Journal of Advances in Microbiology

13(2): 1-12, 2018; Article no.JAMB.45442
ISSN: 2456-7116

Culture-Dependent Analysis of Endophytic Bacterial Community of Sweet Potato (*Ipomoea batatas*) in Different Soils and Climates

Ramesh Raj Puri[1], Fumihiko Adachi[2], Masayuki Omichi[3], Yuichi Saeki[4], Akihiro Yamamoto[4], Shohei Hayashi[2] and Kazuhito Itoh[1,2*]

[1]The United Graduate School of Agricultural Sciences, Tottori University, 4-101 Koyama-Minami, Tottori, 680-8553, Japan.
[2]Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu, Matsue, Shimane, 690-8504, Japan.
[3]Department of Agricultural Science and Business, Hokkaido College, Takushoku University, Memu 4558, Hokkaido, 074-8585, Japan.
[4]Faculty of Agriculture, University of Miyazaki, Gakuen-Kibanadai-Nishi 1-1, Miyazaki, 889-2192, Japan.

Authors' contributions

This work was carried out in collaboration among all authors. Authors FA, MO, YS and AY supported in cultivation and sampling. Author KI designed the study. Author RRP conducted the laboratory experiment and author SH provided technical help. Authors RRP and KI managed the literature search and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/45442
Editor(s):
(1) Dr. Niranjala Perera, Department of Food Science & Technology, Wayamba University of Sri Lanka, Sri Lanka.
Reviewers:
(1) P.N. Yapa Rajarata, University of Sri Lanka, Sri Lanka.
(2) Wei-Liang Chao, Soochow University, China.
(3) Stefan Martyniuk, Institute of Soil Science and Plant Cultivation – State Research Institute, Poland.
(4) Rebecca Yegon, University of Embu, Kenya.
(5) Aba-Toumnou Lucie, University of Bangui, Central African Republic.
Complete Peer review History: http://www.sciencedomain.org/review-history/27455

Original Research Article

Received 11 September 2018
Accepted 26 November 2018
Published 28 November 2018

ABSTRACT

Aims: To examine the effects of soil and climatic conditions on community structure of the sweet potato bacterial endophytes.
Study Design: Sweet potato plants were cultivated in different soils and locations combinations

**Corresponding author: E-mail: itohkz@life.shimane-u.ac.jp;*

and the endophytic bacterial isolates from the tubers were examined to clarify the effect of soil and climatic conditions on the microbial community.
Place and Duration of Study: The plants were cultivated in Fukagawa, Matsue and Miyazaki in Japan for ca. 3 months.
Methodology: Bacterial isolates were characterised based on their morphologies and the representative colonies were purified for identification by analysing the partial 16S rRNA gene sequences. Endophytic bacterial community was analysed based on phylum/class and genus levels.
Results: Sixty two colonies were isolated and identified. γ-Proteobacteria (96%), β-Proteobacteria (87%) and Actinobacteria (88%) dominated in the samples cultivated in Fukagawa, Matsue and Miyazaki soils at the corresponding locations, respectively. When the soil samples were used in the different locations, the above mentioned location-specific phyla increased at the new sites. The endophytic bacterial population was also affected by the cultivating locations. It was suggested that the location rather than the soil influenced on the endophytic community and population.
Conclusion: The cultivating locations were more important factor than the soils to determine the sweet potato endophytic bacterial community and population.

Keywords: Sweet potato; endophytic bacteria; microbial community.

1. INTRODUCTION

Endophytic bacteria are a class of microbes that resides within the interior tissues of plants without harming the host plants, and they have been isolated from a broad range of plants [1]. Many endophytic bacteria have been reported to possess plant growth abilities, anti-plant pathogenic and phytoremediation abilities [2,3,4,5]. Therefore, understanding the effects of environmental conditions on the endophytic community is important to utilise their functions for developing sustainable systems of crop production.

Previous studies have analysed endophytic bacterial community in sweet potato by the culture dependent method and revealed that the plant was colonised by diazotrophic *Pantoea agglomerans* and nondiazotrophic *Enterobacter asburiae* [6]. Similarly, Khan and Doty [7] isolated 11 endophytes belonging to *Enterobacter, Rahnella, Rhodanobacter, Pseudomonas, Stenotrophomonas, Xanthomonas* and *Phyllobacterium* from sweet potatoes collected from grocery store. Likewise, Puri et al. [2] isolated 243 endophytic bacteria belonging to 34 genera in six classes from 12 locations of Nepal.

