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RESEARCH ARTICLE

# The endophytic bacterium *Serratia* sp. PW7 degrades pyrene in wheat

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**Abstract** This research was conducted to isolate polycyclic aromatic hydrocarbon-degrading (PAH-degrading) endophytic bacteria and investigate their potential in protecting plants against PAH contamination. Pyrene-degrading endophytic bacteria were isolated from plants grown in PAH-contaminated soil. Among these endophytic bacteria, strain PW7 (*Serratia* sp.) isolated from *Plantago asiatica* was selected to investigate the suppression of pyrene accumulation in *Triticum aestivum* L. In the in vitro tests, strain PW7 degraded 51.2% of the pyrene in the media within 14 days. The optimal biodegradation conditions were pH 7.0, 30 °C, and MS medium supplemented with additional glucose, maltose, sucrose, and peptones. In the in vivo tests, strain PW7 successfully colonized the roots and shoots of inoculated ($E^+$) wheat plants, and its colonization decreased pyrene accumulation and pyrene transportation from roots to shoots. Remarkably, the concentration of pyrene in shoots decreased much more than that in roots, suggesting that strain PW7 has the potential for protecting wheat against pyrene contamination and mitigating the threat of pyrene to human health via food consumption.

**Keywords** Pyrene · Endophytic bacterium · Isolation of endophytic bacterium · 16S rRNA gene · Plant colonization

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**Abbreviations**

| | |
|---|---|
| EPA | US environmental protection agency |
| HMW | High-molecular weight |
| PAHs | Polycyclic aromatic hydrocarbons |
| LB medium | Luria-Bertani medium |
| MS medium | Mineral salt medium |
| POPs | Persistent organic pollutants |

## Introduction

An increasing accumulation of polycyclic aromatic hydrocarbons (PAHs) has been found in multiple environments (Hu et al. 2013), including arable soils (Tao et al. 2004), urban lands (Tang et al. 2005; Wang et al. 2013), forests, and grasslands (Orecchio 2007). PAHs tend to be accumulated in food chains due to their high hydrophobicity and affinity for fatty tissues. Pyrene, a tetracyclic aromatic hydrocarbon produced by incomplete combustion of organic materials, is one of the 16 PAHs on the US Environmental Protection Agency's (EPA's) priority list. Some properties of pyrene are as follows: molecular weight, 202.26 g $mol^{-1}$; log $K_{OW}$ (*n*-octanol-water partition coefficients), 5.18; log $K_{AW}$ (dimensionless air-water partition coefficient), −3.43; and log $K_{OA}$ (*n*-octanol-air partition coefficients), 8.61(Gao and Collins 2009). Pyrene is persistent and has been found in relatively high levels in coal tar-contaminated sites and polluted aqueous environments. Because it has been widely used as an indicator and model compound to study biodegradation of high-molecular weight (HMW) PAHs (Chen and White 2004), pyrene was selected as a representative HMW PAH to investigate the pyrene detoxification potential of endophyte inoculation.

In China, more than 20% of the main food crops contain PAHs that exceed the control limits (Zhan et al. 2013). Dietary intake of PAHs is a major route of exposure for humans, and

food crops are major sources of dietary PAHs. Thus, the mitigation of the PAH contamination in crops has become an important scientific problem. Usually, there are two main ways to alleviate PAH contamination in plant tissues: one is to reduce the concentration of PAHs in the environment, and the other is to decrease the concentration of PAHs in plant through reducing the uptake of the PAHs and degrading the PAHs in the plant tissues.

Microbial degradation of PAHs is considered to be the key remediation strategy in the soil environment, and many PAH biodegradation studies have been carried out. Various bacterial strains that utilize PAHs as growth substrates have been isolated, but only a few strains are capable of degrading HMW PAHs efficiently (Fu et al. 2014). In addition, it is difficult for bioaugmentation to improve the degradation rate or degradation extent (respiratory activity) of HMW PAH in the field (Silva et al. 2009). Effective PAH-degrading bacteria may be used to remove PAHs in terrestrial systems; however, microorganisms with the potential to degrade PAHs may not be prevalent in soils where PAH remediation is required. In many cases, laboratory-adapted strains are unable to compete with the indigenous microflora, and the degradative performance of most inocula will depend on soil types and environmental conditions, which may not be easily controlled in the field. PAH-contaminated soils are often deficient in nutrients that are necessary to support PAH-degrading bacteria. Due to these inhibitory effects, the bioremediation progress can be very slow (Sayara et al. 2009).

Endophytic bacteria may provide a method for reducing HMW PAH levels in contaminated soils and plants. Endophytic bacteria participate in plant protection either by acting directly on pathogens and herbivores or by enhancing plant responses. Endophytic bacteria have been found in the shoots, leaves, and roots of plants (Selosse et al. 2004). Several persistent organic pollutant (POP)-degrading endophytic bacteria have been isolated from plants grown in POP-contaminated soils. Some bacterial endophytes have been shown to have positive effects on plant growth and survival in soils heavily contaminated with POPs by stimulating the nutrient uptake of host plants (Li et al. 2008), by degrading POPs in plant tissues (Wen et al. 2011; Sun et al. 2014), by improving the plant's tolerance to POPs (Moore et al. 2006; Weyens et al. 2010), by affecting the activities of plant enzymes, and by secreting plant hormones, siderophores, and other organics (Suto et al. 2002; Li et al. 2008). The application of endophytic bacteria to degrading PAHs may offer advantages for several reasons: (i) the host plant provides a stable environment without interference by indigenous microflora, and (ii) due to their abilities to promote the growth of plants and reduce the content of PAHs directly in plant tissues, PAH-degrading endophytic bacteria may have a great capacity to reduce PAH levels in plant tissues.

