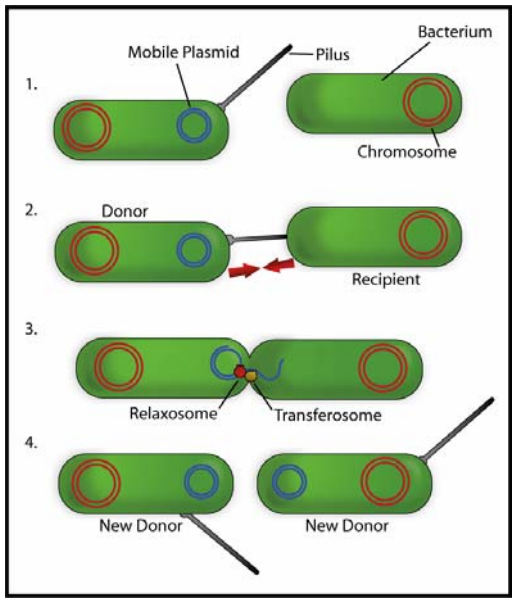
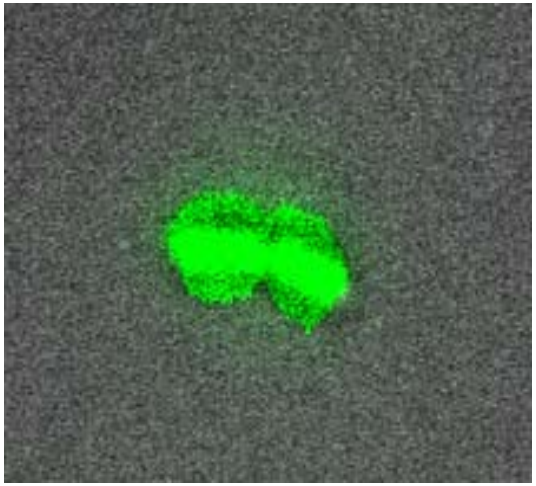
Effect of substrate type on toluene biodegradation following a horizontal gene transfer event in *Escherichia coli* DH5α





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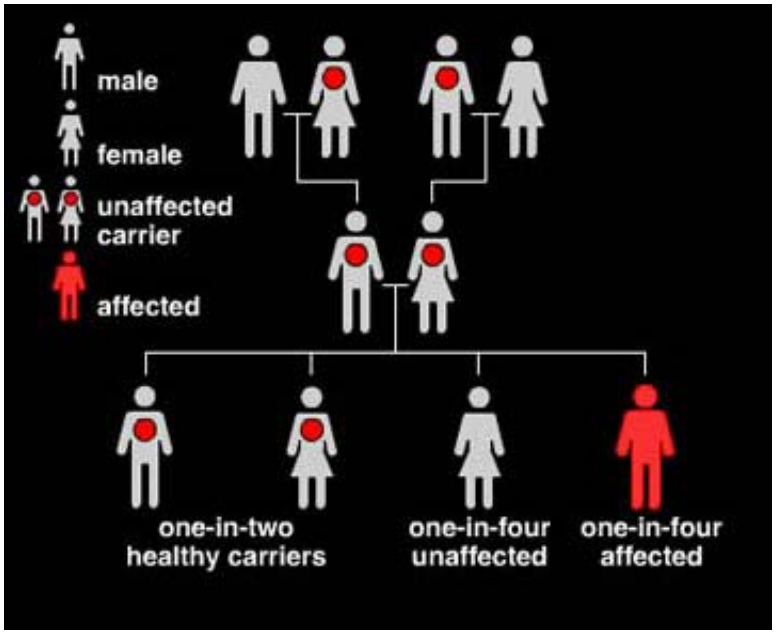
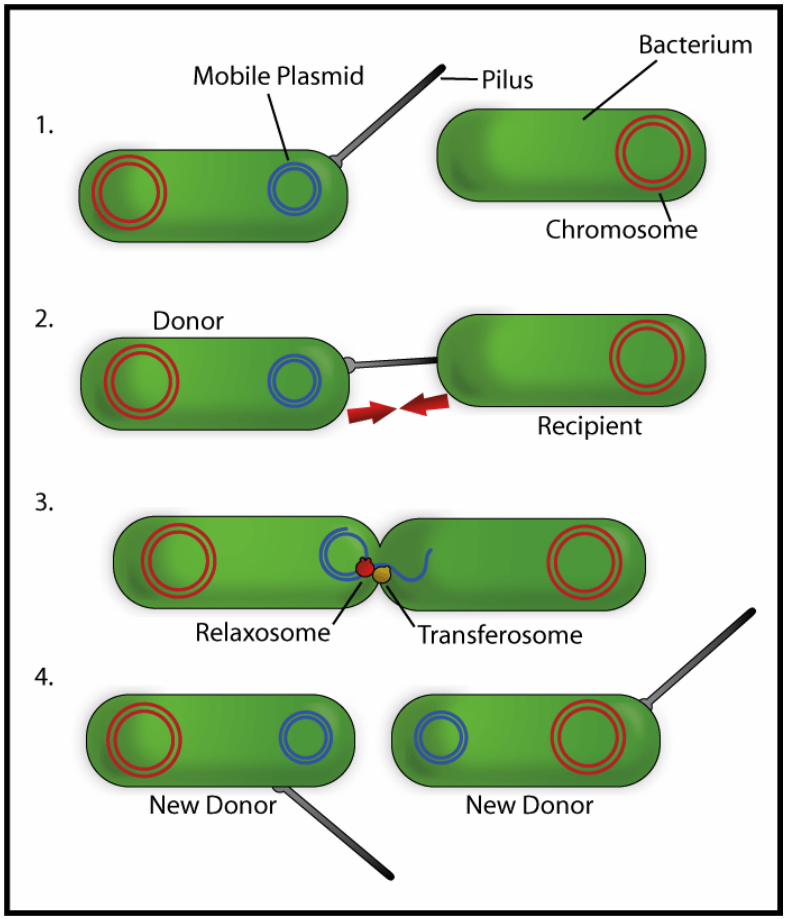
Horizontal gene transfer (HGT) as a means to improve degradation of contaminants by microbes

