

Genetics in *Zymomonas mobilis* ZM4

Piyush Behari Lal,^{1,2*} (plal2@wisc.edu), Fritz Wells,² and Patricia Kiley^{1,2}

¹Great Lakes Bioenergy Research Center, University of Wisconsin – Madison, Madison, WI;

²Department of Biomolecular Chemistry, University of Wisconsin – Madison, Madison, WI.

<http://www.glbrc.org/>

Project Goals:

The goal of this work is to develop genetic tools for *Zymomonas mobilis* ZM4, and create a platform strain with increased efficiency to accept foreign genes.

Abstract

The ethanologenic bacterium *Zymomonas mobilis* ZM4 (*Z. mobilis*) has emerged^{1,2,3} as a promising candidate for microbial conversion of plant biomass (lignocellulosic biomass/hydrolysate) into biofuel and other value-added products. The present work aims to expand genetic techniques for *Z. mobilis* so that strain optimization is feasible and efficient. To remove genes from *Z. mobilis*, we have developed a method for generating markerless gene deletions. This is a two-step process that involves integration of an engineered suicide plasmid into the genome via homologous recombination, and a subsequent recombination event that leads to loss of target gene. A key feature of this strategy is that GFP expressed from integrated vector allows easy identification of cells that have lost the integrated plasmid by fluorescence activated cell sorter (FACS). We have also improved transconjugation of plasmids from *Escherichia coli* into *Z. mobilis* by deleting *Z. mobilis* restriction systems. We propose this work will provide a platform for genetic engineering in *Z. mobilis* to build better biofuel producing strains.

References

1. He et al., 2014.
2. Motamedian et al., 1977.
3. Swings and Ley, 1977.

Funding statement.

This material is based upon work supported by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494.