J. Microbiol. Biotechnol. (2006), **16**(10), 1622–1628

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Plant Growth Substances Produced by *Methylobacterium* spp. and Their Effect on Tomato (*Lycopersicon esculentum* L.) and Red Pepper (*Capsicum annuum* L.) Growth

RYU, JEOUNGHYUN[1], MUNUSAMY MADHAIYAN[1], SELVARAJ POONGUZHALI[1], WOOJONG YIM[1], PANDIYAN INDIRAGANDHI[1], KYOUNGA KIM[1], RANGASAMY ANANDHAM[1], JONGCHUL YUN[2], KYE HOON KIM[3], AND TONGMIN SA[1]*

[1]*Department of Agricultural Chemistry, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Department of Organic Farming Technology, National Institute of Agricultural Science and Technology, RDA, Suwon, Korea*
[3]*Department of Environmental Horticulture, The University of Seoul, Seoul 130-743, Korea*

Received: May 22, 2006
Accepted: June 28, 2006

Abstract Bacteria from the *Methylobacterium* genus, called pink-pigmented facultative methylotrophic bacteria (PPFMs), are common inhabitants of plants, potentially dominating the phyllosphere population, and are also encountered in the rhizosphere, seeds, and other parts of plants, being versatile in nature. The consistent success of the *Methylobacterium* plant association relies on methylotrophy, the ability to utilize the one-carbon compound methanol emitted by plants. However, the efficiency of *Methylobacterium* in plant growth promotion could be better exploited and thus has attracted increasing interest in recent years. Accordingly, the present study investigated the inoculation effects of *Methylobacterium* sp. strains CBMB20 and CBMB110 on seed imbibition to tomato and red pepper on the growth and accumulation of phytohormone levels under gnotobiotic conditions. Seeds treated with the *Methylobacterium* strains showed a significant increase in root length when compared with either the uninoculated control or *Methylobacterium extorquens miaA*$^-$ knockout mutant-treated seeds. Extracts of the plant samples were used for indole-3-acetic acid (IAA), *trans*-zeatin riboside (*t*-ZR), and dihydrozeatin riboside (DHZR) assays by immunoanalysis. The treatment with *Methylobacterium* sp. CBMB20 or CBMB110 produced significant increases in the accumulation of IAA and the cytokinins *t*-ZR and DHZR in the red pepper extracts, whereas no IAA was detected in the tomato extracts, although the cytokinin concentrations were significantly increased. Therefore, this study proved that the versatility of *Methylobacterium* as a plant-growth promoting bacteria could be better exploited.

Key words: *Methylobacterium*, indole-3-acetic acid, cytokinins, immunoanalysis

*Corresponding author
Phone: 82-43-261-2561; Fax: 82-43-271-5921;
E-mail: tomsa@chungbuk.ac.kr

In recent decades, there has been increasing evidence that besides N_2-fixation and increased nutrient uptake, the synthesis and export of phytohormones by plant-associated microorganisms may play an important role in plant growth promotion. Phytohormones, believed to assimilate partitioning patterns in plants and affect growth patterns in roots, are also called plant growth regulators because of their regulatory role in plant growth development. There is also evidence that the growth hormones produced by bacteria can in some instances increase growth rates and improve the yield of host plants [2, 4].

It is well established that many soil and plant-associated bacterial groups, including Gram-negative and Gram-positive, symbiotic, and nitrogen-fixing bacteria, are able to synthesize phytohormones. Many of these bacteria can also produce and excrete more than one phytohormone. Auxins are believed to be essential phytohormones for plant life, as no plant has yet been found that is unable to synthesize auxins [41]. Indole-3-acetic acid (IAA) is the main auxin in plants and controls many important physiological processes, including cell enlargement and division, cell specialization, tissue differentiation, and responses to light and gravity [6, 39]. Thus, bacterial IAA producers can potentially interfere with such processes based on the input of IAA into a plant's auxin pool. The quantity of IAA produced and sensitivity of the plant tissue also play an important role in several functions, such as root elongation and the formation of lateral and adventitious roots [9]. Cytokinins are N^6-substituted adenine derivatives that have diverse effects on important physiological functions in plants [26] and whose level can alter the root functions [36]. Cytokinin production by plant-growth promoting rhizobacteria (PGPR), including *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bacillus*, and

Pseudomonas spp., in pure cultures has already been reported [1, 30, 40].

