Phenol-Degrading Bacteria from Polluted Soil and the Metabolomics of Phenol Biodegradation

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Introduction. Studies show that hydrocarbon polluted areas are main sources of hydrocarbon-degrading microorganisms for bioremediation processes. In the town of São José do Rio Preto in São Paulo state, Brazil, there are no studies of the 47 areas contaminated with petroleum derivatives as sources of hydrocarbon-degrading bacteria. **Objectives**. We aim to isolate and identify phenol-degrading bacteria in these contaminated areas and evaluate the degradation pathways used by such bacteria. **Materials and methods**. Soil samples were taken from 5 gas stations. Physico-chemical characteristics were determined. Populations of hydrocarbon-degrading bacteria by most probable number (MPN) method were estimated. Increasing concentrations of phenol (3-21 mM) were used to determine the tolerance of strains. The culture conditions were 30 °C, total darkness, and pH 7.0 ± 0.1. Three colony-forming units (CFUs) were selected and underwent degradation tests. The isolates were identified by amplifying the 16S rRNA subunit using the Polymerase Chain Reaction (PCR) technique. Metabolomic analysis with High Performance Liquid Chromatography (HPLC) was undertaken. Standard solutions (50 mg L^{-1}) of phenol, catechol and cis,cis-muconic acid were prepared to determine retention time. The HPLC analysis was carried out on an Agilent Infinity LC VL chromatograph. The mobile phase was a mixture of water with phosphoric acid (pH 2.2) as solution A; solution B was acetonitrile. Solutions were filtered and degassed prior to use. Chromatographic conditions were: column temperature 35 °C, flow rate 1.0 mL min^{-1}, injection volume 30 µL, detection wavelength 270 nm, column C18 reverse phase 250 mm x 4.6 mm. **Results**. Soils were acidic (pH 6.2-6.7) and had an organic matter content of 10-44 gm/dm^3. MPN was 2.48 x 10^8 CFU/ml. The maximum concentration of phenol tolerated was 11 mM. Sequences from PCR showed 99% similarity to *Pseudomonas entomophila* L48 record NC008027-1, *Pseudomonas putida* GB-1 record NC0103221, and *Pseudomonas putida* KT2440 record NC0029473. Cultures turned from clear to a yellow color. HPLC-MS analysis showed the presence of muconic acid. **Discussion**. The yellow culture color suggests that cultures incompletely degraded the phenol through meta-cleavage, but HPLC analysis showed muconic acid indicating the ortho-cleavage pathway. **Conclusions**. Predominant culturable strains were Pseudomonads. Pathways used by bacteria were by the ortho-cleavage pathway.

Keywords: Phenol-degrading bacteria, Metabolomics of phenol biodegradation, Aromatic hydrocarbon biodegradation, Ortho-cleavage pathway.

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