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Phyto-ruminal-bioremediation: Grasses to the rescue

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Introduction

The USEPA has defined Super Fund sites for 594 toxins in the United States. The EPA definition of toxins is one that causes acute or chronic human health effects, or causing significant environmental harm. One of the categories of toxins is nitroaromatic molecules, which are used primarily as munitions. In the United States alone there are 16,000 Department of Defense (DoD) sites. The most common munitions there are TNT, RDX, HMX, and 2,4 DNT. In the world, these sites range historically from World War II residues to those from recent Middle East conflicts. Human health effects from these toxins include damage to the renal, nervous system, reproductive system, dermal, and hepatocarcinoma.

In the animal world, a ruminant has four stomachs, one of which the rumen contains obligate ruminant anaerobes. These anaerobic microbes have the unique ability to break down the cellulose bond and other compounds from plant material. An additional fact is that grasses can extract nitroaromatic compounds from the soil and do it far better than dicotyledon plants. A number of people have looked at the uptake of toxins by grasses and other plant material. This paper will present the ability of cool season grasses to remove toxins from the soil and their subsequent degradation by ruminal microbes from sheep.

Materials and Methods

To prove the ability of cool season grasses to extract nitroaromatic compounds and the grasses subsequently detoxified by sheep anaerobic microbes, a series of experiments were conducted.

Experiment one, 14investigated the fate and uptake of [^{14}C]-TNT from soil into orchardgrass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), and tall fescue (*Festuca arundinacea*). The plant materials were grown for one year with clippings when the grass was seven centimeters above soil surface and subsequently 63, 181, 369 days after planting. Extraction and analysis of TNT was done using HPLC (Duringer, et al., 2010).

Experiment two. [^{14}C] radio labeled TNT was administered to sheep and the fate of the TNT followed for 72 hours in tissues and blood. (Smith, et al., 2008).

Experiment three, the role of multiple ruminal bacterial species ability to biodegrade TNT was investigated. Twenty-one ruminal bacteria species were tested for their ability to degrade 2,4,6-trinitrotoluene (TNT) within 24 h.

Two additional **experiments, four and five**, incubated whole rumen fluid with RDX (Eaton, et al., 2013) and HMX (Perumbakkam, and craig, 2012). Analysis was made using HPLC.

Experiment six, finally, a study sought to identify the major genes involved in the breakdown of RDX and TNT by the microbes of the ovine rumen using metagenomic approaches (Li, et al., 2014).

Results and Discussion

Experiment one confirmed continual uptake of TNT into grasses over a one year period with the heaviest concentration in the roots followed by the crown then the grass blades. Of the three grasses, the orchardgrass was the most efficient species in taking up TNT.

Experiment two. In the sheep dosed with radio labeled TNT, parent TNT and the dinitroamino metabolites and diaminonitro metabolites were not detected in excreta even though 76% of the radio activity was present in the feces (Smith *et al.*, 2008). The toulene molecule was not found in muscle or kidney tissue, which further substantiated that the TNT molecule no longer existed beyond the rumen and was not absorbed by the animal. The sheep suffered no ill effects from the TNT. The conclusion is that the ruminal microbes can breakdown the parent compound and its major metabolites into nontoxic moieties.

Experiment three. *Butyrivibriofibrisolvens, Fibrobacter succinogenes, Lactobacillus vitulinus, Selenomonasruminantium, Streptococcus caprinus, and Succinivibriodextrinosolvens* were able to completely degrade 100 mg/L TNT, with <5% of the original TNT recovered as diaminonitrotoluene metabolites in incubation experiments. Other bacteria were also able to partially biodegrade TNT. These results indicate that a variety of rumen bacteria is capable of transforming TNT.

Experiments four and five found similar results using RDX (Eaton, et al., 2013) and HMX (Perumbakkam, and craig, 2012) with incubation of these compounds in sheep whole rumen fluid. Analysis of these nitroaromatic compounds was made by extraction and analysis by HPLC.

Experiment six. Metagenomic approaches found that sequences homologous to at least five RDX-degrading genes cloned from environmental samples (diaA, xenA, xenB, xplA, and xplB) were present in the ovine rumen microbiome. Among them, diaA was the most abundant, likely reflective of the predominance of the genus Clostridium in the ovine rumen. At least ten genera known to harbor RDX degrading microorganisms were detectable. Metagenomic sequences were also annotated using public databases, such as Pfam, COG, and KEGG. Five of the six Pfam protein families known to be responsible for RDX degradation in environmental samples were identified in the ovine rumen.

Conclusion

Phyto-Ruminal-Bioremediation is a new paradigm to cleaning-up environmental toxin molecules as defined by the US Department of Agriculture (USDA). Phyto-Ruminal-Bioremediation is the discovery that grasses are particularly tolerant of nitroaromatic compounds, and their rootlets often extend into the soil for five feet allowing them to absorb the hydrophobic nitroaromatic compounds, which exist in their highest concentration near the surface of the soil. The grasses pull the nitroaromatic compounds to their leaf blades, on which ruminate species eat. As the toxic nitroaromatic compounds are released from the grass cells, ruminant anaerobes degrade the toxic molecules into nontoxic moieties with no harm to the animals.

This abstract discusses the absorption of nitroaromatic compounds by grasses from soil and the ability of the ruminants to consume the grass with subsequent digestion of these toxic molecules by the ruminal microbes to render them nontoxic. This new paradigm combines grassland science with ruminal microbiology to clean up our environment of nitroaromatic compounds.

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