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FEATURE ARTICLE

Distribution of culturable endophytic bacteria in aquatic plants and their potential for bioremediation in polluted waters

Wei-Min Chen[1], Yue-Qin Tang[1], Kazuhiro Mori[2], Xiao-Lei Wu[1,*]

[1]Department of Energy and Resources Engineering, College of Engineering, Peking University, Beijing 100871, PR China
[2]Department of Civil and Environmental Engineering, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi 400-8511, Japan

ABSTRACT: Plant–bacterial asscociations can improve the degradation of organic pollutants in soil. However, little is known about the diversity and distribution of endophytic bacteria associated with aquatic plants and their potential to enhance phytoremediation of aquatic environments. In the present study, endophytic bacteria were isolated from 4 species of aquatic plants: *Phragmites communis*, *Potamogeton crispus*, *Nymphaea tetragona* and *Najas marina.* The isolated bacteria were classified into 12 genera in the *Gammaproteobacteria*, *Bacilli*, *Alphaproteobacteria*, *Flavobacteria* and *Actinobacteria.* In addition, different strains and/or different concentrations of the bacteria were isolated from different parts of the 4 plants, suggesting the different parts of the 4 plants harbored different endophytic bacteria. Some of the isolates degraded naphthalene and pesticides and some showed potential to dissolve insoluble phosphate. This is one of the first studies to isolate and compare culturable endophytic bacteria among different aquatic plants. This research indicates that culturable endophytes in aquatic plants are very diverse, but are dominated by *Gammaproteobacteria*, and have the potential to enhance *in situ* phytoremediation.

Guanting Reservoir shoreline where endophytic bacterial strains were isolated from aquatic plants.

Photo: Yue-Qin Tang

KEY WORDS: Aquatic plants · Endophytic bacteria · Phytoremediation · Organic pollutants · Phosphorus

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INTRODUCTION

Plant endophytic bacteria have been studied since the 1940s. They have been classified into 82 genera within *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Lodewyckx et al. 2002, Rosenblueth & Martínez-Romero 2006), and most belong to *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria.* They can supply nutrients to plants by fixing atmospheric nitrogen and solubilizing iron (Marx 2004, Porras-Soriano et al. 2009) and can protect the host plants from infection by plant pathogens through competition for space and nutrients, the production of hydrolytic enzymes and antibiosis, and by inducing plant defense mechanisms

*Corresponding author. Email: xiaolei_wu@pku.edu.cn

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(Ryan et al. 2008). In addition, recent studies have shown that endophytic bacteria have the potential to enhance the removal of soil contaminants by phytoremediation (Barac et al. 2004, Compant et al. 2005, Germaine et al. 2006, Doty et al. 2009, Taghavi et al. 2009, Janda & Abbott 2010). For example, a genetically modified endophytic strain, *Burkholderia cepacia*, together with yellow lupine *Lupinus luteus*, could improve degradation of toluene (Barac et al. 2004). Addition of petroleum to a sediment doubled the prevalence of naphthalene dioxygenase (ndoB)-positive endophytes in *Scirpus pungens* (Siciliano et al. 2001). Some endophytic bacteria from poplar trees exhibited the ability to degrade benzene, toluene, ethylbenzene and xylene (BTEX) compounds at one contaminated site (Moore et al. 2006). However, reports about the biodegradational abilities of endophytic bacteria were mostly focused on rhizospheric bacteria in terrestrial plants. Apart from Toyama et al. (2009), who showed that an aquatic plant–bacterial collaboration could accelerate contaminant degradation in the aquatic environment, there has been little research into endophytic bacteria associated with aquatic plants.

Environmental water pollution is a serious problem in China and other developing countries because of the discharge of organic pollutants such as pesticides and nutrients including phosphorus (Zhao et al. 2006, H. Wang et al. 2007, Perelo 2010). Because organic pollutants in aquatic environments are generally present in low concentrations, bioremediation and/or phytoremediation may be the most economic and reliable approach to address the problem. In addition, phosphorus, which can stimulate eutrophication, usually precipitates in sediments. This makes phosphorus largely unavailable to plants. Furthermore, the phosphorus precipitate can persist in a lake for a considerable length of time and continue to periodically cause algal blooms. The release of insoluble phosphorus and its removal from the environment using aquatic plants is a so-called eco-engineering approach (van Bohemen 2005), in which released phosphorus is taken up by plants and then removed from the aquatic environment by harvesting the plants. However, little research has been undertaken to understand more about endophytic bacteria in aquatic plants, in particular their diversity, distribution within the plant, bioremediation abilities and their association with the plants.

The present study was therefore undertaken to investigate endophytes found in 4 species of aquatic plants growing in a reservoir in Beijing, China, by using the culture-dependent method.

MATERIALS AND METHODS

Sample collection and isolation of endophytic bacteria

Guanting Reservoir (from 40° 25′ 96″ N, 115° 59′ 74″ E to 40° 45′ 64′ N, 115° 96′ 53″ E) is located in the northwest of Beijing city. It was once Beijing's second largest water source for drinking, agricultural and industrial purposes. Since the 1970s, Guanting Reservoir has been heavily polluted owing to rapid industrialization and intensive application of chemical fertilizers and pesticides to arable fields in upstream regions (T. Wang et al. 2007, Hu et al. 2009). Pollutants detected in Guanting Reservoir include pesticides (organochlorines, OCPs) and persistent organic pollutants (POPs) at concentrations of 16.7 to 791 ng l^{-1} (mean, 234 ng l^{-1}), 275 to 1600 ng l^{-1} (mean, 644 ng l^{-1}) and 5250 to 33 400 ng kg^{-1} (mean, 13 000 ng kg^{-1}) in the surface water, pore water and sediment (dry weight), respectively. An analysis of the sediment yielded the following: chemical oxygen demand, 49.7 mg l^{-1}; total phosphorus, 53.5 mg l^{-1}; total nitrogen, 62.4 mg l^{-1} and chlorophyll *a* (chl *a*), 51.6 mg m^{-3} (Xue et al. 2005, 2006).

