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Selection and application of endophytic bacterium *Achromobacter xylosoxidans* strain F3B for improving phytoremediation of phenolic pollutants

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ABSTRACT

While phytoremediation has been considered as an in situ bioprocess to remediate environmental contaminants, the application of functional endophytic bacteria within plants remains a potential strategy that could enhance the plants' efficiency in phytoremediation. In this study, 219 endophytes were isolated from plants that are predominantly located in a constructed wetland, including reed (*Phragmites australis*) and water spinach (*Ipomoea aquatica*). Twenty-five strains of the isolated endophytes utilize aromatic compounds as sole carbon source; *Achromobacter xylosoxidans* strain F3B was chosen for the *in planta* studies using the model plant *Arabidopsis thaliana*. Phylogenetic analysis indicated that those endophytic isolates of *A. xylosoxidans* formed a cluster within its species, and a specific real-time PCR detection method was developed for confirming the stability of the isolates in plants. In the presence of either catechol or phenol, inoculation of *A. thaliana* with F3B could extend into the root lengths and fresh weights to promote pollutants removal rates. These results demonstrate the potential of the endophytic F3B strain for helping plants to tolerate stress from aromatic compounds and to improve phytoremediation of phenolic pollutants.

1. Introduction

Phytoremediation plants and their associated microorganisms have been long recognized as a cost-effective method to remove or neutralize hazardous environmental contaminants [1,2]. The process often requires a longer time to achieve the remedial goals than other treatment technologies, and it is often limited by the presence of higher levels of contaminants. Furthermore, phytoremediation via a phytoextraction process may accumulate contaminants in plant tissues, which could cause ecological and airborne exposure issues. To enhance the efficiency of phytoremediation, functional rhizobacteria have been introduced in several successful cases of treating a wide variety of organic chemical contaminants [3,4]. In addition, the concept of using endophytic bacteria to promote the removal of organic contaminants and the accumulation of heavy metals has been proposed recently [5,6]. Endophytic bacteria are bacteria that reside within the tissues of their plant host without causing any disease [7]. In some cases, these bacteria can also accelerate seedling emergence, promote plant growth under adverse conditions and enhance plant growth [8]. Inoculation with functional endophytic bacteria could potentially reduce phytotoxic effects, increase the rates of pollutant uptake and removal [9], and reduce the release of toxic volatiles into the atmosphere [6].

Constructed wetlands are engineered systems that are designed to remediate wastewater by processes involving wetland vegetation, soil and their associated microbial assemblage [10]. While the wetland plants could prevent erosion and retard the entry of pollutants, the vegetative component of a constructed wetland becomes one of major factors in the waste treatment process that could function in creating additional environments for microbial activities. Plant tissues, such as roots, stems and leaves, provide numerous attachment sites for microbes that constitute the reactive surfaces for assimilation and transformation [4]. From a microbiological point of view, it is interesting to explore the additional applications for natural endophytic bacterial strains isolated from the vegetative component constructed wetlands for enhancing the efficiency of remediation of aromatic pollutants (such as catechol, phenol and toluene) in wastewater and soil.

In this study, endophytic bacterial strains from the plants in a constructed wetland, such as reeds and water spinach, were isolated and characterized. These strains, which possess the ability to degrade catechol, phenol and toluene [11,12] were inoculated into the model plant *Arabidopsis thaliana* to investigate the bacteria's ability to help plants reduce aromatic compound stress. One of the

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isolates that belongs to *Achromobacter xylosoxidans* showed a great potential for phytoremediation by combining with plants such as vetivers.

2. Materials and methods

2.1. Isolation and characterization of endophytic bacteria strains from different plant species

Root samples were collected from reeds (*Phragmites australis*) and water spinach plants (*Ipomoea aquatica*) in the Daniaopi manmade constructed wetland (for the treatment of organic compound- and heavy metal-contaminated wastewater) in Taipei (24°58′57″N, 121°25′34″E). The root samples were washed free of soil with water and soaked in sterile phosphate-buffered saline (pH 7.0) for 10 min to equilibrate osmotic pressure. Afterwards, the roots were shaken in 1.2% NaOCl for 30 min to sterilize the surface and rinsed 3 times in sterile deionized H_2O before maceration [13]. A 100-μL sample of water from the third rinsing cycle was taken and placed onto an LB agar plate as a sterility check. The macerated root slurry was put into sterilized mortars to be crushed, spread over 4 different media (869, 1/10 869, LB, and 284 agar medium) [6] and incubated for 3 days at 30 °C. All morphologically different bacterial colonies were selected and subcultured 3 times to ensure purity and stability.

