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Diversity and Plant Growth Promoting Ability of Culturable Endophytic Bacteria in Nepalese Sweet Potato

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Abstract

There is no information on Nepalese sweet potato endophytes. We isolated 243 endophytic bacteria belonging to 34 genera in six classes from 12 locations of Nepal. Among them, the predominant classes were Bacilli and Gammaproteobacteria. The principal component analysis revealed that the composition of bacterial classes was unrelated to the environmental parameters of the sampling sites. Regarding their plant growth promoting potentials, 57% of the strains demonstrated indole-3-acetic acid (IAA) producing ability while 5% strains had nitrogen fixing gene (*nifH*) and acetylene reduction assay (ARA) activity. The representative strains in all six classes showed antagonistic effect against bacterial pathogens while only *Bacillus* strain showed the effect against fungal pathogen. For endophytic traits, cellulase activity was observed in 5 classes, while pectinase activity was only in Proteobacteria. Fresh weight and vine length of sweet potato increased by inoculating mixed cultures of the isolates from each location.

Keywords

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is a resilient, easily propagated crop which grows well in marginal lands. The plant can be cultivated in low-fertile soils, takes up more nitrogen than other root crops [1] [2]. The capacity of sweet po-

tato to grow well in low fertile soils might be due to the endophytic bacteria with plant growth promoting traits. Endophytic diazotrophic bacteria such as *Klebsiella*, *Pantoea* and *Gluconacetobacter* have been isolated from sweet potatoes [3] [4] [5]. Similarly, sweet potato bacterial endophytes with auxin production, antagonistic effect, phosphate solubilization and siderophore production abilities have also been isolated [6] [7]. On the other hand, there have been a few studies on endophytic bacterial community from sweet potato crops. For instance, the community was examined for samples collected in Brazil [6] and in USA [7], and it was shown that Gammaproteobacteria was common dominating group in both studies.

Nepal, a small Himalayan country, lies along the southern slopes of the Himalayan Mountains between China and India. It varies greatly in topography, climate and vegetation; the elevation ranges from 68 to 8848 masl in a just 150 to 250-km south-north transect. In Nepal, sweet potato is cultivated from terai (60 - 300 masl) to mid hills (300 - 2000 masl) and the average productivity is 5 - 6 tons ha^{-1} [8], while the world productivity is 12.2 tons ha^{-1} [9]. Till date, there is no information on Nepalese sweet potato endophytes. Adhikari *et al.* reported that the diverse climate and soils in Nepal was suspected to be conducive for the occurrence of diverse soybean rhizobial strains [10]. So, we expect that diverse endophytic bacterial isolates with the potentials for plant growth promotion could be isolated from the Nepalese sweet potato.

Sustainability issues in agriculture are a priority for several countries in the world; in this regard, the use of microbial inoculants to the agriculture farming might contribute to ensure sustainable production. In this study, we aimed to examine bacterial community of sweet potato endophytes in Nepal in relation to the environmental parameters and characterize their plant growth promoting traits. As synergistic effect of mixed cultures of plant growth promoting bacteria was reported [11] [12], we also examined their potential by inoculating combined isolates from each location.

2. Materials and Methods

2.1. Sample Collection and Study Sites

Sweet potato tubers were collected from three months old plants during the autumn of 2015 representing 12 sweet potato growing sites in Nepal, six from subtropical and six from temperate regions. Sampling sites, climate and soil properties are presented in Table 1.

The sweet potato samples were washed with tap water, shade dried and kept at room temperature until the isolation of the endophytic bacteria. Soil samples collected from the same field during the spring of 2016 were air-dried and crushed to pass through a 2 mm sieve. The pH was measured using the glass electrode method with a soil: water ratio of 1:2.5 [13]. Total carbon (TC) and total nitrogen (TN) were determined by the dry combustion method using an NC analyzer (MT-700, J-Science, Kyoto, Japan). Available phosphorus (P) was

Table 1. Climate, land and soil properties of the sweet potato sampling sites in Nepal.

Climate	Location (District)	Temperature[a] Max	Min	Latitude	Longitude	Annual Rainfall (mm)[a]	Altitude (masl)	Soil type[b]	Soil pH	Soil P[c]	Soil C[c]	Soil N[c]
	Rolpa	32	3.4	28.30˚N	82.63˚E	1261	1200	Inceptisols	7.3	331	16	1.5
	Salyan	31	6.5	28.42˚N	82.00˚E	987	1300	Entisols	6.2	4.8	11	0.8
Temperate (Cooler highland)	Gulmi	28	4.3	28.02˚N	83.24˚E	1860	1500	Entisols	6.7	12	7.0	0.7
	Palpa	33	6.1	27.89˚N	83.50˚E	1564	1219	Entisols	6.4	37	20	1.7
	Kavre-a	28	2.8	27.62˚N	85.58˚E	1190	1408	Entisols	5.8	111	8.0	0.6
	Kavre-b	28	2.8	27.61˚N	85.59˚E	1190	1116	Entisols	5.5	87	7.0	0.6
	Banke-a	39	8.0	28.02˚N	81.76˚E	1230	181	Alfisols	8.0	87	11	1.0
	Banke-b	39	8.0	28.11˚N	81.59˚E	1230	179	Alfisols	8.4	6.2	8.0	0.7
Subtropical (warmer foothills & plains)	Rupandehi	38	7.8	27.58˚N	83.31˚E	1572	107	Alfisols	8.3	11	4.0	0.3
	Chitwan	36	6.2	27.65˚N	84.39˚E	1960	228	Alfisols	6.6	137	13	1.0
	Sunsari-a	34	8.0	26.71˚N	87.25˚E	1816	107	Alfisols	6.7	379	20	1.7
	Sunsari-b	34	8.0	26.70˚N	87.28˚E	1816	108	Alfisols	5.9	20	16	1.3

[a]5 years average of maximum, minimum annual temperature and annual rainfall (http://www.dhm.gov.np/); [b]Based on USDA classification [26]; [c]Soil P in mg kg^{-1}, and Soil C and Soil N in g kg^{-1}.

determined by Olsen's bicarbonate method [14].

2.2. Isolation and Identification of Endophytic Bacteria

The sweet potato samples were washed again with running tap water for 10 min. Each sample was cut transversely when its diameter was more than 10 mm otherwise cut longitudinally. Then, the cut surface was stamped on the modified MR agar medium [4], and incubated for 2 days at 26˚C. The appeared colonies were grouped based on their morphologies and the representative colonies reflecting their relative abundance were purified for further analysis as endophytes.

The partial 16S rRNA genes of the isolated endophytic bacteria were amplified using the universal primers (fD1 and rP2) to the domain bacteria [15]. The PCR mixture was prepared by mixing MilliQ water, 10x reaction buffer, 10 mM dNTPs, *Taq* DNA polymerase (GENETBIO Inc., Daejeon, Korea), fD1 and rP2 primers together with the template. The PCR reaction was carried out with a pre-run at 94˚C for 3 min, 30 s at 94˚C, 30 s at 50˚C, 1 min at 72˚C for 30 cycles and final run at 72˚C for 5 min. The PCR products were sequenced as described by Adhikari *et al.* [10]. In brief, the respective PCR products were purified by using SOPE^{TM} resin (Edge Biosystems Inc. USA) and a Performa Dye Terminator Removal (DTR) Gel Filtration Cartridge (Edge Biosystems Inc. USA). Then, their nucleotide sequences were analyzed by an ABI Prism, 3100-Avant-100D2 (3130 xl/Genetic Analyzer, Hitachi, Tokyo, Japan). Close relatives for each isolate was assigned using the data base (https://www.ddbj.nig.ac.jp/index-e.html) by a BLAST search [16].

2.3. Distribution of Endophytic Bacteria in Relation to Environmental Conditions

Correlation between bacterial class compositions and the environmental parameters (Table 1) of the sampling sites was analyzed by the principal component analysis (PCA). Bacterial class compositions as expressed by relative percentage were used for the calculation.

