

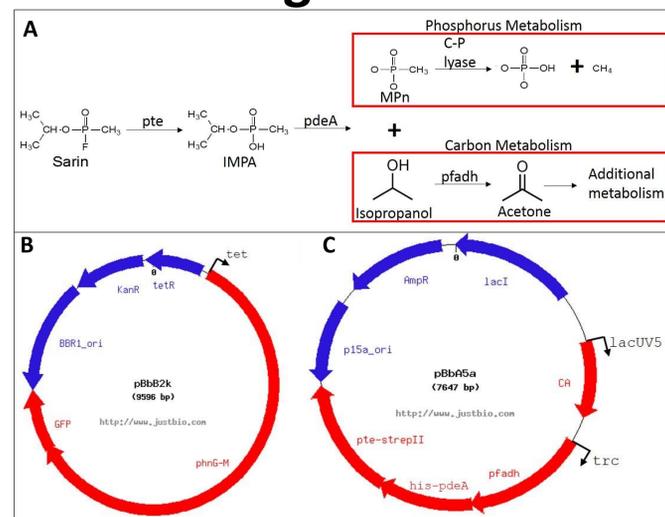
# Evaluation of *Escherichia coli* Utilizing Sarin as a Sole Phosphorus Source

Jennifer A. Gibbons<sup>1,2</sup>, Trevor Glaros<sup>1</sup>, Paul Demond<sup>1,2</sup>, Steve Harvey<sup>1</sup>, Calvin Chue<sup>1</sup>  
<sup>1</sup>Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, <sup>2</sup>Excet, Inc., Springfield, VA

## Introduction

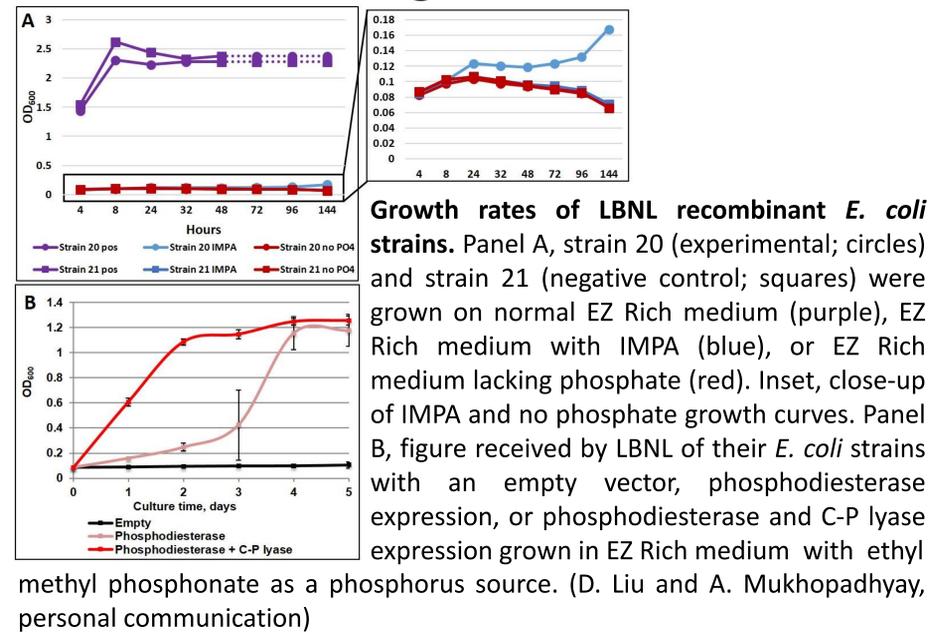
Destruction of chemical agent stockpiles was a concern in the recent past. One method proposed by Lawrence Berkeley National Laboratory (LBNL) is the biological destruction of chemical nerve agents. These organisms would use agents as carbon or phosphate sources. Multiple enzyme classes have been discovered that can detoxify organophosphates by hydrolysis, such as phosphotriesterases (also termed organophosphorus hydrolases) and organophosphorus acid anhydrolases (1). Although these enzymes have been recombinantly engineered for increased effectiveness against chemical nerve agents, the resulting metabolites will remain unless further metabolized by other means. Therefore, an ideal decontamination scheme would involve the total degradation of chemical nerve agents into common small molecules in the environment. Similar efforts have been published in the past, such as activated sludge using sarin as a sole carbon source (2), *Escherichia coli* using the pesticide paraoxon as a sole phosphate source (3), and *Pseudomonas putida* using paraoxon as a sole carbon and phosphorus source (4). The *P. putida* effort synthetically engineered a recombinant bacterial strain by adding a phosphodiesterase from *Delftia acidovorans*, a bacterial species capable of using some organophosphates as sole phosphate sources (5), and a phosphotriesterase from *Flavobacterium* sp. strain ATCC 27551. LBNL proposed to test this method with recombinant *E. coli*, attempting to design a strain that could utilize sarin (GB) as a sole carbon and phosphorus source. ECBC was requested to evaluate the resulting strain(s) for use of sarin as a phosphate source.