Along the shoreline of the reservoir, 4 species of healthy plants, *Phragmites communis*, *Potamogeton crispus*, *Nymphaea tetragona* and *Najas marina*, which are dominant aquatic plants in this region, were sampled from Daying (40° 44′ 30″ N, 115° 90′71″ E), GuiS lake (40° 45′ 16″ N, 115° 96′ 08″ E) and KZ bridge (40° 45′ 00″ N, 115° 88′ 02″ E) where one of the rivers flows into the reservoir. At each sampling site, 4 plants from each species were sampled and cleaned with sterilized water to get rid of sediment and dust. The roots, stems and leaves of the entire plant were sliced into 1 to 2 cm × 1 to 2 cm pieces. The roots, stems and leaves were taken separately from different plants within each species and mixed completely for that species as root, stem and leaf samples, respectively. The mixed root, stem and leaf samples from each plant species were then divided into 3 subsamples (each 6 g), washed in sterilized water for 5 min, surface-sterilized with a solution containing 5% active chloride (w/v, added as a NaOCl solution) for 3 min and 70% ethanol for 1 min and then rinsed 6 to 8 times in sterile distilled water. They were then ground separately with scissors and a glass rod to make plant slurries. The root, stem and leaf subsample slurries were mixed again to make one integrated root sample, stem sample and leaf sample, respectively, for each of the original species of plant. The slurry samples were then diluted with sterilized double-distilled H_2O. The final diluted slur-

ries (100 µl each) from the integrated root, stem and leaf samples of each plant species were respectively spread onto Luria-Bertani (LB) agar plates (12 agar plates in total), which is often used to isolate endophytic bacteria from plants (Bhore et al. 2010, Guo et al. 2010, Luo et al. 2011), and incubated at 28°C for 5 to 7 d. In addition, 100 µl of the last rinse and dilution water were also plated on LB medium as a sterility check. Then 100 morphologically different bacterial colonies from the 12 LB agar plates were selected and subcultured for purification.

Sequencing and analyses of DNA

Genomic DNA was prepared from the pure individual isolates using FastDNA Spin Kit for soils (MP Biomedicals) and PCR was performed with the universal 16S rRNA gene primers: 8f: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 1492r: 5′-GGT TAC CTT GTT ACG ACTT-3′. The PCR mixture (50 µl) contained 5 µl of 10× PCR buffer with 15 mmol l^{-1} $MgCl_2$ (Takara), 200 µmol l^{-1} of each deoxynucleotide triphosphate (Takara), 10 pmol of each primer (Applied Biosystems), 1.5 units of *Taq* DNA polymerase (Takara) and 1 µl of DNA template. The PCR was performed in a thermocycler (PTC-200, Bio-Rad), with a thermal profile of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 45 s and extension at 72°C for 1.5 min and a final elongation at 72°C for 6 min. An approximate 1500 bp PCR fragment of the 16S rRNA gene was obtained for each isolate, and restriction fragment length polymorphism (RFLP) analysis was conducted. The purified PCR product (8 µl) was digested with either *Rsa*I and *Msp*I according to the manufacturer's instructions and separated by electrophoresis in 1.5% agarose gel. Strains were separated into different groups, and those sharing identical restriction digestion profiles were classified into the same group as described previously (Matsuzawa et al. 2010). Representative PCR amplicons were then selected from each group of restriction digestion profiles and sequenced. After performing the Chimera Check as described in the Ribosomal Database Project II (Cole et al. 2005), alignment of the sequences was made with ClustalX 2.0, imported into BioEdit v7.1.3 and manually corrected. A phylogenetic tree was constructed by the neighbor-joining method with Kimura's 2-parameter distance correction and 1000 bootstrap replicates by using Phylo_WIN (Galtier et al. 1996). The tree topology and stability were evaluated with the maximum-likelihood and maximum-parsimony algorithms.

Functional analysis of the isolates

Representative isolates were selected from each genotype in combination with their location in the 4 aquatic plants. After being precultured in LB medium for 5 d at 28°C, cells of the representative isolates were harvested by centrifugation and washed 3 times with sterilized water. They were then inoculated onto the minimal salt agar plates containing 1.5 g l^{-1} K_2HPO_4, 0.5 g l^{-1} KH_2PO_4, 1.0 g l^{-1} NaCl, 1.0 g l^{-1} NH_4NO_3 and 0.20 g l^{-1} $MgSO_4$ at pH 6.8 to 7.0 and supplemented with 20 mg l^{-1} chlorpyrifos, fenpropathrin and bifenthrinas and naphthalene, respectively, which have been detected as serious pollutants in Guanting Reservoir (Xue et al. 2006). The final cell concentration was 10^8 cells ml^{-1} in each culture. The cultures were then incubated at 28°C in the dark and shaken at 150 rpm for 2 wk. Cultures without bacterial inoculation were used as controls.

Residual pesticides and naphthalene were extracted twice from the culture supernatant by using equal volumes of dichloromethane. The organic layers of dichloromethane were aspirated, pooled and evaporated at room temperature in a nitrogen atmosphere. Concentrations of chlorpyrifos, naphthalene, fenpropathrin and bifenthrinas were measured at 230, 250, 275 and 270 nm, respectively, by HPLC (LC-20AT, Shimadzu) equipped with an Agilent column (4.6 mm inside diameter × 150 mm) filled with Kromasil 100-5 C18 and an UV detector (SPD-M20A). A mixture of methanol and water (7:3, v/v) was used as a carrier liquid and had a flow rate 1.0 ml min^{-1}. The removal ratio of the pesticides and naphthalene was then calculated as described by Anwar et al. (2009).

To detect phosphate solubilizing ability, isolates from different groups were streaked onto Pikovskaya's agar that contained 10.0 g l^{-1} glucose, 5.0 g l^{-1} $Ca_3(PO_4)_2$, 0.5 g l^{-1} $(NH_4)_2SO_4$, 0.2 g l^{-1} KCl, 0.1 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.0001 g l^{-1} $MnSO_4 \cdot H_2O$, 0.0001 g l^{-1} $FeSO_4 \cdot 7H_2O$ and 15.0 g l^{-1} agar. The pH was 6.8. After incubation at 28°C for 5 d, strains that induced clear zones around the colonies were considered to be positive for their ability to solubilize phosphate (Naik et al. 2008).

The partial 16S rRNA gene sequences of the isolates were deposited in the NCBI GenBank with the GenBank/EMBL/DDBJ accession numbers FJ683653 to FJ683690.

