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In situ degradation of phenol and promotion of plant growth in contaminated environments by a single *Pseudomonas aeruginosa* strain

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ABSTRACT

For bioremediation of contaminated environments, a bacterial strain, SZH16, was isolated and found to reduce phenol concentration in a selective medium. Using the reaction vessel containing the soil mixed with phenol and bacteria, we found that the single strain degraded efficiently the phenol level in soil samples. The strain was identified as *Pseudomonas aeruginosa* on the basis of biochemical tests and by comparison of 16S rDNA sequences, and phosphate solubilization and IAA production were not observed in the strain. Simultaneous examination of the role of strain SZH16 in the plant growth and phenol biodegradation was performed. Results showed that inoculation of the single strain in the phenol-spiked soil resulted in corn growth promotion and in situ phenol degradation and the increase in plant biomass correlated with the decrease in phenol content. Colonization experiments showed that the population of the SZH16 strain remained relatively constant. All these findings indicated that the corn growth promotion might be due to reduction in phytotoxicity, a result of phenol biodegradation by the single strain SZH16. Furthermore, the strain was found to stimulate corn growth and reduce phenol concentration simultaneously in phenol-containing water, and even historically contaminated field soils. It is attractive for environment remediation and agronomic applications.

1. Introduction

Phenol is an important material and primary pollutant of various industries. Phenol can be found from various industries such as the manufacturing of resins and plastics, petroleum refining, steel production, coal conversion, coal mines, byproducts of agricultural chemicals, dyestuff, textiles, tanning, fiberboard production, painting, pulp and paper, pesticides, medications, pharmaceuticals and even from food processing industries [1,2]. The use of these substances imposes severe risks to human health and the environment and its toxicity and possible accumulation in the environment have been observed [3–6]. Acute exposure to phenol is known to cause skin irritation, gastrointestinal discomfort, and headaches. Phenol is toxic to the nervous system, the heart, the kidneys, and the liver and is readily absorbed through skin and mucosa. Toxicity of phenol towards plants has been proved. The 50–60 mg/L of phenol concentration was found to be inhibitive to seed germination [7]. The willow trees exposed to 1000 mg/L phenol wilted and eventually died [8]. The phenol with concentrations of 0.08 mM had a negative influence on the growth of water lenses (*Lemna gibba*) [9]. Cucumber (*Cucumis sativus* L.) exposed to the phenol with concentrations of 220 mg/L exhibited a significant reduction in growth. Introduction of 130 mg/L of phenol inhibited the growth of lettuce (*Lactuca sativa* L.). The 120 mg/L of phenol was harmful to the growth of Millet (*Panicum milliaceum*) [10].

Chemical and biological technologies have been employed in phenol degradation [11,12]. However, the chemical technologies have been found to have inherent drawback owing to the tendency to form secondary pollutants and also proven to be costly [13]. Phenol biodegradation is an environmentally friendly and cost-effective method, and important in environmental remediation. However, phytoremediation of highly volatile organic xenobiotics, such as toluene and phenol, is often inefficient because plants do not completely degrade these pollutants, potentially resulting in new environmental problems [14]. The biodegradation of phenols by different types of microbial cultures has attracted the attention of many researchers during the past two decades. In our present report, a single bacterial strain was found to degrade efficiently the phenol level in a phenol-spiked soil, phenol-containing water and historically contaminated field soils. At the same time, the strain was observed to promote the plant growth under the above phenol-contaminated environments. Furthermore, the increase in plant biomass correlated with the decrease in phenol content.

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2. Materials and methods

2.1. Isolation of phenol-degrading bacteria from water and soil samples

Water samples (surface layer, 0–3 cm depth) were collected at margins of Suzhou Creek, Shanghai, in 100 ml sterilised bottles. These samples were immediately transported to the laboratory of microbiology, and subjected to serial ten-fold dilutions in sterile distilled water. Soil samples were taken from the rhizosphere of test plants. Ten grams of soil was shaken for 30 min in 100 ml sterile deionized water containing 25 g of sterile glass beads. Serial dilutions were prepared. Convention spread plate technique under aerobic condition was used. The Luria-Bertani (LB) medium was first applied. Selective medium for phenol-degrading bacteria contains per 1000 ml distilled water: 3 g $(NH4)_2SO_4$, 0.5 g KH_2PO_4, 0.5 g Na_2HPO_4, 0.3 g Mg_2SO_4 $7H_2O$, and 1 ml microelement solution. Two hundred milligrams of phenol as a single carbon and energy source was added. The microelement solution contains per 1000 ml distilled water: 0.5 g $FeSO_4$ $7H_2O$, 0.15 g $MnSO_4$ H_2O, 0.14 g $ZnSO_4$, and 0.2 g $CoCl_2$ [15]. Isolated strains were maintained in the Luria-Bertani (LB) medium. If needed, the strains were inoculated in LB medium and the bacterial suspension with the indicated concentration (10^8 CFU/ml) was prepared.

