Fungi for Bioremediation of Hydrocarbon Pollutants

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Fungi are the decomposers in the global cycle of life and death. They are usually there to do the work when anything--animal, plant, or even non-living object--is ready to be broken down again into its molecular constituents. Fungi are found in soil, in fresh and sea water, inside the bodies of plants and animals, and traveling through the air as spores. While they often are found functioning together with bacteria and an array of microorganisms, it is fungi that can especially handle breaking down some of the largest molecules present in nature (Fernandez-Luqueno et al. 2010). Their growth patterns facilitate the investigation of a wide range of environments in search of energy sources. Fungi can either grow in a multicellular form, with the somatic mycelium extending minute root-like structures through the substrate; or it can be present as unicellular yeast. Some fungi exude extracellular enzymes which allow for digestion of energy sources in their surroundings and further diffusion of these molecules through the substrate towards the fungus (Mai et al. 2004). If fungi occupy the niche of decomposers, then what is their particular relationship with the ever-increasing quantity of stuff that humans have manufactured and left to rot?

One of the largest categories of inputs to the environment in need of decomposition is man-made hydrocarbon waste. Large reserves of hydrocarbons that were previously stored deep underground are being brought to the surface, altered, and used. A majority of the pollution that occurs now involves fossil fuels, whether it is the exhaust and byproducts of spent fuel or the accumulated polymer plastics made from these same hydrocarbons. Fossil fuels are composed of polycyclic aromatic hydrocarbons (PAHs), as well as shorter carbon molecules. Many PAHs are naturally occurring (Prenafeta-Boldu et al. 2004) in plants and animals; these were the raw ingredients that first decomposed to form fossil fuel reserves. PAHs are building blocks of life, and they are very common on the planet. However, the accumulation and chemical alteration of these PAHs are following a pattern now dominated by the actions of humans (Prenafeta-Boldu et al. 2004).

PAHs form when carbon materials are not completely burned: for example, sooty exhaust from cars, charbroiled hamburgers, and burnt toast contain them. Furthermore, large amounts of PAHs are extracted, refined, and transported, and contamination of the environment occurs frequently all over the world. Burning fossil fuels, manufacturing gas and coal tar, wood processing, fuel-burning kitchen stoves, and incinerating waste are some of the ways PAHs escape into the environment. This is some of the most widespread pollution in the world, and because of the hydrophobic nature of PAHs, they can easily accumulate in fatty tissue and spread throughout the food chain. Seven of the sixteen PAHs listed as pollutants by the EPA are carcinogenic, teratogenic, and mutagenic. Current ways of dealing with polluted sites include removing the soil or incinerating it (Steffen et al. 2007).

The build-up of waste polymers is also a worldwide problem. Polymers are manufactured from hydrocarbons; a wide variety of chemical additions and structural alterations produce the wide array of plastic products available today. These products need to be resistant to degradation when fulfilling their uses, yet they also need to be able to degrade later or their disposal problems will only increase (Jecu et al. 2010). Global synthetic plastic production is 140 million tons per year, and this rate has been steadily increasing since the 1930's. It is estimated that 11% of landfill volume is made up of waste plastics (Gautam et al. 2006). So can the decomposers of natural detritus also go to work on materials altered and concentrated by humans? As so many polluted sites exist, the opportunity for fungi to evolve in their presence is ongoing. Leahy and Colwell (1990) looked at the fungal populations in areas contaminated with hydrocarbons, such as the Athabasca Oil Sands of Canada, and found the populations in these areas were much better at degrading hydrocarbons. Similarly, Llanos and Kjoller (1976) observed that after oil-waste application to the soil, the resident fungi increased their mycelial biomass, and that the community shifted towards dominance by three genera: *Graphium, Fusarium,* and *Penicillium*. Early studies such as these led to more work on the genetic level. Fernandez-Luqueno et al. (2010) report that expression levels of genes that code for degradive enzymes increase after exposure to hydrocarbon pollution, and that these genes are selected for during adaptation to the new, PAH-rich environment.

Fungi possess these decomposing abilities to deal with the array of naturally-occurring compounds that serve as potential carbon sources. Hydrocarbon pollutants have similar or analogous molecular structures which enable the fungi to act on them as well. When an area is contaminated, the ability to deal with the contamination and turn it into an energy source is selected for within the fungal population and leads to a population that is better able to metabolize the contaminant (Fernandez-Luqueno et al. 2010). Furthermore, Peng et al. (2008) report that the genes responsible for PAH degradation are present as many homologous loci within the genome, which provides a particularly large pool of mutation and rearrangement possibilities within that gene family.

