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Isolation and identification of phenol-degrading bacteria in the industrial wastewater from the coal tar mine of Zarand in Iran

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ABSTRACT

Phenol is one of the major aromatic and carcinogenic pollutants. Phenol degrader bacteria from the coal tar mine of Zarand have been identified with the bacterial growth assayed in different concentrations. Four samples were collected from different sites of the mine. Total heterotrophic and phenol-degrading bacteria were quantified and the results indicated that most of phenol degrader bacteria aggregated in the sludge aggregate site. Eleven phenol degrading bacteria were isolated that were capable of degrading 200 ppm phenol in 7 d, 6 bacterial strains were selected for capability of growth on more concentration of phenol and biochemical characteristics. Finally, two strains named isolates 42P and 53P were selected for analysis of 16S rRNA sequences. Strain 42P belongs to *Chryseobacterium indologenes* AHB42P while Strain P53 belongs to *Pseudomonas putida* AHB53P which is capable of degrading 1,800 ppm phenol. The results support the idea that environments in mines can serve as a source of bacterial strains for purposes of bioremediation.

Keywords: Coal tar mine; Degrading bacteria; Phenol; Aromatic hydrocarbons; Zarand mine

1. Introduction

Aromatic hydrocarbons are toxic and dangerous compounds for the environment. The major way that these compounds enter water and soil is through the wastewater of factories, industries and mines. Phenol is one of the main mono-aromatic pollutants among the variety of toxic and carcinogenic compounds derived from coal mines, gasoline, petrochemicals, wood preservation plants, pesticides, insecticides, herbicides, domestic waste, agricultural run-off, pharmaceuticals, textiles, steel industries and chemical spills [1,2]. The utilization of bacterial strains plays a crucial role in phenol bioremediation. Bioremediation has developed into an important economical method of waste remediation. Biological removal has become a favoured alternative because of lower costs and the possibility of complete mineralization. Various species of bacteria capable of degrading phenol have been isolated, mostly from water and soil environments [3,4]. Phenol is a compound with ArOH formula which is extremely toxic and found in different forms or together with other elements. Phenol is a liquid or a solid with a low melting point, but its boiling point is high because of hydrogen bonds. Phenol is slightly soluble in water due to its ability to make hydrogen bonds with water [5]. The probable technologies for the treatment of wastewater containing phenol include: chlorination, ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment. But physicochemical methods of the treatment of phenolic wastewater have inherent drawbacks due to the tendency of the formation of secondary toxic materials. Moreover,

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the physicochemical treatment processes have proven to be costly [6]. Biodegradation is preferred over the other conventional treatment methods of phenol due to its potential to degrade phenol completely and overcome the disadvantages posed by other processes. It produces no harmful end products, cost effective and most importantly maintains phenol concentration below the toxic limit. The microbes break down phenol completely and utilize it in the TCA cycle for energy production [7]. Many aerobic bacteria can use aromatic compounds as a sole carbon source and energy. Aerobic degradation of phenol with pure cultures, especially *Pseudomonas* sp., has been widely studied. Phenol biodegradation studies with the bacterial species have resulted in bringing out the possible mechanism and also the enzyme involved in the process. These strains are capable of utilizing phenol as a sole carbon source [8,9]. The wide variety of microorganisms that can aerobically degrade phenol include pure bacterial cultures such as: *Rhodococcus* spp. [10], *Bacillus brevis* [11], *Bacillus* sp. [12], *Nocardioides* [13], *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* [14]. Objectives of the present research were to isolate and characterize indigenous bacterial strains from industrial waste in coal mines that can tolerate and degrade high concentration of phenol. In addition, molecular characterization of the strains has also been carried out.

2. Materials and methods

2.1. Collection of samples

Water and sludge samples were collected from four contaminated zones in the Kerman Province coal tar mine of Zarand (Iran) they include: coal vacate site (MC), sump site (IB), sludge aggregate site (SA) and Near sludge aggregate site (NF). The selected four sites are adjacent to coal tar mine located at Zarand (latitude: N30° 47′ 56.206″, Longitude: E56° 35′ 59.910″) (Fig. 1). This site was selected for isolation of bacteria, since the phenol contaminations of coal tar mine are at high levels. The phenol concentrations in the samples were determined by (Gibb's reagent) method as following: MC (550 ppm), IB (650 ppm), SA (660 ppm) and NF (530 ppm). The contaminated samples (water and sludge 300 mL) were taken from 1 to 10 cm below the surface of land using a sterile knife. Afterwards, samples were collected into sterile jars, placed on ice and immediately transported to the laboratory for further experiments [15].

