Degradation of pyridine by one *Rhodococcus* strain in the presence of chromium (VI) or phenol

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ABSTRACT

A *Rhodococcus* strain, Chr-9, which has the ability to degrade pyridine and phenol and reduce chromium (VI) (Cr (VI)) was isolated. The strain could grow with pyridine as the sole carbon and nitrogen source, and its pyridine-degradation capability was enhanced by $100\,mg\,l^{-1}$ phenol; however, the degradation of pyridine was inhibited when the phenol concentration was greater than $400\,mg\,l^{-1}$. The hydroxylation of pyridine suggested that the stimulation and inhibition of phenol to the pyridine degradation may be attributed to competition of phenol and pyridine for the hydroxylase gene. Strain Chr-9 was also able to reduce Cr (VI) when glucose and LB was used as the carbon source; however, the Cr (VI) reduction did not occur when pyridine was the sole carbon and energy source. In addition, strain Chr-9 could reduce Cr (VI) and simultaneously degrade pyridine in the presence of glucose. To the best of our knowledge, strain Chr-9 is the first *Rhodococcus* strain reported to degrade pyridine in the presence of Cr (VI), and the first strain with the pyridine degradation being stimulated by low concentrations of phenol.

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1. Introduction

Pyridine, which is a chemical commonly discharged by the chemical and petrochemical industries, is a serious pollutant worldwide that is also listed as a priority pollutant by the USEPA because of its toxic, teratogenic and carcinogenic effects [1]. In general, the heterocyclic structure of pyridine makes it recalcitrant to microbial degradation and persistent in nature. However, due to the great microbial genetic plasticity and metabolic versatility in nature, some microorganisms are capable of degrading pyridine [2–7] and its derivatives [8–12] under aerobic and anaerobic conditions. These microorganisms include *Arthrobacter* sp. [10], *Alcaligenes* sp. [13,14], *Bacillus* sp. [2], *Brevibacterium* sp., *Corynebacterium* sp. [15], *Gordonia nitida* [11,16], *Micrococcus luteus* [17], *Nocardia* sp. [2,18], *Nocardiodes* sp. [9], *Paracoccus* sp. [3,19], *Pimelobacter* sp. [20], *Pseudomonas* sp. [18,21,22], *Pseudonocardia* sp. [12], *Ralstonia/Burkholderia* sp. [23], *Rhodococcus pyridinivorans* [24], *Shinella zoogloeoides* [5] and *Streptomyces* sp. [7].

However, toxic chemicals such as Cr (VI) and phenol are often found in pyridine polluted environments [20,25–28], which causes the biodegradation of pyridine to be more difficult [14,29]. For example, phenol has been reported to inhibit the pyridine-degradation activities of *Bacillus cereus* ITRCEM1, *Alcaligenes faecalis* ITRCEM2 [14] and *Pseudomonas putida* MK1 [29]. However, Adav et al. [30] demonstrated that phenol could enhance pyridine degradation of aerobic granules. Nevertheless, little is known about how Cr (VI) affects pyridine degradation, despite being of crucial importance for the removal of pyridine and the bioremediation of environments polluted by pyridine injunction with phenol and/or Cr (VI).

In this study, we report a *Rhodococcus* strain capable of utilizing pyridine as the sole carbon and nitrogen source for growth that could also reduce Cr (VI) and degrade pyridine simultaneously. The stimulating and inhibitory effects of phenol on pyridine degradation by the strain are also reported.

2. Materials and methods

2.1. Strain isolation and characterization

Strain Chr-9 was isolated from an activated sludge collected from the aerobic reactor of a tannery in Binzhou, Shandong Province, Eastern China. Samples serially diluted (10^{-4} and 10^{-5}) were plated directly on Laurie–Bertni (LB) (l^{-1}, tryptone (Oxoid), 10.0 g; yeast extract (Oxoid), 5.0 g; NaCl, 10.0 g and pH 7.0) agar

incubated at 30 °C for three days. The strain was then subjected to morphological, physiological and phylogenetic analyses using the previously-described procedure [31].

After growth in LB medium overnight at 35 °C, cells of strain Chr-9 (in 100 ml culture) in the late exponential growth phase ($OD_{600} \approx 5.0$) were harvested by centrifugation at $4000 \times g$ for 5 min and then washed twice with sterilized mineral salt medium without nitrogen source (MSM medium) (l^{-1}, NaCl, 1.0 g; K_2HPO_4, 1.5 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4$, 0.025 g, pH 7.0–7.2). The cell pellet was then resuspended in MSM medium, forming a cell suspension with an OD_{600} of 5.0. This cell suspension was then used as the inoculum for the following experiments. All of the following experiments were conducted in triplicate.