2.2. Phenotypic profiling

Bacterial colonies were collected from the LB, 869, 1/10 869, and 284 media [6]. The ability of the bacterial endophytes to grow in the presence of aromatic compounds was assessed by plating them on a minimal agar medium (0.25 g NH_4Cl, 0.266 g $MgSO_4{\cdot}7H_2O$, 3 g KH_2PO_4, and 11.32 g $Na_2HPO_4{\cdot}7H_2O$/L) and incubating them at 28 °C for 7 days with either 0.4 mM of the test compounds (catechol and phenol) or 1000 mg/L of BTEX compounds (benzene, toluene, ethylbenezene, and xylene). No other carbon source was included in the medium.

2.3. Inoculation of A. thaliana and vetiver with endophytic bacteria

The isolate *A. xylosoxidans* F3B was chosen as an inoculum. Fresh cultures of the inoculum were grown under selective conditions at 30 °C on a rotary shaker to an approximate $A_{600\,nm}$ value of 3.0. The cells were diluted ten-fold in LB medium. Typically, this inoculum contained 10^7–10^9 cells/mL. Seven-day-old *A. thaliana* plants were immersed in the inoculum for 1 min and cultured in half-strength Murashige and Skoog (MS) agar in a growth chamber (constant temperature of 22 °C and 14/10 h light and dark cycle).

Inocula (250 mL) with an $A_{600\,nm}$ value of 3.0 were prepared for inoculation of vetiver plants (*Vetiveria zizanioides*). The cells were collected by centrifugation, washed 3 times in 10 mM $MgSO_4$, and diluted in half-strength sterile Hoagland's solution (1/2 HS) [6] to obtain a cell concentration of 10^{10} CFU/mL. The vetiver plants were placed in a 1-L beaker containing 0.8 L of sterile 1/2 HS. A bacterial inoculum was added to each jar at a final concentration of 10^8 CFU/mL. After 7 days of incubation, the plants were planted in fresh 1/2 HS and were placed in the greenhouse. Every week, the plants' roots were harvested for DNA extraction from the endophytes.

2.4. Aromatic compounds' phytotoxicity test on plate conditions

Aromatic compounds such as toluene, xylenes, and naphthalene are degraded into catechol-like intermediates, followed by cleavage of the benzene ring [14]. According to this, the catechol was selected as reference aromatic compound. To determine whether catechol-degrading endophytes conveyed protection against aromatic compounds, the inoculated plants were exposed to concentrations of 0.2, 0.4, 0.6 and 0.8 mM catechol, and 0.3, 0.6, 0.9 and 1.2 mM phenol. One-week-old *A. thaliana* plants that were inoculated with either *A. xylosoxidans* F3B or *Rhodococcus erythropolis* BC11 (strain involved in remediating petroleum compounds) [15], and control plants, which were not inoculated, were transferred onto 1/2 MS agar containing different concentrations of catechol and phenol. After 7 days, growth parameters of the plants, such as fresh weight and root length, were measured. Five replicates were performed for all experiments.

2.5. Achromobacter xylosoxidans phylogenetic analysis

The endophytic bacterial strains' 16S rRNA sequences were amplified with the universal primers E8F and U1510R [16]. To perform the phylogenetic analysis on the 16S rRNA sequences, similarity searches were carried out with GenBank from the National Center for Biotechnology Information (NCBI) website. The phylogenetic analysis and tree construction were performed using a Neighbor-Joining method. Bootstrap percentages were calculated using 1000 repetitions on the MEGA 4.0 analysis system.