Basile *et al.* [3] isolated some bacteria as covert contaminants from tissue cultures of liverwort *Scapania nemorosa*, where the bacteria were later identified as pink-pigmented facultative methylotrophic bacteria (PPFMs) belonging to the genera *Methylobacterium,* which are ubiquitous on plant surfaces [3, 7, 8, 10, 11]. However, the overall nature of the relationship of these bacteria with plants is still not well understood. The *Methylobacterium* spp., as bacterial symbionts of plants, consume plant waste products and produce metabolites useful to plants [14, 15]. Several beneficial aspects have already been investigated, such as the production of urease [16], stimulation of seed germination and plant growth [14, 25], and production of cytokinins and ACC (1-aminocyclopropane-1-carboxylate) deaminase [24].

Accordingly, this study examined the production of auxins and cytokinins by *Methylobacterium* strains in a pure culture, along with changes in the early stages of tomato and red pepper seedling growth after inoculation with methylotrophic bacterial strains.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

The methylotrophic strains *Methylobacterium* sp. CBMB20 and *Methylobacterium* sp. CBMB110 were isolated from a rice stem and leaf, respectively [24]. Unless otherwise stated, the methylobacteria were grown for 72 h on ammonium mineral salt (AMS) media (per liter of distilled water: 0.5 g NH_4Cl; 0.7 g K_2HPO_4; 0.54 g KH_2PO_4; 1.0 g $MgSO_4 \cdot 7H_2O$; 0.2 g $CaCl_2 \cdot 2H_2O$; 4.0 mg $FeSO_4 \cdot 7H_2O$; 100 μg $ZnSO_4 \cdot 7H_2O$; 30 μg $MnCl_2 \cdot 4H_2O$; 300 μg H_3BO_4; 200 μg $CoCl_2 \cdot 6H_2O$; 10 μg $CuCl_2 \cdot 2H_2O$; 20 μg $NiCl_2 \cdot 6H_2O$; 60 μg $Na_2MoO_4 \cdot 2H_2O$; pH 6.8) supplemented with 0.5% methanol and cycloheximide (30 μg/ml). The mutant *Methylobacterium extorquens miaA*$^-$, kindly provided by Joe C. Polacco (Department of Biochemistry, University of Missouri-Columbia, Columbia, Missouri 65211, U.S.A.), was cultured in the AMS medium containing 15 μg/ml tetracycline at 30°C.

IAA and Cytokinin Production by Methylotrophic Bacteria

The production of IAA by the methylotrophic isolates was determined according to Ivanova *et al.* [18] with slight modifications. Aliquots of 100 μl of the *Methylobacterium* strains grown in the AMS broth were transferred into 50 ml of a minimal medium containing (per liter of distilled water) KH_2PO_4 2.0 g; $(NH_4)_2SO_4$ 2.0 g; $MgSO_4 \cdot 7H_2O$ 0.025 g; $FeSO_4 \cdot 7H_2O$ 0.002 g; pH 7.2, supplemented with 0.5% methanol (v/v) and 500 μg/ml L-tryptophan (Sigma-Aldrich Co., St. Louis, MO, U.S.A.). After incubation for 7 days, the density of each culture was measured spectrophotometrically at 530 nm, and then the bacterial cells were removed from the culture medium by centrifugation (11,336 ×*g*, 15 min). A 2-ml aliquot of the supernatant was then transferred to a fresh tube, followed by the addition of 100 μl of 10 mM orthophosphoric acid and 4 ml of a reagent (1 ml of 0.5 M $FeCl_3 \cdot 6H_2O$ in 50 ml of 35% $HClO_4$). The mixture was incubated at room temperature for 25 min and the absorbance of the pink color that developed was read at 530 nm. The concentration of IAA in each culture medium was determined by comparison with a standard curve of pure indole-3-acetic acid (Sigma-Aldrich Co., St. Louis, MO, U.S.A.).

To analyze the cytokinin production, the AMS medium (750 ml) containing 30 μg of cycloheximide per ml in 2-l flasks was inoculated with 100 ml of a starter culture, and the bacteria grown to the stationary phase (5 days) at 28 to 30°C and 200 rpm [20]. The bacterial growth was assessed by counting the CFU/ml, and the cultures clarified by centrifugation for 5 min at 8,000 ×*g*. The clarified supernatants were transferred to 1-l polypropylene bottles and stored at 80°C until the culture purity was confirmed. Immediately before harvesting, culture aliquots were plated on the AMS medium to check for contamination, and then sterile supernatants of samples taken after 120 h of growth were analyzed for cytokinin production, as described under Immunoanalysis.

