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Endophytes Community of *Populus euphratica* Oliv and the Strains Improving the Salt Tolerance of Plant

Xiangyang Ju[1], Liming Ouyang[1]* and Lili Zhang[2]

[1]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China.
[2]Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Tarim University, Alar 843300, China.

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The diversity of endophytic bacteria associated with wild forest of *Populus euphratica* Oliv located in arid and saline area of northwestern China was investigated for the first time. 197 stable isolates were isolated from the tissues of *P. euphratica* trees and were further classified into 102 operation taxonomic units (OTUs) by amplifed ribosomal DNA restriction analysis (ARDRA) and morphology. Based on the sequence analysis of partial 16S rRNA genes, the 102 representative strains of each OTU belong to 21 genera, in which the *Bacillus* and *Pseudomonas* were the most common consisting of 49.0% and 14.7% of the community respectively. 16.7% of the representative strains were demonstrated to improve the germination of wheat (*Triticum aestivum*) seeds under salinity stress as plant growth promoting bacteria (PGPB). However, most lost the germination promoting effects when salinity stress was revoked. Interestingly, the proportion of PGPB on all endophytes exhibited a marked escalating trend along with the increase of bacterial salt tolerance.

Key words: Endophytes; Bacterial community; Salinity stress; *Populus euphratica*; PGPB; ARDRA.

Endophytes are a specific group of microorganisms (bacterial and fungal) residing within a wide variety of plant tissue types, including seeds and ovules, fruit, stems, roots and tubers. A diverse array of bacterial genera has been reported to be endophytes, including *Acetobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Herbaspirillum* and *Pseudomonas*. Many reports have demonstrated some special endophytes as well as rhizospheric bacteria, acting as plant growth promoting bacteria (PGPB), promote the growth and tolerance of host plant to abiotic stress such as flooding [1], drought [2], and salt [3], but most of them are limited to herbaceous plants in agriculture or horticulture. As stable symbiotic partners of trees with longer life span, endophytes of woody plants may contribute more to the tolerance of their hosts.

Several groups have studied the endophytic community of poplar (*Populus*) trees, which have become more important not only as a future energy source but also as 'the trees of choice' for phytoremediation purposes and as target species for genetic transformations [4]. Moore et al. [5] analyzed the composition of culturable endophytic bacteria in poplar grown on a benzene, toluene, ethylbenzene and xylene (BTEX)-contaminated site and reported a high diversity of isolates dominated by *Gammaproteobacteria*, especially *Pseudomonas* sp. Detailed analysis of endophytic bacteria from different hybrid poplar clones revealed a high phylogenetic diversity of endophytic bacteria with a total of 53 taxa at the genus level that included *Proteobacteria*,

* To whom all correspondence should be addressed.
Tel.: +86 21 64252257; Fax: +86 21 64253019;
E-mail: ouyanglm@ecust.edu.cn

Actinobacteria, *Firmicutes* and *Bacteroidetes* [6]. Some endophytic bacteria isolates of poplar were demonstrated to promote plant growth [7] or alleviate the harm of organic contaminations to their hosts [8]. These studies indicate the endophytic communities of trees have positive impact on the adaptability of hosts to special environments.

Populus euphratica is the only tree species that occurs naturally in the arid and saline areas in northwestern China. It is a halophytic plant, tolerating salt, cold and extreme drought stresses, and has recently been used as a model to study salt resistance mechanisms in plant [9]. Most research were focused on the plant physiology of *P. euphratica* so far, while its endophytic bacteria community structure and the possible rules of endophytes to the tolerance of host plants are still lack of understanding.

Here we present our data on the bacteria community of endophytes isolated from the little studied *P. euphratica*, a tree of the *Populus* genus with a tenacious vitality for surviving in desert conditions. The salt tolerance of the strains and their efficiency on enhancing growth and salt tolerance of plants were further evaluated.

MATERIALS AND METHODS

Sampling

Samples of root, stem, and leaf were collected from seven *P. euphratica* trees (numbered as PE1~PE7 respectively) growing in the arid and saline areas of Tarim river basin (Shaya County, Aksu Prefecture, Xinjiang Uygur Autonomous Region, China) in the autumn of 2012. Root samples were taken from PE3, PE4, PE5, PE6 and PE7. Stem samples were collected from PE1, PE2, PE5, PE6, and PE7. Leaf samples were just taken from PE1 and PE2. Upon collection, healthy samples were carefully packed into an icebox and transported to laboratory within 12h.

