



SYNTHETIC MICROBIAL CONSORTIUM FOR ISOBUTANOL PRODUCTION

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BACKGROUND

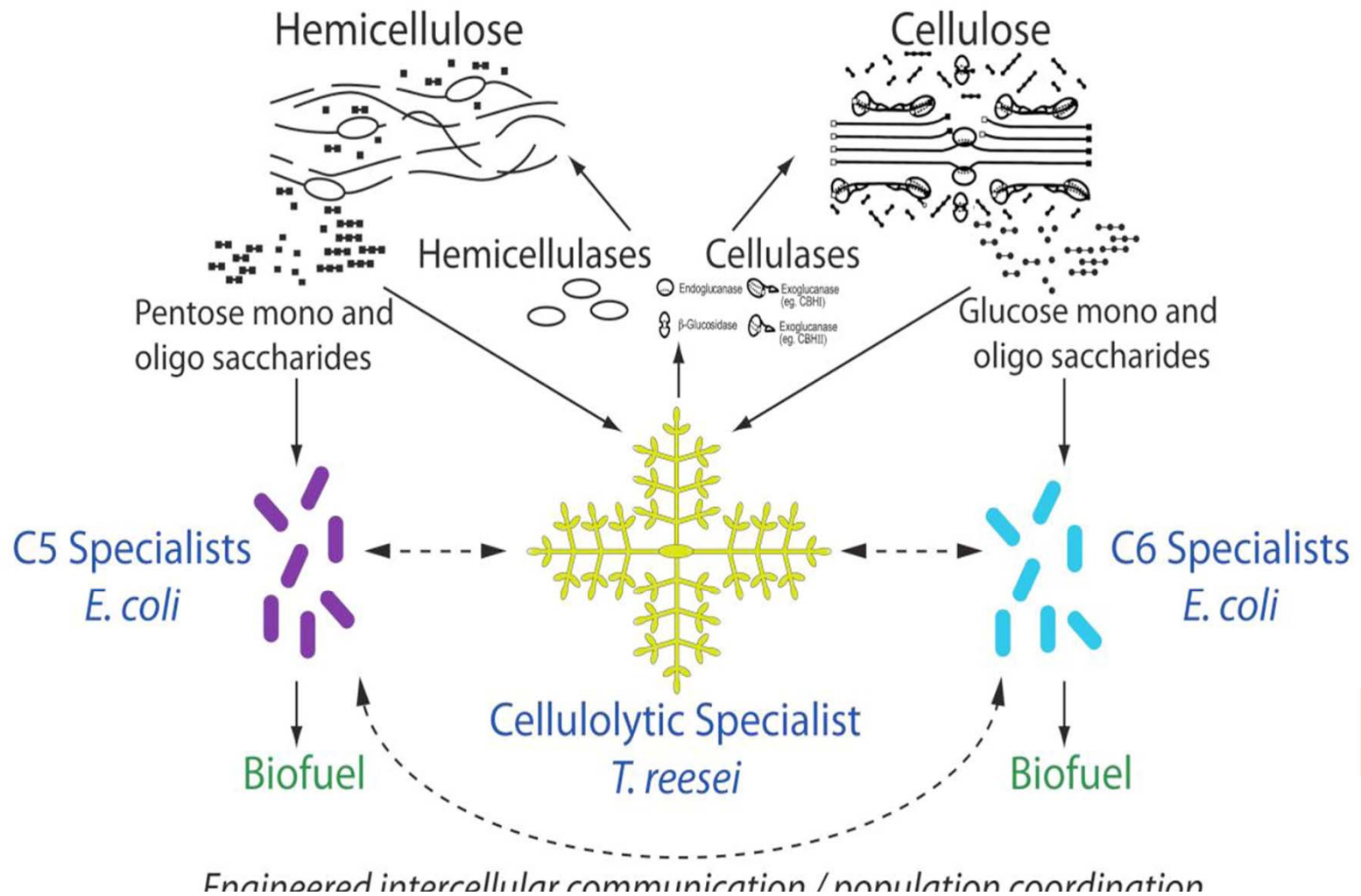
○ Why Isobutanol

- 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



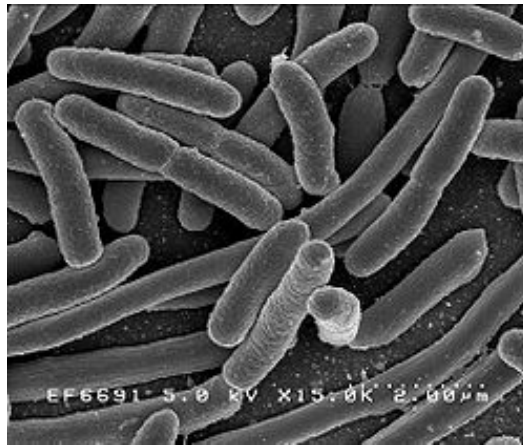
BACKGROUND

- Microbial Consortium



BACKGROUND

- Isobutanol Production Strain JCL260
 - ($rrnB_{T14}\Delta lacZ_{WJ16} hsdR514\Delta araBAD_{AH33}\Delta rhaBAD_{LD78}\Delta adh\Delta ldh\Delta frd\Delta fnr\Delta pta\Delta pflB$)*
 - + plasmids pSA55 & pSA69 from the Liao Lab, UCLA



*Atsumi, S. et al, Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels, *Nature* **451**, 86-90 (2007).