In this study, pyrene was selected as the representative PAH; we searched for the functional endophytic bacteria which could efficiently degrade HMW PAHs. An efficient pyrene-degrading endophytic *Serratia* sp. named strain PW7 was isolated. This isolate, which can use pyrene as the sole carbon source, was selected from 14 strains to investigate the colonization of plants by endophytic bacteria and the effect on pyrene levels. Average rates of pyrene degradation by strain PW7 were evaluated with in vitro tests for different pyrene concentrations and different cultivation conditions. Subsequently, in vivo tests were performed to examine the colonization efficiency of strain PW7 in wheat to determine whether the inoculation with strain PW7 can promote the growth of wheat and whether this strain reduces the pyrene content of wheat grown in pyrene-contaminated soil.

## Materials and methods

### The isolation and identification of pyrene-degrading endophytic bacteria

Some healthy plants (*Plantago asiatica* L., *Trifolium repens* L., and *Setaria viridis* L. *Beauv.*) were collected from PAH-contaminated sites, located near the Sinopec Yangzi Petrochemical Co., Ltd., Nanjing, China. Plant samples were preserved at 4 °C and used within 7 days. Luria-Bertani (LB) medium was used for the enrichment of pyrene-degrading bacteria. Mineral salt (MS) medium was used as the basal medium for isolating pyrene-degrading endophytic bacteria and evaluating their ability to degrade pyrene. A stock solution of pyrene was prepared in methanol and used in all the degradation experiments.

Plant tissues were sterilized by immersion in 75% (*v*/*v*) ethanol-water for 3–5 min and then in 0.1% (*v*/*v*) mercuric chloride solution for 2–5 min. The sterilized plant tissues were washed with sterile deionized water at least three times to remove the surface sterilization agents and were cultivated on LB plates to confirm that all the external bacteria had been eliminated. After being successfully surface disinfected, the plant tissues were ground aseptically with sterile deionized water.

The homogenate was incubated in Erlenmeyer flasks containing 100 mL MS medium supplemented with 50 mg $L^{-1}$ pyrene. Aliquots were transferred weekly to fresh MS medium supplemented with 50 mg $L^{-1}$ pyrene at least four times before the bacterial strains were isolated. All flasks were incubated in the dark on a rotary shaker at 30 °C and 150 rpm. Isolation and purification procedures were carried out on MS medium agar plates coated with a layer of pyrene on the surface. The size and color of the isolated colonies were recorded. Bacterial strains were selected based on colony morphology and color.

The selective enrichment culture procedure resulted in the isolation of 14 pyrene-degrading endophytic bacterial strains that could use pyrene as the sole source of carbon and energy.

The degradation rates of pyrene by those strains were 6.9–47.3% in the medium containing 40 mg $L^{-1}$ pyrene within 10 days. Among them, strain PW7 was selected for further investigation due to its efficient pyrene-degrading ability.

Strain PW7 was classified based on 16S ribosomal RNA (rRNA) gene sequence analysis and its physiological and biochemical characteristics. Fragments of the 16S rRNA gene of each of the isolated strains were prepared according to the method described by Byers et al. (1998). The sequencing was performed by the Nanjing Genscript Biotechnology Company, Limited (Nanjing, China). The 16S rRNA gene sequences were queried against the GenBank database (http://www.ncbi.nlm.nih.gov/), and microgenetic analysis was performed using the MEGA 6.0 programs.

### Tests in vitro: degradation of pyrene by endophytic bacteria

The strains used as the inocula in vitro and in vivo tests were cultured in liquid LB medium containing pyrene. After they reached the stationary phase, the strains were centrifuged, washed twice with MS medium to remove the residual carbon source, resuspended in fresh MS medium, and adjusted to an optical density of 1.0 at 600 nm ($2.5 \times 10^9$ CFU $mL^{-1}$).

The degradation of pyrene was monitored in 50-mL flasks containing 20-mL MS medium containing 40 mg $L^{-1}$ pyrene as the sole carbon source. To the prepared flasks, 1-mL inocula were added. Control flasks were inoculated with sterilized MS medium to assess abiotic effects on pyrene stability. During the 14-day incubation period, triplicate samples from each treatment were retrieved daily for detecting the pyrene residues.

Four independent 10-day incubation experiments were conducted to investigate the effect of initial pyrene levels, additional nutrients, incubation temperature, and pH on pyrene degradation by strain PW7. To assess the effects of the initial pyrene levels on the biodegradation rates of pyrene, 1-mL inocula were added to 20 mL of MS medium supplemented with 20, 40, 60, 80, or 100 mg $L^{-1}$ pyrene. The MS medium supplemented with 40 mg $L^{-1}$ pyrene was used in the experiments for testing the effects of nutrients, temperature, and pH. All cultures were incubated on a rotary shaker (150 rpm) at 30 °C (except for the temperature experiments) in the dark. Additional nutrients were maltose, glucose, sucrose, fructose, lactose, serine, peptones, yeast extract, and $KNO_3$ at 50 mg $L^{-1}$ added separately. Temperature experiments included temperatures of 25, 30, 37, and 42 °C. In the pH experiments, the initial pH value of the MS medium was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. Control flasks were inoculated with sterilized MS medium. After 10 days of incubation, triplicate flasks from each treatment were retrieved for the measurement of bacterial growth and pyrene concentrations.