2.4. Characterization of Endophytic Bacteria

Sixty representative isolates of 34 genera in 6 classes were selected by their phylogenies (Table S1 & Figure S1) and used for the characterization of their plant growth promoting traits, antagonistic effect and endophytic traits.

For indole-3-acetic acid (IAA) production assay, the isolates were cultivated in the modified MR liquid media supplemented with 200 μg/ml of L-tryptophan at 26˚C for 3 days with shaking (150 rpm). After centrifugation at 8000 g for 15 min, the supernatant was applied for quantification of IAA according to the method described by Gordon and Weber [17].

For detection of nitrogen fixing gene (*nifH*), primers PolF and PolR which were designed to match a broad range of bacterial *nifH* gene [18] were used for PCR. The PCR components and conditions were as described by Adhikari *et al.* [10]. Nitrogenase activity of the *nifH* gene containing isolates was evaluated using the acetylene reduction assay (ARA). The isolates were cultivated in N-free modified MR media [19] for 3 days at 26˚C with shaking (150 rpm). Then, the culture was washed and suspended in sterile distilled water at OD_{660nm} 0.2. Then, 50 μl of the suspension was inoculated on a slant of semi-solid (1.3%) N-free modified MR agar media in 60 ml vials in triplicate. The vials were sealed with a butyl-rubber and an aluminium stopper, and 10% of the headspace volume was replaced with pure acetylene. Vials without acetylene and without inoculants served as controls. Ethylene concentrations in the vials were measured after 3 days of incubation in dark at 28^0C, using a gas chromatograph (Shimadzu GC-14B; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and Porapak N (50/80 mesh; GL Sciences, Tokyo, Japan).

For assay of antagonistic effects, the point inoculation method [20] was used against the fungal pathogen (*Pythium ultimum* var *ultimum* Strain OPU744). Briefly, each test strain was streaked on the PDA plate (dextrose: 1 g l^{-1}, peptone: 5 g l^{-1}, yeast extract: 2.5 g l^{-1} and agar: 15 g l^{-1}). After one day of cultivation at 28˚C, a 5 mm of fungal mycelial disk was placed at approximately 30 mm from the bacterial streaked line and continuously cultivated for 7 days. The corresponding fungal disk without endophyte strain served as control. To test antagonistic effect of endophytes against bacterial pathogens, each test isolates were streaked on a half part of PDA plates and incubated for 2 days at 28˚C. Then each bacterial pathogens (ECa: *Erwinia chrysanthemi* Strain NARCB200126, AZ9702, causing stem and root rot in sweet potato, ECb: *Erwinia chrysanthemi* Strain E7725, causing stem rot in potato and ECc: *Erwinia chrysanthemi* Strain

Ech T5-2, causing root rot in Taro); was streaked approximately 5mm to the endophytes and incubated at 28˚C for 9 days. For both assays, antagonistic effects were categorized based on the distance between the test isolates and the pathogen as follows: no (0 mm), weak (1 - 3 mm) and strong (>3 mm) activities.

For the cellulase assay, the isolates were spotted on a carboxymethyl cellulose (CMC) agar medium [21]. Plates were incubated at 28˚C for 6 days. The clear zone around the point of inoculation was examined by staining remaining CMC with Congo red [22]. For the pectinase assay, the test strains were spotted on a nutrient agar (DIFCO laboratories, USA) medium supplemented with 0.5% pectin and incubated at 28˚C for 3 days, then remaining pectin was stained with cetyltrimethylammonium bromide (CTAB) to visualize the clear zone around the bacterial culture [23]. For both assays, the activities were categorized based on the clear zones around the bacterial colony as follows: no (0 mm), weak (1 - 3 mm) and strong (>3 mm) activities.

2.5. Evaluation of Plant Growth Promotion in Sweet Potato with Endophytes

The endophytes within the same location were selected for the inoculation experiment based on their phylogenies (Table S1). Each strain was cultivated separately in nitrogen containing modified MR liquid medium at 26˚C with shaking for 5 days. The cells were harvested by centrifugation (10,000 rpm for 10 minutes at 15˚C), washed twice and suspended with sterile distilled water, and OD_{660} was adjusted to 0.2. Then, the cell suspensions of the same location were mixed together to make the inoculants in 10 ml final volume. The experiment was conducted using a Leonard jar [24]. The upper pot was filled with water-soaked sterile vermiculite and the lower pot was supplied with 150 ml of sterile 1/5N plant medium [25]. The cotton wick was set to connect the upper pot and the lower reservoir. The whole pot was autoclaved before use.

Micro-propagated sweet potato plantlets cv. Koukei (3 - 4 leaves) was used for the experiments. Two consecutive experiments were conducted in duplicate. After measuring initial fresh weight, vine and root lengths (except for root length in the first experiment due to the absence of roots), the root part was dipped into the inoculants for 3 min and transplanted to the sterile pot, and 5 ml inoculant was poured on the vermiculite around the plant. The inoculated plants were aseptically grown in a plant growth chamber (LH240S, Nippon medical and chemical instruments co., ltd, Japan) with a 14 hour photo period, 28˚C/25˚C (day/night) at 7000 lux, which was provided by white fluorescent tubes. The same plant media without nitrogen was supplied to the bottom pot, as per the requirement. For control, sterile distilled water was inoculated. First experiment was conducted with nine inoculants and the better performing six inoculants were used in the second experiment.

The plants were harvested at 30 days after inoculation. The whole plant was carefully pulled to avoid damage and shaken to release loosely attached vermiculite, then strongly adhered vermiculite was manually removed with tweezers.

After blotting excess moisture from the roots with absorbent paper, whole plant fresh weight, vine and root lengths were measured. Then, nitrogenase activity for the fresh roots was assayed by ARA using 100 ml vial. Uninoculated plant roots with/without acetylene served as controls.

2.6. Statistical Analysis

All the statistical analyses were carried out using SPSS software package version 16.

2.7. Nucleotide Sequence Accession Numbers

All sequences are deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers LC389337 to LC389579 (16S rRNA) and LC389580 to LC389582 (*nifH* gene).

3. Results

3.1. Isolation and Identification of Endophytic Bacteria

Different morphologies were observed among the appeared bacterial colonies, ranging from one to nine morphologies in Gulmi and Rupandehi locations, respectively. Two to 43 endophytic bacterial isolates per location, making a total of 243 isolates, were isolated and examined (Table S1).

Based on partial 16S rRNA gene sequencing analysis, 243 endophytic bacterial isolates were assigned to their close relatives, belonging to 34 bacterial genera in 6 classes (Table 2). Among the classes, Bacilli represented the highest relative abundance (28%), and *Bacillus* sp. was the most dominant genus (25%), followed by Gammaproteobacteria (22%)/*Enterobacter* sp. (5.3%), Betaproteobacteria (17%)/*Burkholderia* sp. (8.6%), Actinobacteria (16%)/*Microbacterium* sp. (6.8%), Alphaproteobacteria (14%)/*Rhizobium* sp. (6.3%) and Flavobacteriia (4.4%)/*Flavobacterium* sp. (4.4%).

3.2. Distribution of Bacterial Genera

Endophytic bacterial genera were distributed unevenly among the sweet potato samples (Table 2). Four bacterial genera commonly detected in at least five sampling sites were applied to the distribution analysis (Table 3). *Enterobacter* sp. and *Microbacterium* sp. were detected frequently in nutrient rich (copiotrophic) and poor (oligotrophic) soils, respectively. *Bacillus* sp. showed acidophilic nature while *Rhizobium* sp. and *Microbacterium* sp. were alkaliphilic. Similarly, *Enterobacter* sp. showed neutralophilic property. On the other hand, distribution of these four genera was unaffected by the temperature conditions.

PCA of the environmental parameters explained 41.3% and 40.6% of the variation in the first and second principal component factors, respectively, and showed that there are approximately two groupings, first being the high temperature and alkaline soils (Rupandehi, Banke-a and Banke-b) and second with the others (Figure 1(a)). PCA of bacterial class compositions explained 31.0% and

Table 2. Relative abundance of bacterial endophytes of sweet potato in Nepal.

