Strain KL9 increased the dry weight of root and shoot of tomato plant 78.6 and 120.6%, respectively, compared to those of the uninoculated control, and BL6 also increased them by 11.9 and 46.7%. The ratios of fruit formation from flower were raised 88.6 and 35.9% by KL9 and BL6, respectively.

Effect on yield and lycopene content of tomato by *Rhodopseudomonas* sp.

After 8 weeks of a greenhouse test, the average weight and lycopene content of tomato fruit grown under each condition were determined. The average weight of tomato fruit per plant treated with KL9 strain (82.7 g) was higher than those of others including the uninoculated control (Table 2). Treatment of killed PNSB also showed some positive effects on the increase of tomato weight. The content of lycopene in the ripe tomato fruit increased by 48.3% with the application of *Rhodopseudomonas* sp. KL9, but BL6 did not show any effect on lycopene content although the lycopene content in the cells of KL9 and BL6 were 0.65 and 1.12 mg/g, respectively (Table 2).

Analysis of bacterial community in tomato rhizosphere

During 8 weeks of the greenhouse test, the bacterial community in rhizosphere of tomato plant was analyzed by PCR-DGGE. DGGE band profiles of the native bacterial community in the control soil were similar to those in the soil treated with *Rhodopseudomonas* sp. KL9 (Fig. 2). Distinctive DGGE bands of *Rhodopseudomonas* sp. KL9 could be maintained by the repeated inoculation of bacteria into the soil.

Discussion

Rhodopseudomonas sp. KL9 and BL6 tested in this study

Table 2. Comparison of fresh weight and lycopene content of tomato fruit grown under different inoculation conditions

Inoculation	Average fresh weight of fruit[a] (g/plant)	Content of lycopene[a] (mg/g fruit)
Uninoculated control	41.7±4.2	0.60±0.10
Killed BL6	59.1±3.8	0.61±0.07
BL6	50.4±3.8	0.61±0.14
Killed KL9	62.7±4.6	0.41±0.16
KL9	82.7±4.4	0.89±0.11

[a] Fresh weight and lycopene content of tomato fruit were measured after 8 weeks of cultivation. Data were analyzed by Student 'T'-test (n=10).

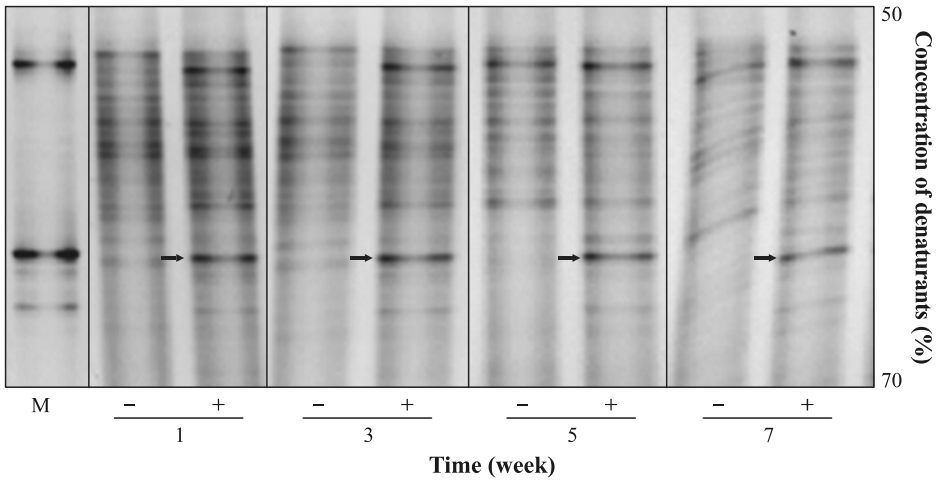

Fig. 2. DGGE profiles of the bacterial populations in the tomato rhizosphere soil in a greenhouse treated with *Rhodopseudomonas* sp. KL9 (M, PCR products from the culture of *Rhodopseudomonas* sp. KL9; lane −, control soil; lane +, soil treated with KL9).

could produce the phytohormones, such as IAA and ALA and solubilize the insoluble phosphates in the culture media, and enhance the germination and growth of tomato seeds under axenic conditions (Koh and Song, 2007). In this study, their plant growth promoting capability was tested to the tomato plants under greenhouse conditions. As shown in Fig. 1, the live cells of strain KL9 and BL6 could enhance the elongation of tomato shoot, but killed bacteria did not have any significant effect on the shoot elongation. This result indicated that the PNSB could exhibit plant growth promoting effects in a real agricultural environment, and those effects were resulted by the activity of live bacteria. As like the results in the previous laboratory test, *Rhodopseudomonas* sp. KL9 showed the higher growth promoting capability than BL6, which may be due to the higher production rates of IAA, ALA, and soluble phosphate by strain KL9 (Koh and Song, 2007). Those reasons were already reported as the major mechanisms for plant growth promotion in other plants (Benizri *et al*., 1998; Rodríguez and Fraga, 1999; Ryu *et al*., 2006; Gravel *et al*., 2007).