2.2. Preparation of phenol-spiked soil and inoculation of bacteria

Phenol-spiked soils were prepared by an incorporation of phenol into sterile sandy loam soil (sand: 74%, silt: 18%, clay: 10%, water holding capacity: 34%, pH: 6.7). The soil was artificially spiked with phenol solution at a concentration of 100 mg phenol/kg soil (dry weight). To examine the in situ phenol-degrading ability of bacteria in soil, the 20 g of phenol-spiked soil was mixed with 1 ml of bacterial suspension (10^8 CFU/ml). The soil samples that did not contain phenol or only contained LB medium without bacteria served as controls. The 20 g of the soil was put into a 100 ml beaker, covered with 8 layers of gauze. The beakers containing the soil were placed in a sterile incubator at 28 °C. Three replicates were used for each treatment. Samples were collected for quantitative analyses of phenol at the indicated time points.

2.3. Phosphate solubilization and IAA production

Mineral phosphate solubilization was assayed on TY medium supplemented with 5 g/L of $Ca_3(PO_4)_2$. Aliquots of fresh culture were spread onto plates, and incubated at 28 °C for 48 h. A clear zone around the colonies indicated solubilization. Qualitative IAA production ability test was performed as previously described [16]. In brief, strain was transferred onto the medium supplemented with 5 mM tryptophan, overlaid with a nitrocellulose membrane until bacterial colonies reach 1–2 mm in diameter. The membrane is removed to a filter paper saturated with Salkowski reagent and incubated until distinct red haloes form around the colonies.

2.4. Quantitative analyses of phenol

The extraction and the quantitative analyses of phenol were performed as previously described with the slight modifications [17]. One gram of soil sample (dry weight) or 10 ml of culture medium was mixed with 3 ml of chloroform and filtered through 0.22 μm filters (Sigma). Each sample was extracted three times, and the filtrate supernatants were combined. Phenol concentrations of the filtrate supernatants were analyzed by high performance liquid chromatography (HPLC) (Hewlett Packard, 1050). A C18 reverse phase HPLC column (Agilent, Zorbax extend-C18) was used. The mobile phase was composed of methanol and 2% acetic acid (60:40, v/v). The detection was carried out at 270 nm. Phenol concentrations were calculated on the basis of peak area measurements by comparison with an external standard of known concentration of pure phenol.

2.5. DNA extraction and 16S rDNA amplification

A single colony was picked from a fresh culture and resuspended in 50 μl sterile deionized water. The genomic deoxyribonucleic acid (DNA) was isolated according to standard method [18]. If needed, DNA solution was re-extracted with phenol and chloroform, precipitated with isopropanol, and washed twice in ethanol. PCR amplification of 16S ribosomal DNA (rDNA) based on the genomic DNA was performed with the universal primer pair: 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-TACCTTGTTACGACTT-3′) [19,20]. The PCR product was purified using the gel extraction kit (Pharmacia), sequenced through dideoxynucleotide sequencing.

2.6. Phenol degradation and plant growth promotion in soils

Seeds of corn (*Zea mays*) were surface sterilized with 0.1% $HgCl_2$ for 5 min and then washed thoroughly with distilled water. Seedlings were placed with their roots in the Hoagland's solution under sterile conditions [21]. Healthy two week-old seedlings, after washing with sterile distilled water and bacteria were not found to adhere to the surface of the corn seedlings, were transferred into the pots containing the above sterile sandy loam soil. The earthenware pots, 20 cm in height and 20 cm in diameter, were used in the experiment, and 4 corn seedlings were grown in each pot containing 5 kg of soil. Ten pots per treatment were used. The pots were covered with 8 layers of gauze containing holes through which the root of the plants was introduced, and placed in the greenhouse at 22–28 °C. The sandy loam containing phenol and bacterial suspension with above concentration were used. The experiment in which corn seedlings grew in the soil without phenol, the phenol-spiked soil without bacterial suspension or the phenol-spiked soil only containing LB medium without bacteria served as control. Three replicates were used for each treatment. Remediation of phenol-contaminated soil was performed as above except that the historically phenol-contaminated soil samples (21 mg/kg) were collected in the field, and finely pulverized.

