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Enabling Fermentation Utilization of Pyrolytic Substrates for the Production of Biorenewable Fuels and Chemicals

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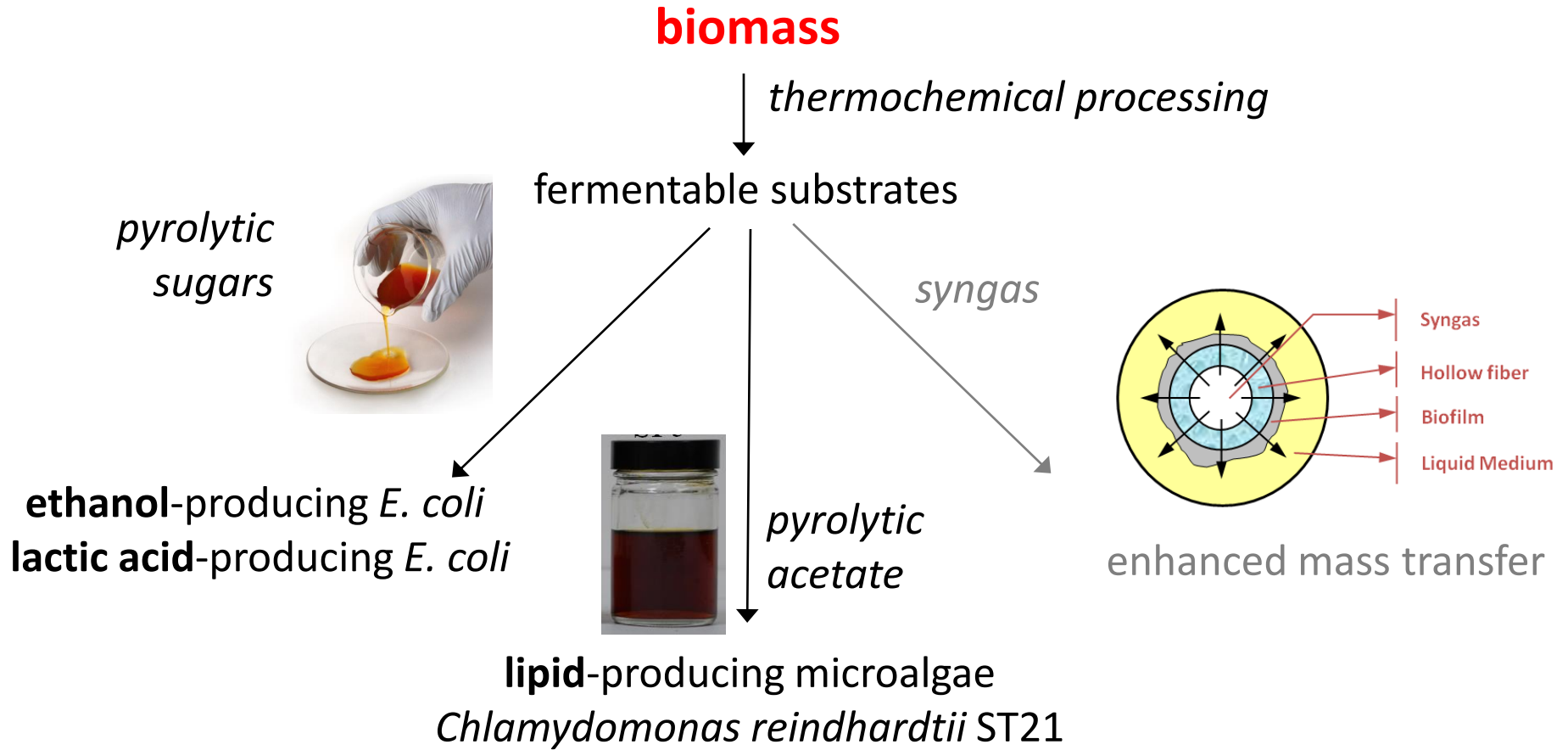
Iowa State University

About Iowa State University



- Named #1 US Institute for biofuels research by Biofuels Digest (2011)
- First US “Land Grant” university
- 31,000 students
- Home of the first electronic computer and George Washington Carver