### Tests in vivo: inoculation of wheat with pyrene-degrading endophytic bacteria

Strain PW7 is resistant to a mixture of ampicillin, streptomycin, and tetracycline hydrochloride each at 100 mg $L^{-1}$.

Seedlings of the wheat Yangmai 16 (*Triticum aestivum* L.), 10-cm in length, were used for inoculation with strain PW7. The roots of seedlings were dipped in the inocula for 6 h, and the non-inoculated, control seedlings were dipped in MS medium with autoclaved strain PW7. After inoculation, all the seedlings including the inoculated and non-inoculated seedlings were washed with sterile water more than four times, dried with sterile filter paper, and planted in Hoagland solution supplemented with pyrene. There were four treatments in the tests in vivo: T1 (inoculated seedlings) and CK1 (non-inoculated seedlings) were cultivated in Hoagland solution supplemented with 100 μg $L^{-1}$ pyrene; T2 (inoculated seedlings) and CK2 (non-inoculated seedlings) were cultivated in Hoagland solution supplemented with 500 μg $L^{-1}$ pyrene. Seedlings were grown in 300-mL brown glass containers sealed with translucent caps. Each glass container contained 250 mL Hoagland solution and 12 seedlings. The Hoagland solution was supplemented during the cultivation period. Plants were kept in a growth chamber for 12 days with a 12 h photoperiod and a light/dark temperature regime of 25:20 °C. During the 12-day inoculation experiment, plant samples were collected every 2 days for evaluating the colonization efficiency and pyrene levels.

The colonization efficiency of strain PW7 on wheat was detected by reisolation of strain PW7 from inoculated seedlings and counting the CFU of strain PW7. After being successfully surface disinfected, the seedlings were ground with sterile deionized water aseptically and then cultivated on pyrene-MS agar plates (50 mg $L^{-1}$ pyrene) containing a mixture of the antibiotics. The identification of the re-isolated PW7 was based on 16S rRNA gene sequence analysis.

### Detection of pyrene

The pyrene was extracted from the MS medium with methyl alcohol, which was added to the medium at a ratio of 1: 1 (*v*/*v*), ultrasonically extracted for 30 min and centrifuged at 12,000×*g* for 10 min, followed by filtration through 0.22-μm filters.

Plant samples were washed, freeze-dried, ground, and homogenized. The pyrene was extracted from the homogenate ultrasonically extracted for 30 min in a 1: 1 (*v*/*v*) solution of acetone and dichloromethane (DCM) with anhydrous $Na_2SO_4$ to remove moisture, followed by centrifugation. This process was repeated three times. Then, the supernatant was filtered through a column with 2 g of silica gel containing a 10 mL 1: 1 (*v*/*v*) elution of DCM and hexane. The solvent fractions were evaporated and exchanged with methanol for a final volume of 10 mL, followed by filtration through 0.22-μm filters.

Pyrene was quantified using an HPLC system (LC-10AT). Chromatography was performed at 40 °C and detection at 245 nm.

## Statistical analyses

The statistical significance of any differences between treatments was subjected to one-way analysis of variance (ANOVA). Differences with $P$ values <0.05 were considered to be statistically significant. The pyrene degradation kinetics equation was determined by regression analysis.

## Results

### Isolation and identification of pyrene-degrading endophytic bacteria

A photograph of colonies and a micrograph of a cell of strain PW7 are shown in Fig. 1. Some physiological and biochemical characteristics of strain PW7 were identified and are presented in Table 1. Colonies of strain PW7 were red, opaque, and convex with a glistening surface and an irregular shape with a slightly serrated border (Fig. 1a). Cells were found to be short rods, non-sporulating, acapsular (Fig. 1b), aerobic without gelatinase, and Gram-negative.

A phylogenetic analysis of strain PW7 is shown in Fig. 2. Accession numbers of 16S rRNA sequences are given in parenthesis. Based on a BLAST sequence comparison, the 16S rRNA of strain PW7 was identical to that of *Serratia* sp.

### Degradation of pyrene in vitro

After 14 days of growth at 30 °C in MS medium containing 40 mg $L^{-1}$ of pyrene, strain PW7 degraded 51.2% of the pyrene (Fig. 3). There was a significant negative correlation between the cell densities and the pyrene residues in the culture medium; the Pearson correlation coefficient was −0.436 ($P < 0.01$). The pyrene removal was more than 47.5% at day 10 when the maximal cell density (approximately $1.21 \times 10^8$ CFU $mL^{-1}$) was observed. The rates of pyrene removal and cell growth were low during the first 2 days, suggesting that strain PW7 required time for adaptation to pyrene as a carbon source. And after day 10, the cell density reduced with the decreasing levels of pyrene residues in the media, suggesting that pyrene residues could not raise more strains.