At the indicated time points, the seedlings were harvested and the rhizosphere soil was carefully collected. The plant biomass was determined (fresh weight) and designated Wt. The plant biomass was determined (fresh weight) before transferred into the pots and designated Wo. The value of Wt/Wo was then calculated. At the same time, quantitative analyses of phenol in the rhizosphere soil were performed and the final contents were obtained. The ratio of the final phenol to the initial content (100 mg/kg) was calculated. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$).

2.7. Colonization of bacterial strains in the corn rhizosphere soil containing phenol

Rifampicin-resistant strains were selected for colonization experiments. The rifampicin concentration gradient was designed as 0–100 μg/ml. Colonies able to grow on LB plate containing 100 μg/ml rifampicin were transferred onto LB culture for further use. The rifampicin-resistant strains derived from their corresponding parent strains were confirmed by match of 16S rDNA sequences. The 20 g of the above phenol-spiked soil was mixed with 1 ml of the indicated rifampicin-resistant bacterial suspension (10^8 CFU/ml), and put into the earthenware pots, 20 cm in height and 20 cm in

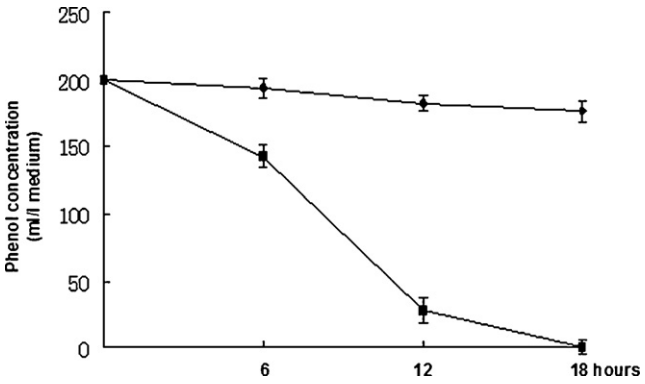

Fig. 1. Degradation of phenol in medium by strain SZH16. Strain SZH16 (■) grew on the selective medium with phenol (initial concentration: 200 ml/L medium) as the sole carbon. The phenol-containing medium without bacteria served as control (♦). Quantitative analyses of phenol were performed 6, 12, and 18 h after inoculation. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$).

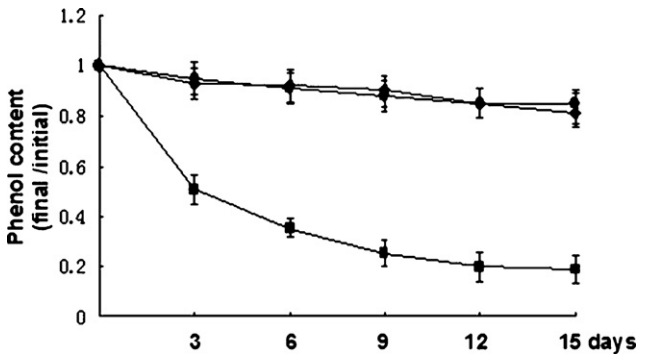

Fig. 2. Degradation of phenol artificially spiked into soil by strain SZH16. One millilitre of bacterial suspension (10^8 CFU/ml) (■) was introduced into the 20 g of phenol-contaminated soil (100 mg phenol/kg soil). The phenol-spiked soil without bacterial suspension (●) or the phenol-spiked soil containing LB medium without bacteria (♦) served as control. Quantitative analyses of phenol were performed 3, 6, 9, 12 and 15 days after inoculation. The ratio of the final phenol to the initial content was calculated. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$).

diameter. Two week-old corn seedlings were transferred into the pots. The rhizosphere soils were carefully collected at the indicated time points, and the bacterial strains were enumerated by plating on LB plate containing 100 μg/ml rifampicin. If needed, the selective medium containing phenol was used. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$). At the same time, examination of the plant growth and phenol biodegradation was performed as described above.