The utilization of aromatic compounds as sole carbon sources by strain Chr-9 was investigated as follows. 2 ml of the inoculum cell suspension was inoculated into 100 ml mineral salt medium (MSMN medium) (l^{-1}, NaCl, 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 1.5 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4$, 0.025 g, pH 7.0–7.2) amended with 100 mg l^{-1} (final concentration) pyridine, 3-hydroxypyridine, phenol, m-cresol, p-cresol, quinoline, o-cresol, p-chlorophenol, 2,4-dichlorophenol, naphthalene, 1-naphthylamine, anthracene, and pyrene. The cultures were then incubated at 35 °C in the dark while shaking at 150 rpm. Growth of the strain was then analyzed based on the optical density at 600 nm using a UV-1700 spectrophotometer (Shimadzu, Japan). At the end of the experiments, the cultures (approximately 97 ml) were mixed with 40 ml methanol, 2 ml of the mixture were filtered through a 0.22 μm membrane, the degradation of these chemicals were then measured by using the protocol of pyridine detection described below.

2.2. Utilization of pyridine as the sole carbon and nitrogen source

Inoculum cell suspension (2 ml) was inoculated into 100 ml MSMN medium and MSM medium in 250 ml flasks supplemented with pyridine (final concentration, 200 mg l^{-1}). Mixtures without cells were used as a control. Cultures were incubated at 35 °C in the dark while shaking at 150 rpm, sampled at different time and analyzed for cell biomass based on the OD600 value and residual pyridine when necessary. Liquid samples (0.5 ml) were mixed with 0.5 ml methanol, after which they were filtered through a 0.22 μm membrane. The filtrate (10 μl) was then injected into a reverse-phase high-performance liquid chromatograph (HPLC) (LC-20AT, Shimadzu, Japan) equipped with an Agilent column (4.6 mm i.d. × 150 mm) filled with Kromasil 100^{-5} C_{18} and an ultraviolet detector (SPD-M20A). Methanol:water (7:3, v:v) was applied as the mobile phase at a flow rate of 0.7 ml min^{-1}. Pyridine was detected at 254 nm and 30 °C with the detection limit being 0.01 mg l^{-1}.

2.3. Effects of phenol on pyridine degradation

To investigate the effects of phenol on pyridine degradation by strain Chr-9, phenol at final concentrations of 100, 200, 400 and 600 mg l^{-1} was added to MSMN and MSM media supplemented with 200 mg l^{-1} (final concentration) pyridine. Two millilitre of the inoculum cell suspension of strain Chr-9 was then inoculated into the above mixtures (100 ml), which were subsequently incubated at 35 °C in the dark while shaking at 150 rpm. The pyridine and phenol concentrations and the growth of the strain based on the OD_{600} value were measured at different times throughout the experimental period. Residual phenol and pyridine were extracted and analyzed using the same methods as described above except that phenol was detected based on the absorbance at 271 nm at 30 °C.

2.4. Effects of Cr (VI) on pyridine degradation

To detect the ability of strain Chr-9 to reduce Cr (VI), 2 ml of the inoculum cell suspension was inoculated into LB medium (100 ml) containing 50 mg l^{-1} Cr (VI) as well as MSM medium containing 2.0 mg l^{-1} Cr (VI), 200 mg l^{-1} pyridine and 5000 mg l^{-1} glucose. The cultures were then incubated at 35 °C in dark and shaken at 150 rpm. The concentrations of pyridine and Cr (VI) were then measured at different times.

In addition, to analyze the effects of Cr (VI) on the pyridine-degradation ability of strain Chr-9, 2 ml of the inoculum cell suspension and Cr (VI) with final concentrations of 10, 20, 30, 40 mg l^{-1} was added into 100 ml MSMN medium supplemented with 200 mg l^{-1} pyridine in a 250 ml flask, followed by inoculation at 35 °C in the dark while shaking at 150 rpm. The cultures were then sampled at different times and analyzed for pyridine and Cr (VI) concentrations, as well as the growth of the strain by measuring the optical density at 600 nm. The residual pyridine was analyzed using the methods described above. The Cr (VI) concentration was analyzed according to the protocol described by Bartlett and James [32].