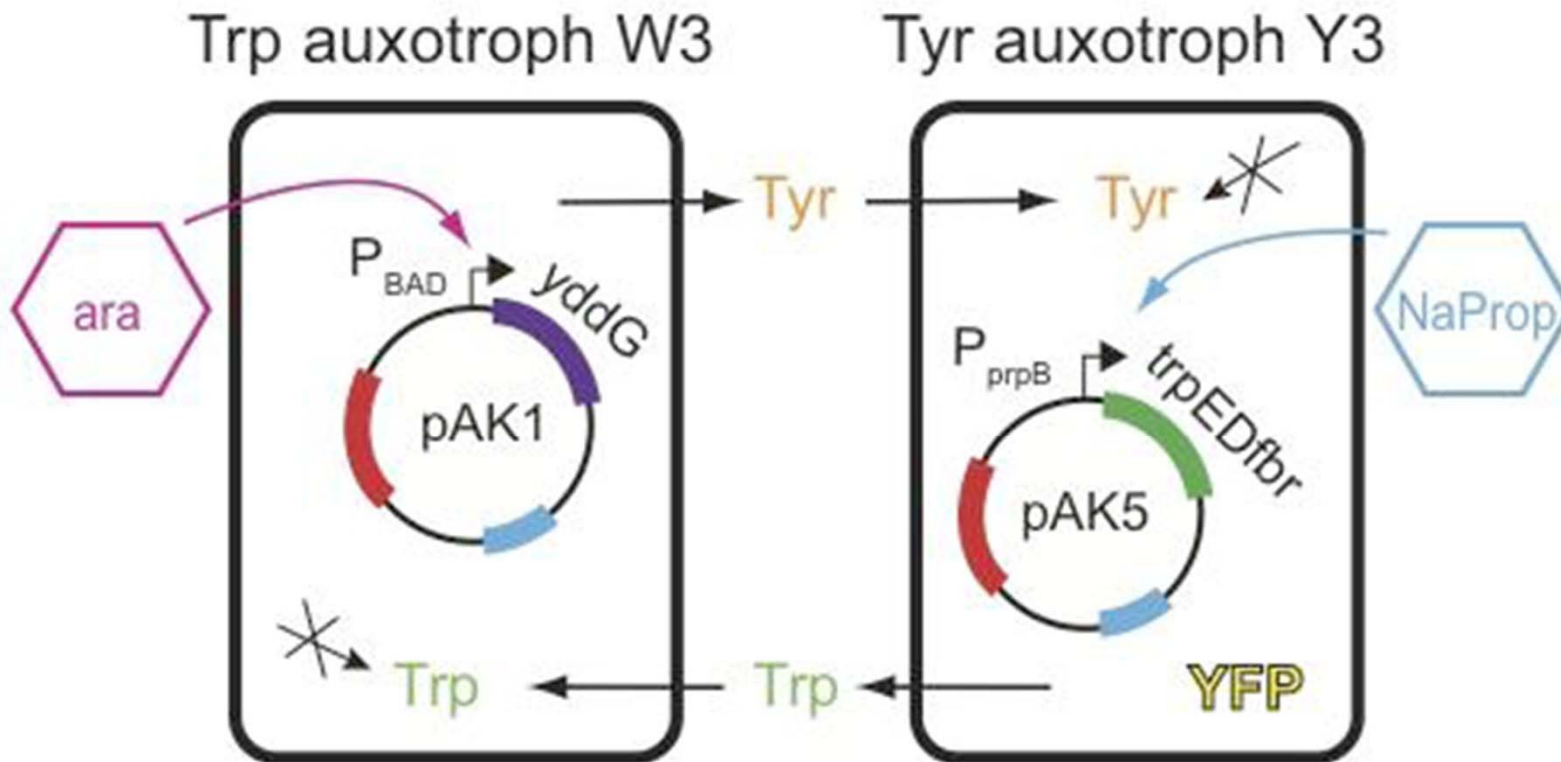


PURPOSES

- 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



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