RESULTS

Isolation of endophytic bacteria

Endophytic bacteria were isolated from different parts of the 4 aquatic plants and the bacterial population ranged from 2.0×10^2 to 8.7×10^3 CFU g^{-1} of plant tissue (wet weight) (Table 1). Among all the isolates on the 12 LB plates, colonies were chosen according to their morphological differences on each LB plate, and more colonies were chosen from the higher population of culturable bacteria on each of the LB plates. In total, 100 morphologically different colonies were selected from different sample LB plates in nearly the same ratios of the total colony number on the same plates for genotypic and phenotypic studies (Appendix 1). In contrast, no colonies appeared in the control plate inoculated with the final washing and dilution water, indicating that aseptic handling and cultivation of the endophytic bacteria were successful.

After PCR amplification, the nearly complete 16S rRNA gene fragments (ca. 1500 bp) were digested by 2 restriction endonucleases, *Rsa* I and *Msp* I. The 100 isolates were then classified into 25 groups according to the digestion patterns of both enzymes (Appendix 1). After a comprehensive comparison among the 16S rRNA genotypes of the different parent plants and plant parts, a total of 39 isolates were selected from the 25 groups, sequenced and phylogenetically analyzed. The 100 isolates could be then be assigned to 12 bacterial genera (Fig. 1): *Pseudomonas*, which contained 27 strains of the 100 isolates (27% of the total isolates), *Enterobacter* (16%), *Aeromonas* (12%), *Klebsiella* (4%) and *Pantoea* (5%) in *Gammaproteobacteria* (64% in total), *Bacillus* (8%), *Paenibacillus* (11%), *Lactococcus* (2%) and *Staphylococcus* (5%) in the *Bacilli* (26% in total), *Delftia* (1%) in the *Alphaproteobacteria*, *Flavobacterium* (1%) in the *Flavobacteria* and *Microbacterium* (8%) in the *Actinobacteria* (Figs. 1 & 2).

Table 1. Mean number of isolates from each part of the plants listed (CFU g^{-1} wet wt)

Plants	Mean number of isolates from part of plant (CFU g^{-1} wet wt)		
	Root	Stem	Leaf
Phragmites communis	8.7×10^3	5.7×10^3	3.4×10^3
Potamogeton crispus	6.2×10^3	4.5×10^3	4.0×10^2
Najas marina	6.0×10^2	2.0×10^2	None
Nymphaea tetragona	4.0×10^2	7.5×10^3	None

The phylogenetic analyses also revealed that the endophytic isolates shared high sequence similarities (greater than 98%) with their recognized relative species. For example, *Pseudomonas* isolates were phylogenetically closely related to *Pseudomonas taiwanensis* (with the 16S rRNA gene sequence similarity of 99.4%), *P. xanthomarina* (99.3%) and *P. jessenii* (98%). The isolates belonging to *Enterobacter* and *Pantoea* were closely related to *Enterobacter hormaechei* (99.3%) and *Pantoea agglomerans* (99.6%), most of which were isolated from roots. The *Aeromonas* isolates were closely related to *Aeromonas salmonicida* (99.9%) and *A. caviae* (98.6%). *Bacilli* isolates were closely related to *Paenibacillus barcinonensis* (99%), *P. lautus* (99.2%), *Staphylococcus epidermidis* (99.9%), *Bacillus simplex* (99.9%), *B. sphaericus* (99.9%), *B. aryabhattai* (99.8%) and *Lactococcus lactis* (100%). The remaining endophytes were related to *Flavobacterium oceanosedimentum* (99.7%), *Microbacterium flavescens* (99%) and *Klebsiella terrigena* (99.2%) (Fig. 2).

Location of the endophytes within the 4 aquatic plant species

Since the 100 isolates were selected from different sample plates with the ratio of isolates similar to the ratio of the total colony numbers on different sample plates, the distribution pattern by the 100 isolates could be roughly used to reflect the distribution and diversity of the culturable endophytic bacteria from the 4 plant species. In general, the 4 aquatic plants harbored different culturable bacteria. Among the 100 isolates, 48 were isolated from *Phragmites communis*, 25 from *Nymphaea tetragona*, 22 from *Potamogeton crispus* and only 5 from *Najas marina*. Among the 10 genera of bacteria isolated from *Phragmites communis*, 9 genera (22 isolates) were isolated from the root, 6 genera (15 isolates) were isolated from the leaf and 7 genera (11 isolates) were isolated from the stem. The leaves of the other 3 aquatic plants harbored few culturable bacteria. Stems of *Potamogeton crispus* and *Nymphaea tetragona* harbored more diverse cultured bacteria than did their roots (Fig. 3).

Among all the isolates, *Pseudomonas* spp. were isolated from the roots and stems of all 4 of the plants. *Enterobacter* spp. and *Paenibacillus* spp. were the second most widely spread endophytic bacteria and were isolated from roots, stems and leaf. Although *Aeromonas* spp. were only isolated from *Phragmites*