Gnotobiotic Root Elongation Assay

A gnotobiotic root elongation assay was performed with tomato and red pepper to study the effects of seed imbibition with methylobacteria. The bacterial strains were grown aerobically to the late-log phase in a liquid AMS medium, and then transferred to an AMS liquid medium containing 3 mM ACC as the sole source of nitrogen, instead of NH_4Cl. The seed treatment and procedure for the gnotobiotic growth pouch assay followed Penrose and Glick [34]. A bacterial cell pellet was suspended in 0.5 ml of sterile 0.03 M $MgSO_4 \cdot 7H_2O$, and then placed on ice. Next, a 0.5-ml sample was removed from the cell suspension and diluted eight to ten times in sterile 0.03 M $MgSO_4 \cdot 7H_2O$ to adjust the absorbance of the bacterial suspension at 600 nm to 0.15. Tomato (*Lycopersicon esculentum* L. cv Mairoku, Sokata Korea, Seoul) and red pepper (*Capsicum annuum* L. cv Barodda, New Seoul Seed Company, Kongju) seeds (approximately 0.2 g per treatment) were soaked in 70% ethanol for 1 min in a glass Petri dish, and then in 2% sodium hypochlorite (NaOCl). After 10 min, the bleach solution was aspirated and the seeds thoroughly rinsed with sterile distilled water at least 5 times. Each dish was incubated at room temperature for 4 h with the appropriate treatment: sterile 0.03 M $MgSO_4 \cdot 7H_2O$ (used as the negative control) or bacterial suspensions in sterile 0.03 M $MgSO_4 \cdot 7H_2O$. The seeds were transferred aseptically to growth pouches (CYG seed germination pouch, Mega International

Manufacturer, U.S.A.) and incubated in a growth chamber maintained at 20±1°C with a cycle beginning with 12 h of dark followed by 12 h of light. Ten pouches were used for each treatment. Seeds that failed to germinate 2 days after sowing were marked, and the roots that subsequently developed from these seeds were not measured. The primary root lengths were measured on days 10 and 15 of growth and the data analyzed. The seedlings were harvested on day 25 and analyzed for auxin and cytokinin production using immunoassays.

Phytohormone Analysis

Plant extraction. The extracts were prepared by homogenizing the seedlings with a TBS buffer (each liter contained 3.03 g Trizma base, 5.84 g NaCl, 0.2 g $MgCl_2 \cdot 6H_2O$, and 0.2 g sodium azide; pH 7.2) in a ratio of 1:5 (w/v). The homogenates were clarified by centrifuging at 3,885 ×*g* for 5 min, two times, and the supernatants collected in 50-ml centrifuge tubes. The resulting extracts were then used for the IAA, *trans*-zeatin riboside (*t*-ZR), and dihydrozeatin riboside (DHZR) assays.

Immunoanalysis

The cytokinin derivatives, *t*-ZR, DHZR, isopentenyladenosine (iPA), and auxin IAA were all purchased from Sigma, U.S.A. The Enzyme-Linked Immunosorbent Assay (ELISA) test kits were purchased from AGDIA (AGDIA Inc., Indiana, U.S.A.) and used according to kit instructions. Stock solutions of the cytokinins and IAA (10 mM) were prepared in methanol and stored at 4°C. Dilutions were then made with sterile AMS media as the diluent to prepare solutions for the standard curves. The samples were diluted as needed to obtain accurate estimates within the standard concentration range. The absorbance was read at 405 nm using an ELISA plate reader (BIO-RAD Model 550, Japan). The standards were prepared according to the instructions given in the AGDIA kit, with the sensitivity optimum between 0.2 to 100 pmol for *t*-ZR ml^{-1}, 78 and 2,500 pmol for IAA ml^{-1}, and 0.2 to 1,000 pmol for DHZR ml^{-1}. The % binding for each standard point or sample was calculated using the following formula: % Binding=[standard or sample O.D.NSB O.D.]/[Bo O.D.NSB O.D.]×100, where Bo (100% Binding)=100 µl Tracer+100 µl of TBS buffer; NSB (0% Binding) (Non Specific Binding)=100 pmol/0.1 ml+100 µl of tracer. The best-fit sigmoid curve was plotted using the % Binding (B/Bo %) versus the concentration (pmol *t*-ZR, DHZR, and IAA), and the hormone concentration determined by interpolation of the sample values.

Statistical Analysis

The treatments were arranged in a randomized design, and the mean, standard error analysis of variance, and LSD were calculated using SAS package, Version 9.1 [38] with arcsine transformations for normalizing the data.