2.8. Plant growth and phenol biodegradation in water

Seed germination and seedling growth referred to the above experiments. Two week-old seedlings, after washing with sterile distilled water and bacteria were not found to adhere to the surface of the corn seedlings, were transferred into the 500 ml beakers with 300 ml of the half-strength sterile Hoagland's solution containing 100 mg/L phenol and 100 mg/L LB medium, supplemented with 1 ml of bacterial suspension (10^8 CFU/ml). One corn seedling was grown in each beaker and 20 beakers were used per treatment. The beakers were covered by a glass plate containing a hole through which the plant root could grow into the Hoagland's solution. Seedlings growing in Hoagland's solution, without phenol and bacteria, served as a control. Seedlings growing in the phenol-contaminated water only containing LB medium without bacteria also served as a control. Three replicates were used for each treatment. The above beakers were placed in a growth chamber at a constant temperature of 22 °C and a cycle of 14 h of light (150 μmol m^{-2} s^{-1}) and 10 h of darkness. At the indicated time points, the seedlings were harvested and the water samples were carefully collected. The plant biomass and phenol content were determined as above description.

3. Results

3.1. Degradation of phenol artificially spiked into soil

A bacterial strain, SZH16, was found to be able to grow on the selective medium with phenol as the sole carbon and energy source and reduce the phenol concentration (Fig. 1). To examine the in situ phenol-degrading ability of the single strain SZH16 in soil, the soil containing phenol was prepared and inoculated with strain SZH16. Results showed that introduction of strain SZH16 significantly reduced the phenol content in the phenol-spiked soil, compared with the uninoculated control (Fig. 2). About 85% the phenol was reduced after 15 days. The slight fluctuation in phenol content was observed in the control experiments, indicating that the volatilization and other factors were not responsible for the loss of phenol. Therefore, the phenol reduction might primarily result from degradation by strain SZH16.

3.2. Identification of strain SZH16

Cell morphology was determined through microscopy, Gram and spore staining. Analysis of biochemical properties was performed according to the standard protocols. The strain was Gram-negative, nonspore forming, rod-shaped and with a single polar flagellum. Bacterial colonies were circular, and convex with entire margins. The strain was catalase negative, oxidase and arginine dihydrolase positive. The strain did not ferment glucose and produced fluorescent pigment on the King's medium B (KMB). These morphological and biochemical properties are characteristic criteria for *Pseudomonas aeruginosa*.

For further identification, genomic DNA was isolated from the above strain cells, 16S rDNA sequences (accession no. GU384267) were obtained by PCR with the putative primers and alignment analysis was performed. Results showed that the 16S rDNA sequence of strain SZH16 was nearly identical to that of *P. aeruginosa* strain S25 (accession no. DQ095913.1). Therefore, strain SZH16 was temporarily classified as *P. aeruginosa*, Furthermore, phosphate solubilization and IAA production were not observed in the strain (results not shown).

3.3. Plant growth and phenol biodegradation in phenol-spiked soil and phenol-containing water

To investigate the effect of the strain on the plant growing in the phenol-spiked soil, corn seedlings were transferred to the soil containing phenol and strain SZH16. At the indicated time points, the seedlings were harvested, and the plant biomass was determined. As shown in Fig. 3A, inoculation of strain SZH16 in the phenol-spiked soil significantly increased the plant growth, compared with the phenol-spiked soil without bacteria inoculation after 15 days. The plant growth promotion in the phenol-spiked soil inoculated with strain SZH16 is similar to that in the soil without phenol after 15 days. The corn seedlings looked healthy and were growing well in the experiments. The plant growth in the

