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Isolation and characterization of phenanthrene-degrading strains *Sphingomonas* sp. ZP1 and *Tistrella* sp. ZP5

He-Ping Zhao, Lei Wang, Jiao-Rong Ren, Zhuo Li, Min Li, Hong-Wen Gao *

State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai 200092, China

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Abstract

Two bacteria strains *Sphingomonas* sp. strain ZP1 and *Tistrella* sp. strain ZP5 were identified as phenanthrene-degrading ones, based on Gram staining, oxydase reaction, biochemical tests, FAME analysis, G + C content and 16S rDNA gene sequence analysis. We isolated these two bacteria strains *Sphingomonas* sp. ZP1 and *Tistrella* sp. ZP5 from soil samples contaminated with polycyclic aromatic hydrocarbon (PAH)-containing waste from oil refinery field in Shanghai, China. Strain *Sphingomonas* sp. ZP1 was able to degrade naphthalene, phenanthrene, toluene, methanol and ethanol, salicylic acid and Tween 80. Moreover, it can remove nearly all the phenanthrene at 0.025% concentration in 8 days. Strain *Tistrella* sp. ZP5 cannot degrade phenanthrene individually but it can increase the speed of phenanthrene degradation together with ZP1. The growth conditions of strain *Sphingomonas* sp. ZP1 were optimized. The result also indicated that the degradation rate of phenanthrene ranged from 250 to 1000 ppm with strain ZP1 remained nearly the same, i.e., a high concentration of phenanthrene did not inhibit both the growth of microbial strains and the phenanthrene-degradation ability. Besides, the effect of non-ionic surfactants such as Brij 30, Triton X-100 and Tween 80 on the phenanthrene degradation was determined. Such two strains may be useful for bioremediation applications.
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Keywords: Phenanthrene; Degradation; Bacteria; *Sphingomonas*; *Tistrella*

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants produced by industrial operations using fossil fuels as well as by natural events such as forest fires [1,2]. PAHs are ubiquitous pollutants found in soil at wood preservation plants, gas works, oil refineries, runoff from asphalt pavement, and combustion process. PAHs are after highly toxic, mutagenic, and carcinogenic [3], so they represent considerable environmental concerns [4–6]. The US Environmental Protection Agency has monitored PAHs as priority pollutants in ecosystems since the 1970s [7]. PAHs released into the environment could be removed though many processes, including volatilization, photo-oxidation, chemical oxidation, bioaccumulation, and adsorption on soil particles. However, the principal process for successful removal and elimination of PAHs from the environment is the microbial transformation and degradation [8,9]. Nowadays, in order to study the fate of these compounds in natural environments, considerable efforts have been focused on the isolation of microorganisms able to degrade them. Bioremediation, based on certain species of microorganisms, is a cheap and effective way to decontaminate PAHs-contaminated soils. During the last few decades, a variety of bacteria capable of degrading PAHs, particularly low-molecular weight compounds, were discovered [10,11]. Most of these bacteria belong to the genera *Agmenellum*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Berjerinckia*, *Burkholderia*, *Corynebacterium*, *Cyclotrophicus*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Nocardioides*, *Pseudomonas*, *Lutibacterium*, *Rhodococcus*, *Streptomyces*, *Sphingomonas*, *Stenotrophomonas*, *Vibrio*, *Paenibacillus* and others [11–15]. Moreover, some studies have shown that bacteria such as *Mycobacterium*, *Rhodococcus*, *Alcaligenes*, *Pseudomonas* and *Sphingomonas* are able to grow on the four-ring PAHs [16–19]. As to the practical application of the research, however, given the importance of sorption in controlling PAH bioavailability, it

* Corresponding author at: College of Environmental Science and Engineering, Tongji University, Siping Road, 1239 Shanghai, China.
Tel.: +86 21 65988598; fax: +86 21 65988598.
E-mail address: emsl@tongji.edu.cn (H.-W. Gao).

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is questionable whether microbial isolates cultivated by using this approach are important in soil and sediment environments. Grosser et al. [32] established enrichment cultures in which solid organic phases were used to reduce phenanthrene bioavailability to different degrees. Their results are consistent with the hypothesis that different phenanthrene-utilizing bacteria inhabiting the same soils may be adapted to different phenanthrene bioavailabilities. While, Yael et al. [33] found that phenanthrene mineralization was substantially enhanced upon sorption to mineral-HA complexes and the degree of enhancement was positively correlated with the fraction of sorbed phenanthrene. The stimulation is thought to be related to sorption of both the microorganisms and phenanthrene to the colloidal surfaces. Their study suggests that when sorbed contaminants are still bioavailable, the presence of surfaces may stimulate mineralization.