Vertical gene transfer



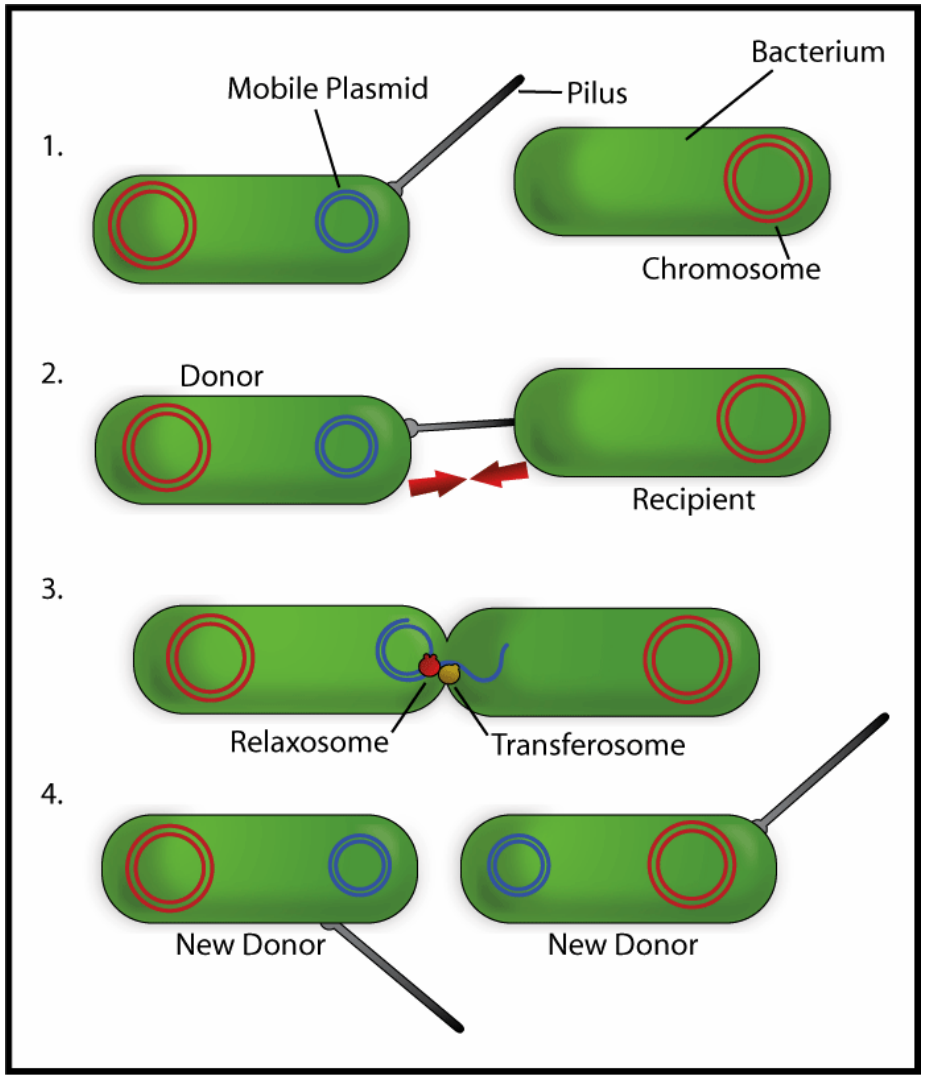
http://www.sciencemuseum.org.uk/on-line/genes/232.asp

Horizontal gene transfer



http://en.wikipedia.org/wiki/Bacterial_conjugation

Plasmids can transform cells that come in contact with donor cells (conjugation)