Hybrid Processing: Thermochemical, then Biological

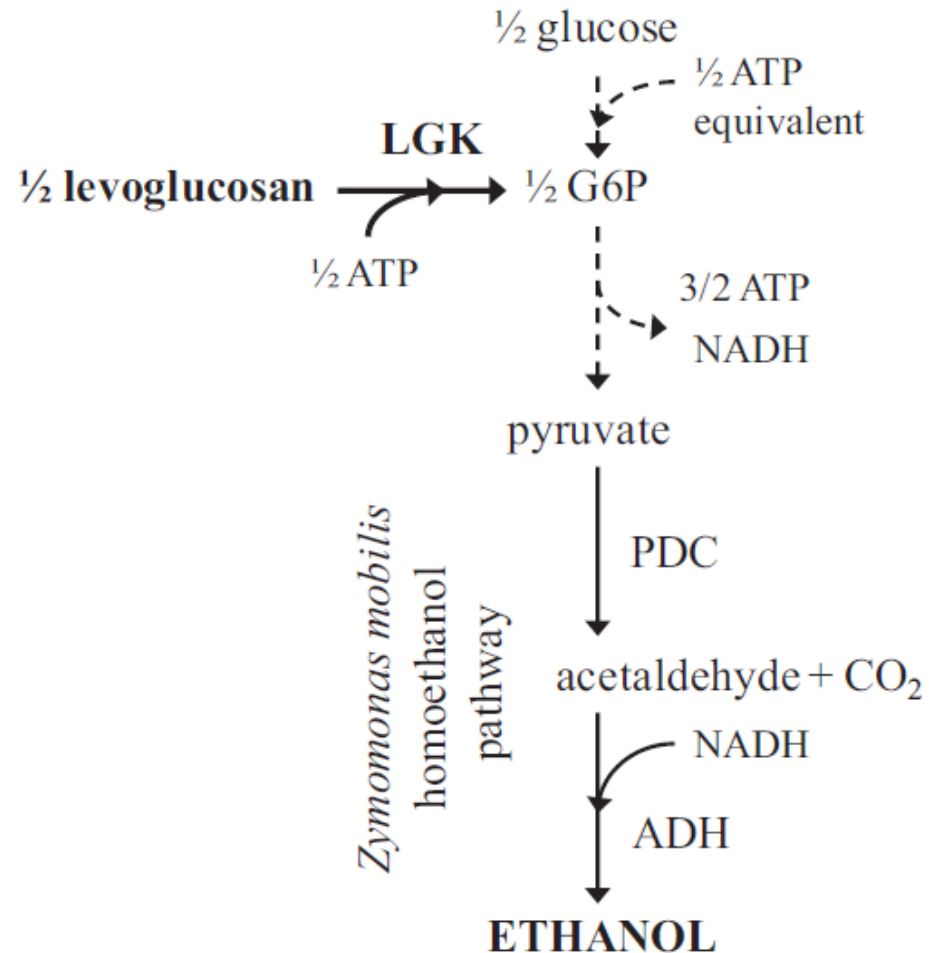


biorenewable fuels and chemicals

Our goal is to identify generic strategies for enabling hybrid processing, not production of specific compounds.

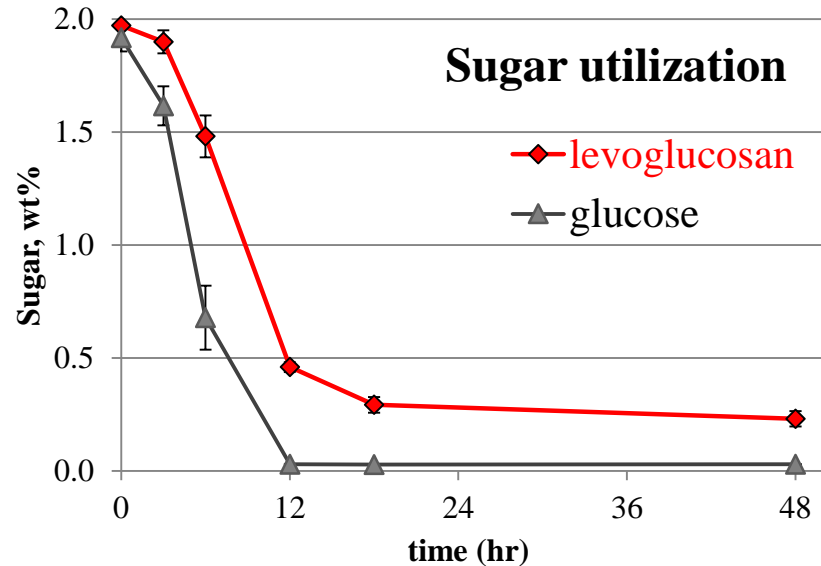
Engineering Pyrolytic-Sugar Utilizing Biocatalysts

Existing biocatalysts can easily be engineered for utilization of levoglucosan as carbon/energy source with same redox, ATP demand as glucose

