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**Title: "Biodegradation of polyaromatic hydrocarbons (PAHs) especially Chrysene by soil bacteria and identification of their genetic determinants.**

## Abstract:

Petroleum hydrocarbons and their toxic effects on humans have become a matter of concern over the years. They are recalcitrant organic pollutants with ubiquitous distribution. They are carcinogenic, mutagenic and genotoxic to humans and other life forms. Chrysene is one of the 16 priority pollutants listed by the USEPA (United States Environmental Protection Agency).To commence the present study soil samples were collected form petroleum hydrocarbon contaminated sites of Mathura petroleum refinery, Utter Pradesh. These soil samples were then processed in the lab and screened for chrysene degrading microbes. MSM media was used and MSM agar plates were prepared where the chrysene was supplied as the sole source of carbon and energy. Two strains 3AS and 5AS were isolated which have the capability to utilize the chrysene. The chrysene biodegradation ability of the strains was further evaluated. It was observed for the isolated strain 3AS that it could degrade 60 % of chrysene supplemented at 100 ppm of chrysene concentration in MSM broth while strain 5AS could degrade only 55 % of chrysene over a period of 7 days. Phenotypic characterization and phylogenetic analysis confirmed strain 3AS to be *Enterobacter cloacae* and 5AS as *Ochrobactrum intermedium.*

During the present study an effort was made to identify the metabolites of the underlying chrysene biodegradation pathway by GC-MS technique. Few compounds were common in the GC-MS spectral analysis of both the strains and these are Propanamide, N-(2-fluorophenyl)-3-(4-morpholyl)-, 12-Octadecadienoic Acid (Z,Z)-, Methyl Ester, 9-Octadecenoic Acid, Methyl Ester, Methyl stearate, Bis(2-ethylhexyl) phthalate and 9-Octadecenamide. **Bis (2-ethylhexyl) phthalate** was the metabolite with great importance regarding the present study observed in the cultures. It is evident from the literature that phthalates were formed as an intermediate product during the bacterial degradation of high molecular weight PAHs like pyrene and flourene.

Naphthalenyl dodecanoate was the metabolite of prime importance observed in the culture of the strain 5AS. Chrysene was broken down in to intermediates metabolites like phthalates and Naphthalenyl dodecanoate. Naphthalenyl dodecanoate was then further metabolized in to the intermediated of TCA (tri carboxylic acid) cycle by a series of enzyme catalyzed reactions. It is conclude that both the isolated strains have the ability to degrade chrysene by the formation of different intermediate and that may be the result of richness of catabolic and anabolic enzymes in their genome.

Further the presence of catechol catabolic genes was investigated in both the chrysene degrading isolated strains. The presence of catechol-1,2-dioxygenase gene was not observed while the presence of catechol-2,3-dioxygenase gene indicates the potential of chrysene degradation via *meta* cleavage pathway in the cultures of the strain 3AS and 5AS. This gene falls under the gloxylase/bleomycin resistance/dioxygenase gene superfamily in *Ochrobactrum intermedium* strain which is a part of the cat E gene super family along with VOC gene superfamily in *Enterobacter clocae*.