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Bacterial Community Variations in Hot Pepper-Sown Soil Using FAME Analysis as an Indicator of Soil Quality

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Abstract The bacterial compositions of seven hot-pepper sown soil were compared in this study. From the 624 isolates, 95 species and 49 genera were identified by fatty acid methyl ester analysis (FAME). The FAME results of seven soil showed two distinct clusters for aerobic and Gram-negative bacteria in the high productivity and monoculture soil samples. While *Arthrobacter* (17%), *Kocuria* (11%), *Pseudomonas* (8%), and *Bacillus* (8%) were predominant among bacteria which were cultured on heterotrophic (YG) agar medium, *Pseudomonas* (58%), *Stenotrophomonas* (16%), and *Burkholderia* (8%) were predominant on crystal violet agar medium. Shannon-Weaver indices (H) indicated that colonies obtained from heterotrophic agar medium (3.1) were found to be more diverse than those obtained from the crystal violet media (1.4). The results suggest that FAME analysis may be a potential indicator of soil quality.

Key words: Bacterial community, FAME, soil quality, diversity

It is important to sustain agro-ecosystems with high soil quality and productivity. Intensive agriculture like monoculture cropping often leads to the deterioration of soil quality because cropping requires added nutrients and pesticides, which often result in fertilizer overdose and salt accumulation, and in turn may lead to poor soil structure. Monocropping has also been known to increase the frequency of plant disease by the pathogens that are selected on the successive cultivation of specific crops [4, 22].

The common approaches to identify soil quality mainly through the use of soil physical and chemical parameters do not consider how the soil biological component may affect the health of its crop [4, 5, 22, 23]. Community-level microbial interactions are complex, with individual species relying on the presence, function, and interaction of many species. Quantitative and qualitative changes in the composition of soil microbial communities may serve as an important and more sensitive indicator of changes in the soil quality [6, 7, 8, 17, 18, 19]. Elliott *et al.* [5] and Turco *et al.* [22] addressed the need for assessing the microbial component that is relevant to soil quality, since soil microorganisms are potentially one of the most sensitive biological markers available. In particular, they discussed methods to address the size and diversity of microbial communities in different soil samples. Methods for assessing soil biological quality include measurements of microbial biomass [24], characterization of soil bacterial composition by using dilution plating [10], measurements of soil enzyme and microbial activity [3], incidence of soilborne diseases [23], and the use of nucleic acid profiles [21] with FAME [15]. However, the relationship between soil biological properties and soil quality remains unclear. All methods based on the isolation of bacteria from soil and environmental samples have inherent limitations, but they are still useful for providing fundamental information on the abundance of culturable bacterial species which are present in the microbial community [6, 9, 21, 26]. Using cluster and factor analysis to determine the relatedness of microbial communities, it may also be possible to link bacterial community compositions not only to crop health, but to physical and chemical factors which are associated with soil quality [22]. The ability to identify bacterial species has also been greatly simplified by the availability of fatty acid analysis procedures that rely on differences in the fatty acid composition of various bacterial species [5, 7, 10, 12, 15, 16, 19, 20, 22]. Herein, we report the use of FAME to identify soil bacterial species which were associated with soil having different background in cultivation.

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MATERIALS AND METHODS

Soil Sampling

Soil samples from each field sown with hot pepper (*Capsicum annum* L.) were taken from 10 cm below the surface. We sampled two high productivity soils (Umsong H, Goesan H soil), a greenhouse soil (Jinju G soil), and four monoculture soils (Suwon M, Umsong M1, Umsong M2, and Goesan M soil). High productivity soil was characterized as soil that consistently produced high crop yields without showing any plant disease. On the other hand, the monoculture soil that was selected had variable yields with low soil chemical and physical qualities. The greenhouse soil was classified as highly productive because the farmer had maintained soil quality effectively every year by using regular organic matter inputs and leaching of accumulated salt with water. Suwon M soil is from a field at the Crop Protection Experiment Station, RDA (Korea), which had examined hot pepper plant in relation to plant pathology regularly. The sites were sampled once at Suwon, Umsong, Goesan, and Jinju, Korea in April, 2000. At these locations, the soil was preserved at 4°C prior to microbial and chemical analyses. Soil samples from each site were combined and passed through a 2-mm sieve. Soil chemical properties of each soil are presented in Table 1.