2.2. Enumeration of heterotrophic and phenol-degrading bacteria in collected samples

Total heterotrophic and phenol-degrading bacteria were quantified with the most probable number (MPN) procedure using the microtiter plates and colony forming unit (CFU) method. In CFU method for enumeration of heterotrophic and phenol-degrading bacteria, cells present in the sludge or water samples were serially diluted and plated (100 μL) on nutrient agar (NA) and Bushnell Haas (BH) agar media containing 200 ppm phenol, respectively. Plates were incubated at 30°C ± 1°C. After 2 d, the numbers of grown colonies were counted. Results were expressed as CFUs per mL of water (CFU mL^{-1}). All experiments were done in triplicate [16]. MPN of heterotrophic and phenol-degrading bacteria was carried out according to the method described by Brown and Braddock [17].

Fig. 1. Location of the sampling sites in coal tar mine of Zarand, Kerman-Iran. (1) Coal vacate site (MC), (2) sump site (IB), (3) sludge aggregate site (SA) and (4) near sludge aggregate site (NF) (satellite position which was taken from Google).

2.3. Isolation and screening of phenol-degrading bacteria

The BH medium consisted of (per liter of distilled water): 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3 and 0.05 g $FeCl_3$. The BH medium also contained trace elements solution which were the following (L): $ZnSO_4$ 5.0 g, $FeCl_3$ 2.3 g, $MnSO_4$ 5.0 g and $(NH_4)_6Mo_7O_{24}$ 1.0 g. Then 200 ppm of phenol as sole carbon source and energy were added to the medium. After that 5 mL of contaminated water and sludge samples were added to Erlenmeyer flasks containing 100 mL of medium and the BH medium was shaken on an orbital shaker (180 rpm, INFORS AG, Switzerland) at 30°C for 7 d. Then 5 mL aliquots were moved to a fresh medium. After a series of two further subcultures, inoculums from the flask were streaked out and phenotypically different colonies purified on BH agar medium. Phenotypically various colonies obtained from the plates were transferred to a fresh medium with phenol to eliminate agar-utilizing bacteria. The procedure was repeated and isolates only exhibiting pronounced growth on phenol were stored in stock media with glycerol at –20°C [18,19].

2.4. Identification of the strains

2.4.1. Biochemical tests

The isolated bacteria were identified based on morphological observation and biochemical characterization. The tests involved were Gram staining, catalase, oxidase, nitrate, TSI, oxidation/fermentation, citrate and indole tests. Bergey's Manual of Determinative of Bacteriology was used as a reference to identify the isolates [20,21].

2.4.2. Molecular identification of bacteria

The strains were identified precisely by a molecular method based on 16S rRNA gene amplification and sequencing. Total DNA extraction of bacterial strains was carried out with the (cetyltrimethylammonium bromide [CTAB]) method [22]. 16S rRNA gene was amplified in thermal cycler using forward primer Bac27-F (5′-AGAGTTTGATCCTGGCTCAG-3′) and universal reverse primer Uni-1492R (5′-TACGYTACCTTGTTACGACTT-3′). The amplification reaction was performed in a total volume of 25 μL consisting, 2 mM $MgCl_2$ (1 μL), 10X PCR reaction buffer (200 mM Tris; 500 mM KCl) (2.5 μL), 2 mM each dNTP (2 μL), 0.15 mM each primer (1 μL), 1U (0.5 μL) Taq DNA polymerase (Qiagen, Hilden, Germany) and 2 μL of template DNA (50 pmol). The distilled water was added for the remainder of the reaction (15 μL). The amplification of 16S rRNA gene was performed according to these conditions: one cycle of pre-denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, primers annealing at 54°C for 1 min, and extension at 72°C for 1.30 min, and also final extension at 72°C for 5 min [23,24]. The PCR product was electrophoresis using a horizontal 2% (w/v) agarose gel in TBE buffer (Tris 0.089 M, boric acid 0.089 M, EDTA 0.002 M, pH 8.5). Gels were stained in a solution of ethidium bromide and visualized with a UVP UV transilluminator (UVP Inc., San Gabriel, CA). The 16S rRNA amplified was sequenced with BigDye terminator V3.1 cycle sequencing kit on an automated capillary sequencer (model 3100 Avant Genetic Analyzer, Applied Biosystems, USA). The assembled sequence was subjected to BLAST Server and DNA Data Bank of NCBI. The sequences of 16S rRNA gene of closely related validly published species were retrieving for phylogenetic analysis of the isolated strains. Alignment of sequences was carried out using Clustal W and the phylogenetic analysis was performed to determine the evolutionary relationship of the strains with other validly published strains. Phylogenetic trees were constructed using neighbor-joining algorithms contained in MEGA-4 software package [25].