Sampling sites	Rolpa	Salyan	Gulmi	Palpa	Kavre-a	Kavre-b	Banke-a	Banke-b	Rupandehi	Chitwan	Sunsari-a	Sunsari-b	Total
No. of colonies	301	440	20	70	54	165	320	327	384	150	172	87	2490
No. of morphologies	7	5	1	3	2	5	7	7	9	4	3	2	55
No. of isolates	30	43	2	6	5	17	31	32	37	14	18	8	243
Class/genera													Average (%)
Flavobacteriia	23	25					4.0						4.4
Flavobacterium sp.	23	25					4.0						4.4
Bacilli	16	3.0	100	32	22	51	3.0		8.0	48	44	12	28
Bacillus sp.		3.0	100	43	22	37			3.0	48	44	12	25
Staphylococcus sp.	16					13	3.0						2.7
Exiguobacterium sp.									3.0				0.3
Paenibacillus sp.									3.0				0.3
Actinobacteria	37	5.0					61	13	67				16
Microbacterium sp.	7.0	5.0					40	3.0	27				6.8
Curtobacterium sp.	23						11	10	12				4.6
Cellulomonas sp.	23						3.0						2.0
Arthrobacter sp.							6.8		10				1.0
Glutamicibacter sp.									13				1.0
Pseudarthrobacter sp.									5.0				0.4
Streptomyces sp.	4.0												0.3
Brachybacterium sp.	3.0												0.2
Alphaproteobacteria		11		16	37	13	24	44	6.0	7.0	6.0		14
Rhizobium sp.		9.0		16		13	6.0	19	6.0		6.0		6.3
Agrobacterium sp.		2.0			37		6.0	13					4.8
Sphingobium sp.								13					1.0
Sphingomonas sp.							12						1.0
Neorhizobium sp.										7.0			0.6
Betaproteobacteria	7.0	7.0			22	32		10	13	22		88	17
Burkholderia sp.										15		88	8.6
Achromobacter sp.						19			13				3.0
Herbaspirillum sp.		7.0			22	7.0							3.0
Xenophilus sp.								10					0.8
Massilia sp.	7.0												0.6
Paraburkholderia sp.										7.0			0.6
Caballeronia sp.						6.0							0.5
Gammaproteobacteria	17	48		51	19	4.2	6.0	33	6.0	23	50		22
Enterobacter sp.	7.0	2.0		33			3.0			7.0	11		5.3
Pseudomonas sp.				19							39		4.8
Stenotrophomonas sp.	3.0	28						12		9.0			4.3
Luteibacter sp.				19	1.0								1.7
Pantoea sp.						3.0	3.0		6.0	7.0			1.6
Klebsiella sp.		18											1.5
Xanthomonas sp.								15					1.3
Pseudoxanthomonas sp.								6.0					0.5
Yokenella sp.	6.0												0.5

Table 3. Detection frequency (%) of the bacterial genera in different environmental conditions[a].

Genera	Phosphorus		Carbon		Nitrogen		pH			Temperature	
	Low	High	Low	High	Low	High	Acidic	Neutral	Alkaline	Low	High
Bacillus sp.	83	67	71	80	83	67	100	83	33	83	67
Rhizobium sp.	67	50	71	40	67	50	33	50	100	50	67
Enterobacter sp.	33	67	29	80	17	83	0	83	33	50	50
Microbacterium sp.	50	33	57	20	50	33	0	33	100	33	50

[a]: Low P (4.8 - 37 mg kg^{-1} soil) and high P (87 - 379 mg kg^{-1} soil), low C (4 - 11 g kg^{-1} soil) and high C (13 - 20 g kg^{-1} soil), low N (0.3 - 0.8 g kg^{-1} soil) and high N (1.0 - 1.7 g kg^{-1} soil), acidic (pH 5.5 - 5.9), neutral (pH 6.2 - 7.3) and alkaline (pH 8.0 - 8.4), high temperature (34˚C - 39˚C) and low temperature (28˚C - 32˚C).
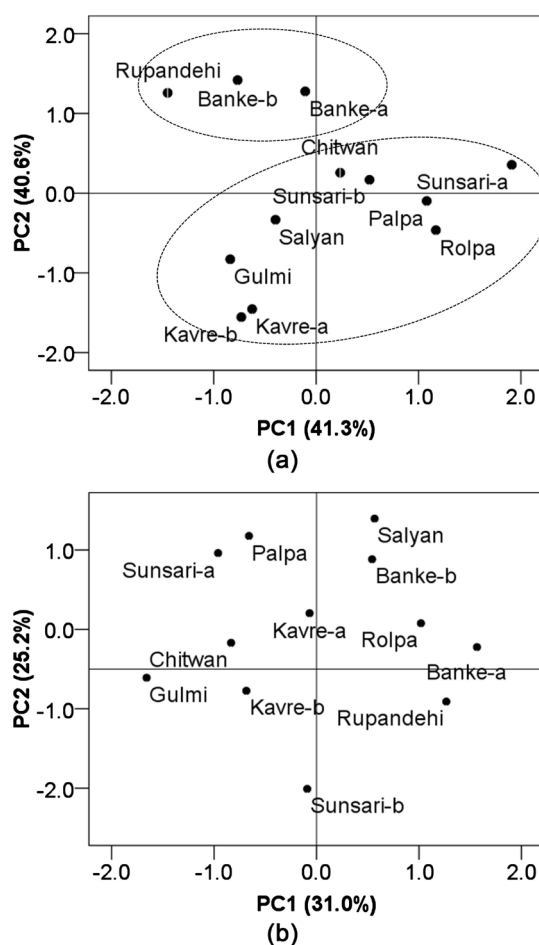

Figure 1. 2-D plot of principal component analysis of sampling locations based on (a) environmental parameters and (b) bacterial class composition in each location.

25.2% of the variation in the first and second principal component factors, respectively, and revealed that the endophytic bacterial composition did not group as the environmental conditions (Figure 1(b)).

3.3. Characterization of the Endophytic Bacterial Isolates

Eighty three percent of the bacterial strains presented at least one of the charac-

teristics examined. Within all the strains, 57% produced IAA, 5.0% had *nifH* gene and showed ARA activity, 37% and 2.0% possessed antagonistic effect against the bacterial and the fungal pathogens, respectively. In addition, 17% and 8.0% showed cellulase and pectinase activities, respectively (Table 4).

Proportions of the bacterial class representing the examined traits were different (Table 4). IAA production was detected in the strains from Bacilli, Actinobacteria, Alpha- and Gamma-proteobacteria classes, and ARA activity was from Beta- and Gamma-proteobacteria. All the classes possessed antagonistic effect against the bacterial pathogens, while only Bacilli class showed the effect against the fungal pathogen. Almost all the bacterial classes showed cellulase activity while Alpa-, Beta- and Gamma-proteobacteria classes had pectinase activity.

Bacterial strains exhibiting at least one of the following plant growth promoting or endophytic traits are presented in Table 5: high IAA production (≥30 µg/ml), ARA activity, strong antagonistic and cellulase/pectinase activities. Among the bacterial strains 8 of them showed higher IAA production potential with Ban-b 4 being the highest (65 µg/ml). Strains belonging to the same genus showed varying levels of IAA producing ability. In addition, Sal 1, Sal 6 and Rol 5 had *nifH* gene and showed the ARA activity with 54.5 ± 7.3 nmol C_2H_4/h/vial, 39.9 ± 1.9 nmol C_2H_4/h/vial and 8.9 ± 0.8 nmol C_2H_4/h/vial, respectively.

Likewise, 11 bacterial strains showed strong antagonistic effect against at least one pathogen tested. Among them, Chi 2 and Gul 1 possessed strong activity against the tested bacterial pathogens and the latter showed strong activity against the fungal pathogen assayed. However, these two isolates did not show IAA producing ability. On the other hand, 5 bacterial strains showed cellulase activity while Ban-b 6 showed both cellulase and pectinase activity.