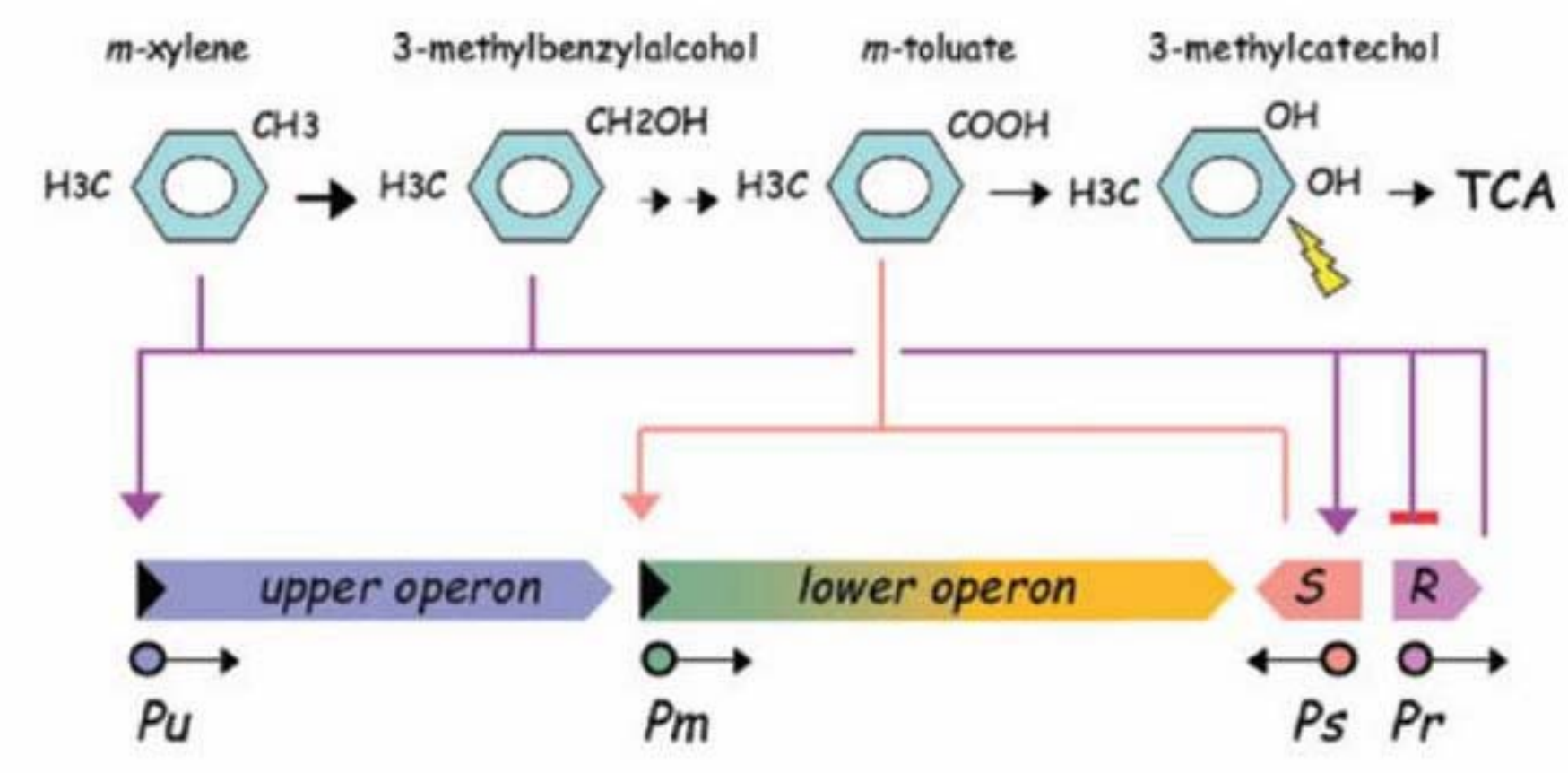


http://en.wikipedia.org/wiki/Bacterial_conjugation

Conjugated cells will not keep the plasmid unless it confers an environmental advantage!

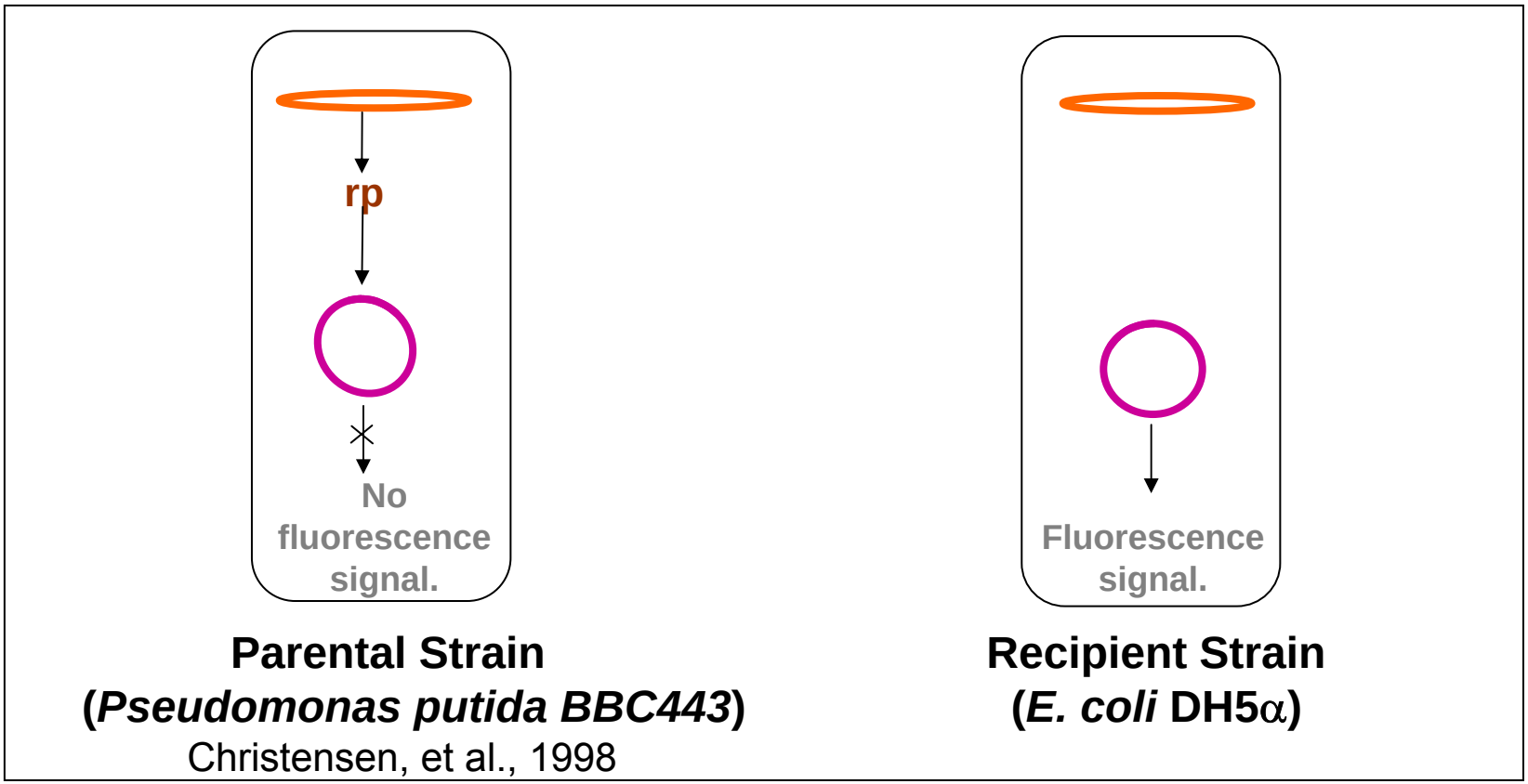
→ Conjugation events may readily occur but many do not result in a **functional phenotype**

