Development of a High Temperature Microbial Fermentation Process for Butanol Production

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Introduction

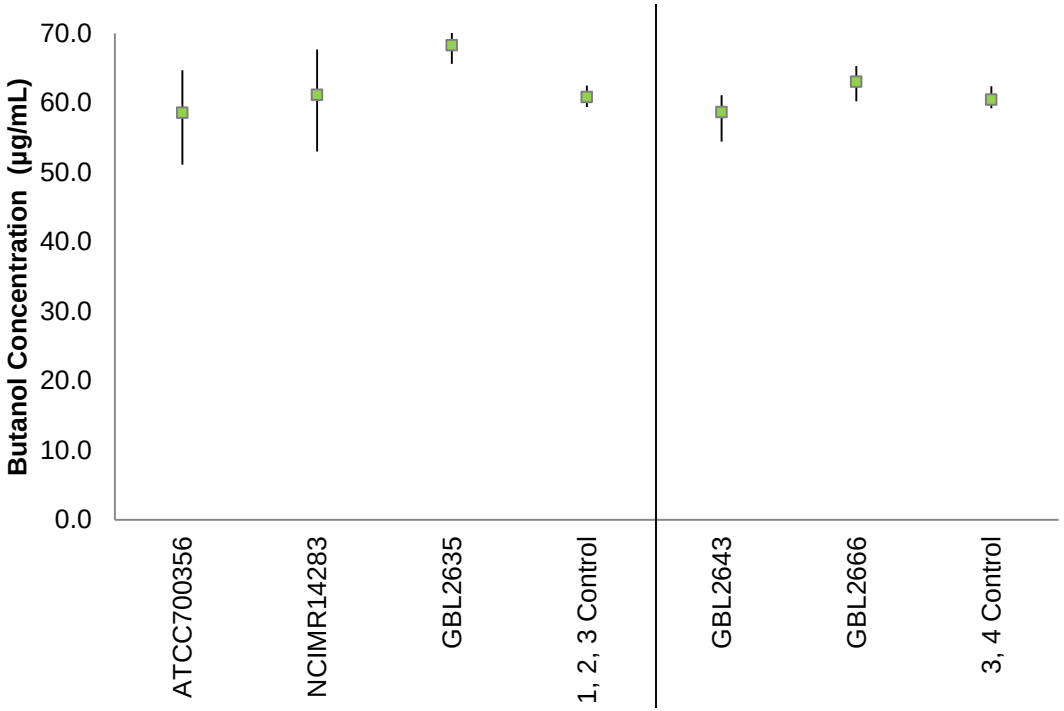
- Transforming renewable biomass into cost competitive high-performance biofuels and bioproducts is key to US energy security
- Butanol production by microbial fermentation and chemical conversion to polyolefins, elastomers, drop-in jet or diesel fuel, and other chemicals is a promising solution
- Butanol fermentations using *Clostridial* microorganisms have production limitations:
  - current strains have low butanol tolerance and low yields resulting in increased product recovery costs
  - strains grow at ambient temperatures with slow growth rates
  - current processes require batch fermentation
- High temperature fermentation process can facilitate butanol recovery up to 20%, by using gas stripping with a continuous flow rate[4]

Benefits of a High Temperature

- Optimal hydrolysis rates in the saccharification of biomass which leads to maximized butanol production
- Decreases energy costs associated with reactor cooling and capital cost associated with reactor design
- Decrease process contamination for maintaining a sterile environment
- Butanol stripping at elevated temperatures gives higher butanol production with constant removal and continuous fermentation

Butanol Production Screening

Bacterial strains were purchased or provided by GreenBiologics (GBL) and screened for butanol production.