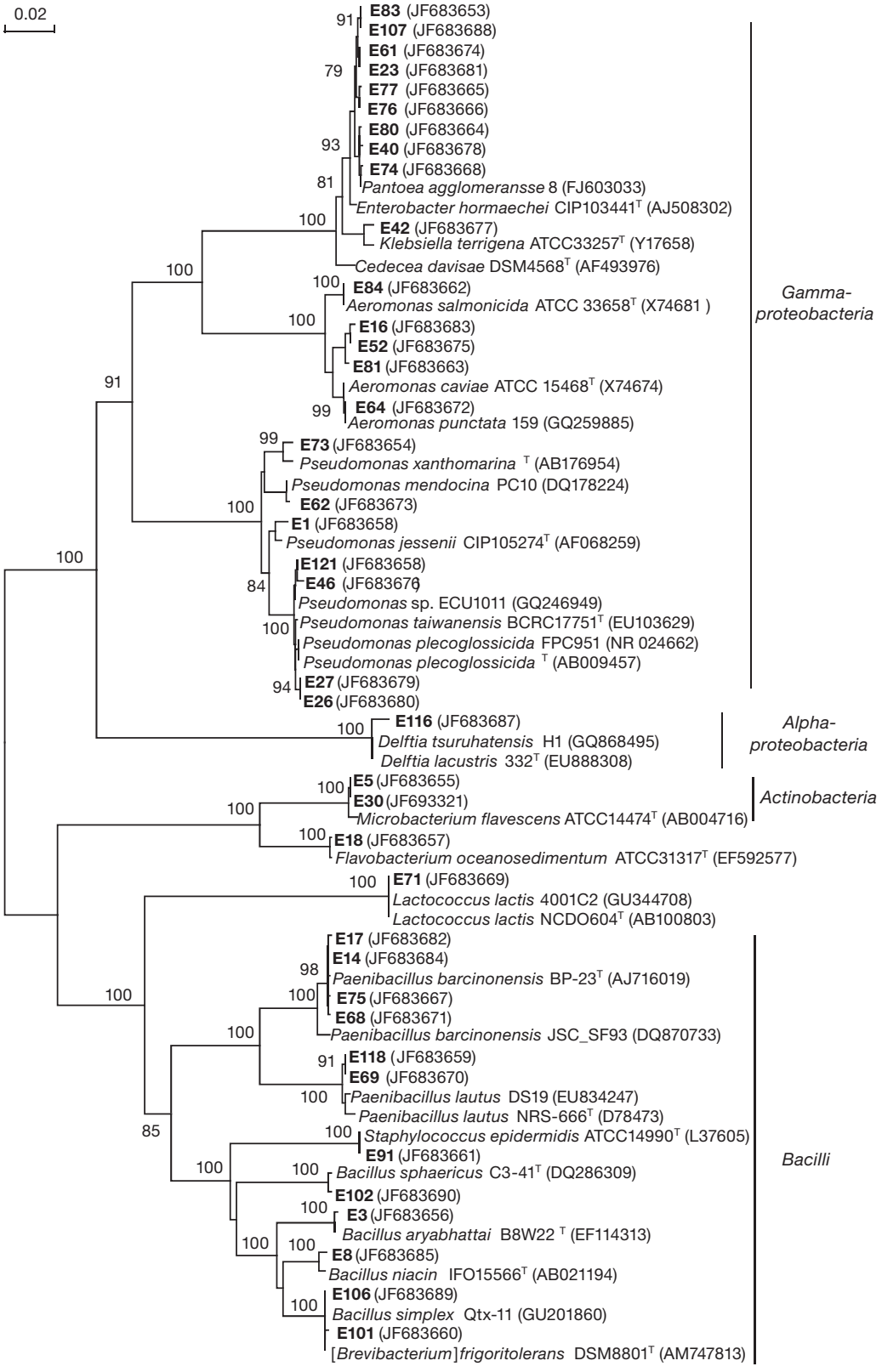

Fig. 1. Neighbor-joining tree of the 16S rRNA gene sequences showing the relationship of strains (accession number in parentheses) isolated from the 4 aquatic plants and reference species. Bootstrap values (1000 replicates) above 70% are shown at nodes. The tree topology was also evaluated with the maximum-likelihood and maximum-parsimony algorithms. Bar: 2 nucleotide substitutions per 100 nucleotides

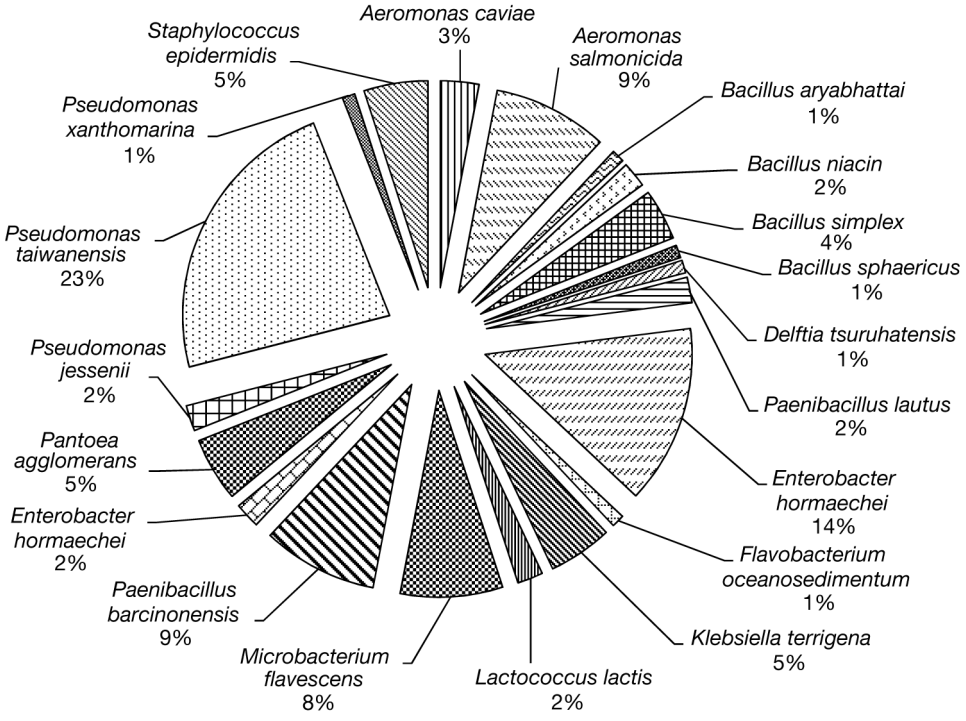

Fig. 2. Relative frequencies of bacterial isolates classified to the species level on the basis of 16S rRNA gene sequence analysis. Data show the percentage contribution of each group of isolates to the total 100 isolates

communis and the stem of *Nymphaea tetragona*, the *Aeromonas* contained more discrete isolates (12 isolates) than the widely isolated *Paenibacillus* spp. *Staphylococcus* and *Delftia* bacteria were only isolated from the root of *Potamogeton crispus*, accounting for 5% and 1% of the total 100 isolates, respectively. *Flavobacterium* spp. were only isolated from the stem of *Phragmites communis* (Fig. 3).