2.5. Detection of the metabolites of pyridine degradation

To evaluate the hydroxylation of pyridine by strain Chr-9, 5 ml of the inoculum cell suspension was inoculated into MSMN medium (100 ml) amended with 200 mg l^{-1} (final concentration) pyridine. The cultures were incubated at 35 °C in the dark while shaking at 150 rpm and sampled. The culture samples were then extracted using equal volume chloroform, followed being dried over anhydrous Na_2SO_4 at room temperature. The extract was then evaporated using a vacuum rotary evaporator at room temperature, and the residue was dissolved in 2 ml methanol, after which they were filtered through a 0.22 μm membrane.

The extract (1 μl) was then injected into a HPLC (1260, Agilent Technologies, USA) equipped with a Eclipse plus C18 column (Agilent, 5 μm, 4.6 × 150 mm), and methanol/water (70/30, v/v) as the mobile phase at the speed of 0.3 ml min^{-1}. The identification of the metabolites was made by using Accurate-Mass-Q-TOF-LC/MS 6250 (Agilent, USA) equipped with a electrospray ionization (ESI). The ionization mode was positive. The electron multiplier was set up to 2500 V. The voltages of fragmentor, skimmer and OCT1 RF Vpp were 100 V, 65 V and 700 V respectively. The nebulizer gas pressure was 45 psi and the gas temperature was 350 °C. Full-scan (+)-ESI-MS mass spectra were obtained by scanning from 50 to 200 m/z. The structures of the metabolites were elucidated by applying multiple-stage fragmentation studies using tandem mass spectrometry (MS/MS). Culture media extracts of the reference control samples (non-inoculated but supplemented with pyridine) were also prepared and analyzed in the same way.

2.6. Analysis of phenol hydroxylase gene

The phenol hydroxylase gene of strain Chr-9 was amplified and sequenced using the following primer sets, PheU-f (CGKATGACSTACGGCTGGATGGGCG) and PheU-r (ACGTCCTGTTC-GATGATCTCCTTGATCCGC), according to a previously described protocol [33]. The gene sequence has been deposited in GenBank under accession No. HM807584.

After strain Chr-9 was grown in MSMN medium supplemented with 1% glucose (final concentration) at 35 °C for 24 h, cells (in 100 ml suspension) were harvested by centrifugation at $4000 \times g$ for 5 min and then washed twice with MSM medium. The pellets were then re-suspended in 100 ml MSM or MSMN medium supplemented with pyridine and phenol, respectively. Next, the cultures were incubated at 35 °C in the dark while shaking at 150 rpm.

After 2 h of incubation, the total RNA of strain Chr-9 was extracted using a RNasio Plus Kit (TaKaRa Biotechnology (Dalian) Co. Ltd., Dalian, China) according to the manufacturer's instructions. The total RNA was evaluated for DNA contamination by using the PCR method. First-strand cDNA was then synthesized using a Rever-Tra Ace-α-First Strand cDNA Synthesis Kit for RT-PCR (TOYOBO Co. Ltd., Shanghai, China) according to the manufacturer's instructions with the following profile: a mixture of 0.5 μg total RNA in 20 μl solution was treated at 65 °C for 5 min, after which it was incubated at 32 °C for 10 min, 42 °C for 20 min, 99 °C for 5 min and finally 4 °C for 5 min. The cDNA was then used as a template for real time PCR to quantify the phenol hydroxylase gene by using a SYBR *Premix Ex* Taq^{TM} kit (TaKaRa Biotechnology (Dalian) Co. Ltd., Dalian, China). The 16S rRNA gene was used as the internal control to normalize the integrity of total RNA as usual. The primers used in real time PCR were: Phe-RT-f (CCCGTACATGAAGACGTTCTCCCA) and Phe-RT-r (CGGTCTGCCGATCAAGAAGAAGGAA) for the phenol hydroxylase gene, and 16S-RT-f (CCACCTTCCTCCGAGTTGACC) and 16S-RT-r (CGAAGAACCTTACCTGGGTTTGAC) for the 16S rRNA gene. All PCR amplification repeated three times. Real time PCR was conducted using a CFX96^{TM} Real-Time System (Bio-Rad, Singapore) according to the manufacturer's instruction, with the following thermal profile: 3 min at 95 °C followed by 50 cycles of 10 s at 95 °C, 10 s at 58 °C and 25 s at 72 °C. Optical data obtained by real time PCR were analyzed using the default and variable parameters available in the Bio-Rad CFX manager (Version 1.1.308.1111).

