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SBRP "Research Brief" - Number 26

**Title:** Microbial Degradation of Polycyclic Aromatic Hydrocarbon Mixtures

Thousands of hazardous waste sites across the United States are contaminated with mixtures of many different compounds, including the class of chemicals called polycyclic aromatic hydrocarbons (PAHs). There are more than 70 uniquely shaped and sized molecules in this chemical group. What all PAH molecules have in common is that each has two or more fused benzene rings. These compounds are an ongoing source of public health concern since many possess toxic properties and some are considered possible human carcinogens. Because PAHs normally degrade very slowly in the environment, strategies are needed to reduce the high levels of these contaminants on hazardous waste sites.

Harnessing natural microbial processes to clean up PAH-contaminated soils is a promising approach to reducing the amount and toxicity of PAHs on waste sites. However, developing a bioremediation technology that can degrade PAHs of all shapes and sizes is difficult because PAHs differ greatly in their degree of biodegradability. Typically, the greater the number of fused rings the more resistant the compounds are to biodegradation. Despite these challenges, many scientists believe it is possible to develop strategies for utilizing microbes to remove PAH mixtures from contaminated soils. What is needed to develop an effective microbial based biodegradative strategy for PAH mixtures is a greater understanding of the kinds of microbes that can degrade the larger PAHs, and the combinations of microbes that result in the most efficient and extensive degradation of these contaminants.

In an effort to develop biological methods to degrade complex PAH mixtures, scientists at the University of Cincinnati have reported the isolation of a bacterium that can degrade different regions of benzo(a)pyrene (BaP), a large five ring PAH compound. The bacterium, *Mycobacterium* sp. RJGII.135 (strain 135), was isolated from a former coal gasification site. Strain 135 can metabolize BaP to 7,8-BaP-dihydrodiol and three cleavage products including 4,5-chrysene-dicarboxylic acid and a dihydro-pyrene-carboxylic acid. Studies are underway to confirm that these metabolic products are less toxic than the original compound. In addition to degrading different regions of the BaP molecule, the bacterium can break down multiple regions of other large ring PAHs.

Other studies with strain 135 provide information concerning the ability of this bacterium to degrade mixtures of PAHs. This bacterium can degrade three ring (anthracene and phenanthrene), four ring (pyrene and benz(a)anthracene), and five ring PAH (BaP) when each compound is present alone. However, when strain 135 is grown with two of these compounds together, the degradation is different depending on which compounds are present. BaP, pyrene, benz(a)anthracene, and anthracene appear to be

degraded through one bacterial pathway, but the bacterium appears to utilize a separate pathway to degrade phenanthrene. Because this bacterium may need to use different degradation pathways for different PAHs, by itself it may not be useful for degrading the mixtures of PAHs commonly found on hazardous waste sites.

Recognizing that combinations of different microbes may be more useful for degrading mixtures of PAHs than individual species, the scientists are studying how different microbes work together to break down recalcitrant PAHs. Experiments were carried out with two bacterial species and one algal species. In these studies BaP was incubated first with the algae followed by one of the bacterial species. For strain 135 the degradation was nearly the same regardless of whether BaP was incubated with the algae first. For the other bacterial species, when BaP was incubated with algae first a greater percentage of the BaP was degraded and mineralized (broken down to carbon dioxide and water). These findings suggest that algae in conjunction with bacteria may lead to a more complete degradation of large PAHs like benzo(a)pyrene.

The results of this research are significant for providing a greater understanding of the microbial degradation of recalcitrant PAHs and PAH mixtures. In addition to elucidating the effectiveness of one bacterial species to degrade several large PAH compounds, these studies suggest that using combinations of microorganisms may be necessary to clean up hazardous waste sites where complex PAH mixtures are present. Altogether, the knowledge acquired in these studies represent important advances toward the long-term goal of developing bioremediation strategies for PAH mixtures.

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To learn more about this area of research please refer to the following articles:

Schneider, J., R. Grosser, K. Jayasimhulu, W. Xue and D. Warshawsky. 1996. Degradation of pyrene, benz(a)anthracene and benzo(a)pyrene by *Mycobacterium* sp. strain RJGII.135, Isolated from a former coal gasification site. *Appl. Envir. Microbiol.* 62:13-19.

Sutton, S., S.L. Pfaller, J.R. Shann, D. Warshawsky, B.L. Kinkle and J.R. Vestal. 1996. Aerobic degradation of 4-methylquinoline by a soil bacterium. *Appl. Environ. Microbiol.* 62:2910-14.

Warshawsky, D., Cody, T., Radike, M., Reilman, R., Schumann, B., LaDow, K. And Schneider, J. 1995. Biotransformation of benzo(a)pyrene and other polycyclic aromatic hydrocarbons and heterocyclic analogs by several green

algae and other algal species under gold and white light. Chem. Biol. Interact. 14:375-382.

Grosser, R.J., D. Warshawsky and J.R. Vestal. 1995. Mineralization of polycyclic and N-heterocyclic aromatic compounds in contaminated soils. Environ. Toxicol. Chem. 14:375-382.

Warshawsky, D., J. Schneider, R. Reilman, K. LaDow, M. Radike, T. Cody, R.J. Grosser and J.R. Vestal. 1995. Detection of polycyclic aromatic hydrocarbon metabolites in aquatic organisms. In M. Munawar, S. Roy, D. Brown, I. Karenlampi and O. Hanninen (eds.). Bioindicators of Environmental Health Ecovision. World Monograph Series, S.P.B. Academic Publishing, The Netherlands, pp. 1-12.

Grosser, R.J., D. Warshawsky, and J.R. Vestal. 1991. Indigenous and enhanced mineralization of pyrene, benzo(a)-pyrene and carbazole in soils. Appl. Envir. Microbiol. 57:3462-69.

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As always, your feedback is welcomed.

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