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Analysis of Microbiota in Bellflower Root, *Platycodon grandiflorum*, Obtained from South Korea

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Bellflower root (*Platycodon grandiflorum*), which belongs to the *Campanulaceae* family, is a perennial grass that grows naturally in Korea, northeastern China, and Japan. Bellflower is widely consumed as both food and medicine owing to its high nutritional value and potential therapeutic effects. Since foodborne disease outbreaks often come from vegetables, understanding the public health risk of microorganisms on fresh vegetables is pivotal to predict and prevent foodborne disease outbreaks. We investigated the microbial communities on the bellflower root (n = 10). 16S rRNA gene amplicon sequencing targeting the V6-V9 regions of 16S rRNA genes was conducted via the 454-Titanium platform. The sequence quality was checked and phylogenetic assessments were performed using the RDP classifier implemented in QIIME with a bootstrap cutoff of 80%. Principal coordinate analysis was performed using the weighted Fast UniFrac distance. The average number of sequence reads generated per sample was 67,192 sequences. At the phylum level, bacterial communities from the bellflower root were composed primarily of Proteobacteria, Firmicutes, and Actinobacteria in March and September samples. Genera *Serratia*, *Pseudomonas*, and *Pantoea* comprised more than 54% of the total bellflower root bacteria. Principal coordinate analysis plots demonstrated that the microbial community of bellflower root in March samples was different from those in September samples. Potential pathogenic genera, such as *Pantoea*, were detected in bellflower root samples. Even though further studies will be required to determine if these species are associated with foodborne illness, our results indicate that the 16S rRNA gene-based sequencing approach can be used to detect pathogenic bacteria on fresh vegetables.

Keywords: Bellflower root, microbiota, *Platycodon grandiflorum*

Introduction

Bellflower root (*Platycodon grandiflorum*), which belongs to the *Campanulaceae* family, is a perennial grass that grows naturally in Korea, northeastern China, and Japan [1]. It has been widely consumed as both food and medicine owing to its high nutritional value and potential therapeutic effects for cough, sore throat, obesity, hyperlipidemia, and diabetes [2–4]. Because bellflower root is harvested from soil, it could be contaminated with pathogens related to foodborne disease [5]. The first step to evaluating the risk of foodborne illnesses associated with bellflower root is to identify the microbial communities in it.

Conventional culturing methods have been used to identify pathogens in food products; however, this method is laborious, time-consuming, and only able to detect bacteria that can be cultured under experimental conditions [6, 7]. To overcome this limitation, the polymerase chain reaction (PCR) has been used as a convenient and rapid strategy to investigate the presence of foodborne pathogens

in food [8, 9].

Meanwhile, metagenomic sequencing techniques, which are non-culture-based methods, have been widely used to identify and characterize microbiota [10]. For instance, this method has been utilized to explore bacterial communities associated with fresh produce, such as sprouts, fruits, and vegetables [11, 12]. With the help of molecular techniques, various plant-associated microbiota have been investigated because they are regarded as important factors for human health as well as plant productivity [5]. Although there are an increasing number of studies related to human pathogens within plants [13–15], information on the bellflower root microbiota is still limited.

Therefore, the aim of this study was to investigate the bacterial community associated with bellflower roots from five South Korean farms between March and September of 2016. Bacterial communities of each bellflower root sample were analyzed using the 454 GS-FLX Titanium pyrosequencing system. The microbial community and the potential risk of foodborne pathogens in bellflower root were characterized in association with sampling time and sampling site.

Materials and Methods

Sampling and Preparation

Bellflower root samples were collected from farms in South Korea (Table 1). Samples were weighed (25 g per each sample), placed in stomacher bags (Labplas, Canada) with 225 ml of buffered peptone water (Oxoid, UK), and homogenized using a stomacher (Interscience, France) for 2 min. Large residues or materials such as soil were filtered using a strainer (pore size, 1.0 mm) and the remaining samples were transferred to clean conical tubes. All samples were then centrifuged at 10,000 ×*g* for 10 min at 4°C and the supernatants were discarded. The pellet was resuspended with 5 ml of TES buffer (0.1 M NaCl) (Sigma, Germany), 1 mM ethylenediaminetetraacetic acid (EDTA) (Amresco, USA) and 10 mM Tris-HCl (pH 8.0) (GeneDEPOT, USA); to wash the pellet [16] and centrifuged at 10,000 ×*g* for 10 min at 4°C. The washing step was repeated three times and the final pellet was stored at −80°C before extraction of total bacterial community DNA.

