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Community of endophytic bacteria colonized in Jerusalem artichoke observed under molecular technique

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Abstract

Plant associated bacteria, named endophytic bacteria, colonize inside plant organs and mostly interact via plant growth promotion. The location of bacteria inside plants is different depending on bacterial species; likely, the bacterial diversity is different depending on plant species and stress environments. The bacterial community was determined in parts of Jerusalem artichoke which grew under well watering and deficient water conditions with inoculation of endophytic bacteria, *Rossellomorea aquimaris* 3.13, *Micrococcus luteus* 4.43 and *Bacillus velezensis* 5.18. The techniques used to determine community and colonization were denaturing gradient gel electrophoresis and transmission electron microscopy, respectively. The root showed the highest bacterial diversity, particularly under drought condition. The endophytic bacteria inoculated in the plants had negative effect on bacterial community. In contrast, the drought stress did have an effect on bacterial community. The majority of endophytic bacteria were members of gamma-*Proteobacteria* and *Firmicutes* (*Bacillus* spp.). The colonization of three endophytic bacteria was observed in the xylem vessel of stem and root of Jerusalem artichoke. Results showed that the endophytic bacteria were localized where they were isolated, leading to an understand of the relationship between endophytic bacteria and plant host.

Keywords: Bacterial community, Colonization, Endophytes, *Helianthus tuberosus* L.

1. Introduction

Endophytic bacteria are bacteria that live in plant tissues for all or part of their life time, with population richness in root tissues up to 10^3–10^4 CFU/g [1]. They provide benefits to host plants both directly and indirectly and do not cause any harm to the plants. As the colonization of endophytes start at the root surface area and penetrate into intracellular tissues, endophytes are able to subsequently transfer into intercellular tissues of the stem and leaf and throughout the plant endosphere [2]. However, the effects of endophytes located in root or stem or leaf on plants remain unclear. The microbial diversity and biomass community are the major regulators in soil ecosystem processes for nutrient cycle, organic compound decomposition and gas fluxes [3]. The relationship of microbial diversity and microbial biomass in soil is very high in arid soil with low carbon content and is very low in cold environments with rich carbon content [4]. The interaction of bacteria and plants indicates the effect of plant species on soil microbial community and antagonistic interactions in *Streptomyces*. Some plant species (*Rhododendron simsii*, *Dasiphora fruticosa*, and *Salix oritrepha*) exhibit a direct effect in decreasing soil bacterial diversity. In contrast, they have an indirect effect in increasing the soil bacterial community via *Streptomyces* antagonistic interaction [5]. The endophyte community has been of more interest recently, in exploring the relationship between microbes and plant hosts. The abundances of endophytic bacteria are attributed as members of *Proteobacteria*, *Firmicutes*, *Actinobacteria* and

Bacteroidetes [6,7]. Different plant species have direct effect on endophytic bacteria community in extreme environments, i.e., cold, dry.

In previous work, three endophytic bacteria (*Rossellomorea aquimaris* 3.13, *Micrococcus luteus* 4.43 and *Bacillus velezensis* 5.18) were isolatFed from Jerusalem artichoke with the property of plant growth promotion involved in auxin production and inulin synthesis [8,9]. They enhanced plant growth and the yield (inulin) accumulating in tubers. Thus, the plants inoculated with these endophytic bacteria were used as samples to determine bacterial diversity compared with the control plants without bacterial inoculation; also compared were planting conditions, between well watering and deficient water, using denaturing gradient gel electrophoresis (DGGE) method. This technique is a molecular method to distinguish nucleotide compositions in DNA sequences; the DNA fragment with one base difference is separated in different positions, appearing in different bands on gel. The 16S rRNA gene is commonly used to identify bacterial species; the V6 region is a part of 16S rRNA that reveals the greatest differences in phyla of *Firmicutes* and *Proteobacteria* [10]. The endophytic bacteria inoculated in the plant were used as bacterial markers to determine their colonization. Moreover, the colonization of the endophytic bacteria was determined under a sterile condition using transmission electron microscopy (TEM).

