



Investigation Of Solvent Toxicity in Bacterial Strains Involved in Butanol Production

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Problem Statement

Reduction in dependency of imported petroleum and the quest to identify renewable energy sources has led to a search for innovative biofuels derived from renewable biomass, that promise long-term reductions in greenhouse gas emissions. Butanol is one possible biofuel. It is an industrial fuel that can be produced from crops using acetone-butanol (AB) fermentation by butanologenic microbes, such as *Clostridium* spp.

However, high yields of butanol production by microbial AB fermentation strategies, has been limited by butanol toxicity. To make AB fermentation economically tractable, two major shortcomings of this microbial fermentation process, that uses *Clostridium* spp, must be overcome:

- Butanol toxicity to the clostridial cells at low concentrations (e.g. $\leq 2\%$ in final fermentation broth)
- The inefficient regulation of electron flow and distribution in *Clostridium acetobutylicum* that leads to loss of reducing equivalents and an incorrect redox balance

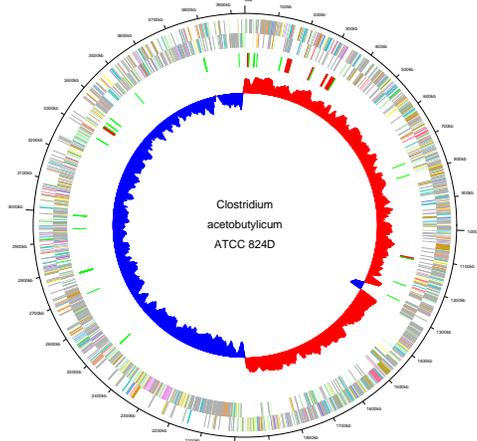


FIGURE 1: Genome map of *Clostridium acetobutylicum* ATCC 824D

Technology Description

This Phase I project aims to identify the gene functions, metabolically reconstruct and compare the genomes of *Clostridium acetobutylicum* and *C. beijerinckii*, two key organisms for butanol production, with specific emphasis on development of a metabolic network map of these organisms to facilitate improvement of solvent tolerance and bioenergetics. This will be performed in-house using the ERGO™ Genome Analysis Suite (Overbeek *et al.*, 2003).

In addition, the genomes of the *Clostridium* spp. studied will be compared to other known microbial genomes capable of survival at high levels of organic solvents (e.g. *Pseudomonas putida*) to identify key differences and similarities in genes relevant to conferring solvent resistance.

Expected Results

A state-of-the-art *in silico* metabolic model of the *Clostridium* spp. genomes used to produce butanol currently will be created. This model will then be used to make predictions of better ways to improve the bacterial strains used for butanol production that may be implemented later by direct metabolic and strain engineering approaches.

The overall goal of this work, in Phase II, is to create a pilot-scale butanol bio-production system that is competitive with the ethanol now being produced for fuel. This will require engineering the bacterial organism(s) to better resist higher levels of butanol (between 1.5-2.0% butanol) and produce higher levels of the biofuel.

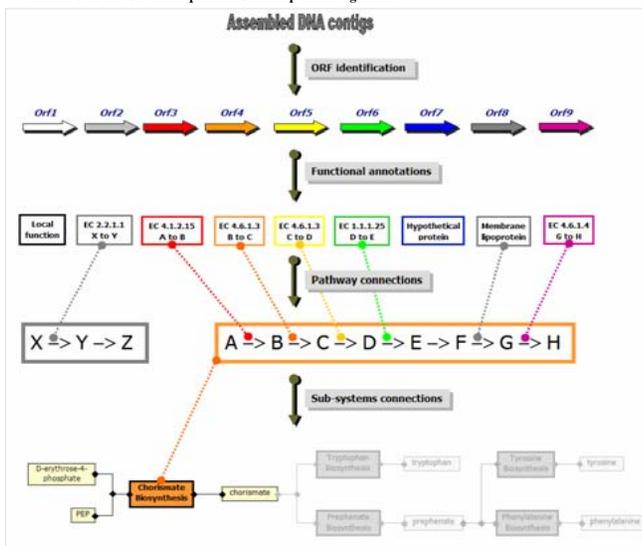
Potential Environmental Benefits

The ultimate target of this research is to create a butanol bio-production system that is competitive with the ethanol now being produced for fuel. There are several advantages of butanol over ethanol as an advanced biofuel:

- Higher energy content
- Miscibility with gasoline
- Octane-improving power
- Low volatility

Per unit volume, there is predicted to be greater energy yield with butanol as a biofuel additive to gasoline than ethanol, contributing to facilitating long-term reductions in greenhouse gas emissions.

FIGURE 2: Overview of workflow during the genome reconstruction process. Genes are identified and assigned functions. Where possible, gene functions are associated with pathways and connected to cellular overviews to develop a metabolic map of the organism.



Reference

Overbeek, R. *et al.* (2003) The ERGO(TM) genome analysis and discovery system *Nucleic Acids Res.* 31:164-71