Within *Phragmites communis*, *Pseudomonas*, *Enterobacter*, *Paenibacillus*, *Microbacterium* and *Aeromonas* were commonly isolated from the roots, stems and leaves (Fig. 4), while *Baccillus*, *Klebsiella*, *Lactococcus* and *Pantoea* isolates were shared differently by the roots, stems and leaves. In *Potamogeton crispus*, 7 distinct genera were isolated, 4 of which colonized the roots; among these 4 genera *Pseudomonas* spp. were also isolated from the stems and leaves and *Enterobacter* spp. were also isolated from the stems but not the leaves. The bacteria isolated from the stems of *Potamogeton crispus* belonged to *Paenibacillus* spp., *Klebsiella* spp., and *Pantoea* spp. In contrast, *Staphylococcus* spp. and *Delftia* spp. were only isolated from the roots. Within *Nymphaea tetragona*, most bacteria were isolated from the stems and were dominated by *Aeromonas* spp. and *Pseudomonas* spp. Only one isolate each was isolated from the roots of *Pseudomonas* and *Bacillus*. *Najas marina* harbored the smallest number of culturable bacteria. Only 4 isolates belonging to the genera *Pseudomonas*, *Bacillus* and *Paenibacillus* were cultured from the roots, and only one *Pseudomonas* isolate was isolated from the stems (Figs. 3 & 4).

Functions of the endophytic bacteria

Among the 100 isolates, 44 were selected from different genera, plants and plant tissues for the functional test (Table 2). Among the 44 isolates, strain *Bacillus* sp. E3 from the roots of *Phragmites communis* could degrade 50.0% of the chlorpyrifos and 33.0% of the bifenthrin, while other *Bacillus* species isolated from roots of *Najas marina* and stems of *Nymphaea tetragona* could not degrade any pesticide. Strain *Microbacterium* sp. E5 from the roots of *P. communis* could degrade 64.0% of the chlorpyrifos and 45.6% of the fenpropathrin, while *Microbacterium* isolates from the stems and leaves of *P. communis* could not. Strain *Paenibacillus* sp. E14 from the roots of *Najas marina* could degrade 64.0% of the fenpropathrin and *Paenibacillus* sp. E68 from the stems of *Nymphaea tetragona* could degrade 39.0% of the chlorpyrifos. In contrast, no pesticide was degraded by the *Paenibacillus* isolates from other parts of *Najas marina* and *Nymphaea tetragona* nor by the strains closely related to *Paenibacillus lautus*

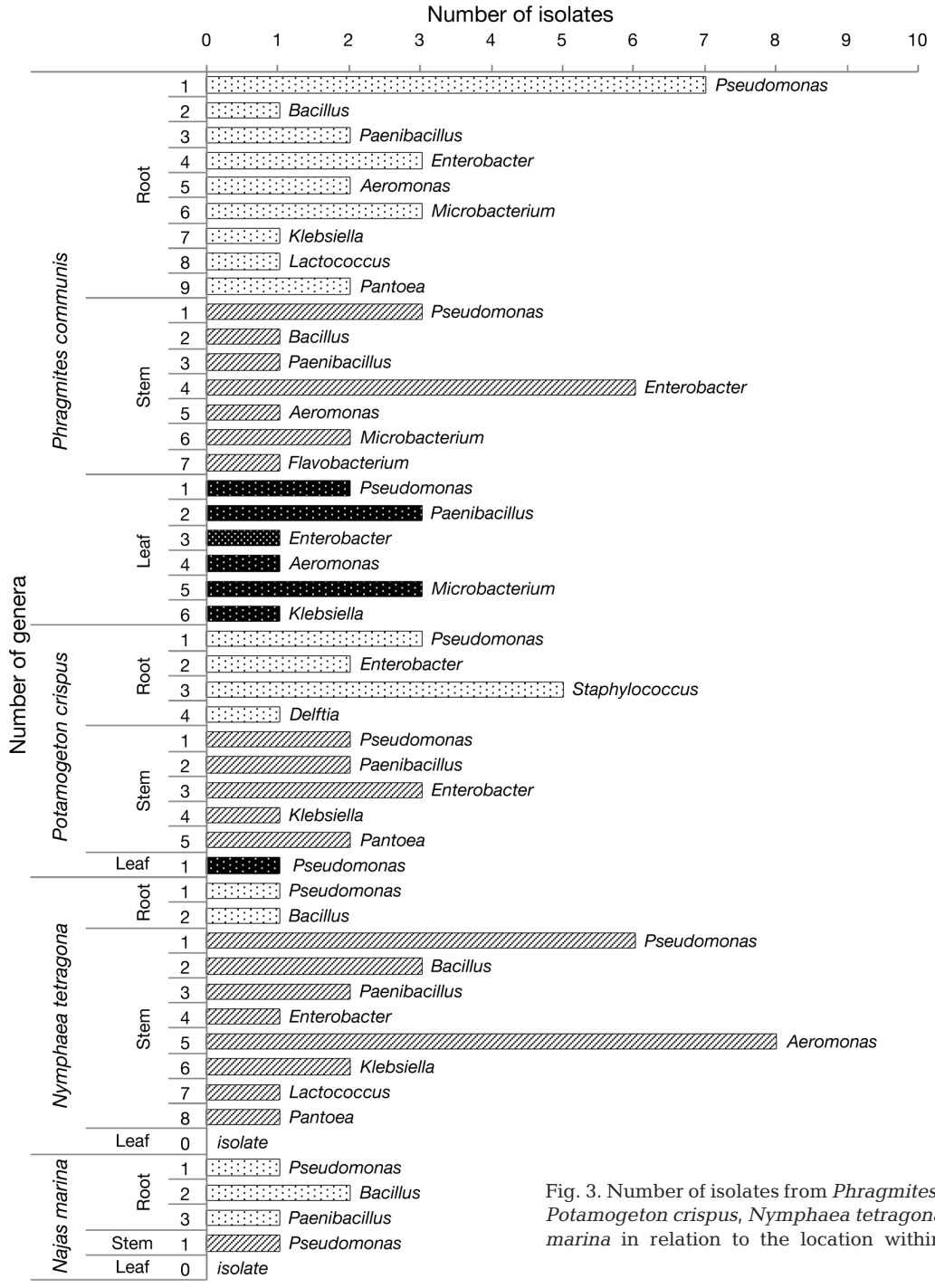

Fig. 3. Number of isolates from *Phragmites communis*, *Potamogeton crispus*, *Nymphaea tetragona* and *Najas marina* in relation to the location within the plant

or from the roots of *Phragmites communis*. Strain *Aeromonas* sp. E16 from the stems of *P. communis* and *Aeromonas* sp. E64 from the roots of *P. communis* could degrade 60.0% of the fenpropathrin and chlorpyrifos. Strain *Flavobacterium* sp. E18 from the stems of *P. communis* could degrade bifenthrin (47.6%). The bifenthrin was also degraded by strain *Klebsiella* sp. E42 from the stems of *Nymphaea tetragona* (70.0%), *Enterobacter* sp. E61 from the roots of *P. communis* (54.0%) as well as *Pseudomonas* sp. E62 from the roots of *Phragmites communis* (65.0%). Strain *Klebsiella* sp. E42 from the stems of *Nymphaea tetragona* could degrade fenpropathrin (70.2%), naphthalene (35.9%) and bifenthrin (70.0%). Strain *Lactococcus* sp. E71 from the stems of *N. tetragona* could degrade fenpropathrin (71.0%).