2.6. A novel real-time PCR monitoring system for A. xylosoxidans F3B

To design new primers, 16S rRNA sequences were obtained from GenBank and the sequences were subjected to multiple alignments using DNAMAN version 6.0 software. We compared the *A. xylosoxidans* F3B 16S rRNA sequence (JQ740156) that was amplified by primers E8F and U1510R with the following 16S rRNA sequences from GenBank (accession numbers are given in parentheses): *Achromobacter ruhlandii* (NR027197), *Advenella incenata* (AM944735), *Alcaligenes aquatilis* (AJ937889), *Alcaligenes faecalis* (FJ982933). The sequences of 16S rRNA-specific primers were as follows: forward primer F3B16s192F: 5′-GCAGGGGATC GCAAGACCTT GCACT-3′ and reverse primer F3B16s441R: 5′-TTTCCCGGGG TATTAACCCG GAACGT-3′. For DNA extraction, *A. thaliana* plants inoculated with *A. xylosoxidans* F3B were taken from agar plates and shaken in 0.5% NaOCl for 3 min to sterilize the surface. The vetiver root tissues were shaken in 0.5% NaOCl for 15 min. The plant tissue was crushed using a mortar and pestle. A Tissue and Genomic DNA Purification Kit (GeneMark) was used to extract DNA. The amplification reactions (20 μL) contained 1 μL of DNA template, 1× Ex Taq™ Buffer, 0.2 mM dNTPs, 0.1 μM of each F3B-specific primer, 0.5 U of TaKaRa Ex Taq™, and sterile-filtered water. Real-time PCR analysis was performed using a SYBR Green-based detection system. This analysis was performed in a Rotor-Gene RG-3000 with Rotor-Gene software version 6.1. Following optimization, each reaction commenced with 5 min at 95 °C, followed by 40 cycles for 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C. The fluorescence signal was measured for 5 s at 89.5 °C. After the amplification, a melting peak analysis with a temperature gradient of 0.1 °C s^{-1} from 55 °C to 99 °C was performed to confirm that only the specific products were amplified [17]. The standard curve for the F3B-specific 16S fragment ranged from 1×10^2 to 1×10^8 copies μL^{-1}. The calibration curve showed significant linearity ($R^2 > 0.98$) within the tested range.

2.7. Catechol removal tests on hydroponics

One-week-old *A. thaliana* plants (controls and those inoculated with *A. xylosoxidans* F3B) were used to evaluate the degradation of catechol. After 3 days, the plants were carefully taken out of

the plate. Their roots were vigorously shaken in 0.5% NaOCl for 3 min to sterilize the surface and rinsed in sterile water to remove bacteria from the surface. Each flask was filled with 50 mL of sterile half-strength MS medium, and 0.4 mM catechol was added. For the purpose of comparison, plants inoculated with *A. xylosoxidans* F3B were exposed to this sublethal concentration of catechol in a growth chamber (constant temperature of 22 °C and 14/10 h light and dark cycle). Uninoculated plants received the same treatment and were used as a control. Samples were taken from the medium every 24 h. The samples were directly analyzed with an Agilent 1100 HPLC using an Agilent Eclipse XDS-C18 column provided with a 277 nm diode array detector. Methanol–water (70:30) was used as the eluent at a flow rate of 0.8 mL/min. A 20-mL aliquot of each sample was injected into the column. The retention times of known standards were used to identify the catechol.

2.8. Total petroleum hydrocarbon (TPH) analysis of rhizosphere soil

The vetiver plants were grown in a 1007 ± 216 mg/kg of $TPH_{C10–C40}$-contaminated clay soil coming from a refinery factory site. After one week of inoculation with F3B, the vetiver plants were carefully removed from the jars. After washing their roots with sterile water, the plants were planted in different pots containing 5 kg of contaminated soil (one seedling per pot). The experiments were performed in duplicate. After one week, the plants were pulled out and the root-adhering soil was collected for TPH analysis. Dichloromethane was used as the extraction solvent. Soil samples (2.5 g) were shaken with 7–10 g of anhydrous Na_2SO_4, until the mixture flowed freely. Next, 25 mL of dichloromethane was added to each tube, and the tubes were sonicated in a water bath for 50 min at room temperature. The supernatants were placed in 2-mL sample vials. The samples were analyzed with an Agilent HP-6890N gas chromatograph with a J&W DB-1HT column and a flame ionization detector. The operating temperature was 250 °C for the injection port and 300 °C for the detector; the initial temperature was set to 50 °C for 5 min, increasing at a rate of 10 °C min^{-1} to 350 °C, where it remained for 15 min. Nitrogen was used as the carrier gas at a flow rate of 50 mL/min. The TPH removal ability was calculated by the following equation: TPH removal ability (%) = [(TPHc − TPHt)/TPHc] × 100. Where TPHc was the control TPH concentration at the time *t* (mg/kg); TPHt was the treatment TPH concentration at the time *t* (mg/kg).