In addition, microorganisms have within them a number of other stress responses that generate more phenotypic and genetic diversity so that they can deal with a new environmental stress (Fernandez-Luqueno et al. 2010). In one study, sexual reproduction in soil fungi was found to increase under ecologically stressful conditions (Grishkan et al. 2003). This shift in reproduction increases genetic diversity, and it can be seen at different levels within the fungal community. At the species level, the sexual ascomycetes increased in their relative abundance to asexual fungi as stress in the environment increased. At the level of the individual, they found the sexual fungi spending more time in a teleomorphic state (sexual ascospore state) versus an anamorphic asexual state when stress levels were higher. They hypothesized that residence of the fungi in an extreme environment that is often changing leads to selection for the ability to adapt. This is expressed through selection for genetic diversity and individuals' responses to stress such as increased sexual reproduction (Grishkan et al. 2003).

These observations point to fungi's ability to adapt to changing environments. Gautam et al. (2006) also point out that fungi have had less than 100 years in which to evolve in the presence of synthetic plastics and many other pollutants. Abilities that have not been found yet in fungi relating to the degradation of pollutants could be evolving right now in some very polluted pocket of soil.

Fungi are especially well-suited to PAH degradation relative to other bacterial decomposers for a few reasons. They can degrade high molecular-weight PAHs, whereas bacteria are best at degrading smaller molecules (Peng et al. 2008). They also function well in non-aqueous environments where hydrophobic PAHs accumulate; a majority of other microbial degradation occurs in aqueous phase. Also, they can function in the very low-oxygen conditions that occur in heavily PAH-contaminated zones (Fernandez-Luqueno et al. 2010). A review of different studies by Fernandez-Luqueno et al. (2010) yielded a list of over 51 fungal species or species groups that are successful at degrading different PAHs. A wide variety of fungi have evolved effective mechanisms to attack specific PAHs. One reason for this ability lies in the similarity between lignin, a long, aromatic family of molecules that is present in wood and PAHs. Lignin is one of the main components of woody tissue in all vascular plants along with cellulose and hemicellulose. It has been described as the cement in woody tissue that adds strength and flexibility to cellulose. This is the substance that gives trees the strength to grow taller towards the light and provides the crunch of vegetables (McCready 1991). Fungi produce extracellular enzymes to degrade lignin, which cannot pass through the cell walls of microorganisms. This process of degradation is called mineralization, and the end product is carbon dioxide. Since lignin is comprised of many different aromatic rings in long varied chains, the fungal enzymes for mineralization are non-specific and frequently can also mineralize PAHs (Mai et al. 2004).

The oyster mushroom, *Pleurotus ostreatus*, can degrade 80-95% of all PAHs present in soil after 80 days (Steffen and Shubert 2007). This is a wood-rotting fungus, part of a group known as white rot fungi. Many species within this group have been studied extensively and found to be ubiquitous PAH degraders: among them *Phanerochaete chrysosporium* and *laevis*. These basidiomycetes have at least two pathways. One pathway is the cyctochrome 450 system, much like the system in mammal livers which break down large molecules into metabolites; however, many of these metabolites are toxic themselves. The lignin extracellular degradation pathway is preferable because the metabolites are fully broken down into carbon dioxide. Peng et al. (2008) suggest that a mixture of white rot fungi and bacteria could function best at PAH degradation, as the fungi break down the largest molecules into low molecular weight PAHs, and the bacteria can then act on those molecules.

Litter-decomposing basidiomycete fungi also have substantial ability to degrade PAHs. Although their performance is not as high as typical white-rot fungi, litter-decomposing fungi are native to the soil environment in which most PAH contamination is found, and so their long term ability to exist and function in PAH-contaminated soil could be greater than that of the wood-inhabiting white-rot fungi (Steffen et al. 2002). *Stropharia rugosoannulata* was found to be the most efficient strain of basidiomycete for the removal of a variety of PAHs, doing away with over 85% of them after six weeks in experimental culture. These results occurred with the addition of manganese (II) to the culture; without this addition, performance was much lower. This shows that manganese peroxidase, one of the extracellular lignolytic enzymes, is an important component of the degradation that *S. rugosoannulata* performs (Steffen et al. 2002). In further testing of eight promising basidiomycetous fungi by Steffen et al. (2007), 60% of all PAH's present were decomposed by *S. rugosoannulata*, again the top-performing species.