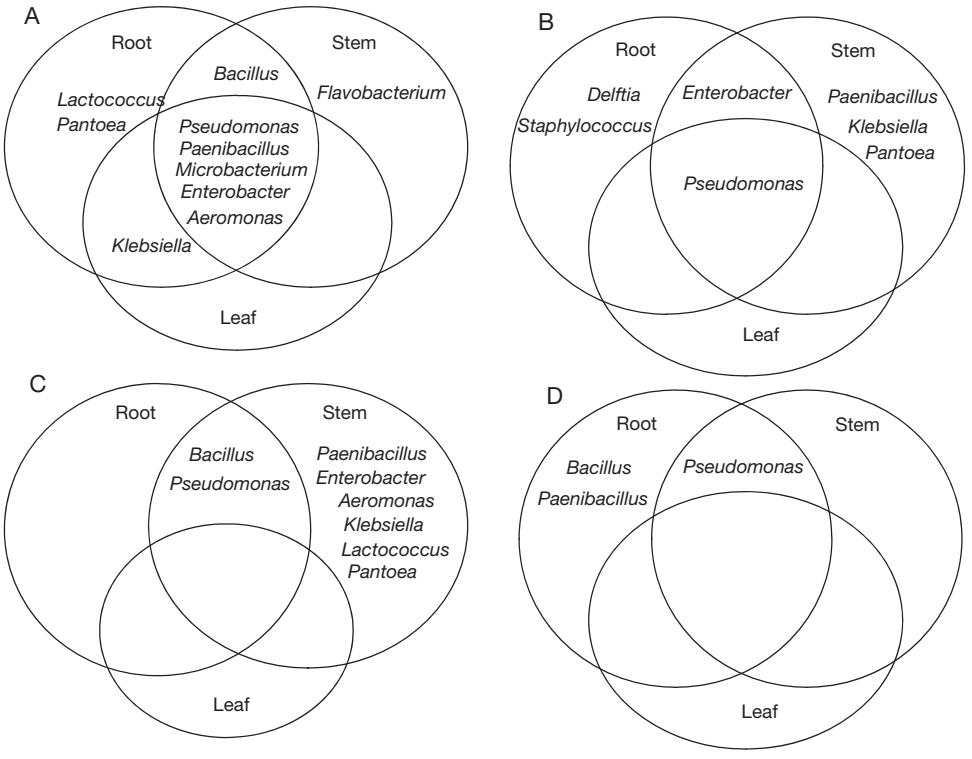

Fig. 4. Distribution of endophytic bacterial isolates in the aquatic plants (A) *Phragmites communis*, (B) *Potamogeton crispus*, (C) *Nymphaea tetragona* and (D) *Najas marina*

In addition, 36 isolates belonging to *Pseudomonas*, *Paenibacillus*, *Enterobacter*, *Aeromonas*, *Flavobacterium*, *Klebsiella* and *Pantoea* could induce an apparent clear zone on Pikovskaya's agar after 5 d incubation, which demonstrated that the bacteria have a potential capacity to transform phosphate from an insoluble form to a soluble form (Table 2).

DISCUSSION

Endophytic bacteria are reported to enhance the phytoremediation of xenobiotic compounds in polluted environments and to increase the solubility of phosphorus and thus promote the growth of the host plants (Ryan et al. 2008). Endophytes in aquatic plants may also therefore be able to remove organic

Table 2. The distribution of endophyte isolates capable of degrading pesticides and solubilizing insoluble phosphorus. Data in parentheses represent the percent of corresponding organic pollutant degraded. For P-solubilization, + is positive activity and – is negative activity

Endophyte species	Plant species	Plant part	Strains with ability to degrade (% degraded)				P-solubilization
			Chlorpyrifos	Fenpropathrin	Naphthalene	Bifenthrin	
Bacillus sp.	*Phragmites communis*	Root	E3 (50)			E3 (66)	–
Microbacterium sp.	*Phragmites communis*	Root	E5 (64)	E5 (46)			–
Paenibacillus sp.	*Najas marina*	Root		E14 (64)			+
Aeromonas sp.	*Phragmites communis*	Stem		E16 (62)			+
Flavobacterium sp.	*Phragmites communis*	Stem			E18 (48)		+
Klebsiella terrigena	*Nymphaea tetragona*	Stem		E42 (70)	E42 (36)	E42 (70)	+
Pantoea sp.	*Phragmites communis*	Root		E61 (35)		E61 (54)	–
Pseudomonas sp.	*Phragmites communis*	Root	E62 (40)			E62 (65)	+
Aeromonas sp.	*Phragmites communis*	Root	E64 (60)	E64 (71)			+
Pseudomonas sp.	*Nymphaea tetragona*	Stem	E73 (70)				+
Paenibacillus sp.	*Nymphaea tetragona*	Stem	E68 (39)				+

pollutants and phosphorus from aquatic environments. However, there has been little investigation into the distribution and functions of endophytic bacteria in aquatic plants. In the present study, endophytic bacteria found in *Phragmites communis*, *Nymphaea tetragona*, *Najas marina* and *Potamogeton crispus* were isolated and their ability to degrade pesticides and to release insoluble phosphorus was studied.

Compared with the endophytes isolated from terrestrial environments where *Alphaproteobacteria* and *Gammaproteobacteria* are the dominant bacteria (Gnanamanickam 2006), the endophytic bacteria isolated from *Phragmites communis*, *Nymphaea tetragona*, *Najas marina* and *Potamogeton crispus* were members of the genera *Pseudomona*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Aeromonas*, *Lactococcus*, *Pantoea*, *Staphylococcus*, *Delftia*, *Flavobacterium*, *Microbacterium* and *Klebsiella*, which mainly belong to *Gammaproteobacteria* and *Baccilli*, with the *Gammaproteobacteria* predominating. One of the reasons for the different dominance of cultured bacterial strains may be because of the different culturing media used. Although most of the endophytic bacteria from the 4 aquatic plants were also species commonly present in water and sediment environments, few studies have been undertaken to examine endophytic bacteria isolated from aquatic plants.