3. Results and discussion

3.1. Isolation and characterization of endophytic bacteria

The selection of morphologically different colonies from cultures inoculated with plant tissue material resulted in 219 isolates. A total of 180 isolates were obtained from reeds (*P. australis*), and 39 strains were isolated from water spinach (*I. aquatica*). Among the twenty-five isolates that could use aromatic compounds as the sole carbon source (Table 1), only one was isolated from water spinach. These endophytic bacteria can grow on minimal medium with aromatic compounds added. The isolates were comprised of 72% functional Gammaproteobacteria, including 64% *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas* spp. and a minor percentage of *Serratia* spp. and *Erwinia* spp. Twenty percent of the remaining isolates constituted gram-positive bacteria, with the predominant strains being *Bacillus* spp. (12%), *Paenibacillus* spp., and *Microbacterium* spp. It was determined that all of these bacterial could use catechol as a sole carbon source.

3.2. Inoculation and stability of F3B within A. thaliana and V. zizanioides

The protective effects of the isolated endophytic strains on *A. thaliana* were examined with catechol as a model compound. The isolates (Table 1) that could degrade aromatic compounds were individually inoculated into *A. thaliana.* These inoculated plants were grown on 0.2 mM catechol agar medium. Out of all of the inoculated endophytes, only the F3B isolate conferred a protective effect against different pollutant stresses in plants and was further characterized (data not shown). Using a 16S rRNA gene database, the F3B isolate was identified as *A. xylosoxidans* strain F3B, and a real-time PCR monitoring system, based on the specificity to its 16S rRNA gene, was developed for observing F3B in plants.

Enumeration of *A. xylosoxidans* F3B cells in the tissues from colonized *A. thaliana* plants showed that the bacteria efficiently colonized the inoculated plants. The colonization of F3B in plants in 0.2 mM catechol agar increased during the first three days, as indicated by the change from 3.8×10^2 to 3.9×10^4 16S rDNA copies. After 6 days, this colonization achieved a maximum population of 6.9×10^5 copies. A population size of 10^5 can be maintained in the presence of 0.2 mM catechol for at least 2 weeks. However, in plants without this pressure, the population size increased from 10^2 to between 10^5 and 10^6 copies after 3 days. Alternatively, *V. zizanioides* was chosen as another model plant for phytoremediation. The population size of *A. xylosoxidans* F3B in vetiver roots can also maintain 3×10^5 copies for more than one month. During the period from week 1 to week 5, the size of the F3B population increased from 1.2×10^5 to 3×10^5 copies. Besides certain bacterial pathogens, there is no evidence for host-specific interactions between endophytic bacteria and the colonized hosts. There are a variety of reports in which bacteria that were originally isolated from one host are able to endophytically colonize one or several different plant species [18,19]. A number of studies used antibiotic-resistant mutants of a wild-type strain and selective plating to screen for bacterial targets [6,20]. During root surface sterilization, chemical agents might affect the endophyte's growth. Counting colonies may not be accurate for lacking duplication of natural conditions. In this study, a quantitative real-time PCR technology was developed to detect the endophyte population, which was observed to be more sensitive than counting the colonies on a plate (data not shown).

3.3. Phylogenetic analysis of A. xylosoxidans

In previous studies, *A. xylosoxidans* was reported to have the ability to degrade catechol, biphenyl, and monoaromatic hydrocarbons (BTEX) [12,21,22]. However, *A. xylosoxidans* was also considered a potential human pathogen that was associated with respiratory diseases, such as cystic fibrosis [23,24]. In 2009, Prabhat Jha and Ashok Kumar first reported the presence of endophytic *A. xylosoxidans* WM234C-3 in the roots and culm of healthy wheat plants [25]. Phylogenetic analysis showed that endophytic *A. xylosoxidans* F3B was similar to endophytic *A. xylosoxidans* WM234C-3, based on the percent divergence, as shown in Fig. 1. The results distinguished the endophytic *A. xylosoxidans* from human- and soil-borne strains. As our results showed that *A. xylosoxidans* F3B could colonize different plant species, the impact on human and environmental safety should be discussed for further applications.

3.4. The effects of inoculation with endophytic bacteria and soil bacteria

R. erythropolis BC11, which was isolated from soil, can degrade high concentrations of diesel and fuel oil. Strain BC11 can also use benzoate, phenol, toluene, and catechol as sole carbon sources.

