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ORIGINAL ARTICLE

Ecology of bacterial endophytes associated with wetland plants growing in textile effluent for pollutant-degradation and plant growth-promotion potentials

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Abstract
In this study, 41 culturable endophytic bacteria were isolated from the roots and shoots of three wetland plants, *Typha domingensis*, *Pistia stratiotes* and *Eichhornia crassipes*, and identified by 16S rRNA gene sequencing. Textile effluent-degrading and plant growth-promoting activities of these endophytes were determined. The analysis of endophytic bacterial communities indicated that plant species had a pronounced effect on endophytic bacterial association and maximum endophytes (56.5%) were associated with *T. domingensis*. These endophytic bacteria mainly belonged to different species of the genera *Bacillus* (39%), *Microbacterium* (12%) and *Halomonas* (12%). Eight of the 41 strains showing maximum efficiency of textile effluent degradation also exhibited plant growth-promoting activities such as production of indole-3-acetic acid and siderophore, presence of 1-amino-cyclopropane-1-carboxylic acid deaminase, and solubilization of inorganic phosphorous. This is the first study describing the diversity and plant-beneficial characteristics of the textile effluent-degrading endophytic bacteria associated with wetland plants. *T. domingensis* showed better growth in textile effluent and also hosted maximum number of endophytic bacteria in roots and shoots. The interactions between *T. domingensis* and its associated endophytic bacteria could be exploited to enhance the efficiency of constructed wetlands during the remediation of industrial effluent.

Keywords: *Wetland plants, ecology, endophytic bacteria, phytoremediation, textile effluent*

Introduction

The knowledge that plants can be used to remediate the polluted soil and water has opened up new avenues for research, and has given a basis for the present-day use of constructed wetlands (CWs) for treating domestic and industrial effluent. Use of CWs is an efficient, cost-effective, environment friendly and esthetically pleasant approach to remediate the domestic and industrial effluent (Chen et al. 2006; Tamura et al. 2007; Zhou et al. 2009; Vymazal 2011). In CWs, plant-associated microorganisms play a vital role in the detoxification of organic pollutants present in wastewater and *in planta* (Kabra et al. 2012; Khandare et al. 2013; Oliveira et al. 2014).

Plants and their associated microbes, especially endophytic bacteria, interact with each other for mutual benefits and these interactions can be exploited to enhance the remediation of polluted soil and water (Newman & Reynolds 2005; Sessitsch et al. 2013; Shehzadi et al. 2014). Endophytic bacteria colonize the internal tissues of the plant without causing pathogenicity to their host (Sessitsch et al. 2005; Compant et al. 2010; Muresu et al. 2011; Afzal et al. 2013). Plants provide residency and nutrients to endophytic bacteria. In return, endophytic bacteria decrease both the toxicity and evapotranspiration of the pollutants due to their pollutant degradation activities (Weyens et al. 2009; Khan et al. 2013). As plants can take up and accumulate organic pollutants in their roots, shoots, and leaves, endophytic bacteria seem to be the best candidates for their degradation *in planta*. Plant growth-promoting activities, such as nitrogen fixation, siderophore production, and phosphorous solubilization, of endophytic bacteria play an important role during the growth of plants in a polluted environment (Arshad et al. 2007; Glick 2010; Afzal et al. 2011). Furthermore, endophytes may protect plants against the inhibitory effects of high concentrations of

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pollutants and may improve plant stress tolerance by, e.g. reducing ethylene levels with 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity.

Although a number of wetland/aquatic plants show potential to remediate domestic and industrial effluents (Mishra & Nautiyal 2009; Rahman & Hasegawa 2011), high concentration of dyes and other toxic compounds in textile effluent inhibits plant growth and phytoremediation efficiency (Davies et al. 2009; Khandare et al. 2011; Shehzadi et al. 2014). Recently, many studies revealed that endophytic bacteria isolated from the plants grown in soil or water contaminated with organic and inorganic pollutants improved plant growth and pollutants degradation/extraction during phytoremediation (Newman & Reynolds 2005; Weyens et al. 2009; Yousaf et al. 2011). However, the population and diversity of endophytic bacteria associated with wetland plants grown in textile effluent and their potential to enhance remediation efficiency of CWs have not been emphasized. Therefore, the aim of the current study was the isolation and characterization of culturable endophytic bacteria associated with three wetland plants, *Typha domingensis*, *Pistia stratiotes*, and *Eichhornia crassipes*, grown in textile effluent. Moreover, their textile effluent-degrading and plant growth-promoting activities were determined.

Materials and methods

Collection and characterization of textile effluent

Wastewater samples were collected from the outlet of different textile industries located in Khurianwala, Faisalabad, Pakistan. Wastewater samples were analyzed for different physico-chemical parameters, such as pH, electrical conductivity (EC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), total dissolved solid (TDS), total settleable solids (TSeS), total organic carbon (TOC), Cl, SO_4, PO_4, NO_3, metals (Na, K, Ca, Mg), and heavy metals (As, Ni, Fe, Cr, Cd), according to standard methods as described earlier (APHA 2005).