**Table 1** Physiological and biochemical characteristics of strain PW7

| Characteristic | Results | Characteristic | Results |
|---|---|---|---|
| Gram staining | − | Catalase activity | + |
| Spore formation | − | Indole production | − |
| Starch hydrolysis | + | Gelatin liquefaction testing | − |
| Methyl red staining | + | Hydrogen sulfide test | − |
| Voges-Proskauer reaction | + | Citrate utilization test | + |

+, positive reaction; −, negative reaction

### Factors influencing pyrene degradation

Degradation of pyrene by strain PW7 was significantly affected by the initial pyrene concentration in the medium ($P < 0.01$) (Fig. 4). When the initial pyrene concentration was more than 60 mg $L^{-1}$, the mass of pyrene lost decreased significantly, and an increasing inhibition on bacterial growth was observed. Strain PW7 performed well in the media containing 20 mg $L^{-1}$ pyrene, 40 mg $L^{-1}$ pyrene, or 60 mg $L^{-1}$ pyrene. The maximal mass of pyrene lost was 22.9 mg $L^{-1}$ in the medium containing 40 mg $L^{-1}$ pyrene, and the maximal relative removal of pyrene by strain PW7 was 70.8% in the medium containing 20 mg $L^{-1}$ pyrene, whereas the minimal mass of pyrene lost was 9.8 mg $L^{-1}$ in the medium containing 100 mg $L^{-1}$ pyrene. In this test, pyrene removal rate was also positively correlated with cell growth (the Pearson correlation coefficient was 0.929) ($P < 0.01$) and negatively correlated with the pyrene content (the Pearson correlation coefficient was −0.960) ($P < 0.01$), suggesting that a high pyrene contamination would require more time to be degraded by strain PW7.

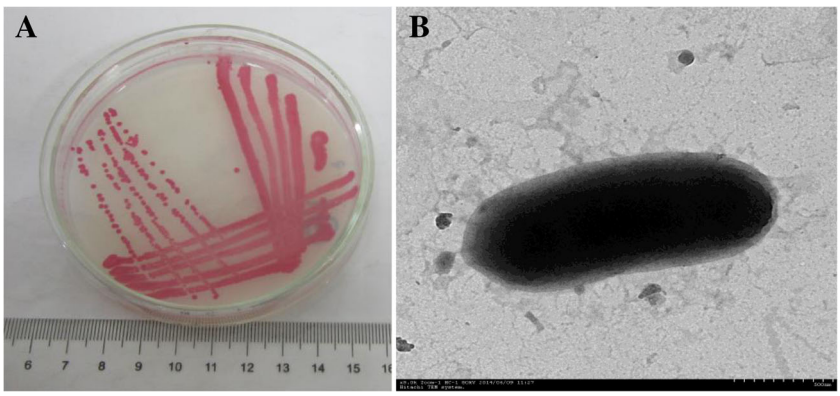


**Fig. 1** A photograph of colonies of strain PW7 on an LB plate (**a**) and an electron micrograph of strain PW7 (**b**)