After the group of *Pseudomonas* spp., bacteria belonging to the family *Enterobacteriaceae*, including *Enterobacter*, *Klebsiella* and *Pantoea*, formed the second largest population. These bacteria are widely found in soil, plants, water, sewage and even in the skin and intestinal tracts of humans and animals. Previous studies suggested that about 30% of *Klebsiella* strains can fix nitrogen in anaerobic conditions (Postgate 1998). *Enterobacter* sp. 638 could produce acetoin and 2,3-butanediol, which are involved in promoting plant growth (Taghavi et al. 2010), and has been found to trigger an increased level of growth in *Arabidopsis*, which also has a biocontrol function in preventing host damage from pathogenic bacteria (Ryu et al. 2003). In addition, the *Paenibacillus*, *Microbacterium* and *Bacillus* species are also well known to be beneficial to host plants as well as promoting the degradation of organic pollutants.

In total, 13 *Aeromonas* strains including *A. caviae* and *A. salmonicida* were isolated from the healthy tissues of *Phragmites communis* and *Nymphaea tetragona* and made up 13% of the total number of isolates from the 4 aquatic plants. This high ratio of *Aeromonas* bacteria as endophytes is not usual in terrestrial plants although *Aeromonas* is widely distributed in soil, marine water, fresh water, sewage water, food and vegetables. The relationship of *Aeromonas* species to their host plants is not well documented; however, some of them are known to cause gastroenteritis and bacteremia in humans and fish (Pianetti et al. 2005). The presence of a large quantity of *Aeromonas* may be a warning that aquatic plants may be a risk to human beings or animals (Rahman et al. 2007). Water from human activities may be one of the sources of the *Aeromonas* endophytes as water from human activity in general flows eventually into surface water bodies including reservoirs and lakes. For example, Guanting Reservior receives discharge from surrounding villages and neighborhoods, which means that human pathogens from sewage may infect the water body (Xue et al. 2005). Opportunistic human pathogens such as *Aeromonas* species may then colonize the aquatic plants in the reservoir. Since *Phragmites communis* and *Nymphaea tetragona* are plants located closer to the reservoir bank than are *Potamogeton crispus* and *Najas marina*, there may be a greater potential for them to come into contact with bacteria in the influent water, leading to these species becoming more likely to be colonized by these opportunistic human and animal pathogenic bacteria than others. In fact, it is natural that environmental conditions select the microbial community. The high concentrations of OCPs and POPs in Guanting Reservoir therefore resulted in the isolation of bacterial strains able to degrade chlorpyrifos, fenpropathrin, naphthalene and bifenthrin. However, further studies, such as culture-independent investigations, are needed to understand the pathways and mechanisms involved.

The plant tissues were colonized by distinct populations of bacteria that showed different degradation abilities. For example, *Aeromans* isolates from *Phragmites communis* showed an ability to degrade pesticides, while those from *Nymphaea tetragona* could not. *Klebsiella terrigena* isolated from *N. tetragona* could degrade pesticides, while those from *P. communis* and *Potamogeton crispus* could not. In addition, pesticide-degrading bacteria were mostly isolated from *Phragmites communis* and *N. tetragona*. The reasons why different plants and plant tissues harbor different bacteria with different degrading abilities are complex. One of the reasons may be that different plants and different parts of the plants could excrete different compounds, such as different aromatic compounds. These compounds, could act as selection pressures or stimulators for attached, as well as endophytic, bacteria. In addition, different parts of the plants have different connections with the aquatic environment. The sediment can be a larger

pool of microorganisms than the water body and the air into which *N. tetragona* leaves are exposed. Therefore, more endophytic bacteria could be found in the plants with larger roots, in particular *N. tetragona*, *Potamogeton crispus* and *Najas marina*. In contrast, as *Nymphaea tetragona* has a larger stem than the others, more endophytes were isolated from the stem than the root (Fig. 3, Appendix 1). Therefore, different plants and even plant parts can be used as potential carriers for endophytes of interest to enhance degradation of organic pollutants.

Organic pollutants in water bodies are generally present in very low but toxic concentrations. *In situ* bioremediation may be the only economic and rational solution to remove the contaminants such as POPs and pesticides. Research into bioremediation has to date usually concentrated on plants or bacteria in the rhizosphere, and research involving endophytes has only been reported recently (Ryan et al. 2008, McGuinness & Dowling 2009, Weyens et al. 2009). Bioremediation of aquatic environments by endophytes from aquatic plants has been studied even less. The present study shows that the isolated endophytic bacteria belonging to 8 genera, including *Paenibacillus* and *Microbacterium*, can achieve relatively high rates of pesticide degradation and phosphorus solubilization. In addition, most of these 'functional bacteria' colonize *Phragmites communis* and *Nymphaea tetragona*. This offers a potential phytoremediation approach in which these plants could be put into polluted aquatic environments to inoculate the polluted water with endophytic bacteria that would then degrade pollutants and solubilize phosphorus.

Phosphorus-solubilizing bacteria could promote plant growth by transforming insoluble forms of phosphorus into accessible forms, which could lead to an increased availability for plants and thus improve plant yields. Little work has been undertaken with this group of bacteria in sediments or in aquatic plants. In the Guanting Reservoir, the endophytic bacteria *Pseudomonas* sp., *Enterobacter* sp., *Aeromonas* sp., *Flavobacterium* sp., *Klebsiella* sp., *Pantoea* sp. and *Paenibacillus* sp., have a P-solubilization capacity and could be used as inoculants and promoters to increase P-uptake by plants, as suggested by Park et al. (2011).

In conclusion, culturable endophytic bacteria from 4 aquatic plant species, were isolated. According to the nearly complete 16S rRNA gene sequences, the bacteria were assigned to 12 genera within the *Gammaproteobacteria*, *Bacilli*, *Actinobacteria*, *Alphaproteobacteria* and *Flavobacteria*, and the *Gammaproteobacteria* dominated the culturable community. In addition, different plants and plant parts harbored different culturable bacteria, which showed different pesticide degradation and phosphorus solubilization abilities. It is noteworthy that these endophytic bacteria contained some populations of opportunistic human and animal pathogenic bacteria. As far as it is known, this is the first study to compare culturable endophytic bacteria among different aquatic plants.