Fungi attack plastic polymers as well; these come in a wide range of structures as lignin and are acted upon by different fungi species for different polymers. This decomposing ability is perhaps even more impressive than PAH decomposition. PAHs are naturally occurring, although altered and concentrated by human activity; plastic polymers on the other hand are thoroughly transformed by human processes, and they are designed to resist degradation. In one experiment, acrylic polymers were joined with lignin polymers to enhance decomposition. It was found that the white rot basidiomycetes known for lignin degradation specifically *P. ostreatus* could effectively break down polyacrylimide. This polymer is the superabsorbent material in diapers and hygiene products (Mai et al. 2004). Filip (1978) tested the ability of garbage landfill leakage fluid, teeming with the microorganisms that could thrive in a landfill environment, for its ability to degrade polyurethane. Polyurethane made from polyester (but not polyether) lost 35% of its mass after three months exposure to the fungi-enriched fluid. Clay in the mixture was found to greatly reduce the efficacy of this process (Filip 1978). *Cladosporium resinae* was found in another study to also degrade polyurethane (Gautam et al. 2006). Jecu et al. (2010) examined polyvinyl alcohol films under a scanning electron microscope and found substantial degradation by fungi, most notably *Aspergillus niger*.

Copolymerization with natural polymers such as starch or collagen increased biodegradation. These provide an additional carbon source for the fungi and may also provide access points for the fungi to invade the synthetic polymer (Jecu et al. 2010).

Sometimes the same fungi that degrade PAHs have been found to remediate toxic metals as well, which are commonly found in the same polluted sites and can reduce the effectiveness of some degradive microorganisms. Hong et al. (2009) surveyed gas station soil and found strains of *Fusarium* and *Hypocrea* that could degrade one carcinogenic high weight PAH, pyrene, as well as uptake copper and zinc. These strains were able to use the pyrene as their sole carbon source. A few papers discuss the potential applications for fungal bioremediation. Fernandez-Luqueno et al. (2010) review the techniques below. Bioaugmentation involves bringing selections of effective fungi to contaminated sites, either from the lab or from older contaminated sites in which fungi have had ample chance to evolve degradive abilities. Biostimulation is adding nutrients such as N, P, K, Cu, or S to stimulate activity of the fungi that are native to the location Steffen and Hatakka (2002) utilized this method when they added manganese to a mixture of litter-decomposing fungi and contaminated soil. A host of organic materials could be added that would contain some of these nutrients as well, such as compost, food waste, and sewage sludge. Additionally, plants (especially grasses) can be added to increase fungal activity. Introduction of genetically modified microorganisms was discussed here as well, though the authors caution that the potential for their escape from the contaminated site makes this approach too dangerous to recommend at this time (Fernandez-Luqueno et al. 2010).

P. ostreatus, or the oyster mushroom, has been utilized in some bioremediation trials involving contaminated public grounds and appears to have been quite successful. It should be noted that the results discussed in this paragraph are from a non-scientific web publication. In one test, the Washington State Department of Transportation invited different groups with bioremediation ideas to try them out on heavy oil-contaminated soil from a truck yard. While other treatments failed to yield impressive results, the berm inoculated with P. ostreatus was covered in mushrooms as large as 12 inches in diameter after 4 weeks. 95% of the PAHs were removed in this time, and the mushrooms did not contain petroleum products. After the fungi used up the available food, a successional regrowth began as flies, then birds, and then seedlings of new plants recolonized the berm. The experimenters hypothesize that the fungi could be a keystone organism in restructuring PAH-contaminated areas. These same kinds of fungi were utilized again to help mop up oil washing up in San Francisco Bay after the tanker Cosco Busan spilled 60,000 gallons in 2007. The oil was soaked up from the shores using an innovative, highly absorbent mat made of human hair. Spores were introduced to the soaked mats and turned the entire product into carbon dioxide, water, and innocuous compost (Stametz 2010).

One paper reviewed here stands alone in its grim warnings of the potential dangers associated with fungi that can consume pollutants. Prenafeta-Boldu et al. (2006) found a correlation over many fungal orders of ability to decompose lignin with the ability to infect the human brain and cause neuropathy. These infections can be quite severe; *Cladophialophora bantiana*, in particular, causes a severe brain infection which is fatal without brain surgery and intensive follow-up treatment. Infections by this group of fungi are mostly fortuitous—the diseases are not passed between people, but are instead associated with environmental exposures. Inhalation of wood dust or coal fumes can bring on some of these infections, and infections have tended to occur in immune-compromised individuals (personal communication with Don Hemmes).

