SYNTHETIC MICROBIAL CONSORTIUM FOR ISOBUTANOL PRODUCTION

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BACKGROUND

Why Isobutanol
  - Energy dilemma VS ethanol
  - can be produced out of different biomass including agricultural residues, green and cheap
  - Isobutanol can blend with a variety of fossil fuel-based materials in higher ratio to produce greener versions of fuel
  - Can be shipped in pipeline since less corrosive and non-hygroscopic (don’t absorb water)
  - Isobutanol also produces 25 percent more energy than ethanol per gallon
BACKGROUND

- Microbial Consortium

**Diagram:**

- Hemicellulose
  - Pentose mono and oligo saccharides
  - C5 Specialists: *E. coli*
  - Biofuel: *T. reesei*

- Cellulose
  - Glucose mono and oligo saccharides
  - C6 Specialists: *E. coli*
  - Biofuel

Engineered intercellular communication / population coordination.
BACKGROUND

- Isobutanol Production Strain JCL260
  - (rrnB\textsubscript{T14}ΔlacZ\textsubscript{WZ15} hsdR514ΔaraBAD\textsubscript{AR33} ΔrhaBAD\textsubscript{LD5}ΔadhΔldhΔfrdΔfnrΔptaΔpflB)*
  - + plasmids pSA55 & pSA69 from the Liao Lab, UCLA

PURPOSES

- Construct genetic circuits in isobutanol production strains based on a similar project
  - Details in a minute

- Analyze isobutanol productivity and sugar consumption of strains
STEPS FOR CONSTRUCTION
**Steps for Construction**

- **Trp auxotroph:**
  - Over-express yddG and knockout tyrR to export more Tyr (finished)
  - Add in $P_{BAD}$ for arabinose tuning (future)

- **Tyr auxotroph:**
  - Over-express trpEDfbr to produce more Trp
  - Add in YFP (fluorescent) as strain marker
  - Knockout tyrA to construct Tyr auxotroph (NOW)
  - Add in $P_{prpB}$ for NaPropionate tuning (future)
**METHODS & RESULTS**

- Insert \( \lambda \text{Red-cat-sacB cassette} \)
  - \( \lambda \text{Red} \) can trigger gene recombination from functioning
METHODS & RESULTS

- Use P1 transduction to insert
  - Lysates of P1 includes particles that are packed with the bacterial chromosome. The fragment will recombine into the host chromosome.

Transduction: lac+ gene from P1 lac+ phage is inserted into lac- bacterium by recombination. The resulting bacteria are lac+.
METHODS & RESULTS

- PCR verification
  - Only #4 JCL260 C5 strain is similar with the positive control, unspecific bands, will be repeated later
METHODS & RESULTS

- OD reads and growth rates studies
  - Optical Density reader, a spectrophotometer measuring light scatter to show cell density
  - We do OD reads on samples of different timepoint from two flask experiments
  - Have data plots that generates the growth rates
METHODS & RESULTS

- HPLC analysis
  - A chromatographic technique used to separate a mixture of compounds
  - Samples from flask experiments
  - Two comparations
METHODS & RESULTS

- **HPLC analysis**
  - C6 strain utilize more glucose compared with K12 and produce decent amount of isobutanol
**METHODS & RESULTS**

- **HPLC analysis**

- **C5 strain behave poorly, even C6 utilize more xylose than C5**
FUTURE WORKS

- Improve C5 strain
- Add in tuning
- Combine with another project (fungi T.reesei), just put in waste and make isobutanol!!!
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