RESULTS

IAA and Cytokinins Production by *Methylobacterium* Isolates

The characteristics of the *Methylobacterium* strains used in this study are listed in Table 1. A quantitative analysis using the Salkowski reagent of the culture liquids of the methylobacteria grown in the defined medium with L-tryptophan and incubated for 5 days produced significantly different amounts of IAA (P=0.05). The production of IAA by the *Methylobacterium* strains CBMB20 and CBMB110 was 2.33 and 4.03 µg/ml, respectively, in the presence of L-tryptophan. Immunoassays using ELISA kits were also performed to determine the cytokinins produced by the *Methylobacterium* strains. The cytokinins *t*-ZR, iPA, and DHZR were all present at detectable and replicable levels in the cultures tested, with *t*-ZR present in smaller quantities. The total amount of cytokinins recovered from the cultures varied, but strain CBMB20 produced a significantly higher amount than CBMB110.

Gnotobiotic Root Elongation Assay of Tomato and Red Pepper Seeds

The germination percentage and root length of the *Methylobacterium* strain-treated tomato and red pepper seeds were comparatively greater when compared with the uninoculated control. CBMB20 recorded the highest percentage of germination, followed by CBMB110 (Fig. 1A). After ten days in the gnotobiotic growth pouches, the roots from the tomato seeds treated with *Methylobacterium* strains

Table 1. Characteristics of selected isolates of *Methylobacterium* spp. used in the present study.

Strain	Location	Crops/cultivar	Accession number	IAA (µg/ml)[c]	Concentration of cytokinin recovered (ng/l)			
					t-ZR[c]	iPA[c]	DHZR[c]	Total
CBMB20	Cheongwon[a]	Rice/Nampeoung	AY683045	2.33±0.11	47.01±0.45	32.92±1.43	23.40±0.94	103.33±3.45
CBMB110	Milyang[b]	Rice/IlMi	AY683046	4.03±0.20	41.87±1.26	26.23±1.24	15.51±0.82	83.61±0.65

[a]Chungbuk Provincial Agricultural Research and Extension Services, Cheongwon.
[b]National Yeongnam Agricultural Experiment Station, RDA, Milyang.
[c]Data obtained from Madhaiyan *et al.* [24]. Each value represents mean±SE of three replicates. IAA, indole-3-acetic acid; *t*-ZR, *trans*-zeatin riboside; iPA, isopentenyladenosine; DHZR, dihydrozeatin riboside.

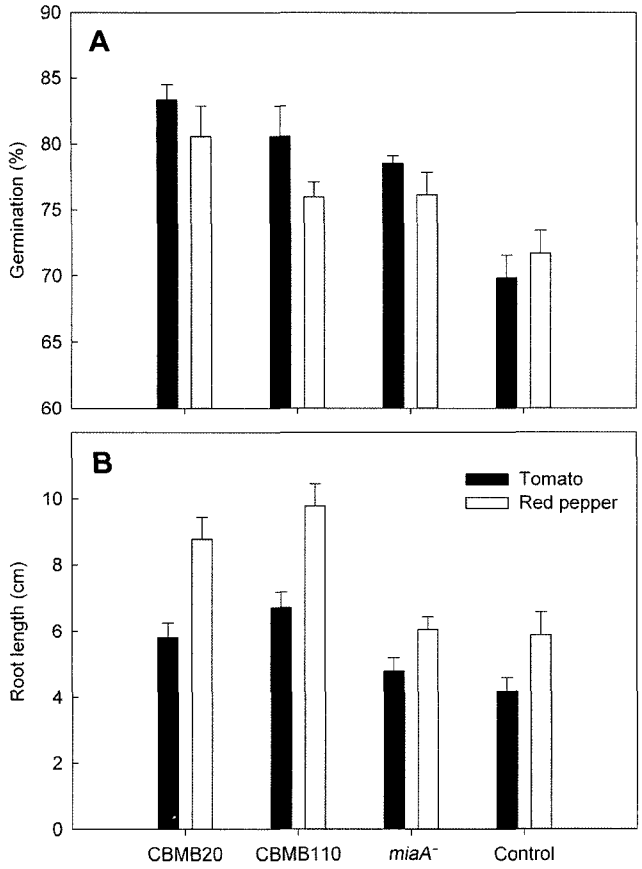

Fig. 1. Effect of *Methylobacterium* spp. inoculation on germination % (**A**) and root length (**B**) under gnotobiotic conditions.
Each value represents±SE, n=3. Mean root lengths of tomato and red pepper seedlings according to root elongation assay based on measurements of 50 seedlings (5 seeds/growth pouch; 10 pouches/treatments).