Recently, attention has been turned toward diverse PAHs-metabolizing bacteria, degradation mechanisms, assimilation of PAHs into bacterial strains and related catabolic genes [20–22]. In order to achieve an efficient bioremediation process, it is important to find new bacteria involved in performing a complete degradation pathway so that potentially toxic metabolites do not accumulate [23]. If this is not the case, it is important to know which metabolites are expected to accumulate so other strains able to degrade those metabolites can be added if necessary. Strains that can degrade PAHs completely and rapidly with good adjustment will be more favored although many bacteria capable of degrading PAHs have been isolated. The present work describes the isolation and characterization of two PAH-degrading bacteria from the polluted Chinese soils, including the first representative of strain *Tistrella* sp. ZP5 able to increase the speed of phenanthrene degradation when inoculated with another phenanthrene-degrading bacterium strain *Sphingomonas* sp. ZP1.

2. Materials and methods

2.1. Media

The minimal medium (MM) was composed of (l^{-1}): $Na_2HPO_4{\cdot}2H_2O$ 8.5 g, KH_2PO_4 3.0 g, NaCl 0.5 g, NH_4Cl 1.0 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $CaCl_2$ 14.7 mg. MM also contained trace elements as follows (l^{-1}): $CuSO_4$ 0.4 mg, KI 1.0 mg, $MnSO_4{\cdot}H_2O$ 4.0 mg, $ZnSO_4{\cdot}7H_2O$ 4.0 mg, H_3BO_3 5.0 mg, $H_2MoO_4{\cdot}2H_2O$ 1.6 mg and $FeCl_3{\cdot}6H_2O$ 2.0 mg. Solid MM plate was composed of (l^{-1} MM): 15 g agar. Solid LB plate was composed of (l^{-1}): 10 g NaCl, 10 g peptone, 5 g yeast extract, 15 g agar.

Ten times SSC (saline-sodium citrate buffer) was composed of (l^{-1}): NaCl 87.66 g, $C_6H_7NaO_7H_2O$ (sodium citrate monobasic) 44.12 g. 0.1× SSC: dilute 10× SSC to 100 times. (All these reagents and chemical were bought from Shanghai Reagent Factory.)

2.2. Isolation of phenanthrene-degrading bacteria

Soil samples were collected from two sites in oil refinery field in Shanghai. PAH-contaminating pollutants had been released in environment without any control there for more than 30 years. Two grams of soil sample was suspended in 200 ml of MM. Into the suspension 0.025% phenanthrene was added as the enrichment substrate and the suspension was incubated with shacking at 180 rpm at 30 °C in the dark. Eight days later, 10-ml of the aliquot was transferred to 200 ml of a fresh MM containing the same phenanthrene as above and incubated under the same conditions. This process was repeated for four times. Pure cultures were obtained by spreading each 100 μl of aliquots on the solid MM plates, before use, 0.025% phenanthrene dissolved in MM with 1% DMSO (dimethyl sulphoxide), was sprayed on the surface of the medium as the sole carbon source [10,11]. After incubation for 7 days, colonies, especially those forming clear zones on the sprayed-coated plates, were selected as the candidate phenanthrene-degrading strains [24]. All isolates were stored at −20 °C in the liquid cultures containing 20% glycerol (v/v).

2.3. Phenanthrene-degradation assay

The effect of strain ZP5 can improve the phenanthrene-degradation speed was performed by determining the phenanthrene-degradation rate of pure ZP1 culture and mixture of ZP1 and ZP5. The ZP1 liquid culture (300 ml of MM with 0.025% phenanthrene, w/v) was inoculated by transferring 3 ml of pre-culture (MM liquid medium, $OD_{600\,nm} = 0.3$, about 3×10^7 cells/ml) of ZP1 (1% v/v), the mixture of *Sphingomonas* sp. ZP1 and *Tistrella* sp. ZP5 was inoculated by transferring 3 ml of ZP1 pre-culture and 3 ml of ZP5 pre culture. ZP1 liquid culture, mixture of ZP1 and ZP5 liquid culture were incubated as described above. The flasks were incubated in darkness at 180 rpm at 30 °C, and initial pH was adjusted to 7.0. All biodegradation experiments were performed in 250 ppm phenanthrene as the sole carbon and energy source. Some factors were tested including other carbon sources, e.g. yeast extract, peptone, glucose at 1000 mg l^{-1}, and non-ionic surfactants such as Brij 30, Triton X-100 and Tween 80 at 1, 20 and 100 critical micelle concentrations (CMC). Effects of various pHs (6.0, 7.0 and 8.0) and temperatures (20 °C, 30 °C and 37 °C) have been investigated too.

Phenanthrene biotransformation by the bacteria under different concentration was determined in parallel by using Erlenmeyer flasks containing phenanthrene range from 250, 500 to 1000 ppm, respectively. Control experiment was performed to inoculate the boiled cells of mixture of ZP1&5.