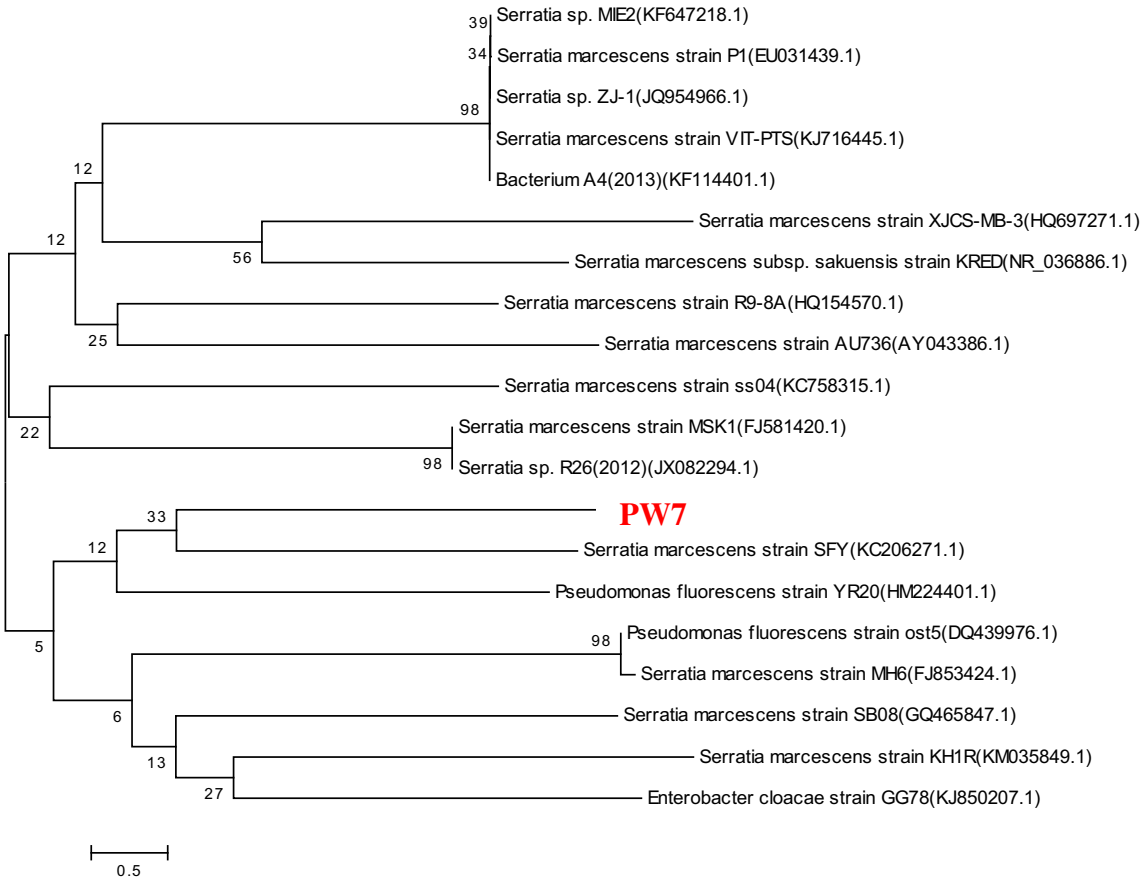


**Fig. 2** The phylogenetic tree of 16S rRNA gene sequences of strain PW7 and related bacteria

To determine the optimal environmental conditions for strain PW7, degradation tests were carried out at pH values of 3.0 to 10.0 and at temperatures of 25 to 42 °C. As shown in Fig. 5, the optimal conditions for pyrene degradation were determined to be pH 7.0 and 30 °C.

The addition of other carbon and nitrogen sources, including glucose, sucrose, maltose, lactose, serine, peptones, and yeast extract, could significantly enhance the degradation of pyrene ($P < 0.05$). When grown in media separately with glucose, sucrose, maltose, lactose, or serine, strain PW7 removed more than 55% of the pyrene from the medium (Fig. 6). However, no significant difference in pyrene degradation was observed in the presence of $KNO_3$ and $NH_4Cl$ ($P > 0.05$). Compared to the control, the greatest increase in degradation rate was observed when 50 mg $L^{-1}$ glucose was added to the medium, suggesting that glucose is an optimal co-substrate for strain PW7 ($P < 0.05$).

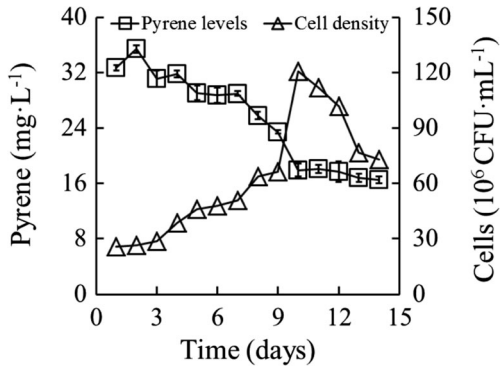


**Fig. 3** Degradation of pyrene by strain PW7. Bacteria were cultivated for 14 days, and pyrene was extracted from the culture medium every day

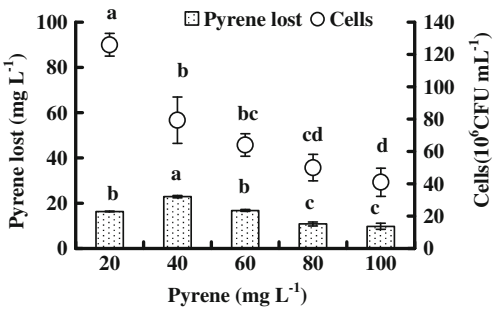


**Fig. 4** Degradation of different concentrations of pyrene by strain PW7 after cultivation for 10 days at 30 °C. *Different lowercase letters* indicate significant differences among treatments ($P < 0.05$)

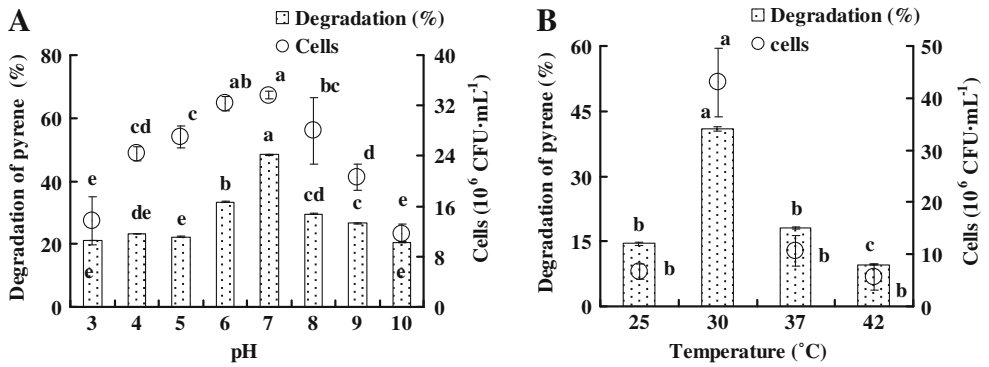


**Fig. 5** Effects of pH (**a**) and temperature (**b**) on the degradation of pyrene by strain PW7. Bacteria were grown in MS medium supplemented with 40 mg $L^{-1}$ pyrene, and the pyrene levels in the culture supernatant were detected after 10 days of incubation. The optimum conditions were 30 °C (**b**) and pH 7.0 (**a**). *Different lowercase letters* indicate significant differences among treatments ($P < 0.05$)

### In vivo tests: inoculation of seedlings with the pyrene-degrading endophytic bacterium

In a 12-day incubation experiment, strain PW7 was isolated from the shoots and roots of inoculated seedlings, but it was not obtained from non-inoculated seedlings (Table 2). The results suggested that strain PW7 successfully colonized seedlings through roots and that it could move from the roots to the shoots.