Table 1
The growth characteristics of different bacterial strains isolated from *Phragmites australis* and *Ipomoea aquatica*.

Seq. ID	Isolate	Phenol	Catechol	Benzene	Toluene	Ethylbenzene	Xylene
Achromobacter xylosoxidans	F3B	+	+	+	+	+	+
Pseudomonas vancouverensis	B10	+	+	+	+	+	+
Pseudomonas putida	F19	+	+	+	+	+	+
Pseudomonas putida	I18	+	+	+	+	+	+
Pseudomonas fluorescens	I17	+	+	–	–	+	+
Pseudomonas fluorescens	J6	+	+	–	–	+	+
Pseudomonas mendocina	G4	+	+	+	/	/	/
Acinetobacter baumannii	4A14	/	+	+	+	+	+
Acinetobacter baumannii	4B16	/	+	+	+	+	+
Acinetobacter baumannii	4D8	/	+	+	+	+	+
Acinetobacter johnsonii	5R8	+	+	+	+	+	+
Erwinia sp.	4B15	/	+	–	–	–	+
Serratia marcescens	6R17	+	+	+	+	+	+
Stenotrophomonas maltophilia	A10	+	+	+	–	+	+
Stenotrophomonas maltophilia	A15	+	+	–	–	–	–
Stenotrophomonas maltophilia	B17	+	+	–	+	+	+
Stenotrophomonas maltophilia	C6	+	+	+	–	–	–
Stenotrophomonas rhizophila	J7	+	+	–	–	+	+
Stenotrophomonas sp.	B7	+	+	–	–	+	+
Bacillus pumilus	C3	+	+	+	+	+	+
Bacillus pumilus	E10	+	+	+	+	+	+
Bacillus subtilis	F17	+	+	+	+	+	+
Paenibacillus sp	I12	+	+	+	+	+	+
Chryseobacterium sp.	B15	+	+	–	–	–	+
Microbacterium sp.	J9	+	+	+	+	+	+

(+) indicates growth, (–) indicates no growth, and (/) indicates that it was not tested.

This strain has the potential to be used as a bioremediation organism [15]. Compared with *A. xylosoxidans* F3B, *R. erythropolis* BC11 has a larger petroleum-degrading ability without inoculation into plants. Plants inoculated with *R. erythropolis* BC11 were significantly different from *A. xylosoxidans* F3B-inoculated plants, which grew on catechol-contaminated plates. This finding demonstrates that in the absence of inoculated bacteria, increasing the catechol concentration caused increased levels of phytotoxicity in plants. The inoculation with the endophytic bacteria *A. xylosoxidans* F3B had a better effect on plant growth than soil bacteria, as shown in Fig. 2. This finding is in agreement with Barac et al., who studied the improvements in phytoprotection and phytoremediation with engineered endophytic bacteria. These researchers also showed that the plants inoculated with toluene-degrading endophytes survived better than those inoculated with soil bacteria [6].

3.5. The effects on plant growth during phytotoxicity tests on plating conditions

Seven-day-old *A. thaliana* were placed on target plates to test phytotoxicity. After 1 week of exposure to catechol, the biomass and root lengths were measured. Fig. 3(A) shows uninoculated control plants, which received no catechol treatment (0 mM). Instead, plants exposed to 0.2 mM catechol showed smaller and shorter roots. For uninoculated control plants in the presence of catechol, the growth indices suggested that increasing levels of catechol resulted in greater phytotoxicity. Plants inoculated with the endophytic strain *A. xylosoxidans* F3B, which can effectively metabolize catechol and phenol, showed better growth compared to the controls. Fig. 3(B) shows plants inoculated with *A. xylosoxidans* F3B received a slight beneficial effect, as the inoculation attenuated