2. Materials and methods

2.1 Plant samples and endophytic bacteria

Endophytic bacteria, *R. aquimaris* 3.13, *M. luteus* 4.43 and *B. velezensis* 5.18 were cultivated in nutrient broth and incubated at 30ºC for 24 h. After that, the bacterial suspension was adjusted to McFarland scale 0.5 and used in 5 ml for plant inoculation. Jerusalem artichoke HEL65 was sourced from Faculty of Agriculture, Khon Kaen University. The plants were grown in pots (15-inch diameter, 11-inch depth) and inoculated twice with the endophytic bacteria by pouring cell suspension into the seedling in each pot at 20 and 30 days of planting; the plants without the endophytic bacteria were run as controls by adding 5 ml of water. There were 2 levels of watering: firstly, Field capacity (FC) for well watering condition; secondly,1/3 available soil water (1/3) for drought condition. The plant samples were collected at 75 and 125 days after transplantation.

2.2 Genomic DNA extraction

Plant samples (leaves, stems and roots of Jerusalem artichoke) were washed three times with sterile distilled water and ground with liquid nitrogen to a fine powder using mortar and pestle. Total genomic DNA was extracted from plant tissues using GeneJET Plant Genomic DNA Purification Mini Kit (Thermo scientific, EU). In parallel, the pure culture of each bacterium was cultivated in 2 ml of nutrient broth and incubated at 30°C, 150 rpm for 18 h. After incubation, the bacterial culture was centrifuged at 12,000 rpm for 5 min to collect the cell pellet for DNA extraction following the instruction of GeneJET genomic DNA Purification kit (Thermo scientific, EU) for maker strains. The purified DNA was kept at -20°C until used in the experiment.

2.3 DNA amplification with polymerase chain reaction

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers, 27F (5' AGA GTT TGA TCC TGG CTC AG 3') and 1492R (5' GGT TAC CTT GTT ACG ACT T 3'). PCR reaction mixture (50 µl) contained 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTP), 0.5 µM each primer, 1 U of Taq DNA polymerase (Thermo scientific, EU), 1 µl (150 ng) DNA template and sterile distilled water. Amplification reactions were performed in an initial denaturation step at 95°C for 3 min; after that 35 cycles of PCR conditions were started at 95°C for 1 min, 55°C for 1 min and 72°C for 3 min and a final extension at 72°C for 12 min. PCR products were purified by GeneJET PCR purification kit (Thermo scientific, EU) and determined by 1% agarose gel electrophoresis.

Consequently, the PCR products of 16S rRNA gene were used as templates for amplification of V6 region [11] using primers of 968F-GC (5' CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG CGG GGG GAA CGC GAA CGC GAA CCT TAC 3'); GC-clamp is underlined, and 1401R (5' CGG TGT GTA CAA GAC CC 3'). The PCR reaction mixture was performed the same as 16S rRNA gene amplification with 1 µl (150 ng) of PCR products as a template. The amplification reaction was done under an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 5 min. The V6 PCR products were analyzed by DGGE technique.

2.4 Denaturing gradient gel electrophoresis (DGGE)

An equal amount of the V6 PCR products was separated on an 8% (w/v) polyacrylamide gels in 1×TAE (Tris–Acetate–EDTA) buffer. An optimum separation was performed with a linear denaturing gradient increasing from 15 to 35% of 7M urea with 40% (v/v) formamide. The gels were run overnight at 80 V, 60°C. After electrophoresis, the gels were stained with ethidium bromide for 15 min at room temperature and photographed under an UV transilluminator. The bands were cut from the gels using surgical blade and placed in 20 µl of sterile ultrapure water to allow DNA release at 4°C overnight. The DNA fragments (5 µl) were reamplified using 968F non-GC-clamped primers (5' GAA CGC GAA CGC GAA CCT TAC 3') and 1401R (5' CGG TGT GTA CAA GAC CC 3'). The PCR products were confirmed by sequencing from ATGC Co., Ltd (Thailand).

2.5 Examination of bacterial colonization in plant using transmission electron microscopy (TEM)

Jerusalem artichoke HEL65 tubers were washed twice with sterile distilled water; the surface sterilization was completed in 70% of ethanol for 1 min and 3% of sodium hypochlorite for 3 min. After that, the tubers were washed with sterile distilled water three times for surface sterilization. Then, the tubers were placed in sterile bottles packed with coconut coir and incubated completely in the dark at room temperature for 10-14 days. The plants were transferred into seedling trays packed with sterile soil and coconut coir under sterile conditions for 10-14 days. The seedlings were transferred into each plastic pot (18 cm diameter, 14.5 cm depth), and then inoculated with 5 ml of bacterial suspension (10^8 CFU/ml) prepared in phosphate buffer (pH 7.4) on the first day, and after 7 days of planting. Sterile water was added to the control.