Isolation of Bacterial DNA

The pellet was resuspended with 400 µl of Tris-EDTA (TE) buffer (pH 8.0) (GeneDEPOT, USA) with 50 µl of lysozyme (100 mg/ml) (Sigma, Germany) and incubated at 37°C for 1 h. After incubation, the mixture was chilled at −80°C for 10 min and incubated again at 37°C for 10 min. The sample was then treated with 200 µl of proteinase K (2 mg/ml) (Sigma, Germany), 2% sodium dodecyl sulfate (Sigma, Germany), and 0.35 M EDTA (pH 8.0) and incubated at 56°C for 1 h. The sample was centrifuged at 21,130 ×*g* for 1 min at room temperature (RT) and the supernatant was transferred to a new microcentrifuge tube. The mixture was treated with an equal volume of phenol-chloroform-isoamyl alcohol (v/v) (25:24:1) (Sigma, Germany), inverted several times, and centrifuged at 21,130 ×*g* for 5 min at RT [17]. The supernatant was collected and treated with an equal volume of chloroform (Sigma, Germany) to remove the residual phenol and centrifuged in the same manner, and the aqueous phase was transferred to a new tube. Then, the aqueous phase was incubated with 3 µl RNase A (100 mg/ml) (Sigma, Germany) at 37°C for 1 h to remove the RNA. The phenol-chloroform extraction was repeatedly performed until no aggregation appeared in the interphase [17]. The resultant aqueous phase was carefully transferred to a new microcentrifuge tube and treated with an equal volume of chloroform to remove the residual phenol. It was centrifuged in the same manner, and the aqueous phase was transferred to a new tube. After transferring, a 10% volume of 3 M sodium acetate (Sigma, Germany) and an equal volume of ice-cold absolute

Table 1. Information of bellflower root samples.

Month (Year)	Sample	Site	ID[a]	Sampling date	Average temperature (°C)[b]	Average humidity (%)[b]
March (2015)	Bellflower root	A	B1	March 17, 2015	3.17	37.03
		B	B2	March 17, 2015	4.24	51.21
		C	B3	March 17, 2015	4.24	51.21
		D	B4	March 17, 2015	3.85	36.52
		E	B5	March 17, 2015	3.85	36.52
September (2015)	Bellflower root	A	B6	September 08, 2015	21.71	75.56
		B	B7	September 08, 2015	22.61	77.42
		C	B8	September 08, 2015	22.61	77.42
		D	B9	September 08, 2015	22.54	64.57
		E	B10	September 08, 2015	22.54	64.57

[a] B1 to 10 indicate bellflower roots.

[b] Average temperature and humidity were calculated using 10-day data prior to the sampling date.

ethanol were added to the solution [18]. After inverting several times, the mixture was centrifuged at 21,130 ×*g* for 20 min at 4°C. The supernatant was discarded and the remaining pellet was washed using 1 ml of ice-cold 70% ethanol. After centrifugation in the same manner, the supernatant was discarded and the pellet was dried at room temperature. The isolated DNA was resuspended with 50 µl of TE buffer and incubated at 55°C for 1 h to completely dissolve the DNA. The DNA obtained was quantified using a Colibri Microvolume Spectrometer (Titertek-Berthold, Germany) and stored at −20°C until use.

Detection of Virulence Genes in Bellflower Root Samples

Conventional PCR was used to detect the virulence genes of foodborne pathogens in all samples [9]. The target virulence genes and their specific primers are presented in Table 2. To improve amplification of the target genes, PCR additive reagent (750 mM trehalose (Sigma, Germany), 1 mg/ml bovine serum albumin (Sigma, Germany), 1% Tween-20 (Sigma, Germany), and 8.5 mM Tris-HCl (pH 8.0)) was added to the reaction mixture [19].

Pyrosequencing

PCR was used to amplify the V6-V9 regions of the 16S rRNA genes using the universal primers 926F (5′- GCACAAGCRGHG GARCATG-3′) and 1505R (5′-ACGGYTACCTTGTTACGACTT-3′) [20]. Multiplex identifier adaptors and 454 adaptors were attached to the primers for the sorting of samples. After amplification, the PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corp., USA).

Sequencing of amplicons was performed at LabGenomics (Korea). The library was prepared using PCR products according to the GS FLX plus library prep guide. Libraries were quantified using the Picogreen assay (Invitrogen, USA), and sequencing was conducted using a Genome Sequencer FLX plus system (454 Life Sciences, USA). Each sample was loaded in a 70−75 mm picotiter plate (454 Life Sciences, USA) fitted with an 8-lane gasket. All procedures were according to the manufacturer's protocol.

