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Determination of Bacterial Diversity in Bulk Soil and Rhizospheres Using Molecular Fingerprintings and Phenotypic Features

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Abstract: The rationale of this study was to determine effects of root exudates on soil bacterial community within a particular period as well as on genetic diversity and phenotypic features of culturable bacteria. Total DNA was extracted directly from 3 soil samples including bulk soil, *Brassica oleracea* rhizosphere and *Phaseolus vulgaris* rhizosphere. Denaturing gradient gel electrophoresis (DGGE) profiles of PCR-amplified 16S rDNA of these soil samples were identical, suggesting that root exudates were unable to alter bacterial community within the 8-week planting period. As bacterial community could be retained after the enrichment of various compounds present in root exudates, this supports the hypotheses that soil contains a unique bacterial community that can adapt to environments and enrichment can affect only a portion of soil population. Even though plants did not cause detectable changes in bacterial community, they increased the numbers of culturable bacteria. Genetic diversity of bacterial isolates derived from 3 soil samples was investigated using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC PCR). Dendrograms constructed from ERIC PCR profiles showed that genetic diversity of culturable strains from 3 soil samples was similar to one another. The 29 strains were obtained and used for plasmid profile and phenotypic feature studies. The 7 strains habored small plasmids range in number from 1 to 2 and size from 8 to 21 kilobase pairs (kb). The phenotypic features, including utilization of carbon and nitrogen sources, antibiotic resistance, vitamin requirement and production of indole acetic acid (IAA), were indistinguishable among strains from different soils. The effect of soil extracts on the growth of 6 representative bacteria was determined. The results show that soil extracts of both rhizospheres had the same effects on the growth of 6 strains tested, which differed from soil extract of bulk soil.

Key words: Denaturing gradient gel electrophoresis (DGGE) · Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC PCR) · Phenotypic feature · Rhizosphere bacterial community · Soil extract

INTRODUCTION

The rhizosphere refers in general to the portion of soil adjacent to the roots of living plants. It supports a diverse and densely populated microbial community and is subjected to chemical transformations caused by the effect of root exudates and metabolites of microbial degradation [1]. Root exudates contain simple carbon substrates, including primary metabolites, such as sugars, amino acids and organic acids, in addition to a diverse array of secondary metabolites that are released into the rhizosphere and surrounding soil [2]. In contrast, bulk soil is soil outside the influence of plant roots, where carbon is the limiting nutrient for growth [3]. As root exudates are an important source of nutrition for many rhizosphere microorganisms, changes in their composition may affect the patterns and activities of rhizobacterial populations [4]. Effects of root exudates on microbial community and

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diversity have been reported in previous studies. Denaturing gradient gel electrophoresis (DGGE) has commonly been used to analyze microbial community without cultivation. In some cases, plant species has been found as the major factor in determining microbial community [5,6], while in other cases, a significant impact of plant species on microbial community was not observed [7,8]. Besides cultivation-independent methods such as DGGE, cultivation-dependent methods could provide remarkable results as reported by Xu *et al.* [8]. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC PCR) has been employed to estimate genetic diversity of culturable bacteria [9,10]. As root exudates provide a broad range of organic and amino acids, sugars and oligosaccharides [11], it is interested to determine the effects of root exudates on phenotypic features of bacteria. Therefore, in this study we determined the effects of root exudates on bacterial diversity in soils. DGGE profiles of PCR-amplified 16S rDNA of bulk soil, *Brassica oleracea* rhizosphere and *Phaseolus vulgaris* rhizosphere were compared. Bacterial strains isolated from these soil samples were compared for ERIC PCR profiles, plasmid profiles and phenotypic features including utilization of carbon and nitrogen sources, antibiotic resistance, vitamin requirement and production of indole acetic acid (IAA).