3. Results

3.1. Characterization of strain Chr-9

Strain Chr-9 was a Gram-positive, strictly aerobic, non-spore-forming, rod-shaped bacterium, forming red, rough hard and dry colonies on LB agar after growth at 30 °C for 2–3 days. The organism was catalase positive and oxidase and amylase negative. Growth in LB medium occurred at 20–45 °C (optimal 35 °C), pH 6.0–8.0 and 0–2% NaCl. Phylogenetic analysis based on the 16S rRNA gene sequence of strain Chr-9 (1229 bp) (GenBank accession No. GU357742) suggested that the strain was a member of the genus *Rhodococcus*, forming a stable clade with *Rhodococcus pyridinivorans* PDB9^T [24] and *Rhodococcus rhodochrous* ATCC271^T [34] with a sequence similarity of 99% for both (Fig. S1). The sequence of the phenol hydroxylase gene in strain Chr-9 (GenBank accession No. HM807584) was closely related to that of *Rhodococcus* sp. T-5 and T-10 (GenBank accession No. FJ392542 and FJ392543) [33]. Therefore, strain Chr-9 was designated as *Rhodococcus* sp. Chr-9. Strain Chr-9 was able to utilize glucose, pyridine, 3-hydroxypyridine, phenol, m-cresol, p-cresol and quinoline for growth in MSMN medium, but it could not degrade o-cresol, p-chlorophenol, 2,4-dichlorophenol, naphthalene 1-naphthylamine, anthracene, or pyrene in MSMN medium (Table 1).

3.2. Degradation of pyridine and phenol

Strain Chr-9 could degrade pyridine at 30–40 °C and pH 6.0–9.0 in MSMN medium with the optimum occurring at 35 °C and pH 8.0. The strain was also able to use phenol as the sole carbon source when it was applied at initial concentrations up to 600 mg l^{-1} in MSMN medium. The optimum phenol concentration for growth was 300 mg l^{-1}, suggesting that lower concentration (<300 mg l^{-1}) could be not sufficient enough for strain Chr-9 to maintain the largest growth, while higher concentrations inhibited the growth of strain Chr-9.

Pyridine could also be used as both the sole carbon source and the nitrogen source for strain Chr-9 (Fig. 1(I, II and III)). The presence

Table 1
Degradation of aromatic compounds and growth of strain Chr-9 in MSMN medium "+" for positive and "−" for negative.

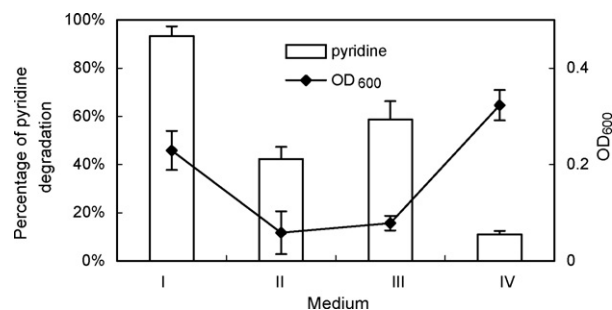| Aromatic compound (100 mg l^{-1})	Degradation	Growth
Phenol	+	+
p-Cresol	+	+
m-Cresol	+	+
o-Cresol	−	−
p-Chlorophenol	−	−
2,4-Dichlorophenol	−	−
Pyridine	+	+
3-Hydroxypyridine	+	+
Quinoline	+	+
8-Hydroxyquinoline	+	+
Indole	+	+
Naphthalene	−	−
1-Naphthylamine	−	−
Anthracene	−	−
Pyrene	−	−

Fig. 1. Pyridine degradation and growth of strain Chr-9 in MSM medium supplemented with 500 mg l^{-1} glucose (I); MSM medium (II); MSMN medium (III); and MSMN medium supplemented with 500 mg l^{-1} glucose (IV). Error bars represent the standard error of three replicates.

of both additional nitrogen (NH$_4$NO$_3$) and carbon (glucose) sources led to the optimum growth of strain Chr-9 and the least degradation of pyridine (Fig. 1(IV)). When pyridine was the sole nitrogen source and additional glucose was added, strain Chr-9 degraded the most pyridine and its growth was better than when pyridine was used as the sole carbon source (Fig. 1(I)). The lowest growth was observed when pyridine was used as the sole source of nitrogen and carbon, while the degradation of pyridine was only better than when both additional nitrogen and carbon sources were used (Fig. 1(II)). These results also suggested that additional nitrogen or carbon sources could stimulate strain Chr-9 to degrade pyridine.