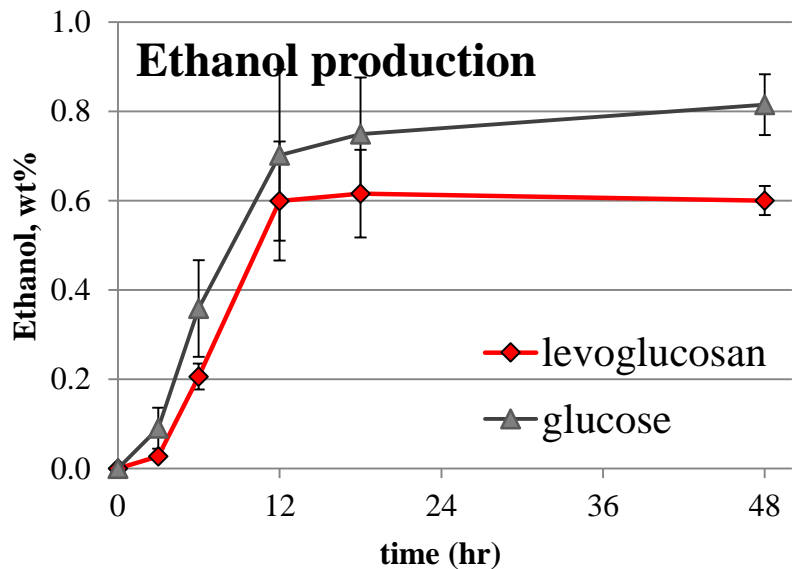


Layton *et al* Bioresource Tech 2011

Sugar utilization

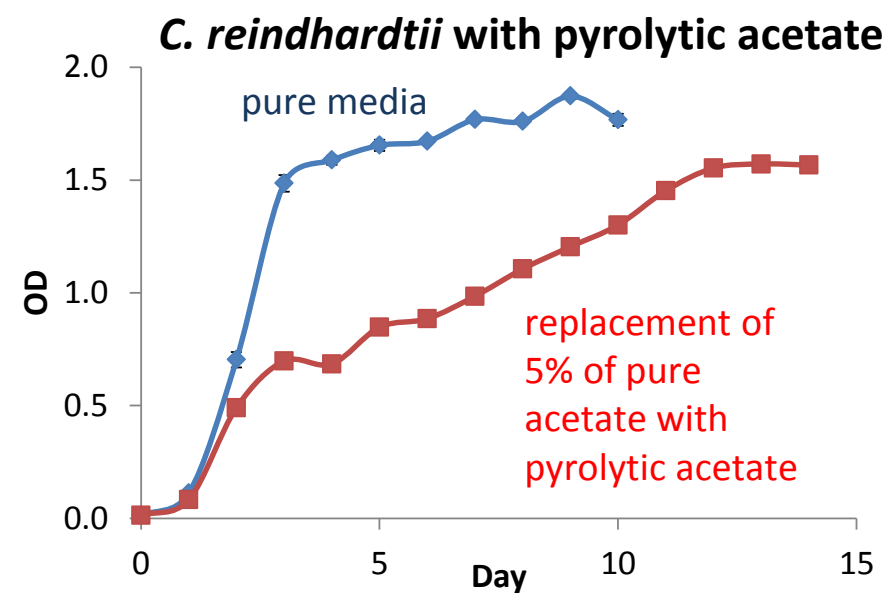
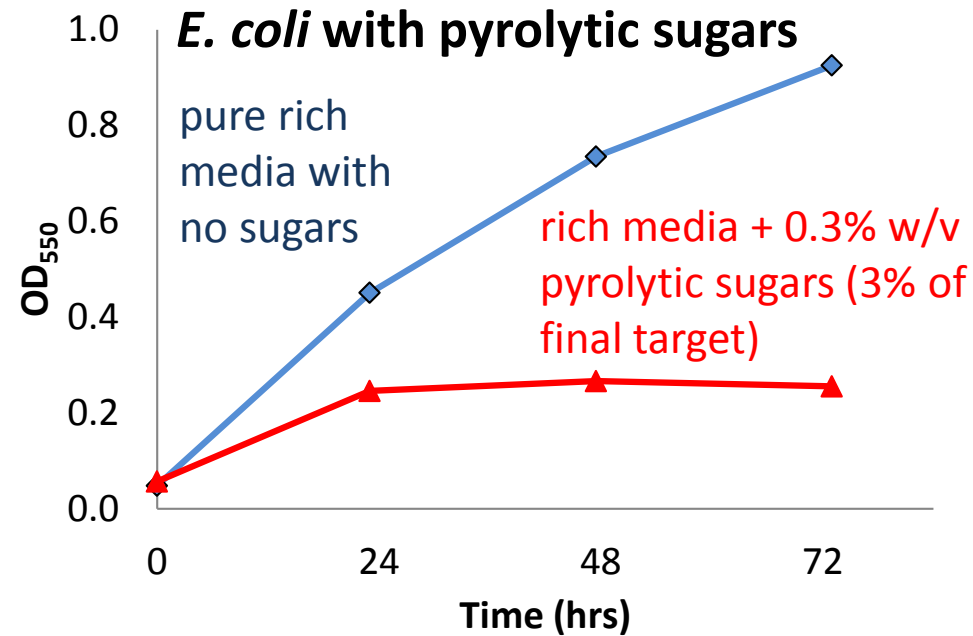


Ethanol production



LB + pure sugars, 37C, pH 6.5

Other Challenges for Hybrid Processing: Toxicity



The presence of trace inhibitory compounds (phenols, acids, others?) is harmful to the microorganisms. This limits how much pyrolytic substrate we can feed to them, which limits productivity. Our strategy is to:

- (1) Improve biocatalyst robustness
- (2) Decrease the toxicity of the pyrolytic substrates through chemical treatments that do not decrease the process economics
- (3) Identify the harmful compounds (and their source) in order to appropriately tailor biomass selection and processing