In addition to the enhancement of shoot elongation during the growth period, *Rhodopseudomonas* sp. KL9 and BL6 could increase the biomass of tomato plant measured by the dry weight of whole root and shoot harvested after 8 weeks of cultivation (Table 1). Both strains enhanced the growth of root and shoot, but the increasing rates of dry weight of root and shoot by KL9 strain (78.6 and 120.6%) were much higher than those of BL6 strain (11.9 and 46.7%). Even killed KL9 could increase the dry weight of both root and shoot. When the dry weight increase of shoot by KL9 was compared to the shoot length increase in Table 1, the dry weight increase was much higher than the length increase. Not only the length but also the diameter of tomato shoot increased by KL9 treatment (data not shown). Since there has been few report on the plant growth promotion by PNSB, it is hard to compare the plant growth promoting capability of *Rhodopseudomonas* sp. KL9 to other PNSB, but the growth promotion by this strain KL9 was higher than those by other PGPB in tomato plant, such as 5.7∼8.0% increases of plant length and 3.7∼5.1% increases of plant fresh weight by *Pseudomonas fluorescens* (Siddiqui *et al*., 2001), 39.42∼61.25% increases of root length by *Methylobacterium* spp. (Ryu *et al*., 2006), 14.9 and 12.8% increases of dry weight by *Enterobacter agglomerans* and *Glomus etunicatum* (Kim *et al*., 1998) and 26.5∼32.2% increases of root dry weight by *Bacillus subtilis* (Mena-Violante and Olalde-Portugal, 2007).

Rhodopseudomonas sp. KL9 and BL6 could also enhance the formation ratio of tomato fruit from the artificially fertilized flower. The fruit formation ratio may be important for some fruit-bearing crops since it affects the fruit yield. However, to date, there has been no report on the effect of microbial application on fruit formation from flower.

Production of tomato fruit per each plant also increased by the application of *Rhodopseudomonas* sp., and KL9 strain almost doubled the yield of tomato fruit (Table 2). This promotion of tomato yield by PNSB was higher than 21.5∼25% increase of yield/plant by *Bacillus subtilis* BEB-13bs (Mena-Violante and Olalde-Portugal, 2007). The killed cells of KL9 and BL6 also showed high yield enhancement of tomato fruit, and it may be due to the secreted phytohormones and metabolites. It has been reported that fruit yield improvement of greenhouse tomato plants might be occurred by IAA produced from *Pseudomonas putida* or *Trichoderma atroviride* (Gravel *et al*., 2007). ALA also showed some promotive effects on the yield of several other crops (Hotta *et al*., 1997).

Not only plant growth and yield but also fruit quality can be improved by inoculation of PGPB. Lycopene content in the ripe tomato fruit was increased by 48.3% with the application of *Rhodopseudomonas* sp. KL9 (Table 2). The lycopene content in the tomato fruit treated with KL9, 0.89 mg/g fruit was much higher than the average content, 0.4∼0.6 mg/g reported by Perkins-Veazie *et al*. (2001) and Toor *et al*. (2006). Although live BL6 and killed cells of KL9 and BL6 showed the positive effects on growth and yield of tomato plant, they could not affect the lycopene content of tomato fruit. There are numerous reports on the effects of PGPB,

but most of them were focused on promotion of growth and yield (Kokalis-Burelle *et al*., 2002; Aslantaş *et al*., 2007). However, growth promotion by PGPB may induce the elevation of the quality of plant products. Mena-Violante and Olalde-Portugal (2007) reported the uplift of tomato texture by PGPR *Bacillus subtilis*, and Kapoor *et al*. (2004) reported the improvement of essential oil quality of *Foeniculum vulgare* by mycorrhizal inoculation. Since *Rhodopseudomonas* sp. KL9 increased the lycopene content in tomato, we measured lycopene content in the cells of strain KL9 and BL6. Goodwin (1956) already reported lycopene in *Rhodopseudomonas* sp. In contrast to the increasing effects, the lycopene content of strain KL9 was lower than that of BL6. It indicated that the lycopene in tomato fruit did not come from *Rhodopseudomonas* sp., but resulted from plant metabolism stimulated by the inoculated bacteria. It is necessary to define the mechanism of lycopene increase in tomato by this bacterium. Nowadays, tomato is becoming more popular as a well-being food since ripe tomato has the antioxidizing, anticalcinogenic, and antiatherogenic effect due to mainly by lycopene pigment (Omoni and Aluko, 2005). Therefore, if PNSB can help to produce tomato with high lycopene content, its value as a biofertilizer will be very high.

The DGGE analysis showed that the population size of *Rhodopseudomonas* sp. KL9 decreased until 2∼3 days after inoculation and the lowered population could be maintained up to 2 weeks (data not shown). When KL9 was inoculated every 7 days, certain level of bacterial population had been maintained during 8 weeks of experimental period (Fig. 2). Moreover, the indigenous soil bacterial community did not show any significant change by the inoculation of *Rhodopseudomonas* sp. KL9. These results indicated that the maintaining population of *Rhodopseudomonas* sp. KL9 could induce the growth promotion of tomato plant in a greenhouse soil without the disturbance of native bacterial community in the soil of tomato rhizosphere.

In this study the enhancements of growth, fruit formation, yield and quality of fruit in tomato plant by application of *Rhodopseudomonas* sp. KL9 were shown in a greenhouse test, and those effects may be due to some phytohormones and soluble phosphate produced by PNSB. However, other direct and indirect mechanisms for those enhancements should be investigated, and the large scale field test is necessary for the commercial development of PNSB as a biofertilizer.

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