Conjugation of the TOL plasmid chosen as model of horizontal gene transfer



Velázquez *et al*., 2005

Pseudomonas putida BBC443 harboring a TOL plasmid tagged with GFP and kanamycin resistance used as donors



E. coli DH5α cells were conjugated with the TOL plasmid using filter mating

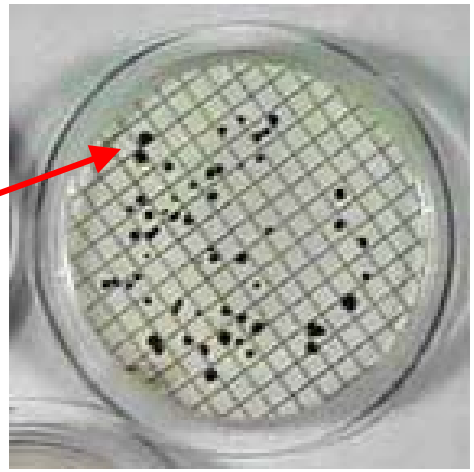
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Drinking_Water/jpgs/Drinking_water.html

Recipient cells (*E. coli* DH5α) on membrane filter

Filter incubated in the presence of donor cells (*P. putida* BBC443)

Donor cells washed off

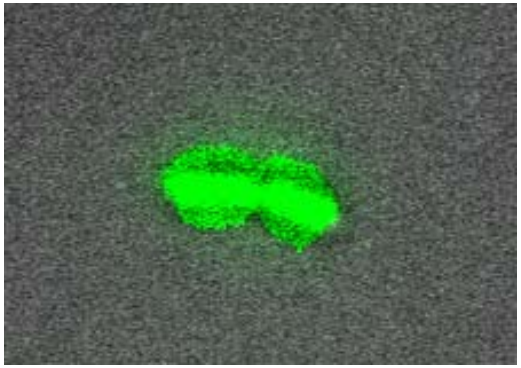
Filter incubated on agar plate



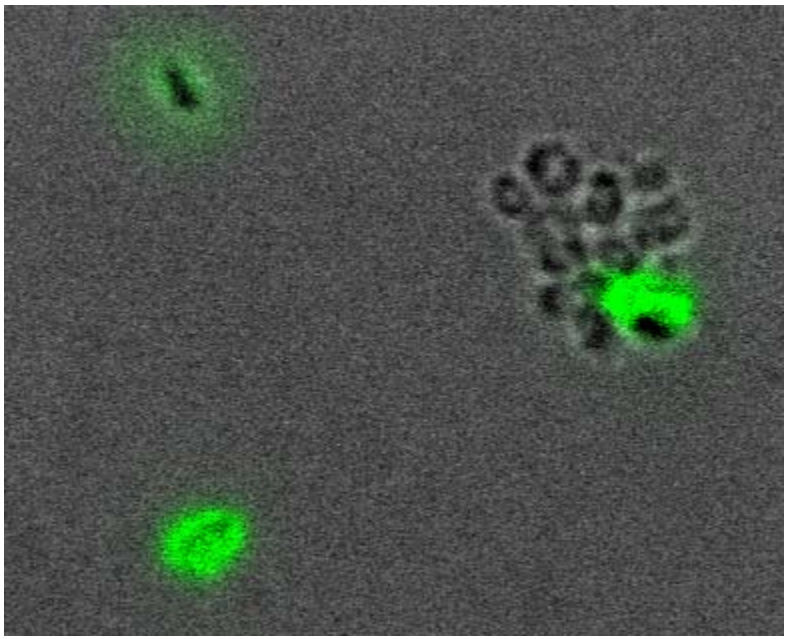
http://filebox.vt.edu/users/chagedor/biol_4684/mfstrep.html

E. coli transconjugants harboring TOL plasmid

Transfer of TOL plasmid into *E. coli* was verified through fluorescence microscopy