Enumeration of Bacterial Populations

Dilution plating on various media was conducted and undertaken to determine microbial counts. For all experiments reported herein, we cultured aerobic bacteria on yeast-glucose (YG) agar (yeast extract, 3.0 g; glucose, 1.0 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; cycloheximide, 0.05 g; agar, 15 g; distilled water, 1 l; pH 6.8) and spore forming bacteria on YG agar after undergoing heat treatment for 10 min at 80°C. Gram-negative bacteria were cultured on modified YG medium (5 ml of 0.1% crystal violet added to 1 l of YG). *Actinomycetes* were grown on humic acid vitamin (HV) agar, and fungi were grown on Rose-Bengal agar [26]. Average CFU (colony-forming units) values were obtained from triplicate plate counts.

Fatty Acid Methyl Ester (FAME) Analysis

FAME was conducted on a total of 624 bacterial strains isolated on YG and crystal violet media. The aerobic bacterial isolates were randomly selected from soil of Suwon M (35 isolates), Umsong M1 (44 isolates), Umsong M2 (39 isolates), Umsong H (39 isolates), Goesan M (34 isolates), Goesan H (51 isolates), and Jinju G (40 isolates), and 50, 53, 48, 51, 46, 43, and 51 isolates of Gram-negative bacteria were selected from the seven soil samples, respectively. The isolates were subsequently subcultured three times on 10% TSA (Tryptic Soy Broth Agar) plates at 28°C for obtaining the pure culture. For the FAME analysis, bacterial colonies were sampled from a single plate at the same dilution for all samples of each soil, and after 24 h of growth, a loopfull of late-log-phase cells were harvested. Fatty acids were extracted and methylated according to the procedure described by the manufacturer (Microbial ID, Inc., Newark, Del, U.S.A.). Extracted samples were analyzed by using a Sherlock Microbial Identification System with a Hewlett-Packard 6890A gas chromatography (Palo Alto, CA, U.S.A.) [7, 10, 15, 19].

Bacterial Diversity Analysis

Bacterial diversity analyses were performed as previously described [1, 7, 19].

Statistical Analysis

Significant differences ($P<0.05$) among the bacterial populations of the seven soil samples were determined by using ANOVA (Minitab, State College, PA, U.S.A.). Based on the frequency of isolates from each sample, community similarities were analyzed by cluster analysis by using the single linkage method with Euclidean distance measure for making a determination of variables between clusters (Minitab, State College, PA, U.S.A.) (Fig. 2).

Table 1. Chemical properties of hot-pepper plant sown soils.

Soil	pH 1:5	OM gkg^{-1}	EC dSm^{-1}	P_2O_5 $mgkg^{-1}$	Exchangeable Cation				Cd	Cr	Cu	Ni	Pb	Zn	Texture
					Ca	K	Mg	Na							
					$cmol+kg^{-1}$				$mgkg^{-1}$						
Suwon M[1]	5.4	13	0.20	549	3.6	0.35	0.74	0.28	0.08	0.22	8.05	0.60	2.7	6.8	sandy loam
Umsong M1[2]	5.7	14	0.20	707	3.8	0.36	0.91	0.30	0.03	0.21	12.20	0.34	2.5	5.8	sandy loam
Umsong M2[3]	7.0	22	3.55	836	13.8	0.42	3.42	0.75	0.07	0.50	3.33	1.00	1.1	11.5	sandy loam
Umsong H[4]	6.8	17	1.35	583	8.7	0.13	2.03	0.66	0.02	0.15	2.37	0.40	1.6	12.6	sandy loam
Goesan M[5]	4.5	13	2.52	541	5.7	0.27	1.63	0.33	0.03	1.29	2.31	1.21	2.4	6.0	sandy loam
Goesan H[6]	5.9	16	0.89	776	10.8	0.30	3.70	0.42	0.04	0.75	4.18	0.99	1.9	6.1	loam
Jinju G[7]	6.7	25	0.35	888	8.9	0.67	1.77	0.30	0.14	1.10	11.03	1.24	0.9	65.3	sandy loam