Endophytic communities are reported to be influenced by several parameters, such as plant genotype [8], growth stage, physiological status and tissue of plant [9], as well as agricultural practices [10]. In addition, climatic conditions are also considered as important factors for determining endophytic community. For example, psychrophilic bacterial endophytes were isolated abundantly in cold environments from the arcto-alpine plant species [11], which seemed to be the selection of psychrophile already adapted in the soil. In a previous study, we examine the diversity of sweet potato endophytes isolated in 12 locations of Nepal, and revealed that the endophytic communities were not related to the climatic conditions. However, it was unclear which factor was more responsible, the soil or the location, in determining the bacterial endophytic community. In this study, we aimed to examine the effects of soil and climatic conditions on the endophytic bacterial communities of sweet potato by using the same soil at different locations and applying culture dependent approach.

2. MATERIALS AND METHODS

2.1 Sweet Potato Cultivation

The experiment was conducted in Fukagawa (Fuk) in Hokkaido prefecture, Matsue (Mat) in Shimane prefecture and Miyazaki (Miy) in Miyazaki prefecture in Japan. Soils of Fukagawa, Matsue and Miyazaki were exchanged and used for cultivation of sweet potato. Briefly, the soils from 3 above mentioned locations were collected in sterile plastic bags and transported to the other locations, and the soils were immediately used for the experiment in the respective sites. The pots were placed in the open field, and placed on a wooden palette or a plastic sheet. Each one sweet potato cv. Beni Azuma slips, received from same nursery farm, were planted

in a plastic pot (25 cm in diameter and 25 cm high) containing each soil sample, fertilised with chemical fertiliser Silicamap 555 (Central Kasei Co. Ube, Japan) containing N : P_2O_5 : K_2O=5:15:15% at 6.6 g/pot, and cultivated in triplicate, from June to September in 2017. After harvesting, the tubers were used for the isolation of endophytic bacteria. The precise location, climatic parameters and soil nutrients of the cultivation sites are presented in Table 1.

2.2 Culturable Endophytic Bacterial Community

One tuber from each cultivation conditions, making a total of 9, was considered for culture dependent analysis. The tubers were washed in a running tap water for 10 min and then rinsed with sterilised distilled water. Then, cork-borer was perpendicularly inserted into the six different parts across the longitudinal axis of the tuber, each ca. 0.5 g making a total of ca. 3 g tuber samples. The samples were then placed in a sterilised mortar and macerated with 6 ml sterilised distilled water under aseptic conditions. Further, serial 10-fold dilutions were prepared up to 10^{-7}, and each 0.1 mL aliquot was taken and spread on modified MR media [12] supplemented with 0.1 g NH_4NO_3/L and incubated at 26^0C. Efficiency of the washing was confirmed by stamping the surface of the washed tubers on the agar media, and a few culturable bacteria (colony forming unit) were expected on the surface of the macerated samples, which was considered to be negligible as the dilutions 10^{-4} to 10^{-6} were used for the endophytic bacterial community analysis (data not shown).

To isolate fast and slow growing bacteria, colonies were selected at two and ten days of cultivation, respectively. From both the groups, appeared colonies were pooled based on their morphologies and one representative colony of each morphology was purified for identification by analysing the partial 16S rRNA gene sequences using universal primers fD1 and rP2 [13]. Then a phylogenetic tree of bacterial genera was constructed using Clustal W [14]. Endophytic bacterial community was analysed based on phylum/class and genus levels.

2.3 Nucleotide Sequence Accession Numbers

The sequence data generated in this study were deposited in the DDBJ Nucleotide Submission System under the accession numbers LC430019 to LC430094.

2.4 Statistical Analysis

Tukey's test after one-way analysis of variance (ANOVA) was used to test the effect of the locations and the soils on the endophytic bacterial populations and compositions. ANOVA was performed by MINITAB (version 14.0).

3. RESULTS

3.1 Isolation and Identification of Endophytic Bacteria

Fast and slow growing endophytic bacterial isolates were detected from 9 sweet potato samples cultivated in different locations and soils (Table S1). For fast growers, 3-9 morphologies in 27-80 colonies per plate, while 1-4 morphologies in 1-9 colonies per plate appeared in slow growers. Due to the smaller numbers of slow growing colonies in a plate, populations were calculated only for the fast growers. The bacterial populations were different among the locations regardless of the soils as the highest at Fukagawa location at 1.1-2.0 × 10^6, then Miyazaki at 8.1-18 × 10^4, and Matsue location possessed the lowest at 1.7-2.4 × 10^4 CFU/g fresh weight (fw) (Fig. 1). The populations of Fukagawa location was significantly higher than Matsue and Miyazaki locations (P=0.001), and Matsue and Miyazaki locations were also significantly different (P=0.017) but not among the soils.