Construction of wetland and plant growth

Three wetland plants, *Typha domingensis*, *Pistia stratiotes*, and *Eichhornia crassipes*, were collected from domestic and industrial wastewater storage ponds and streams located in Faisalabad, Pakistan. These plants were grown in plastic containers (volume 5 l), two-fifth of the volume of the containers contained soil, sand, and stone (1:1:1), whereas the remaining volume of the containers was filled with textile effluent. The containers were placed in a greenhouse at 25 ± 2°C with 16 h light/day. The different treatments were 0%, 25%, 50%, 75%, and 100% textile effluent diluted with tap water. The plants were allowed to grow for two months, harvested, and root and shoot samples were collected. These samples were immediately processed for the isolation of endophytic bacteria.

Isolation and identification of endophytic bacteria

Endophytic bacteria were isolated from the surface-sterilized roots and shoots of the plants as described previously (Afzal et al. 2012; Chen et al. 2012). Roots and cut shoots were washed briefly for 2 min in sterile distilled water before surface sterilization with 70% ethanol for 5 min (shoots) or 10 min (roots), followed by a 1 min wash in 1% NaOCl amended with 0.01% Tween 20 solution, and then a final rinse in sterile distilled water (3 times, 1 min each time). The surface sterility was checked on Luria–Bertani (LB) medium. The LB agar plates were incubated at 30°C for 48 h; no growth was observed. Surface-sterilized roots and shoots (1 g) were homogenized individually with 2 ml 0.9% (w/v) NaCl solution. Aliquots (100 µl) of this suspension were spread onto LB containing 10% filter-sterilized textile effluent. Plates were incubated at 30°C for 24–72 h. A total of 172 colonies were isolated by sub-culturing, and cell morphology was analyzed using a light microscope. On the basis of colony shape and cell morphology, 78 different morphotypes were identified. A restriction fragment length polymorphism (RFLP) analysis of the 16S–23S rRNA intergenic spacer (IGS) region was performed to distinguish these 78 bacterial morphotypes as described earlier (Rasche et al. 2006). On the basis of RFLP analysis, 41 different patterns (IGS-type) were obtained as explained previously (Mastretta et al. 2009).

A representative isolate of each IGS type was identified by partial 16S rRNA gene sequencing. The PCR amplification products were sequenced by the Macrogen (Seoul, Korea). The 16S rRNA sequences were compared with sequences in the GenBank database using NCBI Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were submitted to GenBank database under accession numbers KF227796, KF301604-KF301621, and KF311078-311099. Neighbor joining phylogenetic tree was constructed with the software MEGA5.0 (Tamura et al. 2007). In addition, endophytic bacterial strains were deposited in NIBGE Biological Resource Centre (NBRC).

Determination of textile effluent degradation potential of bacteria

The inoculum of each bacterial strain was prepared by growing these bacteria separately in LB broth at 30 ± 2°C in a shaker (100 rpm) for 24 h. Cells were

harvested by centrifugation at 10,000 rpm, re-suspended in 0.9% (w/v) saline, and OD was adjusted to 0.7 at 600 nm. Twenty milliliter suspension of each strain was inoculated in filter-sterilized textile wastewater (500 ml). Color, COD, and BOD reduction were observed to evaluate the potential of endophytic bacteria for textile effluent degradation. Out of the 41 endophytic bacteria, 8 highly efficient textile-effluent degrading strains were further characterized for plant growth-promoting activities.

Determination of plant growth-promoting properties of bacteria

Different plant growth-promoting activities, such as production of indole-3-acetic acid (IAA), ACC-deaminase activity, solubilization of inorganic phosphate, and production of siderophore, were determined in selected bacteria using the protocols as described earlier (Naveed et al. 2014).

Results

Physico-chemical characteristics of textile effluent

Physico-chemical characteristics of the textile effluent are shown in Table I. Certain parameters such as temperature, pH, COD, BOD, TDS, TSS, and nickel exceeded the permissible limit set by the Environmental Protection Department (EPD), Government of Pakistan, as the National Environmental Quality Standards (National Environmental Quality Standards 1997).

Plant growth in textile effluent

Three wetland plant species, *T. domingensis, P. stratiotis*, and *E. crassipes*, were tested for their survival and growth in textile effluent. Two plant species, *P. stratiotis* and *E. crassipes,* showed poor survival and growth in the textile effluent, particularly when grown in the textile effluent alone (without dilution). The *T. domingensis* plant was able to survive and grow in the textile effluent at all the treatments including undiluted textile effluent.

Diversity of endophytic bacteria

A total of 172 colonies were obtained from the roots and shoots of three plants, of which 78 different morphotypes were selected after analyzing their cell morphology and motility under a light microscope. RFLP analysis further classified these isolates into 41 different IGS-types. Among these 41 different IGS-types, 24 isolates were from *T. domingensis*, 8 from

Table I. Characterization of textile effluent collected from different textile industries.