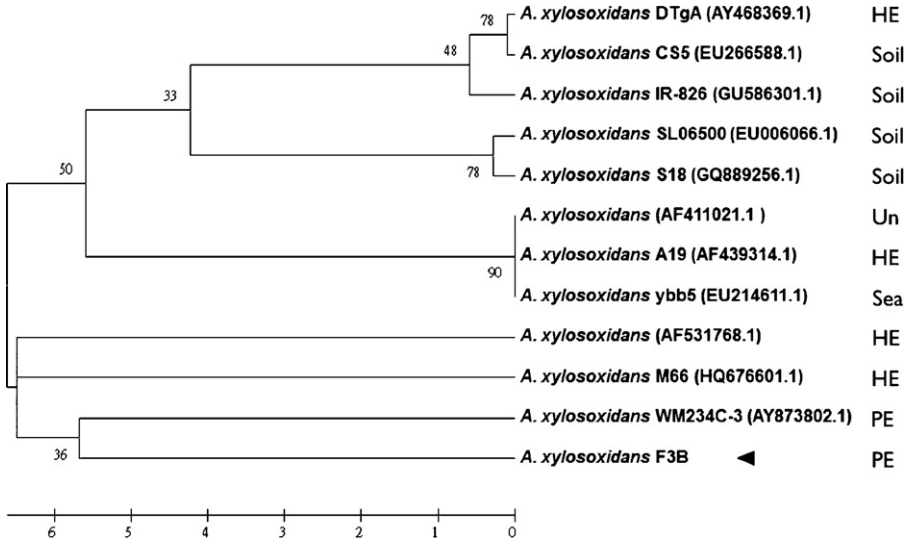

Fig. 1. Phylogenetic analysis shows a genetic relationship between *Achromobacte xylosoxidans* and taxonomically similar strains based on 16S rDNA sequences. The GenBank accession number of each isolate is given in parentheses. The scale indicates the percent distance. HE, bacteria from humans, Soil, bacteria from soil, Sea, bacteria from the sea, PE, endophytes from plant, and Un, unknown.

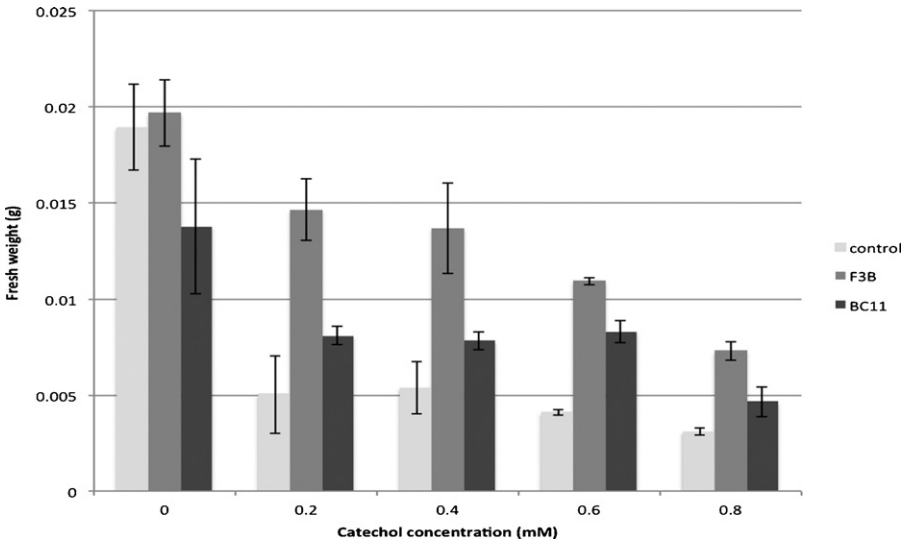

Fig. 2. The effect of various catechol concentrations in soil bacteria- and endophyte-inoculated plants following a 7-day exposure. For this experiment, uninoculated control plants and plants inoculated with *A. xylosoxidans* F3B or *R. erythropolis* BC11 were evaluated. The data are presented as the mean ± SD of three individual experiments.