Isolation of endophytic bacteria from *Populus euphratica*

Healthy samples were rinsed with ethanol: water solution (95% [vol/vol]) for 1 min and then 30 min in a solution containing 2.08% active chloride and finally rinsed three times in sterile distilled water. Sterility checks were performed by plating surface-sterilized plant samples onto 1/5 869 agar medium [10] and successful sterilization was considered as no bacterial growth after 3 days incubation at 30°C. With sterile knife and scissors, sterile samples were cut into pieces prior to plating onto 1/5 869 agar medium and the plates for 3 days at 30°C. All morphologically different bacterial colonies were selected and propagated 3 times to ensure purity and stability. Pure strains were frozen in 25% glycerol at -80°C.

16S rRNA gene amplification, amplifed ribosomal DNA restriction analysis (ARDRA), sequencing, and strain identification

Genomic DNA was crudely extracted from all 197 isolates according to Sudagidan et al. [11], with some modifications. Briefly, isolates were cultured in 2 ml 1/5 869 medium at 30°C for 18 h with shaking. A 50 µl aliquot was pelleted by centrifugation at 4000 g for 2 min. Pellets were resuspended in 25 µl deionized sterile water supplemented with 0.25 µl lysostaphin (2 mg/ml) dissolved in 20 mM sodium acetate, and incubated at 37°C for 10 min. After adding 0.5 µl of proteinase K (5 mg/ml) and 75 µl of 0.1 M Tris–HCl (pH 7.5), samples were further incubated for 10 min. Finally, samples were boiled for 10 min and then stored at -20°C as template for PCR. 16S rRNA genes were amplified using the standard 27F-1492R primer set [12]. PCR reaction was performed using 10 µl of 2 × Taqmix, 2 µl of genomic template, 0.5µl of each primer and supplemented of sterile water to a final reaction volume of 20µl. Amplifications were carried out using the temperature profiles: 94°C for 5 min, followed by 30 cycles at 94°C for 40s, 56°C for 1 min, 72°C for 90s, and a final extension step at 72°C for 10 min. Aliquots of PCR reaction products were separated on a 1% (w/v) agarose gel containing ethidium bromide (1 µg/ ml).

For ARDRA, aliquots of the PCR products were digested overnight at 37°C with *Msp*I in 1× N buffer Tango™ (Fermentas). Isolates with different ARDRA patterns were grouped into different operational taxonomic units (OTUs). Random inspection of the sequences of 16S rRNA genes demonstrated the strains of the same OTU are identical at the level of genus. Representative strains from each OTU were picked randomly for further identification, salt tolerance detection and plant experiments. The PCR products of 16S rRNA genes were purified and sequenced by Biosune Biotechnology Company, Limited (Shanghai, China). Taxonomic classifications were determined

online at the website of Ribosome Database Project II (http://rdp.cme.msu.edu/index.jsp) [13]. The sequences used for identification of the cultivable endophytes are available in the GenBank database (www.ncbi.nlm.nih.gov) under accession numbers KF663767 through KF663868.

Effect of endophytic strains on germination of wheat seeds under salt stress

The effect of isolated endophytic bacteria on improving the germination of wheat seeds under salt stress was investigated as described by Egamberdieva [14] with little modification. Briefly, all test strains were cultured overnight and then diluted with sterilized 10 mM $MgSO_4$ to an OD of 0.5 ± 0.02 at 600 nm. Wheat seeds were sorted to eliminate broken, small, and infected seeds. Sterilized wheat seeds were obtained by rinsing and stirring wheat seeds for 5 min in a sterile flask with a solution containing 0.52% active chloride. After 5 min, the sodium hypochlorite was removed by washing the seeds five times with sterile water. Sterilized seeds were either presoaked with the bacterial suspension or sterilized $MgSO_4$ solution (10 mM) for 2 h prior to seeding. Then the seeds were covered between two pieces of sterile filter paper soaked with a sterilized 100 mM NaCl solution or sterilized water and then plated on plastic Petri dishes (95×20 mm). Fifteen seeds were plated in each plastic Petri dish and three replicates were used for each treatment. NaCl solution or sterile water with equal volume was replenished every two days. The seeds were cultivated in a controlled environment cabinet with a day/night cycle of 16 h/8 h at 30/25°C. After 5 days, germination was checked and the fresh weights of seedlings were determined.

One-way ANOVA test were used to test significant differences of seedling fresh weight between the treatments. A p value of <0.05 was considered to be statistically significant.