Strain Chr-9 could use phenol instead of glucose as carbon source with pyridine as the nitrogen source. In MSM medium containing no carbon and nitrogen compounds, when both pyridine (200 mg l^{-1}) and phenol (<200 mg l^{-1}) were added, the pyridine and phenol were degraded and the growth of strain Chr-9 was better than the control, which contained pyridine, but no phenol. Moreover, the pyridine degradation was stimulated by the addition of 100 mg l^{-1} phenol. However, the degradation of pyridine and phenol as well as the growth of strain Chr-9 were inhibited when the phenol concentration increased to 400 mg l^{-1} (Fig. 2(A)). Comparatively, when cells were cultured in MSMN medium (containing NH$_4$NO$_3$ and no organic carbon) amended with 200 mg l^{-1} pyridine, the addition of phenol (up to 600 mg l^{-1}) led to better growth of strain Chr-9 than no phenol addition. Similarly, in case of MSMN medium, degradation of pyridine was also stimulated by the addition of 100 mg l^{-1} phenol, while it decreased when the phenol was increased to 400 and 600 mg l^{-1} (Fig. 2(B)). When MSM and MSMN media were compared, MSMN medium containing an initial nitrogen source (NH$_4$NO$_3$) resulted in better phenol and pyridine degradation. In MSMN medium, both pyridine and phenol were
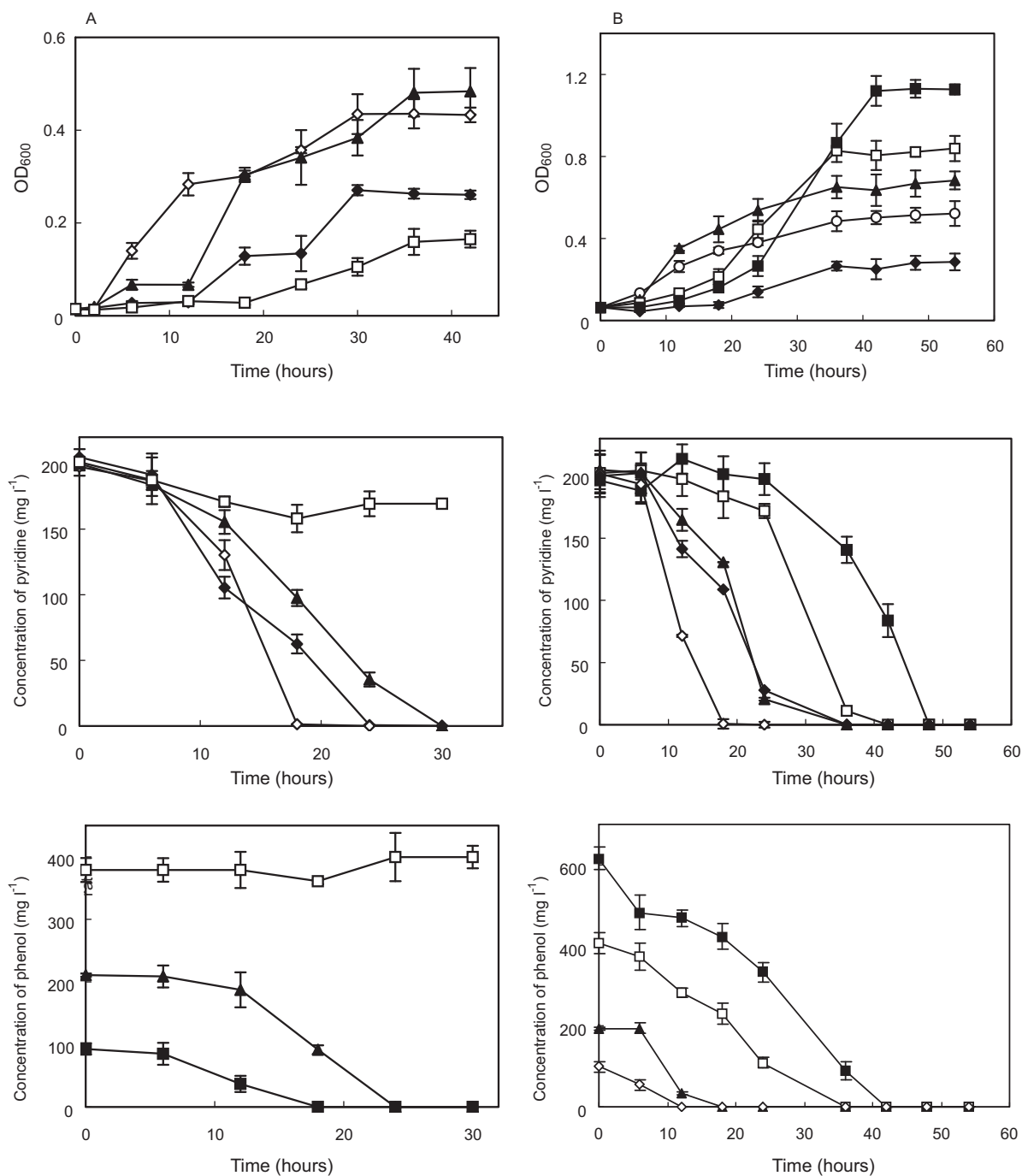
Fig. 2. Degradation of pyridine and phenol and the growth of strain Chr-9 in MSM medium (A) and MSMN medium (B) supplemented with: $200\,mg\,l^{-1}$ pyridine (filled diamond); $200\,mg\,l^{-1}$ pyridine and $100\,mg\,l^{-1}$ phenol (open diamond); $200\,mg\,l^{-1}$ pyridine and $200\,mg\,l^{-1}$ phenol (filled triangle); $200\,mg\,l^{-1}$ pyridine and $400\,mg\,l^{-1}$ phenol (open square); and $200\,mg\,l^{-1}$ pyridine and $600\,mg\,l^{-1}$ phenol (filled square). Error bars represent the standard error of three replicates.