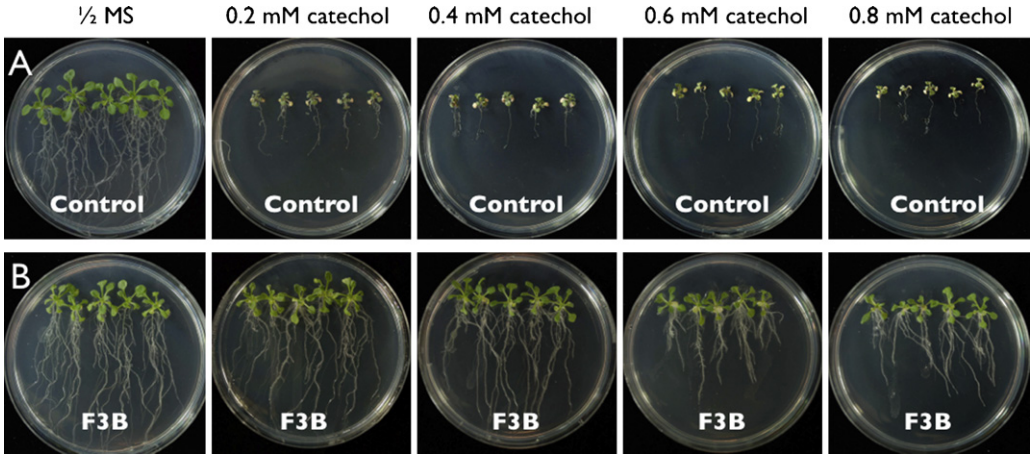

Fig. 3. The effect of a 7-day catechol exposure on plant growth. (A) Uninoculated control plants on agar with different concentrations of catechol. (B) Plants incubated with *A. xylosoxidans* F3B on agar with different concentrations of catechol.

phytotoxic effects induced by 0.2–0.8 mM catechol. The average root length of F3B-inoculated plants was 3.5 times longer than the uninoculated control plants when plants were exposed to 0.2 mM catechol. Plants inoculated with F3B and grown in phenol concentrations between 0.6 mM to 1.2 mM showed significant differences in the root lengths and fresh weight (*t*-test, $p < 0.05$), as shown in Table 2. These results show that the endophytic bacteria *A. xylosoxidans* F3B can potentially help

Table 2
Plant growth on plates containing catechol and phenol after a 7-day exposure. The results are means ± SD of three experiments conducted separately with at least 3 plants under identical conditions.

	Treatment	Catechol (mM)				
		0	0.2	0.4	0.6	0.8
Fresh weight (mg)	Control	28.9 ± 3.55	4.1 ± 0.17	4.3 ± 0.04	2.5 ± 0.16	1 ± 0.38
	F3B	21.7 ± 2.8	21.1 ± 3.86*	12.8 ± 1.73*	12.6 ± 2.86*	7.3 ± 0.77*
Root length (cm)	Control	7.76 ± 0.84	1.98 ± 0.38	2.04 ± 0.44	2.08 ± 0.5	1.89 ± 0.56
	F3B	8.42 ± 0.58*	7.61 ± 0.91*	6.53 ± 0.71*	4.19 ± 0.84*	4.28 ± 1.16*
	Treatment	Phenol (mM)				
		0	0.3	0.6	0.9	1.2
Fresh weight (mg)	Control	17.9 ± 1.72	10 ± 0.96	3.98 ± 1.72	2.68 ± 3.95	2.19 ± 0.32
	F3B	17.9 ± 2.84	11.7 ± 2.85	5.31 ± 1.54	5.31 ± 3.95*	3.83 ± 0.07*
Root length (cm)	Control	6.32 ± 1.13	5.12 ± 0.9	3.75 ± 0.76	2.91 ± 0.34	2.73 ± 0.45
	F3B	6.83 ± 1.63	5.9 ± 0.64*	4.62 ± 0.97*	4.49 ± 0.87*	3.9 ± 1.01*

* Indicates that comparison differences are significant (*t*-test, $p < 0.05$).

