E.R.A.S.E. Explosive Remediation by Applied Synthetic E. coli

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Introduction

Environmental pollution by explosive waste from Trinitrotoluene (TNT) and nitroglycerin (NG) is a widespread, long-term health problem (Fig. 1A and B). Current remediation strategies are impractical and expensive (Fig. 1C).1, 2 Current remediation practices produce its own problems. We propose a bacteria capable of bioremediation that targets the toxic compounds TNT and NG, converting them to harmless products (Fig. 2, below). The bacteria should also monitor the presence of the target pollutant, killing itself as toxicity falls. Discussions with stakeholders indicated a need for improved detection systems. As kill switches and reporter systems are already available we focussed on two aims: 1. Identify enzymes to degrade TNT/NG to harmless products. 2. Identify a genetically encoded detection system.

In addition, we sought to build a model which would help us to design, analyse and debug our biological system. Specifically it must: • Inform the biological design. • Inform the choice of experiments. • Be receptive to empirical observations. • Should explain how our system works.

The Modelled System

We modelled our system on four key levels: 1. The Biochemical Level. The primary determinant of TNT/NG degradation will be the activity of our enzyme(s). 2. The Cellular Level. In this model cells grow and are destroyed at a rate proportional to the TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetic properties of the enzymes.

Figure 3. The influence of enzyme kinetics on degradation rate. In the blue cells, the enzyme reaction rate is slow and the cell density is dependant on TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetic properties of the enzymes.

3. A Stochastic Model

We created a more complex simulation, modelling the life and death of the cell population over time. Although this model did not guide any experiments it contributed to our spatial model.

4. A Spatial Model

Finally, our most sophisticated model also takes into account the position of each individual bacterium, the level and toxicity of TNT at that position, population growth and a kill-switch.

Conclusions

We designed and created a synthetic promoter comprised of two completely separate sequences that has the capability to be used as a biosensor in a bioremediating organisms. We have also successfully characterised two enzymes capable of degrading TNT and NG in vivo which could be used in a bioremediator.

References


Figures:

1. The problem with Trinitrotoluene (TNT) and nitroglycerin (NG). A. TNT is a highly toxic compound with many detrimental health effects. B. The problem is global. C. Current deratination practices produce its own problems.


3. Figure 1. The problem with Trinitrotoluene (TNT) and nitroglycerin (NG) is a widespread, long-term health problem (Fig. 1A and B). Current remediation strategies are impractical and expensive (Fig. 1C).1, 2

4. Figure 2. Degradation

5. Self-cleaning

6. Detection

Degradation

We identified two enzymes, XenB and Nema2, which may degrade both TNT and NG. We constructed two expression cassettes to test this (Fig. 6) and found that cells expressing the enzymes rapidly catalysed TNT (Fig. 7).

Purified NemA protein degraded TNT in the presence of cofactors (Fig. 8).

Detection

We successfully created a synthetic promoter based on the nemR regulatory region which varies in expression depending on the concentration of TNT.

To create it we took the NemR binding “box” found within NemR regulators and inserted it between the -35 and -10 regions of a constitutive Anderson promoter (BBa_J23100).

Our natural promoter, based on the entire nemR region, showed no specificity for TNT.

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Figure 4. Cell growth and TNT concentration over time. Cells grow and are destroyed at a rate proportional to the TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetic properties of the enzymes.

2. The Cellular Level

In this model cells grow and are destroyed at a rate proportional to the TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetic properties of the enzymes.

3. A Stochastic Model

We created a more complex simulation, modelling the life and death of the cell population over time. Although this model did not guide any experiments it contributed to our spatial model.

4. A Spatial Model

Finally, our most sophisticated model also takes into account the position of each individual bacterium, the level and toxicity of TNT at that position, population growth and a kill-switch.

We characterised BBa_K660004, which encodes the fluorescent protein iLOV. iLOV was introduced to the Registry by Glasgow 2011 but was not characterised. We were able to demonstrate its function in E. coli (Fig. 12A, as well as determining its excitation/emission spectra (Fig. 12B).

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iLOV Characterisation

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iLOV is a viable alternative to GFP and has several benefits, including its reduced size compared to GFP, meaning it can be synthesised for a lower cost.