BIODEGRADATION AND DECHLORINATION OF PENTACHLOROPHENOL WITH A PENTACHLOROPHENOL DEGRADING BACTERIUM Sphingomonas Chlorophenolica

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ABSTRACT

Pentachlorophenol (PCP) has long been used as a wood preservative. Because of the adverse environmental effects of PCP, it has widely contaminated soil and groundwater. In treating PCP, a number of bacteria have been isolated from the environment and applied to bioremediate contaminated areas. However, there is some controversy about the efficiency of PCP removal. This controversy originates in part from insufficient understanding of the physiological characteristics of these bacteria. The purpose of this research was to investigate PCP induced and chloride inhibited effects. Moreover, the intermediates of PCP metabolism were detected using a spectrometer. When Sphingomonas chlorophenolica cells were preincubated with PCP, the lag phase of PCP degradation periods became shorter and the PCP concentrations removed became higher. The results indicated that S. chlorophenolica was able to completely degrade 200 mg L⁻¹ PCP within 23.2 h, and to release 130.3 mg L⁻¹ chloride in the same period of time. Furthermore, the results showed that PCP could be dechlorinated 100% by the cell suspensions. The removal efficiency of 250 mg L⁻¹ PCP was not affected in the presence of the various concentrations of the chloride. Using spectrophotometer scanning, there was no significant change in supernatant spectra from the S. chlorophenolica suspension in minimal salt medium during PCP degradation.

INTRODUCTION

The chlorophenolic compounds are common environmental contaminants originating mainly from wide-spectrum biocide compounds used in industry and agriculture. These compounds are formed during pulp bleaching and some direct industrial waste discharge [1]. Among chlorophenols, pentachlorophenol (PCP) is a priority pollutant widely used as a general biocide in commercial wood treatment [2]. The extensive use of PCP has widely contaminated soil and groundwater. Its toxicity seriously affects living organisms. PCP is toxic to all forms of life because it is an oxidative phosphorylation inhibitor. Moreover, PCP is recalcitrant to degradation because of its stable aromatic ring system and high chloro content. It thus persists in the environment [3].

In treating chlorophenols, biological treatment is superior to physicochemical methods because physicochemical treatments have higher treatment costs and the possibility of causing secondary pollution. Aerobic degradation of polychlorinated phenols has been extensively studied during the last few years [4]. Several strains of bacteria that are able to completely mineralize polychlorinated phenols have been described and applied to bioremediate many PCP-contaminated sites [5-7]. Moreover, the pathway of PCP metabolism has been delineated and several careful researches have been published [8-13]. To solve serious PCP pollution in Taiwan, using indigenous pure strains isolated from contaminated sites to degrade PCP is a good choice. According to previous studies, a number of bacteria isolated from the environment were applied to bioremediate some contaminated areas. However, there is some controversy regarding the efficiency of PCP removal. The reason maybe due to insufficient understanding of the bacteria’s physiological and degraded characteristics. Our research group isolated the first pure PCP-degrading bacterium in Taiwan, identified as Sphingomonas chlorophenolica. In the previous study, this strain was able to degrade high PCP concentration and its basic
physiological characteristics were also investigated [14]. However, before this strain was applied to bio-remediate PCP-contaminated sites in Taiwan, it was important to determine its characteristics of PCP degradation in more details. The purpose of this research was to investigate PCP induced and chloride inhibited effects. Moreover, the intermediates of PCP metabolism were detected using a spectrometer.

MATERIALS AND METHODS

1. Organism, Media and Culture Conditions

The S. chlorophenolica used in all tests was obtained from PCP-contaminated soils in Taiwan [14]. This strain was isolated from an acclimated mixed culture, was purified and maintained on R2A agar [15]. All experimental procedures were performed in media containing the inorganic culture media specified by Yang et al. [14].

2. The PCP Induction Effect

To prepare PCP induced cell suspensions, S. chlorophenolica cells were initially grown on R2A agar. After 2-d incubation at 30 °C, the cells grown on R2A agar plates were transferred into a 2-L flask that containing inorganic culture media with 100–150 mg L⁻¹ PCP. The purpose of this transfer was to induce PCP degrading enzymes production. The culture in 2-L flask was shaken (120 rpm) in the dark at 30 °C. After approximately 18–25 h of incubation and induction, the cells were harvested by centrifugation (6000 g at 4 °C for 14 min). The bacterial pellet was washed twice with fresh inorganic culture media and then re-suspended in an appropriate amount of fresh inorganic culture media prior to use. The PCP un-induced cell suspensions were prepared following the same procedures for PCP induced cell suspensions preparations, but cells were not induced using PCP. PCP was added into two types of liquid culture to compare the removal of PCP by measuring variation of PCP concentration.