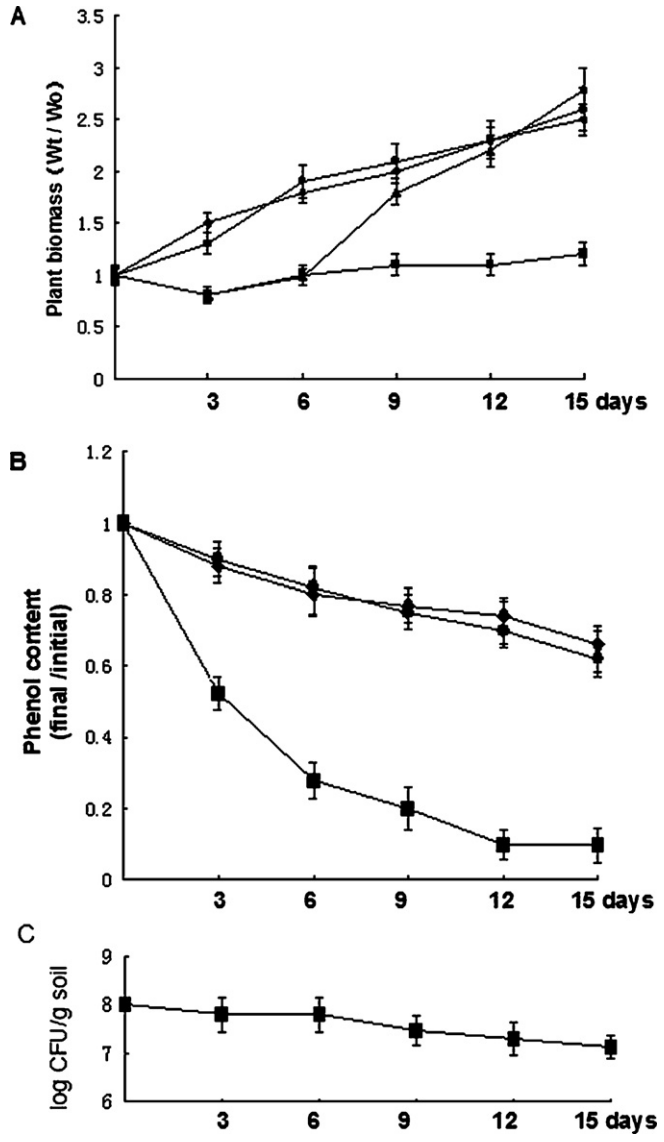

Fig. 3. Plant growth, phenol biodegradation and colonization of strain SZH16 in the phenol-spiked soil. (A) Corn seedlings grew in the soil containing phenol and bacteria (◄), only bacteria (●). The experiment in which corn seedlings grew in the soil without phenol and bacteria (♦) or the phenol-spiked soil only containing LB medium without bacteria (■) served as control. At the indicated time points, the seedlings were harvested, and the plant biomass was determined (fresh weight). The value of Wt/Wo was then calculated. (B) Phenol degradation was performed in the phenol-spiked soil in which bacteria and corn seedlings grew (■). The experiment in which corn seedlings grew in the phenol-spiked soil without bacterial suspension (●) or the phenol-spiked soil containing LB medium without bacteria (♦) served as control. Quantitative analyses of phenol were performed 3, 6, 9, 12 and 15 days after incubation. The ratio of the final phenol to the initial content was calculated. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$). (C) Colonization of strain SZH16 was measured. The rifampicin-resistant strain SZH16 (■) was obtained. The 20 g of the above phenol-spiked soil was mixed with 1 ml of the indicated rifampicin-resistant bacterial suspension (10^8 CFU/ml), and put into the earthenware pots, 20 cm in height and 20 cm in diameter. Two week-old corn seedlings were transferred into the pots. The rhizosphere soils were carefully collected at the indicated time points, and the bacterial strains were enumerated by plating on LB plate containing 100 μg/ml rifampicin.

phenol-spiked soil containing LB medium without bacteria was almost the same as that in the phenol-spiked soil without bacterial suspension, indicating that the nutrients in bacterial culture were not the reason why plant growth was increased (Fig. 3A and results not shown). It was noted that the plant growth in the inoculated soil with phenol was inhibited before 6 days, suggesting that the phytotoxicity of phenol still was present at early time points after inoculation of phenol-degrading bacteria. When strain SZH16 was inoculated in the noncontaminated soil, plant growth promotion was similar to that in the noncontaminated soil without inoculation of the strain, indicating that strain SZH16 could not directly increase plant growth and the corn growth promotion observed in the experiment might be due to reduction in phytotoxicity, a result of phenol biodegradation by strain SZH16. Indeed, when the rhizosphere soil of the corn seedlings was collected, and quantitative analyses of phenol in the soil samples were performed, phenol degradation was significantly observed in the phenol-spiked soil in which bacteria and plants grew, compared with the control experiment in which only plant grew in the phenol-spiked soil (Fig. 3B). For strain SZH16, the increase in plant biomass correlated with the decrease in phenol content (Fig. 3A and B). Colonization experiments showed that the population of the rifampicin-resistant SZH16 strain remained relatively constant in the phenol-spiked soil in the 15 days (Fig. 3C). At the same time, we found that the rifampicin-resistant strain had almost the same influence on the plant growth and phenol biodegradation as its parent strain (results not shown). When the corn seedlings grow in the phenol-containing water without bacteria inoculation, plant growth was negligible. We found that the plant growth significantly increased in the water without phenol and bacteria after 15 days. Inhibition of plant growth might be due to phytotoxicity. Introduction of strain SZH16 into the phenol-containing water significantly promoted plant growth after 15 days, as in the water without phenol and bacteria (Fig. 4A). The corn growth promotion might be due to reduction in phytotoxicity, a result of phenol biodegradation by strain SZH16. Indeed, phenol biodegradation was simultaneously observed in phenol-contaminated water inoculated with strain SZH16, compared with the control experiment without the bacterium (Fig. 4B). From Figs. 3 and 4, we found that the plants exposed to phenol exhibited a reduction in biomass in the soil and the water. Inoculation of strain SZH16 in the phenol-spiked soil and the phenol-containing water enhanced phenol degradation and simultaneously increased the plant growth. Furthermore, the increase in plant biomass correlated with the decrease in phenol content (Figs. 3 and 4). All the results showed that strain SZH16 degraded phenol and reduced phytotoxicity in contaminated environments, ultimately leading to plant growth promotion.