3.4. Effect of Mixture of Endophytes on Plant Growth Promotion

Fresh weight (g), vine and root lengths (cm) were considered for the assessment of plant growth promotion. Plantlets used in the experiments were non-uniform in size, and this might affect the parameters. So, times increase as compared to the control were used for the assessment of the plant growth promotion of the inoculants.

In the first experiment, the inoculated sweet potatoes showed higher values than control (Table 6). Mixture of isolates from Salyan, Palpa, Banke gained 3.18 - 3.51 times their initial weight whereas it was 1.6 in control. Likewise, gain in vine length ranged from 1.33 - 1.50 times for Kavre, Chitwan, Banke and Salyan inoculants, while it was 1.17 in control. Finally, the roots were longer in almost all inoculated plants than control.

Further, we selected six inoculants based on the first experiment and again evaluated. In the second experiment, the growth promoting effects were observed in fresh weight and vine length, but root lengths were shorter in the inoculated plants than control (Table 6). Among all the plants assessed, only Salyan

Table 4. Bacterial class and their proportions having the plant growth promoting traits, antagonistic effect and endophytic traits.

Class	Number of genera	Number of strains	Plant growth promoting traits		Antagonistic effect				Endophytic traits	
			IAA[a]	ARA activity[b]	ECa[c]	ECb[d]	ECc[e]	Fungal[f]	Cellulase	Pectinase
Flavobacteriia	1	1	0	0	100	100	100	0	0	0
Bacilli	4	7	43	0	29	43	28	14	14	0
Actinobacteria	8	20	63	0	21	16	16	0	16	0
Alphaproteobacteria	5	9	78	0	22	33	22	0	11	11
Betaproteobacteria	7	8	0	13	25	25	25	0	13	13
Gammaproteobacteria	9	15	75	13	19	25	6	0	25	19
Total	34	60	57	5	27	58	25	2	17	8

[a]Indole-3-acetic acid (μg ml^{-1}); [b]Acetylene reduction activity (nmol/h/vial); [c]*Erwinia chrysanthemi* NARCB200126, AZ9702; [d]*Erwinia chrysanthemi* E7725; [e]*Erwinia chrysanthemi* T5-2; [f]*Pythium ultimum* var *ultimum* OPU744.

Table 5. Plant growth promoting traits, antagonistic effect and endophytic traits of bacterial endophytes of sweet potato in Nepal.

Class	Strains	Close relatives based on 16S rRNA gene sequencing	Plant growth promoting traits		Antagonistic effect[c]				Endophytic traits[c]	
			IAA/OD[a]	ARA activity[b]	ECa[d]	ECb[e]	ECc[f]	Fungal[g]	Cellulase	Pectinase
Bacilli	Gul 1	*Bacillus* sp.	0	-	++	++	++	++	++	-
	Sun-a 3	*Bacillus* sp.	34	-	-	-	-	-	-	-
Actinobacteria	Rol 1	*Curtobacterium* sp.	0	-	-	-	-	-	++	-
	Sal 8	*Microbacterium* sp.	2	-	+	+	++	-	-	-
	Ban-a 5	*Arthrobacter* sp.	35	-	-	-	-	-	-	-
	Rup 2	*Microbacterium* sp.	11	-	++	+	++	-	++	-
	Rup 6	*Microbacterium* sp.	17	-	++	+	-	-	-	-
Alphaproteobacteria	Sal 7	*Agrobacterium* sp.	30	-	+	+	-	-	-	-
	Ban-a 3	*Agrobacterium* sp.	26	-	-	++	-	-	-	-
	Ban-a 4	*Rhizobium* sp.	28	-	-	++	++	-	-	-
	Ban-a 9	*Sphingomonas* sp.	8	-	-	+	+	-	++	-
	Ban b 4	*Sphingobium* sp.	65	-	++	+	+	-	-	-
Betaproteobacteria	Sal 6	*Herbaspirillum* sp.	0	9	-	+	-	-	-	-
	Chi 2	*Burkholderia* sp.	0	-	++	++	++	-	-	-
	Sun-b 1	*Burkholderia* sp.	0	-	++	+	+	-	+	-
Gammaproteobacteria	Rol 5	*Yokenella* sp.	36	40	-	-	-	-	-	-
	Sal 1	*Klebsiella* sp.	48	55	-	+	-	-	+	-
	Sal 3	*Enterobacter* sp.	61	-	-	+	-	-	-	+
	Kav-b 3	*Luteibacter* sp.	15	-	++	-	-	-	-	-
	Ban-a 7	*Pantoea* sp.	29	-	++	++	-	-	+	-
	Ban-b 6	*Pseudoxanthomonas* sp.	13	-	-	+	-	-	++	++
	Chi 1	*Pantoea* sp.	36	-	-	+	-	-	++	-

[a]Indole-3-acetic acid (μg ml^{-1}) optical density^{-1}; [b]Acetylene reduction activity (nmol/h/vial); [c]-, + and ++ denote no, weak and strong activities, respectively; [d]*Erwinia chrysanthemi* NARCB200126, AZ9702; [e]*Erwinia chrysanthemi* E7725; [f]*Erwinia chrysanthemi* Ech T5-2; [g]*Pythium ultimum* var *ultimum* OPU744.

Table 6. Times increase in growth parameters of sweet potato plants (n = 2).

Inoculants	First Experiment			Second experiment		
	Fresh weight	Vine length	Root length[b]	Fresh weight	Vine length	Root length
Rolpa	2.63	1.17	59	-	-	-
Salyan	3.51	1.33	73	5.70	1.53	7.2
Gulmi	2.31	1.28	68	-	-	-
Palpa	3.33	1.26	76	7.56	1.45	18.7
Kavre	2.59	1.50	66	10.20	2.33	22.4
Banke	3.18	1.33	50	9.45	1.50	34.2
Rupandehi	2.25	1.21	75	5.86	2.14	21.0
Chitwan[a]	2.99	1.35	74	4.98	1.00	25.0
Sunsari[a]	2.80	1.16	70	-	-	-
Control	1.60	1.17	47	4.68	1.24	38.0

[a]Data from one replication is considered for Sunsari and Chitwan in first and second experiment respectively, as one replication plant was dead; [b]Final root length (cm).

isolates inoculated plants showed ARA activity (0.09 nmol/h/g).

4. Discussion

In the present study, culture dependent method was used to learn more about the endophytic bacterial community in sweet potato collected from Nepal. In analyzing endophytic communities by culture dependent methods, most researchers selected dominant single colonies representing distinct morphology and ignored minor ones and therefore their diversity [27] [28] [29] [30] [31]. On the other hand, we examined all colonies in the plates and grouped based on their morphologies, and the representative colony (s) in each group was selected on the basis of their relative abundance for further analysis. Our method would be more reliable in examining the endophytic communities.

In culture dependent methods, media components are the most influential parameter. Marques *et al.* used three media conditions (TSA, PDA and modified RM) and isolated 93 endophytic bacteria belonging to 17 genera for three sweet potato cultivars collected in Brazil [6]. Although the media used were different, the following common genera were mainly detected: *Bacillus* sp. and *Paenibacillus* sp. in Bacilli class, *Arthrobacter* sp. and *Microbacterium* sp. in Actinobacteria, *Sphingomonas* sp. and *Rhizobium* sp. in Alphaproteobacteria and *Enterobacter* sp., *Pantoea* sp. and *Pseudomonas* sp. in Gammaproteobacteria. This result was similar to our result even though the media and cultivation locations were different. Khan and Doty isolated 11 endophytes in seven genera by MS medium from sweet potatoes collected from grocery store in USA [7], where *Stenotrophomonas* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Xanthomonas* sp. in Gammaproteobacteria were dominated. Gammaproteobacteria have been reported as commonly dominant endophyte in plants [32]. On the other hand,

isolates from Bacilli class was not detected [7] demonstrating that the Bacilli might not always be dominated in sweet potato. More studies are necessary to make better conclusions for endophytic dominancy of Bacilli in sweet potato. Hardoim *et al.* reported that Bacilli is not dominantly detected as endophyte in many plants [32], whereas, they have been dominantly detected in several crops as canola [33], banana [34], switch grass [35] and tobacco [36]. The determining factors of Bacilli are still unclear and needs to be explored.

