Extremophiles: Microscopic Exotica

Life can be found almost everywhere on this planet. Much of this life exists in the form of microbes. And the most exotic microorganisms, extremophiles, can live in niches where no other organisms are found. Thermophiles characteristically grow at temperatures greater than 45°C (113°F). Hyperthermophiles can live in environments with temperatures of 80°C or higher. Some extremophiles can tolerate pH levels less than two or greater than ten. And halophiles exist in saturated saline. A number of these microbes belong to the newly defined domain of life, the Archaea. Scientists first believed extremophiles were predominantly archaea, but now they are starting to see bacteria in these extreme environments as well. For example, samples taken from Yellowstone Park's Obsidian Pool showed the ratio of thermophilic bacteria to archaea as 50 to 1 (Pace, 1997). This hot-temperature environment is high in hydrogen sulfide, iron, hydrogen, and carbon dioxide.

Extremophiles can be a boon to bioremediation. Many extreme environments are anaerobic, so these microbes do not need oxygen. They can survive environments that are similar to toxic waste sites and would poison or kill other organisms. Proteins from some of these extremophiles are presently being isolated and characterized in the hopes of learning how they function in such extreme environments. Hopefully, this information will be helpful in re-engineering other microorganisms so that they can tolerate extreme conditions.

Below are profiles of several interesting extremophiles.

Methanococcus jannaschii was the first archaeon whose genome was sequenced (Bult et al., 1996). It was first isolated at the base of a Pacific thermal vent off the coast of Baja California in 1983. *M. jannaschii* possesses a small (about 1.66 Mbp) genome. It is a methanogen (methane producer) and a thermophile. This microbe normally lives at about 8,000 feet below sea level, where the pressure equals about 230 atmospheres (3,380 pounds per square inch). It is strictly anaerobic and autotrophic.

Bacillus infernus (the "bacillus from hell") is a newly identified species of bacteria (Boone et al., 1995). This is the first-known strictly anaerobic member of the bacterial genus *Bacillus*, which prior to this had always been described as aerobic. This thermophile was obtained from a depth of about 9,000 feet below the land surface. Microbes at this depth have been in isolation from the surface for millions of years and have evolved very exotic metabolisms and slow rates of reproduction.

Deinococcus radiodurans species can withstand exposure to radiation levels up to 1.5 million rads (500 rads is lethal to humans). At that point its chromosomes shatter into hundreds of fragments. It is believed that *D. radiodurans* has a more active DNA-repair mechanism than other microorganisms because the conditions under which it is able to survive are so damaging to other species. It isn't exactly clear how *D. radiodurans* obtained its remarkable radiation resistance. It was first observed in the 1950s in cans of meat that had been exposed to supposedly sterilizing doses of radiation. The microbe has certain possibilities for bioremediation. Conceivably, a strain of *D. radiodurans* modified with genes from other organisms having bioremediation ability could be used to treat highly radioactive waste.

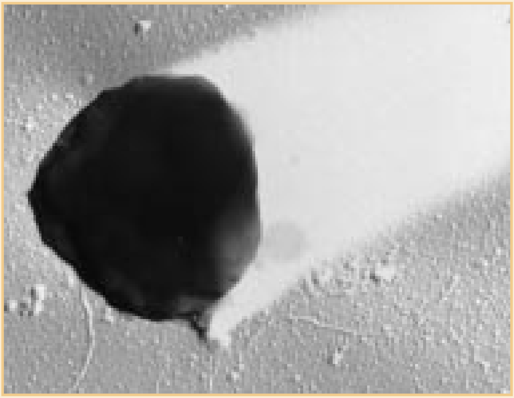

Figure 4.4. ***Methanococcus*** **species. K. O. Stetter, Universität Regensburg, Faculty of Natural Sciences.**