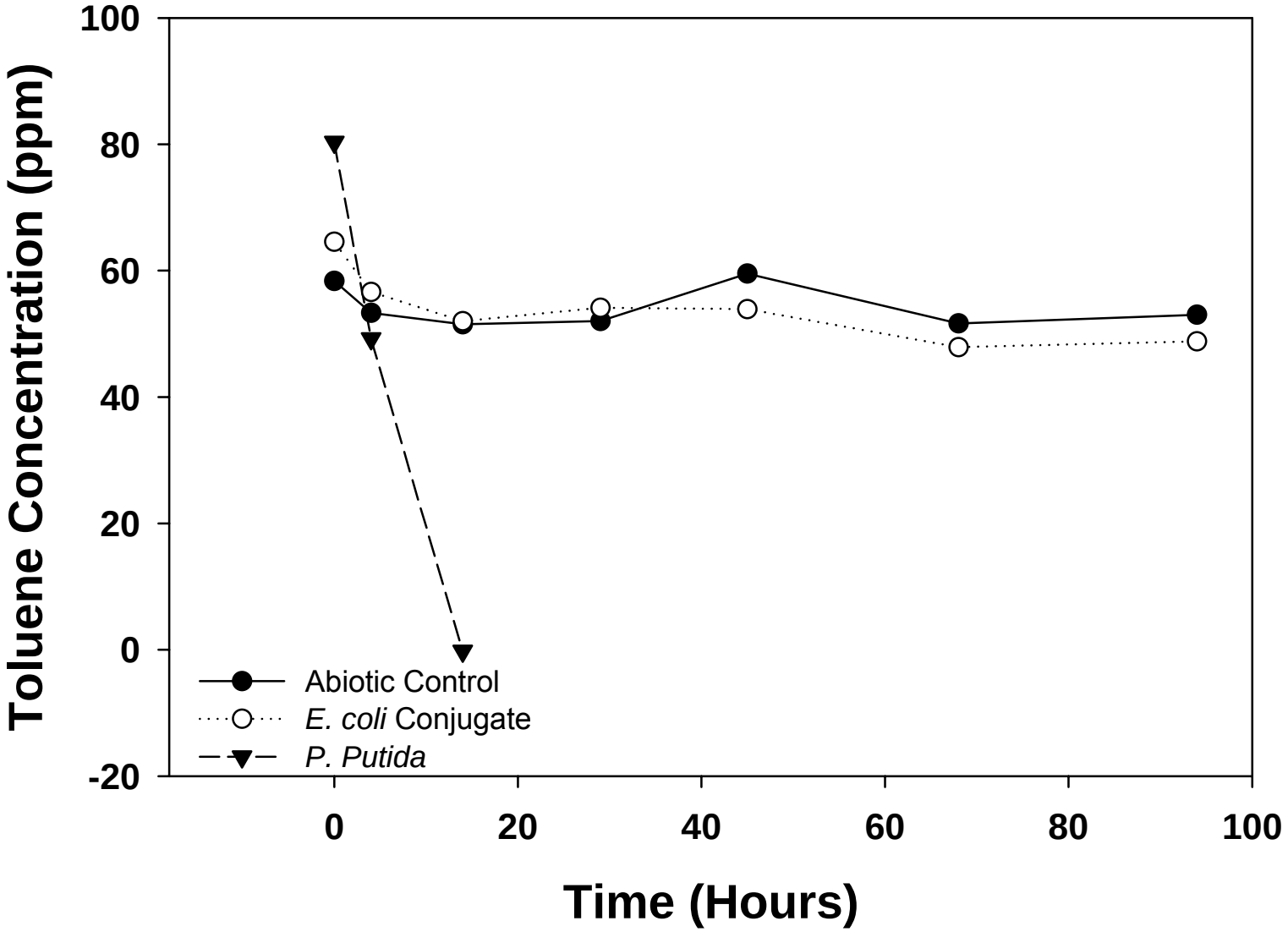


E. coli transconjugant cells

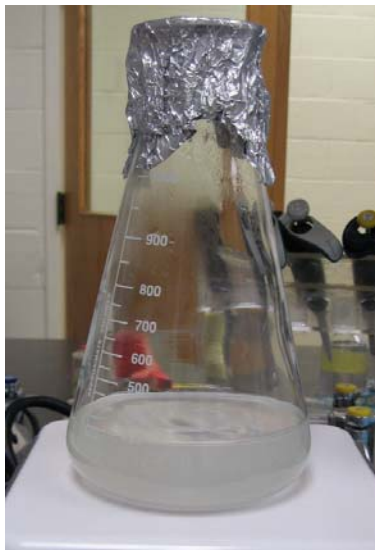


Mixture of *P. putida* cells and *E. coli* transconjugant cells

E. coli transconjugants harboring the TOL plasmid could not grow with toluene as sole carbon source



The effects of the addition of glucose as carbon source along with toluene was tested