degraded even though the phenol concentration was greater than $600\,mg\,l^{-1}$, while phenol and pyridine degradation was completely inhibited in MSM medium when the phenol concentration was $400\,mg\,l^{-1}$.

A metabolite (m/z 112.0986) which has same mass as that of dihydroxypyridines, was detected in the catabolic products of pyridine by strain Chr-9 after 24 h incubation, and the dihydroxypyridines, including 2,3-dihydroxypyridine and 2,6-dihdydroxypyridine, have been determined during the pyridine degradation by *Rhodococcus opacus* strains [35]. Another metabolite with mass 129 (m/z 130.1832) which is same as that of 5-amino-2-oxo-4-pentenoic acid or 5-amino-5-oxo-4-pentenoic

acid which have been detected during the pyridine degradation previous [35], were also detected in the same sample, there was no other metabolites were detected during this degradation (Fig. S2).

The stimulation of pyridine degradation by strain Chr-9 in response to phenol was also supported by the results of real time PCR of the phenol hydroxylase gene. The expression of the phenol hydroxylase gene mRNA was much greater when both pyridine and phenol were present in the MSM medium than when only phenol or pyridine was present (Fig. 3). In addition, the phenol hydroxylase gene mRNA level was greater when pyridine was used as a nitrogen source than as a carbon source. These results indicate that
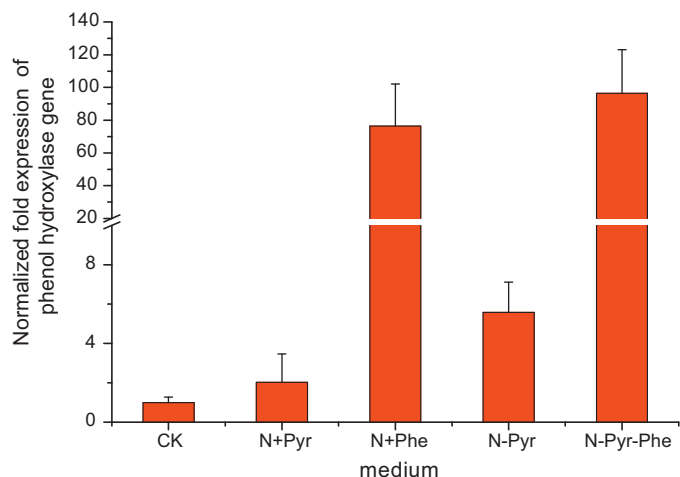
Fig. 3. Expression of phenol hydroxylase gene of strain Chr-9 in: MSMN medium supplemented with $300\,mg\,l^{-1}$ glucose (CK); MSMN medium supplemented with $300\,mg\,l^{-1}$ pyridine (N+Pyr); MSMN medium supplemented with $300\,mg\,l^{-1}$ phenol (N+Phe); MSM medium supplemented with $300\,mg\,l^{-1}$ pyridine (N-Pyr); and MSM medium supplemented with $150\,mg\,l^{-1}$ pyridine and $150\,mg\,l^{-1}$ phenol (N-Pyr-Phe). The vertical coordinates indicate the relative amounts of phenol hydroxylase gene mRNA. Error bars represent the standard error of three replicates.

the phenol hydroxylase may play a key role in the degradation of pyridine.

