Environmental biotechnology

There's a Fungus Among Us

Cookeina tricholoma, this hairy cup fungus, native to tropical regions, belongs to the Ascomycota. Members of this phylum are prime candidates for future bioremediation applications.

Photo: Fotolia/ Manitchakarn Sompoo

Bacteria have long been regarded as the masters of pollutant degradation but also fungi have this amazing ability. Can they be employed for environmentally-friendly and low-cost clean up efforts?

April 20th, 2010 was a black day, literally, for Mother Nature. 4.9 million barrels (780,000 m^3) of crude oil spilled from the BP-operated oil rig, befouling the Gulf of Mexico and surrounding areas. Effects on marine and terrestrial organisms were devastating. There was only one brigade of beings who were chuffed to bits – oil-eating microorganisms who started to munch away the spill and by doing so, began to clean up the polluted ocean. Those 'temporary cleaners' were identified as bacteria *Alcanivorax borkumensis*, *Thalassolituus oleivorans* and representatives of the genera Cycloclasticus, Colwellia and Neptunomonas.

Accidental help

However, it's not only prokaryotes that do a really good job degrading harmful compounds; fungi know how to do it, too – sometimes, even better. "The disadvantage with bacteria is that when you have huge amounts of pollutants, like oil for example, during degradation, the bacteria multiply on a massive scale. That means, we all of a sudden have this immense bacterial bloom in the Gulf of Mexico, or wherever; they are self-replicating catalysts so to speak. With fungi it's different; they profit less from those pollutants or only accidentally. They rather reduce 'en passant' all contaminants in their surroundings; they degrade them," explains Hauke Harms from the Helmholtz Centre for Environmental Research in Leipzig, Germany.

And there are even more instances where fungi beat their prokaryotic opponents, "The flaw with bacteria is that they simply can't move, especially in water-unsaturated soil(s), i.e. soil that contains air. They can't get from point A to point B and thus, can't reach the sources of pollution." In contrast, many fungi have hyphae, which they use to transport nutrients and reach out to (food, pollutant) sources far, far away. An additional drawback is that some specialised bacteria just haven't yet been 'invented'. "With bacteria they, basically, use pollutants as their food, which means that evolution can only occur when pollutants have already been present for a relatively long time and where these pollutants are also suitable as a food source. Therefore, when only trace amounts of pollutants are to be found, then it's not worth bacteria's while to pursue evolution in the direction of using these substances." This is, however, very different with fungi.

Disadvantageous bacteria

Studies date the arrival of the first fungal organisms back to about 760-1,060 million years ago, (*Mycologia*, 101:810). Over those millions of years of evolution, some members of this (taxonomic) kingdom have developed techniques, turning them into one of the top degraders of today – besides bacteria, of course. Actually, in the name of food acquisition, fungi have evolved the ability to break down organic compounds (e.g. lignin or cellulose) into inorganic molecules like CO_2 and water. This is done by special enzymes (e.g. laccases, lignin peroxidases or manganese peroxidases) via extracellular oxidation. The good thing is that those enzymes are not very choosy because, besides their natural substrates, components of the plant cell wall, they don't mind chopping up organic pollutants either. "With fungi we talk about 'biochemical combustion'. Fungi are often specialised to degrade, for example, the woody component of a tree, the lignin, and that's a very unspecific structure. You can't use any specific enzymes to do that because the chemical structures are so irregular and unpredictable that fungi have invented a certain kind of broadband mechanism, which comes in very handy when you have those somewhat unnatural-looking chemical compounds. Like pesticides, contents of cleaning agents, medicines, antibiotics etc," says Harms. Out of the seven proposed fungal phyla, especially members of two, Ascomycota ('Sac Fungi') and Basidiomycota ('Higher Fungi'), excel at this pollutant degradation task.

Beware of the tree-eaters!

Back in the eighties, this very ability was shown for the first time using a species called *Phanerochaete chrysosporium*, a so called 'white rot fungus'. White rot fungi are notorious for devouring a tree's lignin to reach the 'whitish' cellulose. When they are done, the partially decayed tree sports a very nasty, rotten look. But the fungus, as mentioned, can also render many toxic or persistent contaminants harmless. In fact, *P. chrysosporium* is the most 'talented' 'shroom, eating almost everything it is served, ranging from plastics (*Environ. Sci. Technol*, 40, 4196-99) to synthetic dyes, explosives and pesticides (*Science*, 228:1434-36).