Stems, leaves and roots tissues of Jerusalem artichoke HEL65 were collected at 30 days after inoculation and washed twice with sterile distilled water. Plant samples were cut by hand into fragments and then fixed with Karnovsky's fixative solution (2.5% glutaraldehyde, 1mM $CaCl_2$, 2.5% paraformaldehyde) at 4°C for 24 h. The samples were rinsed in triplicate with phosphate buffer (0.1 M, pH 7.2) and post fixed with osmium tetroxide (OsO_4) for 2 h. The fixed fragment tissues were washed in triplicate with phosphate buffer (0.1 M, pH 7.2). The plant fragments were dehydrated in a 50-100% (v/v) gradient absolute ethanol series, and then embedded in EMBED 812 resin 14900. Subsequently, the embedded fragments were cut to ultrathin sections (90 nm thick) using diamond knife and mounted on carbon grids, stained with uranyl acetate and lead acetate. Coated samples were used to determine bacterial cell [12]. Observation and micrograph were made in TECNAI G2 20 transmission electron microscopy (TEM).

3. Results and discussion

3.1 Detection of endophytic bacteria in Jerusalem artichoke using denaturing gradient gel electrophoresis (DGGE)

The V6 PCR products were analyzed for evidence of bacterial community in the plants under well watering and drought by observation of DNA profiles on DGGE gel. Polyacrylamide gels provided a gradient of gel properties from low to high concentration with urea and formamide as denaturants. DNA denaturation occurred at different denaturants concentrations by breaking the hydrogen bonds of double-stranded DNA and splitting into single-stranded DNA. As a result, it was possible to distinguish the same-sized DNA with different base composition. This work studied three endophytic bacteria inoculated into plants, investigating whether they had an effect on the bacterial community. Therefore, the condition of planting was non-sterilization with three endophytic bacteria, *R. aquimaris* 3.13, *M. luteus* 4.43 and *B. velezensis* 5.18 as markers. Community profiles of endophytic bacteria living in different tissues of Jerusalem artichoke were compared between control (without bacterial inoculation) and three strains of the endophytic bacteria inoculated in Jerusalem artichoke which was planted by well watering and deficient water at 75 and 125 days after transplantation.

At the denaturing gradients of 30-70% of formamide and urea, DNA bands of the V6 region were clearly separated. The number of DNA bands was high in the plant roots indicating the highest bacterial community widespread there, then subsequently in stem and leaves (Figure 1). DNA samples were selected for identification as shown in Table 1. Under well watering, the DNA profiles were slightly different between 75 and 125 days of planting; however, there were various faint bands rather than thick band patterns on 125 days (Figure 1A and 1B). This implied that during plant growing (75 days), there were active bacteria with their activity (metabolism) in the plants indicating a bacterial community richer than that at harvest time (125 days); plant growth stages with high nutrient availability tend to increase bacterial diversity [13]. However, under drought condition, a greater number of DNA bands was detected in the root samples compared with well watering condition (Figure 1C and 1D), with a greater number of DNA bands

observed on 125 days. Drought had an important impact on the diversity of the endophytic microbial community in Jerusalem artichoke, particularly in roots. The reason for this was the diversity of endophytic bacteria in performing roles involved in plant response to stress tolerance. Endophytic bacteria produce many compounds to promote plant growth and help plants cope with stress environments. For abiotic stress, several endophytic bacteria produce compatible solutes, such as sugars, amino acids (proline), organic acids and inorganic ions for osmotic adjustment in plants [14]. Also, they are able to produce phytohormones, such as indole acetic acid (IAA), abscisic acid (ABA) and gibberellins for potential signals in several systems of plant growth and response [15].

