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Title: Pyrene Metabolic Pathways, Biosurfactants, And Siderophores In *Pseudomonas Fluorescens* Strain 29L

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ABSTRACT

Pseudomonas fluorescens Strain 29L, isolated from a microbial consortium from a creosote-contaminated soil, is capable of degrading pyrene, among other polyaromatic hydrocarbons (PAHs) as a sole carbon and energy source. This research tested the following hypotheses: (i) *Pseudomonas fluorescens* Strain 29L metabolizes pyrene by multiple pathways, (ii) siderophores may be involved for iron acquisition, and (iii) biosurfactants may be involved in pyrene metabolism. The objectives of this research work were to identify the pyrene metabolic pathways in Strain 29L as well as to investigate the production of siderophores and biosurfactants.

Strain 29L was grown with 50 mg pyrene per liter of medium, as the sole carbon and energy source. The GC-MS analysis showed the presence of two and three fused-ring PAHs which accounted for 21.8% of the carbon from pyrene in tine mid-log phase. These decreased to 9.48% into the late log phase with an increase in the hydroxyl and carboxylic acid substituted benzenes accounting for 15% of the carbon from pyrene. By day 6, in the stationary phase when 91 % of

the pyrene had been degraded, citric, oxalic, and pyruvic acids were present in higher concentrations compared to the other intermediates. These three acids accounted for 10.4% of the carbon from pyrene on day 6. Results from the enzyme assays showed the presence of several monooxygenases and dioxygenases involved in pyrene metabolism by Strain 29L in the wild type (WT) and mutants, M15 and M38, from this strain. Interestingly, Strain 29L and mutant 38 did not have any gentisate 1, 2 dioxygenase activity. Mutant 38 also did not have any catechol 2, 3 dioxygenase activity while mutant 15 did not have any catechol 1, 2 dioxygenase activity. This implies the existence of alternative, concurrent pathways in strain 29L. All the enzymes are induced by pyrene and peaked in the log phase where most of the intermediates were detected. There was no correlation in the ability to grow on pyrene and siderophore production. However, 9 1% of the mutants capable of growth on pyrene produced biosurfactants. Biosurfactant production was significantly lower for growth on acenaphthene, naphthalene and salicylate.

In conclusion, *Pseudomonas fluorescens* Strain 29L degrades pyrene by various pathways which include the upper, lower, ortho, meta, and the Evans pathways. Siderophores are not involved in pyrene biodegradation in this strain which indicates that there are other mechanisms for iron acquisition. Biosurfactants aid in pyrene biodegradation. This research sheds light on the pyrene metabolic pathways and emphasizes that this organism has alternate, concurrent routes for pyrene metabolism.