Development of Tools for the Shift to Isobutanol as a Biofuel

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Abstract

There are several issues in the switch from petroleum to alternative fuels. One problem is what to do with existing wastes including leaking coal ash ponds. Another issue is the efficient production of an alcohol that is effective as a fuel, easy and inexpensive to produce, and will function within the current infrastructure. This year, we improved the sensitivity of our cadmium detector using sensitivity tuners. We are developing a strain of *E. coli* resistant to isobutanol and cloning GlmY, GlmZ, and IlvM which are genes involved in the isobutanol production pathway. Addition of sensitivity tuners has improved the cadmium detector approximately 4-fold, from 10mM to 2.5mM, still over the federal limit. The isobutanol resistant strain of *E. coli* can tolerate 3 times as much isobutanol as the original strain. Finally, we have successfully isolated the GlmY coding region and have cloned it into the BioBrick vector.

Background

Cadmium

- Common in coal ash produced by power plants
- Spills result in groundwater contamination
- Ingestion can lead to cardiovascular and digestive problems including cancer
- EPA limit is 5 ppb
- Will remain a problem even after the shift to a biofuels-based economy

Biofuels - Isobutanol

- Renewable alternative energy source
- Contains 82% of the energy of gasoline
- Can be used in current infrastructure
- Will not harm current engine parts

Cadmium Methods

Cadmium

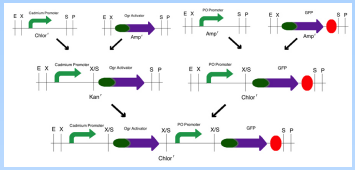

Figure 1: Illustration of the construction of K1320000

- Grow cultures overnight in 37°C with concentrations ranging from 0-100mm
- Resuspend samples in 1x PBS
- Check OD600 and fluorescence using Vernier SpectroVis Plus

Biofuels Methods

GlmY cloning

- Genomic DNA PCR to amplify the gene
- Increases Isobutanol resistance in *E. coli*
- GlmZ and IlvM cloning in process

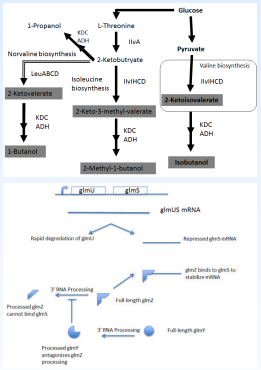

Figure 2: Isobutanol production pathway in bacteria. Genes involved in each step are noted.

Biofuels Methods

Isobutanol Resistant Strain

- Grow *E. coli* in varying concentrations of alcohol overnight
- If control and experimental strain show equivalent growth, increase alcohol concentration.
- If experimental growth is lower than control growth, repeat with same alcohol concentrations

Cadmium Results

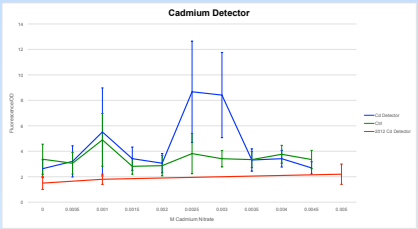

Figure 3: Comparison of K824008 (2012, red), K1320000 (2014, blue) and control. The 2014 Cadmium detector using tuners developed by the 2007 Cambridge team increases the detection level from 10mM (2012) to 2.5mM (2014).

Biofuels Results

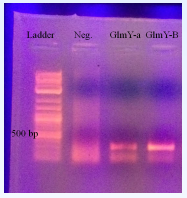

Figure 4: Gel of PCR amplification of the submitted plasmid K1320100.

GlmY will antagonize the breakdown of GlmZ to increase resistance to isobutanol.

Safety Considerations

Cadmium

- Add kill switch before testing in the wild

Biofuels

- Particularly important for isobutanol resistant strain
- Change cleaning procedures in lab to 10% bleach
- Determine specificity of resistance
  - Is it resistant to ethanol or isopropanol?
  - Atsumi, et. al report that isobutanol resistance is separate from ethanol resisitance
- Addition of a kill switch before commercial use

Conclusions and Future

1. Cadmium detector is 4-fold more sensitive than 2012 version
   - Still not sensitive enough for commercial use
   - Try different tuners or different reporter
   - Add kill switch
2. GlmY is cloned and in BioBrick format
   - Clone additional genes involved in resistance and in production
3. Isobutanol resistant strain can grow in 3 times as much as the original strain
   - Selection of more resistant strains continues
   - Specificity testing underway

References

Atsumi, S., Wu, T., Machado, I. M., Huang, W., Chen, P., Pellegrini, M., and Liao, J.C., (2010). Evolution, Genomic Analysis, and Reconstruction of Isobutanol Tolerance in E. coli. Molec. Says. Bio 6:449.

Atsumi, S., Hanai, T., and Liao, J.C., (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451(3):86.

Urban, J.H., and Vogel, J., (2008). Two Seemingly Homologous Noncoding RNAs Act Hierarchically to Activate glmS mRNA Translation. PLoS Biology 6(3):631.