Bacterial tolerance to salt

To determine the bacterial tolerance to salt, a 1 µl aliquot of an overnight culture was inoculated onto the 1/5 869 plate with supplement of extra NaCl of 5, 8, 10 or 15% (w/v).The plates were then incubated at 30°C for 5 days. Based on data obtained, acceptable salt concentration (ASC) of endophyte was determined as the highest salt concentration upon which the endophytic bacteria grew.

RESULTS

Isolation and classification of community composition of cuturable endophytic bacteria of *P. euphratica*

Endophytic bacteria were isolated from surface-sterilized samples taken from *P. euphratica* growing in the arid and salty area of Tarim River watershed of china. Based on morphological characteristics, 197 strains were selected for classification by ARDRA. Then they were grouped into 102 OTUs by their ARDRA pattern. 102 representative strains from each OTU were selected for the further study. After 16S rRNA gene sequence analysis of the 102 representative strains, the community composition of culturable endophytes residing in *P. euphratica* were revealed (Fig. 1). All the representative endophytic strains obtained from *P. euphratica* can be assigned to 21 different genera except for seven strains which were only identified to the level of families (Table 1). The majority of the isolated strains (52%) belonged to *Firmicutes*, including *Bacillus* sp. (49%), *Staphylococcus* sp. (2%) and *Penibacillus* sp. (1%). *Gammaproteobacteria* were the second most abundant class (21.6% of total isolates) dominated by *Pseudomonas* sp. (14.7%). Other genera were *Acinetobacter* sp. (2%), *Enterobacter* sp. (1%), *Serratia* sp. (1%), *Stenotrophomonas* sp. (1%) and two strains belong to *Enterobacteriaceae*. *Alphaproteobacteria* comprised 9.8% of the total isolates, represented by *Mesorhizobium* sp. (2.9%), *Phyllobacterium* sp. (1%), *Rhizobium* sp. (2.9%), *Novosphingobium* sp. (1%) and two strains of *Phyllobacteriaceae* and *Rhizobiuaceae*. *Betaproteobacteria* which made up with *Massilia* sp. (4.9%) and *Cupriavidus* sp. (2%) comprised 6.9% of all the representative strains. Another dominated class was *Actinobacteria* (5.9%), represented by six strains as *Agrococcus* sp., *Curbobacterium* sp., *Mycobacterium* sp., *Arthrobacter* sp., *Nocardiopsis* sp., and *Streptomyces* sp. Bacteria belonging to *Bacteroidetes* were represented only by the family *Sphingobacteriaceae* and formed only 2.9% of the total isolates. *Deinococcus-thermus* comprised only 1% of the total isolates.

To compare the differences among the individual trees, the distributions of entophytic bacteria from root samples of 5 *P. euphratica* clones

were illustrated as Fig. 2. *Firmicutes* again accounts for relative bigger proportion of bacterial species in all the root samples although their ratios among trees are different. However, the proportions of *Gammaproteobacteria, Alphaproteobacteria* and *Betaproteobacteria* varied a lot which reflected the specificity of endophytic community associated with individual trees.

Effect of the 102 representative strains on the growth of wheat seedlings

Wheat seeds inoculated with the bacteria suspensions were exposed to a solution of 100 mM NaCl in germination station. Uninoculated seeds treated with or without salt were set as negative or positive control groups. After five days of culture under salt stress, the fresh weight of the negative control decreased dramatically compared to that of the positive control (Fig. S1-S12). Among the 102 Fig 3 strains used in inoculation, 17 from genera of *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Cupriavidus., Serratia. Curbubacterium* and 2 from families of *Sphingobacteriaceae* promote the growth of seedlings significantly compared to negative control. These 17 strains were defined as PGPB. While 6 strains belonging to *Bacillus sp.*, *Pesudomonas sp.* and *Paenibacillus sp.* significantly inhibited the growth of seedlings under salt stress in fresh weight. The other 79 strains demonstrated non-significant effects on growth of host plants under salt stress. Strains which could significantly increase or decrease the fresh weight of seedlings under salt stress were shown in Fig. 3a.