MATERIALS AND METHODS

Soil Samples Preparation: Rhizosphere soils were prepared by placing bulk soil into pots. Seeds of *Brassica oleracea* var. *alboglabra* (wild cabbage) and *Phaseolus vulgaris* (common bean) were scarified and surface-sterilized with 3% hydrogen peroxide as described previously [12]. Seeds were germinated on moistened cotton plates at 25°C in the dark for 2 days. The germinated seeds were heavily planted in pots. Rhizospheres and bulk soil were watered regularly and plants were grown for 8 weeks. After the planting period, these soil samples were collected.

Soil DNA Extraction and rDNA Amplification: The total DNA was extracted from soil samples by using a GF-1 soil DNA extraction kit (Vivantis, Malaysia) according to the manufacture's instruction. Total DNA was amplified by PCR with a MyCycler™ Thermal Cycler (Bio-Rad, Hercules, CA). The variable region 3 (V3) within the 16S rDNA (corresponding to positions 341-534 of the 16S rRNA gene in *Escherichia coli*; [13]) were amplified by using a set of primer F341 (5'-CCT ACG GGA GGC AGC AG-3') with the GC-clamp (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G-3') and primer R534 (5'-ATT ACC GCG GCT GCT GG-3'). PCR reactions and conditions were performed as described previously [14]. The amplified products were separated on 1% agarose gels containing ethidium bromide (EtBr) and visualized under UV light.

Denaturing Gradient Gel Electrophoresis (DGGE): The PCR fragments were separated by using DGGE performed with the BioRad DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA). Twenty-five µl of mixed PCR products from each soil sample were applied to 8% polyacrylamide gel with a liner gradient of 25-50 % denaturant (100% denaturant corresponds to 40% [v/v] of formamide plus 7 M of urea). Electrophoresis was performed at 200 V for 4 h at a constant temperature of 60°C. Gels were then stained with GelStar® Nucleic Acid Gel Stain (Cambrex Bio Science, Rockland, ME) for 30 min and visualized.

Isolation and Enumeration of Bacteria in Bulk Soil and Rhizospheres: Bacteria were isolated from soil samples by using a stomacher blender as described by Nimnoi and Pongsilp [10]. Nutrient agar (NA) medium was used for enumeration of total culturable bacteria by the total plate count method. Plates were incubated at 30°C for 2 weeks. The 15 single colonies were selected from each soil and pure cultures were maintained on NA slants at 4°C.

ERIC PCR Fingerprintings of the Isolated Bacteria: Total of 45 bacterial isolates were selected from 3 soil samples to investigate genetic diversity based on ERIC PCR fingerprints. Genomic DNA was extracted from exponentially grown culture by boiling cell pellets in deionized (DI) water. The PCR reactions were carried out using a pair of primers ERIC2 (5' AAG TAA GTG ACT GGG GTG AGC G 3') and ERIC1R (5' ATG TAA GCT CCT GGG GAT TAC 3') as described by Versalovic *et al.* [15]. Negative controls (no DNA added) were included in all sets of reactions. The presence and size of the amplified fragments were determined by 1% agarose gel electrophoresis and unweighted pair groups using mathematical averages (UPGMA) dendrograms were constructed using the Image Master 1D Elite Software version 5.20 (Amersham, UK). The strains with different ERIC PCR profiles were selected for determination of plasmid profiles and phenotypic features.

Plasmid Profiles of the Isolated Bacteria: The selected strains were examined for the presence of small plasmids. Small plasmids were extracted from exponentially grown culture by using a GF-1 plasmid extraction kit (Vivantis, Malaysia) according to the manufacture's instruction. The presence and size of small plasmids were determined by 1% agarose gel electrophoresis.