There are many more fungi that don't shy away from doing inconsiderate humankind a huge favour. *Pleurotus ostreatus*, for example, the oyster mushroom, degrades certain classes of polycyclic aromatic hydrocarbons (PAHs) – toxic pollutants released during fuel burning – and is able to decolourise several dyes present in the waste water of textile manufacturing plants. Also, model fungus *Neurospora crassa* proved its usefulness beyond genetic analysis studies by absorbing lead and copper from metal-contaminated aqueous solutions. Furthermore, several *Aspergillus* species were found to biotransform certain insecticides like endosulfan, which has recently been added to the Stockholm Convention's list of Persistent Organic Pollutants, POPs. Even though the pesticide has already been abandoned by many countries and the aim is to achieve a global ban by mid-2012, the compound, along with its similarly persistent metabolites, will pollute the soil for a much longer time (ranging from a few weeks to several months or even years, depending on climate conditions and soil type).

Fungus vs. TNT

So, the contamination is there and when taking all the biodegrading potential of fungi into account, it doesn't take a wise man to consider them for bioremediation approaches. In their recent paper, Naresh Magan *et al.* from the Cranfield University in Bedford, UK define bioremediation as "techniques [that] aim to accelerate the naturally occurring biodegradation process by optimising the conditions under which it occurs" (*Mycobiology*, 38:238-48). This can be achieved through different methods including biostimulation (improving the 'living conditions' of native species by adding nutrients or optimising the pH value) or through bioaugmentation, in which species with certain preferences are brought in to do the clean-up job.

Fungi, as it seems, are well suited to do that job. But are there actually fungi-based bioremediation techniques out there already? During the last few decades, there have been many attempts to 'tame' our little myco-friends and make them do as we please. What looked promising in the lab, under sterile conditions, has turned out to be rather difficult in the field. All-round degrading king *P. chrysasporium* was, of course, the hottest candidate to be tested in the "real world". It failed! Dawen Gao and his co-authors from the Northeast Forestry University in China and Stanford University, USA write, "*Phanerochaete chrysosporium* […] has not shown positive results in any large-scale tests." They go on to describe its application in the nineties to treat TNT-contaminated soil at a "former ordnance area of a USA Naval submarine base". After 120 days of treatment, TNT concentrations hadn't met the target level and so "the test was considered a failure. No further testing was reported" (*Crit Rev Biotechnol*, 30(1):70-7).

Wrong strategy

The oyster mushroom, *P. ostreatus*, was put to the bioremediation field test, too and indeed, scientists found that during a 62-day incubation period, concentrations of TNT, HMX and RDX (all explosives) were significantly reduced. Oddly, though, concentrations were also reduced when no fungus was added. The effect was attributed to the addition of growth substrate that promoted the activity of native species. Gao *et al.*, therefore concluded, "The role of the fungus in this field test was not clear."

So, what went wrong in the search to find an environmentally-friendly waste disposal technique? Harms thinks that, in the past, scientists investigating the pollutant-degrading poten-


Photo: Fotolia/ fergregory

Besides the usual suspects, explosives like TNT (from e.g. military activities) also contaminate our precious soil.

tial of fungi have put the cart before the horse, so to speak. They focussed too much on biochemical features and forgot about the ecology. Thus, it was attempted to use a tree-dwelling fungus, known for its wood-decaying abilities, for the degradation of pollutants in the soil. "They have simply mixed the fungi into the soil and didn't think about the fact that it's, of course, a totally alien environment for them," says Harms.

No shake and plough

Additionally, because it was known from bacteria that it has a positive effect on bioavailability of the pollutant and oxygenation capacity to shake and plough, the same was thought to be true and raise the 'working moral' of a fungus, too. WRONG! As we already know, most fungi have hyphae and this filamentous network needs to be intact for a fungus to perform its obligations. "That just wasn't taken properly into consideration and, thus, it led to the fungi being damaged in the process. They were used as 'displaced organisms' in a wrong setting."

Trees – cosy home and delicious food, all in one.

Subsequently, it became apparent that many more things have to be considered before fungi are let loose on the contaminated environment. There are several factors that can influence a fungus' degradation abilities like habitat preferences, temperature (for example *P. chrysosporium* prefers it a bit warmer than *P.ostreatus*), pH value or moisture (*Mycobiology*, 38:238-48). Most importantly, fungi have to stand up to indigenous microorganisms that, of course, were there first and, understandably, don't want to share their food sources and living space voluntarily. However, many white rot fungi have "low competitive capabilities" and hence sound retreat rather than nestle down (*Crit Rev Biotechnol*, 30(1):70-7).

Moving along

But all hope is not yet lost, as Harms and colleagues wrote in their latest article "Untapped Potential: Exploiting Fungi in Bioremediation of Hazardous Chemicals" (*Nat Rev Microbiol*, 9:177-92). The potential only needs to be exploited the right way. "One should give much more attention to the ecological role of fun-

Plastic Pollution

Finnish researchers and their laboratory fungi want to put a stop to bisphenol A's harmful game.

"If you don't have BPA in your body, you're not living in the modern world", wrote the *TIME* magazine in an article a year ago. BPA aka bisphenol A, used in the process of making polycarbonate plastics, seems to be found in almost everything from water bottles over dental fillings to sales receipts. Even though it's not a Persistent Organic Pollutant (POP) – it has a half-life of only a few days – it's nevertheless considered a harmful contaminant because of its sheer omnipresence. In 2009, more than 2.2 million tonnes were produced worldwide.