Interestingly, when these 17 PGPB strains were then tested for their effects on wheat seedlings without salt stress, none of them demonstrated their growth promoting effect and 2 (strain 45 and 136) actually appeared harmful as

Table 1. Numbers and classifications of representative strains isolated from different plant tissue types of *P. euphratica*

Classification of strains		Plant tissue type		
Family	Genus	Root	Shoot	Leave
Moraxellaceae	*Acinetobacter sp.*		1	2
Penibacillaceae	*Penibacillus sp.*		1	1
Bacillaceae	*Bacillus sp.*	41	12	1
Xanthomonadaceae	*Stenotrophomonas sp.*		1	
Sphingomonadaceae	*Novosphingobium sp.*		1	
Sphingobacteriaceae	-	3		
Enterobacteriaceae	*Eterobacter sp.*	1		
	Serratia sp.	1		
	-	1	1	
Microbacteriaceae	*Agrococcus sp.*	1		
	Curbobacterium sp.		1	
Pseudomonadaceae	*Pseudomonas sp.*	10	5	
Rhizobiaceae	*Rhizobium sp.*	3		
	-	1		
Deinococaceae	*Deinococcus sp.*	1		
Burkholderiaceae	*Cupriavidus sp.*	2		
Oxalobacteriaceae	*Massillia sp.*	5		
Mycobacteriaceae	*Mycobacterium sp.*	1		
Nocardiopsaceae	*Nocardiopsis sp.*	1		
Micrococaceae	*Arthrobacter sp.*	1		
Staphylococcaceae	*Staphylococcus sp.*	2		
Streptomycetaceae	*Streptomyces sp.*	1		
Phyllobacteriaceae	*Mesorhizobium sp.*	3		
	Phyllobacterium sp.	1		
	-	1		

(–) Zone represents the OTUs unclassified to genera level but only to family level

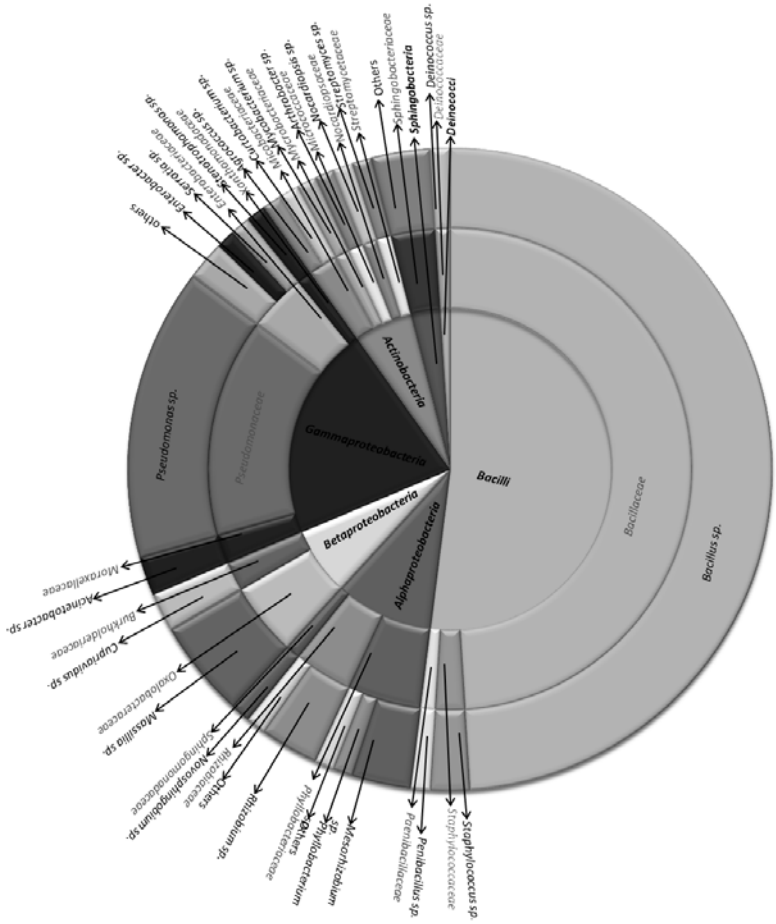

Fig. 1. Taxonomic breakdown of the culturable endophytic community composition identified by 16S rRNA gene sequences. The central pie details percentages by class; each outer annulus progressively breaks these down to finer taxonomic levels, with families and genus in the outermost annuli