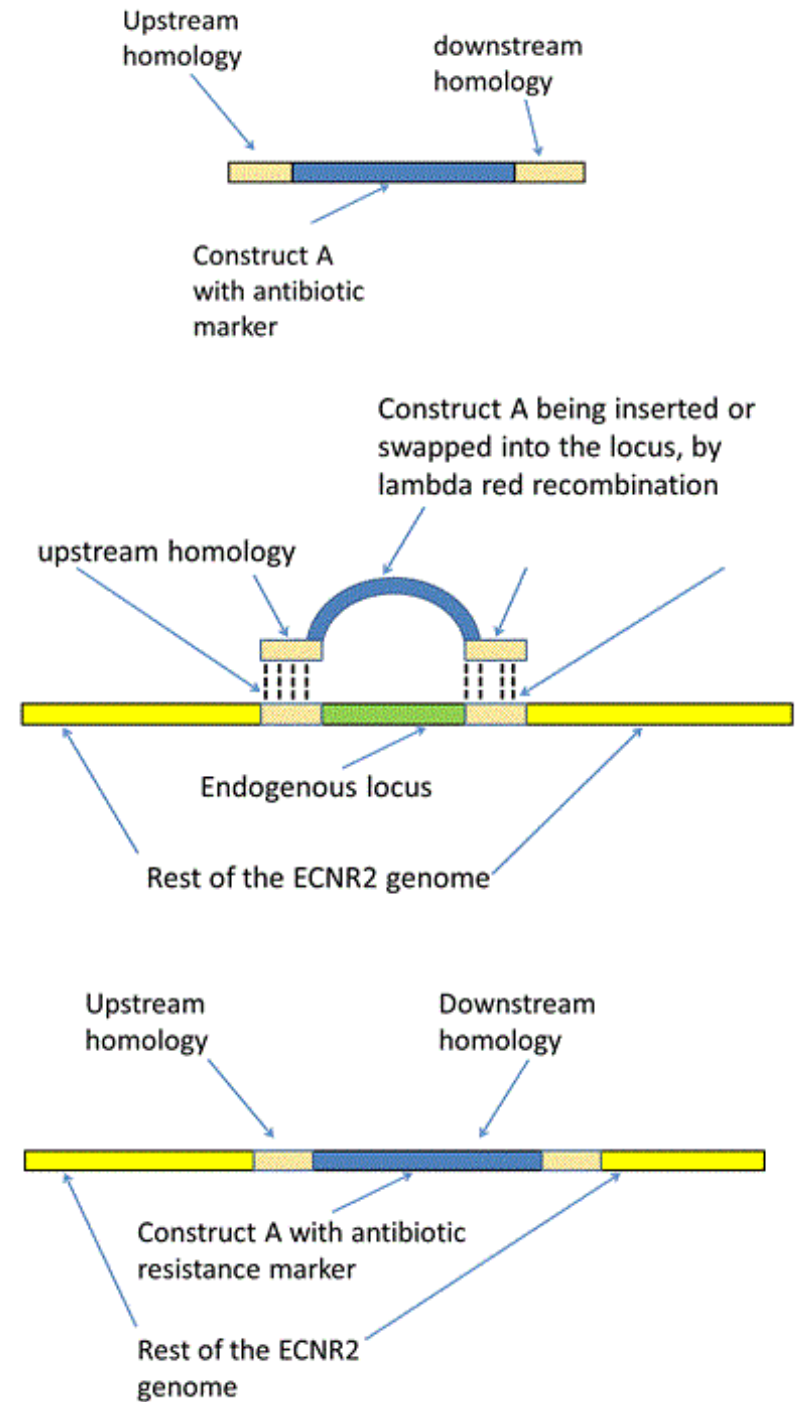
- Trp auxotroph:
 - Over-express yddG and knockout tyrR to export more Tyr (finished)
 - Add in P_{BAD} for arabinose tuning (future)