High productivity soils - Umsong H soil[4], Goesan H soil[6].
Monoculture soils - Suwon M soil[1], Umsong M1 soil[2], Umsong M2 soil[3], Goesan M soil[5].
Green house soil - Jinju G soil[7].

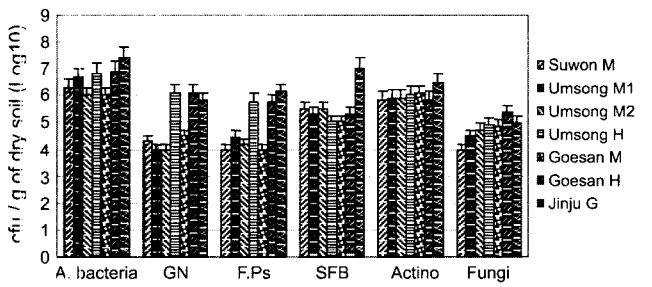

Fig. 1. Bacterial population analysis of hot pepper high productivity and monoculture soil samples.
A. Bacteria, aerobic bacteria; GN, Gram-negative bacteria; F. Ps, fluorescent *Pseudomonas*; SFB, spore-forming bacteria; Actino, *Actinomycetes*; Fungi, fungi.

RESULTS AND DISCUSSION

Soil Properties

As shown in Table 1, the Umsong M1 soil had the highest EC level (electrical conductivity, 3.55 ds/m), the Goesan M soil the lowest pH (4.5), and Jinju G soil the highest zinc concentration level (65.3 mg/kg) among the seven soil samples. The high zinc level in Jinju G (greenhouse) soil might have been due to the primary minerals, amendment with zinc-containing sewage sludge, and overfertilization with zinc.

Bacterial Community of the Seven Soil Samples

In this study, a total of 624 isolates which consisted of 282 isolates from YG medium and 342 isolates from crystal violet medium were identified by the FAME analysis. Among them, 26 isolates (7.6%) could not be grouped into any major bacterial division. The distribution of the isolates obtained from each individual soil samples are presented in Table 2. High G+C Gram-positive bacteria were predominant among total aerobic bacteria regardless of soil types. On the other hand, the gamma *Proteobacteria*