3.4. Plant growth and phenol biodegradation in phenol-contaminated soils

To analyze the role of the strain in environmental remediation, the phenol-contaminated soil samples (21 mg/kg) were collected from the field with a history of phenol pollution, finely pulverized, inoculated with the strain. The corn seedlings were transferred into the soil, and plant biomass was determined after 15 days. As shown in Fig. 5A, the corn seedlings exposed to phenol exhibited a significant reduction in biomass (Fig. 5A, lane 2), compared with the plants in the noncontaminated soil (Fig. 5A, lane 1). Inoculation of strain SZH16 in the contaminated soil increased the plant growth (Fig. 5A, lane 3). Inoculation of strain SZH16 in the noncontaminated soil also promoted the plant growth (Fig. 5A, lane 4), as in the noncontaminated soil without inoculation of strain SZH16 (Fig. 5A, lane 1), indicating that strain SZH16 could not directly increase plant growth. Similarly, phenol content in the rhizosphere soil of these seedlings was measured. Introduction of the strain into the phenol-contaminated soil in which the corn seedlings grew significantly reduced the phenol concentration (Fig. 5B, lane 3), compared with the uninoculated controls (Fig. 5B, lanes 1 and 2). We noted that the increase in plant biomass correlated with the decrease

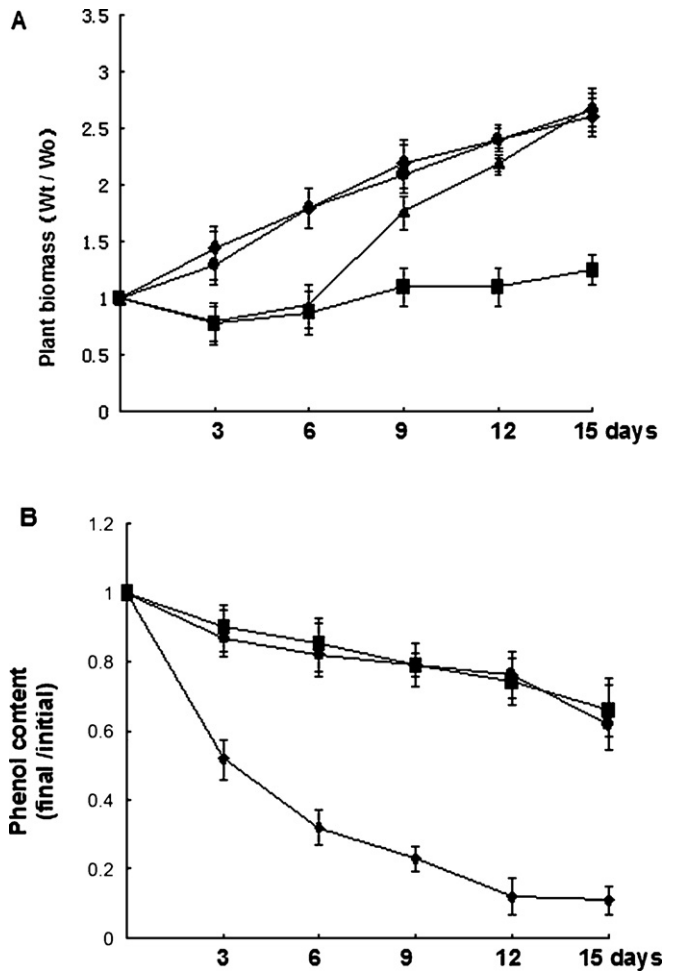

Fig. 4. Plant growth and phenol biodegradation under hydroponic condition. (A) Corn seedlings grew in the water containing phenol and bacteria (◄), only bacteria (●). The experiment in which corn seedlings grew in the water without phenol and bacteria (♦) or the phenol-contaminated water only containing LB medium without bacteria (■) served as control. At the indicated time points, the seedlings were harvested, and the water samples were carefully collected. The plant biomass including roots