Improving Biocatalyst Robustness

biomass-derived
("dirty") sugars



biorenewable fuel
or chemical

furfural
5-HMF
acetate
methoxyphenols

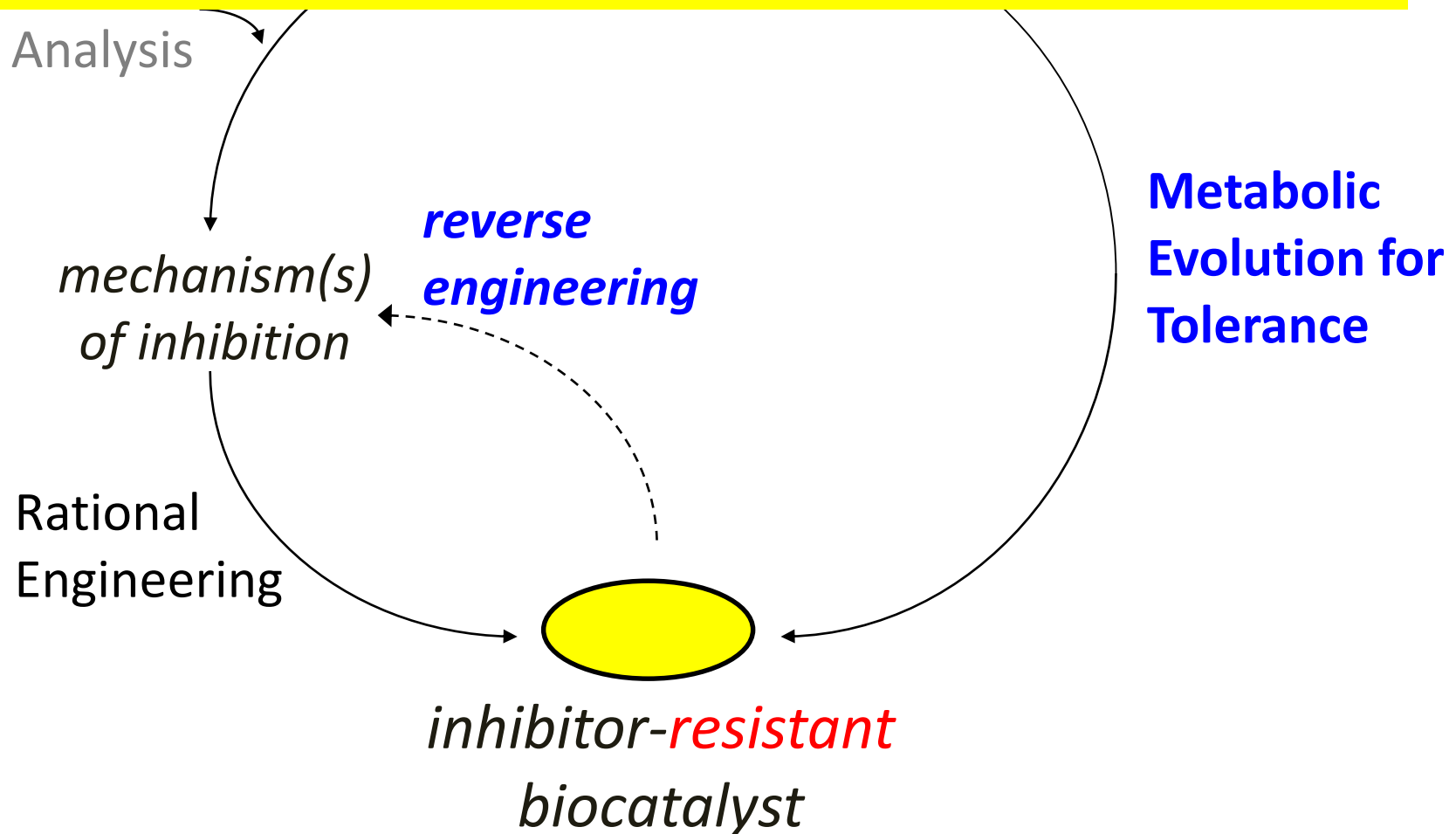
Both ends of biocatalyst
metabolism are affected by
inhibitory compounds

ethanol
butanol
carboxylic acids
styrene
limonene

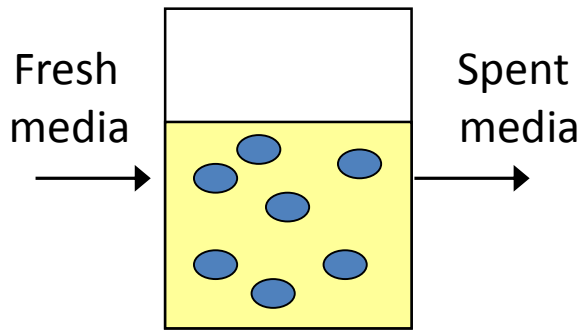
Improving Biocatalyst Robustness

inhibitor-sensitive
biocatalyst

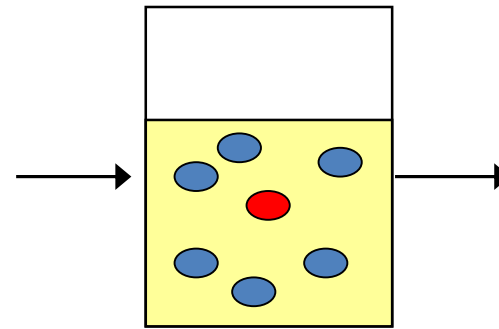
Project outcome: A list of modifications to implement in existing bacterial biocatalysts to enable pyrolytic sugar utilization.