2.5. Assay the growth of phenol-degrading bacteria and analysis of residual phenol

The pre-culture of bacteria was done in NB medium. The bacteria added to experiment was equal to the number that calibrated to McFarland standard 0.5 (1×10^8 CFU mL^{-1}). After incubation of strains at 30°C ± 1°C for 1 week on rotator shaker (180 rpm INFORS AG, Switzerland) growth curves of the isolates were routinely assessed indirectly by turbidity measured as optical density (OD 600 nm) in a UV–visible spectrophotometer (Shimadzu UV-160, Japan) [26]. Also, the OD was checked for viable cell by culture of last growth on NA. The phenol concentrations in the samples were determined using 2,4-dichloroquinone-4-chloroimide dyes (Gibb's reagent) method, which reacted with phenol and produced a blue colour compound. For assay of residual phenol, the medium was centrifuged and 150 μL of medium supernatant (adjusted to pH = 8) was mixed with 30 μL of $NaHCO_3$ (adjusted to pH = 8). Then, 20 μL of Gibb's indicator was added to the mixture and vortexed, then kept for 15–45 min at room temperature with a thermo-mixer. The mixture absorbance was read at 630 nm [27,28].

2.6. Emulsification index and bacterial adhesion to hydrocarbon

The emulsification activity (E_{24}) of culture samples were determined by adding 2 mL of hydrocarbon to the same amount of culture, vortexed for 2 min, and left to stand for 24 h. The E_{24} index is given as percentage of height of emulsified layer (mm) divided by total height of the liquid column (mm). Bacterial adhesion to hydrocarbon was carried out according to the procedure that was explained by Priya and Usharani [29], and Pruthi and Cameotra [30].

2.7. Growth assessment of isolated bacteria in different concentrations of phenol

The pre-culture of bacteria was done in NB medium. The bacteria added to experiment was equal to the number that calibrated to McFarland standard 0.5 (1×10^8 CFU mL^{-1}). In order to determine the bacterial growth curve in different concentrations of phenol, the BH medium was supplemented with various concentrations of phenol (200, 300, 400 and 500 ppm). The flasks were incubated for 7 d at 30°C ± 1°C on an orbital shaker, operating at 180 rpm (Shaker INFORS AG, Switzerland). Cell growth was monitored by measuring OD (600 nm) of overnight culture [31,32].

2.8. Extraction of residual phenol from culture media and gas chromatography

In this research, phenol was extracted from samples by the method that was described by Amzad et al. [11] with some modification as follows: after 7 d of incubation, 10 mL of BH broth was centrifuged and supernatants was used to determine residual phenol. After that, 10 mL of dichloromethane was added and the mixture was then shaken in a vigorous manner for 1 h. After that, the water–solvent was transferred to the separator funnel and then allowed to stand in a rack for 10 min. The dichloromethane layer (extract) was left in the separator funnel. The extract was transferred into a volumetric flask and kept in a cool place. For removal of residual water from the sample extract, the extract was treated with anhydrous sodium sulfate (2 g) and placed in a funnel and slightly watered to make a layer of solid form that does not mix with the extract [33,34]. The GC analysis of the crude dichloromethane water extract of the samples was performed using a Varian GC (Model Shimadzu GC-14B, Japan). 1 μL was injected in Shimadzu GC-14B, equipped with a flame ionization detector. An electron ionization system with the ionization energy of 70 eV was used for the detection of GC. Helium was used as a carrier gas with a constant flow rate of 1 mL min^{-1}. The capillary column used was a silicone Ph Me (12 m × 0.2 mm i.d × 0.33 μm film thickness). The initial column temperature was 40°C, to 130°C (6°C min^{-1}). Temperatures of the injector and detector were 300°C and 330°C, respectively. The percentage concentration of phenol biodegradation (*D*%) was calculated by using the following equation: $D\% = 100\ (CI-CF)\ CI^{-1}$ that CI was the initial concentration of phenol, CF was the final concentration of phenol after 7 d of incubation [35].

3. Results

3.1. Quantity of heterotrophic and phenol-degrading bacteria in collected samples

The quantity of heterotrophic and phenol-degrading bacteria was determined in all collected samples by two enumeration method: CFU and MPN. The results are presented in Table 1.

As shown in this table, the highest quantity of heterotrophic and phenol-degrading bacteria in Zarand coal tar mine relate to SA site. Although, in the coal vacate site (MC), the quantity of heterotrophic and degrading bacteria was low in comparison with other mine sites.