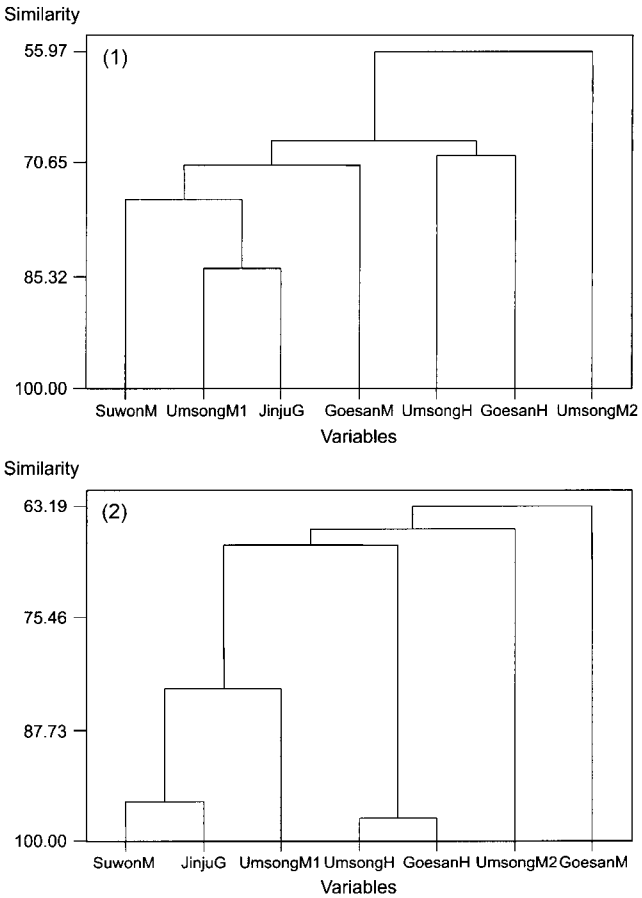

Fig. 2. Cluster analysis of isolates from hot pepper high productivity and monoculture soil samples.
(1) Aerobic bacteria, (2) Gram-negative bacteria.

subdivision was predominant among Gram-negative bacteria in 6 of the 7 soil samples (Table 2). In Table 3, *Pseudomonas* spp. were represented by several species, including *P. chlororaphis*, *P. fluorescens*, *P. putida*, and *P. syringae*. Each *Rodococcus* and *Variovorax* represented 2

Table 2. Major bacterial division distribution among isolates from high productivity and monoculture hot pepper-sown soil samples.

Division	No. of aerobic bacteria isolates[a]								No. of Gram -negative bacteria isolates[b]							
	Suwon M	Umsong M1	Umsong M2	Umsong H	Goesan M	Goesan H	Jinju G	Total	Suwon M	Umsong M1	Umsong M2	Umsong H	Goesan M	Goesan H	Jinju G	Total
High G+C[1]	20	34	11	22	21	17	23	148	0	0	1	0	0	0	0	1
Low G+C[2]	9	8	9	4	6	7	11	54	0	0	0	0	0	0	0	0
Pro-alp[3]	0	0	0	1	0	6	0	7	2	0	1	1	0	2	2	8
Pro-bet[4]	0	0	0	1	4	2	2	9	2	3	0	1	32	3	3	44
Pro-gam[5]	3	0	18	9	0	9	2	41	46	49	35	48	2	37	45	262
CFB[6]	0	0	0	2	0	7	0	9	0	1	0	0	0	0	0	1
Deinoco[7]	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
No match[8]	2	2	1	0	3	3	2	13	0	0	11	1	12	1	1	26
Total	35	44	39	39	34	51	40	282	50	53	48	51	46	43	51	342

[1]High G+C: High G+C Gram-positive bacteria; [2]Low G+C; Low G+C Gram-positive bacteria; [3]Pro-alp; alpha *Proteobacteria*; [4]Pro-bet; beta *Proteobacteria*.
[5]Pro-gam; gamma *Proteobacteria*; [6]CFB; *Cytophaga-Flexibacter-Bacteroides*; [7]Deinoco; *Deinococcus*; [8]No match; unidentified.
[a]Bacterial isolates cultured on YG media.
[b]Bacterial isolates cultured on crystal violet media.

Table 3. Chemical identification of soil quality, diversity index (H), and dominant bacteria.

Soil	Soil quality	Diversity Index (H)		Dominant bacteria	
		YG[1]	CV[2]	YG	CV
Suwon M	Low pH	3	1.6	*Kocuria, Bacillus*	*Pseudomonas***
Umsong M1		2.5	1.3	*Arthrobacter**, *Kocuria*	*Pseudomonas****
Umsong M2	High EC	2.7	1.7	*Pseudomonas**	*Pseudomonas***
Umsong H		2.4	1.6	*Arthrobacter, Stenotrophomonas*	*Stenotrophomonas***, *Pseudomonas*
Goesan M	Low pH	2.6	1.9	*Cellulomonas*	*Burkholderia***
Goesan H		3	1.6	*Arthrobacter, Flavobacter*	*Stenotrophomonas***, *Pseudomonas**
Jinju G		3.1	1.4	*Bacillus, Arthrobacter*	*Pseudomonas****

[1]YG, yeast-glucose agar; [2]CV, yeast-glucose agar added crystal violet.
*30-50%; **50-80%; ***more than 80%; no asterisk: less than 30%.

bacterial species composition for culturable bacteria from each soil. No identified isolates were found for Umsong M2 soil (11 isolates, 22%) and Goesan M soil (12 isolates, 27%).