What's even more worrying, hardly a day goes by without 'sickening' news of its effects on health. Constantly, new reports are emerging that connect this organic compound to obesity, neurological issues or cancer in humans, and which also led to the recent ban of its use in baby bottles. But humans are not the only species to be affected. Aquatic organisms, especially fish, are heavily influenced in their growth, reproduction and development, too. These effects can be traced back to the endocrine-disrupting activity of BPA, mimicking the action of natural hormones and thereby interfering with the endocrine system. In 2007, Matsushima *et al.* from Kyushu University, Japan found that BPA binds to human oestrogen-related receptor γ (ERR-γ), preserving the receptor's basal constitutive activity (*J Biochem*, 142: 517-524).

So, there are plenty of reasons to get rid of this nasty substance; but how? Maybe fungi can help? Yes, they can! Grit Kabiersch, doing her PhD in the group of Annele Hatakka at the University of Helsinki, uses the two white rot fungi, *Stropharia rugosoannulata* and *Stropharia coronilla*, to get down to the real root of trouble. "When I was still at school, my chemistry teacher told me that 'biological degradation' simply means that the parental compound has disappeared after treatment with some biological agent. However, this does not necessarily solve the problem since there are still lots of possibly harmful metabolites left and with them, their biological activity. This is why we also focus on the removal of the oestrogenic activity and on possible metabolites." In their recent paper, Kabiersch *et al.*, showed exactly that. Seven-day old cultures of one of the studied fungi, the litter-decomposing and slightly hallucinogenic *Stropharia coronilla*, were able to remove BPA's oestrogenic activity "rapidly and enduringly" (*Chemosphere*, 83:226-32).

At the moment, the research is still at "bottle level" but Grit and her colleagues, Kari Steffen and Marja Tuomela, already "have ideas on how to design experiments at the pilot scale".

Photo: Fotolia/Friedberg

gi with pollutant degradation. Such as the ability of fungi to put continuous networks through soils, also through soils that are water-unsaturated. In this way, microorganisms like bacteria, for example, are able to move along these fungi." This is what researchers of the Helmholtz Centre for Environmental Research in Leipzig, including Harms, found a few years back. In the laboratory, they studied the movement of PAH-degrading bacteria (*Pseudomonas putida*) along the hyphal network, the mycelium, of the plant pathogenic fungus *Pythium ultimum* (*Environ Microbiol*, 12:1391-8). The authors suggest that "fungi improve the accessibility of contaminants in water-unsaturated environments" and by doing so, enhance biodegradation by bacteria. This knowledge could be put to good use in future bioremediation strategies that are "based on ecological principles" – an approach favoured by Harms and co-workers. "Apart from water treatment, where it is possible to work with pure fungal biomass, under normal circumstances the fungus won't act by itself. With all the experience we have gathered and with all the theoretical knowledge, we can assume that the fungus, when it is doing alright, will actively support bacteria and plants in their activities." And it's exactly this interaction that scientists need to delve into further.

Ecology first

For now, the most important thing seems to be to first understand the fungal organism, focussing primarily on its ecology. And only later should one consider directly inoculating contaminated areas with certain specialist species. "We have just started a project with petroleum-contaminated soils. In this project we are working together with a company and here, we also want to test different fungi for their effectiveness. This will be a laboratory-based study but it will also be taken as far as pilot scale. As we have noticed, there's a lot of interest from the industry to warrant continuing work with this kind of approach," says Harms. Interestingly, genetic engineering of fungi won't play a bigger role with such approaches, at least not in the near future, as, according to Harms, "Biochemical performance is not the limiting factor in the real world."

To come back to the oil spill in the Gulf of Mexico; according to Christopher Haney, chief scientist for the US-based wildlife conservation organisation Defenders of Wildlife, "Up to 75 percent of the oil from the disaster still remains in the environment." He continues, "Whether buried under beaches or settling on the ocean floor, residue from the spill will remain toxic for decades." So, there's lots of clean up work still left to do and a call for more effective, efficient methods can be heard loud and clear. Also, fungi could one day be part of those strategies, helping to get everything back to normal after disaster has struck. One thing is already sure: a world without fungi would be a very different one. "It would be totally boring," says Harms and continues to speculate, "If, all of a sudden, fungi were to disappear, then all woody plants would become sick." And, clearly, plants wouldn't be the only ones to suffer.

Pathogenic and helpful

And so, we have learnt that there are not only fungi of the delicious or pathogenic kind, some of them are very helpful, too. They are, as tv mafia boss Tony Soprano would say, in the "waste management business", taking special care of human-made, ecologically harmful pollutants. Even though it's only 'accidental'.

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