3.2. Screening and identification of phenol-degrading bacteria strains

In this study, 11 phenol-degrading bacteria were isolated from enrichment cultures BH that were incubated at 30°C ± 1°C for 7 d. Six bacterial strains were selected for capability of growth on more concentration of phenol 300–400 ppm. Then, bacteria were first classified by biochemical tests. The results of the biochemical tests are shown in Table 2.

According to this table, all isolated bacteria were gram negative and most of the isolates lack motility. Two isolates strains 42P and 53P were capable of utilizing different concentrations of phenol. As, 42P degraded maximum up to 400 ppm, 53P was capable degrading high concentration of phenol (1,800 ppm). Molecular identification of these two isolates was performed by amplification and sequencing the 16S rRNA gene and comparing them with the database of known 16S rRNA sequences (Table 3).

Table 1
Quantity of heterotrophic and phenol-degrading bacteria in collected samples from the coal tar mine

Sample	MPN cell g^{-1} phenol-degrading bacteria	MPN cell g^{-1} heterotrophic bacteria	CFU g^{-1} phenol-degrading bacteria	CFU g^{-1} heterotrophic bacteria
SA	4.6×10^6	1.1×10^{12}	1.9×10^4	1.9×10^6
IB	2×10^5	1.5×10^{10}	1.5×10^4	1.7×10^6
NF	1.9×10^6	2.1×10^{11}	1.6×10^4	1.6×10^6
MC	1.7×10^6	1.5×10^{10}	1.2×10^4	2.5×10^4

Table 2
Results of biochemical tests for strains

Strain name	Gram stain	Catalase	Oxidase	O	F	Nitrate	Citrate	S	I	M	TSI
P54	−	−	+	+	+	+	+	−	−	+	Alkali/alkali
P52	−	+	−	+	+	−	−	−	−	−	Alkali/alkali
P42	−	+	+	+	+	+	+	−	+	−	Alkali/alkali
P57	−	+	−	−	−	−	−	−	−	−	Acid/alkali
P41	−	+	+	+	+	+	+	−	+	−	Alkali/alkali
P53	−	+	+	+	+	−	+	−	−	+	Alkali/alkali

Symbol used: +, positive response; −, negative response; O, oxidation; F, fermentation; S, sulfide gas; I, indole and M, motility tests.

Table 3
Percentage of similarity of our sequences with other sequences in the DNA data bank

Strain description	Ident	Accession	Query cover
Chryseobacterium sp. H2(2016) 16S ribosomal RNA gene, partial sequence	97%	KU359255.1	94%
Chryseobacterium sp. LDVH 42/00 16S ribosomal RNA gene, partial sequence	97%	AY468475.1	94%
Chryseobacterium indologenes strain N6 16S ribosomal RNA gene, partial sequence	97%	KC189901.1	94%
Pseudomonas sp. VS-74 16S ribosomal RNA gene, partial sequence	93%	FJ497688.1	82%
Pseudomonas sp. VS05_23 16S ribosomal RNA gene, partial sequence	94%	FJ662884.1	78%
Pseudomonas sp. VS-77 16S ribosomal RNA gene, partial sequence	94%	FJ497690.1	77%

The molecular identification shows that these two isolated bacteria (strains 42P and 53P) belong to *Chryseobacterium indologenes* AHB42P and *Pseudomonas putida* AHB53P, respectively. The sequences of these two strains were submitted to the genetic sequence database at the National Center for Biotechnology Information (NCBI). The Gene bank ID of the strains in NCBI is: LN866620 (Strain AHB42) and LN866621 for (Strain AHB53P). The phylogenetic trees of these two strains are shown in Fig. 2.

3.3. Bacterial adhesion to hydrocarbons test and emulsification activity (E_{24}%) of isolated strains

The emulsification activity and bacterial adhesion to hydrocarbon (BATH) test were examined for all isolated bacteria to selection of prevalent strains. The results for BATH and E_{24}% tests are shown in Table 4.

The high percentage of BATH belong to the 53P (52%) and the high percentage of E_{24} related to 42P strains (23%).

3.4. Growth curve and phenol degradation by selected isolated strains in different concentrations of phenol

Isolated strains were evaluated for their phenol-degrading potential in different concentrations of phenol. The results showed 11 isolated bacteria capable of utilizing phenol at 200 ppm concentrations. However, six strains could grow and degraded phenol at 300 ppm concentrations. Between all isolated bacteria, two strains (42P and 53P) showed phenol biodegradation at 400 and 1,800 ppm concentrations after 7 d. The results for phenol degradation and growth of six isolated bacteria are presented in Table 4. According to this table, strain 53P reached maximum OD (1.06) after 7 d and approximately degraded 98% of phenol at 200 ppm concentrations. Growth curves of the strains 53P and 42P are presented in Fig. 3, According to this figure, strain 53P is more effective in biodegradation than strain 42P. The growth curve and phenol degradation by *P. putida* strain 53P at 600 ppm phenol concentrations during 7 d is presented in Fig. 4. As shown in this figure, the highest growth occurred after 72 h of inoculation.