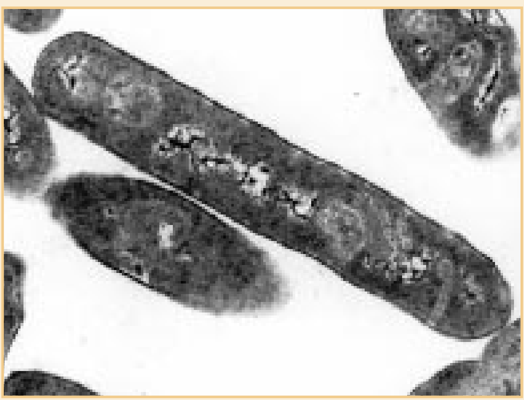

Figure 4.5. New species ***Bacillus infernus*****, "the bacillus from hell," magnified 50,000 times. Transmission electron micrograph taken by Henry C. Aldrich, University of Florida.**

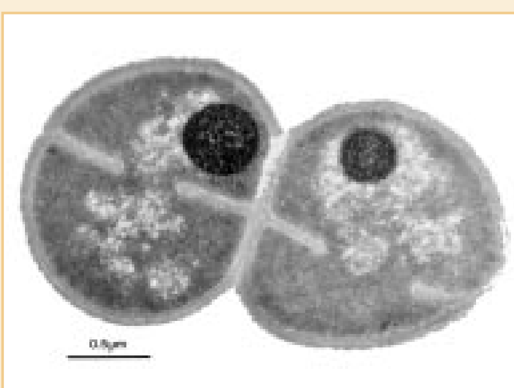

Figure 4.6. ***Deinococcus radiodurans*****, magnified 60,000 times. Taken by John Battista and Peggy O'Cain of Louisiana State University.**

Microbial Consortia

Microbial biotransformation and biodegradation can occur only if microorganisms are present that can metabolize the contaminant. In particular, there must be microbial enzymes that can act as catalysts for the oxidation–reduction reactions that will degrade or transform the compound. However, knowing and capitalizing on the relationship of the organisms to the substrate is only one aspect of bioremediation. Another aspect is understanding the interrelationships of the microorganisms in the microbial community, or consortium. A consortium is a relatively stable but loose-knit association of microorganisms in an environment. Microbe-to-microbe interactions are complex, and may run the gamut from symbiosis to predator–prey relationships.

One type of microbial consortium is a biofilm (Figure 4.7). Biofilms are created by groups of microorganisms adhering to a sediment particle or other surface and releasing exopolysaccharides. In fact, this phenomenon may create small "stagnant" areas in the sediment pore spaces where all of the oxygen is depleted, even though the fast groundwater flow path areas and therefore the bulk environment are saturated with oxygen or are aerobic. These biofilms also allow organisms to come into juxtaposition so that a variety of complex relationships, as discussed below, can develop.

In symbiosis, two species form an association whereby the individuals of one or both species are benefited. Two of the most common symbiotic relationships are commensalism and mutualism. Commensalism is a symbiotic relationship in which a one-sided association is formed between two species. The individuals of one provide sustenance to those of the other. Neither group, however, is harmed. In mutualism, both species benefit from each other's products.

Syntrophy ("mutual feeding") is a well-known form of mutualism in which members of two species are nutritionally dependent on one another. In a syntrophic relationship, the organisms together can degrade a substance that neither can degrade separately. For example, in the coupled reaction of ethanol fermentation with methanogenesis, a syntrophic relationship is formed between an ethanol fermenter and a methanogen. The ethanol fermenter produces hydrogen (H_2) and acetate, but the energy yield from that reaction is low. The methanogen then consumes the H_2 from the fermentation half reaction to produce methane with a relatively high energy yield. The coupled reaction produces a higher energy yield for the fermentation half reaction. Therefore, the ethanol fermenter utilizes more of the ethanol in the coupled reaction than it would without the syntrophic relationship with the methanogen. And the methanogen gets the H_2 it needs to produce the methane.

