



Screening of extremotolerant fungi for the bioremediation of hydrocarbon contaminated sites

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Bioremediation can be used to treat contaminated sites, by taking advantage of microorganisms which have the potential to degrade a wide range of contaminants. While research has been focused mainly on bacteria, the knowledge on other microorganisms, especially fungal communities, is still limited. However, the use of fungi may have advantages compared to bacteria. Extremophile fungi like the black yeasts can withstand high levels of environmental stress (e.g. range of pH, water availability and temperature, presence of toxic chemicals). Therefore they might be applicable in situations, where bacterial communities show limited performance.

In order to identify fungi which are good candidates for bioremediation application, a selection of 163 fungal strains, mostly from the group of the black yeasts, was tested for their capability to degrade three different pollutants: hexadecane, toluene, and polychlorinated biphenyl 126, which were used as model compounds for aliphatic hydrocarbons, aromatic hydrocarbons and polychlorinated biphenyls. These chemicals are frequently found in sites contaminated by oil, gas and coal. The screening was based on a two-step selection approach. As a first step, a high throughput method was developed to screen the relatively large amount of fungal strains regarding their tolerance to the contaminants. A microtiter plate based method was developed for monitoring fungal growth in the presence of the selected contaminants photometrically with a Tecan reader. Twenty five strains out of 163, being species of the genera *Cladophilaophora*, *Scedosporium* and *Exophiala*, showed the ability to grow on at least 2 hydrocarbons, and are therefore the most promising candidates for further tests. In a second step, degradation of the contaminants was investigated in more detail for a subset of the screened fungi. This was done by closing the carbon balance in sealed liquid cultures in which the selected pollutant was introduced as the sole source of carbon and energy. Substrate depletion and CO₂ accumulation in the headspace were monitored chromatographically. In the course of these experiments, two strains showed a good capacity to grow on toluene.

In summary, the presented screening method can be used to identify potential candidates for the fungal degradation of contaminants. Further research is necessary to investigate the potential use of the identified fungal strains for remediation purposes.