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Appendix 1. 16S rRNA genotypes of endophytic bacteria from 4 host aquatic plant species and sequence similarity with related species

Endophyte strain	Host plant	Position	16S rRNA			Related species (sequence similarity, %)
			*Msp*I	*Rsa*I	Genotypes	
E1, E112, E113, E114	*Phragmites communis*	Root	1	1	1	*Pseudomonas jessenii* (98.0)
E12, E111	*Phragmites communis*	Stem	1	1	1	
E26, E27, E28	*Nymphaea tetragona*	Stem	1	1	1	*Pseudomonas taiwanensis* (99.7)
E87	*Nymphaea tetragona*	Root	1	1	1	
E36	*Phragmites communis*	Leaf	1	1	1	
E88	*Potamogeton crispus*	Root	1	1	1	
E104	*Potamogeton crispus*	Stem	1	1	1	
E3	*Phragmites communis*	Root	2	1	2	*Bacillus aryabhattai* (99.8)
E5, E4, E6	*Phragmites communis*	Root	2	3	3	*Microbacterium flavescens* (99)
E10, E21	*Phragmites communis*	Stem	2	3	3	
E30, E32, E35	*Phragmites communis*	Leaf	2	3	3	*Microbacterium flavescens* (99)
E8, E11	*Najas marina*	Root	2	1	4	*Bacillus niacini* (99.4)
E14	*Najas marina*	Root	12	4	5	*Paenibacillus barcinonensis* (99)
E17	*Phragmites communis*	Stem	12	4	5	*Paenibacillus barcinonensis* (99)
E37, E38, E39	*Phragmites communis*	Leaf	12	4	5	
E54, E58	*Potamogeton crispus*	Stem	12	4	5	
E68	*Nymphaea tetragona*	Stem	12	4	5	*Paenibacillus barcinonensis* (99)
E75	*Phragmites communis*	Root	12	4	5	*Paenibacillus barcinonensis* (99)
E15, E24, E29	*Phragmites communis*	Stem	3	5	6	
E77, E56, E78	*Potamogeton crispus*	Stem	3	5	6	*Enterobacter hormaechei* (99.6)
E85, E108	*Potamogeton crispus*	Root	3	5	6	
E34	*Phragmites communis*	Leaf	3	5	6	
E76	*Nymphaea tetragona*	Stem	3	5	6	*Enterobacter hormaechei* (99.6)
E86	*Phragmites communis*	Root	3	5	6	
E16	*Phragmites communis*	Stem	3	1	7	*Aeromonas caviae* (98.6)
E25	*Nymphaea tetragona*	Stem	3	1	7	
E33	*Phragmites communis*	Leaf	3	1	7	
E18	*Phragmites communis*	Stem	3	7	8	*Flavobacterium oceanosedimentum* (99.7)
E19, E22	*Phragmites communis*	Stem	5	5	9	*Enterobacter hormaechei* (99.6)
E107	*Phragmites communis*	Root	5	5	9	*Enterobacter hormaechei*
E20	*Phragmites communis*	Stem	1	8	10	
E44	*Phragmites communis*	Root	1	8	10	
E46, E51	*Nymphaea tetragona*	Stem	1	8	10	*Pseudomonas taiwanensis* (99.4)
E55	*Najas marina*	Stem	1	8	10	
E103	*Najas marina*	Root	1	8	10	
E59	*Potamogeton crispus*	Stem	1	8	10	
E115, E90	*Potamogeton crispus*	Root	1	8	10	
E117	*Phragmites communis*	Leaf	1	8	10	
E116	*Potamogeton crispus*	Root	1	9	11	*Delftia tsuruhatensis* (99.3)
E42, E40	*Nymphaea tetragona*	Stem	8	5	12	*Klebsiella terrigena* (99.2)
E57	*Potamogeton crispus*	Stem	8	5	12	
E120	*Phragmites communis*	Leaf	8	5	12	
E47, E60	*Nymphaea tetragona*	Stem	5	1	13	
E52, E63	*Phragmites communis*	Root	5	1	13	*Aeromonas salmonicida* (100)
E64	*Phragmites communis*	Root	5	1	13	*Aeromonas salmonicida* (99.9)
E65, E66, E81, E84, E89	*Nymphaea tetragona*	Stem	5	1	13	
E23	*Phragmites communis*	Stem	6	5	14	*Enterobacter hormaechei* (99.6)
E61	*Phragmites communis*	Root	6	5	14	*Enterobacter hormaechei* (99.6)
E62	*Phragmites communis*	Root	12	8	15	*Pseudomonas jessenii* (99.9)
E69	*Nymphaea tetragona*	Stem	11	4	16	*Paenibacillus lautus* (99.2)
E118	*Phragmites communis*	Root	11	8	16	*Paenibacillus lautus*
E70	*Phragmites communis*	Root	13	2	17	
E71	*Nymphaea tetragona*	Stem	13	2	17	*Lactococcus lactis* (100)
E74, E79	*Potamogeton crispus*	Stem	7	5	18	*Pantoea agglomerans* (100)
E80	*Nymphaea tetragona*	Stem	7	5	18	*Pantoea agglomerans* (99.8)
E73	*Nymphaea tetragona*	Stem	5	2	19	*Pseudomonas xanthomarina* (99.3)
E82, E83	*Phragmites communis*	Root	6	5	20	*Pantoea agglomerans* (99.3)
E91, E93, E95, E96, E97	*Potamogeton crispus*	Root	2	6	21	*Staphylococcus epidermidis* (99.9)
E102	*Nymphaea tetragona*	Stem	2	10	22	*Bacillus sphaericus* (99.9)
E101	*Nymphaea tetragona*	Root	2	4	23	*Bacillus simplex* (99.9)
E105, E106	*Nymphaea tetragona*	Stem	2	4	23	*Bacillus simplex* (100)
E110	*Phragmites communis*	Stem	15	11	24	*Bacillus simplex* (99)
E121	*Potamogeton crispus*	Leaf	10	12	25	*Pseudomonas taiwanensis* (99.7)

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