

Background

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.

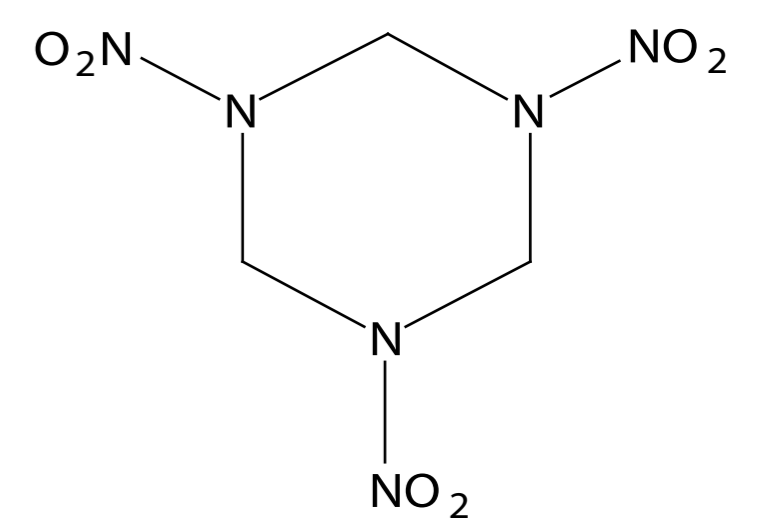


Figure 1: The structure of RDX

AIMS

- To isolate aerobic bacteria able to degrade RDX
- To identify and compare the isolated bacteria
- To find the most efficient RDX degrading bacterium
- To investigate the products of RDX biodegradation

Results: Isolating and Identifying Bacteria

Soil samples from several explosive contaminated sites were obtained from DERA. 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. Figure 3 shows these strains on a plate containing RDX as a sole nitrogen source. A "Zone of Clearance" appears round the colonies indicating uptake and utilisation of RDX. This is a reliable screen for RDX degrading ability.

The bacteria were identified using 16S ribosomal DNA analysis (Figure 4). All are aerobic, Gram positive organisms. Comparisons of bacteria using 16S sequence, appearance and specific activity against RDX show that all strains are distinct.