Determination on Phenotypic Features of the Isolated Bacteria: The selected strains were determined for the following features: 1) utilization of 30 compounds (each at a concentration of 0.1% w/v) as sole carbon sources; 2) utilization of 20 compounds (each at a concentration of 0.1% w/v) as sole nitrogen sources; 3) resistance to 10 antibiotics; 4) requirement for 10 vitamins and 5) production of IAA. Utilization of compounds as sole carbon and nitrogen sources was performed on basal medium [16] without yeast extract and agar was replaced with agarose by the method described elsewhere [17]. To test antibiotic resistance, bacterial cultures were spread on NA plates and antibiotic discs (Oxoid, Basingstoke, UK) were placed on the agar surfaces. Antibiotic susceptibility was observed as clear zones around antibiotic discs. Requirement for vitamins was assessed as described by Watson *et al.* [18] on Bergersen's synthetic medium (BSM) [19] in which thiamine and biotin were replaced with various vitamins and agar was replaced with agarose. For measurement of IAA, strains were propagated in Tris-TMRT broth [20]. The amounts of IAA produced by strains were determined by the colorimetric assay [21]. All tests were conducted in 3 replicates.

Determination of Effects of Soil extracts on the Growth of Representative Bacteria: One strain of each gram-positive bacteria and gram-negative bacteria from each soil sample were selected as representative strains to examine effects of soil extracts on bacterial growth. Soil extracts were prepared from each soil sample. Ten grams of soil was suspended in 100 ml of sterile DI water. Soil suspension was shaken at 180 rpm for 30 min and centrifuged at 600 rpm for 10 min. The supernatant was sterilized by membrane filtration. The volume 500 µl of soil extract was added into 5 ml of BSM broth. Each strain was grown at 30°C at 200 rpm for 1-5 days and used as inoculum. The total cell numbers of inoculum were examined by the total plate count method. The desired amount of inoculum was inoculated into BSM broth with and without soil extract. The initial cell density of each strain was 1.00×10^5 colony forming unit (CFU)/ml. The cultures were shaken at 200 rpm for 1-5 days. The cell numbers were measured by the total plate count method.

Statistical Analysis: The standard deviation was calculated using Microsoft Excel 2003 software.

RESULTS AND DISCUSSION

DGGE Community Fingerprinting of Bulk Soil and Rhizospheres: To obtain the total bacterial community without cultivation, total DNA was directly extracted from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere. PCR-DGGE was performed by amplification of V3 region of 16S rDNA. Amplification and electrophoresis of samples were performed in three replicates. As displayed in Fig. 1, three replicates of bulk soil and two rhizospheres generated identical DGGE patterns, suggesting that root exudates were unable to alter bacterial community and diversity within the planting period. The patterns exhibited the dominant species and the intensity of each band indicated its relative abundance. The patterns consisted of only one stronger band and several less intensive bands. These results indicated that these soil samples consisted of only one dominant ribotypes, whereas other ribotypes which varied in relative band intensity seemed to be equally abundant.

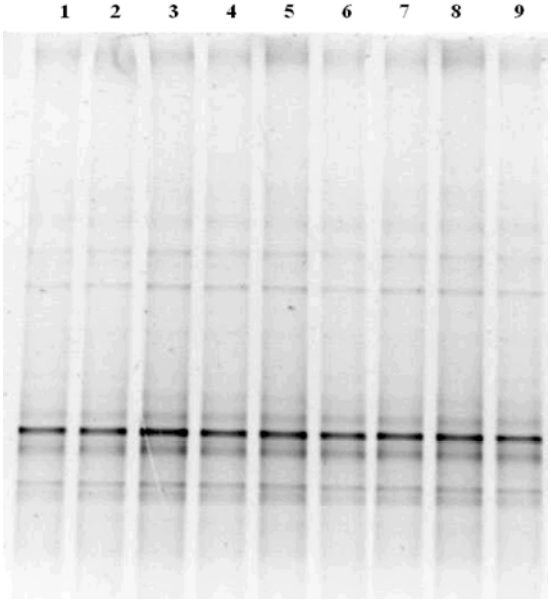

Fig. 1: DGGE patterns of bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere. Lane 1-3, 3 replicates of bulk soil; 4-6, 3 replicates of *B. oleracea* rhizosphere; 7-9, 3 replicates of *P. vulgaris* rhizosphere.