3.3. Degradation of pyridine and reduction of Cr (VI)

Strain Chr-9 could grow in LB medium amended with $50\,mg\,l^{-1}$ Cr (VI) and reduce the Cr (VI) (Fig. 4(A)). In addition, it could degrade pyridine (initial concentration $200\,mg\,l^{-1}$) and reduce Cr (VI) (initial concentration $2\,mg\,l^{-1}$) when MSM medium amended with glucose ($5000\,mg\,l^{-1}$) was used (Fig. 4(B)). However, when pyridine ($200\,mg\,l^{-1}$) was used as the sole carbon source for strain Chr-9 in MSMN medium, pyridine was degraded, but Cr (VI) was not reduced. The higher concentration of Cr (VI) resulted in lower rates of pyridine degradation and growth of strain Chr-9 (Fig. 4(C)). The growth of strain Chr-9 was almost completely inhibited when the Cr (VI) concentration was increased to $30\,mg\,l^{-1}$.

4. Discussion

Strain Chr-9 could grow in the presence of pyridine when phenol and Cr (VI) were present. In general, phenol hydroxylase, which is a key enzyme included in the degradation of aromatic compounds, plays important roles in catalyzing the first step of the conversion of aromatic compounds to their o-diol derivatives [33]. Owing to the
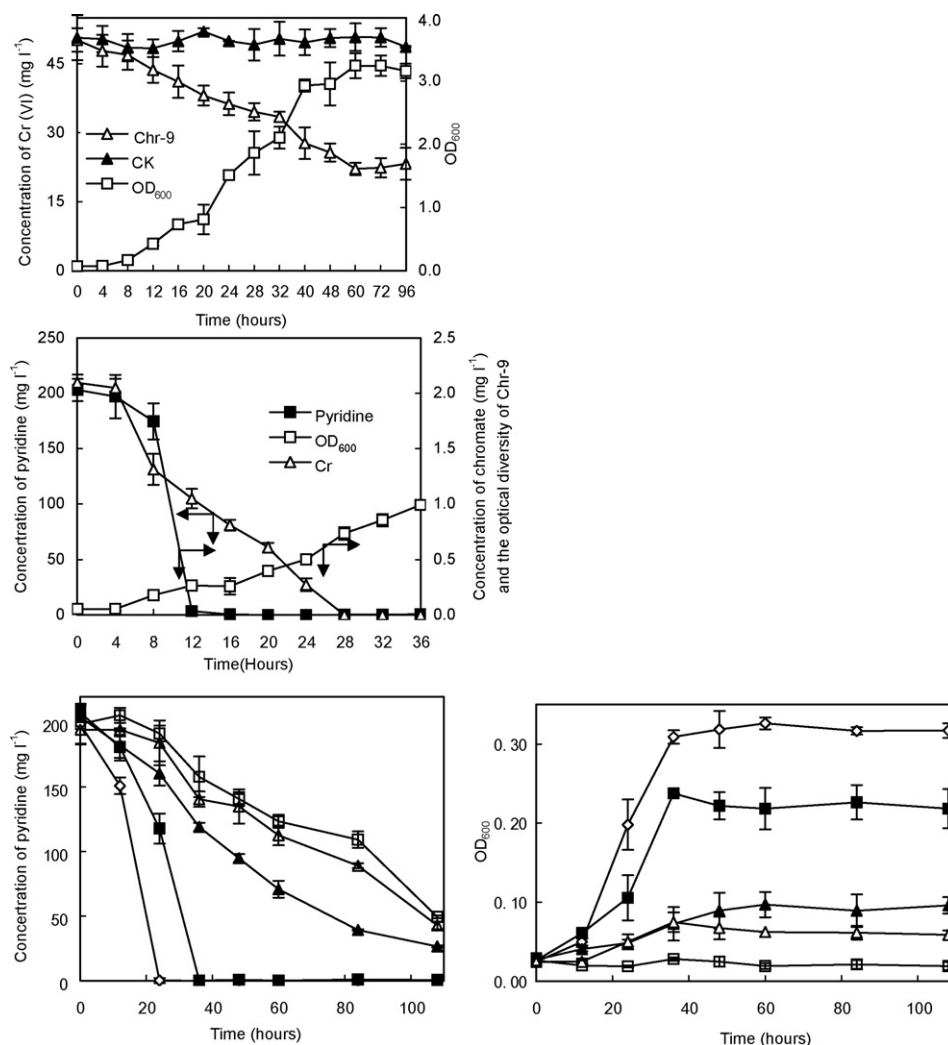