and shoots was determined (fresh weight). The value of Wt/Wo was then calculated. (B) Phenol degradation was performed in the phenol-contaminated water in which bacteria and corn seedlings grew (♦). The experiment in which corn seedlings grew in the phenol-contaminated water without bacterial suspension (●) or the phenol-contaminated water containing LB medium without bacteria (■) served as control. Quantitative analyses of phenol were performed at the indicated time points. The ratio of the final phenol to the initial content was calculated. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$).

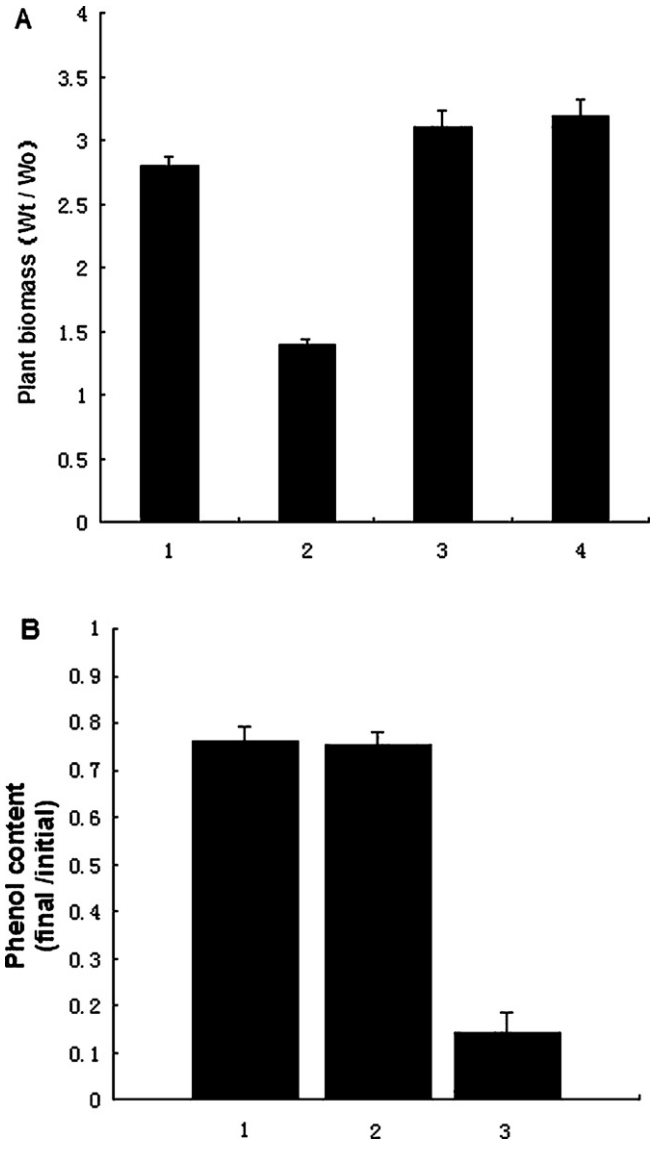

Fig. 5. Plant growth and phenol biodegradation in the phenol-contaminated soil. (A) Corn seedlings grew in the soil containing phenol and bacteria (3), only bacteria (4). The experiment in which corn seedlings grew in the soil without phenol and bacteria (1) or the phenol-contaminated soil only containing LB medium without bacteria (2) served as control. After 15 days, the seedlings were harvested, and the plant biomass was determined (fresh weight). The value of Wt/Wo was then calculated. (B) Phenol degradation was performed in the phenol-contaminated soil in which bacteria and corn seedlings grew (3). The experiment in which corn seedlings grew in the phenol-contaminated soil without bacterial suspension (2) or the phenol-contaminated soil only containing LB medium without bacteria served as control (1). Quantitative analyses of phenol were performed 15 days after inoculation. The ratio of the final phenol to the initial content was calculated. The data are the means of 3 replicates and the error bars indicate the standard deviation from the mean. Two-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$).

in phenol content in the contaminated soil inoculated with strain SZH16 (Fig. 5A and B, lane 3).