- 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

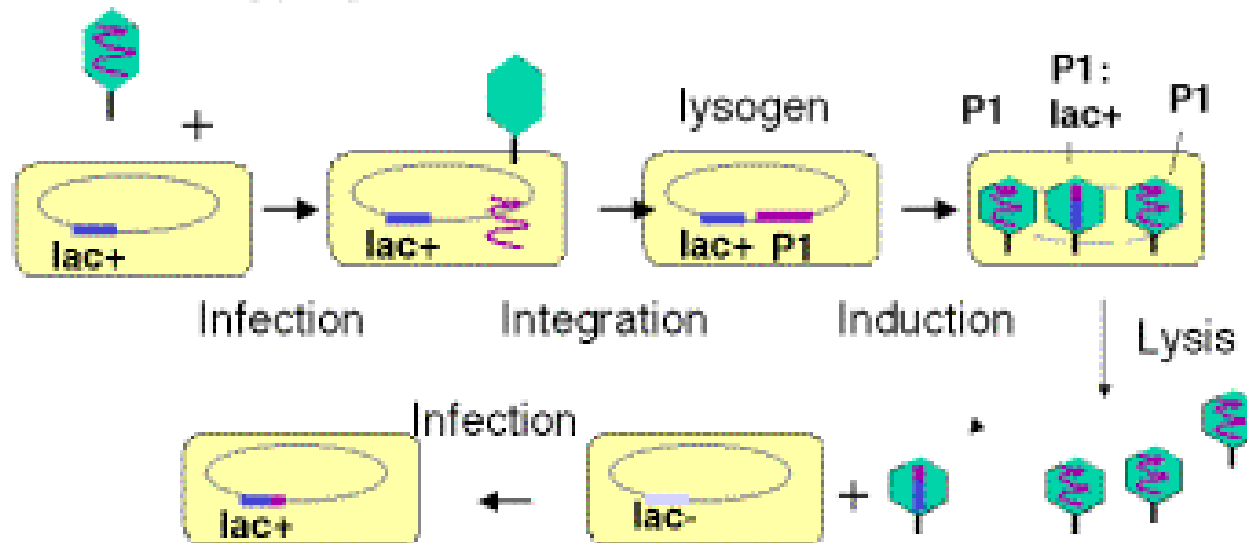
- Insert λ Red-cat-sacB cassette
 - λ Red can trigger gene recoml from functioning