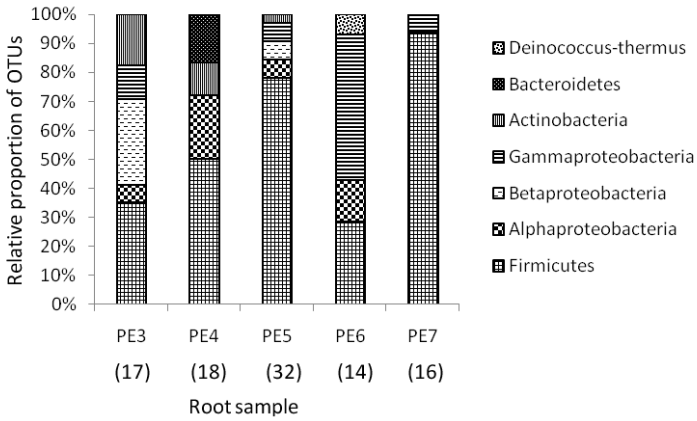

Fig. 2. Distribution of the bacterial classes of endophytic bacteria isolated from the root samples of different *P. euphratica* clones. The number of representative strains isolated from each sample is given in brackets

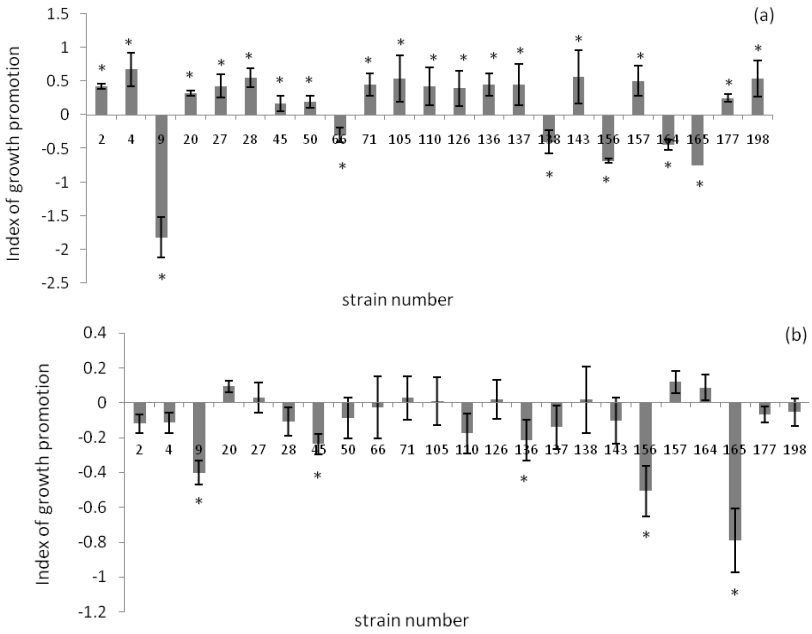

Fig. 3. Effects of 23 endophytic strains on growth of wheat seedlings compared to uninoculated control. The index was counted by following formula: Index= {fresh weight (inoculated) - fresh weight (uninoculated control) / fresh weight (uninoculated control). Within each value, bars having a * indicated significant ($P < 0.05$) differences as determined by one-way ANOVA test. (a) under salt stress; (b) without salt stress

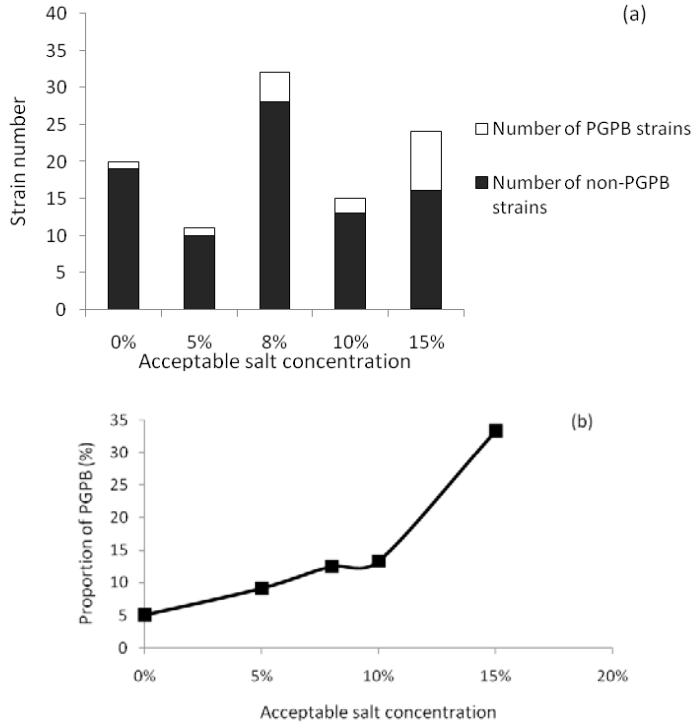

Fig. 4. Numbers and proportions of PGPB in bacterial group with different ASC. (a) The PGPB and non-PGPB strain numbers; (b) the proportions of PGPB within certain ASC

they significantly inhibiting the growth of seedlings compared to control. Of the 6 strains detrimental to germinations of wheat seeds under salt stress, 3 of them (strain 9, 156 and 164) still exhibited significant adverse effects while the 3 remaining strains lost their malign effect on seedling growth (Fig 3b).