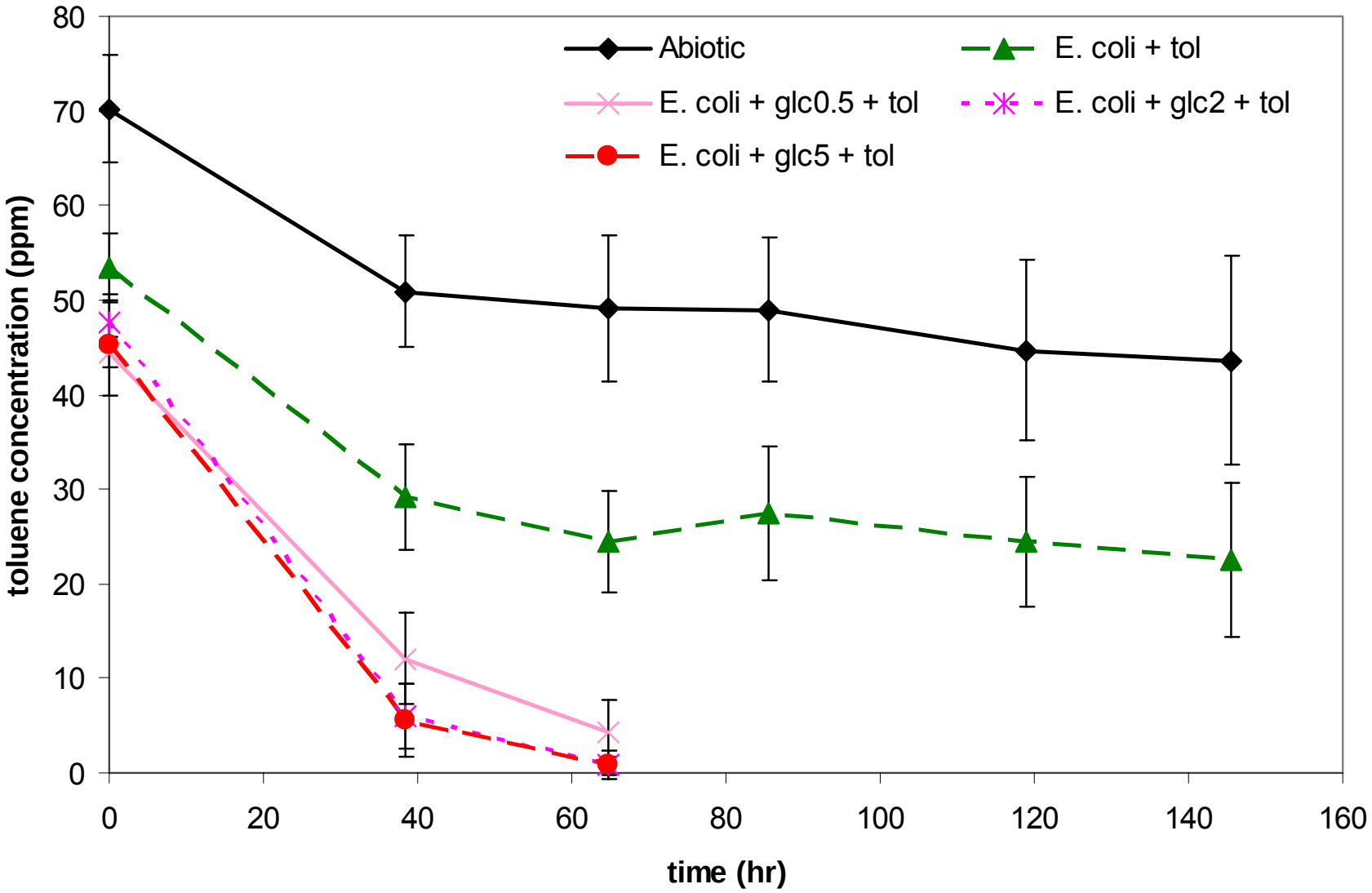


Pre-grown *E. coli* transconjugant cells

Incubated in the presence of toluene and glucose

- Toluene degradation over time
- Cloning of TOL plasmid genes
- Catechol-2,3-dioxygenase (C23O) enzyme assay

The addition of glucose significantly increased the rate of toluene degradation in *E. coli* transconjugants



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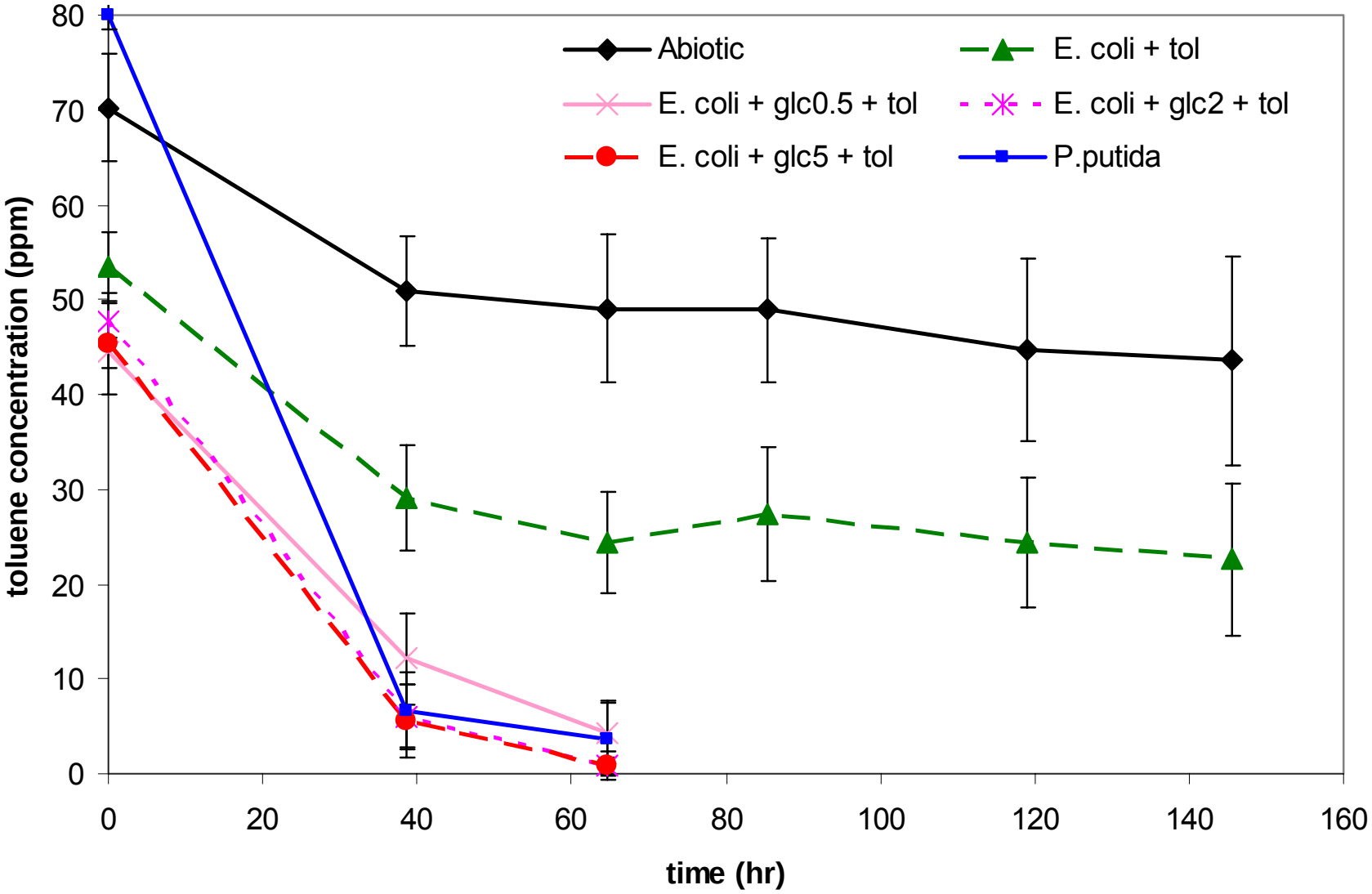
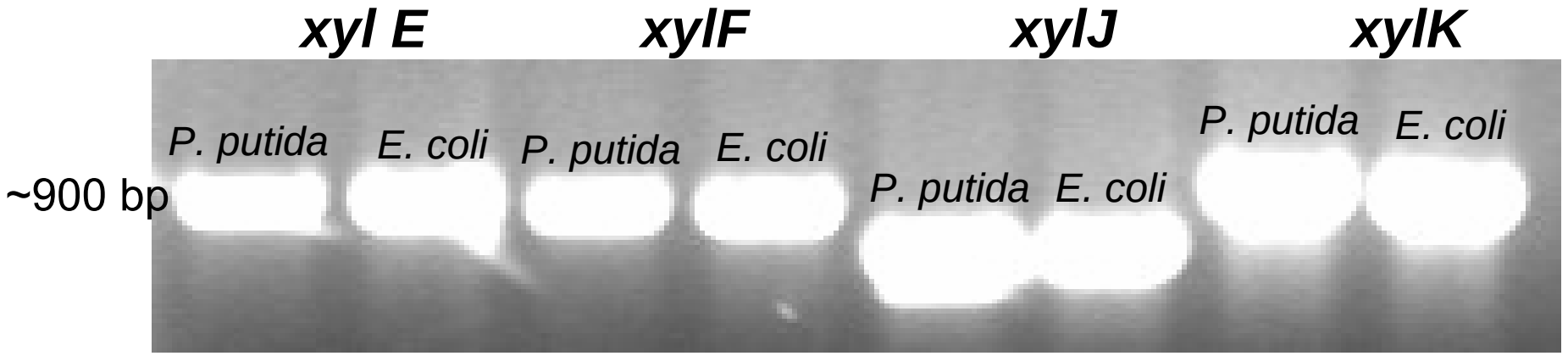