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

P1 transducing phage

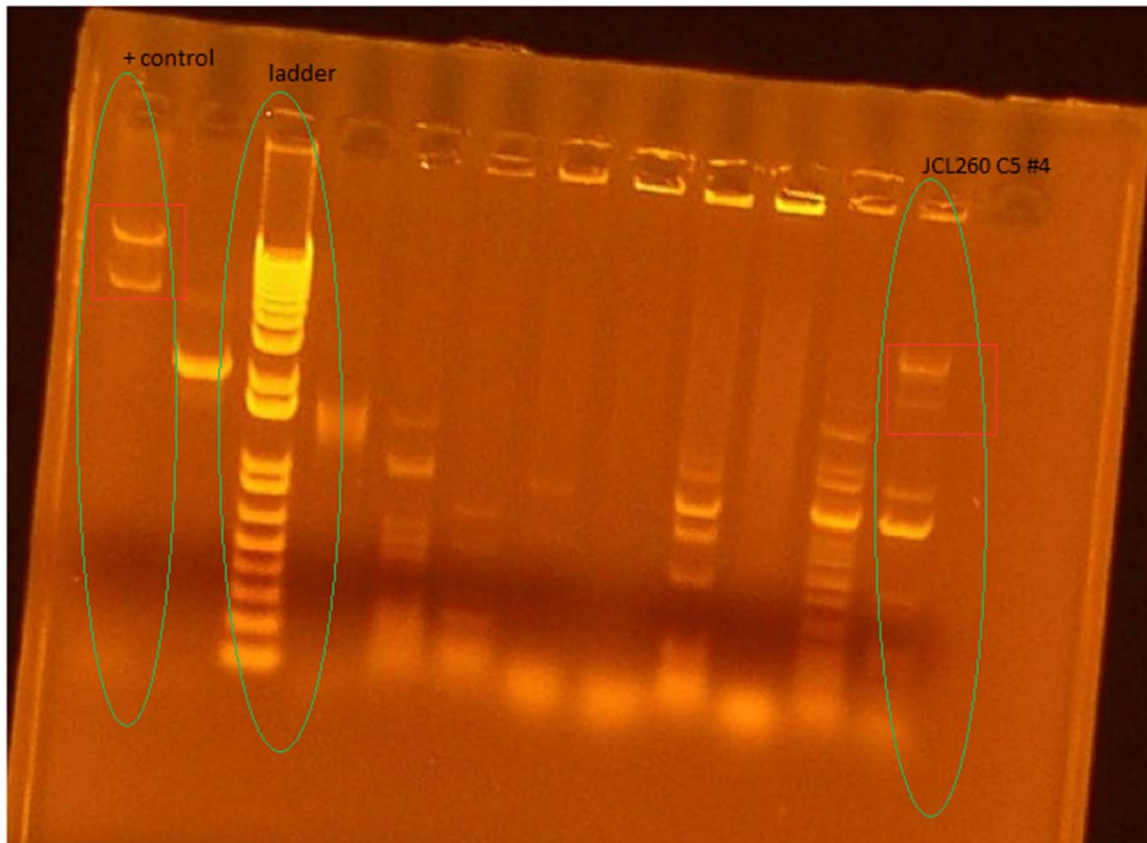


Transduction: *lac+* gene from P1 *lac+* phage is inserted into *lac-* bacterium by recombination. The resulting bacteria are *lac+*.

FIGURE 10.7

METHODS & RESULTS

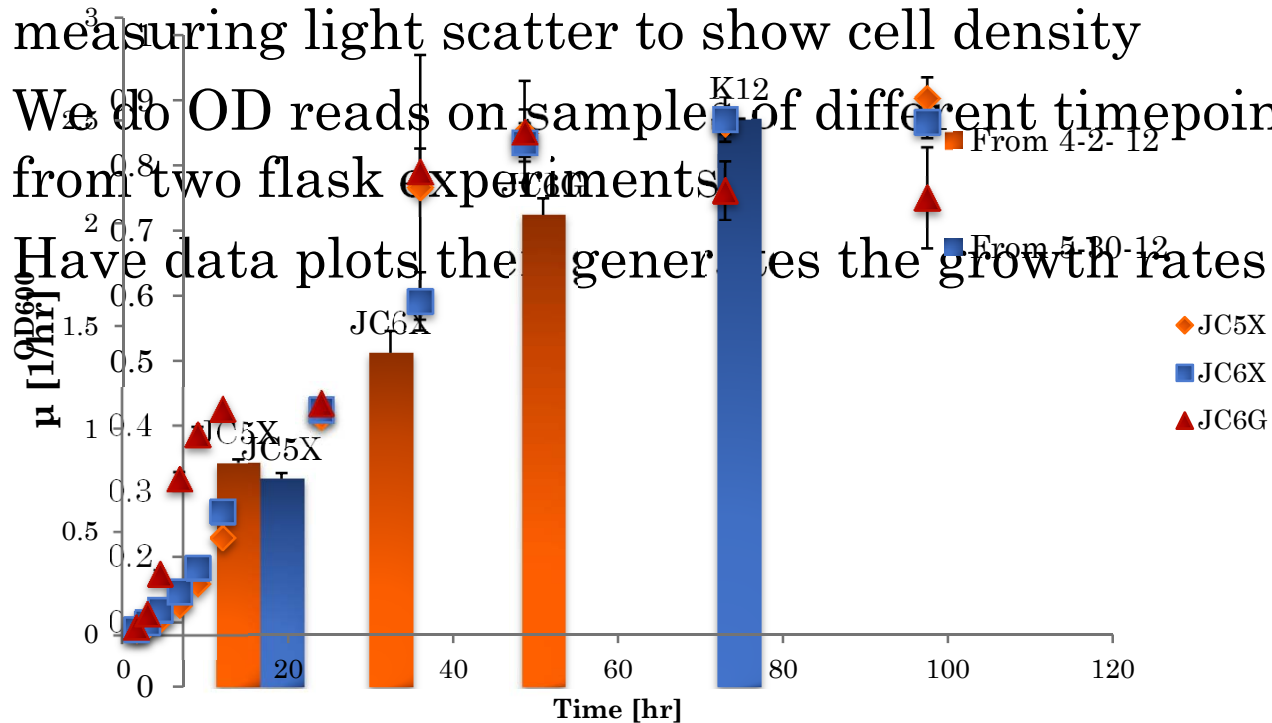
- PCR verification
 - Only #4 JCL260 C5 strain is similar with the positive control, unspecific bands, will be repeated later