Based on the partial 16S rRNA gene sequence, 47 endophytes belonged to four bacterial phyla representing 25 genera. The endophytic compositions clearly showed that the phyla and genera differed among samples and shifted by changing the cultivating locations (Table 2 and Fig. 2).

Proteobacteria was the most dominant in 8 samples in Fukagawa (92-100%), Matsue (63-94%) and Miyazaki (56-63%) locations. For the Miyazaki location and Miyazaki soil sample, it was 10%. Compositions of Proteobacteria were dominated by only 1 or 2 classes in each sample. In Fukagawa location, γ-Proteobacteria (Fuk-Fuk, Fuk-Mat) or γ- and β-Proteobacteria (Fuk-Miy) dominated. In Matsue and Miyazaki locations, β-Proteobacteria (Mat-Fuk, Mat-Mat, Miy- Fuk) or α- and β-Proteobacteria (Mat-Miy, Miy-Mat) dominated. In the Miy-Miy sample, Actinobacteria dominated (88%) under the lower composition of Proteobacteria and this phylum

was detected as second highest component in the other samples of Miyazaki location (23-26%) and 2 samples of Matsue location (11-23%). Phylum Firmicutes was detected in 7 samples as a minor component (2-18%). Bacteroidetes was detected only from Mat-Fuk sample, representing 14%.

The relative abundance of γ-Proteobacteria in Fukagawa location was significantly higher than those in Matsue and Miyazaki locations (*P*=0.003). The relative abundance of β-Proteobacteria and Actinobacteria were relatively higher in Matsue and Miyazaki locations, respectively, but the differences were not significant. The difference was also not significant among the soils.

3.2 Shift in Composition of Endophytic Bacterial Phyla

The endophytic bacterial compositions showed dominancy of specific bacterial phyla at the original sites but changed when the soils were used in the different locations (Fig. 2).

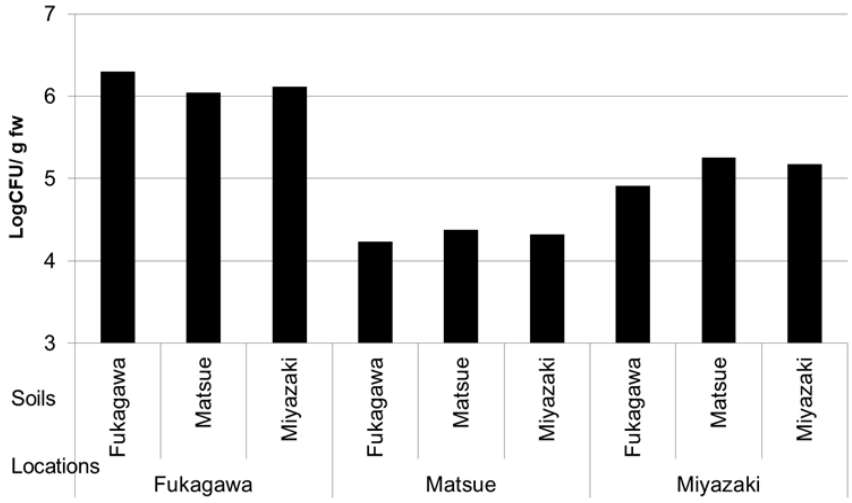

Fig. 1. Endophytic bacterial populations in different locations and soils conditions

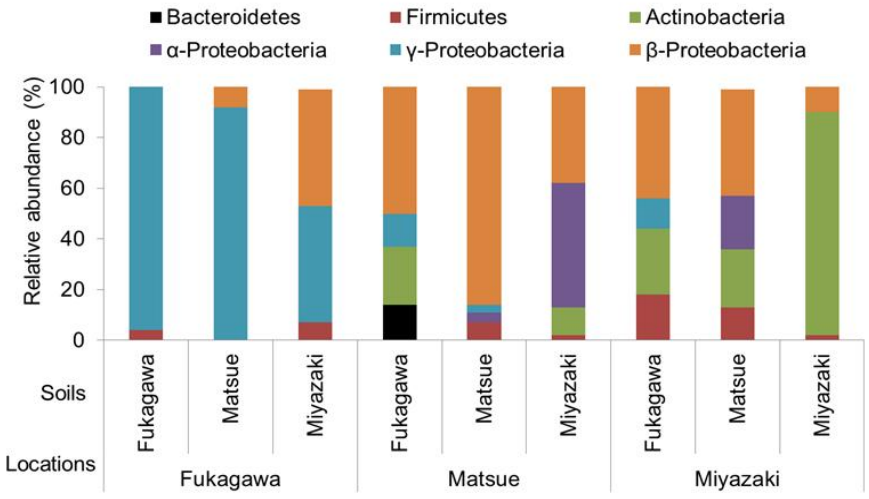

Fig. 2. Sweet potato endophytic bacterial composition cultivated in different location and soil combinations

