154d Proteome Analysis for Biphasic Growth Pattern in a Co-Metabolic Mixture of Phenol, Sodium Glutamate, and 4-Chlorophenol

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In previous research on biodegradation by *Pseudomonas putida* ATCC 49451 in a ternary co-metabolic system containing two growth substrates, phenol, sodium glutamate (SG) and a non-growth substrate, 4-chlorophenol (4-CP), a new cell growth pattern with two exponential phases separated by an intermediate lag phase was observed at certain unique concentrations of the substrates. It was found that cells preferentially utilized phenol in the first growth phase while SG was mainly utilized in the second growth phase. The results suggested that the presence of 4-CP inhibited the degradation of phenol and SG to different extent due to the disparity of its toxicity to different sets of proteins associated with the different growth substrates. In this research, we used two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to further study the physiology of *P. putida* ATCC 49451 in terms of the protein profiles during cell growth under the various culture conditions. The protein profiles of *P. putida* during growth on phenol, SG, and the mixture of phenol, SG, and 4-CP were examined by 2-D PAGE. Proteins differentially expressed in cells harvested from different exponential growth phases in the biphasic growth pattern were annotated and classified into three groups: phenol degradation associated proteins (Group P), SG degradation associated proteins (Group S), and proteins associated only with degradation of the ternary mixture of phenol, SG, and 4-CP (Group M). In the biphasic ternary system, during the first exponential growth phase, the protein profile was Group P protein dominated; while Group S dominated protein profile was obtained during the second exponential growth phase. This correlates well with the preferential utilization of substrates in previous research. In addition to certain catabolic enzymes, stress response related proteins were also included in the proteins grouped above. All these differentially expressed protein spots were excised from Coomassie blue stained gels, followed by in-gel digestion and MALDI-TOF MS analysis. The MS data were then subjected to appropriate databases using different search engines for protein identification. Directly at the protein level, our results lead to the comprehensive understanding of the biphasic growth pattern, at the same time, demonstrate that cells growing in mixed pollutants undergo significant physiological responses.