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RESEARCH ARTICLE

Effective remediation of aged HMW-PAHs polluted agricultural soil by the combination of *Fusarium* sp. and smooth bromegrass (*Bromus inermis* Leyss.)

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Abstract

Fusarium sp. strain ZH-H2 is capable to degrade high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs), smooth bromegrass (*Bromus inermis* Leyss.) can also degrade 4- to 6-ring PAHs. Pot experiments were conducted to investigate how bromegrass and different inoculum sizes of ZH-H2 clean up HMW-PAHs in agricultural soil derived from a coal mine area. The results showed that, compared with control, different sizes of inocula of ZH-H2 effectively degraded HMW-PAHs, with removal rates of 19.01, 34.25 and 29.26% for 4-, 5- and 6-ring PAHs in the treatment with 1.0 g kg^{-1} ZH-H2 incubation after 90 d. After 5 mon of cultivation, bromegrass reached degradation rate of these compounds by 12.66, 36.26 and 36.24%, respectively. By adding strain ZH-H2 to bromegrass, HMW-PAHs degradation was further improved up to 4.24 times greater than bromegrass (W), in addition to the degradation rate of Bbf decrease. For removal rates of both 5- and 6-ring PAHs, addition of 0.5 g kg^{-1} *Fusarium* ZH-H2 to pots with bromegrass performed better than addition of 0.1 g kg^{-1}, while the highest concentration of 1.0 g kg^{-1} *Fusarium* ZH-H2 did not further improve degradation. Degradation of 4-ring PAHs showed no significant difference among different ZH-H2 incubations with bromegrass treatments. We found that the degradation rates of 4-, 5- and 6-ring PAHs in all treatments are significantly correlated in a positive, linear manner with activity of lignin peroxidase (LiP) (r=0.8065, 0.9350 and 0.9165, respectively), while degradation of 5- and 6-ring PAHs is correlated to polyphenoloxidase (PPO) activity (r=0.7577 and 07806). Our findings suggest that the combination of *Fusarium* sp. ZH-H2 and bromegrass offers a suitable alternative for phytoremediation of aged PAH-contaminated soil in coal mining areas, with a recommended inoculation size of 0.5 g *Fusarium* sp. ZH-H2 per kg soil.

Keywords: coal mining area, HMW-PAHs, *Fusarium*, smooth bromegrass, enzymatic activities

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic pollutants whose molecules contain two or more fused benzene rings in linear, angular or cluster arrangements (Shree and Rudra 2007). As PAHs are carcinogenic, teratogenic and mutagenic, they are listed as priority persistent organic pollutants by the United States

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Environmental Protection Agency. Low molecular weight (2- or 3-ring) aromatic hydrocarbons such as naphthalene, phenanthrene and anthracene can be rapidly degraded by microbes which use these compounds as a carbon source. In contrast, high molecular weight PAHs (HMW-PAHs) with four or more benzene rings are relatively resistant to microbial degradation due to their lipophilicity, low water solubility and high stability (Carl 1992; Hughes *et al.* 1997; Shree and Rudra 2007). As a consequence, such HMW-PAHs persist in the environment where they are considered serious pollutants. Due to their high hydrophobicity, HMW-PAHs concentrate in non-aqueous phases and strongly adhere to particulate matters, resulting in their accumulation in soils (Wilson and Jones 1993). Such pollution affects not only the health of local soil ecosystems but also that of humans when HMW-PAHs enter the food chain (Boonchan *et al.* 2000). Therefore, it is highly necessary and urgent to remediate soils polluted by HMW-PAHs. In the mining areas of Hebei Province, China, the combination of coal mines, steel works and coking plants poses a serious threat to the environmental quality of the surrounding agricultural soils. A previous investigation showed that 89% of agricultural soils in this area are polluted by HMW-PAHs (Zhao *et al.* 2015).