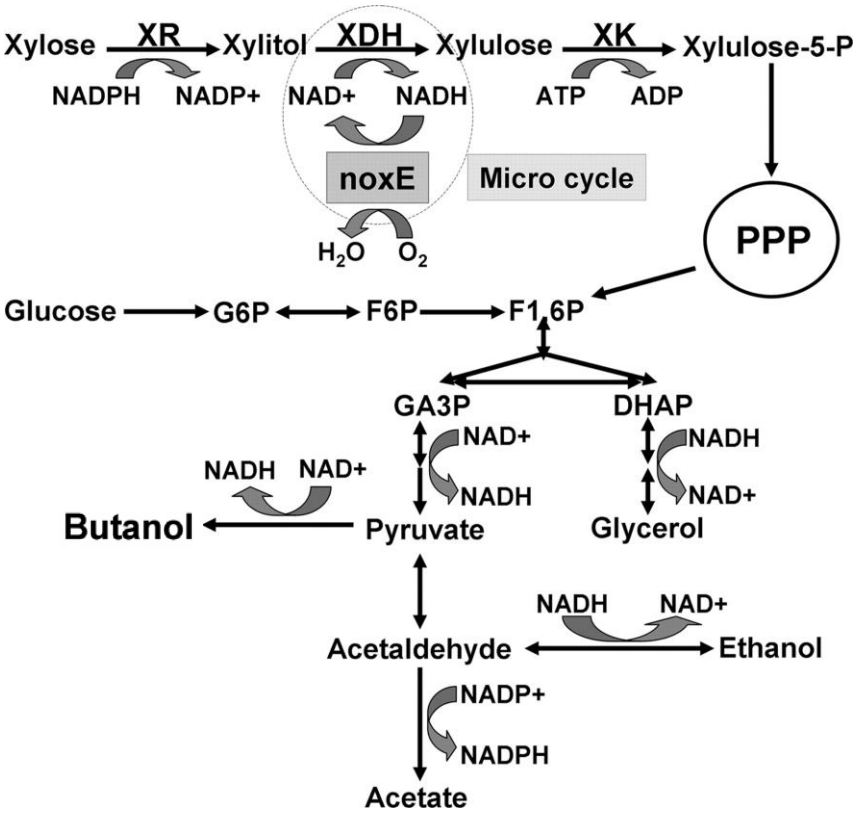
Butanol production on 2TY media with xylose as the carbon source. Strains: Geobacillus caldosylosiliticus (ATCC700356), Geobacillus caldoxyloliticus TK4 (NCIMR14283), Bacillus Licheniformans (GBL2635), Ureibacillus thermophaericus (GBL2643), Ureibacillus thermophaericus (GBL2666).

Geobacillus caldoxylosilyticus (ATCC700356) strain selection

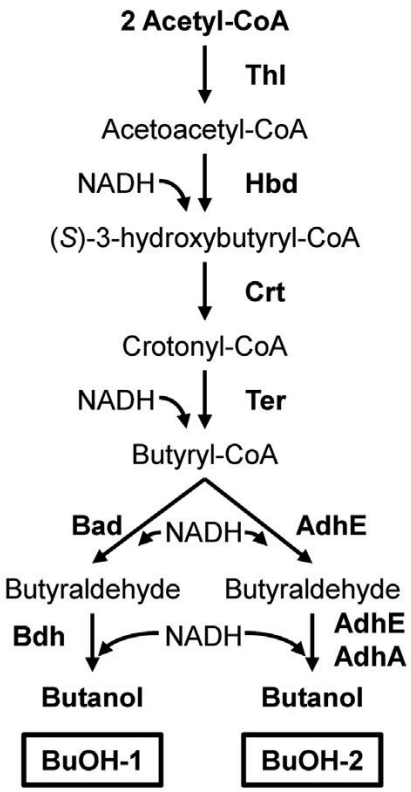
- Capable of anaerobic growth on xylose and glucose.
- Optimum growth temperature at 65°C.
- Enzymes for xylose fermentation produced at high levels when fermenting glucose, suggesting simultaneous consumption.
- No alcohol dehydrogenase activity detected anaerobically, suggesting lower alcohol production.