Culture dependent methods have a limitation of analyzing microbial communities due to unknown conditions for growth requirements of many bacteria and presence of the viable but noncultivable state [37]. As a result, the dominant bacterial endophytes could not always be isolated. For example, *Ralstonia* sp. was dominant in culture independent methods in salad crops [31] and sweet potato [6], but it was not isolated from the samples. Likewise, *Enterobacter* sp. dominantly detected in culture independent methods in maize was not isolated [38]. In our study, we could not successfully amplify the bacterial DNA from the sweet potato DNA using LNA-PCR technique [39] and the possible reason is unknown. The culture dependent method has its own limitation on determining the bacterial community but it is only the option to isolate the bacteria for their functional analysis. It is important to find the suitable culture conditions for the endophytes. Modification of media components considering their natural habitat could be one of the options.

There have been relatively a few studies that have analyzed the effects of environmental variables on endophyte diversity [40]. For example, culturable endophytic bacterial communities were more diverse in tobacco roots from organic soils compared to those grown in mineral soils [36]. Similarly, psychrophilic bacterial endophytes were isolated abundantly in cold environments from the arcto-alpine plant species [41]. Likewise, culturable endophytic bacterial communities in four vegetable crops were more diverse in organic farming practices as compared to conventional ones [42].

The colonization of endophytic bacterial community could be influenced by environmental conditions through the following two processes. Firstly, environmental variables affect the plant physiology thereby influencing the root exudates which might determine the microbial communities in the rhizosphere, the potential endophytic candidates [33] [43] [38]. It was reported that environmental factors such as temperatures and photon flux density influenced root exudates of tomato and clover [44]. Likewise, low photon flux density increased the release of carbon in root exudates of rye grass [45]. In addition, it was also reported that oxalate in the root exudates enriched Oxalobacteraceae family in the rhizosphere of stiff brome plant [46]. Similarly, Haichar *et al.* reported that the rape plant root exudates enriched the rhizospheric zone with Alpha-, Delta-, Beta- and Gamma-proteobacteria and Actinobacteria, barrel clover with Alpha- and Gamma-proteobacteria, and maize with Alpha-, Beta-, Gamma-proteobacteria and Actinobacteria [47].

Secondly, environmental variables influence the bacterial composition in the bulk soil which ultimately determines the possible endophytic community [48]. Although several environmental factors are responsible in determining the soil bacterial community, soil pH is one of the influencing parameters. In a diverse set of ecosystems across South and North America, soil bacterial community was strongly shaped by soil pH at the continental scale, where bacterial diversity was highest in neutral soils and lower in acidic soils [49]. Similarly, the relative abundance of Actinobacteria, Bacterioidetes, Fibrobacteres and Firmicutes was higher at near-neutral pH and lower at acidic and alkaline pH [50]. In addition, temperature is one of the environmental factors determining the composition of the soil bacterial community. Studies conducted applying culture independent methods revealed that the relative abundances of Actinobacteria and Firmicutes increased at higher temperatures, while Bacteroidetes and Deltaproteobacteria showed the opposite pattern when the soils collected from an alpine meadow were incubated at different temperatures [51]. Similarly, Lin *et al.* reported that the relative abundance of soil Acidobacteria decreased with increasing temperature while Gammaproteobacteria increased [52]. Thus, environmental conditions influenced on the endophytic bacterial community by changing the profile of plant exudates resulting in selection of distinct rhizobacterial community and by influencing the soil microbial community; the main sources for endophytic community.

There are reports that the plant determines the endophytic bacterial communities and soil factors played a minor role [33]. Other researchers, however, have reported that soil type [53] [54] and environmental factors [53] determine the endophytic communities. Our results indicate that endophytic diversity is independent on soil and environmental factors. Hence, it was suggested that the plant and other unknown factors would be responsible in determining the endophytic bacterial community.

Plant growth promoting and endophytic characteristics of the selected bacterial isolates were analyzed in this study using *in vitro* tests. IAA is the main phytohormone in plants, regulating many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light [17]. In our study, 57% of endophytic bacteria isolated from sweet potato synthesized IAA from tryptophan, and the ability was distributed to Bacilli, Actinobacteria, Alpha- and Gamma-proteobacteria classes. Similarly, IAA producing endophytes in the same classes have been reported in sweet potato [6] [7] and the other crops as rice [55], ginseng [56], semi-aquatic grass [57] and poplar trees [58]. The IAA producing endophytes can be used as plant growth promoting agent, but the ability should be confirmed in *in situ* conditions.

Among the tested endophytes, only three strains (5%) showed the N_2-fixing potential. Similarly, detection of *nifH* in endophytic isolates was negative in sweet potato [6] or not often in rice [55] and ginseng [56], representing 2% and 4% of the total isolates, respectively. As their presence might play a role in the

growth of sweet potato plants, it is thus, necessary to determine how much nitrogen they fix in the host plant.

Endophytic bacteria from all the classes demonstrated the antagonistic effect against the bacterial pathogens, while antagonism against the fungal pathogen was observed only for Bacilli class represented by *Bacillus* sp. Gul 1. In congruent to our findings, Marques *et al.* reported that *Bacillus* sp. isolated as endophytes from sweet potato tubers showed antimicrobial activity against a fungal pathogen, *Plenodomus destruens* [6]. Because it was reported that some of the *Bacillus* strains produce antimicrobial compounds as iturins that affect fungal signaling pathways [59] and surfactin, an antifungal lipopeptide [60], *Bacillus* sp. Gul 1 might produce antifungal compounds.

Besides all these plant growth promoting properties, endophytes need to colonize inside the host plants. Except for already established seed endophytes [61], common points of entry are through stomata [62], primary and lateral root cracks and tissues wounds created as a result of plant growth [63]. Besides these pathways, presence of the hydrolytic enzymes in sweet potato endophytes suggests enzyme based penetration of these endophytes to the plant.

The inoculation of crop plants with beneficial microbes is a practice used in agriculture and provides advantages to crops by enhancing plant growth and triggering protection to diseases [55]. It was also reported that the inoculation with multiple beneficial bacteria have higher potential than inoculation with a single bacterial inoculant [12] [64]. Our study also showed the positive effects when the isolates were applied as a mixture from each location. Mixture of inoculants might interact synergistically to provide nutrients, remove inhibitory products and stimulate one another.

Plant growth promotions by the IAA producing endophytes have been reported in sweet potato [7] and other crops as tomato [65] and strawberry [66]. In our study, all inoculants with plant growth promoting activity included the IAA producing endophytic bacteria suggesting that IAA produced influenced the growth. In addition, sweet potato inoculated with Salyan mixture including *nifH* gene containing *Klebsiella* sp. Sal 1 and *Herbaspirillum* sp. Sal 6 showed ARA activity suggesting that N-fixation would be one of the reasons for the higher fresh weight of the inoculated plants.

In this study, it was difficult to prepare the test plants with similar initial size, and the bigger the initial size produced the bigger plant. Therefore, times increase of fresh weight, vine length and root length were used to compare the plant growth promoting effect. Although, the times increase were not constant in the repeated experiments, we could observe the positive effect of the inoculants. This suggests the endophytic community possess potential for plant growth promotion.

5. Conclusion

Further studies will be necessary to determine the responsible endophytes and

their mechanisms of the plant growth promotion, but community might be important and necessary for the ability. Besides this, scope still exists to unravel the endophytic community structure by culture independent method and to cultivate the uncultured endophytes by modifying the culture conditions. Although the *in vitro* assays used may not reproduce exactly the conditions of natural environment, they can provide rapid screening of the potential strains, which can save time and costs, and further screening for the candidates *in situ* is necessary.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table S1. List of endophytic bacterial strains isolated from Nepalese sweet potato tubers.