## Figure 1

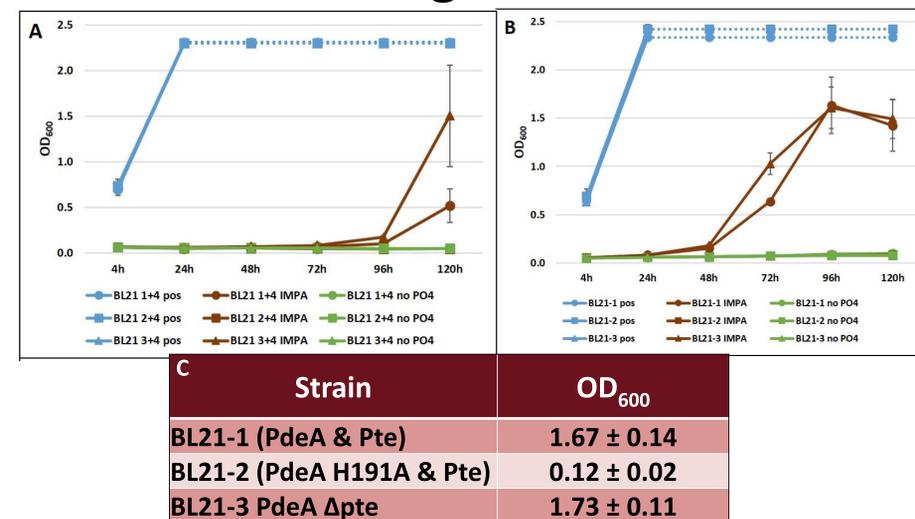


**Proposed sarin degradation by recombinant *E. coli*.** Panel A, proposed sarin metabolism pathway for carbon and phosphorus mineralization. Abbreviations: pte (phosphotriesterase), pdeA (phosphodiesterase), pfadh (alcohol dehydrogenase). IMPA (isopropylmethylphosphonic acid), MPn (methylphosphonic acid). Panel B, overexpression plasmid for endogenous phnG-M. Panel C, overexpression plasmid for initial metabolism steps and part of carbon metabolism (three versions). Strain 1 (above), strain 2 expresses catalytically dead pdeA (H191A), and strain 3 does not express pte.

## Figure 2

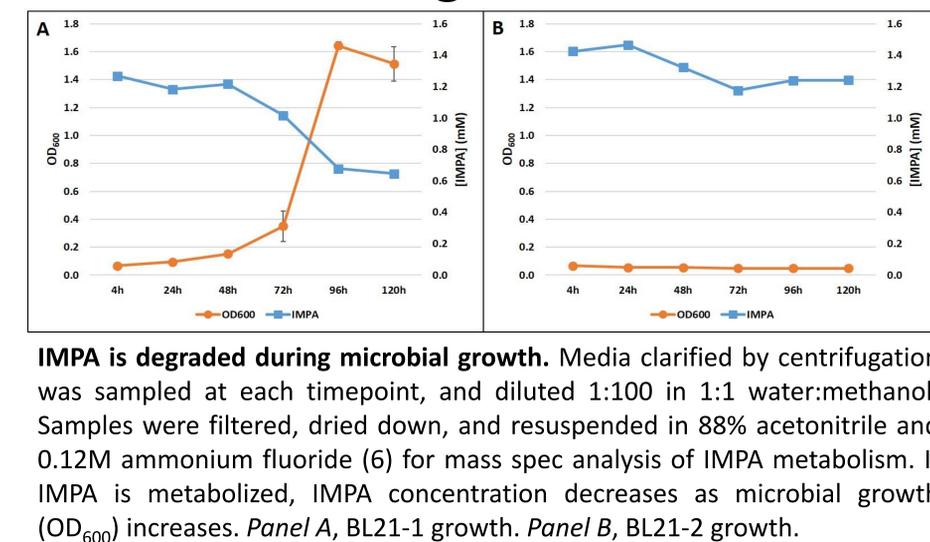


## Figure 3



**Growth rates of LBNL plasmids in BL21 T7 Express background.** Panel A, BL21 T7 Express strains expressing both pBbB2k and pBbA5a. Panel B, BL21 T7 Express strains expressing only pBbA5a. Blue lines, growth in complete EZ Rich medium; brown lines, growth in EZ Rich medium with IMPA as a phosphorus source, and green lines, growth in EZ Rich medium lacking a phosphorus source. Circles, strains expressing pdeA and pte; squares, strains expressing pdeA H191A and pte; triangles, strains expressing pdeA and no pte. Results shown in triplicate. Panel C, Results were similar when strains were grown in sarin (Triplicate OD<sub>600</sub> results shown for 36 hour growth).

## Figure 4



## Conclusions

- LBNL recombinant plasmids are more effective with a "phase-on" *E. coli* strain.
- LBNL plasmids allow a recombinant *E. coli* strain to utilize sarin as a phosphorus source

## Future Directions

- Optimization of PdeA expression could enhance sarin degradation.
- Other chemical nerve agents could be tested with this recombinant system.

## References

1. Theriot CM, Grunden AM. 2011. *Appl Microbiol Biotechnol* 89: 35-43.
2. Harvey SP, Carey LF, Haley MV, Bossle PC, Gillitt ND, Bunton CA. 2003. *Bioremediation Journal*, 7: 179-85.
3. McLoughlin SY, Jackson C, Liu JW, Ollis DL. 2004. *Appl Environ Microbiol* 70: 404-12.
4. de la Pena Mattozzi M, Tehara SK, Hong T, Keasling JD. 2006. *Appl Environ Microbiol* 72: 6699-706.
5. Tehara SK, Keasling JD. 2003. *Appl Environ Microbiol* 69: 504-8.
6. Noort D, Hulst AG, Platenburg DH, Polhuijs M, Benschop HP. 1998. *Arch Toxicol* 72: 671-5.