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The characteristics of rhizosphere microbes associated with plants in arsenic-contaminated soils from cattle dip sites

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Abstract

Soil microorganisms and plants were studied in samples of arsenic-contaminated soil from two cattle dip sites. The aim was to delineate the parameters that will determine the feasibility of future remediation by growing arsenic-accumulating plants, including the identity and characteristics of some rhizosphere soil microbes. The soil samples contained high total, but low soluble arsenic concentrations which, together with other properties, resembled the previously reported characteristics of dip-site soils from this region of rural Australia. A glasshouse trial demonstrated that dip-site rhizosphere microbes promoted arsenic accumulation by the grass *Agrostis tenuis* on contaminated dip-site soil without inhibition of growth. The arsenic content of the shoots was increased by 45%. We studied the colonization of roots of dip-site plants by mycorrhizal fungi and tentatively identified six genera of other fungi present in the soil samples. Two plant species growing at the sites, Kikuyu grass (the most abundant plant) and Rainbow fern, exhibited mixed infections of their roots by endomycorrhizal fungi (tentatively identified as *Acaulospora* and *Gigaspora*) and by soil-born pathogens. Five rhizosphere bacteria were identified to genus level and we determined the effect of arsenic on their growth. The two most prevalent strains differed greatly in their growth sensitivity to arsenate; *Arthrobacter* sp. being the most sensitive while *Ochrobactrum* sp. exhibited exceptional resistance to arsenate. Of the other, less prevalent strains, two were *Bacillus* spp. and the last, *Serratia* sp., was the most resistant to arsenite. These findings show the importance of understanding plant–soil microbe interactions for developing future strategies aimed at a phytoremediation-based approach to removing arsenic from soil at dip sites.

Keywords: Arsenic; Soil contamination; Cattle dip site; Rhizosphere; Bacteria; Mycorrhizae

1. Introduction

Contamination of soil and water by arsenic is a global problem (Walter and Wenzel, 2002). In Australia, cattle ticks jeopardize the beef and dairy industries and a cattle-dipping program for eradication and containment using arsenic as the major pesticide commenced in the

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early 1900s. Fifty years later, arsenic was replaced by DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane]. In one region of Australia alone (northeastern New South Wales and southern Queensland), at least 1600 cattle dips were constructed (Edvantoro et al., 2003). This region of Australia contains extensive areas of fertile farmland. Used pesticides, disposed by burying in the vicinity of the dip bath, have left a legacy of high level, localized contaminated sites. A recent study of soil contaminants in 12 dip sites found that 80% of soil samples had concentrations between 1 and 1000 mg arsenic kg^{-1} soil while the remaining 20% ranged from 1000 to 4540 mg arsenic kg^{-1}, all co-contaminated with DDT (Van Zwieten et al., 2003). Farming and housing development around the contaminated sites have been prohibited. These sites present both a challenge and an opportunity for developing new techniques using microorganisms and/or plants for future remediation.

In addition to anthropogenic sources, arsenic occurs in soils in some regions naturally from weathering of arsenic-rich mineral deposits. Inorganic arsenic exists primarily as pentavalent arsenate and trivalent arsenite. These oxyacids interconvert and equilibrate depending on the prevailing redox potential and pH. The toxic effects of arsenic on living organisms are well documented (Niragu, 1994), with each valence state having distinct toxic properties. The structural similarity between arsenate and inorganic phosphate permits arsenate to enter cells on membrane inorganic phosphate transporters (Walter and Wenzel, 2002) and to interfere with metabolic reactions involving phosphate. In contrast, much of the toxicity of arsenite is associated with the ability of this trivalent oxyanion to form bonds with functional groups of proteins. Metabolism of inorganic arsenic to methylated forms by living organisms adds to many soil environments a range of organic arsenic compounds of generally lower toxicity (Cullen and Reimer, 1989).

Organisms living in environments with naturally high arsenic concentrations have evolved mechanisms to coexist with, and even benefit from arsenic compounds. Much of the extensive arsenic metabolism of bacteria and fungi (Cullen and Reimer, 1989) is associated with minimizing the concentration of arsenic within the cells, by arsenic-exporting mechanisms and by improving the specificity of phosphate uptake (Cervantes et al., 1994). Arsenic is also utilized in metabolism as a terminal electron acceptor in dissimilatory arsenate respiration or as an electron donor in chemoautotrophic arsenite oxidation. The *arsC* gene, which codes for an arsenate reductase, transforming arsenate into arsenite, is essential for resistance by extruding arsenate from the cell (Jackson and Dugas, 2003).

Microbes in the soil are important in providing nutrients to plant roots. Soil bacteria degrade organic compounds and modify the inorganic products. Soil fungi provide a large surface area for scavenging soil-bound nutrients such as inorganic phosphate and, through ectomycorrhizal and endomycorrhizal associations with roots, transport these to the plant. Toxic compounds in soils are often modified by microbes (Van Zwieten et al., 2003), but many such toxins also may hinder growth of soil microbes and impair their ability to promote plant growth. Additionally, soil fungi associated with roots have the potential to either increase or ameliorate the uptake of inorganic contaminants by plants. Consequently, mycorrhizal fungi in polluted soils are crucial in maintaining diverse populations of indigenous vegetation and act as a barrier to the uptake of toxic heavy metals by plants (Leyval et al., 1997). Sharples et al. (2000) presented evidence that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* acts as a filter to maintain low arsenic uptake rates by roots of the plant *Calluna vulgaris* when growing in arsenic-contaminated soil. In a study of evolved arsenate resistance in cultivars of the grass *Holcus lanatus*, Gonzalez-Chavez et al. (2002) found that colonization by the arbuscular-mycorrhizal fungus *Glomus* suppressed high-affinity arsenate and phosphate transport into the roots. Conversely, mycorrhizal association with the fern *Pteris vittata* has been reported to stimulate arsenic accumulation by the host (Liu et al., 2005).

Several recent publications have addressed the microbial population in cattle dip-site soils and their interaction with the contaminants. The co-contamination of these soils with arsenic and DDT has been shown to have distinct effects on the soil microbial populations. Edvantoro et al. (2003) showed that in contaminated dip-site soils, fungal counts, microbial biomass, carbon and respiration rates were significantly lower compared to uncontaminated controls. However, the bacterial population was not significantly different. They attributed the effects on the fungal population to arsenic rather than DDT. Van Zwieten et al. (2003) measured the concentrations of DDT and two products of its natural slow degradation in dip-site soils, for which soil bacteria are responsible. They found that, in soils with high arsenic concentrations, the breakdown of DDT was impaired. In a study of arsenic loss from similar soils, Edvantoro et al. (2004) made the symmetrical finding that volatilization of arsenic, attributed to microbial activity, was inhibited by the DDT contaminant.

The possibility of using arsenic-accumulating plants to extract arsenic from soil for remediation of contaminated land areas such as dip sites has attracted attention. The