After desired period of incubation, 5 ml of aliquots were picked out every day and the culture was extracted twice with 5 ml of chloroform. Every aliquot has three duplicates. The organic phase extraction were combined and dried over with anhydrous sodium sulfate. About 1.0 μl of the organic phase was analyzed by gas chromatography (GC) (Thermo FINNIGAN, TRACE GC Ultra), with FID mode, using a fused silica capillary column (30-m length × 0.25-mm i.d., 0.25-μm film thickness) and nitrogen as the carrier gas. The temperature program was set as follows: 80 °C for 1 min, then increasing by15 °C/min up to 240 °C for 1 min. Degradation rate was estimated by calculating the GC profile of substrate PAH. Cultures inoculated with boiled

Table 1
Utilization of carbon substrates by phenanthrene-degrading strains

Substrate	ZP1	ZP5
Naphthalene	++	–
Phenanthrene	++	–
Anthracene	–	–
Fluorene	–	–
Benzene	–	–
Toluene	+	–
Xylene	–	–
Phenol	+	–
DMSO	–	–
Methanol	++	+
Ethanol	++	+
Salicylic acid	++	–
Tween 80	+	+
Cyclohexane	–	–

Growth was followed by measuring the increase of $OD_{600\,nm}$ of the culture for 10 days. (++) Good growth: $OD_{600\,nm} > 0.2$; (+) growth: $OD_{600\,nm} > 0.1$; (–) no growth: $OD_{600\,nm} < 0.02$.

dead cells were used in parallel as the abiotic negative controls. Strain cell growth was followed by measuring the increase of OD600 nm of the culture. Each value represents the mean of three repeats with a standard error < 5%.

2.4. Carbon-source utilization

In addition to phenanthrene, the purified strains were also tested for growth on one of the following compounds at 0.01%: naphthalene, phenanthrene, anthracene, fluorene, and benzene, toluene, xylene and other related substrates, which were added as sole carbon sources to liquid MM according to Table 1. Sterilized MM medium containing the appropriate PAH or other carbon source was inoculated with the test strains and incubated in an orbital shaker as described above. Growth was followed by measuring the increase of $OD_{600\,nm}$ of the culture.

All chemicals were of analytical grade and obtained from Sigma or Fluka.

2.5. Biochemical test

The cells morphological properties were examined by transmission electron microscopy (TEM), and included the shape of cell, Gram-stain, the presence of spores, and colony morphology on solid LB plate. Biochemical reactions, catalase reaction, oxidase reaction, acid or gas production from carbohydrates and oxidation or fermentation from carbohydrates were determined by using the API system according to the manufacturer's instructions (Biotech, China).

Whole cell fatty acids analyses were performed by harvesting the cells after culturing at 30 °C for 72 h in MM culture with 0.025% phenanthrene. The cell pellets obtained were washed with 1 ml of *N*,*N*-dimethylformamide (DMF) to remove the undegraded phenanthrene and finally were washed twice with 0.85% NaCl to remove residue of culture medium. Further fatty acids isolation and identification was conducted by the MIDI-MIS method advanced by Sakai [25].

2.6. Determination of G + C content

G + C content of genomic DNA form the isolates was determined by making sure the degeneration temperature of DNA (T_m), respectively. DNA has its different T_m because of the different G + C% content. The DNA of the isolates were solved in 0.1× SSC solvent, G + C content can be calculated from the empirical equation: 0.1× SSC G + C% = 2.44 (T_m − 53.9).

2.7. Analyses of 16S rDNA sequences

The chromosomal DNA was isolated by using a method described by Yoon et al. [26]. 16S rDNA was amplified using two primers according to Stackebrandt and Liesack [27]: the forward primer BSF8/20: 5′-AGAGTTTGATCCTGGCTCAG-3′ (primering site corresponding to 8-27 of 16S rDNA of *Eschericha coli*) and the reverse primer BSR1521/20: 5′-AAGGAGGTGATCCAGCCGCA-3′ (primering site corresponding to 1541–1522 of 16S rDNA of *Eschericha coli*). The PCR mixtures were preheated at 94 °C for 2 min prior to running the following cycles: 94 °C for 1 min, 56 °C for 1 min, 72 °C for 2 min. A PCR was run for 30 cycles in a DNA thermal cycle (BIOER TC25/H; BIOER TEC, Hangzhou, China), employing the thermal profile [26]. At the end of the final cycle, a chain-elongation step at 72 °C for 10 min was programmed. The 16S rDNA sequences of the stains were aligned with the BLAST program on the NCBI website (http://www.ncbi.nlm.nih.gov). Phylogenetic trees were constructed using the MegAlign program (DNASTAR).