Parameters	Textile industries				NEQS limit
	T-1	T-2	T-3	T-4	
Temperature (°C)	**42 ± 2.1**	40 ± 2.4	38 ± 2.6	39 ± 3.1	40°C
pH	**12.93 ± 0.77**	8.02 ± 0.64	7.42 ± 0.51	7.94 ± 0.47	6–10
EC (mS/cm)	8.07 ± 0.40	4.84 ± 0.38	4.67 ± 0.46	3.20 ± 0.27	NG
Color (m^{-1}) (visual)	53 ± 5.3 (Black)	47 ± 2.35 (Pink)	42 ± 2.52 (Blue)	61 ± 5.49 (Red)	NG
COD (mg/l)	**813 ± 56.91**	**532 ± 42.56**	**320 ± 28.8**	**925 ± 92.6**	150
BOD (mg/l)	**422 ± 42.2**	**284 ± 25.56**	**172 ± 13.84**	**450 ± 31.5**	80
TDS (mg/l)	**4834 ± 241.7**	3252 ± 195.1	2912 ± 203.8	2976 ± 201.3	3500
TS (mg/l)	5125 ± 307.5	3419 ± 170.9	3112 ± 217.8	3274 ± 229.1	NG
TSS (mg/l)	**391 ± 23.28**	**217 ± 16.7**	**200 ± 18**	**298 ± 35.82**	150
TSeS (mg/l)	24 ± 2.4	15 ± 1.2	13 ± 1.17	50 ± 3.5	NG
TOC (mg/l)	301 ± 15.05	194 ± 11.64	124 ± 11.16	324 ± 16.2	NG
Hardness (mg/l)	410 ± 24	380 ± 36.4	500 ± 35	520 ± 31.2	NG
Na (mg/l)	1656 ± 115.9	2944 ± 176.6	3243 ± 227.0	2852 ± 256.6	NG
K (mg/l)	858 ± 60.06	975 ± 48.75	624 ± 31.2	1092 ±	NG
Ca (mg/l)	80.16 ± 4.8	96.19 ± 6.73	88.17 ± 5.29	104.20 ±	NG
Mg (mg/l)	48.6 ± 3.88	68.04 ± 0.7	68.04 ± 6.12	63.18 ±	NG
Cl (mg/l)	600 ± 42	90 ± 9	120 ± 10.8	800 ±	1000
SO_4 (mg/l)	412.54 ± 20.6	362.92 ± 28.9	215.64 ± 10.8	**672.8 ± 33.6**	600
As (mg/l)	Nil	Nil	Nil	Nil	1
Ni (mg/l)	**2.0 ± 0.18**	**2.4 ± 0.24**	**1.1 ± 0.08**	**2.7 ± 0.27**	1
Fe (mg/l)	**3.3 ± 0.26**	1.6 ± 0.11	**2.7 ± 0.16**	**2.9 ± 0.29**	2
Cr (mg/l)	0.21 ± 0.014	0.08 ± 0.004	0.25 ± 0.015	0.11 ± 0.008	1
Cd (mg/l)	**0.27 ± 0.016**	**0.11 ± 0.007**	**0.14 ± 0.007**	0.10 ± 0.009	0.1
PO_4 (mg/l)	10.08 ± 0.50	11.15 ± 0.55	9.32 ± 0.55	19.02 ± 1.33	NG
No_3^- (mg/l)	24 ± 1.68	21 ± 1.26	20 ± 1.20	24 ± 1.68	NG

Notes: Each value is a mean of three replicates, bold values are higher than wastewater discharge standards. NG, not given in NEQS list; NEQS, National Environmental Quality Standards for wastewater discharge, set by Government of Pakistan. T1, T2, T3, and T4 were four different textile industries.

E. crassipes, and 9 from *P. stratiotis* (Table II). This shows that maximum diversity was associated with *T. domingensis* plant. Representative isolate of each IGS-type was identified by sequence analysis of 16S rRNA gene. Gene sequencing analysis showed that most of these endophytic bacteria belonged to species of the genera *Bacillus*, *Microbacterium*, and *Halomonas*. *Bacillus* spp. were found to be the most prevalent shoot and root endophytes (39%) in these plants. The host plant, bacterial morphological characters, and accession numbers are mentioned in Table II. The strains showed 98–100% sequence similarity with the submitted species to the NCBI database except *Chryseobacterium* sp. strain obtained from *Pistia roots* which showed 94% similarity to 16S rRNA gene sequences in the database. The phylogenetic analysis of these and other related bacteria from the database revealed two distinct monophyletic clusters forming five groups (Figure 1). The strains which were identified as *Bacillus* spp. grouped with the representative type strains of the same genus within Group I. The bacterial strains identified as *Rhizobium*, *Planococcus*, *Panniobacter*, and *Paracoccus* were grouped into representative strains of the respective genus in Group II. This group contained different genera and was found to be more diverse. Group III

Table II. Endophytic bacterial strains isolated from the roots and shoots of selected plants grown in textile wastewater.