3.5. Effect of different concentrations of phenol on growth of selected strains

The effect of different concentrations of phenol (700–1,800 ppm) on the growth of selected bacterial strain 53P was measured. The results are shown in Figs. 5 and 6. As depicted in Fig. 5, the maximum growth occurred for strain 53P at 1,000 ppm with OD (1.85) after 7 d. Although strain 53P could degrade higher concentrations of phenol compared with strain 42P, according to Fig. 6 this strain had better growth at 1,800 ppm OD (1.55).

3.6. Analysis of residual phenol degradation by strain 53P with GC

The results of gas chromatography (GC) indicated that the strain *P. putida* AHB53P could remove more than 95% of phenol at 1,000 ppm concentrations in the culture medium after 7 d of incubation. The results of GC showed that phenol dramatically decreased by strain 53P compared with blank (Fig. 7).

4. Discussion

Isolation of phenol-degrading bacteria from industrial waste/effluent is well documented. Due to the presence of phenol in industrial wastewater, persistent bacteria are often well adopted. The coal tar mines produce the highest percentage of phenol and are a source of phenol pollution in the environment. Subsequently, we selected this site due to the isolation of reach high phenol degrader bacteria. The isolation and identification of bacterial genera capable of degrading phenol demonstrated the dominance of genus *Pseudomonas*, which may be because of its spread distribution in water and soils. Other genera of bacteria including *Burkholderia*, *Acinetobacter*, *Ralstonia*, *Klebsiella*, *Bacillus* and *Rhodococcus* were illustrated as the degraders of phenol [36]. Gonzáles et al. [37] showed degradation capacity of more than 90% of 500 ppm phenol in 25 h by immobilized cells of *Pseudomonas putida* ATCC 17484 [37]. Yang and Lee [38] have reported that the strain *Pseudomonas resinovorans* was