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 generate the growth rates



METHODS & RESULTS

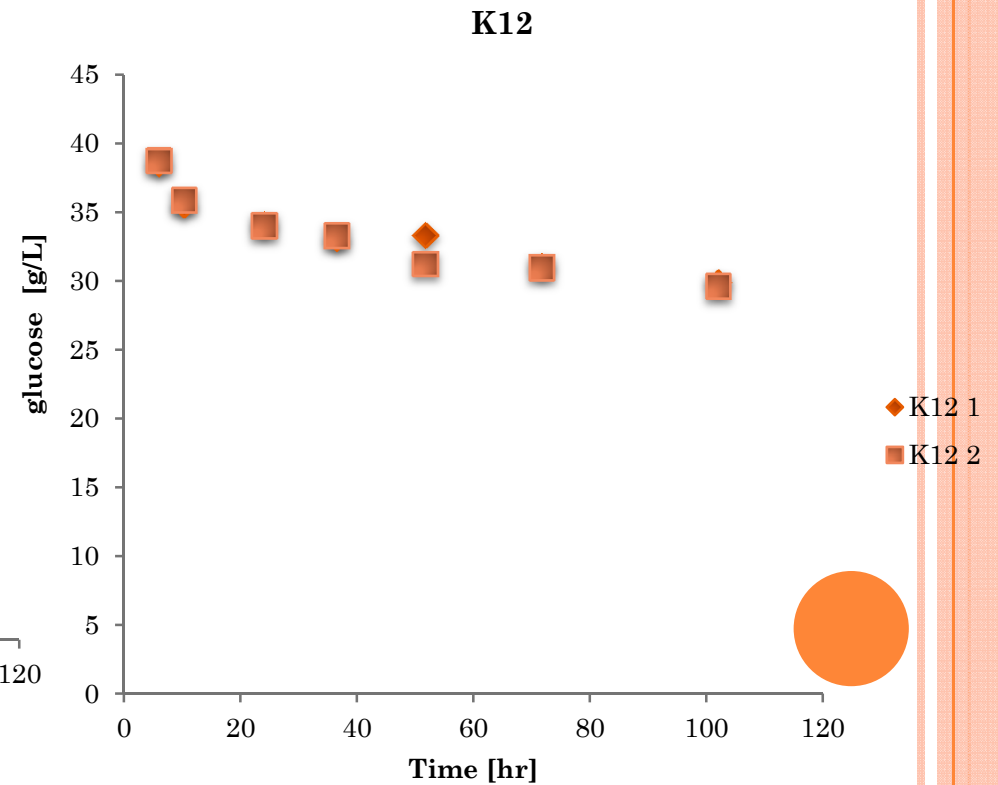
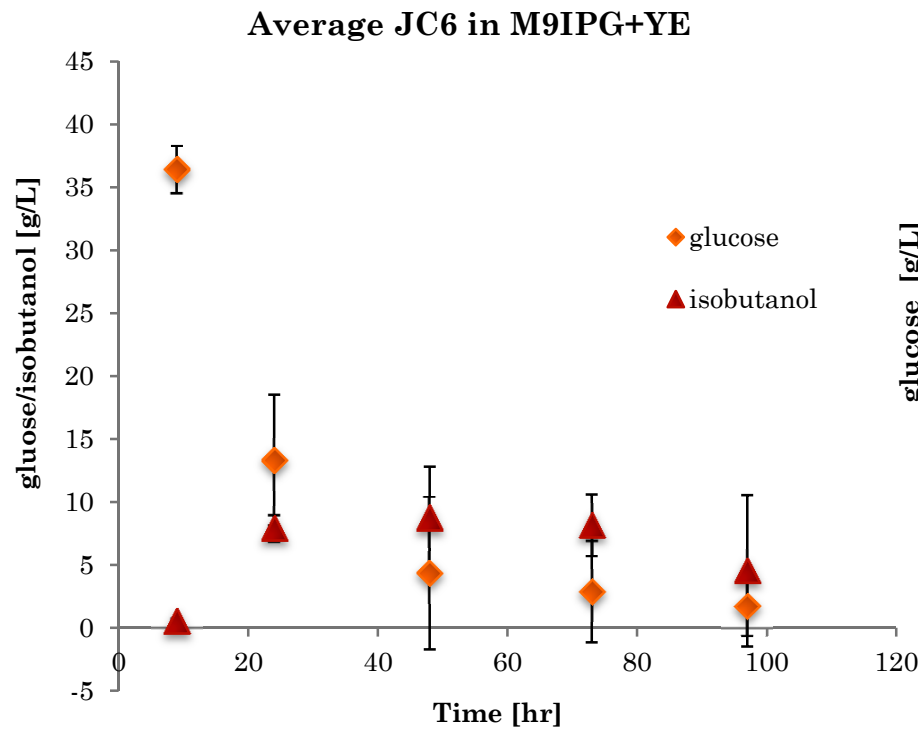
- HPLC analysis
 - A chromatographic technique used to separate a mixture of compounds
 - Samples from flask experiments
 - Two comparisons