In this study we intended to determine effects of root exudates on bacterial community and diversity in soils apart from soil type effect, therefore a bulk soil was used to prepare rhizospheres. The result showed that root exudates of *B. oleracea* and *P. vulgaris* were unable to cause variability in bacterial community within 8 weeks. The planting period is considered as one of major factors in determining bacterial community structure in soils. As also reported in the previous studies, root exudates of tomato had no effect on bacterial community structure, while arbuscular mycorrhizal (AM) fungi induced significant changes within a 6-week period [22]. Three grain legumes induced significant differences in bacterial community structure, indicating plant dependent rhizosphere effects, after 5 months of planting [23].

Isolation and Enumeration of Bacteria in Bulk Soil and Rhizospheres: Bacterial numbers obtained from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere were $9.47 \times 10^6 \pm 1.41 \times 10^6$, $4.50 \times 10^8 \pm 3.26 \times 10^8$ and $2.57 \times 10^7 \pm 5.20 \times 10^6$ CFU/g soil, respectively. The numbers of total culturable bacteria were significantly higher in both rhizosphere soils than that of bulk soil, suggesting that plants contributed to an increased bacterial abundance. Root exudates and rhizodeposition are important sources of nutrition that promote bacterial growth. Pure cultures of 15 isolates were obtained from each soil sample and examined by ERIC PCR fingerprintings. These isolates were designated by abbreviations: BUL, RBO and RPV are used to refer to the isolates obtained from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere, respectively.

ERIC PCR Fingerprinting of the Isolated Bacteria: The 14, 14 and 10 ERIC PCR fingerprints were generated from isolates derived from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere, respectively. The amplified fragment ranged between <500 bp to 3,000 bp in size. These isolates can be identified as the individual strains because they generated specific individual patterns. The dendrograms constructed from ERIC PCR fingerprints of strains obtained from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere are shown in Figs. 2-4, respectively. The genetic diversity of the selected strains from 3 soil samples was similar to one another (approx. 10% similarity). The 10, 10 and 9 strains from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere, respectively, were selected as representative strains for each cluster and used for examination of plasmid profiles and phenotypic features.

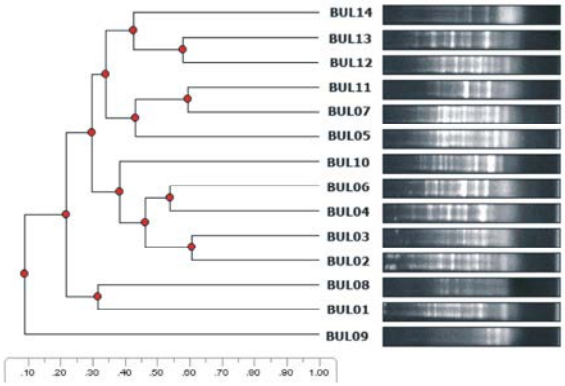

Fig. 2: Dendrogram generated from ERIC PCR fingerprints of the selected bacteria from bulk soil.

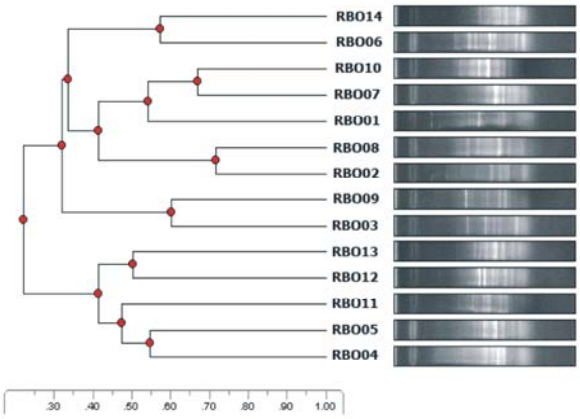

Fig. 3: Dendrogram generated from ERIC PCR fingerprints of the selected bacteria from *B. oleracea* rhizosphere.