IGS type	Strain name	Bacterial species	Host plant	Cell morphology	NCBI accession number
EISI01	MS1	*Escherichia hermannii*	*Eichornia* [S]	Rod-shaped, rods occur singly or in pairs, motile	KF301604
PISI02	MS2	*Planococcus rifietoensis*	*Pistia* [S]	Coccus-shaped cells, motile	KF301605
EIRI03	MS3	*Kocuria rosea*	*Eichornia* [R]	Mostly two cells joined together, non-motile	KF301606
TYSI04	MS4	*Microbacterium arborescens*	*Typha* [S]	Mostly two round cells joined together, non-motile	KF227796
PISI05	MS5	*Microbacterium* sp.	*Pistia* [S]	Very short to round rods, motile	KF301607
TYRI06	MS6	*Rhizobium* sp.	*Typha* [R]	Medium to short rods, motile	KF301608
TYSI07	MS7	*Halomonas stevensii*	*Typha* [S]	Medium to long plump rods, some cells quite thick, non-motile	KF301609
TYSI08	MS8	*Microbacterium* sp.	*Typha* [S]	Very short motile rods	KF301610
EIRI09	MS9	*Bacillus marisflavi*	*Eichornia* [R]	Medium to large plump rods, some cells thick with irregular shape	KF301611
TYRI10	MS10	*Cloacibacterium normanense*	*Typha* [R]	Medium to large irregular shape	KF301612
TYSI11	MS12	*Microbacterium schleiferi*	*Typha* [S]	Medium-sized plump rods, some are very long, some showed budging	KF301613
EISI13	MS13	*Jonesia* sp.	*Eichornia* [S]	Very short thin motile rods	KF301614
PIRI14	MS14	*Chryseobacterium* sp.	*Pistia* [R]	Round plump short rods slightly motile	KF301615
TYRI15	MS15	*Pantoea* sp.	*Typha* [R]	Medium-sized plump rods	KF301616
TYSI16	MS16	*Bacillus safensis*	*Typha* [S]	Thin medium to long smoothed surface cells, motile	KF301617
TYSI17	MS17	*Bacillus* sp.	*Typha* [S]	Thick rods, three to four cells joined together, non motile	KF301618
TYRI18	MS18	*Bacillus* sp.	*Typha* [R]	Thin smooth surfaced motile rods	KF301619
TYRI19	MS20	*Halomonas hamiltonii*	*Typha* [R]	Medium-sized plump smooth surfaced rods	KF301620
PISI20	MS21	*Rhodobacter* sp.	*Pistia* [S]	Medium-sized plump rods	KF301621
TYRI21	MS22	*Sphingobium* sp.	*Typha* [R]	Very short highly motile rods	KF311078
TYRI22	MS23	*Pannonibacter phragmitetus*	*Typha* [R]	Medium to long slightly motile rods	KF311079
TYSI23	MS24	*Bacillus safensis*	*Typha* [S]	Smooth surfaced short rods with shiny contents	KF311080
TYRI24	MS25	*Microbacterium oleivorans*	*Typha* [R]	Round non-motile cells four to five cells clumped together	KF311081
PISI25	MS26	*Bacillus endophyticus*	*Pistia* [S]	Thick rods three to four cells joined together, non-motile	KF311082
TYRI26	MS27	*Bacillus safensis*	*Typha* [R]	Medium- to short-sized slightly motile rods	KF311083
EISI27	MS28	*Halomonas venusta*	*Eichornia* [S]	Smooth surface short plump rods slightly motile	KF311084
PIRI28	MS29	*Paracoccus* sp.	*Pistia* [R]	Medium-sized rods, joined together, showed bulging	KF311085
TYSI29	MS30	*Janibacter melonis*	*Typha* [S]	Round cells, three or four cells joined together	KF311086
PIRI30	MS31	*Bacillus pumilus*	*Pistia* [R]	Very long thin medium-sized rods, motile	KF311087
TYSI31	MS32	*Bacillus* sp.	*Typha* [S]	Medium-sized smooth surface rods	KF311088
TYRI32	MS33	*Bacillus pumilus*	*Typha* [R]	Thin rods with black contents	KF311089
TYRI33	MS34	*Psychrobacter alimentarius*	*Typha* [R]	Thick round or slightly short cells, motile	KF311090
TYSI34	MS35	*Halomonas stevensii*	*Typha* [S]	Medium-sized thick motile rods	KF311091
TYSI35	MS37	*Pseudomonas fluorescens*	*Typha* [S]	Medium-sized thin highly motile rods	KF311092
TYRI36	MS38	*Bacillus subtilis*	*Typha* [R]	Thin rods with blackish shiny contents	KF311093
TYSI37	MS39	*Bacillus aerophilus*	*Typha* [S]	Thick rods with blackish shiny content inside and outside	KF311094
TYSI38	MS40	*Bacillus safensis*	*Typha* [S]	Thin motile rods with black contents	KF311095
PIRI39	MS41	*Bacillus licheniformis*	*Pistia* [R]	Short thin motile rods	KF311096
PISI40	MS42	*Bacillus pumilus*	*Pistia* [S]	Thin rods with black contents	KF311097
EIRI41	MS43	*Bacillus marisflavi*	*Eichornia* [R]	Thin rods slightly motile	KF311098
EISI42	MS44	*Alishewanella* sp.	*Eichornia* [S]	Round to very short motile rods	KF311099

Note: [R]Root, [S]Shoot.