Bioinformatics Analysis

Raw sequences were trimmed and those with a maximum number of eight homopolymers were discarded [21]. Chimeras were removed from the sequences using UCHIME [22]. Operational taxonomic unit (OTU) picking, taxonomic assignment, diversity analysis, and visualization were performed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline [23]. OTUs were defined and clustered using UCLUST at 3% divergence (97% similarity) [24]. Phylogenetic assignment and classification were

Table 2. Primers used in this study.

Bacteria	Target gene	Oligonucleotide sequence (5′ – 3′)	Size of PCR product (bp)	Ref.
E. coli (EHEC)	*stxI*-1	ACC TCA CTG ACG CAG TCT GTG G	350	[48]
	stxI-2	TCT GCC GGA CAC ATA GAA GGA AA		
E. coli (EHEC)	*stxII*-1	ACT GTC TGA AAC TGC TCC TGT G	262	[48]
	stxII-2	TTA TTT TTA TAA CGG GCC TGT TCG C		
E. coli (EPEC)	*bfpA*-1	GAT TGA ATC TGC AAT GGT GC	597	[49]
	bfpA-2	GGA TTA CTG TCC TCA CAT AT		
E. coli (ETEC)	*lt*-1	TCT CTA TGT GCA CAC GGA GC	322	[48]
	lt-2	CCA TAC TGA TTG CCG CAA T		
E. coli	*fimH*-1	TCA GGG AAC CAT TCA GGC A	900	[50]
	fimH-2	CGT GCA GGT TTT TAG CTT CA		
C. perfringens	*cpe*-1	GGT ACC TTT AGC CAA TCA	600	[51]
	cpe-2	TCC ATC ACC TAA GGA CTG		
B. cereus	*nheA*-1	TAA GGA GGG GCA AAC AGA AG	500	[52]
	nheA-2	TGA ATG CGA AGA GCT GCT TC		
B. cereus	*nheB*-1	CAA GCT CCA GTT CAT GCG G	770	[52]
	nheB-2	GAT CCC ATT GTG TAC CAT TG		
B. cereus	*nheC*-1	ACA TCC TTT TGC AGC AGA AC	582	[52]
	nheC-2	CCA CCA GCA ATG ACC ATA TC		
S. aureus	*sea*-1	TTG GAA ACG GTT AAA ACG AA	120	[53]
	sea-2	GAA CCT TCC CAT CAA AAA CA		
S. Typhimurium	*invA*-1	ACA GTG CTC GTT TAC GAC CTG AAT	244	[54]
	invA-2	AGA CGA CTG GTA CTG ATC GAT AAT		

Table 3. Results of PCR screening for the detection of virulence genes in the samples.

Month (Year)	Site	ID	*stxI*	*stxII*	*bfpA*	*lt*	*fimH*	*sea*	*invA*	*cpe*	*nhe*
March (2015)	A	B1	-	-	-	-	-	-	-	-	+
	B	B2	-	-	-	-	+	-	-	-	-
	C	B3	-	-	-	-	-	-	-	-	-
	D	B4	-	-	-	-	-	-	-	-	-
	E	B5	-	-	-	+	-	-	-	-	-
September (2015)	A	B6	-	-	-	-	-	-	-	-	-
	B	B7	-	-	-	-	-	-	-	-	-
	C	B8	-	-	-	-	-	-	-	-	-
	D	B9	-	-	-	-	-	-	-	-	-
	E	B10	-	-	-	-	-	-	-	-	-

performed using the RDP classifier implemented in QIIME [25]. Before estimating the alpha-diversity measurements, data were normalized by a random selection of the same sequence number per sample. The sequence number was determined on the basis of the sample with the least number of sequences. Shannon and Simpson indices and Chao1 values were calculated to verify the diversity of the microbiota obtained from samples. The differences of microbiota among samples were visualized using the principal coordinate analysis (PCoA) plot. A two-sided Welch's *t* test in Statistical Analysis of Metagenomic Profiles (STAMP) software was used to identify significant differences in the relative abundance of microbial taxa between March and September bellflower root samples, with $p < 0.05$ considered significant.

Results

Detection of Virulence Genes in the Samples

The presence of virulence genes in bellflower roots in March and September samples were checked by PCR amplification with specific primers. Results of the detection of virulence genes in the samples are shown in Table 3. In March samples, virulence genes, including *nhe*, *fimH*, and *lt* genes, were detected in B1, B2, and B5 samples, respectively. Moreover, there were no virulence genes detected in B3 and B4 samples. By comparison, all bellflower samples in the September group were all negative for virulence genes as confirmed using PCR.