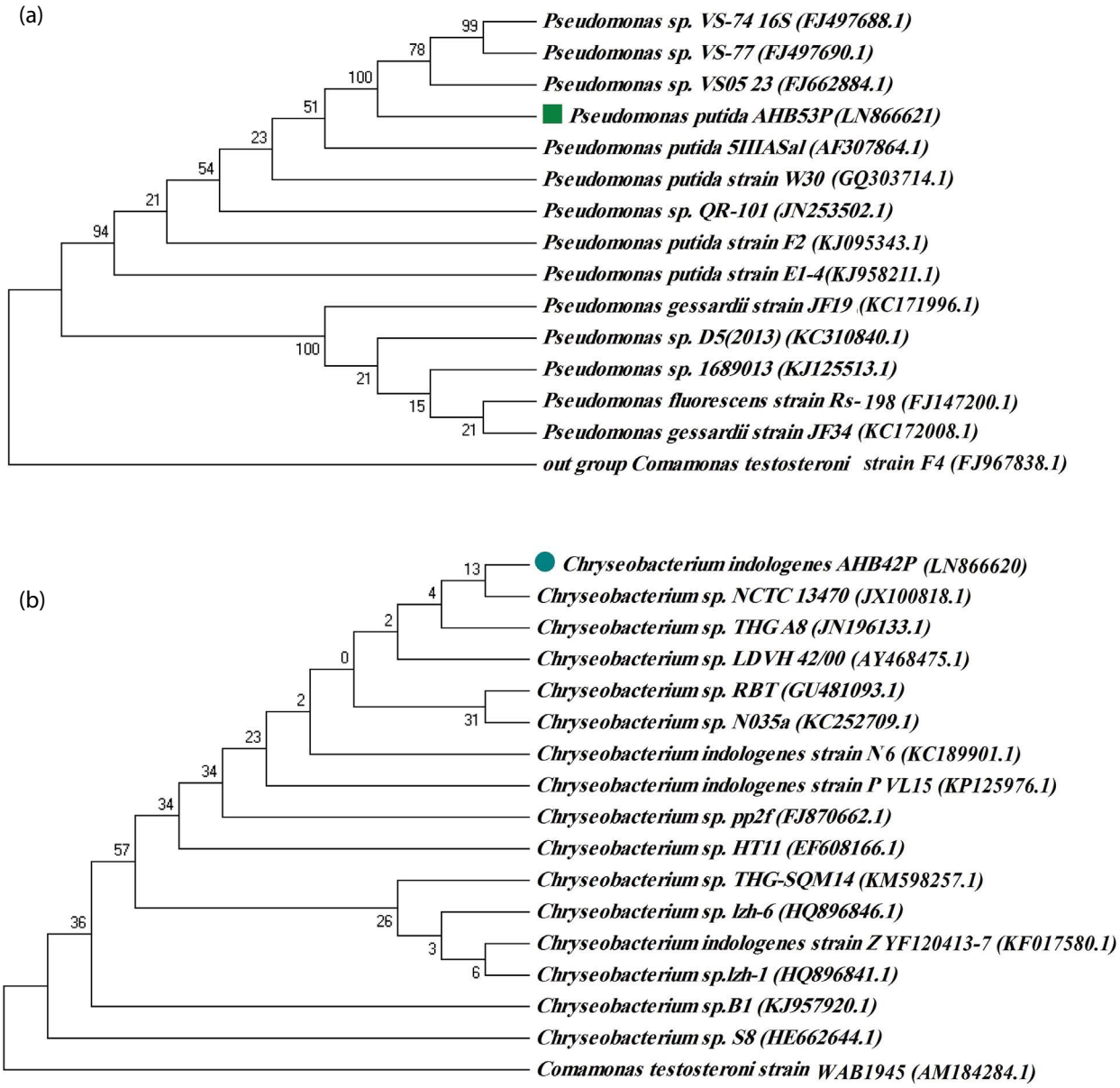

Fig. 2. Phylogenetic tree showing the inter-relationships of strains AHB42P and AHB53P with the most closely related type strains of the genus *Pseudomonas* inferred from sequences of 16S rRNA gene. The tree was generated using the neighbor-joining method. Bootstrap values, expressed as a percentage of 1,000 replications, are given at the branching point. The accession number of each strain is shown in parenthesis.

Table 4
Qualitative degradation, rate of phenol removal % at 200 ppm, growth (value of O.D600), emulsification activity (E_{24}%) and cell surface hydrophobicity (BATH %)

Strain	Qualitative degradation	Rate of phenol degradation (%)	Value of O.D600	(E_{24} %)	(BATH %)
P54	+++	86	0.722	14	5
P52	+++	97	0.809	6	22
P42	+++	98	0.630	23	24
P57	++	62	0.6	5	4
P41	+++	96	0.76	5	5
P53	+++	98	1.06	16	52

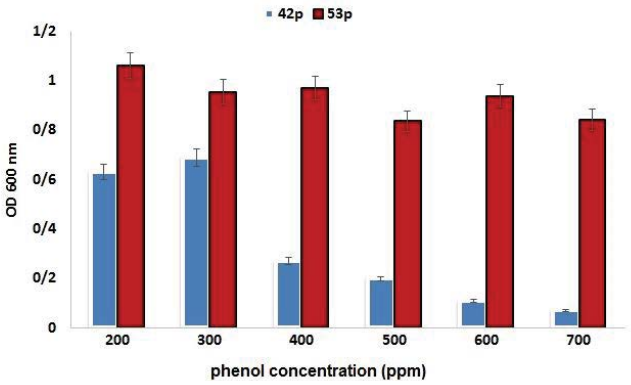

Fig. 3. Optical density (OD) of bacteria strains (53P and 42P) in different concentrations of phenol.

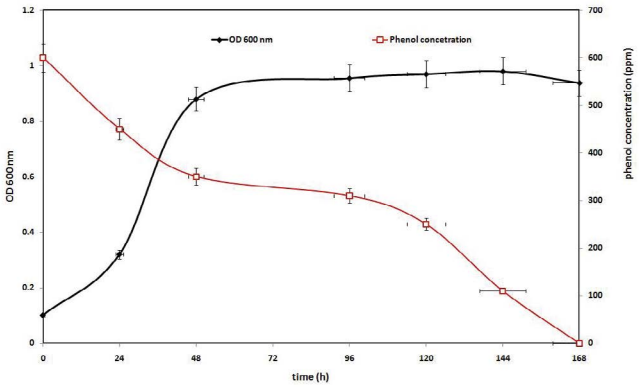

Fig. 4. Growth (as OD 600 nm) and phenol degradation in the culture of *Pseudomonas putida* AHB53P at 30°C in 600 ppm phenol concentrations during 168 h.