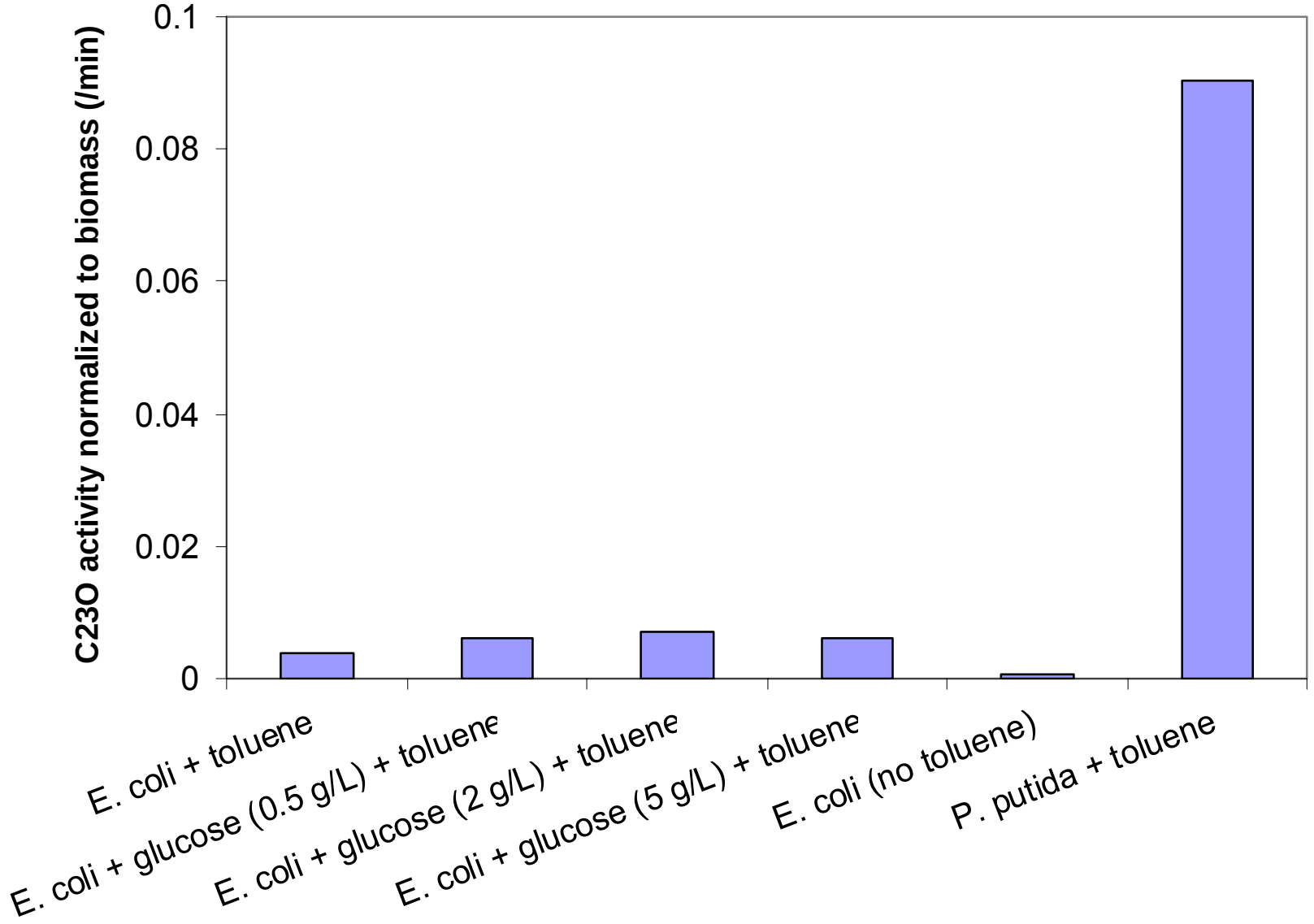
PCR confirmed the presence of TOL plasmid genes in the *E. coli* transconjugant cells