Summary of Sequencing Data

To examine the bacterial community composition, total DNA ($n = 10$) extracted from bellflower roots was processed to amplify and sequence the 16S rRNA gene using 454 GS-FLX Titanium pyrosequencing. A total of 77,970 and 594,837 DNA sequence reads were generated from the bellflower roots harvested in March and September,

Table 4. Alpha-diversity measurements of bellflower root microbiota in March and September.

Month (Year)	Site	ID	DNA sequence reads[a]	OTUs	Shannon	Chao1
March (2015)	A	B1	19,578	958	5.82	2,396.89
	B	B2	19,739	446	3.91	1,138.52
	C	B3	21,060	2,336	6.75	5,880.01
	D	B4	7,957	760	2.43	1,980.75
	E	B5	9,636	1,188	3.8	3,315.38
September (2015)	A	B6	79,711	9,885	11.85	34,655.70
	B	B7	94,192	7,188	11.25	24,614.17
	C	B8	105,832	16,272	11.5	49,479.70
	D	B9	171,361	16,324	12.82	57,969.48
	E	B10	143,741	18,615	7.4	51,037.50

[a]DNA sequence read counts of bellflower root samples before normalization. Alpha-diversity was performed on normalized reads that were randomly selected on the basis of the lowest number of sequences among the samples ($n = 7,957$).
Alpha-diversity measures were calculated on the basis of normalized read counts using QIIME.

respectively. The average number of sequence reads generated per bellflower root was 15,594 for March and 594,837 for September (Table 4). OTUs were analyzed for each sample at 97% identity to compare the bacterial diversity and species richness in March and September. The diversity and richness values of B3 was the highest for all five samples in March, whereas B9 was higher than other samples in September. Moreover, the lowest species diversity was found in B4 with a Shannon value of 2.43, whereas B2 had the lowest species richness with a Chao1 value of 1,138.52 in March samples. By comparison, in September, B10 and B7 had the lowest Shannon value (7.4) and Chao1 value (24,614.17), respectively.

Difference of Microbiota in Bellflower Roots between Sampling Times and among Sampling Sites in South Korea

A total of 32 phyla, 87 classes, 186 families, and 401 genera were identified in all bellflower roots. The relative abundance of sequences at the phylum level is shown in Fig. 1A. Proteobacteria, Firmicutes, and Actinobacteria were the three main phyla dominating the bacterial communities of the bellflower roots. While the relative abundance of Proteobacteria was greater in March than in September samples, Firmicutes were more abundant in the September than in the March samples. The relative abundance of Proteobacteria was most abundant in site C