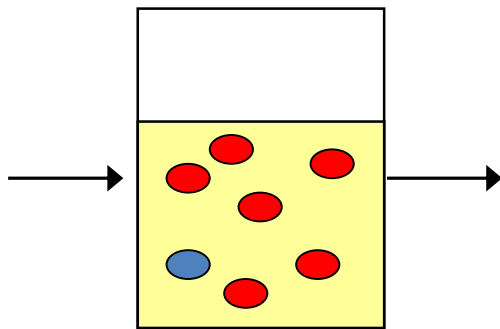
Metabolic Evolution



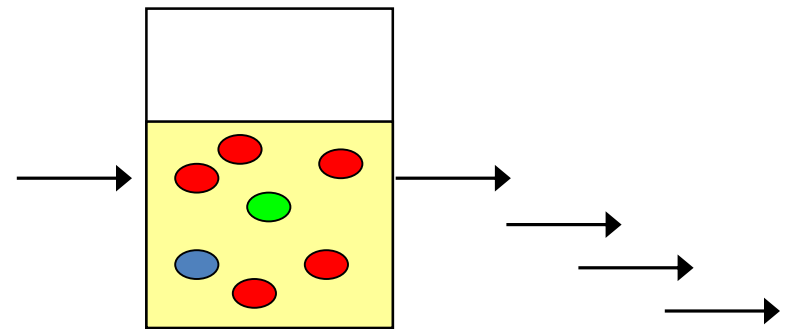
Stressful condition, cells grow poorly



A random mutation confers increased stress tolerance