3. Dechlorination of PCP by S. Chlorophenolica

In order to find out whether chlorides were released during the degrading process, a series of 125-mL batch reactors were used to perform the experiment. Each reactor contained 40 mL of PCP un-induced cell suspensions. 200 mg L⁻¹ PCP was added to serve as the sole carbon and energy source. The reactors were sealed with cotton stoppers and shaken at 120 rpm in the dark at 30 °C and then sampled periodically to observe PCP removal and chloride release under aerobic conditions.

4. PCP Degradation in the Presence of Various Concentrations of Chloride

For testing the effects of various concentrations of chloride on PCP degradation by S. chlorophenolica, experiments were conducted with a series of 250-mL batch reactors, and each reactor contained 100 mL of PCP induced cell suspensions. After adjusting the initial amount of pure bacteria (cell concentration yielding 0.1 optical density (OD) unit), 250 mg L⁻¹ PCP and various concentrations of chloride (100–500 mg L⁻¹) were added into batch reactors. Then the reactors were sealed with cotton stoppers and shaken at 120 rpm in the dark at 30 °C. The flasks were sampled periodically to measure PCP removal, growth of cells and variation of pH under aerobic conditions.

5. PCP Degradation Intermediate Scanning

In the high performance liquid chromatography (HPLC) analysis, no special peak appeared in the full spectrum. The metabolites could not be detected in our HPLC condition. For this reason, spectrometer scanning may be one way to determine whether any detectable intermediate was produced by S. chlorophenolica. Determining the optical wavelength of the intermediates might be useful for modifying the analytical conditions. After un-induced cell suspensions were prepared, 200 mg L⁻¹ PCP was added to serve as the carbon source. Sampling of 10 mL liquid culture was then scanned using a spectrometer (Beckman Du® 530). The scanning wavelength range was 190-450 nm.

6. Analytical Methods

The cell suspensions were clarified by centrifugation at 8000 rpm for 3 min. The cell-free supernatant fraction was analyzed by HPLC with ultraviolet detector. HPLC was performed with a Hitachi system equipped with a Merck Lichrospher 100 PR-18 end-capped (5 mm) column at a flow rate of 0.8 mL min⁻¹. The solvent system ratio was acetonitrile: water: phosphoric acid = 65: 35: 0.1. The UV detector absorbency wavelength was fixed at 284 nm. The pH and OD were measured using a pH meter and Spectrophotometer at 600 nm, respectively.

RESULTS AND DISCUSSION

1. PCP Induction Effect

Figure 1 shows the PCP induction results. Without PCP induction, as the initial PCP concentration was lower than 100 mg L⁻¹, S. chlorophenolica could
Fig. 2. The PCP degradation and the chloride release by un-induced cell suspensions. Fig. 1. The degradation of PCP by (a) un-induced cell suspensions, and (b) induced cells of *S. chlorophenolica*. 

Fig. 2. The PCP degradation and the chloride release by un-induced cell suspensions.

remove PCP completely within 24.4 h. However, the removal efficiency became 89% in 111 h, when the initial PCP concentration was 400 mg L\(^{-1}\). If the initial PCP concentration was increased to 600 mg L\(^{-1}\), *S. chlorophenolica* could not degrade PCP. At any PCP concentration, a significant lag phase occurred in the initial period when the cell suspension was not induced with PCP. The induced *S. chlorophenolica* cells, however, could degrade relatively higher PCP concentrations (Fig. 1b). When the initial PCP concentration was lower than 380 mg L\(^{-1}\), the *S. chlorophenolica* could completely degrade the PCP within 46 h. Increasing the PCP concentration to 560 mg L\(^{-1}\), the efficient PCP removal decreased to 59% within 165 h. Furthermore, there was no lag phase in the initial period. This suggested that the PCP degrading activity was inducible.