Why would there be this association? Fungi have evolved to decompose lignin; lignin's variable and large structure is very similar to polycyclic aromatic hydrocarbons, and the enzymes the fungi produce are nonspecific. The chemical make-up of the brain is also high in aliphatic and phenolic compounds and lipids (50% of the brain is composed of aliphatic lipids), which are similarly affected by the nonspecific lignin-degrading enzymes. Furthermore, dopamine, one of the main neurotransmitters throughout the brain, polymerizes into polycyclic aromatic rings and degrades into the same byproducts as lignin, making it another target of the fungi's enzymes within the brain.

A further correlation exists between fungi that can handle extreme conditions and neuropathogenesis. Fungi must be able to handle the high body temperature inside the body to survive in the brain; some of these infectious fungi have been found in saunas, hot tubs, hot springs, and coal waste piles. These same extreme conditions, as well as other extreme conditions such as low oxygen, are also present in PAH-contaminated soils. Hence, Prenafeta-Boldu et al. (2006) warn that the natural spread of these fungi due to widespread PAH pollution, as well as bioremediation efforts that utilize these species, could inadvertently spread disease agents to the human population. Fortunately, none of the species named within this review as species of great bioremediation potential were in genera mentioned by Prenafeta-Boldu (2006) as causes of human disease. Nevertheless this presents a serious reason for caution before any experimental use of fungal bioremediation is initiated.

Fungi have an astonishing potential to clean up contaminated environments. After looking at the list of fungi that can degrade different PAHs, one could imagine that there is a fungus out there to degrade every type of persistent pollutant, and each one only has to be found. Polluted sites can naturally become incubators for the few species that can consume the pollutant. This is welcome news for a species that creates these polluted areas yet still needs a clean environment to survive. Furthermore, if this decomposing potential is real, should it be exploited by humans to aid in environmental clean-up? The notion of breeding effective populations from the epicenters of contamination is attractive, and it gives some redeeming value to those compromised sites. There are some risks as well. Regardless of human decisions, fungal communities will continue to interface with and attempt to consume pollutants. Research indicates that new populations of fungi can and will arise out of stressful environments to face the challenges of decomposition posed by a human-dominated world.

References Cited

Fernández-Luqueño F., Valenzuela-Encinas C., Marsch R., Martínez-Suárez, C. Vázquez-Núñez E., Dendooven L. 2010. Microbial communities to mitigate contamination of PAHs in soil—possibilities and challenges: a review. Environmental Pollution Science Research 10:11-30.

Filip, Z. 1978. Decomposition of Polyurethane in a garbage landfill leakage water and by soil microorganisms. European Journal of Applied Microbiology and Biotechnology 5: 225-231.

Grishkan I., Korol A.B., Nevo E., Wasser S.P. 2003. Ecological stress and sex evolution in soil microfungi. Proceedings of Royal Society of London 270:13-18.

Gautam R., Bassi A.S., Yanful E.K. 2006. A review of biodegradation of synthetic plastic and foams. Applied Biochemistry and Biotechnology 141: 85-109.

Hong, J.W., Park, J.Y., Gadd, G.M. 2009. Pyrene degradation and copper and zinc uptake by Fusarium solani and Hypocrea lixii isolated from petrol station soil. Journal of Applied Microbiology 108:2030-2040.

Leahy J.G. and Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews 54:305-315.

Llanos C. and Kjøller A. 1976. Changes in the flora of soil fungi following oil waste application. Oikos 27:377-382.

Jecu L, Gheorghe A., Rosu A., Raut I., Grosu E., Ghuirea M. 2010. Ability of fungal strains to degrade PVA based materials.Journal Polymers Environment 18:284-290.

Mai C., Schormann W., Majcherczyk A, Hutterman A. 2004. Degradation of acrylic copolymers by white-rot fungi. Applied Microbiology and Biotechnology 65: 479-487.

McCrady E. 1991. The nature of lignin. At http://cool.conservation-us.org/byorg/abbey/ap/ap04/ap04-4/ap04-402.html, accessed on Nov. 4, 2010.

Peng R., Xiong A., Xue Y., Fu X., Gao F., Zhao W., Tian Y., Yao Q. 2008. Microbial biodegradation of polyaromatic hydrocarbons. FEMS 32: 927-955.

Prenafeta-Boldú, F. X., Summerbell, R., Sybren de Hoog, G. 2006. Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? FEMS Microbiology Reviews 30: 109–130.

Stametz, P. 2010. The petroleum problem. At http://www.fungi.com/mycotech/petroleum_problem.html, accessed on Nov. 2, 2010.

Steffen K.T., Hatakka A., Hofrichter M. 2002. Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Applied Microbiology Biotechnology 60:212–217.

Steffen K.T., Schubert S., Tuomela M., Hatakka A., Hofrichter M. 2007. Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi. Biodegradation 18: 359-369.