As shown in Fig. 7, inoculation with strain PW7 had little effect on the growth of seedlings. During 12 days of incubation, no significant effects were observed on the growth of seedlings exposed to 100 μg $L^{-1}$ pyrene by inoculated with strain PW7 ($P > 0.05$). Only the growth of the seedlings was promoted by the inoculation of strain PW7 on seedlings exposed to 500 μg $L^{-1}$ pyrene on day 10 ($P < 0.05$). According to the high standard deviation of the growth of shoots in group T2 on day 10, the promotion of the growth of shoots by inoculation with strain PW7 needs to be clarified in the future research.

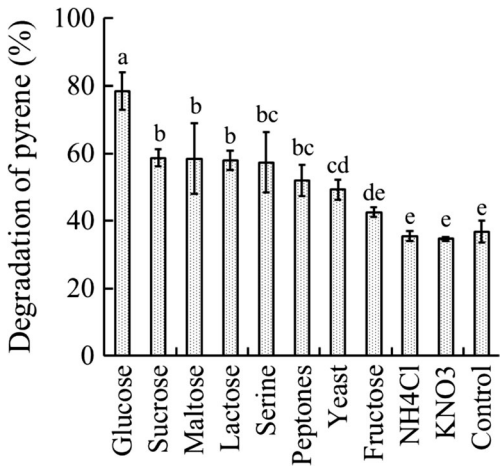


**Fig. 6** Effects of additional nutrients on the degradation of pyrene by strain PW7. Bacteria were grown in MS medium supplemented with 40 mg $L^{-1}$ pyrene, and pyrene levels in the culture supernatant were detected after 10 days of incubation (at 30 °C, pH 7.0). *Different lowercase letters* indicate significant differences among treatments ($P < 0.05$)

Remarkably, inoculation of wheat with strain PW7 significantly decreased the pyrene levels in wheat seedlings. During the 12-day incubation experiment, the pyrene contents in group T2 were significantly decreased by inoculation with strain PW7 ($P < 0.05$) (Fig. 7). Compared to non-inoculated seedlings, the pyrene contents of group T1 decreased by 20.6–70.9% in shoots and by 4.4–60.4% in roots, and the pyrene contents of group T2 decreased by 29.5–50.7% in shoots and by 5.5–24.0% in roots, indicating that inoculation of strain PW7 decreased the pyrene contents in shoots much more than in roots. The pyrene concentrations in the seedlings from T1 to T2 were significantly decreased on day 4, day 6, and day 8, when the growth of seedlings was not significantly affected by inoculation with strain PW7. These results suggested that the reduction of pyrene concentration in the seedlings did not result from the dilution of higher biomass.

Meanwhile, inoculation of wheat with strain PW7 had some effects on the residual pyrene in Hoagland solution (Fig. 8). For group T2, the pyrene residues in the solution were significantly reduced by inoculation with strain PW7. However, for the

**Table 2** Reisolation of strain PW7 from inoculated seedlings during the incubation for 12 days

| Treatments | Pyrene (μg $L^{-1}$) | Tissue | Cultivation time (days) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 |
| T1 (inoculated) | 100 | Shoots | + | + | + | + | + | + |
| | | Roots | + | + | + | + | + | + |
| CK1 (not inoculated) | 100 | Shoots | − | − | − | − | − | − |
| | | Roots | − | − | − | − | − | − |
| T2 (inoculated) | 500 | Shoots | + | + | + | + | + | + |
| | | Roots | + | + | + | + | + | + |
| CK2 (not inoculated) | 500 | Shoots | − | − | − | − | − | − |
| | | Roots | − | − | − | − | − | − |