Table 1. Climate and soil nutrients of the sweet potato cultivation sites

Location	Latitude (^{0}N)	Longitude (^{0}E)	Temperature (°C)[a]		Rainfall (mm)[a]	Soil type[b]	Soil nutrients						pH (H_2O)
			Max	Min			NH_4-N (mg/kg)	P_2O_5 (mg/kg)	K_2O (mg/kg)	Available P (mg/kg)	Total C (g/kg)	Total N (g/kg)	
Fukagawa	43.71	142.01	23	13	501	Andisol	16	472	369	3.3	5.2	0.4	6.0
Matsue	35.48	133.06	29	21	611	Inceptisol	12	288	86	2.5	1.2	0.1	6.2
Miyazaki	31.82	131.41	30	23	1252	Andisol	22	160	220	2.2	4.4	0.3	6.4

[a] *Average maximum and minimum monthly temperatures and total rainfall during the cultivation period (https://www.jma.go.jp),*
[b] *Based on USDA classification [15]*

Table 2. Relative abundance of culturable sweet potato endophytic bacteria isolated from different location and soil conditions

Phyla/Genus	Location-Soil								
	Fuk-Fuk	Fuk-Mat	Fuk-Miy	Mat-Fuk	Mat-Mat	Mat-Miy	Miy-Fuk	Miy-Mat	Miy-Miy
Firmicutes	4		8		6	2	18	13	2
Bacillus sp.	4		8		6	2	11	13	2
Exiguobacterium sp.							7		
Actinobacteria				23		11	26	23	88
Streptomyces sp.						11			38
Microbacterium sp.							26		50
Curtobacterium sp.				23					
Paenarthrobacter sp.								23	
Bacteroidetes				14					
Chryseobacterium sp.				14					
Proteobacteria	96	100	92	63	94	87	56	63	10
α-Proteobacteria					4	49		21	
Sphingobium sp.					4	49			
Caulobacter sp.								21	
β-Proteobacteria		8	46	50	87	38	44	42	10
Variovorax sp.				39	31				
Roseateles sp.					29			21	4

Phyla/Genus	Location-Soil								
	Fuk-Fuk	**Fuk-Mat**	**Fuk-Miy**	**Mat-Fuk**	**Mat-Mat**	**Mat-Miy**	**Miy-Fuk**	**Miy-Mat**	**Miy-Miy**
Janthinobacterium sp.			46						
Mitsuaria sp.						38			
Acidovorax sp.							37		
Paraburkholderia sp.					25				
Pelomonas sp.				11			7		4
Ralstonia sp.								21	
Burkholderia sp.		8							
Herbaspirillum sp.					2				
Chitinimonas sp.									2
γ-Proteobacteria	96	92	46	13	3		12		
Stenotrophomonas sp.	69		2						
Pseudomonas sp.	27		44						
Luteibacter sp.		58							
Pantoea sp.		34			3		12		
Kosakonia sp.				13					

For Fukagawa soil, the endophytic bacterial populations was dominated by γ-Proteobacteria (96%) in original Fukagawa location, but it reduced when used in Matsue (13%) and Miyazaki (12%) locations, while β-Proteobacteria (50% and 44%) and Actinobacteria (23% and 26%) increased in Matsue and Miyazaki locations, respectively.

Similarly, Matsue soil was dominated by β-Proteobacteria (87%) in Matsue location, but it reduced when the soil was used in Fukagawa (8%) and Miyazaki (42%) locations, while γ-Proteobacteria dominated in Fukagawa location (92%), and Actinobacteria (23%) and α-Proteobacteria (21%) increased in Miyazaki location.

Finally, Miyazaki soil was dominated by Actinobacteria (88%) in Miyazaki location, while it was absent in Fukagawa and minor in Matsue (11%) locations, whereas β-Proteobacteria increased when Miyazaki soil was used in Fukagawa (46%) and Matsue (38%) locations. In addition γ- (46%) and α- (49%) Proteobacteria were dominant in Fukagawa and Matsue locations, respectively.

In summary, when the soil samples were used in different locations, γ-, β-Proteobacteria and Actinobacteria showed tendency to dominate in Fukagawa, Matsue and Miyazaki locations, respectively.

3.3 Shift in Composition of Endophytic Bacterial Genera

While the same phyla and class increased by changing the cultivating locations, the genera appeared were not the same among the samples (Fig. 3).

In γ-Proteobacteria, *Stenotrophomonas* (69%) and *Pseudomonas* (27%) were detected as major genera in tubers cultivated in Fuk-Fuk. When Miyazaki soil was used in Fukagawa location γ-Proteobacteria increased, and the main component was *Pseudomonas* (44%). On the other hand, in the case of Matsue soil, *Dyella* (58%) and *Pantoea* (34%) dominated (Fig. 3a).