4. Discussion

The focus on the microbial degradation of phenols in recent years has resulted in the isolation of a number of microorganisms that can grow on phenol as a sole carbon and energy source. These include: *Pseudomonas* species [22–24], *Serratia marcescens* [25], *Bacillus subtilis* [26], *Bacillus brevis* [27], *Rhodococcus erythropolis* [28], *Alcaligenes faecalis* [29], *Burkholderia cepacia* [30], and *Candida tropicalis* [31]. Amongst all the microorganisms listed as good degraders of phenol, the pure culture of Pseudomonads are the most utilised purposely for metabolic pathway studies [13]. The *P. aeruginosa* strain SZH16 present in the report was added to the list, and in situ biodegradation of phenol and reduction of phytotoxicity were observed in it. The bacterial strain was found to degrade the phenol level in the phenol-spiked soil, the phenol-containing water and the phenol-contaminated soil collected from a field with a history of phenol pollution. At the same time, the corn plant in the phenol-contaminated environments inoculated with the strain was observed to obtain almost same biomass as in the noncontaminated environments at the end of experiment although the strain did not directly promote the plant growth. Furthermore, our data showed that in situ biodegradation of phenol was correlated with the increase in plant biomass. Therefore, the normal growth of corn

in the contaminated environments might be due to reduction in phytotoxicity, a result of phenol biodegradation by strain SZH16.

When inoculated with the strain, about 80% of the phenol in a constant temperature incubator (Fig. 2), about 90% of the phenol in phenol-spiked soil and water with corn seedlings (Figs. 3 and 4), and about 85% of the phenol in phenol-contaminated soil with corn seedlings was decreased (Fig. 5), inconsistent with the previous report in which a very small amount of phenol was reduced 10 days after introduction of bacteria into the phenol-contaminated soil [32]. The discrepancy might primarily result from different experiment systems. We observed that the previous experiments were performed in an enclosed system, in contradiction with our system in which the reaction vessel containing the soil mixed with phenol and bacteria was covered by the gauze with many holes.

Phenol-degrading bacteria can be separated into aerobic types, which require oxygen to live, and anaerobic, which can live without oxygen. Aerobic processes were in all cases faster than anaerobic ones [33,34]. Many strains of *Pseudomonas* can aerobically degrade phenol [13,27]. Under aerobic conditions, degradation of phenol was shown to be initiated by oxygenation into catechols as intermediates followed by a ring cleavage, in which a dioxygenase is need [13,35]. The dioxygenase is an enzyme which catalyzes the incorporation of both atoms of dioxygen into its substrate, and widely distributed in nature [35]. In our previous works, phenol-degrading aerobic bacteria, *Pseudomonas fluorescens* and *Burkholderia cepacia*, have been isolated. The plasmids isolated from these strains were found to be loaded with a gene encoding the catechol 2, 3-dioxygenase (C23O) [27]. We have not known if *P. aeruginosa* strain SZH16 contains the gene.

Our results showed that strain SZH16 promoted the growth of corn in the contaminated environments by reduction of phytotoxicity. Many bacteria which promote the plant growth have been isolated [36–38]. *Bacillus* strain EPB22 is able to promote the growth of banana plants through reducing the damage caused by banana bunchy top virus [37]. Some endophytic bacteria have been found to enhance plant growth by induction of systemic resistance in plants against pathogenic microorganisms [39]. Many soil bacteria facilitate plant growth by production of phytohormone [40]. It has been reported that the microorganisms have a positive influence on plant growth by reduction of phytotoxicity [41]. Phytotoxicity is reduced by some bacteria because they owe the enzymes responsible for phytotoxicity degradation, or obtain some genetic determinants for the degradation by horizontal gene transfer, or endowed with a degradation pathway by engineered methods [15,30,42,43]. However, we have not known the mechanism by which strain SZH16 degrade phenol to promote the growth of corn in the contaminated environments.

Using our experiment system, we found the normal growth of corn plant and also detected phenol biodegradation at the same time. To our knowledge, simultaneous examination of the plant growth and phenol biodegradation in a system is difficult. It was dependent on understanding phenol tolerance of plant and phenol saturation of soil, and needed inhibiting excessive evaporation of water and phenol from system, and providing aerobic condition. The system is very useful in microbial bioremediation research. *P. aeruginosa* strain SZH16, with the ability of promotion of corn plant growth in phenol-contaminated environments by reduction of phytotoxicity, remains to be tested in the field scale.

Acknowledgements

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