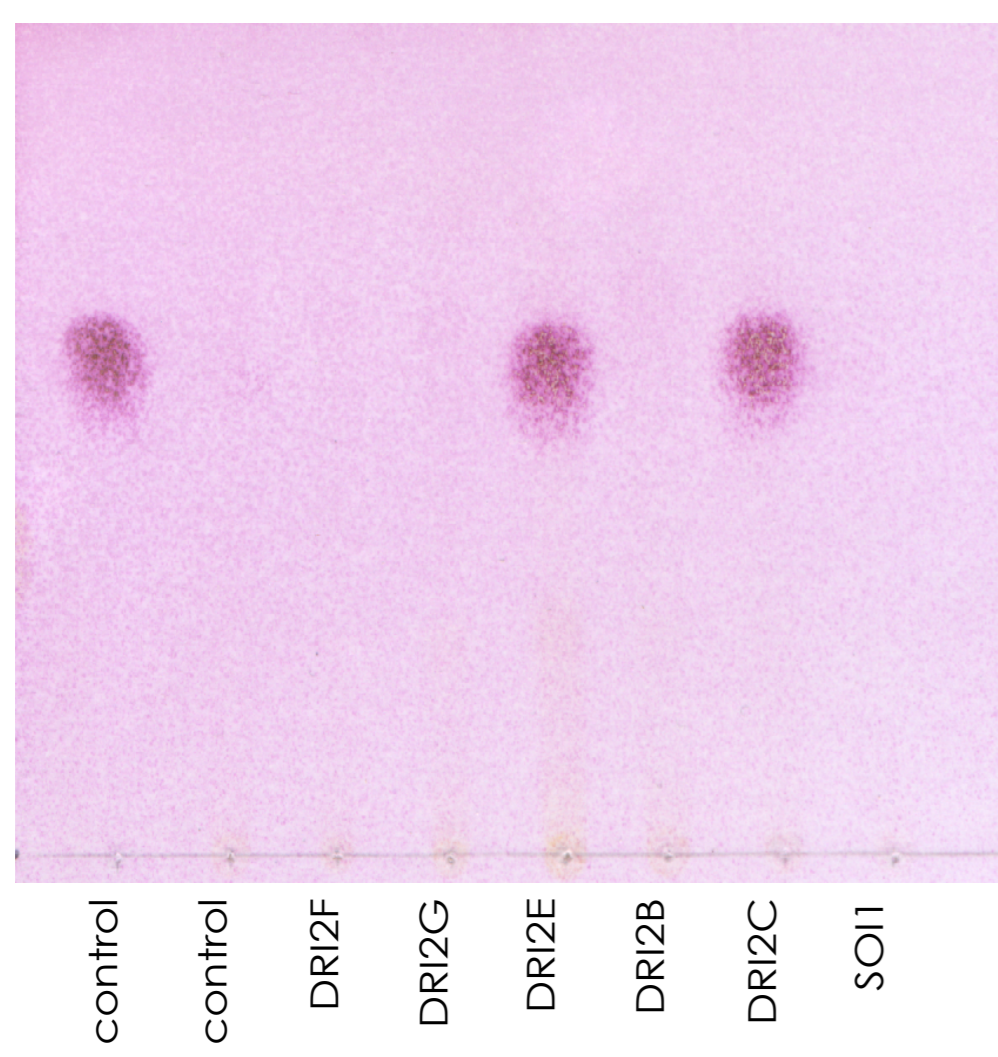


Figure 2: TLC analysis of RDX disappearance
RDX was extracted from media in which the isolated bacteria had been grown. The pink spots indicate RDX in the medium, so absence of pink spots indicates the presence of an RDX degrading bacterium.