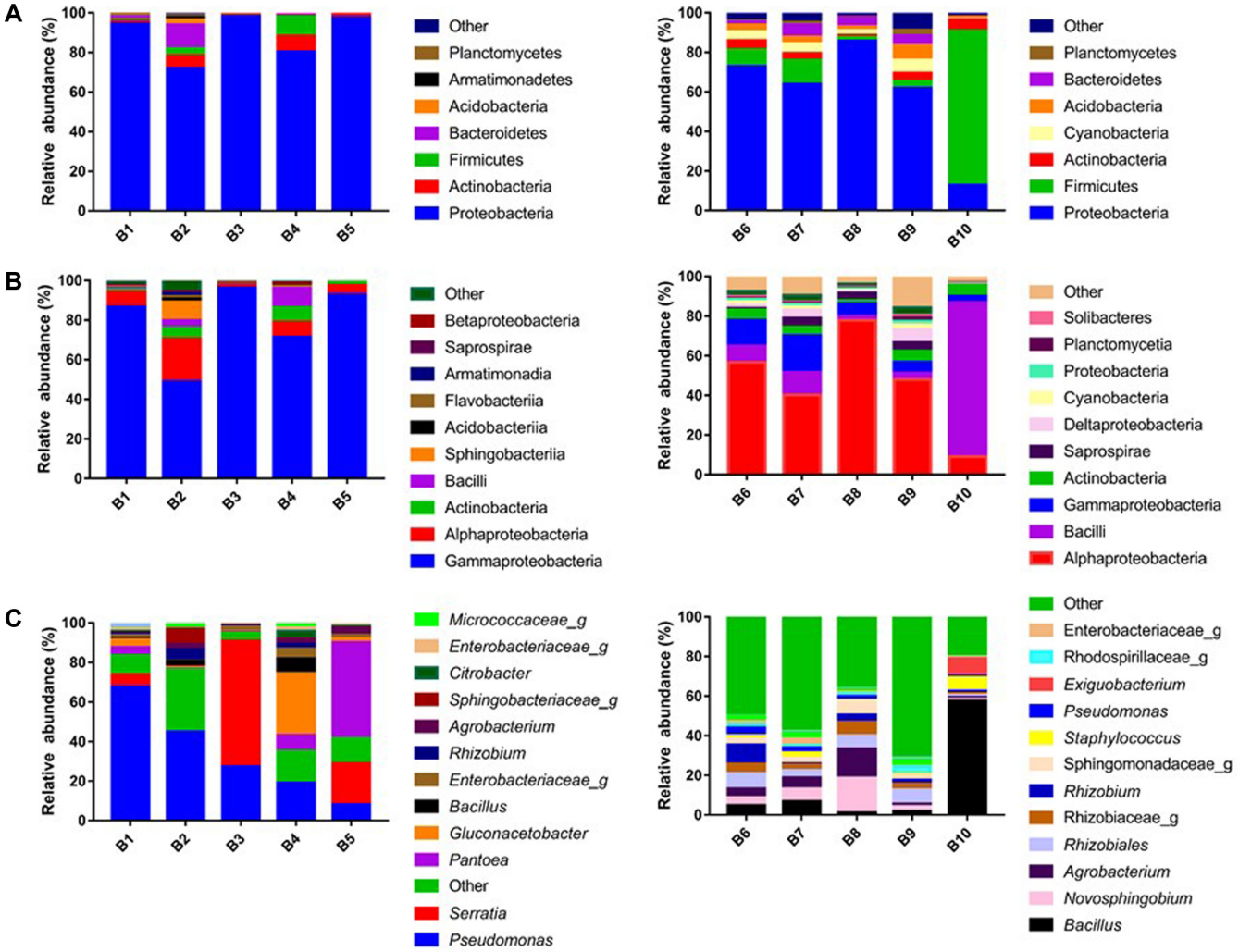

Fig. 1. Relative abundance of taxa at the (**A**) phylum, (**B**) class, and (**C**) genus levels between the bellflower root samples in March (B1, B2, B3, B4, B5) and September (B6, B7, B8, B9, B10).
Phylogenetic assignment and classification based on 16S rRNA sequence similarity were achieved using the RDP database and the QIIME software package.

in both March (B3) (94.58%) and September (B8) (85.74%). Moreover, the relative abundance of Firmicutes was highest in site E in September (B10) (78%). (Fig. 1A).

At the class level, a large percentage of sequences in the bellflower root microbiota consisted of Alphaproteobacteria, Gammaproteobacteria, and Bacilli. The relative abundance of Alphaproteobacteria and Bacilli increased with the change of season from March to September, while the abundance of Gammaproteobacteria decreased. The relative abundance of Gammaproteobacteria was highest in site C in March while its abundance was highest in site B in September. Moreover, the relative abundance of Alphaproteobacteria was highest in site B (B2) (21.5%) among the samples in March while it was highest in site C (B8) (77.92%) in September. Notably, bacteria belonging to class Bacilli was highest in site D (B4) (9.8%) and site E (B10) (77.7%) in March and September, respectively (Fig. 1B).

We also compared the results of taxonomic classification of sequences in bellflower root among sampling sites in March and September at the genus level (Fig. 1C). The most abundant genera in the March group were *Pseudomonas, Serratia,* and *Pantoea.* In September, *Bacillus, Novosphingobium, Agrobacterium*, and Rhizobiales were dominantly present. The relative abundance of *Pseudomonas* was highest in site A among the samples in both March (B1) (67.67%) and September (B6) (3.93%). Moreover, in March the relative abundance of *Serratia* and *Pantoea* were predominant in the C site (B3) (63.78%) and E site (B5) (48.47%), respectively. By comparison, in September, *Bacillus* represented 57% of sequences in site E (B10). In addition, the proportion of *Novosphingobium* was highest in site C (B8) (17.51%). Notably, the genus *Agrobacterium* was highest in the E site (B5) (3.6%) and C site (B8) (14.74%) among the samples in March and September, respectively. Furthermore, various potential pathogens that are related with pathogenesis (include opportunistic pathogens) were detected from numerous samples (Table 5).

