Field: Green Chemistry

Title: Impact of Transgenic Tobacco on Trinitrotoluene (TNT) Contaminated Soil Community

Abstract

Environmental contamination with recalcitrant toxic chemicals presents a serious and widespread problem to the functional capacity of soil. Soil bacteria play an essential role in ecosystem processes, such as nutrient cycling and decomposition; thus a decrease in their biomass and community diversity, resulting from exposure to toxic chemicals, negatively affects the functioning of soil. Plants provide the primary energy source to soil microorganisms and affect the size and composition of microbial communities, which in turn have an effect on vegetation dynamics. We have found that transgenic tobacco plants overexpressing a bacterial nitroreductase gene detoxify soil contaminated with the high explosive 2,4,6-trinitrotoluene (TNT), with a significantly increased microbial community biomass and metabolic activity in the rhizosphere of transgenic plants compared with wild type plants. This is the first report to demonstrate that transgenic plants engineered for the phytoremediation of organic pollutants can increase the functional and genetic diversity of the rhizosphere bacterial community in acutely polluted soil compared to wild type plants.

Introduction

Pollution of the environment with recalcitrant chemicals impacts severely on the microbial community (*1*−*4*). Microorganisms are vital for soil fertility and for the degradation of organic matter in soils and sediments. Bacterial communities in soil are likely to respond to pollution by changing their structure to one that favors organisms that are able to thrive under the new selective conditions at the expense of other organisms that are suppressed. The loss of sensitive microorganisms, often performing specific functions, can have serious ecological consequences (*5*), for instance impacting on nitrogen cycling (*6*).

Explosives are a major cause of organic pollution, with TNT pollution being the most widespread. Historically TNT was the most widely used military explosive (*7, 8*), with a large number of sites used in World War II still heavily contaminated (*9*). TNT also contaminates military training ranges and sites of explosives manufacture. TNT, as with most explosive compounds, is toxic, mutagenic, and highly energetic (*10*), having a serious impact on the environment, and threatening human health (*11, 12*). TNT is a highly recalcitrant xenobiotic compound and, although soil bacteria have been isolated that can metabolize this explosive (*13*), the chemical intransigence of TNT at contaminated sites suggests that there is insufficient microbial biomass and activity present in soil to degrade TNT at any appreciable rate. To address this issue we investigated whether plants could be genetically engineered to yield an optimal system for *in situ* bioremediation of toxic explosives residues in soil. Progress has been made toward this goal by successfully combining the biodegradative capabilities of soil bacteria with the high biomass, stability, and sequestration properties inherent in plants.

Increased tolerance to TNT, in sterile liquid cultures, was shown for *Nicotiana tabacum*(tobacco) engineered to express TNT-transforming bacterial enzymes (*14, 15*). Two bacterial enzymes were separately expressed in tobacco, pentaerythritol tetranitrate (PETN) reductase (*16*) and a classical type I nitroreductase (NR) (*15*), both enzymes deriving from strains of*Enterobacter cloacae* (*17, 18*) which have been shown to catalyze a two electron reduction of TNT to produce hydroxylamino- and amino-dinitrotoluene derivatives (*13*). Vila et al. recently demonstrated that these TNT metabolites are conjugated to glycose in tobacco as part of the detoxification process (*19*). While the experiments conducted in sterile culture provided valuable information, it is how the plants perform in the more complex soil environment that is of relevance to assess the phytoremediation potential. In soil, both abiotic and biotic factors could influence plant growth, health, and response to TNT. As well as the microbial community potentially influencing the plants, the tobacco plants are predicted to impact on the microbial community of the soil.

One important aim was to determine whether NR-expressing tobacco plants were more tolerant to TNT

positively influence the functional and genetic diversity of the microbial community of contaminated soil.

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