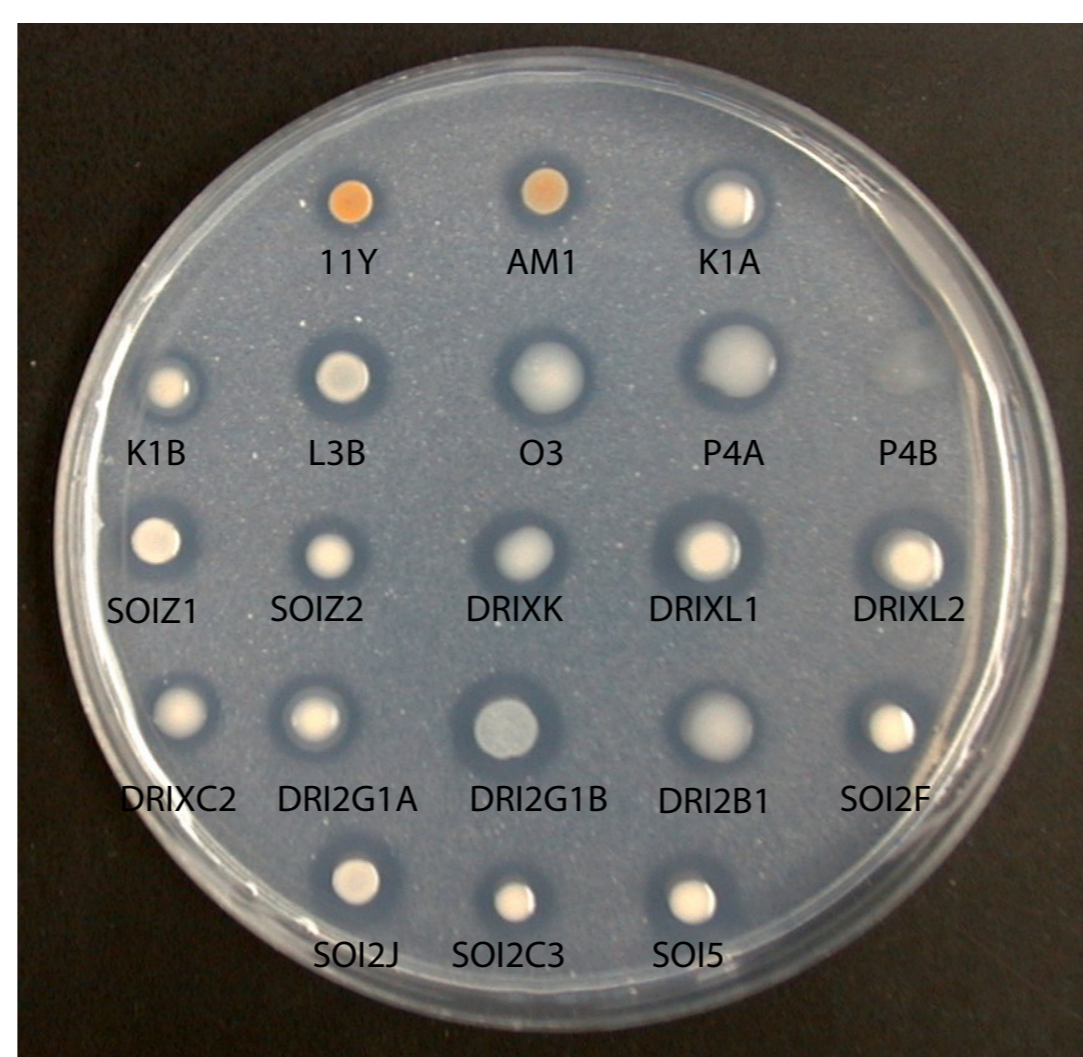


Figure 3: RDX plate of bacterial strains
The RDX is present at a concentration exceeding its solubility limit [4], so a precipitate is visible. Colonies show Zones of Clearance in varying degrees.