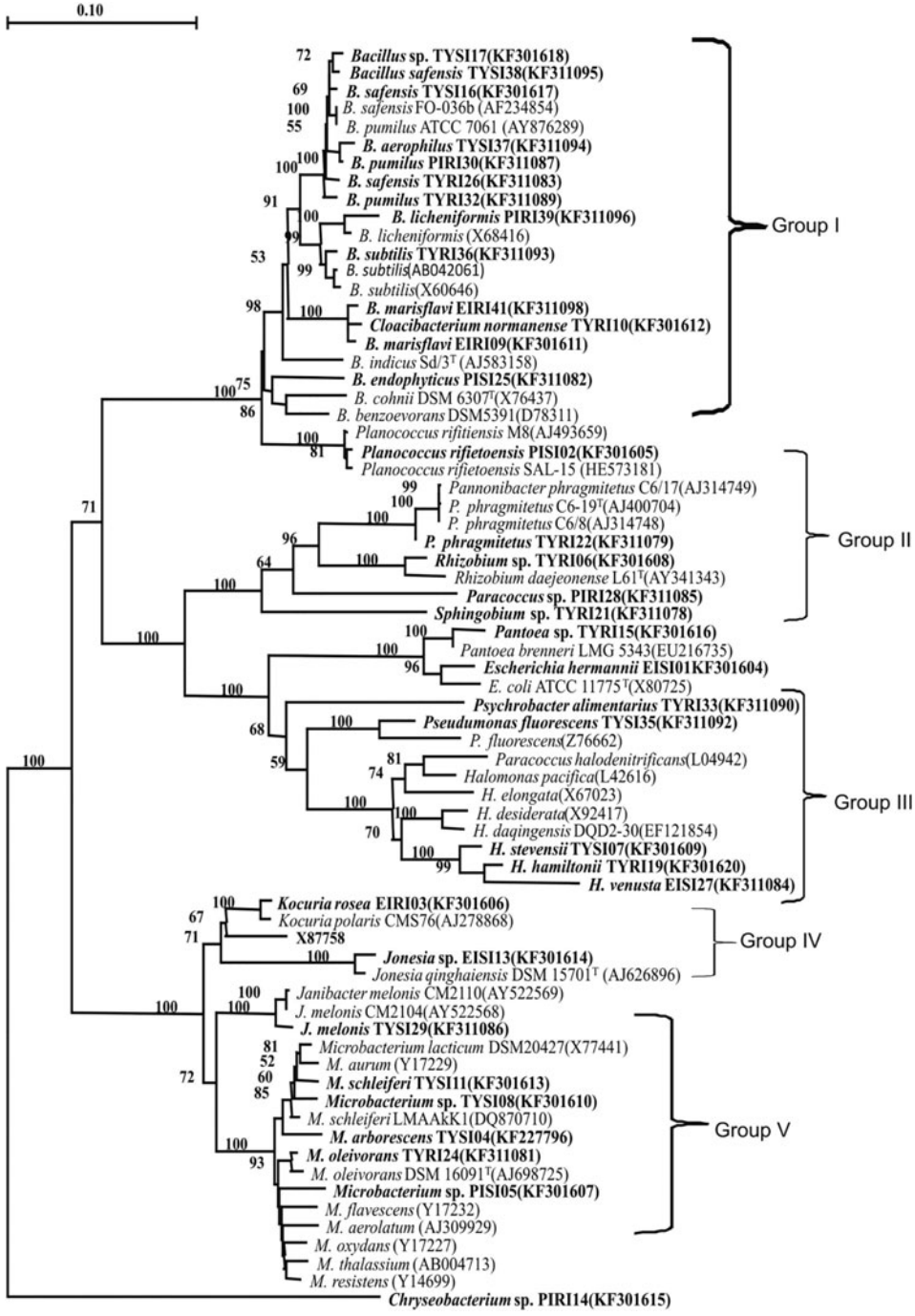

Figure 1. Neighbor joining tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of bacterial endophytes isolated from different wetland plants growing in textile effluent among recognized members of the relative species and genera. Bootstrap values >50%, based on 1000 replications, are shown at branch points. Tree was routed with *Chryseobcterium* sp. PIRI14 (accession number KF301615). Bar, 0.10 substitutions per nucleotide position.

mainly included *Pseudomonas* and *Halomonas* spp. Group IV included *Kocuria* and *Jonesia* spp. while group V included *Microbacterium* spp.