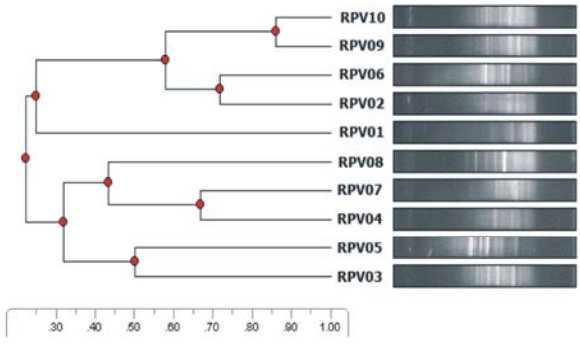

Fig. 4: Dendrogram generated from ERIC PCR fingerprints of the selected bacteria from *P. vulgaris* rhizosphere.

Plasmid Profiles of the Isolated Bacteria: Among 29 bacterial strains, 4, 2 and 1 strains obtained from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere, respectively, harbored small plasmids. The strains from bulk soil BUL 02 and BUL 05 harbored one small plasmid

Table 1: Summary of phenotypic features of the selected strains obtained from bulk soil, *B. oleracea* rhizosphere and *P. vulgaris* rhizosphere

	Bacterial strains from		
Phenotypic features	bulk soil (n=10)*	*B. oleracea* rhizosphere (n=10)*	*P. vulgaris* rhizosphere (n=9)*
Utilization of C sources:			
D-glucose	+	+	+
glycerol and sucrose	+	+/-	+
arabinose, aspartic acid, isoleucine, leucine, malic acid,			
D-maltose, phynylalanine, serine and D-xylose	+/-	+/-	+/-
cellobiose, D-fructose, D-galactose, mannitol,			
D-sorbitol and trehalose	+/-	+/-	+
alanine, casein and proline	+/-	+/-	-
valine	+/-	-	+/-
sorbose and potassium acetate	+/-	-	-
lactose	-	+/-	+/-
xylitol	-	+/-	-
methionine and succinic acid	-	-	+/-
tartaric acid and threonine	-	-	-
Utilization of N sources:			
ammonium dihydrogen orthophosphate	+	+	+
L-asparagine, L-histidine, leucine, DL-lysine,			
L-ornithine, serine, DL-tryptophan and valine	+/-	+/-	+/-
L-tyrosine	+	+/-	+/-
DL-alanine, L-alanine, DL-phenyl alanine, proline and DL-threonine	+/-	+/-	-
DL-aspartic acid and ammonium dihydrogen nitrate	-	+/-	-
cysteine	-	-	+/-
galacturic acid and L-glutamic acid	-	-	-
Tolerance to antibiotics:			
ampicillin (10 µg)	+/-(4)	+/-(6)	+/-(2)
spectinomycin (10 µg)	+/-(2)	+/-(4)	+/-(1)
cefotaxine (30 µg)	+/-(2)	+/-(3)	-
chloramphenicol (30 µg)	+/-(2)	+/-(7)	-
gentamycin (120 µg)	+/-(1)	+/-(1)	-
kanamycin (30 µg)	+/-(1)	+/-(1)	-
streptomycin (10 µg)	+/-(1)	+/-(1)	-
ceftazdime (30 µg)	+/-(3)	-	-
tetracycline (30 µg)	+/-(1)	-	-
novobiocin (30 µg)	+/-(2)	-	-
Vitamins requirement:			
aminobenzoic acid (vitamin B), thiamine hydrochloride (vitamin B1), riboflavin (vitamin B2), nicotonic acid (vitamin B3), panthothenate (vitamin B5), pyridoxine hydrochloride (vitamin B6), biotin (vitamin B7), myo-inositol (vitamin B8), folic acid (vitamin B9) and cyanocobalamin (vitamin B12)	-	-	-
Production of IAA:			
Amount of IAA produced (µg/ml)	0.15 ± 0.08 to 12.60 ± 0.15	0.87 ± 0.37 to 27.50 ± 2.08	0.12 ± 0.02 to 4.66 ± 0.17
Average amount of IAA produced per strain (µg/ml)	2.47 ± 0.02	4.06 ± 0.18	1.26 ± 0.03

*n = number of strains tested

Utilization of C and N sources: +, all strains utilized C or N sources;-, none strains utilized C or N sources; +/-some strains utilized C or N sources