+, detection of strain PW7 in tissues; −, no detection of strain PW7

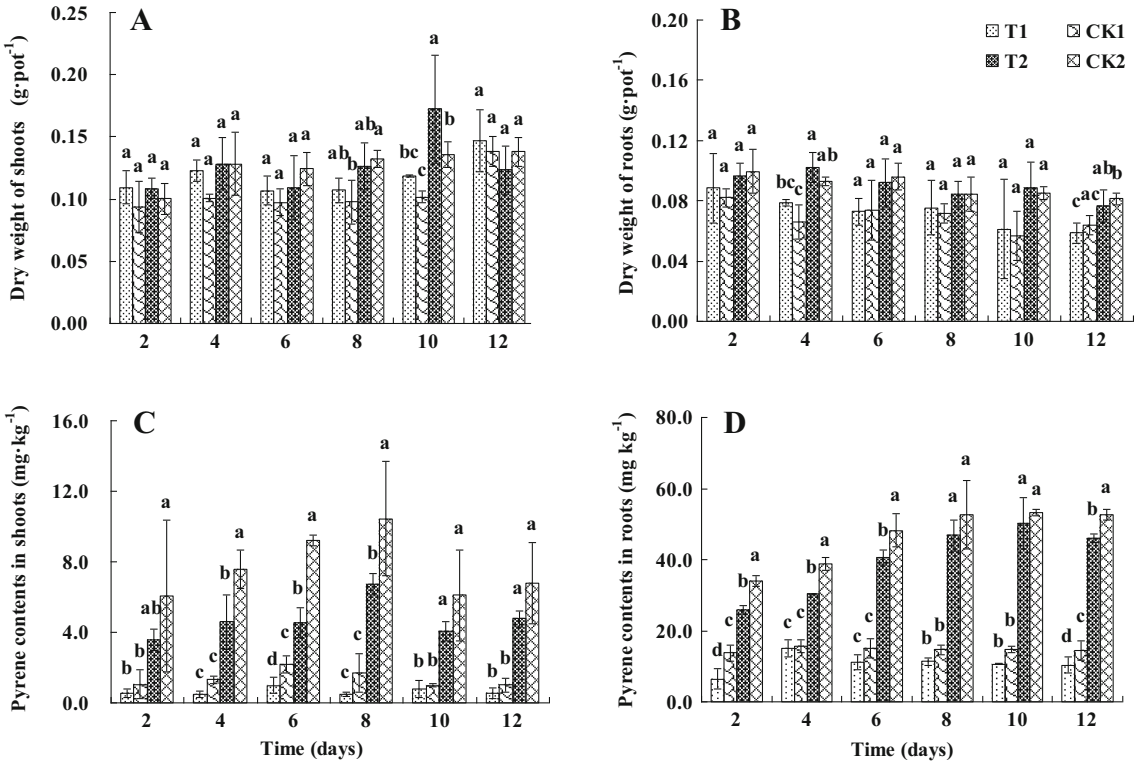


**Fig. 7** The effects of inoculating strain PW7 on the growth (**A**, **B**) and pyrene content of seedlings (**C**, **D**) exposed to 100 μg $L^{-1}$ (*CK1* and *T1*) or 500 μg $L^{-1}$ (*CK2* and *T2*) pyrene after 12 days of incubation (at 30 °C, pH 7.0). *Different lowercase letters* indicate significant differences among treatments at the same cultivation time ($P < 0.05$)

Hoagland solution with 100 μg $L^{-1}$ pyrene, the pyrene residues in T1 and CK1 were not significantly different.

The mass balance of pyrene on day 6, when the pyrene concentration significant decreased both in the seedlings and in the medium, was calculated for all the groups. In T1, 7.71 μg pyrene and 0.73 μg pyrene were detected in the solution and seedlings, respectively. In CK1, 10.97 μg pyrene and 1.28 μg pyrene were detected in the solution and seedlings, respectively; 3.82 μg pyrene was removed by inoculation with strain PW7, and 9.54 μg pyrene was removed by seedlings through uptaking, phyto-degrading, and phyto-volatilization. In T2, 35.39 μg pyrene and 4.22 μg pyrene were detected in the solution and seedlings, respectively. In CK2, 45.47 μg pyrene and 5.77 μg pyrene were detected in the solution and seedlings, respectively; 11.63 μg pyrene was removed by inoculation with strain PW7, and 133.02 μg pyrene was removed by seedlings through uptaking, phyto-degrading, and phyto-volatilization. These results suggested that endophytic strain PW7 could decrease the pyrene concentration in plants and help plants to remove the pyrene from the solution.

Inoculation of seedlings with strain PW7 decreased the shoot/root ratio of pyrene levels in seedlings. The greatest decrease was observed at 6 or 8 days after inoculation (Fig. 9). Moreover, the reduction of the ratio was greatest in those seedlings exposed to 500 μg $L^{-1}$ pyrene.

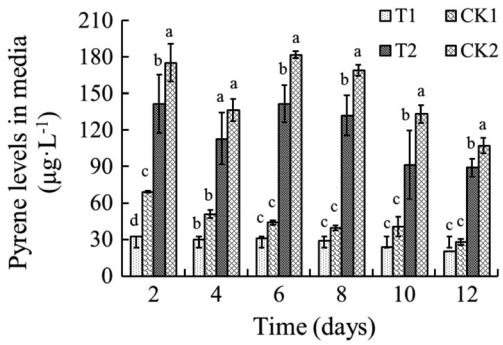


**Fig. 8** Pyrene residues in Hoagland solution. All the data result from 12 days of incubation. *Different lowercase letters* indicate significant differences among treatments at the same cultivation time ($P < 0.05$)

## Discussion

The pyrene degradation tests showed that endophytic strain PW7 had great ability to degrade pyrene. The half-life of pyrene was approximately 11 days in the cultures of strain PW7. Compared to the research reported by Wang et al. (2010), the half-life of pyrene was only 2 days longer than in a two-liquid-phase bioreactor. Moreover, the ability of degrading pyrene by strain PW7 could be great enhanced through adding other carbon sources. Strain PW7 may, therefore, have the potential to be used for pyrene degradation on