CBMB20 and CBMB110 showed a significant increase in the root length when compared with either the control or the *miaA*⁻ knockout mutant-treated seeds, recording 5.81 and 6.72 cm, respectively (Fig. 1B). The percentage increase in root length compared with the control was 39.42% when treated with CBMB20, whereas CBMB110 recorded higher increases over the control amounting to 61.25%. Similar results were also obtained with the red pepper seeds, where the *Methylobacterium* treatments produced a significant increase in the root length when compared with either the control or the *miaA*⁻ knockout mutant-treated seeds, based on measurements taken on day 15 of the gnotobiotic assay (Fig. 2). The roots of the *Methylobacterium* strain-treated red pepper seeds were, on average, nearly twice as long as those of the untreated plants, recording a 49.29 and 66.39% increase over the control with CBMB20 and CBMB110, respectively.

Plant Hormone Analysis

The effect of *Methylobacterium* inoculation on plant growth hormones was assessed by detecting IAA and cytokinins

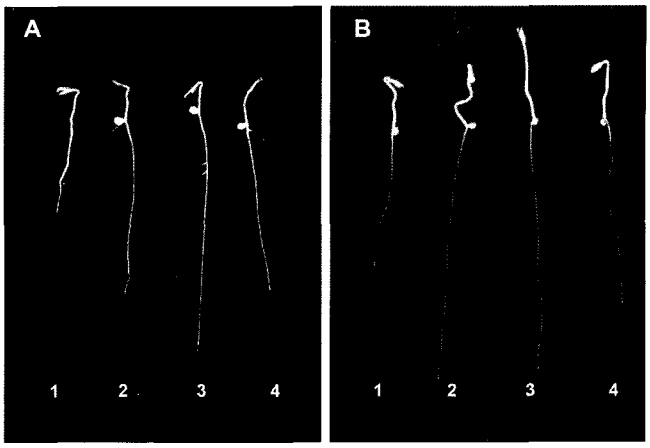

Fig. 2. Response of root systems for red pepper (**A**) and tomato (**B**) seedlings after inoculation with *Methylobacterium* spp.
1, Uninoculated control; 2, *Methylobacterium* sp. CBMB20; 3, *Methylobacterium* sp. CBMB110; 4, *Methylobacterium extorquens miaA*⁻. Photos were taken 3 weeks after gnotobiotic root elongation assay of growth pouch experiments.

in tissue extracts from tomato and red pepper seedlings. The plants were grown under gnotobiotic conditions and the hormones detected by immunoassays using an ELISA kit. For the tomato seedlings, no detectable amounts of IAA were found, although the presence of *t*-ZR and DHZR was recorded. The *Methylobacterium* strains produced significantly higher amounts of *t*-ZR than the control, with CBMB20 recording the highest, amounting to 0.0125 pmol/ g FW. A similar trend was also seen with DHZR, but the differences were not significant (Table 2). The effect of *Methylobacterium* inoculation on the plant growth hormones was more prominent in the red pepper seedlings compared with the tomato seedlings. The amount of IAA in the treated seedlings was significantly different compared with that in the control. *M. extorquens miaA*⁻, which is unable to produce *t*-ZR, produced detectable amounts of IAA, amounting to 56.69 pmol/g FW. The cytokinins in the red pepper tissue

Table 2. Cytokinin accumulation in tomato seedlings inoculated with *Methylobacterium* spp. under gnotobiotic conditions.

Treatment	Concentration of cytokinin derivatives (pmol/g fresh weight)		
	t-ZR	DHZR	Total
Control	0.007 c	0.468 ba	0.475 b
Methylobacterium sp. CBMB20	0.013 a	0.475 ba	0.488 cb
Methylobacterium sp. CBMB110	0.012 b	0.431 b	0.443 c
M. extorquens miaA⁻	0.008 b	0.501 a	0.509 a
LSD ($P \leq 0.05$)	0.001	0.082	0.052

Each value represents the mean of six replicates per treatment. In the same column, significant differences according to the LSD at $P \leq 0.05$ levels are indicated by different letters. *t*-ZR, *trans*-zeatin riboside; DHZR, dihydrozeatin riboside.

Table 3. Auxin and cytokinin accumulation in red pepper seedlings inoculated with *Methylobacterium* spp. under gnotobiotic conditions.