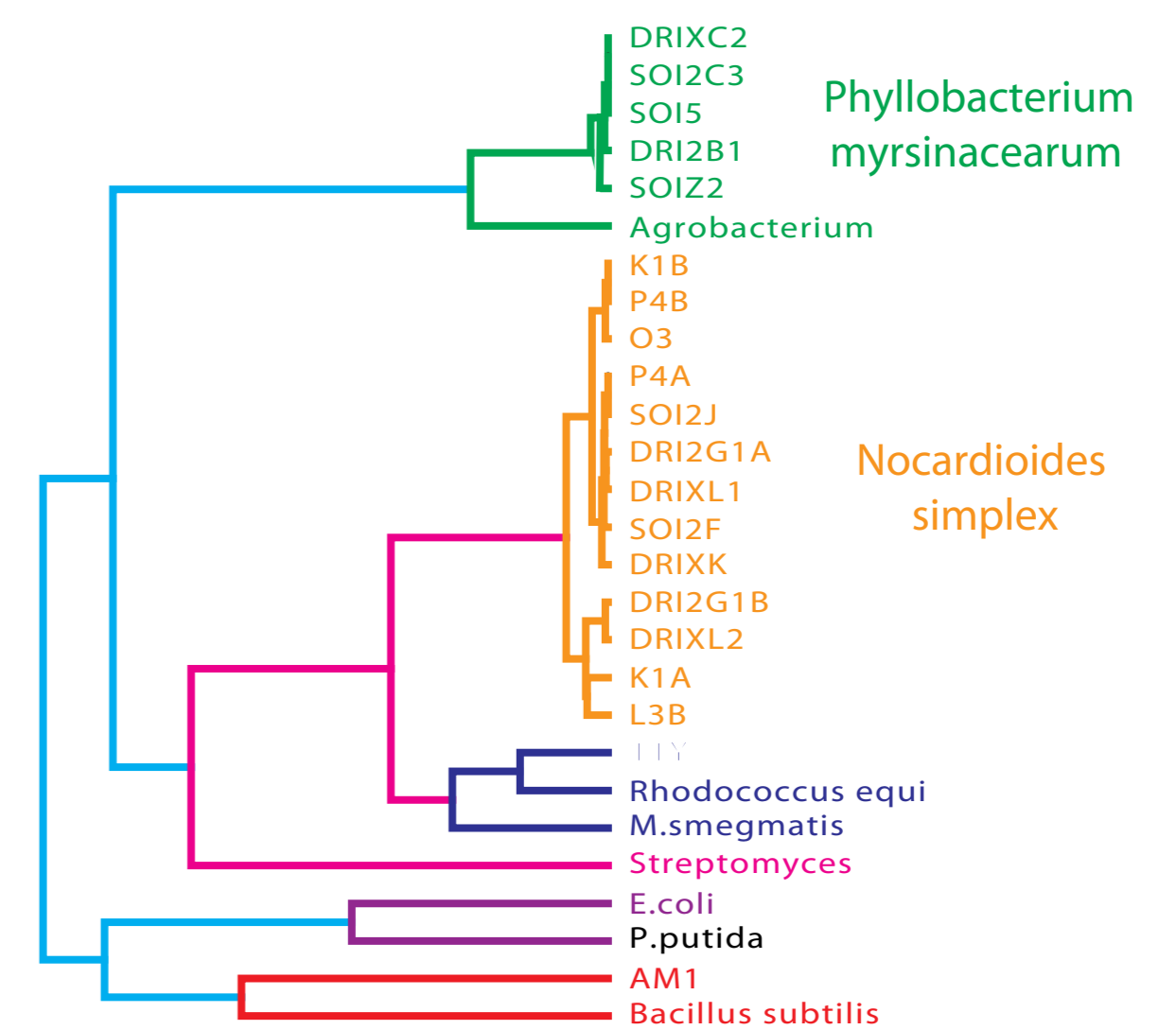


Figure 4: Phylogenetic tree comparing RDX degrading strains with common bacteria

Results: Analysis of RDX Breakdown

RDX degradation rates were calculated in crude cell extracts. Figure 5 shows disappearance of RDX over time using one bacterial strain, measured by HPLC.

Specific Activities of crude cell extracts were calculated (Figure 6). Nitrite, produced during aerobic RDX degradation, was detected using the colorimetric Greiss assay [5].

The observed breakdown products of RDX biodegradation are similar to those seen in RDX alkaline hydrolysis [6] (Figure 7). These products indicate RDX mineralisation.