To visualize the relative distances between bacterial communities of each sample, a three-dimensional PCoA plot was constructed (Fig. 2). The plot was generated by analyzing the 16S rRNA gene sequencing data of bellflower root ($n = 10$) harvested in March and September. The results showed that the bellflower root microbiota from samples harvested in each season consisted of distinct microbial compositions (Fig. 2). We found that 14 genera significantly shifted the microbiota of bellflower root samples between March and September ($p < 0.05$) (Fig. 3). Interestingly, bacteria belonging to genus *Pseudomonas* were significantly more abundant in March than in September ($p = 0.043$). The 13 other genera were significantly higher in

Table 5. Number of reads and relative abundance of bacterial genera that can be related with pathogens (including opportunistic pathogens) in bellflower roots in March and September.

Month (Year)	ID	Total read count	*Pseudomonas*	*Pantoea*	*Erwinia*	*Klebsiella*	*Bacillus*	*Salmonella*	*Clostridium*
March (2015)	B1	19,578	13,249 (67.67)	801 (4.09)	7 (0.04)	0 (0.00)	129 (0.66)	0 (0.00)	1 (0.01)
	B2	19,739	8,873 (44.95)	71 (0.36)	3 (0.02)	0 (0.00)	593 (3.00)	0 (0.00)	3 (0.02)
	B3	21,060	5,749 (27.29)	137 (0.65)	0 (0.00)	0 (0.00)	38 (0.18)	0 (0.00)	0 (0.00)
	B4	7,957	1,514 (19.02)	636 (7.99)	10 (0.13)	0 (0.00)	593 (7.45)	0 (0.00)	0 (0.00)
	B5	9,636	774 (8.03)	4671 (48.47)	19 (0.20)	0 (0.00)	6 (0.06)	0 (0.00)	1 (0.01)
September (2015)	B6	79,711	3,140 (3.93)	379 (0.48)	297 (0.37)	4 (0.01)	3845 (4.82)	0 (0.00)	4 (0.01)
	B7	94,192	2,264 (2.40)	1,935 (2.05)	210 (0.22)	0 (0.00)	6362 (6.75)	12 (0.01)	168 (0.18)
	B8	105,832	1,924 (1.82)	203 (0.19)	40 (0.04)	45 (0.04)	1157 (1.09)	0 (0.00)	52 (0.05)
	B9	171,361	300 (0.18)	44 (0.03)	16 (0.01)	0 (0.00)	3061 (1.79)	0 (0.00)	65 (0.04)
	B10	143,741	1,136 (0.79)	541 (0.38)	339 (0.24)	0 (0.00)	82775 (57.59)	3 (0.00)	9 (0.01)

Relative abundance within the parentheses are expressed in percentage (%).

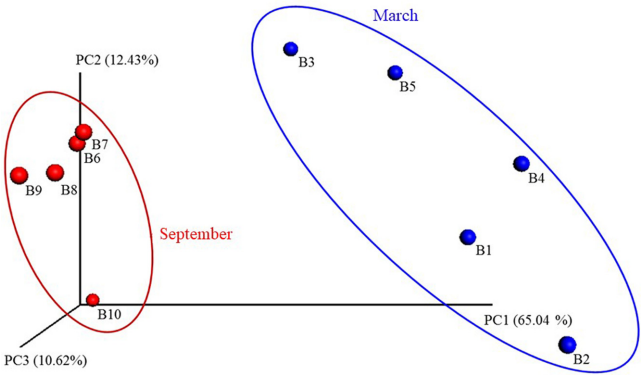

Fig. 2. Principal coordinates analysis (PCoA) plot for the differences of bellflower microbiota between sampling time and site.

A three-dimensional PCoA plot of the FLX pyrosequencing data from bellflower root ($n = 10$) was constructed using the software package QIIME. Each axis on the plot indicates percentage of the diversity distribution. Samples associated with the March (clustered by blue ellipse) and September (clustered by red ellipse) seasons are shown as single points.

September than in March samples ($p < 0.05$); namely, *Rhodoplanes*, *Kaistobacter*, *Sphingosinicella*, *Terrimonas*, *Solirubrobacter*, *Blastomonas*, *Sinorhizobium*, *Nocardioides*, *Erythrobacter*, *Cystobacter*, *Plesiocystis*, *Agromyces*, and *Amycolatopsis* (Fig. 3).