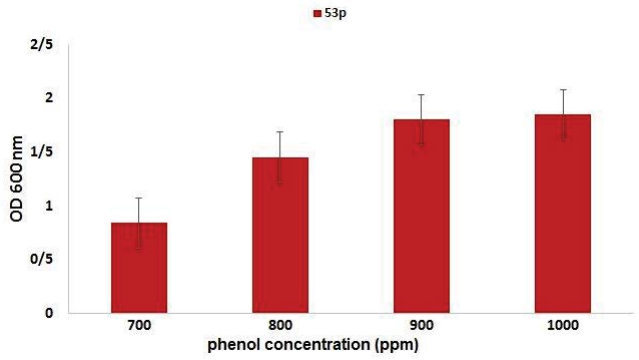

Fig. 5. Effect of different concentration of phenol 700–1,000 ppm on growth of strain 53P.

unable to tolerate an initial concentration of phenol more than 600 ppm. Li et al. [39] examined the growth kinetics of a psychrotroph, *Pseudomonas putida* LY1, while growing on phenol as the sole carbon and energy source. These bacterial strains could completely biodegrade 200 ppm phenol across

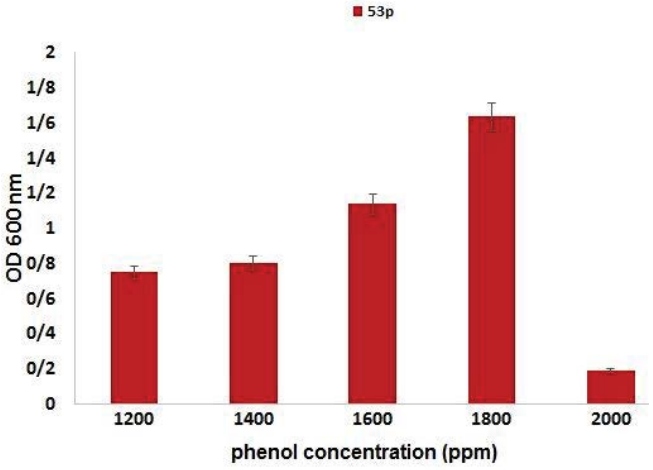

Fig. 6. Effect of different concentration of phenol 1,200–1,800 ppm on growth of strain 53P.

a temperature range from 2.5°C to 35°C, with an optimum temperature of 25°C. High initial phenol concentrations 800 ppm were inhibitory to bacterial growth. Zheng et al. [40] have reported that the microbial strain *Pseudomonas aeruginosa* HSD38 is able of degrading up to 500 ppm of phenol below the detection level but unable to tolerate more than 700 ppm of initial concentration of phenol. In the present investigation, attempts were made to isolate phenol-degrading bacterial strains from contaminated sites at coal tar mine of Zarand in Iran. Eleven phenol-degrading bacteria were isolated that were capable of degrading 200 ppm phenol during 168 h. Six isolated strains showed more degradation up to 300 ppm. The two isolated bacteria (strains 42P and 53P) belonging to *C. indologenes* AHB42P and *P. putida* AHB53P could tolerate more phenol concentration up to 400 and 1,800 ppm, respectively, but high phenol concentrations 2,000 ppm have inhibitory effect on bacterial growth. The strain *P. putida* AHB53P can degrade 95% of phenol 1,000 ppm after 7 d incubation. In this study, it is confirmed that bacterial strains that have high hydrophobicity can produce more bio emulsifier and these mechanisms lead to better biodegradation of phenol. The emulsification activity (E_{24}) and the BATH were done because of these factors related to the surface of degrading bacteria and bacteria that have these characteristic better degraded and absorbed pollutants. Since strain 42P has high emulsification activity (23%) and strain 53P has high cell surface hydrophobicity (52%), can degrade phenol in more quantity and the higher concentration than other strains. In the study of Hassanshahian et al. [41] for isolation of alkane-degrading bacteria from petroleum reservoir wastewater, *Rodococcuserythropolis* strain G2 and *Acintobacterpiechaudii* strain O1 have high cell surface hydrophobicity (27, 32 BATH%) and emulsification activity (30.8, 35.7 E_{24}%), can degrade alkane in more quantity and higher concentration than other strains. In the research of Talaie et al. [42], Hassanshahian and Mohamadian [43], and Hassanshahian [44], for screening of oil-degrading bacteria from the oil-contaminated soils, the A-12 strain resulted in the best emulsification index that was about 36% and could remove