2.8. Sequence access numbers

The sequences obtained in this study were deposited in the GenBank database. The accession numbers for the three short fragments are: DQ659593 (strain ZP1), DQ659596 (strain ZP5).

3. Results and discussion

3.1. Isolation of PAH-degrading bacteria

Strains ZP1 and strain ZP5 were isolated from PAHs contaminated soil samples in oil refinery fields in Shanghai, essentially on the basis of the formation of clear zones on solid MM with sprayed phenanthrene as the sole carbon source. Compared with other strains isolated in this experiment which lost their degradation abilities after more than 3 weeks preservation, one of these three microorganisms, strain ZP1 showed a high degree of degradation of phenanthrene, while strain ZP5 can increase the speed of phenanthrene degradation when incubated together with strain ZP1. Based on Gram staining, oxydase reaction, biochemical tests, FAME analysis, G + C content and 16S rDNA gene sequence analysis, Bacteria strain ZP1 and ZP5 were characterized belonging to genus *Sphingomonas* and *Tistrella*, respectively. The photographs of cells of ZP1 and ZP2 were shown as Fig. 1. ZP5 belongs to α-*Proteobacteria* by genotypic analysis. Some bacteria strains of genus *Rhodoccus* and *Acetobacter* have been reported that can degrade phenanthrene which

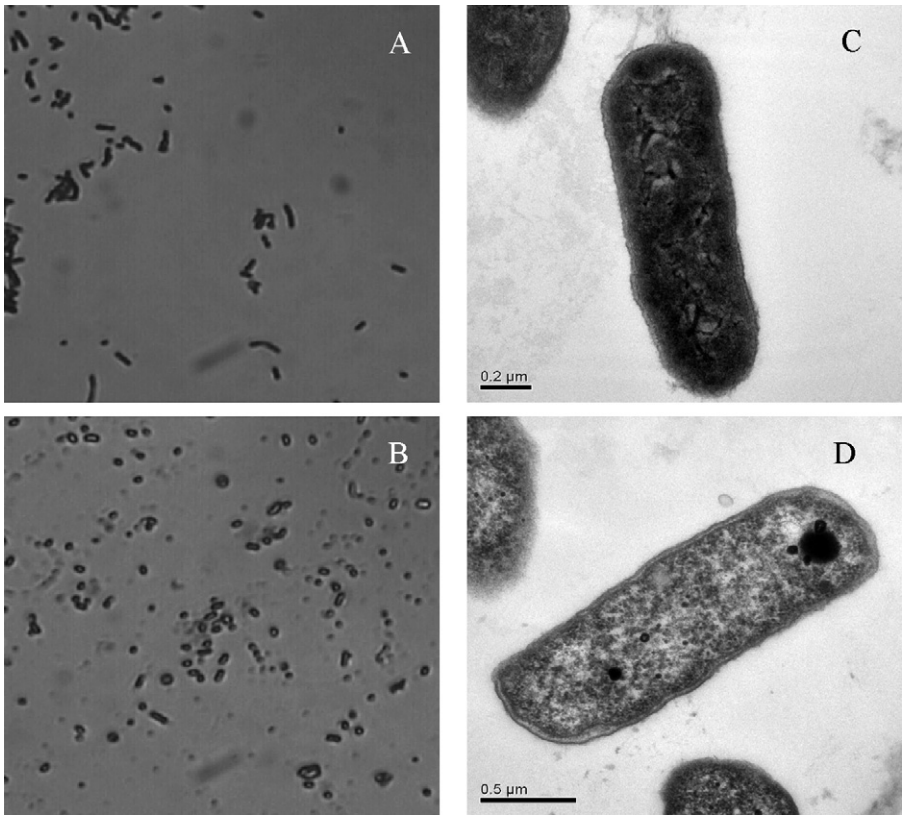

Fig. 1. Photographs of cell of *Sphingomonas* sp. ZP1 and *Tistrella* sp. ZP5. (A) Gram stain photo of ZP1 (10 × 100); (B) Gram stain photo of ZP5 (10 × 100); (C) TEM photo of ZP1 (80× k); (D) TEM photo of ZP5 (60× k).

belongs to α-*Proteobacteria*. Furthermore, many strains from genus *Pseudomonas* which belong to β-*Proteobacteria* have been reported with the phenanthrene-degradation ability.

3.2. Carbon-source utilization

More then ten carbon sources were tested as carbon substrates of these two isolates, including various low and high molecular weight PAHs and other *n*-alkanes. These chemicals were main components of crude oil and ubiquitous in contaminated soil. Strain ZP1 was able to degrade naphthalene, phenanthrene, toluene, methanol and ethanol, salicylic acid and Tween 80, which exhibited a very broad substrate profile. Strain ZP5 was found that cannot use naphthalene, phenanthrene and other PAHs as it's sole carbon and energy source after 3 weeks preservation besides methanol, ethanol and Tween 80. But accidentally, Strain ZP5 was found that can increase the speed of phenanthrene degradation when inoculated with Strain ZP1 and incubated together. The result of carbon-source utilization of these isolates was shown in Table 1.