Production Pathway

- Glycosyl hydrolases available for deconstructing xylan.
  - *Beta*-xylosidase and *alpha*-L-
  - Arabinofuranosidase homologs



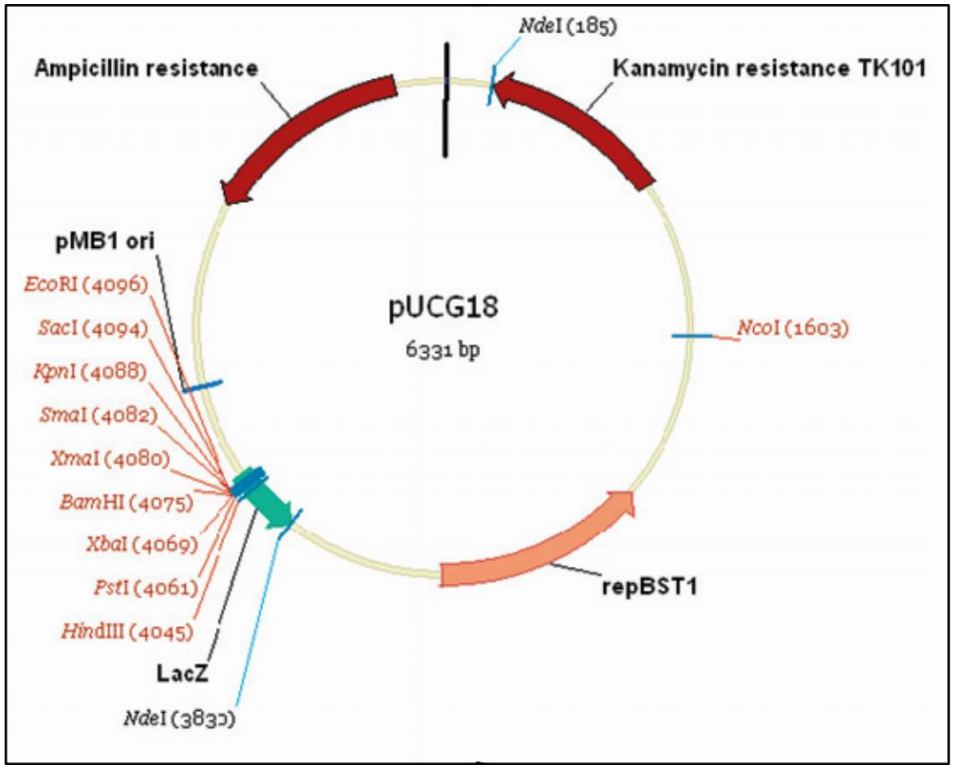
Butanol Pathway



- A butanol pathway may be present
  - *3-hydroxybutyryl-CoA dehydratase homolog*

pUCG18 Plasmid

- Shuttle vector for *Geobacillus* strains grown up to 68°C.
- Thermal stable positive selectable antibiotic marker, conferring resistance to kanamycin.
- Several methods reported in the literature for *Geobacillus* transformation.