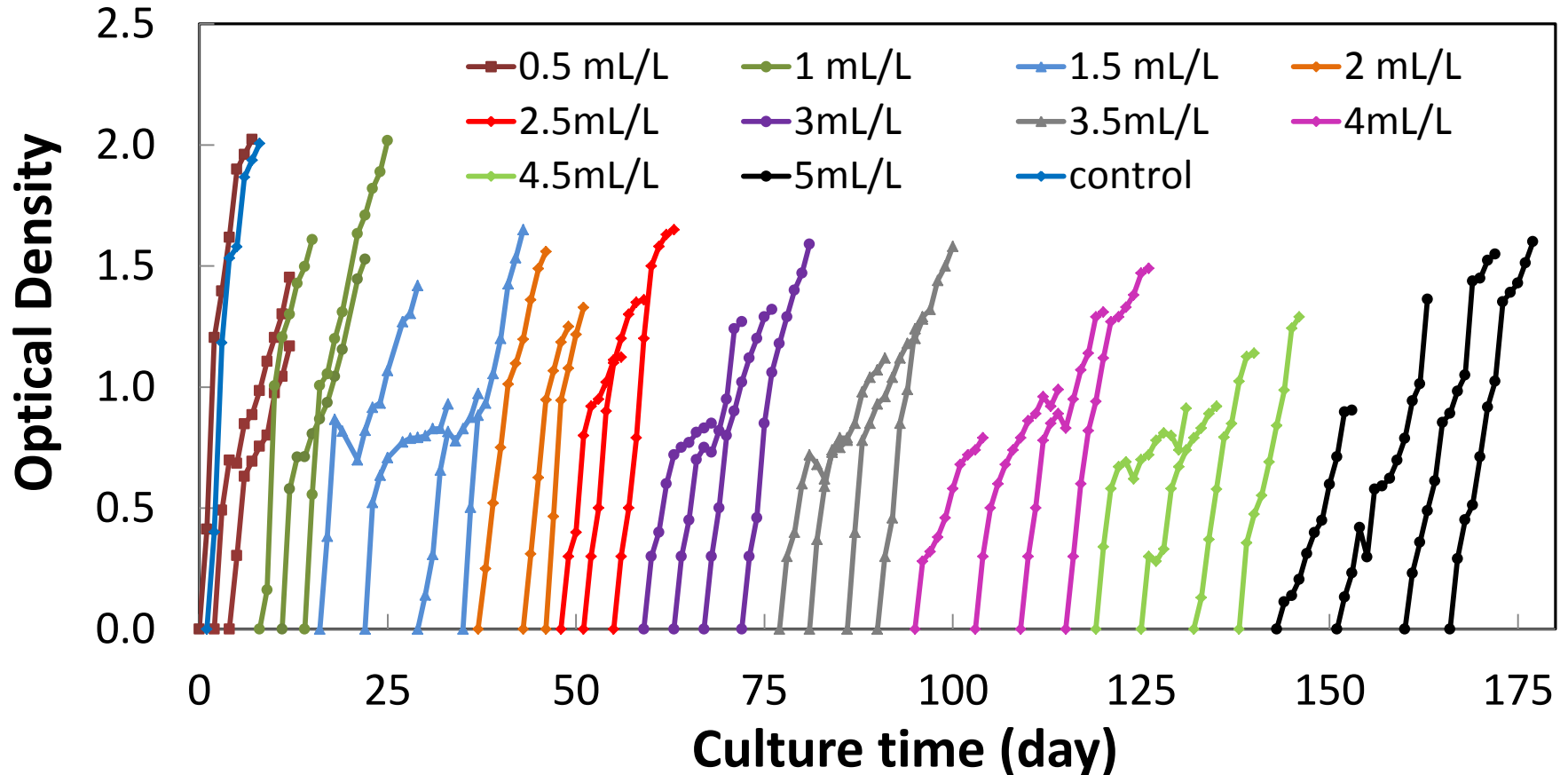


The mutated cell grows faster, its progeny dominate the population



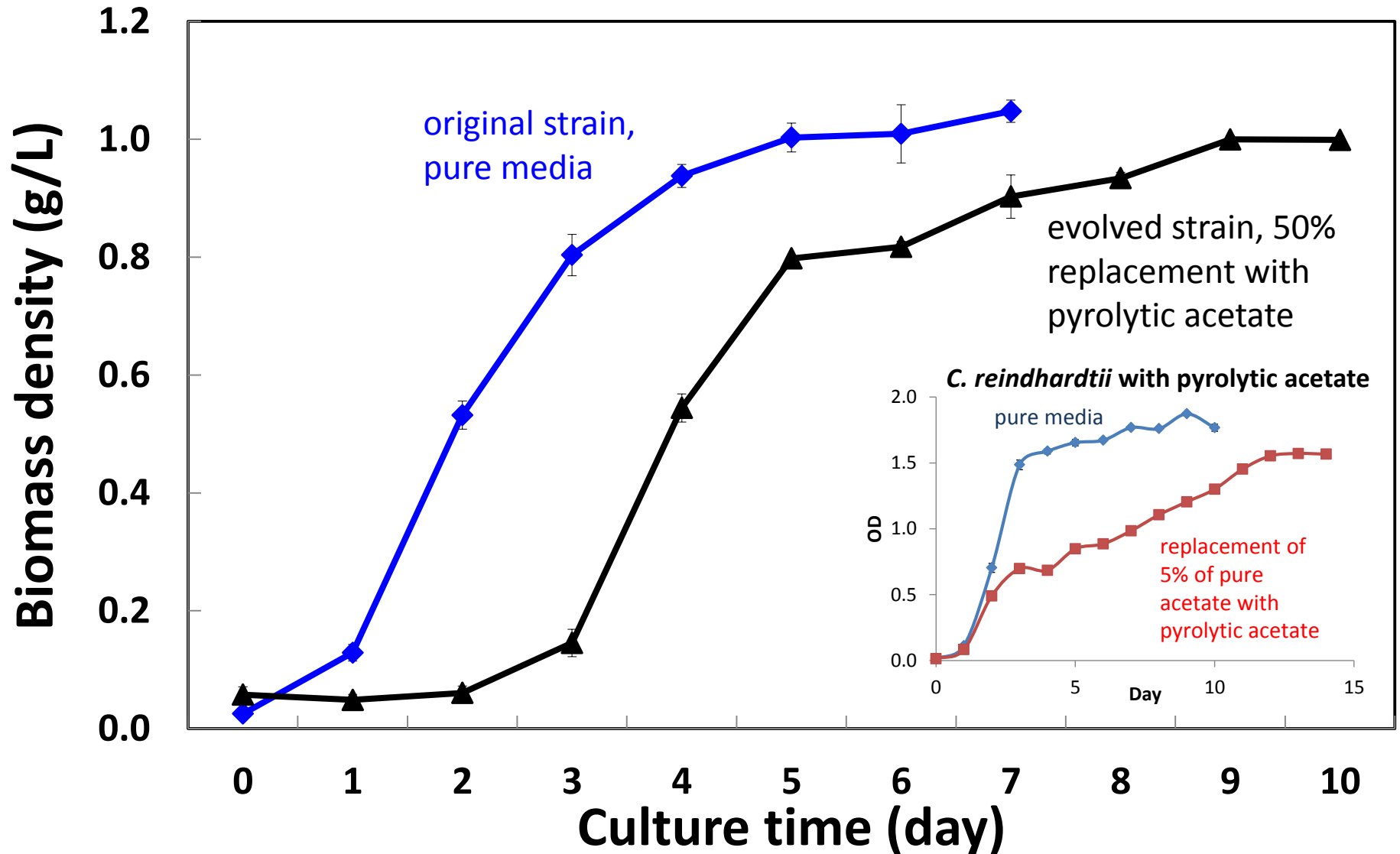
Another random mutation confers even more tolerance