Textile effluent degrading efficiency of endophytic bacteria

All the endophytic bacterial strains showed textile effluent degrading activity (Table III). The color, COD, and BOD of the effluent were significantly reduced by bacterial inoculation after 48 h. Maximum color removal, COD, and BOD reduction were observed by *Pantoea* sp. strain TYRI15, which were 57%, 72%, and 78% more than the control treatment (without bacterial inoculation), respectively. Among 41 strains, 8 bacterial strains performed better than others in terms of their efficiency to remove color,

Table III. Textile effluent degradation by endophytic bacterial strains.

Treatment	pH	Color (m^{-1})	COD (mg/l)	BOD (mg/l)
Control (without inoculum)	7.48 ± 0.85	51 ± 5.10	658 ± 52.6	319 ± 25.5
Escherichia hermannii EISI01	7.18 ± 0.45	33 ± 2.15	355 ± 40.9	147 ± 17.2
Planococcus rifietoensis PISI02	7.59 ± 0.60	36 ± 2.76	216 ± 25.2	105 ± 11.5
Kocuria rosea EIRI03	7.41 ± 0.43	31 ± 2.46	260 ± 25.2	110 ± 7.2
Microbacterium arborescens TYSI04	7.32 ± 0.51	22 ± 1.60	214 ± 18.8	76 ± 10.5
Microbacterium sp. PISI05	7.35 ± 0.60	26 ± 2.52	243 ± 27.4	110 ± 8.8
Rhizobium sp. TYRI06	7.45 ± 0.43	18 ± 1.96	210 ± 21.7	82 ± 10.9
Halomonas stevensii TYSI07	7.38 ± 0.50	28 ± 1.90	312 ± 24.7	122 ± 15.5
Microbacterium sp. TYSI08	7.35 ± 0.41	20 ± 1.80	216 ± 18.9	86 ± 10.2
Bacillus marisflavi strain EIRI09	7.29 ± 0.60	40 ± 2.50	265 ± 18.2	110 ± 10.8
Cloacibacterium normanense TYRI10	7.34 ± 0.69	30 ± 2.80	335 ± 21.7	127 ± 15.8
Microbacterium schleiferi TYSI11	7.37 ± 0.78	29 ± 2.34	289 ± 35.0	120 ± 12.0
Jonesia sp. EISI13	7.45 ± 0.87	31 ± 2.05	381 ± 40.0	129 ± 17.3
Chryseobacterium sp. PIRI14	7.32 ± 0.43	27 ± 2.22	371 ± 48.8	124 ± 17.7
Pantoea sp. TYRI15	7.22 ± 0.57	22 ± 2.24	180 ± 19.6	70 ± 13.3
Bacillus safensis TYSI16	7.36 ± 0.70	33 ± 2.15	253 ± 21.9	96 ± 9.6
Bacillus sp. TYSI17	7.30 ± 0.40	20 ± 2.10	224 ± 19.4	79 ± 17.9
Bacillus firmus TYRI18	7.42 ± 0.52	33 ± 2.15	300 ± 20.0	132 ± 18.5
Halomonas hamiltonii TYRI19	7.23 ± 0.52	25 ± 1.75	335 ± 26.7	130 ± 20.3
Rhodobacter sp. PISI20	7.44 ± 0.60	31 ± 2.46	367 ± 23.3	149 ± 15.5
Sphingobium sp. TYRI21	7.43 ± 0.69	29 ± 2.03	382 ± 28.9	168 ± 16.0
Pannonibacter phragmitetus TYRI22	7.33 ± 0.87	41 ± 3.57	371 ± 39.9	147 ± 14.7
Bacillus safensis TYSI23	7.35 ± 0.70	30 ± 2.43	397 ± 24.8	158 ± 25.9
Microbacterium oleivorans TYRI24	7.30 ± 0.61	32 ± 4.20	269 ± 22.1	101 ± 11.1
Bacillus endophyticus PISI25	7.43 ± 0.52	19 ± 2.32	202 ± 21.1	81 ± 9.09
Bacillus safensi TYRI26	7.35 ± 0.61	23 ± 2.97	272 ± 33.4	106 ± 13.0
Halomonas venusta EISI27	7.29 ± 0.60	27 ± 1.85	331 ± 31.8	109 ± 17.9
Paracoccus sp. PIRI28	7.41 ± 0.51	23 ± 2.64	279 ± 22.7	110 ± 16.8
Janibacter melonis TYSI29	7.33 ± 0.43	30 ± 3.60	322 ± 36.5	141 ± 18.9
Bacillus pumilus PIRI30	7.46 ± 0.77	18 ± 2.80	187 ± 17.2	63 ± 11.4
Bacillus sp. TYSI31	7.22 ± 0.68	32 ± 2.94	344 ± 26.6	134 ± 18.7
Bacillus pumilus TYRI32	7.50 ± 0.60	29 ± 2.32	318 ± 36.3	109 ± 25.1
Psychrobacter alimentarius TYRI33	7.40 ± 0.45	30 ± 3.60	302 ± 48.2	102 ± 29.8
Halomonas stevensii TYSI34	7.45 ± 0.51	31 ± 3.36	354 ± 33.2	175 ± 19.2
Pseudomonas fluorescens TYSI35	7.44 ± 0.50	21 ± 2.17	256 ± 21.3	87 ± 11.2
Bacillus subtilis TYRI36	7.02 ± 0.45	25 ± 1.75	378 ± 23.9	139 ± 21.5
Bacillus aerophilus TYSI37	7.26 ± 0.52	22 ± 2.24	356 ± 31.9	127 ± 15.8
Bacillus safensis TYSI38	7.41 ± 0.62	28 ± 3.04	318 ± 29.2	123 ± 13.3
Bacillus licheniformis PIRI39	7.38 ± 0.70	33 ± 3.01	392 ± 41.4	104 ± 15.2
Bacillus pumilus PISI40	7.29 ± 0.44	31 ± 3.36	266 ± 21.9	123 ± 17.3
Bacillus marisflavi EIRI41	7.42 ± 0.46	33 ± 3.44	282 ± 29	102 ± 21
Alishewanella sp. EISI42	7.38 ± 0.52	29 ± 2.73	312 ± 41.2	136 ± 16.5