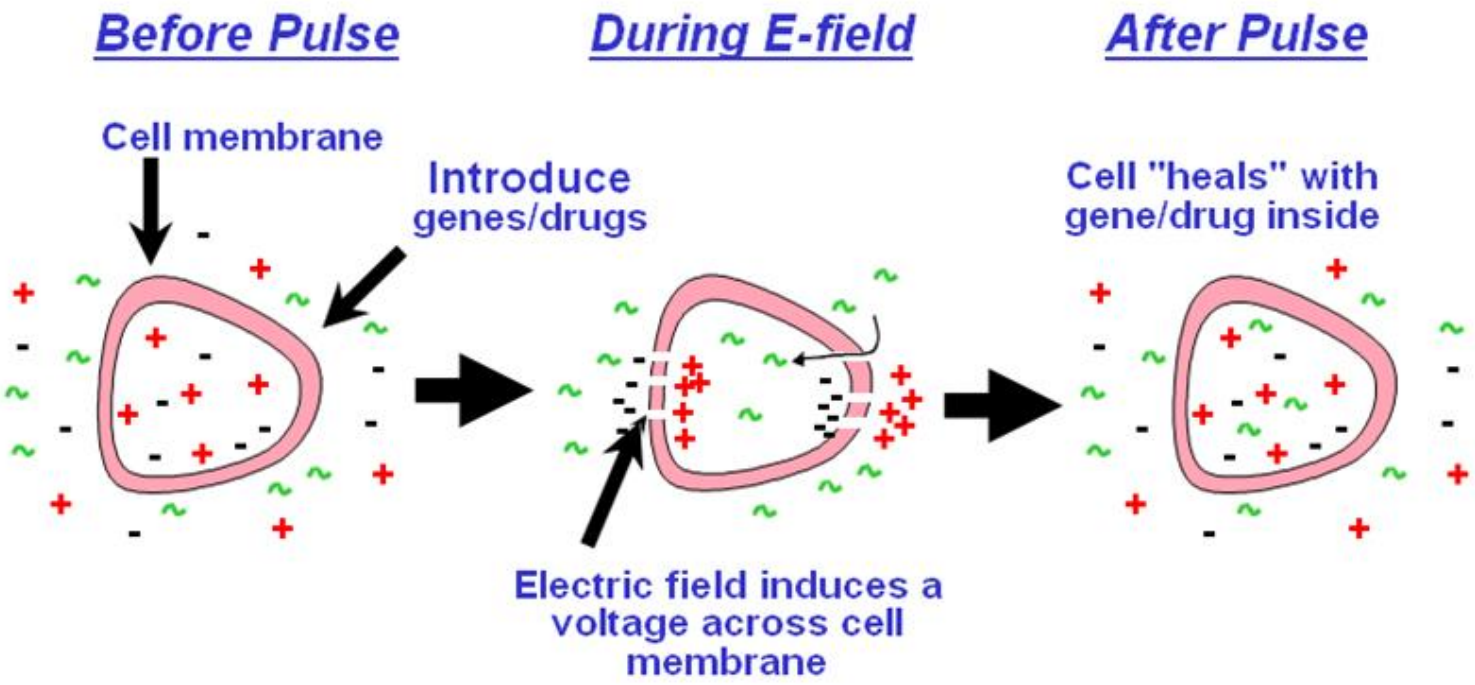
Potential Transformation Methods

1. Electroporation of electrocompetent cells

2. Conjugation for ternary transfer of plasmid from *E. coli*

3. Protoplast fusion

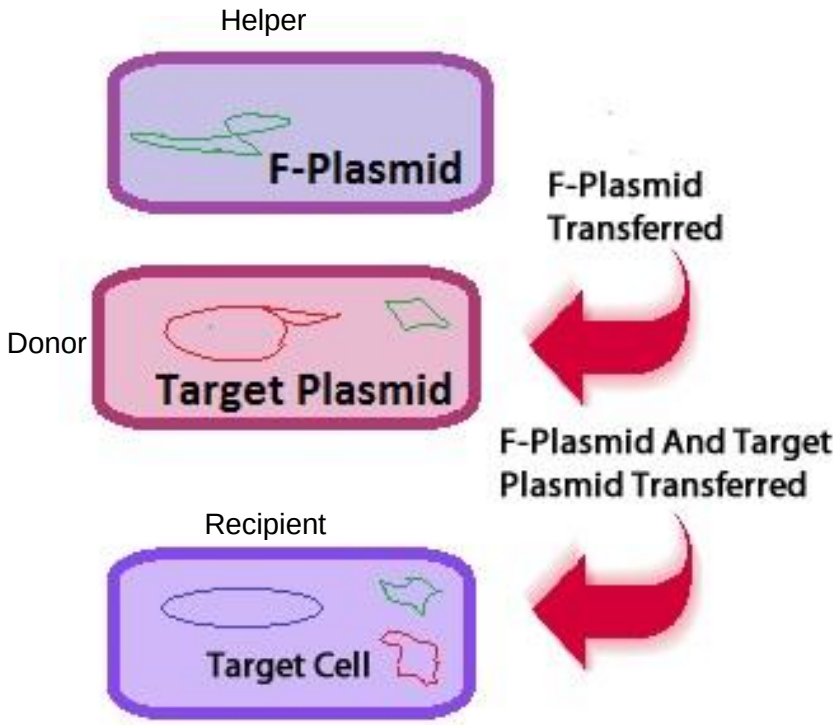
Electroporation



Electroporation

- *Geobacillus* was subcultured and grown to an OD_{600} of 1.4 at 55°C and 220 rpm
- Cells prepared to be electrocompetent
- Mixed with 1-5 µL of plasmid (either methylated or unmethylated)
- Exponential decay program was used
  - 10-25 µF
  - 1500-2500 V
  - 600 Ω
- Recovered for 4 hours at 55°C and 220 rpm
- Grown on TBAB with kanamyacin