In Goesan M soil, *Burkholderia* was the dominant genus (26 isolates), *Ralstonia* was represented by 5 isolates, and *Pseudomonas* was not present. Twenty-nine isolates (58%) from Umsong H soil and 24 isolates (56%) from Goesan H soil were identified as *Stenotrophomonas*, a predominant genera of these two soils. Thus, it was supposed that *Stenotrophomonas* is a potential indicator of soil quality. ANOVA confirmed the differences ($P<0.05$) in the occurrence of aerobic bacteria and Gram-negative bacteria in Umsong H and Goesan H soil, as compared to other soil.

Relationships between Bacterial Communities and Soil Properties

Regarding the relationship of soil properties and bacterial community composition, Umsong H soil showed a high frequency level of some genera, including *Arthrobacter, Stenotrophomonas*, and *Pseudomonas* (Table 3). In contrast, the adverse soil properties of the Umsong M2 (high EC) and Goesan M (low pH) soils were reflected by the clustering pattern of the microorganisms which were associated with each soil (Fig. 2). In Fig. 2, Umsong M2 soil deviated from the main group for both YG and crystal violet media. As shown in Fig. 1, the aerobic bacteria and fluorescent *Pseudomonas* were dominant, whereas spore-forming bacteria had a relatively low population size. On comparison with the other soil, Umsong M2 soil was different in aerobic bacterial composition and Goesan M soil in Gram-negative bacterial composition (Fig 2, Table 2). It is also common to observe a reduction of diversity on low pH [24], high EC [7, 8], herbicide treatment [25], and high concentration level of chemical substances such as heavy metals [2, 11].

On a cluster analysis, the (YG) bacteria obtained from Umsong H soil and Goesan H soil formed a single cluster, suggesting that these two soils had very similar bacterial compositions. The Umsong M2 soil deviated from all other groups, and its bacterial community appeared to be associated with a high EC, and high calcium and magnesium concentrations. *Pseudomonas* was dominant in this sample, unlike the other samples (Table 3). Also, bacteria of the Goesan M soil cultured on Gram-negative medium were differentiated from the other group. Goesan M soil, which was characterized by a low pH and relatively high chromium concentration, revealed the genera *Cellulomonas* and *Burkholderia* as the dominant members of its bacterial community. Along with the results of diversity analyses, correlations of soil properties and dominant bacteria are given in Table 3. The Shannon-Weaver index (H) of Goesan H soil and Jinju G soil, based on bacterial isolates which were cultured on aerobic bacteria medium, had high values. The H of bacteria cultured on a crystal violet medium from both Goesan M soil and Umsong H soil were also high. The indices of species diversity relate the number of species and the relative importance of individual species. Aerobic bacteria had the highest diversity (3.1) in Jinju G soil, and Gram-negative bacteria had the highest diversity (1.9) in Goesan M soil.

Based on this analysis, we can make a conclusion that fluorescent *Pseudomonas* [13, 17], *Stenotrophomonas*, *Burkholderia*, and *Arthrobacter* are potential indicators of soil quality (Fig. 1; Table 3). In Umsong H, Goesan H, and Jinju G soils, the populations of these bacteria were higher than those in the other soil. This was also shown in the cluster analysis, in which aerobic bacteria as well as Gram-negative bacteria from Umsong H soil and Goesan H soil were classified in the same group. Since new methods are being developed for identifying different bacterial species compositions for large numbers of samples, the relationships between crop productivity, soil quality, and bacterial communities may further be confirmed for different crops and soil types.

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