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Hydrocarbon Degradation in Mixed Environments

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Hydrocarbon degradation is a form of bioremediation that involves the breaking down of substance such as benzene and toluene, in a contaminated environment, into carbon dioxide or other useful forms by a microorganism. Much research has been done, using numerous forms of aerobic bacteria, which has led to the identification of several genes responsible for the ability to break down the hydrocarbons. These aerobic bacteria have been able to “clean” up high levels of hydrocarbons in a lab setting in a short period of time. By testing the genes of genetic material found in environmental samples, anaerobic microorganisms containing the necessary genes have been found. But efforts to culture these bacteria in the lab and have them break down hydrocarbons have been unsuccessful to this point. At this time the use of microorganisms in the cleanup of spills or waste sites is not practical because many of the environments are anaerobic and have high levels of other chemicals that are detrimental to the bacteria. It is not know if it will ever be practical to use microorganism for cleaning up these environments.

Introduction

Numerous surface and subsurface environments are affected by hydrocarbons, such as benzene and toluene. Extensive research has been done on the bacteria living on contaminated surface area, including their ability to break down the hydrocarbons. Multiple bacteria and Archaea species that are able to degrade hydrocarbons in aerobic environments are known, but it is the ones that are able to survive in high saline environments and live off hydrocarbons as their sole carbon source that are particularly important. Research is being done to isolate species that are able to survive these extreme environments for the potential to clean up polluted environments. In addition to contaminated surfaces environments, subsurface environments face the same threats but it is harder to find bacteria able to degrade the hydrocarbons do to the anaerobic environment. Core samples taken from such areas, show that bacteria present in the soil have the genes necessary for breaking down hydrocarbons. Bacteria have been attempted to be used in bioremediation of oil spills such as the Exxon Valdez spill, but so far they have proved unsuccessful due to the environments present outside of the laboratory. One of the biggest problems using microorganisms to clean up oil spills is that they die in the unusual environments where

these spills take place. In addition to death degrading on hydrocarbons requires different biological pathways depending on the length and structure of the chain. Finding a microorganism that has the ability to degrade a wide range of hydrocarbons in addition to thriving in extreme environments is the only way to make bioremediation of oil spills a real possibility.

Recent Progress

Samples that were taken from deep-sea sediment at a Mediterranean station produced alkane-degrading bacteria in the genera *Alcanivorax*, *Pseudomonas*, *Marinobacter*, and *Rhodococcus*. These bacteria contained the genes necessary to degrade hydrocarbons in an anaerobic environment and were able to be grown and isolated in a lab setting. There were strands of these bacteria that had been isolated before that was known to have the genes necessary. But this was the first time these strains of bacteria were able to be isolated and cultured from a deep-sea sample and show the genes necessary for hydrocarbon degradation.

Aerobic samples for hydrocarbon bacteria are usually pulled from area that have had long contaminated areas, or are around some kind of hydrocarbon producing plant. Recently a group pulled a sample from an aquifer that was

below a petrochemical plant to look for more aerobic hydrocarbon degrading bacteria. The bacteria that were found and grown from the sample showed an ability to degrade hydrocarbons at fast rates and in mixed environments. The samples included *Pseudomonas fluoresces*, *Acidovorax*, *arthrobacter*, *Hydrogenophaga*, and *Rhodoferrax*. *Pseudomonas* is common hydrocarbon degrading bacteria that has been the subject of extensive research. Almost all of the strains were able to completely degrade benzene and toluene within 7 days. *Hydrogenophaga* was only able to degrade 50% benzene and 25% of toluene within 7 days.

Recent studies in hydrocarbon degradation have focused on Archaea because of their ability to survive in the most extreme of environments. Four strains have recently been isolated from a hyper saline coastal area of the Arabian Gulf. Two strains were of the genus *Haloferax*, while the other two were *Halobacterium* and *Halococcus*. The strains were able to grow in a wide range of salinity but had optimum growth at 4M. The strains were also able to degrade a wide range of hydrocarbons in a high saline environment with between 15-70% degradation being shown after just 3 weeks, with optimum degradation occurring at 3M NaCl.

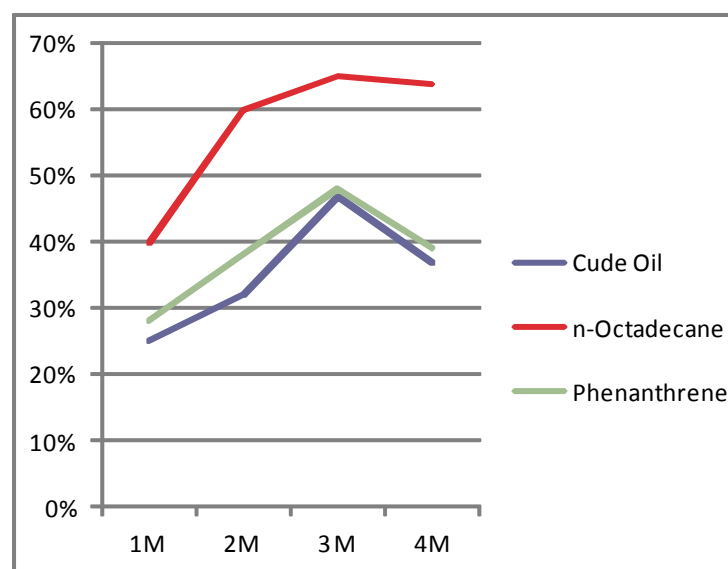
Discussion

Not very many anaerobic hydrocarbon degrading bacteria have been found, let alone grown in a lab setting. Finding these bacteria is important to eventually being able to grow them and modify them in a lab setting. Since these bacteria found have the ability to degrade hydrocarbons, if they can be grown, the most proficient one can be studied and then potentially used to treat oil spills. When BP had their oil spill a lot of oil set at the bottom of the ocean in an anaerobic, high salinity environment, which would not have been treatable by currently known aerobic bacteria. But since these bacteria were found in an anaerobic environment with high salinity they could have been used in attempt of cleaning up the oil if they had been available. Isolating and growing these bacteria was also a major step in the process. Although they had previously been identified by genetic material pulled from different samples, they had not been isolated and grown in a lab setting. Isolating them is necessary for testing their proficiency in degradation outside of a mixed culture.

The bacteria found in the aquifer were important because they provided more sample of aerobic degraders that could be used to clean up around factories. They are already able to survive in areas that would need to be cleaned up. Because of their versatility due to the environments that they came from, they could be a good choice for mass production and use in the bioremediation, replacing other bacteria that are not able to survive. These strains were also of particular importance because of the speed at which they could degrade hydrocarbons. Normal

degradation can take as long as a month for just 25 micro liters of hydrocarbons in 1 liter of solution. The ability for these bacteria to quickly degrade hydrocarbons could be used for quick clean up of an oil spill. Once the genes that they use are identified they could be transferred into another species that has a higher tolerance for salinity and be used as an even more useful tool for bioremediation.

The ability of the archaea to thrive in environments with over 4M NaCl and still be able to degrade hydrocarbons is a major breakthrough. 4M NaCl is almost 8 times higher concentration than that of normal seawater. Figure 1 shows the percent of different hydrocarbons that were degraded by a combination of the archaea strains over a 3 week period, compared to the concentration (M) of NaCl.



Although the highest percent of degradation was only close to 65%, if the test had been allowed to run for a longer period of time degradation would have continued until 100% had been degraded. Because of the ability of these strains of Archaea to degrade such a wide range of hydrocarbons in a wide range of environments, it has been concluded that they could contribute to bioremediation of oil-polluted hyper saline environments.

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