Treatment	IAA (pmol/g FW)	Concentration of cytokinin derivatives (pmol/g FW)		
		t-ZR	DHZR	Total
Control	60.80 c	0.011 b	0.253 c	0.270 c
Methylobacterium sp. CBMB20	61.65 b	0.022 a	0.562 ba	0.584 b
Methylobacterium sp. CBMB110	68.27 a	0.013 c	0.658 a	0.671 a
M. extorquens miaA$^-$	56.69 d	0.012 d	0.511 b	0.523 b
LSD ($P \leq 0.05$)	0.00	0.004	0.097	0.070

Each value represents the mean of six replicates per treatment. In the same column, significant differences according to the LSD at $P \leq 0.05$ levels are indicated by different letters. *t*-ZR, *trans*-zeatin riboside; DHZR, dihydrozeatin riboside; IAA, indole-3-acetic acid.

extract increased with *Methylobacterium* inoculation. The *t*-ZR concentration in the *Methylobacterium*-treated seedlings was significantly increased compared with that in the control and *miaA*$^-$ mutant. The concentration results for DHZR were also similar, although the inverse of *t*-ZR, as CBMB110 produced more than CBMB20 at 0.562 and 0.658 pmol/g FW of DHZR, respectively (Table 3). Thus, the total amount of cytokinins in the seedlings greatly varied according to the treatment, recording significant increases of more than 30% compared with the control.

DISCUSSION

Methylotrophic *Methylobacterium*, commonly called PPFMs, are Gram-negative aerobic rods normally found in the phyllosphere of many plant species. PPFMs are also encountered in the rhizosphere, seeds, and other parts of plants, being versatile in nature, and their role in plant growth promotion has recently attracted interest and been proven in several studies [23, 25, 32]. The advantage for plant-associated methylotrophic bacteria is a rich supply of plant hormones, as most of the metabolic products of the methanol released by plants are lost from leaves during leaf expansion, which is catalyzed by pectin methylesterase [12, 29]. In the present study, the two *Methylobacterium* strains used, CBMB20 and CBMB110, produced significant amounts of IAA, and immunoassays using ELISA kits were performed to determine whether these strains also produced cytokinins. The cytokinins *t*-ZR and DHZR were both present at detectable and replicable levels in the cultures tested, with *t*-ZR present in smaller quantities. Thus, the plant growth promotion observed after inoculation with the *Methylobacterium* spp. may be mainly attributed to the biosynthesis and secretion of cytokinins and IAA [18, 20, 25, 31]. PPFMs synthesize IAA predominantly by an alternate tryptophan-dependant pathway, through indole-3-pyruvic acid [18]; however, the role of bacterial IAA in plant growth promotion remains undetermined.

The promotion of root growth is one of the major markers used to measure the beneficial effect of plant growth-promoting bacteria [13, 19, 21, 27, 33, 35, 37]. The rapid establishment of roots, whether by the elongation of primary roots or proliferation of lateral and adventitious roots, is advantageous for young seedlings, as it increases their ability to anchor themselves in the soil and obtain water and nutrients from their environment, thereby enhancing their chances for survival. Holland [14, 15] reported that PPFMs could be used as a seed inoculum or in seed coatings designed to enhance the germinability, storability, or vigor of seeds.

In the present investigation, gnotobiotic assays were conducted to test the effects of inoculation with *Methylobacterium* strains on root elongation in the case of tomato and red pepper seeds. Following ten days in gnotobiotic growth pouches, the roots from the tomato seeds treated with the *Methylobacterium* strains showed a significant increase in length when compared with either the control or the *miaA*$^-$ knockout mutant-treated seeds. Similar results were also obtained for the red pepper seeds, where the *Methylobacterium* treatment produced a significant increase in the root length when compared with either the control or the *miaA*$^-$ knockout mutant-treated seeds. These results also matched the results of previous studies with rice and sugarcane crops, where treatment with certain cytokinin-producing *Methylobacterium* strains increased growth [23, 25]. The differences recorded between the strains may be related to their ability to promote the early germination of seeds. The results observed for the spent culture media, which promoted better seed germination than the bacterial cells, may have been due to diffusible substances secreted into the medium by the methylotrophic bacteria [20]. However, this requires an understanding of the nature of the substances, other than plant growth hormones, available in the spent culture.

It has already been reported that the production of growth-promoting substances, like IAA and cytokinins, on the phyllosphere contributes to the survival of PPFMs [7, 14, 16, 17, 22, 23]. Thus, in the present study, the amount of IAA and cytokinins in the tissue extracts of the *Methylobacterium*-treated tomato and red pepper seedlings detected using an ELISA immunoassay kit provided support

to the speculative theory proposed by Holland [14] that cytokinins are produced by epiphytic bacteria that rapidly colonize the juvenile plant after germination and are not a product of the metabolism of plant cells.

In the treated tomato seedlings, no detectable amounts of IAA were found, although the presence of *t*-ZR and DHZR was recorded. The effect of *Methylobacterium* inoculation on the plant growth hormones was more prominent in the case of the red pepper seedlings, where significant increases in the amount of IAA were observed, as well as an increase in the amount of cytokinins. Therefore, the total amount of cytokinins in the seedlings varied greatly according to the treatment, recording significant increases of more than 30% compared with the control. Concurrent studies by Butler *et al.* [5] showed that the application of PPFMs to plants produced significantly higher levels of *t*-ZR in plants with high numbers of PPFMs than in plants with small PPFM populations.