β-Proteobacteria was dominant as *Variovorax* (31%), *Roseateles* (29%) and *Paraburkholderia* (25%) in Mat-Mat. When Matsue soil was used in Miyazaki location *Roseateles* (21%) was re-isolated but the other genera disappeared, and *Ralstonia* (21%) was newly detected. In Fukagawa and Miyazaki soils endophytic β-Proteobacteria were not detected and minor (10%) in each site, respectively. But, when Fukagawa soil was used in Matsue and Miyazaki locations, *Variovorax* (39%) and *Pelomonas* (11%), and *Acidovorax* (37%) appeared, respectively. Appearance of different genus in different locations was also observed as *Mitsuaria* (38%) in Matsue and *Janthinobacterium* (46%) in Fukagawa when Miyazaki soil was used in each location (Fig. 3b).

Actinobacteria was dominant in tubers cultivated in Miy-Miy location, representing *Streptomyces* (38%) and *Microbacterium* (50%). When Fukagawa soil was used in Matsue and Miyazaki locations, *Curtobacterium* (23%) and *Microbacterium* (26%) were detected, respectively. *Paenarthrobacter* (23%) was detected when Matsue soil was used in Miyazaki location (Fig. 3c).

3.4 Phylogenetic Relationships of Endophytic Bacterial Genera

Although the quantitative information on the slow growing endophytes was less due to the small number of colonies on the plate and lower populations than the fast growers, whole community of the isolates was expressed in phylogenetic tree (Fig. 4).

β-, γ-Proteobacteria, Bacilli and Flavobacteriia consisted mainly of the fast growers while α-Proteobacteria of the slow growers, and the fast and slow growers were phylogenetically separated in Actinobacteria.

4. DISCUSSION

To our knowledge, this is the first implementation of culture dependent approach to investigate the effects of environmental conditions on the endophytic bacterial community in sweet potato tubers cultivated in the different combinations of soil and location.

The sweet potato endophytic population was affected by the cultivating location rather than the soil, ranging from around 10^6 CFU/g fw to 10^5 and 10^4 CFU/g fw in Fukagawa, Miyazaki and Matsue locations, respectively (Fig. 1). Information on endophytic population and affecting factors is limited. Nissinen et al. [11] reported that the endophytic populations were 10^4-10^6 CFU/g fw in arcto-alpine plants depending on their species, and suggested that

the plant type affected on the population. In our study, we expected that the unknown location-specific factors affected on the plant physiology, which could determine the endophytic and/or rhizospheric population, a major source from which endophytic bacterial populations originate.