Salt tolerance of the 102 representative strains

When culturing the 102 representative strains on 1/5 869 medium containing extra NaCl concentration of 15%, 10%, 8%, 5% and 0% (v/w), they showed different salt tolerance in cell growth. The number of strains with ACS of 15%, 10%, 8%, 5% and 0% were 24, 15, 32, 11 and 20 respectively (Fig. 4a), which means about 70% of the representative strains were tolerant to NaCl with concentration of over 8%.

Relationship between the salt tolerance and plant growth-promoting effects of strains

As for the 17 PGPB strains relieving the damage of salt on wheat seedlings during germination, the biggest proportion of PGPB was within ASC of over 15% ; whereas the smallest proportion is found in the strain group of which bacteria can only grow in medium with additional NaCl of less than 5% (Fig. 4b). It seemed that bacteria strains that survived in higher salt concentration were more likely to be beneficial to the growth of host plant under salt stress.

DISCUSSION

The objective of this study was to examine the community composition of endophytic bacteria from wild *P. euphratica* forest growing in the arid area of northwestern china, and investigate the PGP potential of these endophytes. A high phylogenetic diversity of endophytic bacteria of *P. euphratica* was identified with a total of 102 strains belonging to phyla of *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *deinococcus-thermus*. With the exception of *deinococcus-thermus*, other phyla have previously been detected in other studies in the endophytic bacteria community of populous[5,7,15] .

The distribution of the bacterial genera reflects adaptation to the different microhabitats. The *Bacillus sp*. genus was isolated in all *P. euphratica* samples, with higher prevalence in the roots and shoots. Garbeva et al. [16] demonstrated that the majority of Gram-positive bacteria in soils under different types of management regimes (permanent grassland, grassland turned into arable land and arable land), were putative *Bacillus* species. *Bacillus sp.* is also commonly found in arid land as a consequence of their ability to form endospores that allow bacterial survival for extended time periods under adverse environmental conditions [17]. For endophytes, a study by Marasco et al. [18] indicated an enrichment of *Bacillus* in a drought-sensitive pepper plant (Capsicum annuum L.) cultivated in a traditional Egyptian farm dependent on the presence of drought. Many strains of *Bacillus* and related genera also have been reported to promote the growth of a wide range of plants [19].

The dominance of the *gammaproteobacteria* among the endophytes from poplar was repeatedly reported. Among 78 endophytic bacteria isolated from surface sterilized root and stem samples taken from hybrid poplar H11–11 and native willow, the majority of the isolated strains (71%) belonged to the *gammaproteobacteria* such as *Serratia sp.*, *Serratia plymuthica*, *Serratia proteamaculans* and *Rahnella sp*. Other dominant *gammaproteobacteria* included *Pseudomonas sp.* and *Enterobacter sp*. [7]. High numbers of *Gammaproteobacteria* (61%) were also found in the isolate collection of poplar endophytes, which was dominated by *Pseudomonas sp*. with a frequency of up to 53% of the total isolates in the study of Moore et al. [5]. In contrast, *Gammaproteobacteria* made up 21.6% of the total isolates, with *Pseudomonas sp.* species comprising 14.7% of the isolates in this study. The same result were found with Ulrich et al. [6] who previously reported that *Gammaproteobacteria* made up only 28% of the total isolates, with *Pseudomonas sp.* species comprising 19% of the isolates. The high abundance of *Pseudomonas sp.* isolates in poplar grown in the organic-contaminated field may possibly be explained by their diverse metabolisms of chemicals and high tolerance to a variety of physical conditions. In this context, *Pseudomonas sp.* strains have been reported to be involved in the biodegradation of 2.5-dichlorobenzoate [20], toluene [21] and naphthalene [22]. Besides the metabolism diversity, many *Pseudomonas sp.*

strains also possess characteristics related to plant growth-promotion such as the production of plant growth regulator and nitrogen fixation, and so can be applied as PGPB in phytoremediation [23,24].