Notes: Each value is the mean of three replicates, ± indicates standard deviation. Incubation period was 48 h.

COD, and BOD. The most efficient textile effluent-degrading endophytic bacteria included three *Bacillus* spp. strains (*Bacillus* sp. TYSI17, *B. endophyticus* PISI25, and *B. pumilus* PIRI30), two *Microbacterium* spp. strains (*M. arborescens* TYSI04 and *Microbacterium* sp. TYSI08), one strain of each of *Rhizobium* spp. (*Rhizobium* sp. TYRI06), *Pantoea* spp. (*Pantoea* sp. TYRI15), and *Pseudomonas* spp. (*Pseudomonas fluorescens* TYSI35) (Table III).

Plant growth-promoting properties of selected textile effluent-degrading bacteria

The plant growth-promoting activities of eight efficient textile effluent-degrading bacterial strains are given in Table IV. Among these bacterial strains, only two strains, *Rhizobium* sp. TYRI06 (root endophyte of *T. domingensis* plant) and *P. fluorescens* TYSI35 (shoot endophyte of *E. crassipes* plant) produced IAA. ACC-deaminase activity was exhibited by all except *Rhizobium* sp. TYRI06, *Pantoea* sp. TYRI15, and *P. fluorescens* TYSI35. The ability to solubilize phosphate was exhibited by *Pantoea* sp. TYRI15 and *P. fluorescens* TYSI35, while siderophore production was exhibited by *Pantoea* sp. TYRI15 only, a strain which was isolated from the roots of *T. domengensis*.

Discussion

Knowledge of the diversity, abundance, and ecological function of pollutant-degrading endophytic

Table IV. Plant growth-promoting properties of textile effluent degrading endophytic bacteria.

Strain	IAA production	Phosphorous solubilization	ACC deaminase	Siderophore production
Microbacterium arborescens TYSI04	−	−	+	−
Rhizobium sp. strain TYRI06	+	−	−	−
Microbacterium sp. strain TYSI08	−	−	+	−
Pantoea sp. strain TYRI15	−	+	−	+
Bacillus sp. strain TYSI17	−	−	+	−
Bacillus endophyticus PISI25	−	−	+	−
Bacillus pumilus TYSI31	−	−	+	−
Pseudomonas fluorescens TYSI37	+	+	−	−

bacteria associated with wetland plants is thought to be necessary for controlling the mechanisms that evaluate the efficacy of a wetland system for effluent degradation (Kabra et al. 2012; Khandare et al. 2013). In this study, three wetland plants were assessed for their survival and growth in the textile effluent. *T. domengensis*, *P. stratiotis*, and *E. crassipes* plants were able to survive and grow in the textile effluent. One of the possible reasons of survival and growth of these plants in textile effluent might be that these plants were collected from domestic/industrial wastewater ponds/streams and have adapted to wastewater. Several earlier studies also indicated that these plant species (*Typha*, *Pistia*, and *Eichhornia*) showed survival and growth when grown in wastewater (Sharma et al. 2007; Dipu et al. 2010; Hegazy et al. 2011). However, these plant species respond differently to different effluent concentrations. Among the tested plants, *Typha* was found more efficient and showed good growth even when it was grown in the undiluted textile effluent. However, the other two plants exhibited toxicity symptoms when grown in the undiluted textile effluent. Many endophytic bacteria have been isolated from *Typha* which may have enhanced this plant tolerance and growth in the textile effluent. Many of these endophytic bacteria possessed textile effluent degradation and plant growth-promoting activities, and ACC-deaminase activity. Recently, many studies revealed that endophytic bacteria can improve plant health and growth in a contaminated environment (Chen et al. 2009; Yousaf et al. 2011; Afzal et al. 2014b). ACC-deaminase activity of endophytic bacteria reduces the stress symptoms in a developing plant and improves plant growth, especially root growth (Glick 2010; Afzal et al. 2014a). ACC is the precursor of ethylene, a phytohormone normally associated with stress symptoms (Glick 2003; Toklikishvili et al. 2010).