The inoculated plants with endophytic bacteria had a negative effect on indigenous endophytic bacteria in both planting conditions (well watering and drought). The results did not indicate either higher or lower levels of bacterial community compared with the control. Some microorganisms, e.g. *Streptomyces* spp., demonstrate antagonistic activity which has a role in the formation and maintenance of soil microbial community [16]; *Streptomyces*'s antagonism increased indirectly in soil bacterial community via different plant species [5]. The factors that strongly influence the endophyte community are plant genotype (species), plant age and geographical location [7,17]. Moreover, seed microbiota contributes greatly to endophyte communities by obtaining and conserving a bulk of microbiome species [18]. In contrast, sterilized and non-sterilized soil have an effect on microbial community in roots, stems and leaves, demonstrating high community levels in the sterilized soil [19].

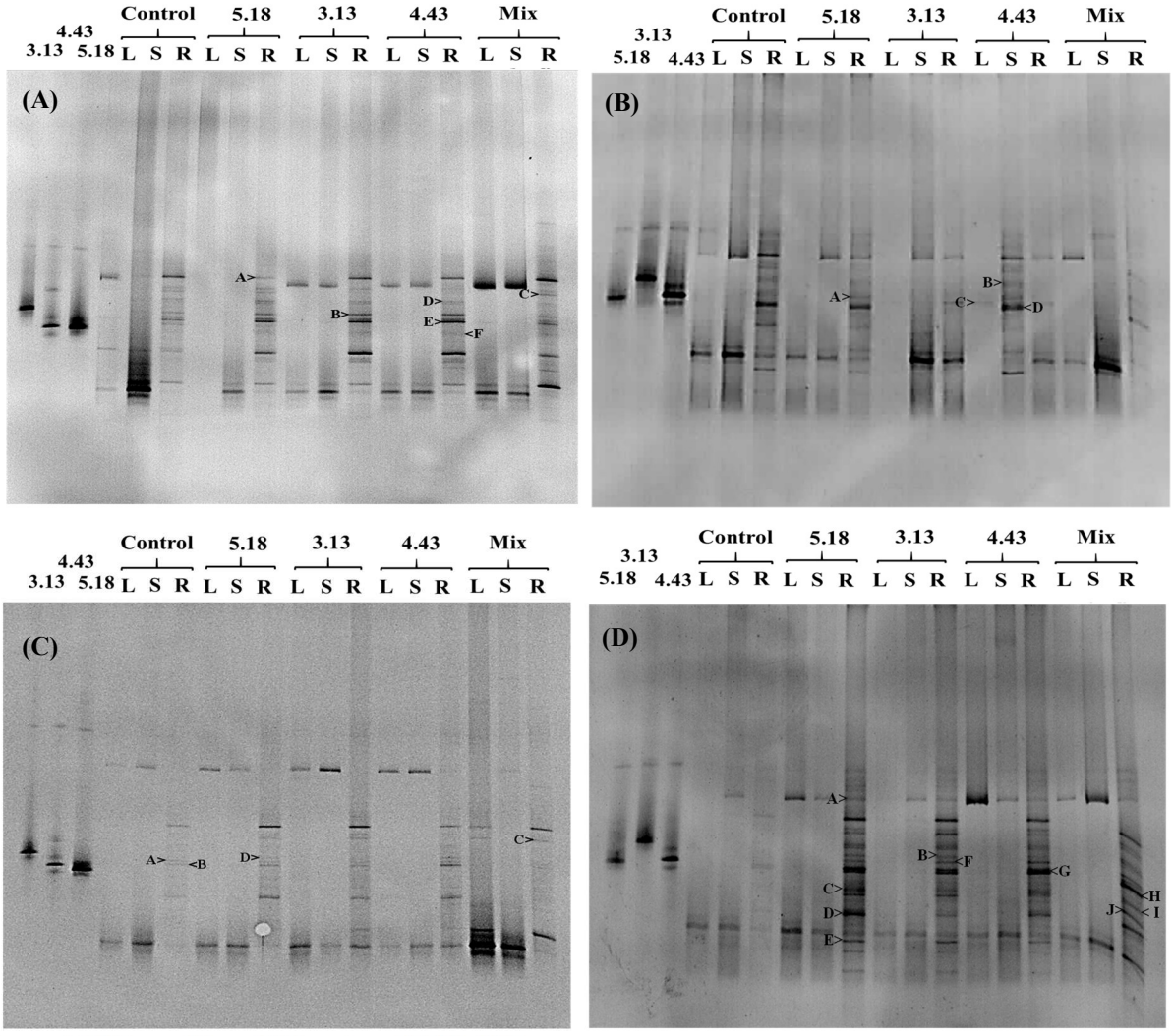

Figure 1 DNA profiles of V6 region of 16S rRNA gene using DGGE technique. DNA extracted from Jerusalem artichoke inoculated with the endophytic bacteria, the isolate 5.18, 3.13 and 4.43; (A) at 75 days (B) at 125 days under well watering and (C) at 75 days (D) at 125 days under deficient water. Lane 1, 2 and 3 showed DNA markers of three endophytic bacteria; Control means DNA of uninoculated plants; Mix means the plants inoculated with the isolate 3.13+5.18; L, S and R represent leaves, stems and roots, respectively.