Tolerance to antibiotics: +/-, some strains were resistant to antibiotics (numbers in parentheses indicate numbers of resistant strains);-, none strains were resistant to antibiotics

Requirement for vitamins:-, none strains required vitamins

Table 2: Cell numbers of 6 representative strains in BSM medium supplemented with and without soil extracts

The origins of strains	Strains tested	Cell numbers (CFU/ml)* of strains in BSM			
		without soil extract	with soil extract of bulk soil	with soil extract of *B. oleracea* rhizosphere	with soil extract of *P. vulgaris* rhizosphere
Bulk soil	BUL 02 (gram negative)	$5.10\times10^{7}\pm8.78\times10^{6}$ a	$4.40\times10^{7}\pm6.00\times10^{6}$ a	$1.10\times10^{8}\pm5.17\times10^{6}$ b	$2.29\times10^{8}\pm9.10\times10^{7}$ c
	BUL 09 (gram positive)	$1.13\times10^{8}\pm1.37\times10^{7}$ b	$6.10\times10^{7}\pm3.57\times10^{6}$ a	$9.50\times10^{7}\pm4.50\times10^{6}$ b	$1.50\times10^{8}\pm9.41\times10^{7}$ b
B. oleracea rhizosphere	RBO 06 (gram negative)	$2.60\times10^{7}\pm4.40\times10^{6}$ a	$2.04\times10^{8}\pm9.62\times10^{6}$ c	$2.30\times10^{8}\pm2.20\times10^{7}$ c	$4.10\times10^{7}\pm1.53\times10^{6}$ b
	RBO 05 (gram positive)	$1.80\times10^{8}\pm2.11\times10^{6}$ d	$4.80\times10^{7}\pm3.19\times10^{6}$ b	$1.17\times10^{8}\pm2.97\times10^{7}$ c	$7.24\times10^{6}\pm9.34\times10^{5}$ a
P. vulgaris rhizosphere	RPV 05 (gram negative)	$2.31\times10^{8}\pm8.05\times10^{6}$ b	$2.17\times10^{8}\pm2.11\times10^{7}$ b	$4.30\times10^{7}\pm5.20\times10^{6}$ a	$3.90\times10^{7}\pm4.74\times10^{6}$ a
	RPV 02 (gram positive)	$2.10\times10^{7}\pm4.11\times10^{6}$ a	$5.60\times10^{7}\pm4.02\times10^{6}$ b	$5.30\times10^{7}\pm5.06\times10^{6}$ b	$1.38\times10^{8}\pm9.90\times10^{6}$ c

* The values shown are means of three replicates ± standard deviation. Means follows by the same letter in a row are not significantly different.

with a size of 9 kb, BUL 09 harbored one plasmid with a size of 8 kb, BUL 04 harbored 2 plasmids with sizes of 9 kb and 21 kb. The strains from *B. oleracea* rhizosphere RBO 02 harbored one plasmid with a size of 18 kb, RBO 05 harbored one plasmid with a size of 15 kb. The strains from *P. vulgaris* rhizosphere RPV 10 harbored one plasmid with a size of 13 kb (Figure not shown).