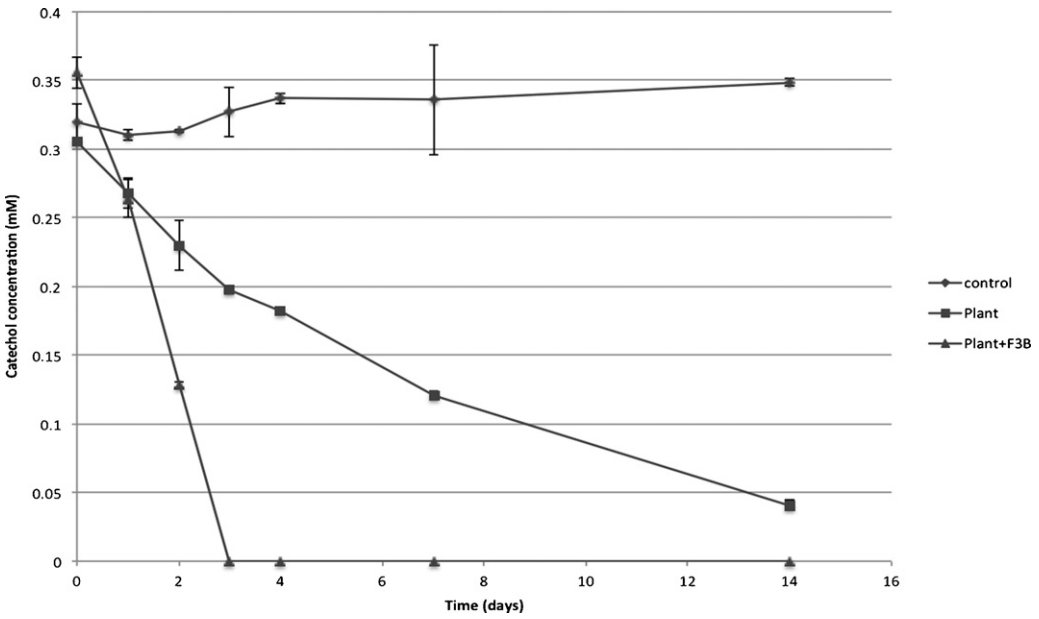

Fig. 4. The amount of catechol remaining in the half-strength MS medium. For this experiment, control medium without plants, uninoculated plants, and plants inoculated with *A. xylosoxidans* F3B were used. The data are presented as the mean ± SD of three individual experiments.

plants survive under conditions of elevated aromatic compound contamination.

A. xylosoxidans has been reported to have a gene encoding catechol dioxygenase, which helps in the aerobic degradation of monoaromatic hydrocarbons (BTEX) [12,22,26], bisphenol A [27], and decolorizing activity of malachite green dye [28]. It can also use endosulfan and 2- and 2,5-(di)chlorobenzoic acid (2-CB; 2,5-DCB) as sole carbon sources and enhance copper phytoextraction [29,30]. Additionally, the gene for *Plesiomonas*-derived chlorocatechol 1,2-dioxygenase (TfdC), which is an intradiol dioxygenase for opening aromatic rings, was introduced into *Arabidopsis* (*A. thaliana*). The transgenic plant line showed an enhanced tolerance to 0.2 mM catechol [31]. In this study, the inoculated endophytic bacteria *A. xylosoxidans* F3B could enhance the tolerance up to 0.8 mM catechol. These results suggest that functional endophytic bacteria provide equal or better effects in the protection of *Arabidopsis* against aromatic compound stress.

3.6. Catechol removal on hydroponics test

Ten plants were immersed in a flask that contained 1/2 MS liquid medium with 0.4 mM catechol. The control medium without plants was also added to a flask with 0.4 mM catechol. The concentrations of catechol in the medium were determined by HPLC. Both the uninoculated and F3B- inoculated plants were able to remove catechol from the medium after 2 weeks. F3B-inoculated plants were extremely efficient in removing catechol from the plants (100% removal), compared to uninoculated plants (41% removal) (Fig. 4).

3.7. TPH removal in a greenhouse test

The removal of the endophytic strain *A. xylosoxidans* F3B on vetiver in greenhouse studies was examined further. Plants were grown on clay soil contaminated with 1007 ± 216 mg/kg $TPH_{C10–C40}$. After 1 week, the root-adhering soil samples were collected and extracted for TPH analysis. The uninoculated control plants removed 405 ± 360 mg/kg (49%) of TPH from the rhizosphere soil compared with TPH-contaminated soil where no vetiver was planted (826 ± 173 mg/kg). The F3B-inoculated plants enhanced the removal (removed 601 ± 98 mg/kg or 72.7%) of TPH from the soil compared with the unplanted soil. Plants inoculated with F3B can enhance TPH removal in comparison to uninoculated plants. Though the p value indicates that it is not statistically significant, the results suggest that *A. xylosoxidans* F3B has the potential to help plants increase their efficiency of TPH removal.

4. Conclusions

A. xylosoxidans F3B was successfully inoculated in *A. thaliana*. F3B can also help the plant tolerate lethal concentrations of aromatic compounds and decrease catechol- and phenol-induced phytotoxicity. In hydroponic and soil tests, the endophytic bacteria *A. xylosoxidans* F3B can enhance the efficiency of phytoremediation. This study is the first to show the effective use of *A. xylosoxidans* F3B as an endophyte in phytoremediation. These types of functional endophytes could play important roles in phytoprotection and phytoremediation.

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