In predator–prey relationships, the first microbe consumes a substrate, and then the second microbe consumes the first microbe. The interactions of bacteria and protozoa (unicellular eukaroytic microorganisms) are an example of such a relationship. Protozoa that consume bacteria and excrete material that is readily utilizable by the same or other bacteria in the biofilm can have a dramatic effect on the rate and type of biodegradation/bioremediation. High rates of protozoa predation at sites being bioremediated by injection of bacteria could decrease the effectiveness of the treatment. However, other sites may benefit from high rates of predation by increasing turnover rate and thereby the biodegradation rate of the contaminant. High rates of predation may also lower the overall numbers of bacteria, even though the activity has increased. This gives the false impression that bacterial densities have decreased and therefore the bioremediation of that subsurface environment has declined. Other relationships between two species can also have both positive and negative effects on bioremediation.

In most situations, the microbes capable of metabolizing the contaminant are already present in the targeted area. However, if the contamination is recent or if the contaminants are complex, anthropogenic compounds (xenobiotics), or compound mixtures, there is a greater chance that capable microorganisms will not be present. And even if the right microbes are present, there may not be enough for a successful cleanup. This can be due to environmental conditions unsuitable for microbial proliferation and activity within the desired time frame to comply with government regulations.

If that is the case, commercially available microbial inoculants can be added through bioaugmentation (discussed in Section II). Inoculants usually consist of a sample of this microbial community that is extracted and cultivated in the laboratory. Conditions are then manipulated ex situ to encourage the growth of the suitable microbes, and then the conditions are duplicated in the field. In this way, organisms that are dormant or are in insufficient quantities but are specifically suited for the bioremediation of a particular contaminant can be selected. One of the major challenges to bioaugmentation is survival of the introduced microorganisms in the contaminated environment. Native or indigenous microbes may out-compete the introduced organisms for limited nutrients.

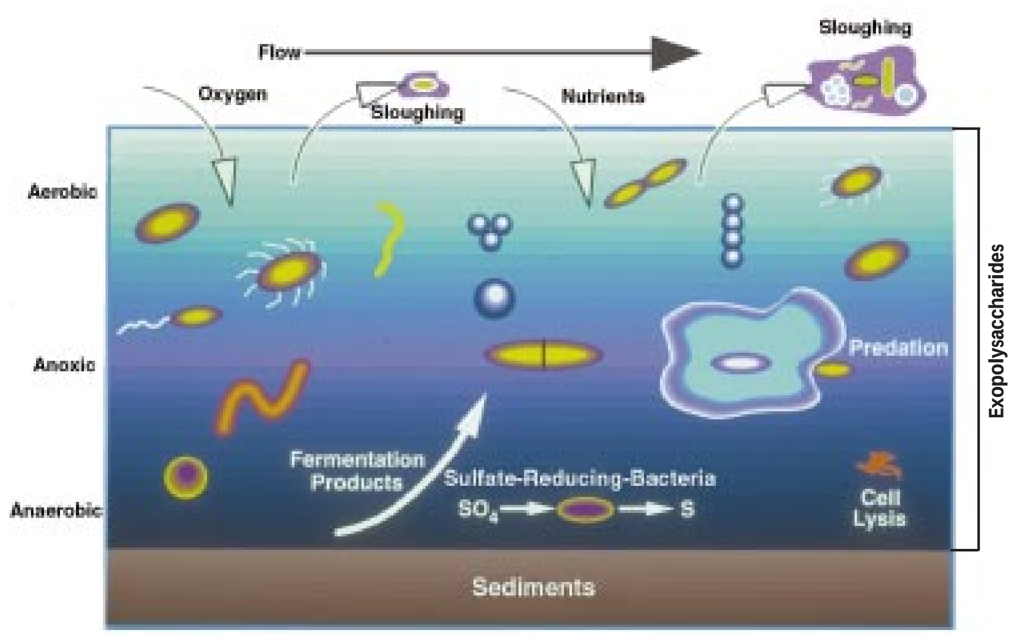

Figure 4.7. Mature biofilm.