Evolution of *C. reinhardtii* for Utilization of Pyrolytic Acetate

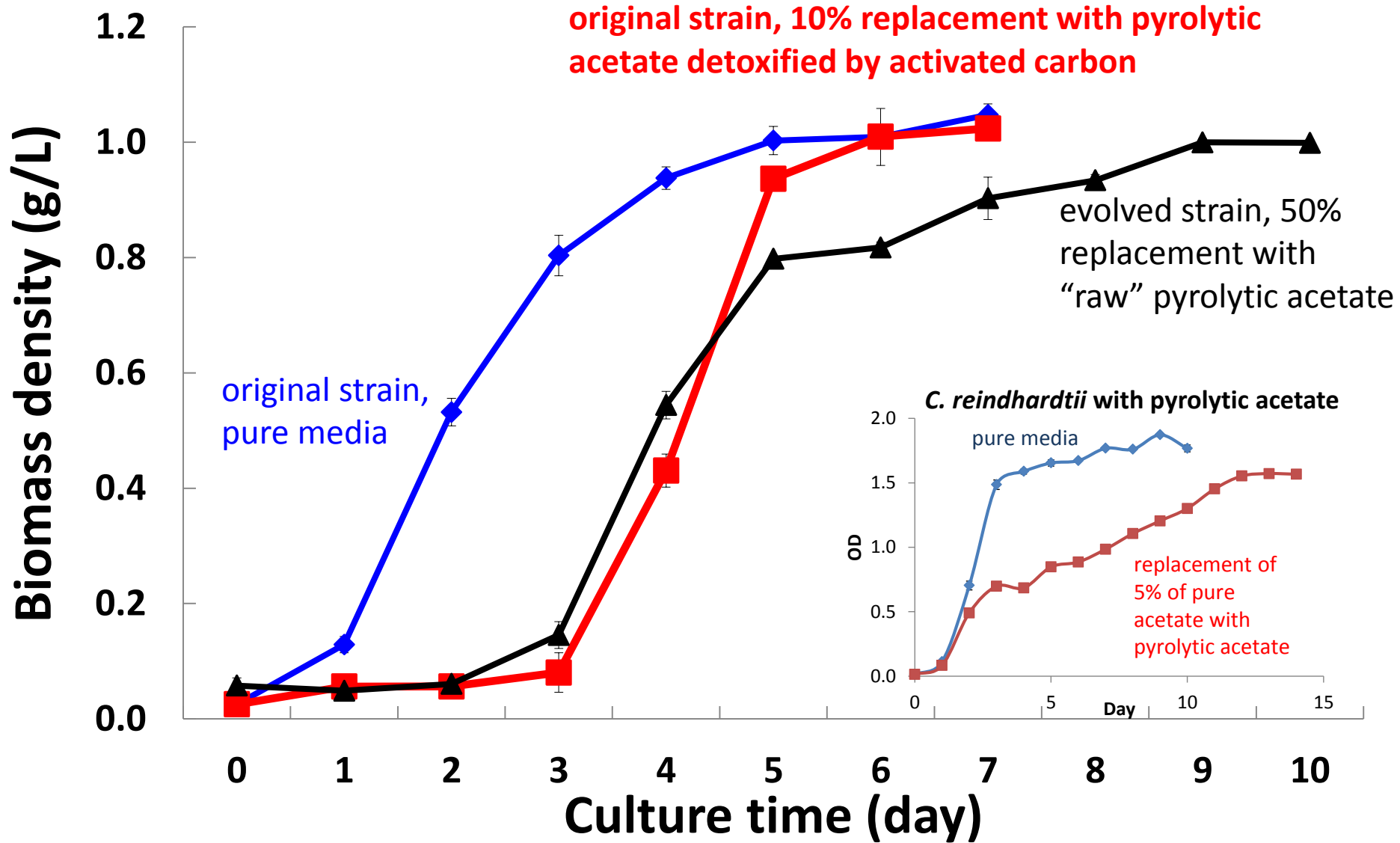


Metabolic evolution was performed over 175 days with gradual increases in pyrolytic acetate content

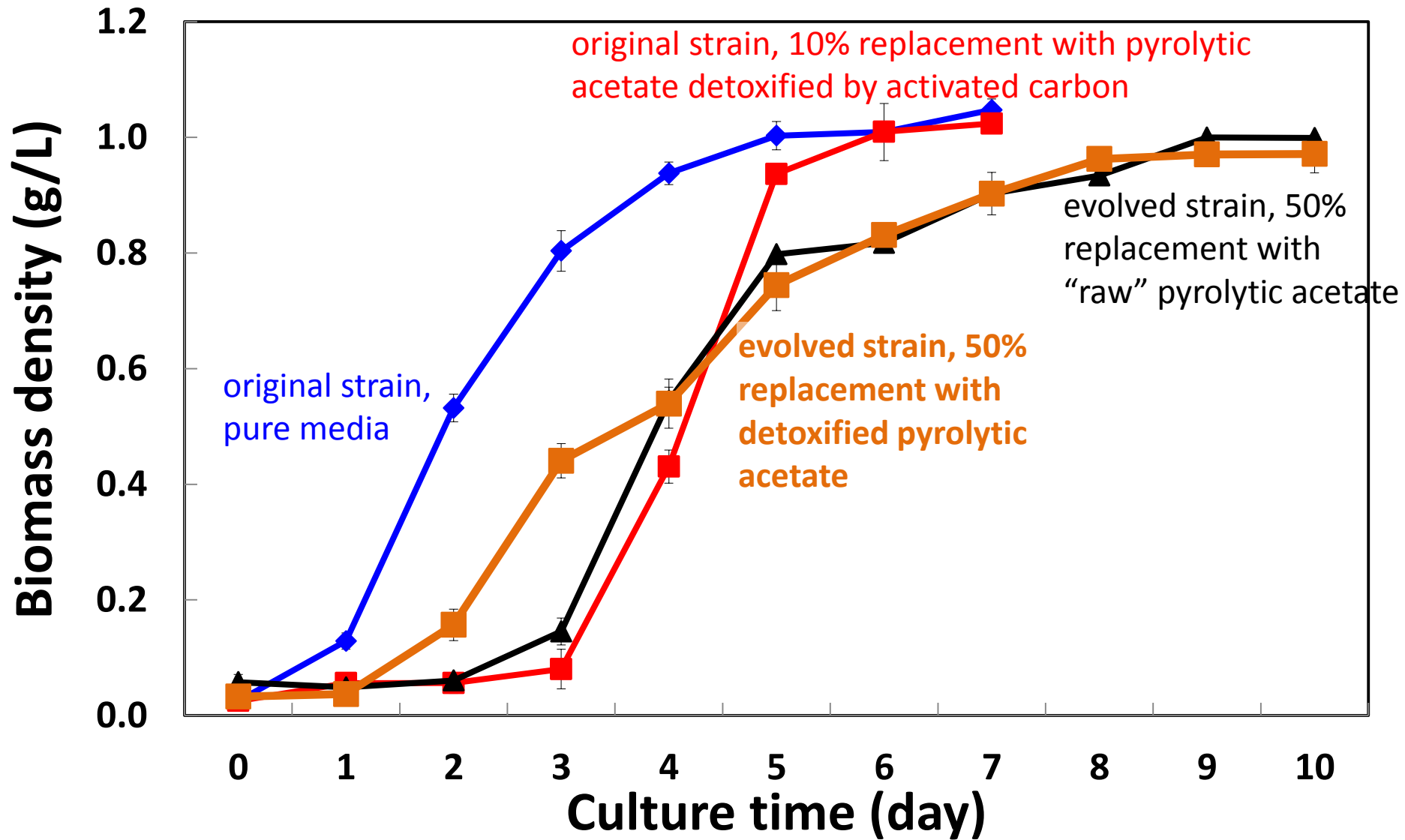
Evolution of *C. reinhardtii* for Utilization of Pyrolytic Acetate