The bacterial inoculation enhanced color removal from the textile effluent and it was significantly more in the inoculated effluent than in the uninoculated effluent. The decreasing color intensity of the effluent has been associated with absorption/degradation of dyes by microorganisms (Asad et al. 2007). Similarly, more COD and BOD reduction was observed in the inoculated effluent than in the non-inoculated effluent. Microorganisms can proliferate in textile effluent and play a vital role in the degradation of organic pollutants present in textile wastewater (Senan & Abraham 2004; Olukanni et al. 2006). In this study, endophytic bacteria were inoculated to filter-sterilized textile effluent, hence, color removal and reduction in COD and BOD were attributed only to endophytic bacteria. Many studies revealed that endophytes are able to degrade organic pollutants present in aquatic and terrestrial environments (Barac et al. 2004; Newman & Reynolds 2005).

Plants maintain a complex ecosystem where bacterial communities interact continuously, competing for nutrients and water in the rhizosphere and endosphere of the host plant. Isolation and characterization of pollutant-degrading endophytic bacteria are thought to be important for improving the efficiency of phytoremediation of polluted sites (Weyens et al. 2009; Sessitsch et al. 2013). In this study, out of 172 bacterial isolates obtained from the roots and shoots of three wetland plants, 41 different endophytic bacterial strains were selected for further analysis. These strains possessed textile effluent-degrading potential, and among these, eight were found highly efficient for textile effluent degradation.

The shoots/roots endophytic bacterial strains isolated from the wetland plants during the present study belonged mainly to *Bacillus*, *Halomonas*, *Microbacterium*, *Pantoea*, *Pseudomonas*, and *Rhizobium* genus. Similarly, many other studies reported that plants host different strains of *Pseudomonas*, *Microbacterium*, *Pantoea*, and *Bacillus* species in their roots and shoots (Bacon & Hinton 2002; Bai et al. 2002; Yousaf et al. 2010). Opportunistic human pathogen, *Halomonas* strains were also isolated from the roots and shoots of these tested wetland plants. In an earlier study, a hydrocarbon-degrading and ACC-deaminase positive *Enterobacter ludwigii* strain (a human pathogen) was isolated from the ryegrass endosphere (Yousaf et al. 2011).

Although endophytic bacteria colonize the roots and shoots of plants to different extents, they are

often reported to enhance plant growth and development (Khan et al. 2013; Sessitsch et al. 2013). Recently, bacteria have been isolated from the roots and shoots of different plant species (Chen et al. 2010; Yousaf et al. 2010; Fatima et al. 2015), many of these showed the potential to enhance plant tolerance to organic pollutants/heavy metal presence in soil and water and may improve plant health and growth through several mechanisms involving mineralization of organic pollutants, production of plant growth-promoting and stress-removing hormones, and improvement in the uptake of water and mineral nutrients (Ryan et al. 2008).

One of the main bacterial activities which reduces plant stress (due to pollutants) and improves plant growth during phytoremediation is ACC deaminase (Glick & Stearns 2011). Among the eight tested bacterial strains, five showed ACC-deaminase activity, and most of these were isolated from the shoots and roots of *Typha*. This shows that ACC-deaminase activity of the endophytic bacteria might be one of the reasons for better growth of *Typha* in textile effluent. Endophytic bacteria can alleviate the stress-mediated impact on plants by enzymatic hydrolysis of ACC (Glick et al. 2007). In this study, the isolated bacterial strains also exhibited IAA production, phosphorous solubilization, and siderophore production activities. Similarly, endophytic bacteria were reported to increase plant growth by virtue of their different plant growth-promoting activities (Chung et al. 2005; Chen et al. 2010).

The diversity of endophytic bacteria isolated from the roots and shoots of selected plants gave an indication of the variation among the endophytes. The closest relatives matched in the Gene Bank database are shown in Table III. A phylogenetic analysis further revealed that the bacterial communities of *Bacillus, Microbacterium* and *Halomonas* showed 99–100% homology and are closest relatives of other strains of the same species. Among the 41 isolated endophytic bacterial strains, 3 species of endophytic bacteria, i.e., *Bacillus*, (39% of isolated endophytic bacterial population), *Microbacterium* (12%), and *Halomonas* (10%) were selected on the basis of abundance and studied by constructing their phylogenetic trees. Phylogenetic trees demonstrated that our isolated strains cluster with closely related strains present in the database.

In conclusion, 41 textile effluent-degrading endophytic bacteria were isolated from three wetland plants. The dominant bacterial genera were *Bacillus*, *Microbacterium*, and *Halomonas*. In addition to textile effluent-degrading activity, these endophytic bacteria showed plant growth-promoting activities such as IAA production, phosphorous solubilization, ACC deaminase and siderophore production. These endophytic bacteria possessed textile effluent-degrading and plant growth-promoting activities and can be applied to improve plant biomass production and remediation of industrial effluent.

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