Accordingly, the present investigation of the inoculation effects of plant-growth promoting methylotrophic bacteria on tomato and red pepper seeds produced satisfactory results, recording significant increases in plant growth and plant hormone concentrations over the uninoculated control. Therefore, this study proved that the versatility of *Methylobacterium* as a Plant-growth Promoting bacteria could be better exploited.

Acknowledgments

This work was supported by grants from the Rural Development Administration (RDA). M.M also acknowledges support from the Korea Research Foundation (KRF) in the form of financial grants under the Foreign Scientist and Engineers Program, Republic of Korea.

REFERENCES

1. Arshad, M. and W. T. Frankenberger. 1993. Microbial production of plant growth regulators, pp. 307–343. *In* F. B. Metting, Jr. (ed.). *Soil Microbial Ecology. Applications in Agricultural and Environmental Management*. Marcel Dekker, Inc., New York.
2. Barea, J. M. and M. E. Brown. 1974. Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *J. Appl. Bacteriol.* **40:** 583–593.
3. Basile, D. V., L. L. Slade, and W. A. Corpe. 1969. An association between a bacterium and a liverwort, *Scapania nemorosa*. *Bull. Torrey Bot. Club* **96:** 6711–6714.
4. Brown, M. E. 1976. Role of *Azotobacter paspali* in association with *Paspalum notatum*. *J. Appl. Bacteriol.* **40:** 341–348.
5. Butler, H. K., R. Dadson, and M. A. Holland. 2000. Evidence that *trans*-zeatin riboside produced by a microbial symbiont is physiologically meaningful to its host plant. (abstract available at http://abstracts.aspb.org/aspp2000/public /P43/0604.html).
6. Callis, J. 2005. Auxin action. *Nature* **435:** 436–437.
7. Corpe, W. A. and D. V. Basile. 1982. Methanol-utilizing bacteria associated with green plants. *Dev. Indust. Microbiol.* **23:** 483–493.
8. Corpe, W. A. and S. Rheem. 1989. Ecology of the methylotrophic bacteria on living leaf surfaces. *Microbiol. Ecol.* **62:** 243–248.
9. Davies, P. J. 1995. The plant hormone concept: Concentration, sensitivity, and transport, pp. 13–18. *In* P. J. Davies (ed.), *Plant Hormones: Physiology, Biochemistry, and Molecular Biology*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. Dunleavy, J. M. 1988. *Curtobacterium plantarum* sp. nov. is ubiquitous in plant leaves and is seed transmitted in soybean and corn. *Int. J. Syst. Bacteriol.* **39:** 240–249.
11. Dunleavy, J. M. 1990. Urease production by *Methylobacterium mesophilicum*, a seed transmitted bacterium ubiquitous in soybean. Presented at *3rd Biennial Conf. Mol. Cell. Biol. Soybean*, Ames. Iowa, July 23–25.
12. Fall, R. and A. A. Benson. 1996. Leaf methanol - the simplest natural product from plants. *Trends Plant Sci.* **1:** 296–301.
13. Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **41:** 109–117.
14. Holland, M. A. 1997. Occam's razor applied to hormonology. Are cytokinins produced by plants? *Plant Physiol.* **115:** 865–868.
15. Holland, M. A. 1997. *Methylobacterium* and plants. *Rec. Res. Dev. Plant Physiol.* **1:** 207–213.
16. Holland, M. A. and J. C. Polacco. 1992. Urease-null and hydrogenase-null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. *Plant Physiol.* **98:** 942–948.
17. Holland, M. A. and J. C. Polacco. 1994. PPFMs and other contaminants: Is there more to plant physiology than just plant? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45:** 197–209.
18. Ivanova, E. G., N. V. Doronina, and Y. A. Trotsenko. 2001. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70:** 392–397.
19. Katiyar, V. and R. Goel. 2004. Improved plant growth from seed bacterization using siderophore overproducing cold resistant mutant of *Pseudomonas fluorescens*. *J. Microbiol. Biotechnol.* **14:** 653–657.
20. Koenig, R. L., R. O. Morris, and J. C. Polacco. 2002. tRNA is the source of low-level *trans*-zeatin production in *Methylobacterium* spp. *J. Bacteriol.* **184:** 1832–1842.
21. Lee, H. Y., K. H. Park, J. H. Shim, R. D. Park, Y. W. Kim, J. Y. Cho, H. B. Hoon, Y. C. Kim, G. S. Cha, H. B. Krishnan, and K. Y. Kim. 2005. Quantitative changes of plant defense enzymes in biocontrol of pepper (*Capsicium annuum* L.) late blight by antagonistic *Bacillus subtilis* HJ927. *J. Microbiol. Biotechnol.* **15:** 1073–1079.
22. Long, R., R. Morris, and J. Polacco. 1997. Cytokinin production by plant-associated methylotrophic bacteria. *Plant Physiol.* Abstract No. 1168.