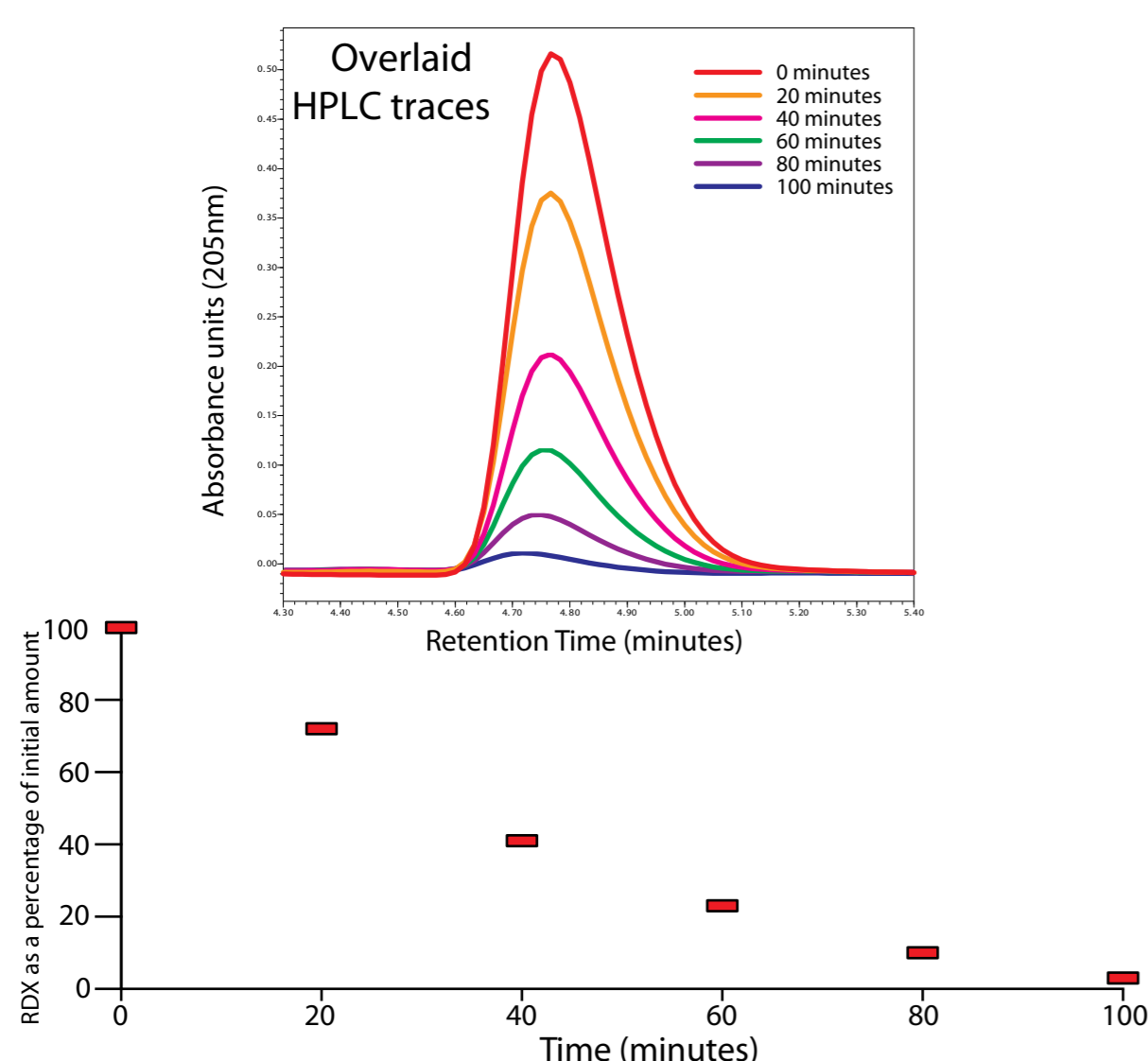


Figure 5: RDX disappearance in 11Y crude extract

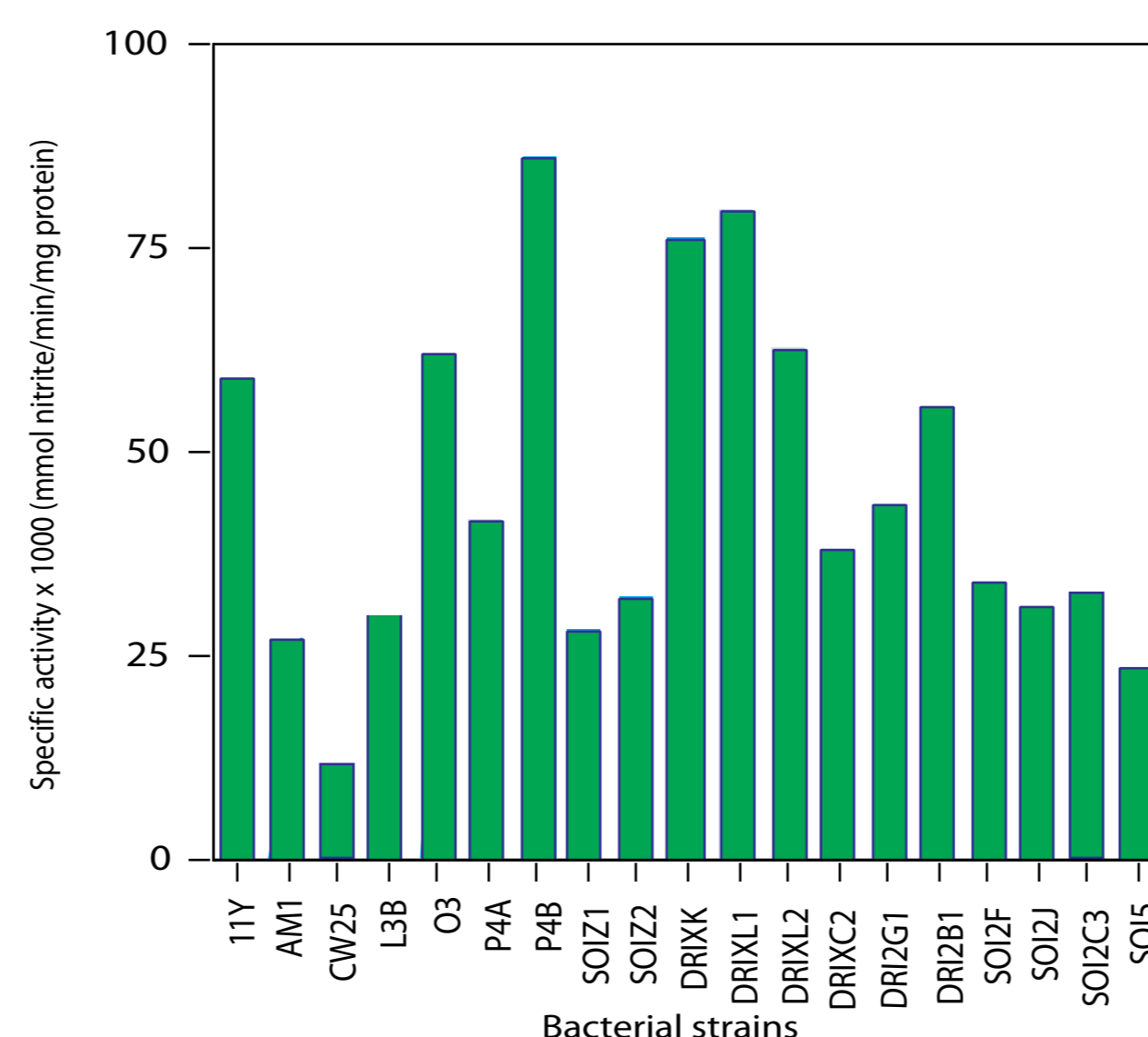


Figure 6: Specific activities of bacterial crude cell extracts against RDX

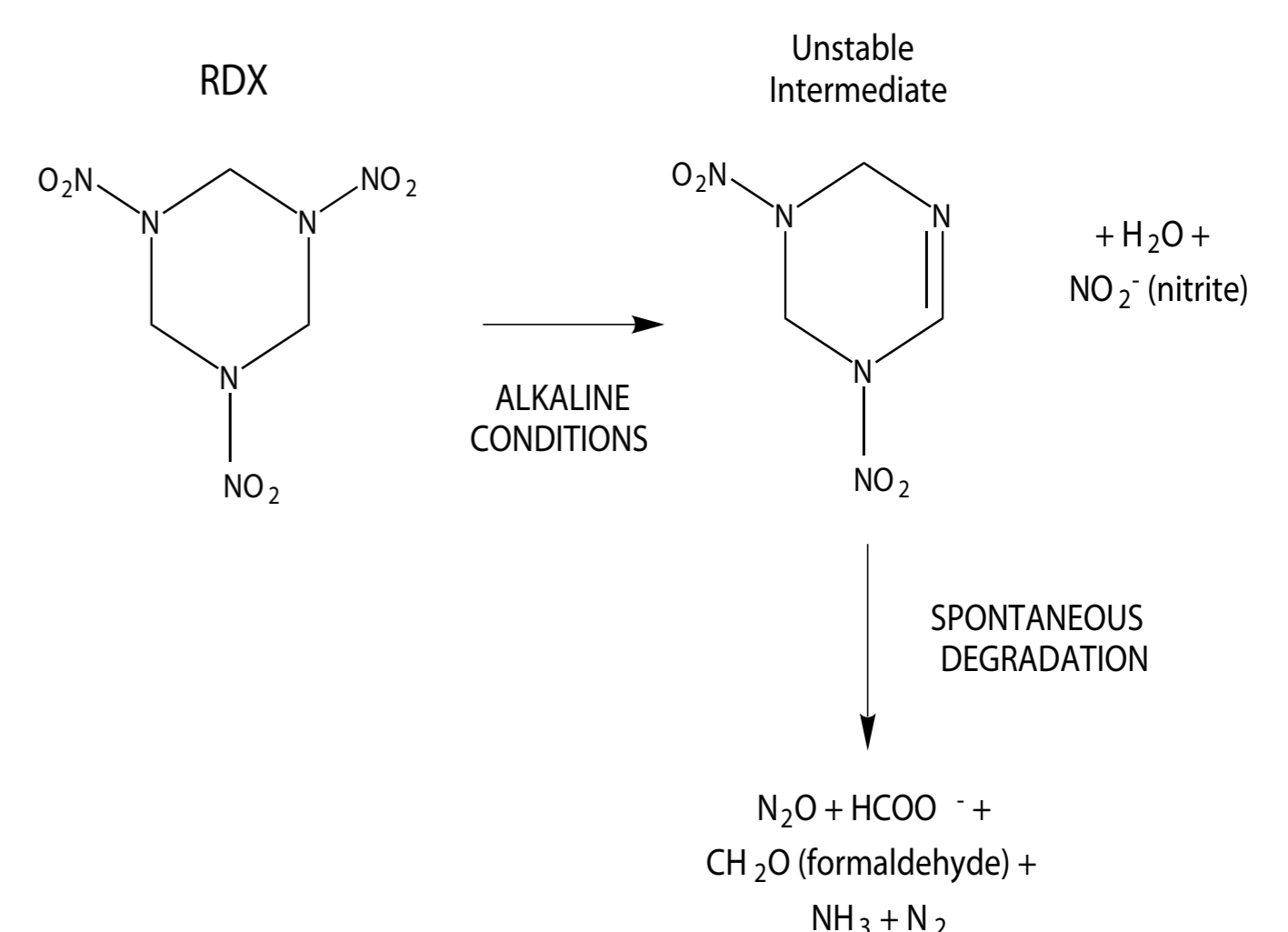


Figure 7: Proposed mechanism for the alkaline hydrolysis of RDX

Summary

- Twenty-one bacteria which can mineralise RDX have been isolated
- Identification shows that all strains are distinct and fall predominantly into two groups
- Comparisons of the specific activities have been performed

References

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Acknowledgements

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