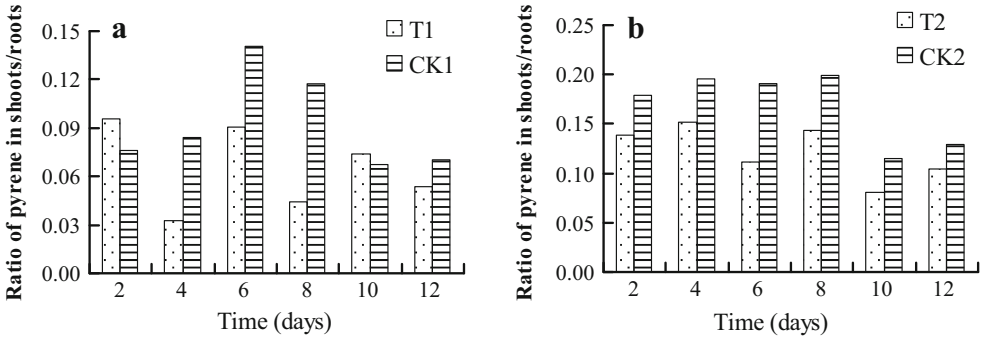


**Fig. 9** Shoot/root ratios of pyrene levels in seedlings inoculated with strain PW7 or not inoculated. **A** is for the seedlings (*CK1* and *T1*) cultivated in the solution containing 100 μg $L^{-1}$ pyrene, and **B** is for the seedlings (*CK2* and *T2*) cultivated in the solution containing 500 μg $L^{-1}$ pyrene. All data result from 12 days of incubation

an identical scale. In the previous reports, the reduction of the PAH concentration was linked to bacterial communities represented by Gram-negative bacterial strains (Lors et al. 2012). As a Gram-negative bacterium, strain PW7 has a thinner cell wall and shorter growth cycle, which results in easier mass transfer and a more efficient degradation of pyrene compared with Gram-positive strains (Ma et al. 2013). However, pyrene may also have toxic effects on strain PW7 as other bacteria reported by Maliszewska-Kordybach and Smreczak (2003). The positive correlation between pyrene removal and cell number in this study indicates that a low cell density might be the main reason for poor pyrene degradation during the first 2 days of culture, and strain PW7 seems to need time to adapt to pyrene as a carbon source. An optimal condition would help strain PW7 to adapt the toxicity of pyrene in a short period. The optimal conditions for strain PW7 were determined to be pH 7.0 and 30 °C, which is reminiscent of other strains reported by Zhang et al. (2009).

Taking advantage of endophytic bacteria might be a good way to resolve the main problems of HMW PAHs in crop plants. Some endophytes could induce the secretion of catabolic enzymes (Wen et al. 2011). In the previous research of our lab, some pyrene-degrading endophytic bacteria could degrade pyrene efficiently in the medium, but not all of them could efficiently decrease pyrene contents in plant tissues. Remarkably, endophytic strain PW7 was observed to have great ability of efficiently degrading pyrene in the medium and decreasing pyrene contents in plant tissues, and the degradation could be great enhanced through co-metabolism. Glucose was found to be the optimal co-substrate for strain PW7 to degrade pyrene ($P < 0.05$). Ambrosoli et al. (2005) also suggested that glucose could enhance the biodegradation of fluorene, phenanthrene, and pyrene. It is known that many other organic carbon sources from plants and yeast extract could improve the biodegradation of HMV PAHs (Ahmed and Ahmed 2014; Chen and Yuan 2012). After successfully colonizing interior plant tissues, strain PW7 would easily obtain optimal co-substrates and live under suitable conditions, including pH, temperature, and glucose, which are factors that likely promote the degradation of HMW PAHs in wheat.

Once successfully colonized on wheat through the roots, strain PW7 also could mitigate pyrene transfer from roots to the aerial parts of the plant. Several PAHs, including phenanthrene and anthracene, are concentrated in specific locations within the cell walls of most cortex cells and travel slowly axially towards the shoots (Wild et al. 2007). Meanwhile, Grall and Manceau (2003) and Gasser et al. (2011) reported that endophytic bacteria could colonize roots and move along the apoplastic spaces and xylem vessels. As an endophytic bacterium, strain PW7 was detected in shoots of inoculated seedlings; it is likely that the bacteria moved from the root to the shoot via the xylem. Strain PW7 has chances to get pyrene interior plant tissues after inoculated through roots. Whether strain PW7 preferentially degrades pyrene in the root remains an open question.

Wheat is an important crop in China. Thus, inoculating wheat with functional endophytic bacteria against wheat PAH contamination is of great significance. More works need to be performed on isolation functional endophytic strains and to test how these strains perform interior plant tissues. In our study, the contents of pyrene in wheat increased with increasing concentrations of pyrene in the solution. Interestingly, the content of pyrene in wheat was reduced by inoculating the wheat with endophytic strain PW7, and the risk of pyrene contamination in shoots was reduced much more than in the roots. Our results warrant further investigation of pyrene-degrading endophytic bacteria, which may have a potential to protect crops from pyrene contamination and may be used for the phyto-remediation of pyrene-contaminated soil.

## Conclusions

In our study, a pyrene-degrading endophytic bacterium *Serratia* sp. PW7 was isolated from a PAH-contaminated plant (*P. asiatica*). After the roots of wheat (*T. aestivum* L.) were inoculated, strain PW7 actively colonized the plant roots and moved

to the shoots. More importantly, strain PW7 had a natural capacity to reduce the content of pyrene in vitro and in vivo. Clearly, these findings indicate that endophytic bacteria could be used to protect crops against organic contamination and mitigate the threat of organic contamination to human health.

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