3.3. Degradation of PAHs

The results of GC analyses show that strain ZP1 can nearly degraded all phenanthrene within 8 days, as shown in Fig. 2 for one substrate for each bacterium, the biomass increased as the remaining concentration of the substrate decreased. Accidentally, ZP5 was found that can increase the speed of phenanthrene degradation when inoculated with ZP1 and incubated together. The concentration of phenanthrene in MM culture inoculated with ZP1 and 5 almost could not be detected after 6 days. It is an interesting phenomenon because strain ZP5 was supposed that can use the intermediates which are expected to accumulate in the ZP1 culture. Strains that can degrade PAHs completely and rapidly with good adjustment will be more favored although many bacteria capable of degrading PAHs have been isolated.

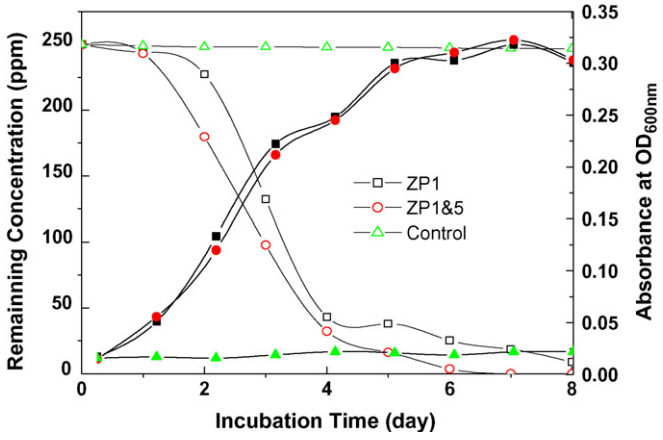

Fig. 2. Comparison of growth of ZP1 and ZP1 and 5 in phenanthrene at 250 ppm and phenanthrene biodegradation by ZP1 and ZP1 and 5, respectively. Control was performed by inoculating with dead cells (solid symbol is about degradation and hollow symbol is about the absorbance value at $OD_{600\,nm}$ which indicate the growth of bacterium).

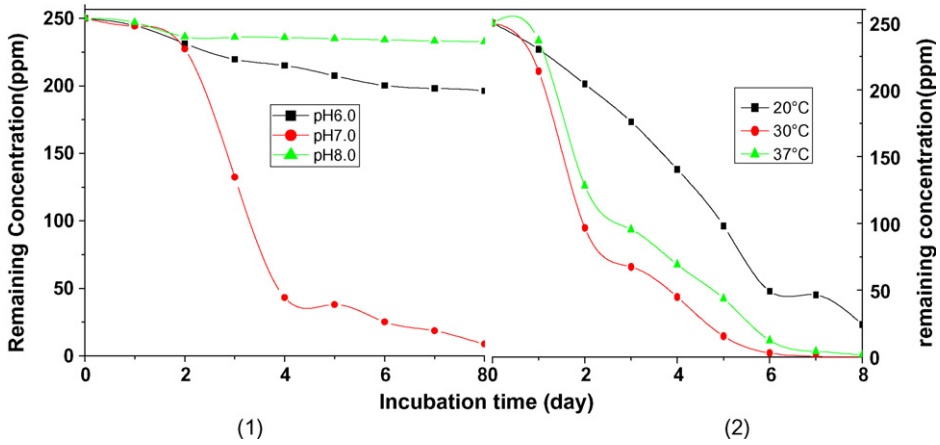

Fig. 3. Effect of different pH value (1) and change in temperature (2) on biodegradation of phenanthrene by strain ZP1 (250 ppm).

3.4. Effect of environmental condition on degradation of phenanthrene

A series of phenanthrene-degradation tests were carried out at various pH from 6.0 to 8.0 and temperatures from 20 to 37 °C. As shown in Fig. 3(1) and (2), the optimal conditions were determined to be at pH 7.0 and 30 °C. This result is similar to those reported by Kim [11]. The result still indicated that the degradation rate of strain ZP1 decreased while phenanthrene ranged from 250 to 1000 ppm (Fig. 4) which is nearly the same with the report of Yuan et al. [28]. So we suppose that biodegradation rate decreased at higher phenanthrene concentrations due to supposed increased toxicity of phenanthrene metabolites. The next study of biodegradation of phenanthrene by these two strains will focus on the microbial transformation and metabolism. Fig. 5 shows the effects of different carbon sources on the phenanthrene degradation. All these tested carbon sources can improve the speed of phenanthrene degradation. This result is accord with earlier reports of organic carbon-source supplementation, which elevated the transformation of PAHs. Zaidi and Iman [29] also indicated that supplementation of glucose did not improve degradation of phenanthrene, as carbon was not a limiting factor. In the present study, when these carbon sources were added, the phenanthrene can be moved nearly 2 days earlier than without them.