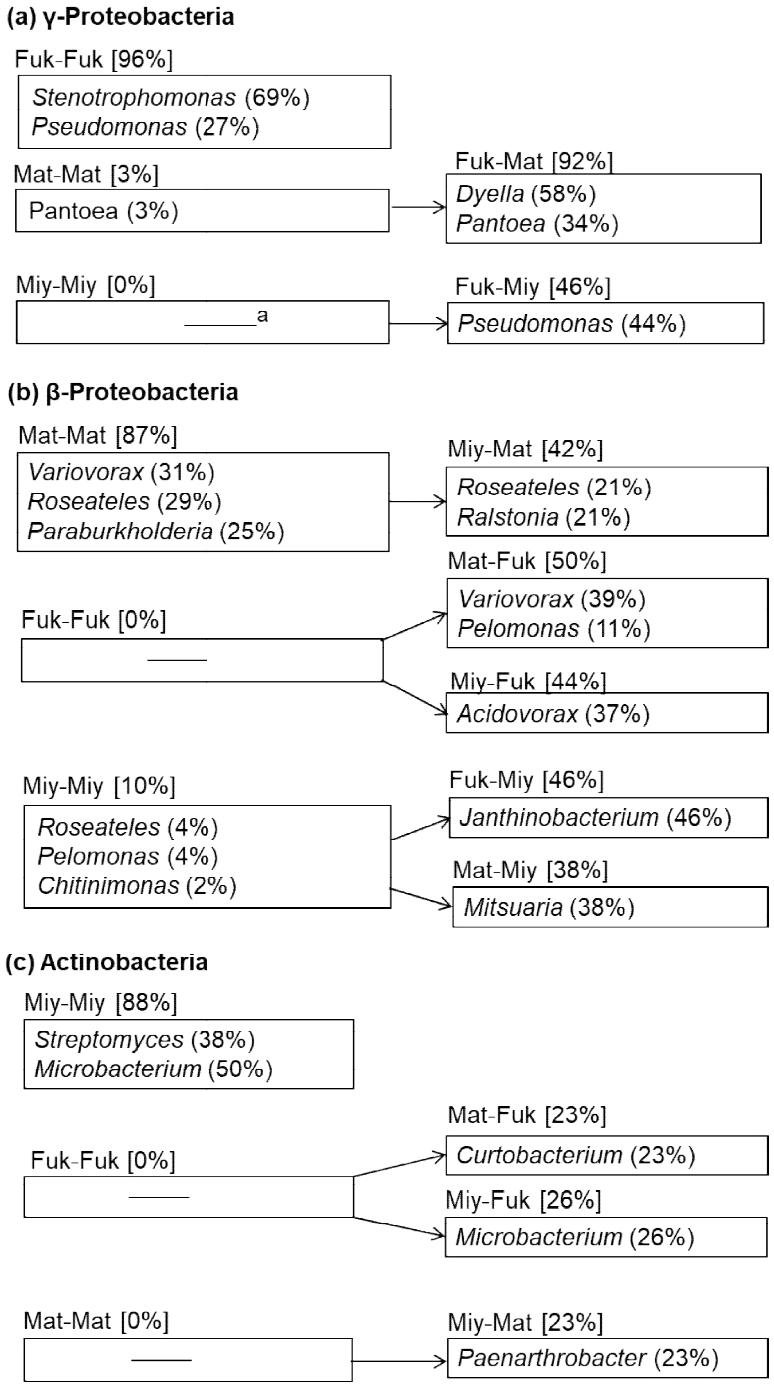

Fig. 3. Shift in endophytic genera composition under different location-soil conditions. [ ] and ( ) indicate relative percentages of class and genera, respectively