In this study, the proportion of some bacteria genera varied greatly among different *P. euphratica* clones (Fig. 2). It was reported that the community structure of endophytic bacteria was shown to be strongly affected by different hybrid poplar clones [6], pointing to individual-specific associations between endophytes and their poplar host. The diversity of endophytic bacteria was also influenced by different plant genotypes of citrus and poplar [5,25]. Thus, endophytic bacterial communities depend on the genotypes of plants, climate and soil environments, and other random factors.

Interestingly, as the ASC of bacteria increased, the proportion of PGPB to the bacteria of the same ASC also increased (Fig. 4b) while 4 of 6 plant growth-inhibiting endophytes were identified in the 0% ASC group. The consistency indicates salt resistance mechanisms of most endophytes may link to some PGP related mechanisms such as producing plant growth hormones or polysaccharides as osmotic regulator, and so benefit the growth of their host plant under salt stress. In addition, with the absence of salt stress, no significant increase in fresh weight of seedlings was observed for all the 23 endophytes. These results strongly suggest that salt stress may change the metabolism of bacteria and introduce expression of substances which improve the plant tolerance to salt stress. Rojas-Tapias [26] also reported the inoculation with *Azotobacter sp.* strains C5 and C9 increased plant growth, but only under saline stress ($p < 0.05$) [26]. Cheng et al. [27] reported that the shoot fresh and dry weights of canola plant treated with salt plus *P. putida* UW4 were 1.7-fold and nearly 2-fold, respectively, greater than those of plants treated with salt only ($p < 0.05$). But in the absence of salinity stress, no statistically significant differences in shoot weights were observed between the uninoculated controls and bacterially treated plants. A few studies confirmed the hypothesis in last decade. Cohen et al. [28] observed that strain *A. brasilense* cultures produced higher amounts of ABA when NaCl was incorporated in the culture medium; also Rojas-Tapias et al. [26] found that *Azotobacter sp.* strain C9 have more phosphate solubilization activity with higher NaCl concentration and C5 synthesized indole acetic acid only under saline stress. The mechanism underlying the association among environmental stress, bacterial productions of plant hormones and salt tolerance of plant-microbe symbiotic system is however unclear and should be studied further.

CONCLUSION

In General, *Bacillus sp.* tolerant to adverse environments and *Pseudomonas* with diverse metabolism pathways are predominant genera in the endophyte community of *Populus euphratica* living in arid and salty area. This phenomenon suggests that the structure and diversity of endophytes are mainly determined by environmental conditions. In addition, the community structure also depends on the specialty of plant clone. The bacteria with higher salt-tolerance are more likely to perform a plant growth-promoting effect under salt stress, suggesting that there is complicated association among environment, bacterial metabolisms and plant growth. Identification of new PGPB to help plant resisting to abiotic stress from wood plants living in adverse environments may be more applicable because the associated bacteria are more adapted to strict environments and can establish longer and better interaction with host plants.

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REFERENCES

1. Grichko, V.P., Glick, B.R. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiol. Bioch.,* 2001; **39**(1): 11-17.
2. Cohen, A.C., Travaglia, C.N., Bottini, R., Piccoli, P.N. Participation of abscisic acid and gibberellins produced by endophytic

Azospirillum in the alleviation of drought effects in maize. *Botany.*, 2009; **87**(5): 455-462.