Possibility of mutations in these genes introduced during the conjugation process

→ Sequencing of xyl genes in progress

Preliminary results show slight increase in TOL enzyme activity with glucose addition



Conclusions and future work

Conjugation of *E. coli* with the TOL plasmid did not result in a functional phenotype (significant toluene degradation capabilities)

Glucose addition increased toluene degradation in *E. coli* transconjugants

- Reducing power?
- Cometabolism?
- Other mechanisms?

Difference in TOL plasmid gene expression (qRT-PCR) under different conditions

HGT of TOL plasmid into strains other than *E. coli* and in mixed cultures

- GC content of recipient genome may play a significant role in transfer efficiency/functional phenotype (Sorek *et al.*, 2007)

Acknowledgements

Department of Civil and Environmental Engineering, Duke University

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Special thanks to Dr. Søren Molin (Denmark Technical University) for the kind donation of *P. putida* strain BBC443

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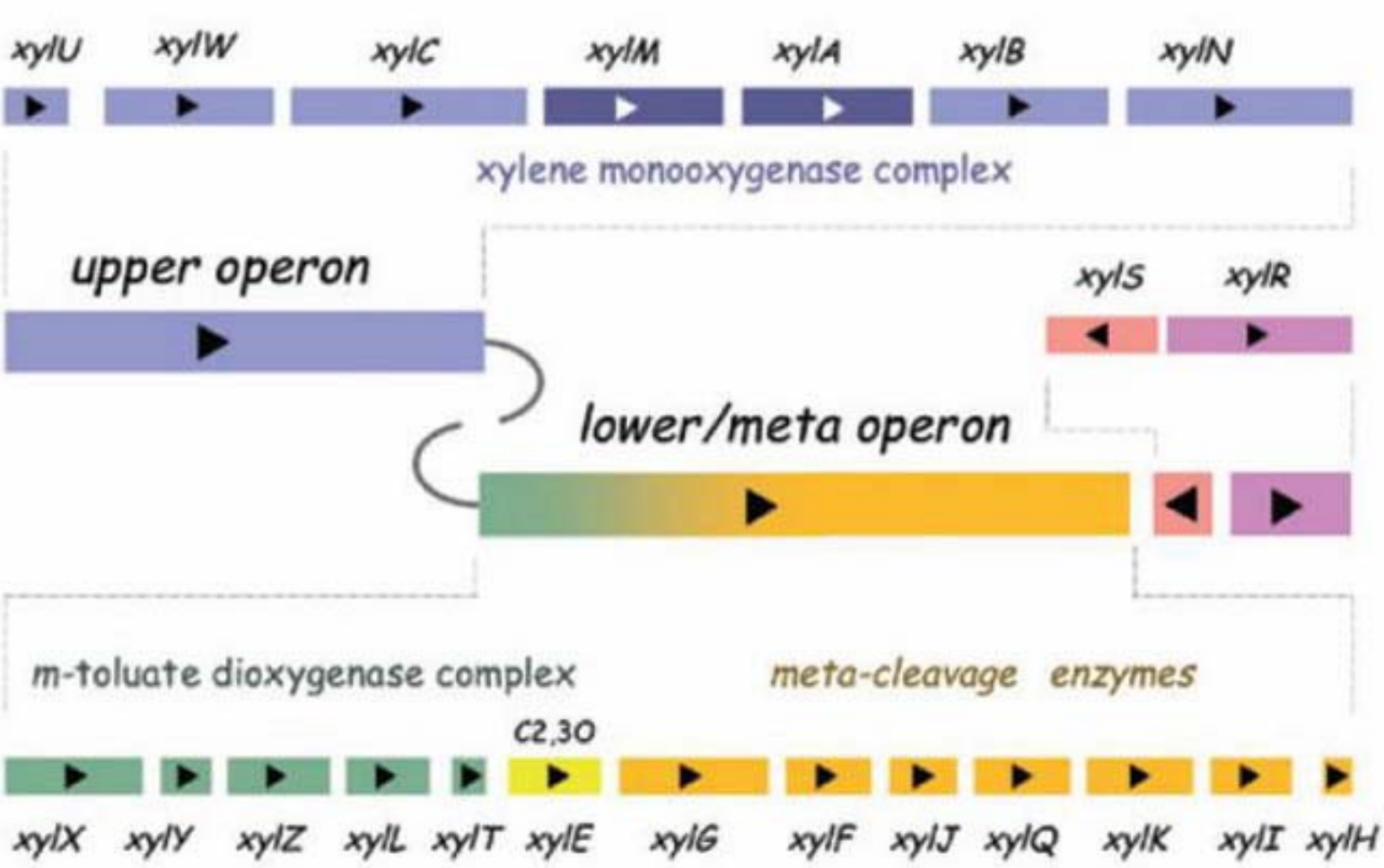
TOL plasmid contains genes encoding proteins that convert toluene to TCA cycle intermediates



Velázquez, et al., 2005

Toluene degradation curve