[a] the bar indicates absence of corresponding isolates

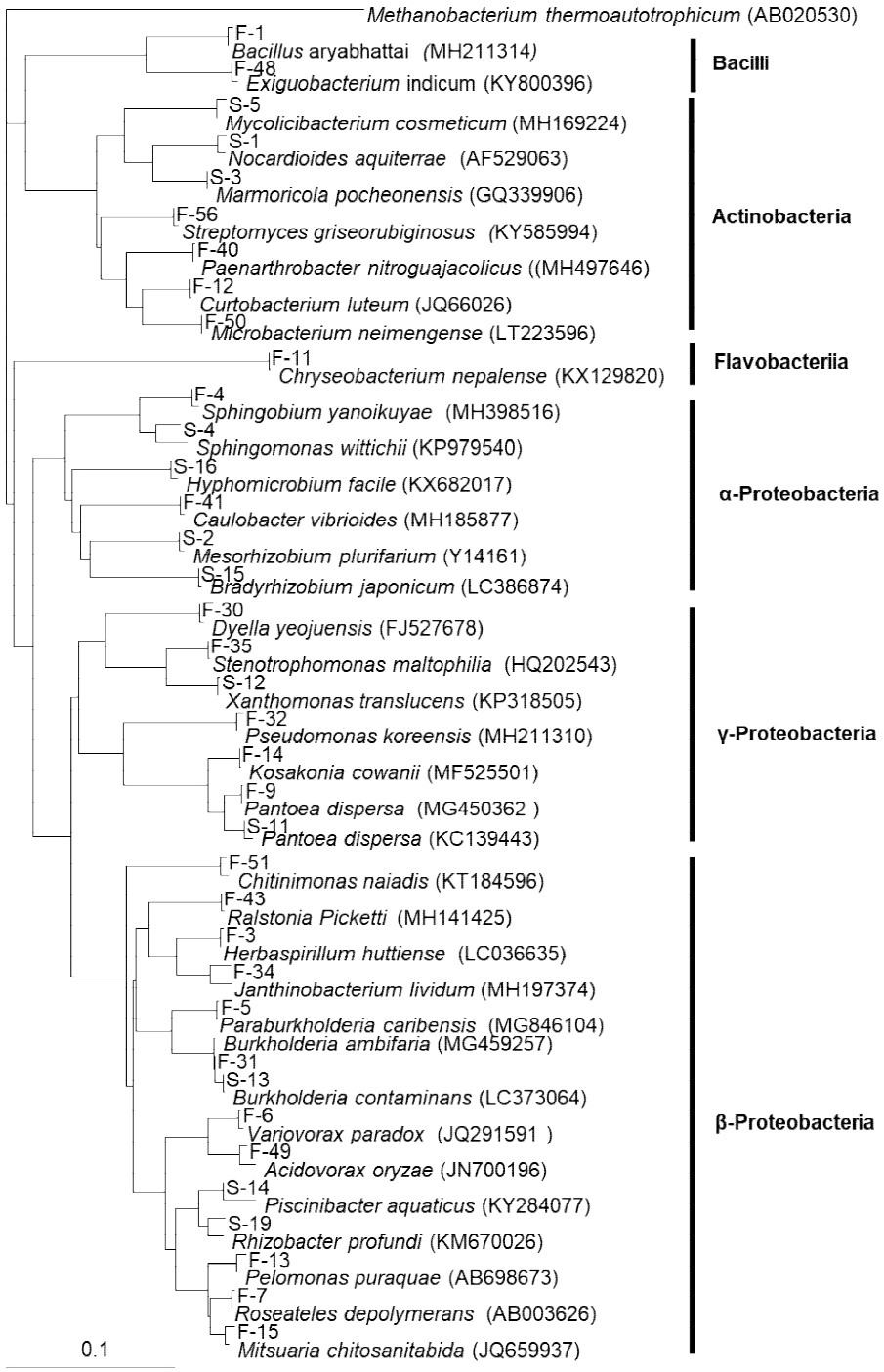

Fig. 4. Phylogenetic relationship of the fast and slow growing sweet potato endophytic bacteria. The sequence of *Methanobacterium thermoautotrophicum* (AB020530) served as an outgroup. Strain names are listed in Table S1 and the name of the strains designated as F and S for the fast and slow growers, respectively. The scale bar indicates the number of substitutions per site

In the present study, some specific phyla, γ- and β-Proteobacteria, and Actinobacteria were dominantly isolated in sweet potato tubers collected from Fukagawa, Matsue and Miyazaki locations, respectively (Fig. 2). It was reported that endophytic bacteria generally belonged to Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, among which γ-Proteobacteria was reported as the most dominant endophytes [16]. This has also been the case for the endophytes of sweet potato, as reported by Khan and Doty [7], Marques et al. [8], Puri et al. [2], and also in this study. When the soil samples were used in the different locations, the above mentioned location-specific phyla increased in the new sites (Fig. 2). From these results, we assumed that the climatic conditions as, temperature and rainfall (Table 1) of the specific locations might influence the physiology of sweet potato plant. Then the physiological changes might effect on the internal plant environment and/or root exudates profile. The former would directly impact on the endophytic community and the later on the rhizospheric conditions, which influence on the rhizospheric community, the potential endophytes. It was reported that temperature influenced root exudates profile of tomato and clover [17]. In a previous study, it was unclear to specify which factor, the soil or the climate, was more important in determining the endophytic community [2]. In this study, by exchanging the soil samples among the different locations, it was suggested that the climatic conditions would determine the endophytic community. The mechanisms and the determining factors of the specific domination have been unclear and need to be investigated.

While the endophytic community is characterised by the location-specific phyla, dissimilar genera generally appeared among the samples (Fig. 3). It was reported that some microbial characteristics were phyla basis. For example, the soil Acidobacteria had a negative relationship with carbon concentration and were classified as oligotrophs, while β-Proteobacteria and Bacteroidetes had an opposite relationship and classified as copiotrophs [18]. In another example, Kurm et al. [19] reported that γ-Proteobacteria grew faster and used more substrates in high nutrient tryptone soy broth media, whereas α-Proteobacteria grew slowly and used fewer substrates among the soil bacterial isolates. In relation to the cultivation conditions, the relative abundances of Acidobacteria, Verrucomicrobia and Gemmatimonadetes decreased in the soil with N-fertilisation [20]. Thus, it was expected that these characteristics might be responsible for the phyla-specific endophytic community.

Culture-dependent methods have been used to characterise the endophytic bacterial community. However, the community is influenced mainly by the media conditions [8], and a limited number of populations are culturable [21,22,23]. Therefore, the use of culture-independent methods, such as next generation sequencing technologies using DNA extracted from the plant sample, are the possible options to provide additional information on the endophytic bacterial communities.

5. CONCLUSION

The bacterial phyla, γ-Proteobacteria, β-Proteobacteria and Actinobacteria, dominated in the sweet potato tubers cultivated in Fukagawa, Matsue and Miyazaki soils at the corresponding locations, respectively. When effects of the location-soil combinations were examined, the above mentioned location-specific phyla increased at respective sites regardless of the soils used, and the endophytic bacterial population was also affected by the locations. The results suggested that the cultivating locations were more important factor than the soils to determine the sweet potato endophytic bacterial community and population.