Locations	Strains	Close relatives based on 16S rRNA gene sequencing	Class	a	b
Rolpa	Rol 1	*Curtobacterium luteum*	Actinobacteria	×	×
	Rol 2	*Stenotrophomonas rhizophila*	Gammaproteobacteria	×	×
	Rol 3	*Brachybacterium rhamnosum*	Actinobacteria	×	×
	Rol 4	*Staphylococcus sciuri*	Bacilli	×	×
	Rol 5	*Yokenella regensburgei*	Gammaproteobacteria	×	×
	Rol 6	*Streptomyces viridochromogenes*	Actinobacteria	×	×
	Rol 7	*Massilia haematophila*	Betaproteobacteria	×	×
	Rol 8	*Enterobacter cloacae*	Gammaproteobacteria	×	
	Rol 9	*Enterobacter asburiae*	Gammaproteobacteria		
	Rol 10	*Curtobacterium luteum*	Actinobacteria		
	Rol 11	*Curtobacterium luteum*	Actinobacteria		
	Rol 12	*Curtobacterium luteum*	Actinobacteria		
	Rol 13	*Flavobacterium johnsoniae*	Flavobacteriia		
	Rol 14	*Yokenella regensburgei*	Gammaproteobacteria		
	Rol 15	*Microbacterium paraoxydans*	Actinobacteria	×	
	Rol 16	*Massilia haematophila*	Betaproteobacteria		
	Rol 17	*Flavobacterium johnsoniae*	Flavobacteriia		
	Rol 18	*Flavobacterium johnsoniae*	Flavobacteriia		
	Rol 19	*Curtobacterium luteum*	Actinobacteria		
	Rol 20	*Curtobacterium luteum*	Actinobacteria		
	Rol 21	*Curtobacterium luteum*	Actinobacteria		
	Rol 22	*Microbacterium paraoxydans*	Actinobacteria		
	Rol 23	*Staphylococcus xylosus*	Bacilli		
	Rol 24	*Staphylococcus saprophyticus*	Bacilli		
	Rol 25	*Staphylococcus saprophyticus*	Bacilli		
	Rol 26	*Staphylococcus saprophyticus*	Bacilli		
	Rol 27	*Flavobacterium johnsoniae*	Flavobacteriia		
	Rol 28	*Flavobacterium johnsoniae*	Flavobacteriia	×	
	Rol 29	*Flavobacterium johnsoniae*	Flavobacteriia		
	Rol 30	*Flavobacterium johnsoniae*	Flavobacteriia		
Salyan	Sal 1	*Klebsiella variicola*	Gammaproteobacteria	×	×
	Sal 2	*Flavobacterium johnsoniae*	Flavobacteriia	×	×
	Sal 3	*Enterobacter asburiae*	Gammaproteobacteria	×	×
	Sal 4	*Rhizobium pusense*	Alphaproteobacteria	×	×

Continued

Sal 5	*Stenotrophomonas rhizophila*	Gammaproteobacteria		×	×
Sal 6	*Herbaspirillum huttiense*	Betaproteobacteria		×	×
Sal 7	*Agrobacterium larrymoorei*	Alphaproteobacteria		×	×
Sal 8	*Microbacterium testaceum*	Actinobacteria		×	×
Sal 9	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 10	*Flavobacterium johnsoniae*	Flavobacteriia			
Sal 11	*Klebsiella variicola*	Gammaproteobacteria			
Sal 12	*Rhizobium cellulosilyticum*	Alphaproteobacteria			
Sal 13	*Bacillus megaterium*	Bacilli		×	
Sal 14	*Flavobacterium anhuiense*	Flavobacteriia			
Sal 15	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 16	*Klebsiella variicola*	Gammaproteobacteria			
Sal 17	*Stenotrophomonas maltophilia*	Gammaproteobacteria			
Sal 18	*Stenotrophomonas maltophilia*	Gammaproteobacteria			
Sal 19	*Stenotrophomonas maltophilia*	Gammaproteobacteria			
Sal 20	*Flavobacterium johnsoniae*	Flavobacteriia			
Sal 21	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 22	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 23	*Flavobacterium johnsoniae*	Flavobacteriia			
Sal 24	*Klebsiella variicola*	Gammaproteobacteria			
Sal 25	*Microbacterium testaceum*	Actinobacteria			
Sal 26	*Rhizobium pusense*	Alphaproteobacteria			
Sal 27	*Herbaspirillum huttiense*	Betaproteobacteria			
Sal 28	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 29	*Flavobacterium johnsoniae*	Flavobacteriia			
Sal 30	*Klebsiella pneumoniae*	Gammaproteobacteria			
Sal 31	*Klebsiella pneumoniae*	Gammaproteobacteria			
Sal 32	*Klebsiella variicola*	Gammaproteobacteria			
Sal 33	*Flavobacterium johnsoniae*	Flavobacteriia			
Sal 34	*Flavobacterium anhuiense*	Flavobacteriia			
Sal 35	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 36	*Herbaspirillum huttiense*	Betaproteobacteria			
Sal 37	*Klebsiella pneumoniae*	Gammaproteobacteria			
Sal 38	*Rhizobium pusense*	Alphaproteobacteria			
Sal 39	*Stenotrophomonas maltophilia*	Gammaproteobacteria			
Sal 40	*Stenotrophomonas rhizophila*	Gammaproteobacteria			
Sal 41	*Flavobacterium johnsoniae*	Flavobacteriia			

Continued

	Sal 42	*Flavobacterium johnsoniae*	Flavobacteriia		
	Sal 43	*Flavobacterium johnsoniae*	Flavobacteriia		
Gulmi	Gul 1	*Bacillus pumilus*	Bacilli	×	×
	Gul 2	*Bacillus safensis*	Bacilli		
Palpa	Pal 1	*Rhizobium cellulosilyticum*	Alphaproteobacteria	×	
	Pal 2	*Enterobacter cloacae*	Gammaproteobacteria		
	Pal 3	*Luteibacter yeojuensis*	Gammaproteobacteria	×	
	Pal 4	*Enterobacter cloacae*	Gammaproteobacteria	×	
	Pal 5	*Bacillus wiedmannii*	Bacilli	×	
	Pal 6	*Bacillus thuringiensis*	Bacilli		
Kavre-a	Kav-a 1	*Herbaspirillum seropedicae*	Betaproteobacteria	×	×
	Kav-a 2	*Pseudomonas oryzihabitans*	Gammaproteobacteria	×	×
	Kav-a 3	*Agrobacterium tumefaciens*	Alphaproteobacteria	×	
	Kav-a 4	*Agrobacterium fabrum*	Alphaproteobacteria		
	Kav-a 5	*Bacillus wiedmannii*	Bacilli	×	
Kavre-b	Kav-b 1	*Caballeronia temeraria*	Betaproteobacteria	×	×
	Kav-b 2	*Staphylococcus succinus*	Bacilli	×	×
	Kav-b 3	*Luteibacter yeojuensis*	Gammaproteobacteria	×	×
	Kav-b 4	*Rhizobium miluonense*	Alphaproteobacteria	×	×
	Kav-b 5	*Bacillus cereus*	Bacilli	×	×
	Kav-b 6	*Caballeronia temeraria*	Betaproteobacteria		
	Kav-b 7	*Herbaspirillum huttiense*	Betaproteobacteria	×	
	Kav-b 8	*Staphylococcus saprophyticus*	Bacilli		
	Kav-b 9	*Rhizobium pusense*	Alphaproteobacteria		
	Kav-b 10	*Achromobacter xylosoxidans*	Betaproteobacteria	×	
	Kav-b 11	*Pantoea stewartii*	Gammaproteobacteria	×	
	Kav-b 12	*Achromobacter xylosoxidans*	Betaproteobacteria		
	Kav-b 13	*Bacillus cereus*	Bacilli		
	Kav-b 14	*Bacillus cereus*	Bacilli		
	Kav-b 15	*Bacillus cereus*	Bacilli		
	Kav-b 16	*Bacillus wiedmannii*	Bacilli		
	Kav-b 17	*Bacillus cereus*	Bacilli		
Banke-a	Ban-a 1	*Microbacterium radiodurans*	Actinobacteria	×	×
	Ban-a 2	*Curtobacterium citreum*	Actinobacteria	×	×
	Ban-a 3	*Agrobacterium larrymoorei*	Alphaproteobacteria	×	×
	Ban-a 4	*Rhizobium cellulosilyticum*	Alphaproteobacteria	×	×
	Ban-a 5	*Arthrobacter pokkalii*	Actinobacteria	×	×

