
Metabolic Engineering of *Zymomonas mobilis* ZM4

The Problem

Use of *Zymomonas mobilis* ZM4 has been problematic due to a poor understanding of the putative DNA restriction modification system.

However, researchers from the School of Biotechnology and Biomolecular Science at the University of New South Wales (UNSW) have overcome these difficulties to develop a platform technology that could be used to express exogenous DNA. Applications may include the production of ethanol from waste plant material and also other valuable metabolites.

The Technology Solution

This technology inactivates the gene encoding putative R-M system by homologous recombination suicide vectors and transforms the mutant strain using a plasmid transformation system in *E. coli*.

The gene encoding putative R-M system knockout mutant strain exhibits higher transformation efficiency compared to the wild type. Transformation efficiencies for the knockout mutants have been shown to work 500-times more efficiently.

The usefulness of the mutant strains are in harbouring this higher transformation efficiency, and the plasmid conditioned by the methylases from the mutants. These contribute to the transformation of the wild type with industrially interested genetic traits.

This versatile technology finds utility in:

Biofuel production: *Why use Zymomonas for ethanol production?*

Zymomonas is a bacterium that has a near theoretical ethanol yield from glucose, no oxygen requirement thus negating the need for expensive oxygen transfer, and high ethanol tolerance making it an effective microbe for ethanol biofuel production.

Despite the suitability of *Z. mobilis* for use in ethanol production, this organism is still restricted in that it lacks a functional pentose metabolism pathway required for the fermentation of pentoses such as xylose and arabinose present in the hemicellulose fraction of cellular biomass. Accordingly, the production of recombinant *Z. mobilis* strains with additional desirable characteristics such as an ability to metabolise pentose, cellulose, and cellobiose represents an important commercial objective. This

technology will allow engineered *Zymomonas* to directly ferment waste biomass material to ethanol.

Platform system for the expression of exogenous DNA: *Why use Zymomonas when existing expression systems can do the job?*

Zymomonas is one of the smallest free living organisms known. It has a relatively small genome (about 2 Mbp) compared to *E. coli* (about 4Mbp) which is commonly used to express exogenous DNA. This presents great potential to create a platform host with a minimal genome to produce maximum commercial commodities. It has been known that this bacterium does not produce any bioactive compounds associated with cell growth. This could be advantageous for expressing exogenous DNA associated with bioactive compounds and/or secondary metabolites production. The expression of such DNA is often problematic in platform hosts producing secondary metabolites such as *E. coli*, which tend to cause host lethal effects. *Zymomonas* also harbours a unique carbon metabolic pathway. Enzymes associated with this pathway constitute up to 50% of a cell's total protein leading to a carbon metabolism highway. The unique pathway and robust metabolic machinery associated with efficient ethanol production capability including high sugar uptake rate, ethanol tolerance and high osmotolerance could provide alternative options for synthetic biology to express exogenous metabolic pathways associated with the production of commercial commodities.

The Team

This technology was developed by a team of researchers from the School of Biotechnology and Biomolecular Science headed by Prof. Brett Neilan.

Investment Opportunity

NSi seeks an industry partner to further support the research, either by advancing the proof of concept in exchange for a license option, or to license the technology.

Further Information:

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