The results revealed that the abundant species were *Bacillus* (gram positive bacteria) and gamma-proteobacteria (gram negative bacteria). This accorded to many reports that the abundances of endophytes were members of the phyla *Proteobacteria* and *Firmicutes* [7,17,19]. The target bands specific to bacterial markers (3 strains of the endophytic bacteria) were observed in Figure 1A, at the D band indicated as *R. aquimaris* 3.13 and Figure 1B, at the A band indicated as *B. velezensis* 5.18. However, these 2 bands were identified as uncultured bacterium and *B. megaterium*, respectively. *R. aquimaris* 3.13 (former named *Bacillus aquimaris*) and *B. velezensis* 5.18 are gram positive bacteria in phylum *Firmicutes,* closest in relation to those identified bands. Therefore, the short fragment (V6 fragments) might not be distinguished in species. Another possibility is that these bacteria isolated from plant stems might be located in stem tissue, where identification was not possible as the DNA bands were faint and failed in PCR amplification. *Micrococcus luteus* observed in leaves was similar to *M. luteus* 4.43 that was inoculated into plants. These results demonstrated that the DGGE technique could be used to detect the inoculum of endophytic bacteria inside the plants.

Several reports revealed the common genera of beneficial endophytes were *Azoarcus*, *Burkholderia*, *Gluconobacter*, *Herbaspirillum*, *Klebsiella*, *Pantoea*, and *Rahnella* [20,21]; some genera were observed under drought, e.g. *Bacillus*, *Enterobacter*, *Pseudomonas*, *Azotobacter*, *Arthrobacter*, *Streptomyces*, and *Isoptericola* [22,23]. This study identified *Steroidobacter* sp., a rod-shaped bacterium belonging to gamma-proteobacterium that degraded steroids and was a denitrifying bacterium. It was firstly isolated from anoxic digested sludge [24]. The aerobic, gram-positive, mesophilic *Ktedonobacteria* was identified in the results; it was classified in new family, *Ktedonobacteraceae* which was first isolated from soil in Italy [25], forming mycelia of irregularly branched filaments, produced spores or sporangia [26]. *Reticulibacter mediterranei* which was identified in the new family *Reticulibacteraceae,* was in the same group with *Ktedonobacteria* [26]. Likely, *Chloroflexi* was phototrophic bacteria and produced filamentous forms in activated sludge wastewater treatment. The members of *Chloroflexi* had a role forming flocs by scaffolding filament around and was able to degrade other complex polymeric organic compounds [27]. The results revealed the presence of different genera of endophytic bacteria compared to other plants; it is possible that different plant species and environments have an influence on bacterial community. This study points to a new resource to isolate and culture potential bacteria for niche applications.

Table 1 Identification of DNA fragments from DGGE patterns of plant-inoculated and uninoculated with the endophytic bacteria (the isolate 3.13, 4.43, 5.18 and Mix (3.13+5.18) growing under well watering and deficient water for 75 and 125 days after transplantation.