3.5. Effect of surfactants

The effects of non-ionic surfactants on phenanthrene degradation were also tested by adding Brij 30, Triton X-100 and Tween 80 at 1 CMC, 20 CMC and 100 CMC, respectively, Fig. 6 (1–3). The phenanthrene biodegradation was nearly inhibited by adding Brij 30 at all tested concentrations. As Triton X-100, it can greatly delay the phenanthrene-degradation speed at 20 CMC and 100 CMC and no significant difference was observed from the phenanthrene degradation at 1 CMC. Different from such two non-ionic surfactants mentioned above, Tween 80 can greatly improve the phenanthrene degradation whatever it is was added in any concentration. In general, the higher the CMC of surfactants added, the greater the inhibition of phenanthrene biodegradation. Surfactants are known to produce toxicity and decrease the activity of microorganisms [30]. Non-ionic surfactants contain polyoxyethylene molecules, an indication of the greater presence of hydrophilic molecules and a known inhibitor of microorganism activity.

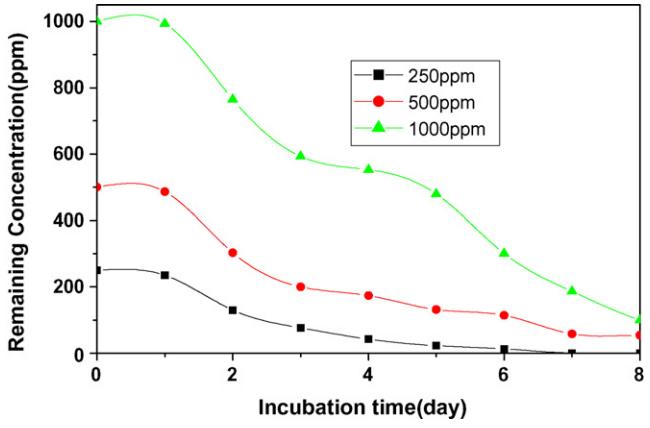

Fig. 4. Effects of changes in phenanthrene concentration on biodegradation of phenanthrene by strain ZP1.

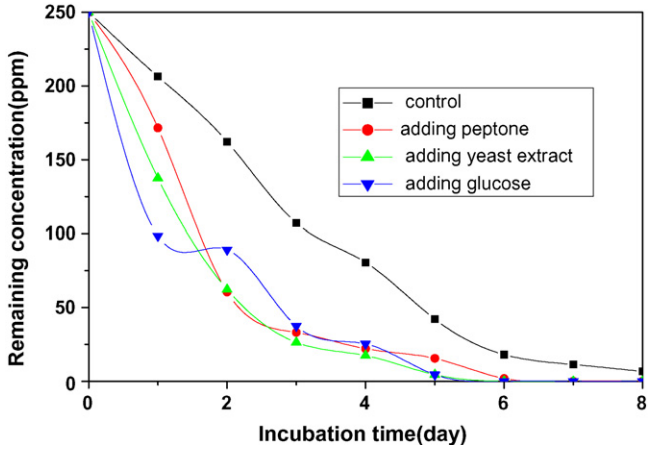

Fig. 5. Effect of various carbon sources on biodegradation of phenanthrene by strain ZP1 (250 ppm).

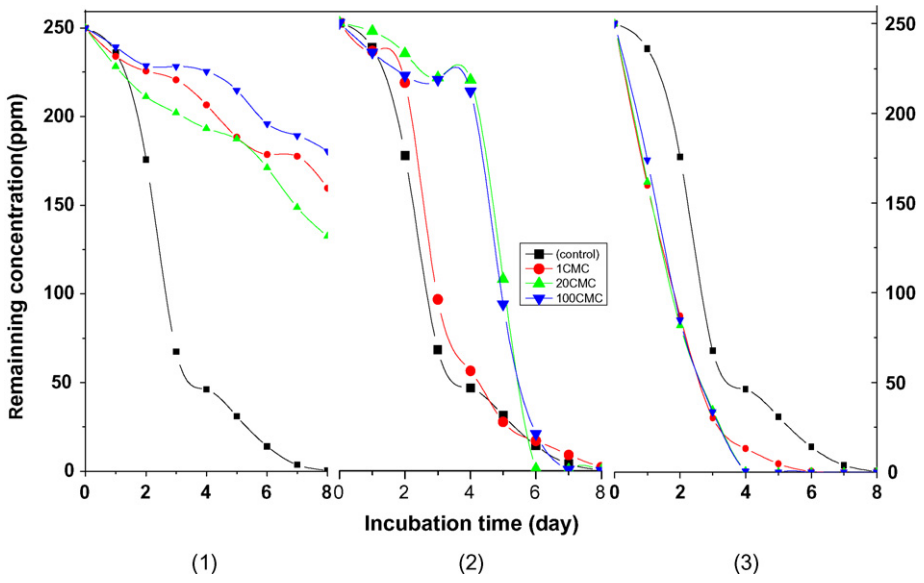

Fig. 6. Phenanthrene degradation by strain ZP1 in the presence of surfactant Brij30 (1) and Triton X-100 (2) and Tween 80 (3).