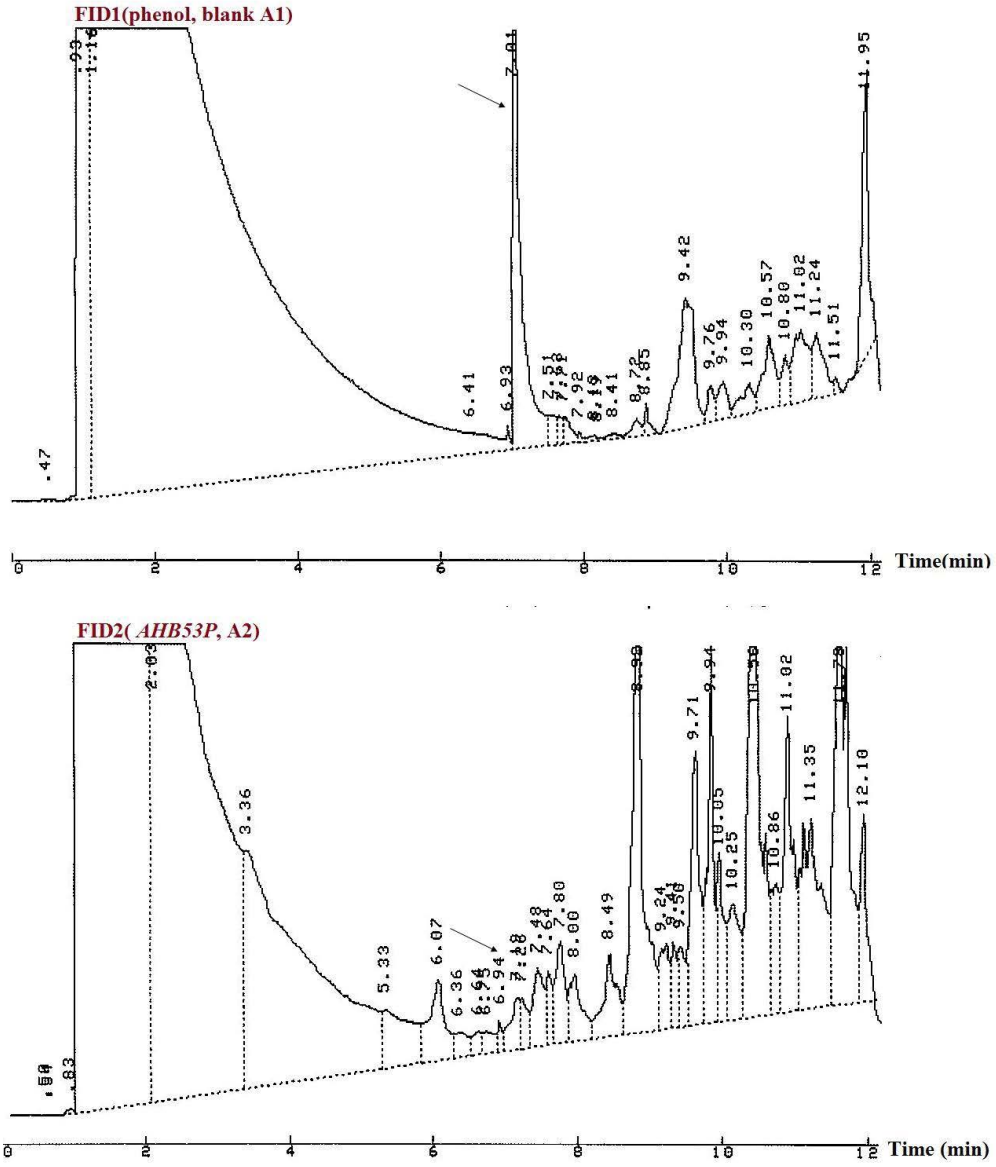

Fig. 7. Analysis of phenol 1,000 ppm by GC after 168 h incubation. FID1 (A1): As blank, and FID2 (B2): By strain AHB53P.

46% and 37% of the total petroleum hydrocarbons and aromatic compound. This study showed that a bacterium *P. putida* AHB53P had the ability to degrade phenol which is a common environmental pollutant with toxic, genotoxic, mutagenic and/or carcinogenic properties. This work has provided a useful guideline in evaluating potential phenol degraders isolated from coal mine environment [45,46].

5. Conclusion

Environments of coal tar mines polluted with some chemical pollutants. A few works done in the environments of coal tar mines for isolation and characterization of phenol-degrading bacteria. In the present study, phenol-degrading bacteria were isolated from coal tar mine at Zarand. Two strains *Chryseobacterium indologenes* AHB42P and *P. putida* AHB53P were obtained as prevalent degrading bacteria. This work describes *P. putida* AHB53P strain efficiency to degrade phenol up to 1,800 ppm, which is very rapid comparatively with other published strains evaluated for phenol degradation. This bacterium may be further exploited in the aromatic contaminated areas after careful investigations.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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