METHODS & RESULTS

○ HPLC analysis

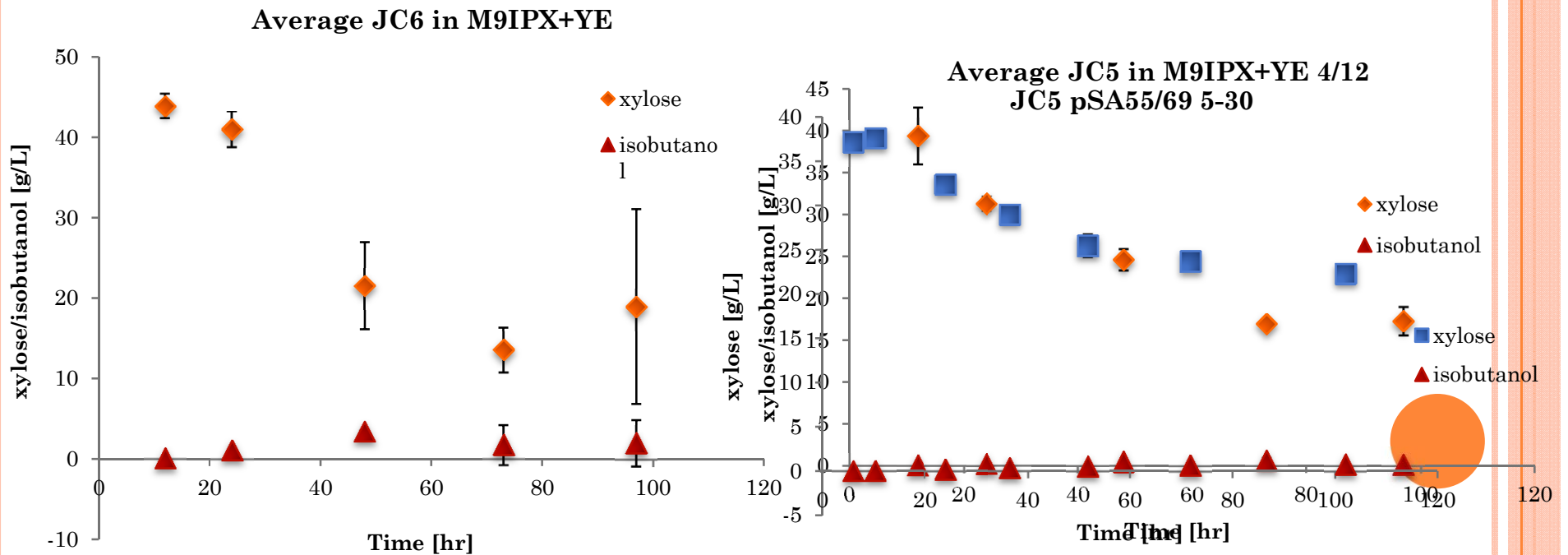
- C6 strain utilize more glucose compared with K12 and produce decent amount of isobutanol



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!!!



ACKNOWLEDGE

- The Lin Lab especially Alissa
- Wang Chu Chien-Wen Funds (Mr. Wang & Mik)
- Thank you for listening