Discussion

A PCR-based method was used to detect virulence genes in bellflower roots in March and September using specific primers associated with potential foodborne pathogens. In March samples, we detected the *nhe*, *fimH*, and *lt* genes in B1, B2 and B5 samples, respectively. Notably, no virulence genes were detected in all samples in September. The non-hemolytic gene (*nhe*) gene was detected in the A site (B1 sample). The *nhe* gene is one of the two-three component enterotoxins responsible for the diarrheal food-poisoning syndrome caused by *Bacillus cereus* [26]. The genus *Bacillus* was abundant in sample B10 in September, but PCR screening showed no virulence genes. The *nhe* gene has been shown to possess a variety of biological effects, including cytotoxic, dermonecrotic, and vascular permeability activities [27]. In this study, the relative abundance of genus *Bacillus* was detected using 16S rRNA gene sequencing in all the samples regardless of the sampling time. Moreover, PCR results showed that the type 1 fimbriae (*fimH*) gene

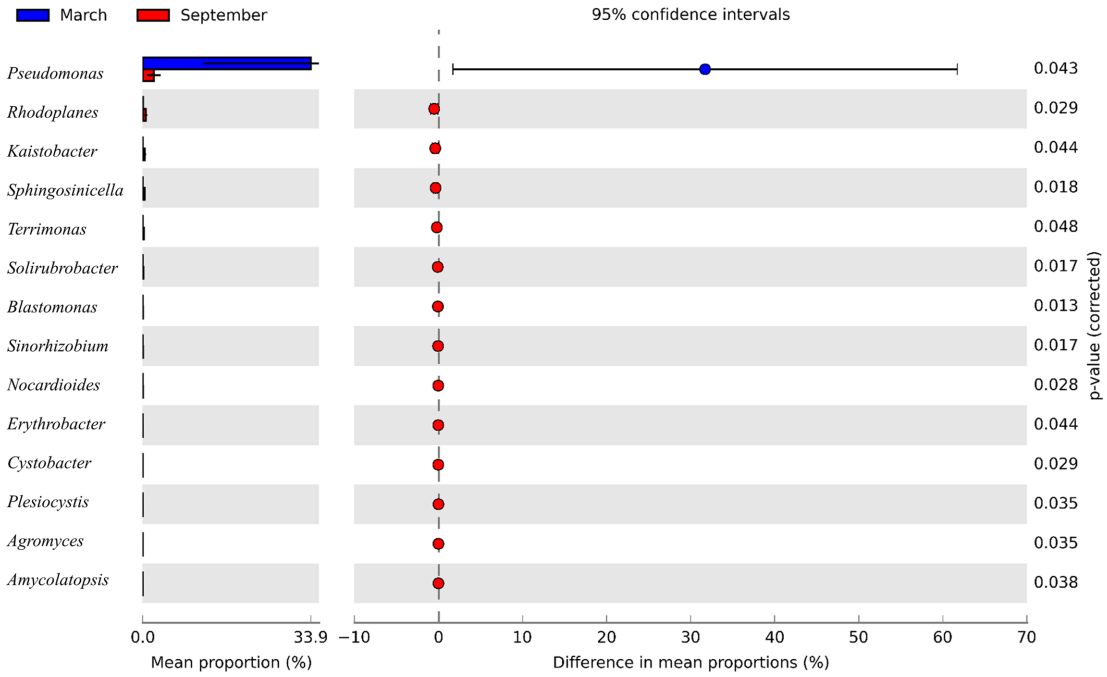

Fig. 3. Extended error plot identifying significantly different genera of the bellflower root microbial community in March and September samples at the genus level.

The corrected *p*-values are shown at the right. The differences in microbial community structure were measured using a two-sided Welch's *t*-test and $p < 0.05$ was considered significant.

was present in one of the samples in March (B2). The *fimH* gene was encoded by uropathogenic *Escherichia coli* (UPEC), which is the most frequent pathogen associated with many diseases, including urinary tract infection [26]. The *fimH* adhesion is important in colonizing different niches of *E. coli* [27]. The type 1 fimbriae protein in mice is a particularly important UPEC virulence factor as its expression greatly enriches UPEC binding and colonization of the murine bladder during experimental urinary tract infection [28]. Furthermore, this study was able to detect another important virulence gene factor in bellflower root, which is the heat-labile (LT) gene. This gene is produced by enterotoxigenic *E. coli* (ETEC) and is a major cause of diarrhea in infants in many developing countries [29]. These results can be reflected in the taxonomic-dependent analysis of the bellflower root microbiota, where *E. coli* is classified under the family Enterobacteriaceae that was detected in small proportions in B2 and B5 samples. Overall, these results suggest that a combination of PCR-based method and 16S rRNA gene sequencing can be used to detect pathogens in bellflower root. Our results have important implications for the prevention and treatment of foodborne diseases in bellflower root.

