

**STUDY TITLE:** Survival of a hydrocarbon-utilizing bacterium when introduced into native and foreign environments

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**BACKGROUND:** Bioremediation is emerging as an attractive means to restore inaccessible or environmentally sensitive areas. The degradation of spilled hydrocarbons is one application where the seeding of bacteria is particularly attractive since it is the microbes who ultimately consume and remove these materials, whether they occur naturally (as in marsh plant cuticles) or as an anthropogenic input. Increasingly we are seeing technological developments in which fluids of diverse origins are brought together, either by design or by accident. We are seeing renewed interest in the use of bioremediation for the purpose of remediating spilled oil.

**OBJECTIVES:** Methods used by the molecular biologist were utilized to, 1) quantitatively amplify the DNA of target species, and 2) develop molecular probes that would unequivocally and rapidly identify the test species. The upshot of these efforts was the development of a methodology that may be used to assess changes in any microbial assemblage as a result of changes in the ecosystem, or for tracking introduced microbial species during bioremediation efforts.

**DESCRIPTION:** In general, bioremediation consists of either seeding bacteria that have been adapted in the laboratory to rapidly degrade the material in question, or modification of the environment with ‘fertilizers’ to promote the development of natural microbial assemblages that are associated with the contaminant. The seeded bacteria might begin the degradation process immediately and more efficiently, whereas fertilizer addition might entail a relatively long lag period before effects are seen. The critical question, however, is whether the seeded microbes can compete and survive against native species in a foreign environment.

**SIGNIFICANT CONCLUSIONS:** A new species of *Acinetobacter* that is capable of using hexadecane as its sole carbon source was isolated from coastal wetlands in Louisiana. When laboratory grown cultures of the *Acinetobacter* were re-introduced to an experimental flow-through marsh environment and hexadecane added, the microbe quickly displaced all other bacteria and represented essentially 99% of the microbial community. When *Acinetobacter* was added to Mississippi River water supplemented with hexadecane in a chemostat, it was completely displaced after six days at which time an indigenous microflora developed that reached a community density of  $2 \times 10^6$  CFU per ml. One of the controls in this experiment was simply the addition of hexadecane to a chemostat that had only river water passed through it, and a microbial community of approximately  $10^6$  developed right from the start.

**STUDY RESULTS:** The results are that 1) Seed bacteria may readily be introduced back into the environment from which they originated, 2) Seed bacteria do not necessarily survive in foreign environments, and 3) Seeded bacteria may actually inhibit the development of normal microflora capable of utilizing the contaminating material. A methodology was developed that may be used to assess changes in any microbial assemblages as a result of changes in the ecosystem, or for tracking introduced microbial species during bioremediation efforts.

**STUDY PRODUCTS:** LaRock, P.A. and L.S. Donovan. 2001. Survival of a hydrocarbon-utilizing bacterium when introduced into native and foreign environments. US Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans LA. OCS Study MMS 2001-094. 70 pp.