Fig. 4. Cr (VI) reduction and/or pyridine degradation and growth of strain Chr-9 in LB medium (A); MSM medium supplemented with $5000\,mg\,l^{-1}$ glucose (B); and (C) MSMN medium supplemented with 200 pyridine and $0\,mg\,l^{-1}$ (open diamonds), $10\,mg\,l^{-1}$ (filled square), $20\,mg\,l^{-1}$ (filled triangle), $30\,mg\,l^{-1}$ (open triangle), $40\,mg\,l^{-1}$ (open square) Cr (VI) respectively. Error bars represent the standard error of three replicates.

structural similarity of pyridine and phenol, the enzyme responsible for phenol degradation may also play an important role in the degradation of pyridine [35]. In fact, two metabolites with same mass as that of dihydroxypyridine (MW 111) and 5-amino-2-oxo-4-pentenoic acid and 5-amino-5-oxo-4-pentenoic acid (MW 129) respectively which have been proved as the metabolite of pyridine by *Rhodococcus* strain [35] were detected, and the MS/MS results also proved the metabolite (m/z 112.0986) has the similarity structure to dihydroxypyridines. Meanwhile the phenol hydroxylase gene was induced by pyridine, especially when pyridine served as the nitrogen source (Fig. 3). In the presence of phenol, the addition of pyridine also induced increased expression of phenol hydroxylase mRNA when compared to phenol alone. However, the quantity of the mRNA induced by pyridine was much lower than that induced by phenol, suggesting that phenol could be a better carbon source for the growth of strain Chr-9. Since both the pyridine and phenol competed for the phenol hydroxylase, phenol had the potential to inhibit the affinity of pyridine to phenol hydroxylase. When low concentrations of phenol were present and the competition for the enzyme between phenol and pyridine was small, the phenol hydroxylase induced by phenol could stimulate the degradation of pyridine, which was used as nitrogen for the growth of Chr-9. However, due to the higher affinity of phenol to phenol hydroxylase, pyridine was out-competed by the high concentrations of phenol for the enzyme, which resulted in a decrease in its degradation. The toxicity of the high concentration of phenol may also have inhibited the growth of Chr-9, consequently inhibiting the degradation of pyridine.

Cr (VI) is acutely toxic and a known carcinogen [36]. Cr (VI) has become a common environmental metal contaminant through the widespread use of Cr (VI) compounds by various industries as well as their incorrect disposal [37]. Strain Chr-9 was able to reduce Cr (VI) when glucose and LB were used; however, it could not reduce Cr (VI) when pyridine was applied as the sole carbon and energy source. Moreover, the increase of Cr (VI) led to decreased pyridine degradation. It is well known that the reduction of Cr (VI) requires enough reducing power (i.e. NADH or NADPH), and that this energy can be provided by organic compounds such as glucose and LB. However, low concentrations of pyridine obviously could not afford the energy required for growth and higher concentrations of pyridine itself would inhibit the growth of strain Chr-9. Therefore, the addition of easily-degradable organic compounds should be a reasonable approach to enhance Cr (VI) reduction and pyridine degradation in cases in which both Cr (VI) and pyridine are present.

5. Conclusion

One *Rhodococcus* strain, strain Chr-9 could grow with pyridine as sole carbon and nitrogen source. It was also able to degrade phenol and reduce Cr (VI). Different from previous reports, low concentration (100 mg l^{-1}) of phenol could stimulate strain Chr-9 to degrade pyridine, however the degradation of pyridine was inhibited when phenol concentration was higher than 400 mg l^{-1}. Two metabolites with same mass as that of dihydroxypyridine (m/z 112) and 5-amino-2-oxo-4-pentenoic acid (m/z 130) have been detected. The stimulation and inhabitation of phenol to pyridine degradation could be attributed to the competition of phenol and pyridine to the expression of hydroxylase gene. Strain Chr-9 was also able to reduce Cr (VI) when glucose and LB was used as carbon source, however the Cr (VI) reduction could not happen when pyridine was the sole carbon and energy source. In addition, glucose could stimulate strain Chr-9 to simultaneously reduce Cr (VI) and degrade pyridine. To the best of our knowledge, strain Chr-9 is the first *Rhodococcus* strain reported to degrade pyridine in presence of Cr (VI), whose degradation of pyridine could be stimulated by low concentration of phenol.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.04.034.

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