Pseudomonas spp. were detected in all bellflower roots. *Pseudomonas* is a gram-negative, aerobic Gammaproteobacteria, which causes opportunistic infections and is widely distributed in soil and water [30]. This bacterium causes many kinds of infections, such as urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections, and a variety of systemic infections, particularly in immunosuppressed patients with severe burns, cancer, and acquired immune deficiency syndrome [31]. All species and strains of *Pseudomonas* have historically been classified as strict aerobes [32]. Exceptions to this classification have recently been discovered in *Pseudomonas* biofilms [33]. A significant number of cells can produce exopolysaccharides associated with biofilm formation. Secretion of exopolysaccharides such as alginate makes it difficult for *Pseudomonas* to be phagocytosed by mammalian white blood cells [32]. Exopolysaccharide production also contributes to surface-colonizing biofilms that are difficult to remove from food preparation surfaces. Growth of pseudomonads on spoiling foods can generate a "fruity" odor [34]. *Pseudomonas syringae* is a prolific plant pathogen that exists as over 50 different pathovars, many of which demonstrate a high degree of host-plant specificity [35]. Numerous other *Pseudomonas* spp. can act as plant pathogens, notably all of the other members of the *P. syringae* subgroup, but *P. syringae* is the most widespread and best studied [36]. Although not strictly a plant pathogen, *P. tolaasii* can be a major agricultural problem, as it can cause bacterial blotch of cultivated mushrooms [37]. Similarly, *P. agarici* can cause drippy gill in cultivated mushrooms [37, 38]. However, not all species of *Pseudomonas* are pathogenic. Many plant-associated *Pseudomonas* species promote plant growth by suppressing pathogenic microorganism, synthesizing growth-stimulating hormones, and promoting increased plant disease resistance [39].

Serratia was detected in several March bellflower root samples. *Serratia* species, in particular, *Serratia marcescens*, are significant human pathogens. *S. marcescens* is a gram-negative, facultative anaerobe, endospore-forming, rod-shaped bacterium and the causative agent of numerous nosocomial infections [40]. It is classified in the large family Enterobacteriaceae. *S. marcescens* is associated with many infections, such as septicemia, meningitis, emphysema, osteomyelitis, urinary tract infections, septic arthritis, peritonitis, and sinusitis [41]. *S. marcescens* is transmitted through hand-to-hand transmission by hospital personnel [42]. *S. marcescens* is commonly found in soil, water, plants, and animals [2, 43]. Our results detected *Serratia* in bellflower roots using 16S rRNA gene sequencing, suggesting that plant-associated *Serratia* may play a possible role in the contamination of humans and animals.

Pantoea was detected across all samples in March and September among all the sampling sites. *Pantoea* species are gram-negative aerobic bacilli in the family Enterobacteriaceae [44]. All species of the genus *Pantoea* can be isolated from feculent material, plants, and soil, where they can be either pathogens or commensals [45]. A number of *Pantoea* spp. are well-known agents of disease in plants. In humans, *Pantoea* spp. are opportunistic pathogens and are among the most common organisms causing nosocomial infections, such as pneumonia, urinary tract infection, surgical wound infection, and catheter-related bloodstream infection [46]. In humans, the most common species of *Pantoea* that can be isolated is *P. agglomerans*, which causes soft tissue, bone, and joint infections [44].

In this study, we observed that the alpha-diversity measurement of bellflower root microbiota was higher in September than in March samples. This could be attributed to the higher relative humidity and temperature in September, which favors the growth of bacteria. We expected to see higher species diversity and richness in September with higher humidity under higher temperature, since these conditions seem optimal for microbial proliferation due to the presence of water on the surface of

the bellflower root samples. Seasonal variation can alter microbial growth and activity that result in differences in the microbial community structure. On the other hand, in March samples, the average temperature recorded was around 4°C. It has been known that many bacteria grow maximally at temperatures around 37°C. At temperatures below their optimum for growth, bacteria will become increasingly unable to sequester substrates from their environment because of lowered affinity [47]. Hence, the lower temperature limits their growth, which consequently lowers the microbial alpha-diversity.

In this study, we investigated the microbial community structure of bellflower roots. Although further studies will be required to determine whether the identified species are associated with foodborne illness, our results indicate that 16S rRNA gene-based sequencing approach can be used to detect pathogenic bacteria on fresh vegetables.

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