3.6. Taxonomic identification of the isolates

Strain ZP1 was found to be a Gram-negative, oxidase-positive, rod-shaped coccus and resistant to ampicillin sodium at a concentration of 25 μg/ml. Its growth temperature on phenanthrene ranged from 20 to 37 °C with an optimum at 30 °C. The fatty acids were main composed by hexadecanoic (16:0) (16.6%), 9-*cis*-hexadecenoic ($16:1^{9}$) (22.8%) and 9-*cis*-octadecenoic ($18:1^{9}$) (55.8%). The G + C content of ZP1 genomic DNA was 64.4%. Analysis of 16S rDNA gene sequence indicated that strain ZP1 belongs to the genus *Sphingomonas* (99% identity) with nearest type strain *P. xenophaga* AY611716 (Fig. 7A). *Sphingomonas* is the most well studied PAH-degrading genus as *Pseudomonas*. Our results demonstrate that

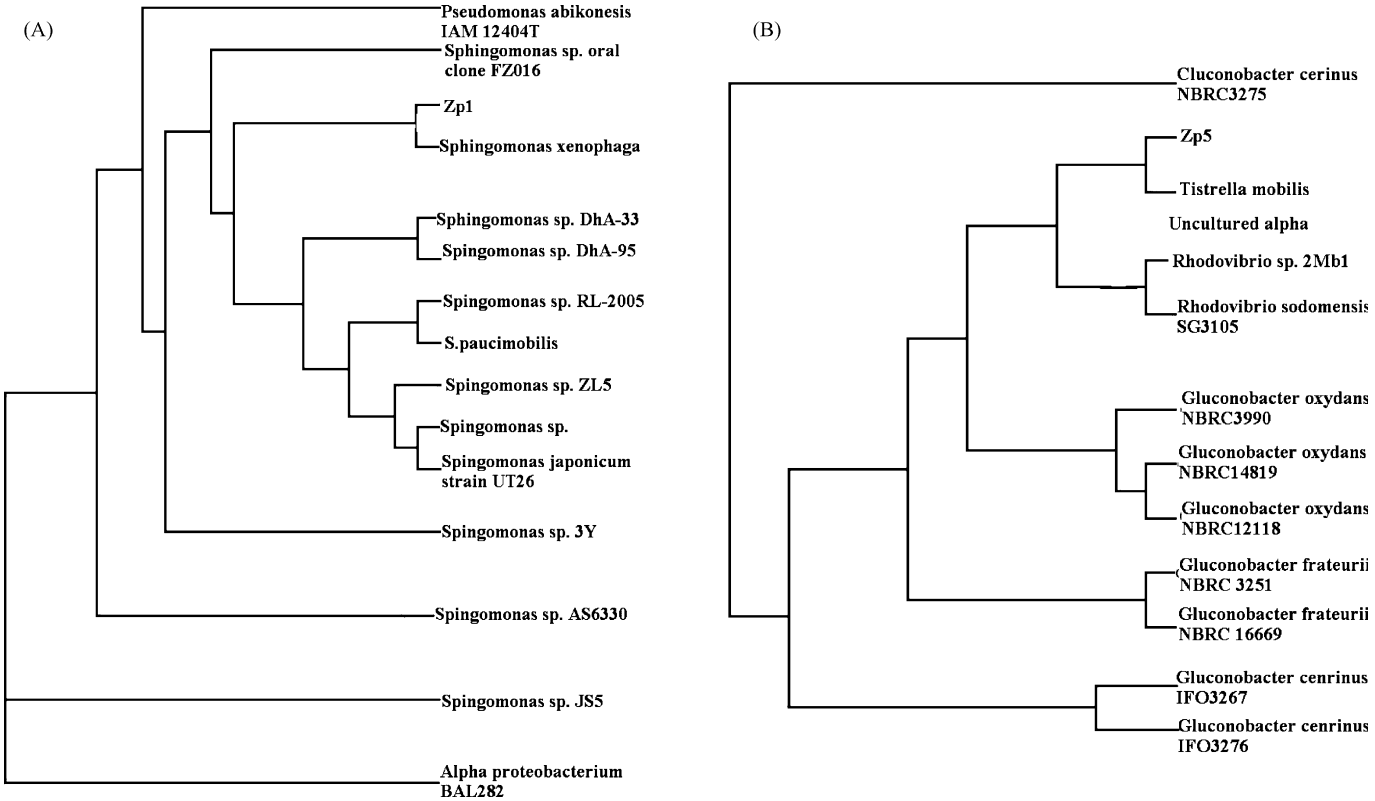

Fig. 7. Phylogenetic trees for the taxonomic location of strains ZP1 and ZP5. This dendrogram was produced by the MegAlign software program of DNASTAR ((a) ZP1; (b) ZP5.).