23. Madhaiyan, M., S. Poonguzhali, H. S. Lee, K. Hari, S. P. Sundaram, and T. M. Sa. 2005. Pink-pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugarcane clone Co86032 (*Saccharum officinarum* L.). *Biol. Fertil. Soils* **41:** 350–358.
24. Madhaiyan, M., S. Poonguzhali, J. H. Ryu, and T. M. Sa. 2006. Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta* **224:** 268–278.
25. Madhaiyan, M., S. Poonguzhali, M. Senthilkumar, S. Seshadri, H. Y. Chung, J. C. Yang, S. Sundaram, and T. M. Sa. 2004. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by *Methylobacterium* spp. *Bot. Bull. Acad. Sin.* **45:** 315–324.
26. Mok, M. C. 1994. Cytokinins and plant development - an overview, pp. 155–166. *In* M. C. Mok (ed.). *Cytokinins - Chemistry, Activity, and Function*. CRC Press, Boca Raton.
27. Nautiyal, C. S., S. Mehta, and H. B. Singh. 2006. Biological control and plant-growth promotion by *Bacillus* strains from milk. *J. Microbiol. Biotechnol.* **16:** 184–192.
28. Napoli, C. A., C. A. Beveridge, and K. C. Snowden. 1999. Reevaluating concepts of apical dominance and the control of auxiliary bud outgrowth. *Curr. Top. Dev. Biol.* **44:** 127–169
29. Nemecek-Marshall, M., R. C. MacDonald, J. J. Franzen, C. L. Wojciechowski, and R. Fall. 1995. Methanol emission from leaves: Enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development. *Plant Physiol.* **108:** 1359–1368.
30. Nieto, K. F. and W. T. Frankenberger. 1989. Biosynthesis of cytokinins by *Azotobacter chroococcum*. *Soil Biol. Biochem.* **21:** 967–972.
31. Omer, Z. S., R. Tombolini, A. Broberg, and B. Gerhardson. 2004. Indole-3-acetic acid production by pink-pigmented facultative methylotrophic bacteria. *Plant Growth Regul.* **43:** 93–96.
32. Omer, Z. S., R. Tombolini, and B. Gerhardson. 2004. Plant colonization by pink-pigmented facultative methylotrophic bacteria (PPFMs). *FEMS Microbiol. Ecol.* **47:** 319–326.
33. Patten, C. L. and B. R. Glick. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* **68:** 3795–3801.
34. Penrose, D. M. and B. R. Glick. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* **118:** 10–15.
35. Poonguzhali, S., M. Madhaiyan, M. Thangaraju, J. H. Ryu, K. Y. Chung, and T. M. Sa. 2005. Rhizobacteria-based bio-formulations to enhanced growth and yield of pearl millet (*Pennisetum glaucum* (L.) R.Br.) and blackgram (*Vigna mungo* L.). *J. Microbiol. Biotechnol.* **15:** 903–908.
36. Rubery, P. H. 1987. Manipulation of hormone transport in physiological and development studies, pp. 161–174. *In* G. V. Hoad, J. R. Lenton, M. B. Jackson, and R. K. Atkin (eds.). *Hormone Action in Plant Development: A Critical Appraisal.* Butterworths Co. Ltd., Long Ashton, U.K.
37. Ryu, C. M., J. W. Kim, O. K. Choi, S. Y. Park, and S. H. Park. 2005. Nature of a root-associated *Paenibacillus polymyxa* from field-grown winter barley in Korea. *J. Microbiol. Biotechnol.* **15:** 984–991.
38. SAS Institute Inc. 2004. SAS user's guide, Version 9.1. SAS Institute Inc., Cary, North Carolina, USA.
39. Taiz, L. and E. Zeiger. 1998. *Plant Physiology*, 2nd Ed. Sinauer Associates, Inc., Sunderland, MA.
40. Timmusk, S., B. Nicander, U. Granhall, and E. Tillberg. 1999. Cytokinin production by *Paenobacillus polymyxa*. *Soil Biol. Biochem.* **31:** 1847–1852.
41. Woodward, A. W. and B. Bartel. 2005. Auxin: Regulation, action and interaction. *Ann. Bot.* **95:** 707–735.