Sample	DGGE Band No.[a]	Highest match	Identity (%)	Accession number[b]
75 days under well watering	A	*Bacillus megaterium* strain p99_A10	94.44	JQ831477.1
	B	*Bacillus pumilus* strain p65_H02	100.00	JQ830483.1
	C	*Steroidobacter* sp. JC2986	90.96	KP185148.1
	D	Uncultured bacterium	81.09	KJ718906.1
	E	*Reticulibacter mediterranei*	87.83	NR_173686.1
	F	*Bacillus flexus* strain p54_E05	93.18	JQ834795.1
125 days under well watering	A	*Bacillus megaterium* strain p16_C07	81.77	JQ833401.1
	B	*Steroidobacter* sp.	88.26	KY992631.1
	C	*Micrococcus luteus* strain AB321	97.56	MT436103.1
	D	*Bacillus megaterium* strain p10_G02	79.59	JQ832221.1
75 days under deficient water	A	*Bacillus megaterium* strain p99_A10	97.22	JQ831477.1
	B	gamma proteobacterium	80.86	KP078361.1
	C	*Lysinibacillus fusiformis* strain p35_B11	100.00	JQ830173.1
	D	gamma proteobacterium YNPRH65B	94.00	AF465652.1
125 days under deficient water	A	gamma proteobacterium	99.00	AB679942.1
	B	*Chloroflexi* sp.	90.86	KP079816.1
	C	*Chloroflexi* sp.	80.97	JX094459.1
	D	*Ktedobacteria* sp.	89.69	HQ674974.1
	E	*Pseudomonas pseudoalcaligenes* strain ML-A04	90.28	AF140011.1
	F	*Bacillus megaterium* strain p58_A04	92.54	JQ834795.1
	G	*Streptomyces* sp. strain H2	85.33	MK511849.1
	H	*Reticulibacter mediterranei* strain 150040	89.81	NR_173686.1
	I	*Chloroflexi* sp.	87.95	EF522531.1
	J	*Ktedonobacter* sp.	80.65	KY992650.1

[a]DGGE band number as indicated position in DGGE gels as shown in Figure 1.

[b]Accession number of bacterial matches in NCBI database using Blast nucleotide software.

3.2 Colonization of endophytic bacteria in plants using transmission electron microscopy (TEM)

Three strains of endophytic bacteria (*R. aquimaris* 3.13, *M. luteus* 4.43 and *B. velezensis* 5.18), isolated from Jerusalem artichoke, were inoculated into plants for promotion of plant growth. This work was to investigate the colonization of these endophytic bacteria in Jerusalem artichoke HEL65 under a sterile condition. The findings indicated that all endophytes colonized in the host plant's tissues of water transport system (xylem vessel) as follows, *R. aquimaris* 3.13 (Figure 2A-B) and *B. velezensis* 5.18 in stems (Figure 2E-F), *M. luteus* 4.43 in roots (Figure 2C-D). Generally, the bacilli cell sizes range from 0.2 to 1.0 μm in diameter and 1 to 10 μm in length; the cocci cell size is 1 μm [28]. This was in accordance with the location inside the plants where three bacteria were isolated. Moreover, the results of *R. aquimaris* 3.13 and *B. velezensis* 5.18 colonization in stems related to their activity of inulin synthesis and enzyme degradation involved in inulin accumulation within the tubers of Jerusalem artichoke [8]. *M. luteus* 4.43 produced IAA phytohormone [9], which aids plant root elongation, was colonized in root tissues. The intercellular spaces of the plant parts were habitats for bacterial endophytes colonization, e.g., roots, leaves, stems, flowers, and seeds. These parts possessed carbohydrates, amino acids, and inorganic nutrients to stimulate endophytes growth. [29]. The bacterial endophytes communities spread to stem and leaf due to endophytes mobility and being accompanied by other endophytes which may help to transfer into other tissues by the synthesis of cellulolytic enzyme. Thus, the different stages of the plant's life cycle affect the community of endophytic bacteria, indicating difference in species. Moreover, the endophyte community is distinct in different environments; this group of bacteria seem to have functions in response to stress [2].

Other types of bacteria were not observed under TEM; this may be caused by the sterilization condition of planting and also the immature plant age (only 30 days of planting). The population of the target bacteria were high; despite high inoculation levels, and small results of populations led to difficult observation. However, the results demonstrated that three endophytic bacteria were able to colonize into plant tissues. As a result of their properties, they produced phytohormone (IAA), ACC deaminase and inulin synthesis pathway which were involved in plant response to drought [30]. Moreover, molecular mechanisms were important in controlling the plant-endophyte relationship. Therefore, a bacterial transcriptome analysis of the plant endosphere and microbial population using NGS are worth discussing and further exploring.