According to previous researches, PCP 4-monoxygenase (PcpB), the first enzyme involved in PCP degradation, oxidizes PCP to 2,3,5,6-tetrachloro-hydroquinone (TeCH), and PcpB activity was not detectable without induction [8-9]. Comparing the PCP degradation results using induced and un-induced cells, the time needed to degrade the PCP by un-induced cells was 2 times longer than the time needed to degrade PCP using induced cells. If *S. chlorophenolica* is applied in bioremediation, a better way to use it is to pre-incubate the cells with PCP. After induction, *S. chlorophenolica* cells will have better ability to degrade PCP with a shorter reaction time.

2. Dechlorination of PCP by *S. Chlorophenolica*

The removal and dechlorination of the PCP by PCP un-induced cells in 125-mL flasks are shown in Fig. 2. The results indicated PCP un-induced cells were able to completely degrade 200 mg L\(^{-1}\) PCP within 23 h, and to release concentration of 130 mg L\(^{-1}\) chloride in the same period of time. The chloride release accompanied the decreasing of the pH. The variation of the O.D. value was not significant during the experiment period and it might be caused by the low PCP concentration. According to previous study, the aerobic pathway of PCP degradation is \(C_6Cl_5OH + 4.5O_2 + 2H_2O \rightarrow 6CO_2 + 5HCl\) [16]. After calculating the dechlorination of PCP, PCP was dechlorinated approximately 100% by PCP un-induced cells. Thus strain *S. chlorophenolica* has the ability of degrading PCP by dechlorination.

3. Effect of Various Concentrations of the Chloride

The removal of 250 mg L\(^{-1}\) PCP in the presence of various concentrations of the chloride using PCP induced cells is shown in Fig. 3. The results revealed PCP induced cells were able to completely degrade 250 mg L\(^{-1}\) PCP within 21 h, and to release concentration 160 mg L\(^{-1}\) chloride at the same period of time. The trends of the PCP removal and the chloride release were the same in the presence of the different concentrations of chloride. Thus there was no effect in the presence of various chloride concentrations for PCP induced cells to degrade 250 mg L\(^{-1}\) PCP. On the other hand, the chloride producing from the PCP degradation was not inhibiting the PCP removal.

4. PCP Degradation Intermediate Scanning

In our early study, the analytical conditions of the first intermediate-Tech with HPLC were identified. However, the result of the analysis was undesirable. Although the standard compound TeCH peak appeared clearly in the spectrum, nothing appeared in the supernatant spectrum from the suspension. The reason might be that the intermediate metabolism was very quick and could not be detected.
In order to analyze other metabolites accumulated, spectrum scanning was selected. Figure 4 reveals the PCP removal and the spectral changes during PCP degradation by un-induced *S. chlorophenolica* cells. As shown in Fig. 4b, there was no significant change in supernatant spectrum from the *S. chlorophenolica* suspension in minimal salt medium during PCP degradation (Fig. 4a). At the beginning, two peaks appeared at the 255 and 320 nm wavelengths, indication of the existence of PCP. As the PCP was removed, these two peaks became smaller and disappeared when PCP was completely removed. The intermediates could not be detected by scanning. The reason could be that the intermediates were unstable or could not be analyzed within the wavelength range used.

**CONCLUSIONS**

The purpose of this research was to study the characteristics of a PCP degrading bacteria *S. chlorophenolica*, and its ability to degrade and dechlorinate PCP. Comparing the PCP degradation results using induced and un-induced cells, the time needed to degrade the PCP using un-induced cells was 2 times longer than that needed to degrade PCP using induced cells. If *S. chlorophenolica* is applied in bioremediation, a better way to use it is to preincubate the cells with PCP. After induction, *S. chlorophenolica* cells will have better ability to degrade PCP with a shorter reaction time. The results indicated that *S. chlorophenolica* was able to completely de degrade 200 mg L⁻¹ PCP within 23 h, and to release concentration 130 mg L⁻¹ chloride at the same period of time. Furthermore, the results showed that PCP had been dechlorinated approximately 100% by the cell suspensions. The removal efficiency of 250 mg L⁻¹ PCP was not influenced in the presence of the various chloride concentrations. Spectrophotometer scanning showed that there was no significant change in supernatant spectra from *S. chlorophenolica* suspension in minimal salt medium during PCP degradation.

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