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Biodegradation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by Rhodococcal species

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Abstract

Large amounts of land and groundwater are contaminated with explosives as a result of their manufacture and disposal. Explosives are xenobiotic compounds, highly recalcitrant in the environment, and have been shown to be toxic to biological systems.† One of the main pollutants is RDX. Current methods used for the remediation of contaminated sites are uneconomical and there is evidence that toxic compounds remain.† Both these problems could be addressed with the development of a biodegradation-based system.† Previous work has demonstrated biotransformation of RDX by anaerobes and mixed cultures.†

Selective enrichments were performed on cultures from RDX contaminated soils, which resulted in the isolation of 21 strains of bacteria that possess the ability to utilise RDX as a sole nitrogen source for growth.† The bacterial strains were investigated using 16S rDNA analysis and were identified as species of Rhodococcus and Phyllobacterium.† One strain, identified as Rhodococcus rhodochrous, was chosen for further investigation.† The products of RDX degradation by this strain have been identified and indicate the potential for mineralisation of RDX.†

Genetic studies are underway to determine the basis for the ability of this strain to degrade RDX.†

Introduction

A significant amount of land and groundwater is polluted as a result of the manufacture, detonation and disposal of explosives [1]. Explosives are xenobiotic compounds, toxic to biological systems [2], and their recalcitrant nature leads to persistence in the environment [1].

RDX (Royal demolition explosive, hexahydro-1,3,5-trinitro-1,3,5-triazine) is the most commonly used military explosive [2], and a major pollutant (Figure 1). Several methods exist for the remediation of explosive contaminated sites, of which biodegradation has the most potential, environmentally and economically.

Many organisms have been reported as being able to biotransform RDX [3]. However, most of these bacteria are anaerobic, and produce compounds as toxic as, or more toxic than, RDX.

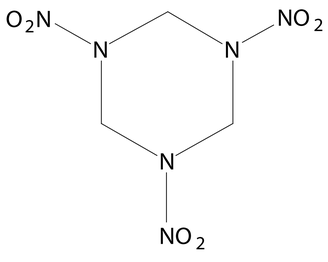

Figure 1: The structure of RDX

Results - Isolation and identification of bacteria

Soil samples from several explosive contaminated sites were obtained from dstl. Bacterial cultures capable of degrading RDX were isolated and purified. Disappearance of RDX from growth medium was measured by TLC (Figure 2).

Twenty one pure new bacterial strains, able to degrade RDX, were isolated.

The bacteria were identified using 16S ribosomal DNA analysis (Figure 3). All are aerobic, Gram positive organisms. Comparisons of bacteria using 16S sequence show that all strains are distinct.

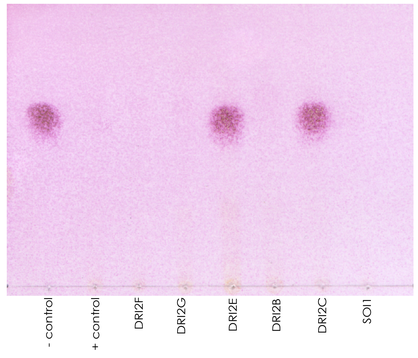

Figure 2: TLC analysis of RDX disappearance
RDX was extracted from media in which the isolated bacteria had been grown. Alkaline hydrolysis released nitrite from RDX, which was detected by the Greiss assay [4]. Pink spots indicate presence of nitrite.

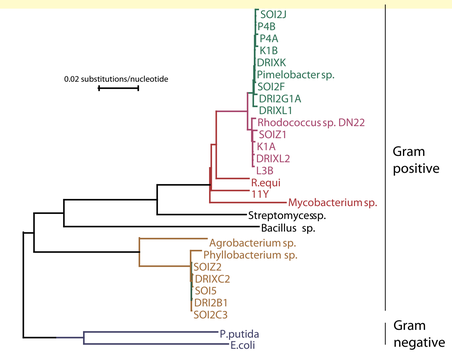

Figure 3: Phylogenetic tree comparing RDX degrading strains with common bacteria

Results - Products of RDX breakdown

One of the bacteria, Rhodococcus rhodochrous 11Y, has been chosen for further study. Growth curves using RDX as the sole nitrogen source have shown RDX disappearance within 35 hours (Figure 4). Compounds such as nitrite and ammonium are produced during RDX biodegradation. Hawari et. al. [5] have suggested a number of mechanisms which may result in the formation of these products. These mechanisms include the formation of an unstable intermediate, similar to that seen in alkaline hydrolysis of RDX (Figure 5). These products indicate RDX mineralisation.

Figure 4: Growth curve showing 11Y growth compared to RDX depletion

Figure 5: Proposed mechanism for the alkaline hydrolysis of RDX [6]

Results - Conferring RDX degradation to another strain

A genomic library technique has been used to confer the RDX degrading activity from 11Y to a non-RDX utilising Rhodococcal host. This clone, p1B, is able to degrade RDX and use it as a sole nitrogen source for growth. This has been confirmed using TLC, Zone of Clearance (Figure 6) and growth studies using HPLC to assay RDX concentrations (Figure 7).

Recent work has shown that the gene(s) responsible for the activity reside in an 8 kb DNA fragment.

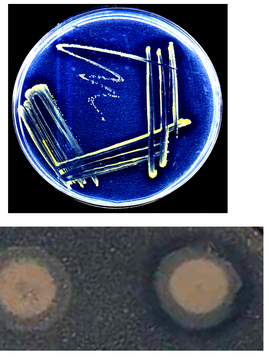

Figure 6: Zone of Clearance plate showing (a) 11Y growth and (b) a non-RDX degrader and the clone p1B
The RDX is present at a concentration exceeding its solubility limit [4], so a precipitate is visible. A "Zone of Clearance" appears round the RDX degrading colonies, indicating uptake and utilisation of RDX.

Figure 7: Growth curve comparing 11Y and clone p1B as RDX degraders.
Clone p1B appears to utilise RDX more slowly and less efficiently than 11Y

Discussion

Very few RDX degrading bacteria have been described to date. In this study, many bacterial strains capable of degrading RDX have been isolated, demonstrating that they are not rare in contaminated soil. Interestingly, all are gram positive organisms. Many of the strains are similar, but all are distinct.

Growth studies have confirmed that all the strains can use RDX as a sole nitrogen source.

Rhodococcus rhodochrous strain 11Y has been chosen for further study as it is one of the fastest at degrading RDX. Intermediates detected during its breakdown of RDX include nitrite and ammonium, which would indicate complete breakdown of RDX and mineralisation. This fits in with various models for RDX destruction, in which any action on the RDX molecule leads to an unstable intermediate and products similar to those seen.

The transfer of the RDX degrading ability to a non-RDX utilising host is a major step in the cloning of the gene(s) responsible. No genes or proteins involved in RDX degradation have been isolated to date. Identifying a gene would have great potential in terms of practical bioremediation.

Summary

- Twenty-one bacteria which can break down RDX have been isolated
- Identification shows that all strains are distinct and fall predominantly into 4 classes
- Products formed during RDX breakdown indicate mineralisation
- Ability to degrade RDX has been transferred to another host
- Genetic study is ongoing

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Acknowledgments

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