Bioremediation is attracting more and more attention due to its advantages of low costs, no secondary pollution and its suitability for large area remediation, with phytoremediation and microbial remediation as the major forms of bioremediation under investigation. Over 40 plant species have been reported to be able to enhance the degradation of aromatic hydrocarbons, such as *Populus*, *Salix*, *Pinus*, *Agropyron*, *Medicago* and *Lolium* species (Gaboriau and Saada 2001). Studies on remediation of soils polluted by aromatic hydrocarbons have shown that high removal efficiencies can be achieved using *Medicago* sp. (*Trifoliae* family) and *Lolium multiflorum* Lam (known as ryegrass). However, it has been pointed out that these plants are not very efficient to degrade HMW-PAHs with 4 or more rings (Shen *et al.* 2011). In addition, the application of phytoremediation is limited because it also requires long incubation time, and the efficiency is to a large extent dependent on the soil and climate conditions and constrained by pollutant concentrations compared with conventional remediation methods. Bioremediation by use of microorganisms has also been recognized as an effective approach for restoration of PAH-contaminated soil, however, it is difficult to generate and maintain the required microbial populations with PAH-degrading activity in natural soils to achieve a high removal rate of PAH (Lu and Lu 2015).

As a solution to these restrictions, plant-microbe remediation technologies combine the advantages of phyto- and microbial remediation and enhance the degradation of organic pollutants in the rhizosphere. Plant-microbe remediation has become a hot topic in the field of soil remediation. For instance, it was reported that the total amount of aromatic hydrocarbons in soil decreased by nearly 60% within half a year when plant species like *Medicago* sp. was combined with selected microbes (Chen and Chen 2005). Yao *et al.* (2014) documented that both *Trichoderma ressei* FS10-C and *Rhizobium meliloti* improved the growth of *Medicago sativa* L. and enhanced the degradation of PAHs in soil; compared to remediation by *M. sativa* on its own, addition of *T. ressei* FS10-C increased removal rates of PAHs from approximately 20 to 25%, while in combination with *Rhizobium meliloti*, *M. sativa* increased removal rates to nearly 33%. Yang *et al.* (2009) demonstrated that inoculation with *Glomus caledonium* significantly raised the remediation efficiency of *Lolium perenne* L. (Yang *et al.* 2009). Liu *et al.* (2010) reported that the combination of *Medicago* sp. with PAH-degrading bacteria enhanced the degradation of 5- and 6-ring PAHs in polluted agricultural soils. However, as polycyclic aromatic hydrocarbons have a complex composition and soil properties vary, current plant-microbe remediation technologies need to be optimized for each condition. To achieve the best effect of joint remediation for a given pollution, stress-resistant plant species and matching microbial inocula need to be screened and optimized. In recent years, a number of studies have been performed on the remediation of soils artificially polluted with PAHs using plants or/and microbes (Liu *et al.* 2004; Zhang *et al.* 2010; Xiao *et al.* 2015), but few investigations have been conducted on the remediation of agricultural soils highly polluted by HMW-PAHs.

When 21 filamentous fungi were tested for remediation of aged aromatic hydrocarbon-polluted soils, *Fusarium* sp. produced the most significant effect on degradation of HMW-PAHs, giving an overall removal rate of over 30% for 5- and 6-ring PAHs (Potin *et al.* 2004). Therefore, it is evident that *Fusarium* sp. has a great potential in remediating soils polluted by HMW-PAHs. Recently, our group isolated an efficient PAH-degrading fungal strain, *Fusarium* sp. ZH-H2, from polluted soil taken from a coal mining area in Hebei Province. Cultured in a mineral salt medium supplemented with 9 different HMW-PAHs (all with more than 4 rings) as the sole carbon source, ZH-H2 degraded 48% of the PAHs. This was increased to 89% degradation when starch was added to the medium (Zhao *et al.* 2016). Other experiments showed a high resistance of bromegrass (*Bromus inermis* Leyss.) against the toxicity of HMW-PAHs. The plant effectively remediated polluted sewage/soil with particularly high removal rate of HMW-PAHs (Wang *et al.* 2015). These findings identified the potential of bromegrass in remediating HMW-PAHs polluted soils. Here, we report experiments conducted to investigate the combination of bromegrass and *Fusarium* sp. ZH-H2 for remediation of aged HMW-PAHs polluted soils. To that end, pot experiments were performed