Continued

	Ban-a 6	*Cellulomonas hominis*	Actinobacteria	×	×
	Ban-a 7	*Pantoea stewartii*	Gammaproteobacteria	×	×
	Ban-a 8	*Microbacterium radiodurans*	Actinobacteria	×	×
	Ban-a 9	*Sphingomonas yantingensis*	Alphaproteobacteria	×	×
	Ban-a 10	*Enterobacter cloacae*	Gammaproteobacteria	×	
	Ban-a 11	*Curtobacterium citreum*	Actinobacteria		
	Ban-a 12	*Curtobacterium citreum*	Actinobacteria		
	Ban-a 13	*Staphylococcus gallinarum*	Bacilli	×	
	Ban-a 14	*Sphingomonas koreensis*	Alphaproteobacteria		
	Ban-a 15	*Flavobacterium johnsoniae*	Flavobacteriia	×	
	Ban-a 16	*Microbacterium radiodurans*	Actinobacteria		
	Ban-a 17	*Microbacterium radiodurans*	Actinobacteria		
	Ban-a 18	*Microbacterium oleivorans*	Actinobacteria		
	Ban-a 19	*Microbacterium oleivorans*	Actinobacteria		
	Ban-a 20	*Rhizobium cellulosilyticum*	Alphaproteobacteria		
	Ban-a 21	*Arthrobacter enclensis*	Actinobacteria		
	Ban-a 22	*Microbacterium radiodurans*	Actinobacteria		
	Ban-a 23	*Microbacterium radiodurans*	Actinobacteria		
	Ban-a 24	*Microbacterium radiodurans*	Actinobacteria		
	Ban-a 25	*Sphingomonas yantingensis*	Alphaproteobacteria		
	Ban-a 26	*Agrobacterium larrymoorei*	Alphaproteobacteria		
	Ban-a 27	*Sphingomonas yantingensis*	Alphaproteobacteria		
	Ban-a 28	*Microbacterium paraoxydans*	Actinobacteria		
	Ban-a 29	*Microbacterium oleivorans*	Actinobacteria		
	Ban-a 30	*Microbacterium oxydans*	Actinobacteria		
	Ban-a 31	*Microbacterium oleivorans*	Actinobacteria		
Banke-b	Ban-b 1	*Xenophilus aerolatus*	Betaproteobacteria	×	×
	Ban-b 2	*Stenotrophomonas maltophilia*	Gammaproteobacteria	×	×
	Ban-b 3	*Xanthomonas campestris*	Gammaproteobacteria	×	×
	Ban-b 4	*Sphingobium yanoikuyae*	Alphaproteobacteria	×	×
	Ban-b 5	*Microbacterium lemovicicum*	Actinobacteria	×	×
	Ban-b 6	*Pseudoxanthomonas spadix*	Gammaproteobacteria	×	×
	Ban-b 7	*Xanthomonas translucens*	Gammaproteobacteria	×	×
	Ban-b 8	*Rhizobium pusense*	Alphaproteobacteria	×	
	Ban-b 9	*Curtobacterium luteum*	Actinobacteria	×	
	Ban-b 10	*Stenotrophomonas maltophilia*	Gammaproteobacteria		
	Ban-b 11	*Rhizobium giardinii*	Alphaproteobacteria		

Continued

	Ban-b 12	*Rhizobium pusense*	Alphaproteobacteria		
	Ban-b 13	*Xanthomonas campestris*	Gammaproteobacteria		
	Ban-b 14	*Agrobacterium fabrum*	Alphaproteobacteria		
	Ban-b 15	*Agrobacterium larrymoorei*	Alphaproteobacteria		
	Ban-b 16	*Stenotrophomonas panacihumi*	Gammaproteobacteria		
	Ban-b 17	*Sphingobium yanoikuyae*	Alphaproteobacteria		
	Ban-b 18	*Curtobacterium citreum strain*	Actinobacteria		
	Ban-b 19	*Rhizobium cellulosilyticum*	Alphaproteobacteria		
	Ban-b 20	*Xenophilus aerolatus*	Betaproteobacteria		
	Ban-b 21	*Sphingobium yanoikuyae*	Alphaproteobacteria		
	Ban-b 22	*Sphingobium yanoikuyae*	Alphaproteobacteria		
	Ban-b 23	*Curtobacterium luteum*	Actinobacteria		
	Ban-b 24	*Agrobacterium tumefaciens*	Alphaproteobacteria		
	Ban-b 25	*Rhizobium pusense*	Alphaproteobacteria		
	Ban-b 26	*Stenotrophomonas maltophilia*	Gammaproteobacteria		
	Ban-b 27	*Pseudoxanthomonas spadix*	Gammaproteobacteria	×	
	Ban-b 28	*Xanthomonas translucens*	Gammaproteobacteria		
	Ban-b 29	*Agrobacterium larrymoorei*	Alphaproteobacteria	×	
	Ban-b 30	*Rhizobium pusense*	Alphaproteobacteria		
	Ban-b 31	*Xanthomonas translucens*	Gammaproteobacteria		
	Ban-b 32	*Xenophilus aerolatus*	Betaproteobacteria		
Rupandehi	Rup 1	*Microbacterium binotii*	Actinobacteria	×	×
	Rup 2	*Microbacterium arborescens*	Actinobacteria	×	×
	Rup 3	*Microbacterium hydrothermale*	Actinobacteria	×	×
	Rup 4	*Achromobacter xylosoxidans*	Betaproteobacteria	×	×
	Rup 5	*Curtobacterium citreum*	Actinobacteria	×	×
	Rup 6	*Microbacterium oleivorans*	Actinobacteria	×	×
	Rup 7	*Glutamicibacter nicotianae*	Actinobacteria	×	×
	Rup 8	*Microbacterium phyllosphaerae*	Actinobacteria	×	×
	Rup 9	*Rhizobium vallis*	Alphaproteobacteria	×	×
	Rup 10	*Paenibacillus taichungensis*	Bacilli	×	×
	Rup 11	*Microbacterium paraoxydans*	Actinobacteria	×	×
	Rup 12	*Exiguobacterium indicum*	Bacilli	×	×
	Rup 13	*Glutamicibacter nicotianae*	Actinobacteria	×	×
	Rup 14	*Curtobacterium luteum*	Actinobacteria		
	Rup 15	*Glutamicibacter nicotianae*	Actinobacteria		
	Rup 16	*Curtobacterium citreum*	Actinobacteria		