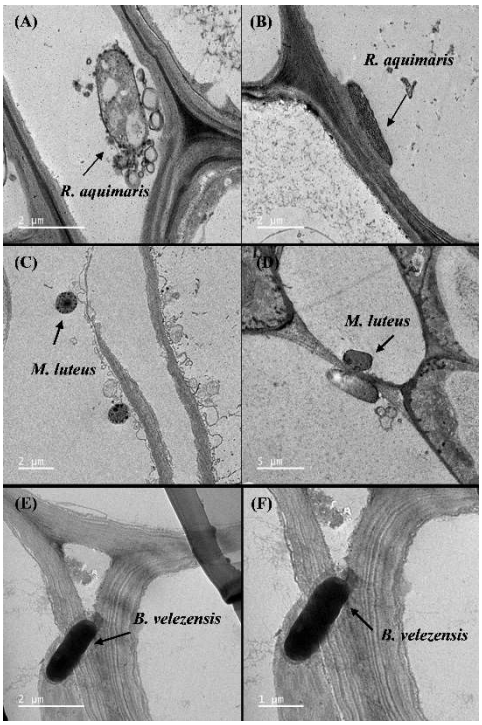

Figure 2 Endophytic bacteria colonization in Jerusalem artichoke HEL65 under TEM by transverse section though a xylem vessel of tissues after transplanting 30 days with the bacteria (10^8 CFU/mL). (A, B) represent *R. aquimaris* strain 3.13 located in the xylem vessel of stem tissues; (C, D) represent *M. luteus* strain 4.43 located in the xylem vessel of root tissues; (E, F) represent *B. velezensis* strain 5.18 located in the xylem vessel of stem tissues. The bacterial cells indicated at arrows.

4. Conclusion

The bacterial endophyte communities demonstrated higher populations in root tissues than in stem and leaf and high diversity in drought condition. *Bacillus* and gamma-proteobacteria were the main groups of endophytic bacteria observed in Jerusalem artichoke. Three endophytic bacteria colonized the stem and root of the host plant. The results showed the closest related species to be *R. aquimaris* 3.13 and *B. velezensis* 5.18 by DGGE and they were located in xylem vessel of the stems under TEM. In addition, *M. luteus* 4.43 was observed in the xylem vessel of the root and matched with the species identified from DNA.

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6. References

[1] Hallmann J, Berg G. Spectrum and population dynamics of bacterial root endophytes. In microbial root endophytes. Springer, Berlin, Heidelberg. 2006:15-31.
[2] Kandel SL, Joubert PM, Doty SL. Bacterial endophyte colonization and distribution within Plants. Microorganisms. 2017;5(4):77.
[3] Delgado-Baquerizo M, Reich PB, Trivedi C, Eldridge DJ, Abades S, Alfaro FD, et al. Multiple elements of soil biodiversity drive ecosystem functions across biomes. Nat Ecol Evol. 2020;4:210-220.
[4] Bastida F, Eldridge DJ, García C, Kenny Png G, Bardgett RD, Delgado-Baquerizo M. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. ISME J. 2021;15:2081-2091.
[5] Wang Y, Ma L, Liu Z, Chen J, Song H, Wang J, et al. (2022) Microbial interactions play an important role in regulating the effects of plant species on soil bacterial diversity. Front Microbiol. 2022;13:984200.
[6] Alibrandi P, Schnell S, Perotto S, Cardinale M. Diversity and structure of the endophytic bacterial communities associated with three terrestrial orchid species as revealed by 16S rRNA gene metabarcoding. Front Microbiol. 2020;11:604964.
[7] Zhang Q, Acuña JJ, Inostroza NG, Mora ML, Radic S, Sadowsky MJ, et al. Endophytic bacterial communities associated with roots and leaves of plants growing in Chilean extreme environments. Sci Rep. 2019;9:4950.
[8] Khamwan S, Boonlue S, Mongkolthanaruk W. Production of fructan synthesis/hydrolysis of endophytic bacteria involved in inulin production in Jerusalem artichoke. 3 Biotech. 2022;12(11):296.
[9] Namwongsa J, Jogloy S, Vorasoot N, Boonlue S, Riddech N, Mongkolthanaruk W. Endophytic bacteria improve root traits, biomass and yield of *Helianthus tuberosus* L. under normal and deficit water conditions. J Microbiol Biotechnol. 2019;29(11):1777-1789.
[10] Brons JK, van Elsas JD. Analysis of bacterial communities in soil by use of denaturing gradient gel electrophoresis and clone libraries, as influenced by different reverse primers. Appl Environ Microbiol. 2008;74(9):2717-2727.
[11] Muyzer G, Teske A, Wirsen CO, Jannasch HW. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol. 1995;164:165-172.
[12] Ferreira Filho AS, Quecine MC, Bogas AC, Rossetto PB, Lima AO, Lacava PT, et al. Endophytic *Methylobacterium extorquens* expresses a heterologous β-1,4-endoglucanase A (EglA) in *Catharanthus roseus* seedlings, a model host plant for *Xylella fastidiosa*. World J Microbiol Biotechnol. 2012;28:1475-1481.
[13] Shi Y, Yang H, Zhang T, Sun J, Lou K. Illumina-based analysis of endophytic bacterial diversity and space-time dynamics in sugar beet on the north slope of Tianshan mountain. Appl Microbiol Biotechnol. 2014;98: 6375-6385.
[14] Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA, et al. Drought tolerance improvement in plants: An endophytic bacterial approach. Appl Microbiol Biotechnol. 2019;103:7385-7397.
[15] Salvi P, Mahawar H, Agarrwal R, Kajal K, Gautam V, Deshmukh R. Advancement in the molecular perspective of plant-endophytic interaction to mitigate drought stress in plants. Front Microbiol. 2022;13:981355.
[16] Sun PP, Otto-Hanson LK, Arenz BE, Ma Q, Kinkel LL. Molecular and functional characteristics of streptomycete communities in relation to soil factors and potato common scab. Eur J Soil Biol. 2015;70:58-66.