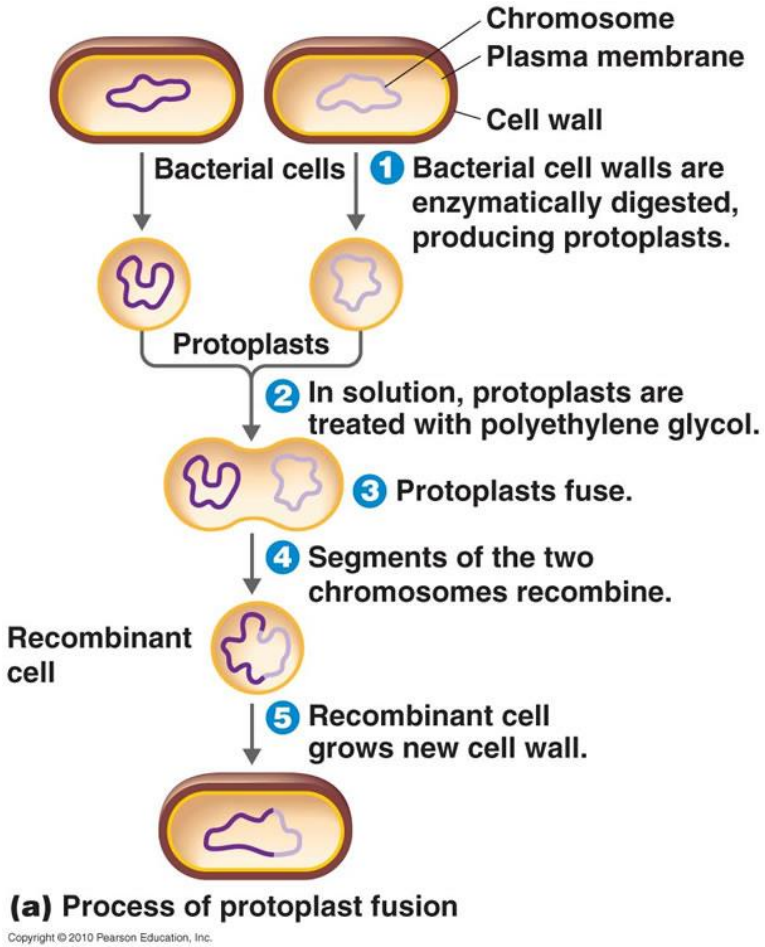
Ternary Conjugation



Ternary Conjugation

- *dam*$^{-}$/*dcm*$^{-}$ *E. coli*, HB101, and *Geobacillus* were subcultured and grown to an OD_{600} of 0.3
- All three cells were combined in an 8:1:1 ratio of *Geobacillus*, *dam*$^{-}$/*dcm*$^{-}$ *E. coli*, and HB101
- Pellet was placed on LB plate and incubated at 37°C for 6-16 hours
- Resultant colonies were plated on LB with kanamycin and grown overnight at 55°C

Protoplast Fusion



(a) Process of protoplast fusion

Protoplast Fusion

- *Geobacillus* was subcultured and grown to an OD_{600} of 0.8-1.0
- Lysozyme added to 10 µg/mL
- 5-20 µL of plasmid was mixed protoplast suspension
- Added PEG 600 or 10,000
- Protoplasts were plated on regeneration plates with kanamycin
- Incubated at 50°C for 12 hours then at 60°C for 24-48
- Colonies transferred to TBAB plates with kanamycin

Discussion

- Electroporation
  - Setting have been adjusted to give a pulse time of 5-6 milliseconds which gives best effeciency
  - Controls showing cells can withstand the shock
  - Both fresh and frozen electrocompetent cells were used
- Ternary Conjugation
  - Cells were plated with no dilution in an attempt to get any amount of growth
- Protoplast
  - Problem with antibiotic selection
- Moving to a small plasmid pUCG3.8
  - Same essential genes
  - Higher transformation efficiency

References

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