Continued

	Rup 17	*Pseudarthrobacter niigatensis*	Actinobacteria		
	Rup 18	*Rhizobium pusense*	Alphaproteobacteria		
	Rup 19	*Achromobacter xylosoxidans*	Betaproteobacteria		
	Rup 20	*Pseudarthrobacter niigatensis*	Actinobacteria	×	
	Rup 21	*Achromobacter xylosoxidans*	Betaproteobacteria		
	Rup 22	*Bacillus aryabhattai*	Bacilli	×	
	Rup 23	*Pantoea dispersa*	Gammaproteobacteria	×	
	Rup 24	*Pantoea dispersa*	Gammaproteobacteria		
	Rup 25	*Glutamicibacter nicotianae*	Actinobacteria		
	Rup 26	*Glutamicibacter nicotianae*	Actinobacteria		
	Rup 27	*Curtobacterium luteum*	Actinobacteria		
	Rup 28	*Microbacterium paraoxydans*	Actinobacteria		
	Rup 29	*Microbacterium hydrocarbonox-ydans*	Actinobacteria		
	Rup 30	*Microbacterium hydrocarbonox-ydans*	Actinobacteria		
	Rup 31	*Arthrobacter nicotianae*	Actinobacteria		
	Rup 32	*Arthrobacter nicotianae*	Actinobacteria		
	Rup 33	*Arthrobacter nicotianae*	Actinobacteria		
	Rup 34	*Microbacterium hydrocarbonox-ydans*	Actinobacteria		
	Rup 35	*Achromobacter xylosoxidans*	Betaproteobacteria		
	Rup 36	*Achromobacter xylosoxidans*	Betaproteobacteria		
	Rup 37	*Achromobacter xylosoxidans*	Betaproteobacteria		
Chitwan	Chi 1	*Pantoea dispersa*	Gammaproteobacteria	×	×
	Chi 2	*Paraburkholderia caribensis*	Betaproteobacteria	×	×
	Chi 3	*Neorhizobium alkalisoli*	Alphaproteobacteria	×	×
	Chi 4	*Bacillus megaterium*	Bacilli	×	
	Chi 5	*Bacillus aryabhattai*	Bacilli		
	Chi 6	*Stenotrophomonas maltophilia*	Gammaproteobacteria	×	
	Chi 7	*Enterobacter cloacae*	Gammaproteobacteria	×	
	Chi 8	*Burkholderia vietnamiensis*	Betaproteobacteria		
	Chi 9	*Bacillus safensis*	Bacilli		
	Chi 10	*Burkholderia caribensis*	Betaproteobacteria		
	Chi 11	*Bacillus pumilus*	Bacilli		
	Chi 12	*Bacillus safensis*	Bacilli		
	Chi 13	*Bacillus safensis*	Bacilli		
	Chi 14	*Bacillus safensis*	Bacilli		

Continued

Sunsari-a	Sun-a 1	*Pseudomonas nitroreducens*	Gammaproteobacteria	×	×
	Sun-a 2	*Pseudomonas nitroreducens*	Gammaproteobacteria	×	×
	Sun-a 3	*Bacillus megaterium*	Bacilli	×	×
	Sun-a 4	*Pseudomonas nitroreducens*	Gammaproteobacteria	×	×
	Sun-a 5	*Pseudomonas nitroreducens*	Gammaproteobacteria		
	Sun-a 6	*Enterobacter asburiae*	Gammaproteobacteria	×	
	Sun-a 7	*Pseudomonas aeruginosa*	Gammaproteobacteria		
	Sun-a 8	*Enterobacter cloacae*	Gammaproteobacteria		
	Sun-a 9	*Bacillus safensis*	Bacilli		
	Sun-a 10	*Rhizobium etli*	Alphaproteobacteria	×	
	Sun-a 11	*Bacillus megaterium*	Bacilli		
	Sun-a 12	*Pseudomonas nitritireducens*	Gammaproteobacteria		
	Sun-a 13	*Pseudomonas nitroreducens*	Gammaproteobacteria		
	Sun-a 14	*Bacillus megaterium*	Bacilli		
	Sun-a 15	*Bacillus megaterium*	Bacilli		
	Sun-a 16	*Bacillus megaterium*	Bacilli		
	Sun-a 17	*Bacillus megaterium*	Bacilli		
	Sun-a 18	*Bacillus megaterium*	Bacilli		
Sunsari-b	Sun-b 1	*Burkholderia cenocepacia*	Betaproteobacteria	×	×
	Sun-b 2	*Burkholderia cepacia*	Betaproteobacteria		
	Sun-b 3	*Burkholderia ambifaria*	Betaproteobacteria		
	Sun-b 4	*Burkholderia cenocepacia*	Betaproteobacteria		
	Sun-b 5	*Burkholderia cenocepacia*	Betaproteobacteria		
	Sun-b 6	*Bacillus safensis*	Bacilli		
	Sun-b 7	*Burkholderia cepacia*	Betaproteobacteria		
	Sun-b 8	*Burkholderia territorii*	Betaproteobacteria		

a: Strains used for the inoculation experiment, b: Strains selected for characterizing their plant growth promoting and endophytic traits.
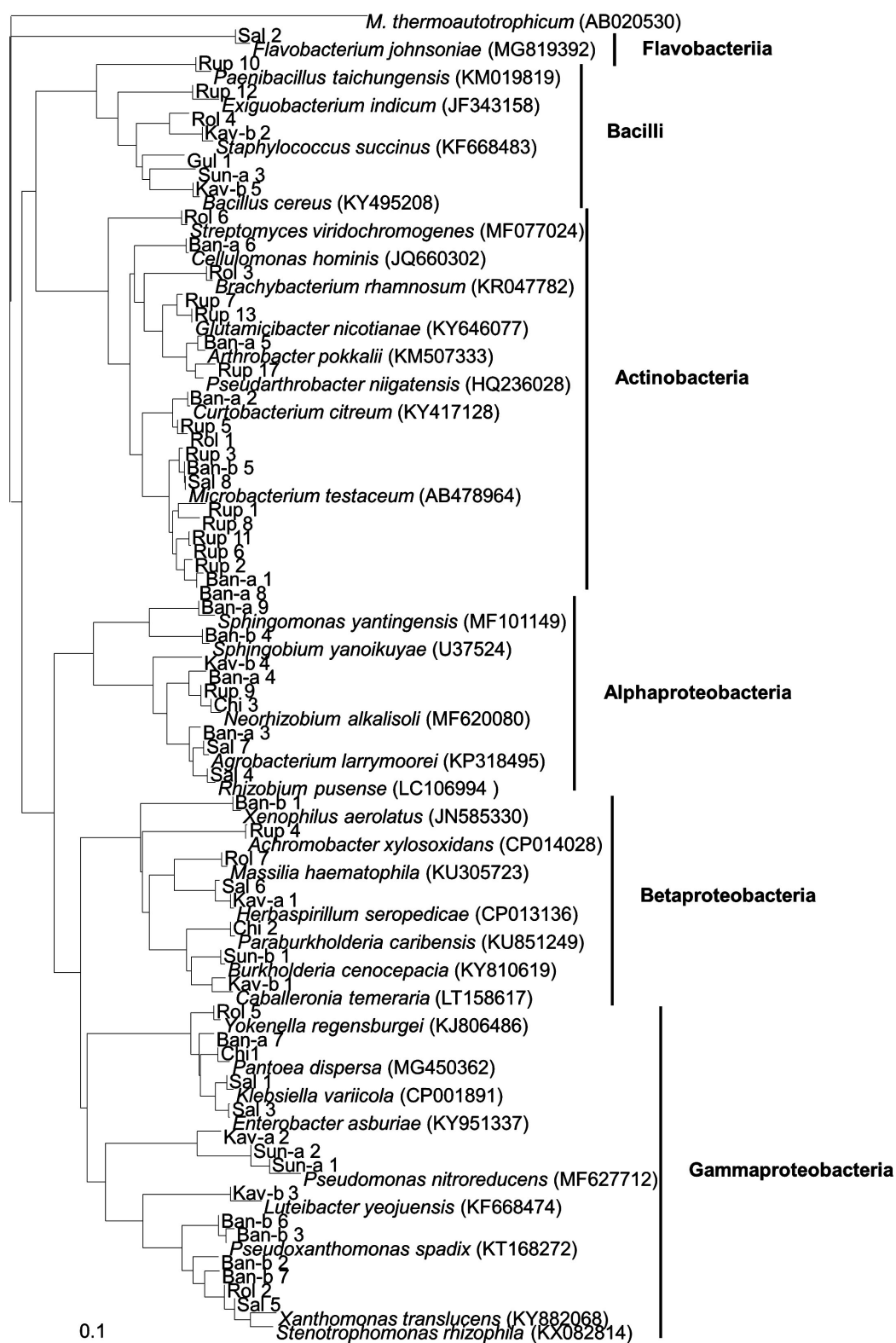
Figure S1. Phylogenetic relationship of 60 selected endophytic bacterial strains from Nepalese sweet potato based on partial 16S rRNA gene sequences. The sequence of *Methanobacterium thermoautotrophicum* (AB020530) served as an outgroup. Strain names are listed in Table S1. Strain names followed by accession numbers represent the sequences from database. The scale bar indicates the number of substitutions per site.