ZP1 can degrade phenanthrene over a wide range of temperature. This ability makes the strain attractive for field bioremediation applications.

Strain ZP5 was found to be a gram-negative, oxidase-negative, rod and resistant to cephalexin and ampicillin sodium at concentration of 20 μg/ml. Its optimum growth temperature is 30 °C. Fatty acids mainly contained 9-*cis*-octadecenoic (18:1) (37.9%), 2-hydroxy stearic (18:0) (2-OH) (35.8%) and 3-hydroxy tetradecanoic acid (14:0) (3-OH) (26.6%). The G + C content of ZP5 genomic DNA was 67.6%. Analysis of 16S rDNA gene sequence indicated that strain ZP5 belongs to the genus *Tistrella* (99% identity) with nearest type strain *T. mobilis* AB071665 (Fig. 7B). This strain is reported that with the capability of producing polyhydroxyalkanoate but never has been reported with the PAH-degradation ability. It was found accidentally that can increase the phenanthrene-degradation ability when inoculated with ZP1 and incubated together. This ability makes the strain attractive for degrading phenanthrene.

Following the enrichment procedure described above, two bacteria capable of degrading PAHs were isolated and the phenanthrene biodegradation ability is affected by changes in pH, temperature, substrate concentration, and addition of carbon sources or non-ionic surfactant. Strain ZP1 and ZP5 were belonging to *Sphingomonas* sp. and *Tistrella* sp., respectively. They were of interest because of their broad substrate and growth temperature range. Accidentally, we found that ZP5 can increase the speed of phenanthrene degradation when inoculated with ZP1 and incubated together. Actually, ZP5 was isolated from the same soil sample at beginning, but lost its phenanthrene-degradation ability after 3 weeks preservation. ZP5 belongs to α-*Proteobacteria* by genotypic analysis. *Rhodoccus* has been reported that can degrade phenanthrene which belongs to α-*Proteobacteria*. Furthermore, α-*Proteobacteria* is near to ZP1 which belongs to the genus *Sphingomonas* mentioned above by phylogenetic trees. ZP5 was reported that with the capability of producing polyhydroxyalkanoate but never with the PAH-degradation ability. In this paper, strain ZP5 was found can increase the phenanthrene-degradation speed when inoculated with ZP1 and incubated together. It has been reported that phenanthrene was metabolized by *Sphingomonas paucimobilis* via salicylate pathway [31]. Form carbon-source utilization experimental results, we suppose that the phenanthrene-degradation speed is faster by mixture of ZP1&5 than by ZP1 because ZP5 consumes the metabolized products of ZP1 in the mixed culture. These two strains may prove to be promising microorganisms for bioremediation to remove PAH-containing pollutants from contaminated sites.

4. Conclusion

Bacteria strain ZP1 with the phenanthrene-degradation ability was isolated and characterized belonging to *Sphingomonas.* Another bacteria strain which can increase the phenanthrene-degradation incubated with ZP1 together was identified as *Tistrella* sp. Their phenanthrene biodegradation ability is affected by changes in pH, temperature, substrate concentration, and addition of carbon sources or non-ionic surfactant. They were of interest because of their broad substrate and growth temperature range. Actually, ZP5 was isolated from the same soil sample at beginning, but lost its phenanthrene-degradation ability after 3 weeks preservation. ZP5 belongs to α-*Proteobacteria* by genotypic analysis. *Rhodoccus* sp. and *Acetobacter* sp. were reported that can degrade phenanthrene which belongs to α-*Proteobacteria*. Furthermore, many bacteria strains from genus *Pseudomonas* which belong to *β-Proteobacteria* have been reported with the phenanthrene-degradation ability. In this paper, strain ZP5 was found can increase the phenanthrene-degradation speed inoculated with ZP1 and incubated together. Phenanthrene can be metabolized by *S. paucimobilis* via salicylate pathway [31]. Form carbon-source utilization experimental results, we suppose that the phenanthrene-degradation speed is faster by mixture of ZP1&5 than by ZP1 because ZP5 consumes the metabolized products of ZP1 in the mixed culture. These two strains may prove to be promising microorganisms for bioremediation to remove PAH-containing pollutants from contaminated sites.

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