ACKNOWLEDGEMENT

This study was supported in part by a Grant-in-Aid for Scientific Research (B) (16KT0032) from the Japan Society for the Promotion of Science (JSPS). We are thankful to the Laboratory of soil and ecological engineering, Shimane University for soil analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, et al. Endophytic bacteria and their potential applications. Critical Reviews in Plant Sciences. 2002;21(6):583–606. DOI: 10.1080/0735-260291044377
2. Puri RR, Dangi SR, Dhungana SA, Itoh K. Diversity and plant growth promoting ability

of culturable endophytic bacteria in Nepalese sweet potato. Advances in Microbiology. 2018;8(9):734–761.
DOI: 10.4236/aim.2018.89049

3. Feng Y, Shen D, Song W. Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. Journal of Applied Microbiology. 2006;100(5):938–945.
DOI: 10.1111/j.1365-2672.2006.02843.x
4. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: Recent developments and applications. FEMS Microbiology Letters 2008;278(1):1–9. DOI: 10.1111/j.1574-6968.2007.00918.x
5. Kandel S, Joubert P, Doty S. Bacterial endophyte colonization and distribution within plants. Microorganisms. 2017;5(4): 77.
DOI: 10.3390/microorganisms5040077
6. Asis JR CA, Adachi K. Isolation of endophytic diazotroph *Pantoea agglomerans* and nondiazotroph *Enterobacter asburiae* from sweetpotato stem in Japan. Letters in Applied Microbiology. 2003;38:19–23.
DOI: 10.1046/j.1472-765X.2003.01434.x
7. Khan Z, Doty SL. Characterization of bacterial endophytes of sweet potato plants. Plant and Soil. 2009;322(1):197–207.
DOI: 10.1007/s11104-009-9908-1
8. Marques JM, da Silva TF, Vollu RE, de Lacerda JRM, Blank AF, Smalla K, et al. Bacterial endophytes of sweet potato tuberous roots affected by the plant genotype and growth stage. Applied Soil Ecology. 2015;96:273–281.
DOI: 10.1016/j.apsoil.2015.08.020
9. Yang R, Liu P, Ye W. Illumina-based analysis of endophytic bacterial diversity of tree peony (*Paeonia* Sect. *Moutan*) roots and leaves. Brazilian Journal of Microbiology. 2017;48(4):695–705.
DOI: 10.1016/j.bjm.2017.02.009
10. Xia Y, DeBolt S, Dreyer J, Scott D, Williams MA. Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Frontiers in Plant Science. 2015;6:1–10. DOI: 10.3389/fpls.2015.00490
11. Nissinen RM, Männistö MK, van Elsas JD. Endophytic bacterial communities in three arctic plants from low arctic fell tundra are cold-adapted and host-plant specific. *FEMS* Microbiology Ecology. 2012;82(2): 510–522.
DOI: 10.1111/j.1574-6941.2012.01464.x
12. Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, et al. Endophytic colonization and in planta Nitrogen fixation by a *Herbaspirillum* sp. Applied and Environmental Microbiology. 2001;67(11): 5285–5293.
DOI: 10.1128/AEM.67.11.5285.
13. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S Ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173(2):697–703.
PMID: 1987160.
14. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 1994; 22(22):4673–4680.
PMID: 7984417.
15. Staff SS. Soil Taxonomy. Soil Taxonomy A Basic system of soil classification for making and interpreting soil surveys USDA Agriculture Handbook No 436 US Government Print Office, Washington DC. 1999;1–754.
16. Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, et al. The Hidden World within Plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews. 2015;79(3):293–320. DOI: 10.1128/MMBR.00050-14
17. Rovira AD. Root excretions in relation to the rhizosphere effect - IV. Influence of plant species, age of plant, light, temperature, and calcium nutrition on exudation. Plant and Soil. 1959;11(1):53–64.
DOI: 10.1007/BF01394753
18. Fierer N, Bradford MA, Jackson RB. Toward an ecological classification of soil bacteria. Ecology. 2007;88(6):1354–1364. DOI: 10.1890/05-1839
19. Kurm V, Van Der Putten WH, De Boer W, Naus-Wiezer S, Gera Hol WH. Low abundant soil bacteria can be metabolically versatile and fast growing. Ecology. 2017; 98(2):555–564.
DOI: 10.1002/ecy.1670

20. Cederlund H, Wessén E, Enwall K, Jones CM, Juhanson J, Pell M, et al. Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Applied Soil Ecology. 2014;84:62–68.
DOI: 10.1016/j.apsoil.2014.06.003
21. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews. 1995;59(1):143–169.
DOI: 10.1016/j.jip.2007.09.009
22. Torsvik V, Salte K, SØrheim R, GoksØyr J. Comparison of phenotypic diversity and DNA hererogeneity in a population of soil bacteria. Appled and Environmental Microbiology. 1990;56(3):776–781.
PMID: 2180371.
23. Oliver JD. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiology Reviews. 2010;34(4):415–425.
DOI: 10.1111/j.1574-6976.2009.00200.x

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27455