Determination on Phenotypic Features of the Isolated Bacteria: The phenotypic features of the isolated bacteria are summarized in Table 1. Twenty nine strains from different soil samples could not be distinguished from each other based on the phenotypic features including utilization of carbon and nitrogen sources, antibiotic resistance, vitamin requirement and production of IAA. Of 30 carbon sources tested, D-glucose was the only one compound that all strains from 3 soil samples were able to utilize as a sole carbon source. None could utilize tartaric acid and threonine as sole carbon sources. Of twenty nitrogen sources tested, ammonium dihydrogen orthophosphate was the only one compound that all strains were able to utilize as a sole nitrogen source. None could utilize galacturic acid and L-glutamic acid as sole nitrogen sources. Root exudates are known to be an important source of nutrition for many rhizosphere microorganisms [4] because they contain simple carbon substrates, including primary metabolites, such as sugars, amino acids and organic acids, as well as a diverse array of secondary metabolites [2]. Changes in their composition may affect the patterns and activities of rhizobacterial populations [4]. The strains from bulk soil exhibited a higher level of antibiotic resistance than the strains from rhizosphere soils. Of ten antibiotic tested, two strains from bulk soil were susceptible to all antibiotics. Eight strains from bulk soil were resistant to 1-7 antibiotics. Two strains from *B. oleracea* rhizosphere were susceptible to all antibiotics. Eight strains of *B. oleracea* rhizosphere were resistant to 1-5 antibiotics. Seven strains from *P. vulgaris* rhizosphere were susceptible to all antibiotics. Two strains of *P. vulgaris* rhizosphere were resistant to 1-2 antibiotics. All strains from *B. oleracea* rhizosphere were susceptible to three antibiotics including tetracycline, ceftazdime and novobiocin. All strains from *P. vulgaris* rhizosphere were susceptible to eight antibiotics including tetracycline, ceftazdime, novobiocin, cefotaxine, chloramphenicol, gentamycin, kanamycin and streptomycin. While, no antibiotic that all strains from bulk soil were susceptible. However, the strains from different soils were indistinguishable from each other based their antibiotic resistance patterns. None of the antibiotics tested were found as selective substances in soil samples since there was no antibiotic to which all strains derived from the same soil were resistant. All strains from 3 soil samples were vitamin prototrophs since they could grow in the absence of vitamins. Vitamins have been found to be important growth factors for soil bacteria and presented in root exudates [24]. Even though all 29 strains could grow on the minimal medium lacking any vitamins, the growth of 14 strains was enhanced when some vitamins were added. All vitamins tested except cyanocobalamin and pyridoxine hydrochloride enhanced the growth of at least one bacterial strain. Nicotinamide was the one that could enhance the growth of most strains (7 strains). The strains varied in the ability to produce IAA. The average amount of IAA produced per strain was highest in strains from *B. oleracea* rhizosphere (4.06 ± 0.18 mg IAA/ml supernatant), followed by bulk soil (2.47 ± 0.02 mg IAA/ml supernatant) and *P. vulgaris* rhizosphere (1.26 ± 0.03 mg IAA/ml supernatant), respectively.

Determination of Effects of Soil Extracts on Growth of Representative Bacteria: Effects of soil extracts on bacterial growth were examined with one representative strain of each gram-positive bacteria and gram-negative bacteria from each soil sample. Cell numbers of 6 strains grown in a minimal medium, BSM, with and without soil extracts are shown in Table 2. Soil extract of bulk soil had

the inhibitory effect on the growth of two strains (BUL 09 and RBO 05), the stimulatory effect on the growth of two strains (RBO 06 and RPV 02) but did not cause significant changes in cell numbers of the remaining strains (BUL 02 and RPV 05). Soil extracts of both rhizospheres had the same effects on the growth of 6 strains tested. Both soil extracts had the inhibitory effect on the growth of two strains (RBO 05 and RPV 05), the stimulatory effect on the growth of three strains (BUL 02, RBO 06 and RPV 02) but did not cause significant change for BUL 09. As root exudates contain both bacterial stimulators and inhibitors that are released into rhizosphere soils. These compounds are likely to be present in soil extracts that vary depending on plant species. Effects of soil extracts on bacterial growth have been studied in previous reports. The growth of *Bacillus cereus* could be supported by filter-sterilized soil-extracted soluble organic matter, but not by humic substances alone [25]. In contrast, the inhibitory activity of crude bean root extracts on the growth of several polysaccharide mutants of *Rhizobium etli* was found [26]. In this study, both stimulatory effect and inhibitory effect of soil extracts on bacterial growth were observed due to the difference in composition of stimulators and inhibitors present in soil extracts as well as nutritional requirement and tolerance to inhibitors of each strain. Moreover, soil extracts of both rhizospheres had the same effects on the growth of 6 strains tested, suggesting similar composition in root exudates from both plant species.

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