3. Mayak, S., Tirosh, T., Glick, B.R. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Bioch*., 2004; **42**(6): 565-572.
4. Van Aken B, Y.J., Schnoor JL. Biodegradation of nitro-substituted explosives 2,4,6-Trinitrotoluene, Hexahydro-1,3,5-Trinitro-1,3,5-Triazine, and Octahydro-1,3,5,7-Tetranitro-1,3,5-Tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (Populus deltoides x nigra DN34). Appl. Environ. Microbiol., 2004b; **l70**: 508–517.
5. Moore, F.P., Barac, T., Borremans, B., Oeyen, L., Vangronsveld, J., van der Lelie, D., Campbell, C.D., Moore, E.R. Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Syst. Appl. Microbiol.*, 2006; **29**(7): 539-56.
6. Ulrich, K., Stauber, T., Ewald, D. *Paenibacillus*—a predominant endophytic bacterium colonising tissue cultures of woody plants. *Plant Cell Tiss. Org.*, 2008; **93**(3): 347-351.
7. Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J., van der Lelie, D. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl. Environ. Microbiol.*, 2009; **75**(3): 748-57.
8. Van Aken, B., Peres, C.M., Doty, S.L., Yoon, J.M., Schnoor, J.L. *Methylobacterium populi* sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoides x nigra* DN34). *Int. J. Syst. Evol. Microbiol*., 2004a; **54**(4): 1191-6.
9. Silva, P., Façanha, A.R., Tavares, R.M., Gerós, H. Role of Tonoplast Proton Pumps and Na+/H+ Antiport System in Salt Tolerance of *Populus euphratica* Oliv. *J. Plant Growth Regul.*, 2009; **29**(1): 23-34.
10. Mergeay, M., Nies, D., Schlegel, H.G., Gerits, J., Charles, P., Van Gijsegem, F. *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. *J. bacteriol.*, 1985; **162**(1): 328-34.
11. Sudagidan, M., Yenidunya, A.F., Gunes, H. Identification of *staphylococci* by 16S internal transcribed spacer rRNA gene restriction fragment length polymorphism. *J. Med. Microbiol.*, 2005; **54**(9): 823-826.
12. Byers, H.K., Stackebrandt, E., Hayward, C., Blackall, L.L. Molecular investigation of a microbial mat associated with the Great Artesian Basin. *FEMS Microbiol. Ecol.*, 1998; **25**(4): 391-403.
13. Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.*, 2007; **73**(16): 5261-5267.
14. Egamberdieva, D. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol. Plant.*, 2009; **31**(4): 861-864.
15. Ulrich, K., Ulrich, A., Ewald, D. Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS Microbiol. Ecol.*, 2008; **63**(2): 169-80.
16. Garbeva, P., van Veen, J.A., van Elsas, J.D. Predominant *Bacillus* spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microb. Ecol.*, 2003; **45**(3): 302-316.
17. Benardini, J.N., Sawyer, J., Venkateswaran, K., Nicholson, W.L. Spore UV and acceleration resistance of endolithic *Bacillus pumilus* and *Bacillus subtilis* isolates obtained from Sonoran desert basalt: Implications for lithopanspermia. *Astrobiology*., 2003; **3**(4): 709-717.
18. Marasco R., Rolli E., Ettoumi B., Vigani G., Mapelli, F., Borin, S., Abou-Hadid, A.F., El-Behairy, U.A., Sorlini, C., Cherif, A., Zocchi, G., Daffonchio, D. A Drought Resistance-Promoting Microbiome Is Selected by Root System under Desert Farming. *PLOS ONE.*, 2012; **7**(10): e48479.
19. Francis, I., Holsters, M., Vereecke, D. The Gram-positive side of plant-microbe interactions. *Environ. Microbiol*., 2010; **12**(1): 1-12.
20. Crowley, D.E., Brennerova, M.V., Irwin, C., Brenner, V., Focht, D.D. Rhizosphere effects on biodegradation of 2,5-dichlorobenzoate by a bioluminescent strain of root-colonizing *Pseudomonas fluorescens*. *FEMS Microbiol. Ecol.*, 1996; **20**(2): 79-89.
21. Parales, J.V., Kumar, A., Parales, R.E., Gibson, D.T. Cloning and sequencing of the genes encoding 2-nitrotoluene dioxygenase from *Pseudomonas* sp. JS42. Gene., 1996; **181**(1-2): 57-61.
22. Simon, M.J., Osslund, T.D., Saunders, R., Ensley, B.D., Suggs, S., Harcourt, A., Wen-chen, S., Cruder, D.L., Gibson, D.T., Zylstra, G.J. Sequences of genes encoding naphthalene

dioxygenase in *Pseudomonas putida* strains G7 and NCIB 9816-4. Gene., 1993; **127**(1): 31-37.
23. Mohamed, H.I., Gomaa, E.Z. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (Raphanus sativus) under NaCl stress. *Photosynthetica.*, 2012; **50**(2): 263-272.
24. Sturz, A.V., Christie, B.R., Matheson, B.G. Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Can. J. Microbiol.*, 1998; **44**(2): 162-167.
25. Araujo, W.L., Marcon, J., Maccheroni, W., van Elsas, J.D., van Vuurde, J.W.L., Azevedo, J.L. Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Appl. Environ. Microbiol*., 2002; **68**(10): 4906-4914.
26. Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D., Bonilla, R. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Appl. Soil Ecol*., 2012; **61**(264-272.
27. Cheng, Z., Woody, O.Z., McConkey, B.J., Glick, B.R. Combined effects of the plant growth-promoting bacterium *Pseudomonas putida* UW4 and salinity stress on the *Brassica napus* proteome. *Appl. Soil Ecol*., 2012; 61(255-263.
28. Cohen, A.C., Bottini, R., Piccoli, P.N. *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. *Plant Growth Regul*., 2007; **54**(2): 97-103.