[17] Afzal I, Shinwari ZK, Sikandar S, Shahzad S. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res. 2019;221:36-49.
[18] Links MG, Demeke T, Gräfenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica Seeds. New Phytol. 2014;202:542-553.
[19] Liu X, Zhu X, Dong Y, Chen Y, Li M, Li C. Limited impact of soil microorganisms on the endophytic bacteria of Tartary Buckwheat (*Fagopyrum tataricum*). Microorganisms. 2023;11:2085.
[20] Rosenblueth M, Martínez-Romero E. Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact. 2006;19:827-837.
[21] Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW. Enhanced maize productivity by inoculation with diazotrophic bacteria. Aust J Plant Physiol. 2001;28:829-836.
[22] Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A. Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. Environ Exp Bot. 2014;97:30-39.
[23] Yaish MW, Antony I, Glick BR. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek. 2015;107:1519-1532.
[24] Fahrbach M, Kuever J, Remesch M, Huber BE, Kämpfer P, Dott W, et al. *Steroidobacter denitrificans* gen. nov., sp. nov., a steroidal hormone-degrading gammaproteobacterium. Int J Syst Evol Microbiol. 2008;58(9):2215-2223.
[25] Chang Y, Land M, Hauser L, Chertkov O, Del Rio T, Nolan M, et al. Non-contiguous finished genome sequence and contextual data of the filamentous soil bacterium *Ktedonobacter racemifer* type strain (SOSP1-21). Stand Genomic Sci. 2011;5(1):97-111.
[26] Yabe S, Zheng Y, Wang CM, Sakai Y, Abe K, Yokota A, et al. *Reticulibacter mediterranei* gen. nov., sp. nov., within the new family *Reticulibacteraceae* fam. nov., and *Ktedonospora formicarum* gen. nov., sp. nov., *Ktedonobacter robiniae* sp. nov., *Dictyobacter formicarum* sp. nov. and *Dictyobacter arantiisoli* sp. nov., belonging to the class *Ktedonobacteria*. Int J Syst Evol Microbiol. 2021;71(7).
[27] Speirs LBM, Rice DTF, Petrovski S, Seviour RJ. The phylogeny, biodiversity, and ecology of the *Chloroflexi* in activated sludge. Front Microbiol. 2019;10:2015.
[28] Atlas of oral microbiology. Basic Biology of Oral Microbes. 2015:1-14.
[29] 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. isolated from wild rice species. Appl Environ Microbiol. 2001;67:5285-5293.
[30] Boonmahome P, Namwongsa J, Vorasoot N, Jogloy S, Riddech N, Boonlue S, Mongkolthanaruk W. Single and co-inoculum of endophytic bacteria promote growth and yield of Jerusalem artichoke through upregulation of plant genes under drought stress. PLoS ONE 2023;18(6):e0286625.