Note

Isolation and Characterization of Phenol-catabolizing Bacteria from a Coking Plant

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New phenol degrading bacteria with high biodegradation activity and high tolerance were isolated as Burkholderia cepacia PW3 and Pseudomonas aeruginosa AT2. Both isolates could grow aerobically on phenol as a sole carbon source even at 3 g/l. The whole-cell kinetic properties for phenol degradation by strains PW3 and AT2 showed a $V_{\text{max}}$ of 0.321 and 0.253 mg/l/min/(mg protein), respectively. The metabolic pathways for phenol biodegradation in both strains were assigned to the meta-cleavage activity of catechol 2,3-dioxygenase.

Key words: phenol; degradation; tolerance; meta-pathway

Phenol biodegradation has been chosen as a method to remediate environments contaminated by phenol, which is massively discharged from uncontrolled industrial waste disposal. A number of aerobic phenol-degrading bacteria have been described previously,1–3) however, little information on bacteria with a high phenol tolerance with high metabolizing activity is available. The kinetic constants for aerobic phenol degradation were reported for several Pseudomonads in the range from 94.11 mg/l to 470 mg/l.2,3) The phenol tolerance of the genera Pseudomonas and Vibrio under growth conditions were reported to be up to 700–800 mg/l.4,5) The best-known phenol-tolerant bacteria was Pseudomonas putida MTCC 1194 which could tolerate phenol up to 1000 mg/l. The aerobic metabolism of phenol in these degraders is known to be initiated by two different pathways, either the ortho- or the meta-pathway.6–8) We are particularly interested in isolating a phenol-degrading bacterium that can aerobically grow at elevated concentrations of phenol.

We have screened phenolic wastes of coke processing units (El-Tabbin, Helwan, Egypt) for phenol-degrading bacteria. Samples were enriched in 150 ml of basal mineral medium8) with 100 mg/l phenol for 24 h at 30°C. Portions from each culture were transferred onto basal mineral agar plates containing 100 mg/l phenol. As a result of the single-colony purification, six bacterial strains (AT1, AT2, AS1, PW3, SW4, and SW5) were able to grow aerobically on phenol as a sole carbon source. The collected cells of each isolate were incubated at $6 \times 10^8$ cells/ml in the medium containing phenol at 100 mg/ml to compare the efficiency of phenol biodegradation. The residual phenol concentration was monitored at different times. Maximum metabolic use of phenol was found to be at 30°C and pH 7.0 for all isolates. The time-dependent changes of phenol concentrations in the media were thus monitored under these conditions (Fig. 1A). Strains AT2 and PW3 were able to degrade phenol completely within a relatively short time. Strains AT1 and AS1 took 300 min to decrease the phenol concentration completely. Strains SW4 and SW5 failed to degrade phenol completely even after extended periods of incubation. Thus, strains AT2 and PW3 were selected as efficient phenol degraders among the isolates. Next, the phenol tolerance as well as the ability to grow at elevated phenol concentrations was investigated with the selected isolates (Fig. 1B). Each strain was respectively inoculated in the medium containing phenol at 1000, 2000, and 3000 mg/l. At such high phenol concentrations, strains PW3 and AT2 could initiate growth with a longer lag time. Cells growing at high phenol concentrations showed a longer lag time compared to those growing at low concentrations. In contrast, the other four isolates did not grow in the presence of phenol at 1000 mg/l, and their growth was permitted at 50 to 200 mg/l (Data not shown). Therefore, isolates PW3 and AT2 were defined as highly phenol-tolerant strains.

To clarify the phenol degradation rates of these two isolates, kinetic experiments were done (Fig. 2). In order to start the phenol-degrading reaction by the
bacterial cells, phenol was injected into a dense cell suspension (OD$_{600}$ of 2.5) at varied concentrations from 5 to 50 mg/l. The phenol disappearance in the reaction mixture was measured time-by-time to measure the degradation rates by the cells at different initial phenol concentrations. The measured rates of phenol degradation were fit to Haldane's equation (equation 1). The Lineweaver-Burk double reciprocal plot was used for graphing kinetic data to estimate $K_I$ (Michaelis constant for cellular kinetics) and $V_{max}$ (equation 2).
Hyde (2-HMS) as a result of the extradiol cleavage has taken place, resulting in the formation of 2-hydroxymuconate semialdehyde (2-HMS), which decreases in absorption at 375 nm and 268 nm indicating the presence of either phenol or catechol. Both strains showed the meta-pathway for phenol biodegradation.

We identified the isolates because of the unusual phenol biodegradation properties. The BILOG GN MicroPlate assay was used for phenotypic identification to test the ability of bacterial isolates to oxidize certain carbon sources and therefore assist in the identification of bacterial isolates. Genotypic identification of isolates was done by analysis of 16S rRNA gene with a pair of forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-ACGGCTACCTTGTTACGACT-3′) primers after the genomic DNA extraction and the corresponding PCR. 16S rDNA-containing plasmid vectors were constructed using the pGEM-T Easy vector and used to transform E. coli cells for the nucleotide sequence analysis of the selected clones. The identified 16S rRNA gene sequences of both strains have been deposited in the GenBank database (DNA Data Bank of Japan) under accession numbers AB091760 (AT2), and AB091761 (PW3). The BILOG Strain metabolic fingerprints of strains PW3 and AT2 were matched at 99.0%

**References**

3) Futamata, H., Harayama, S., and Watanabe, K., Diversity in kinetics of trichloroethylene-degrading