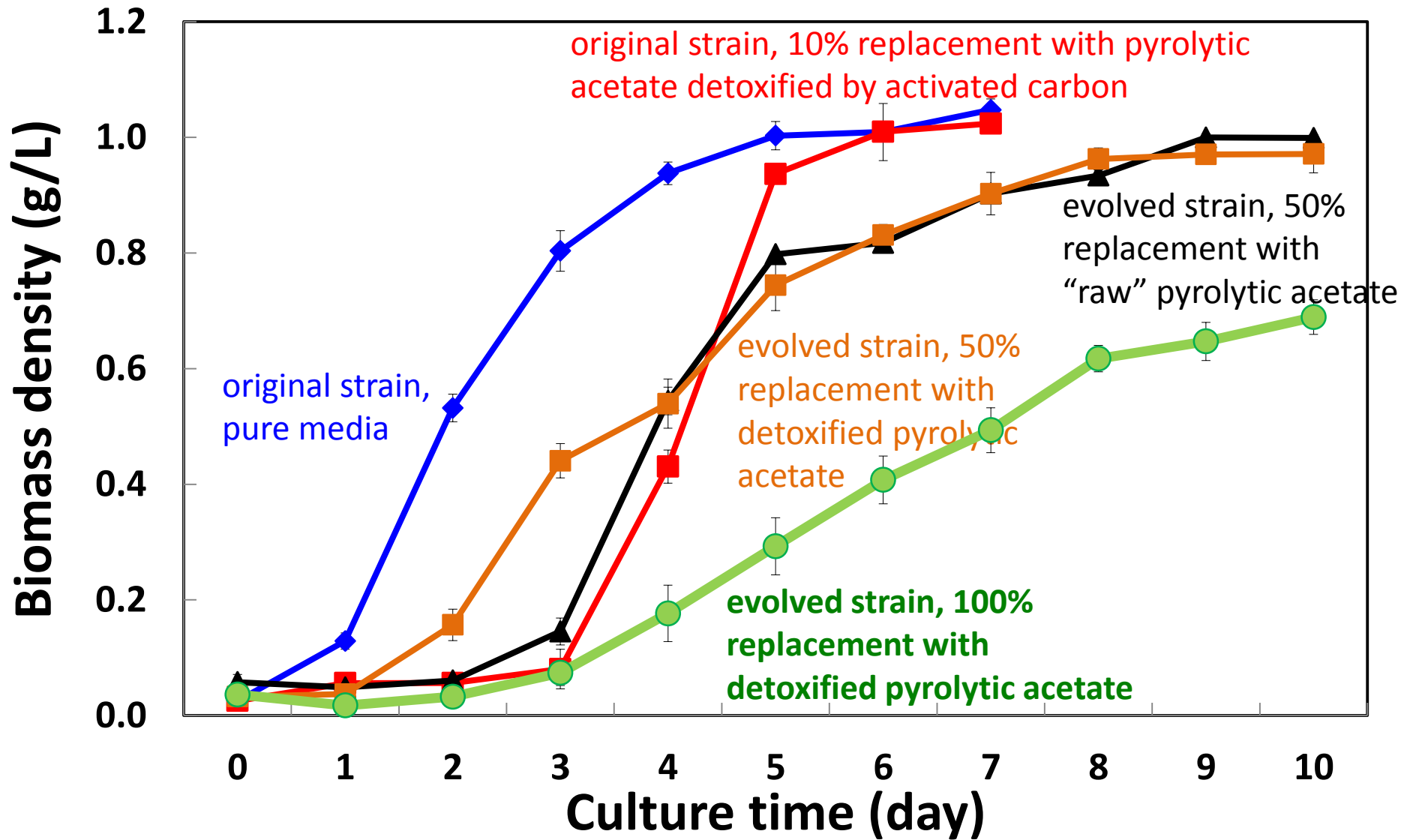
Detoxification of Pyrolytic Acetate



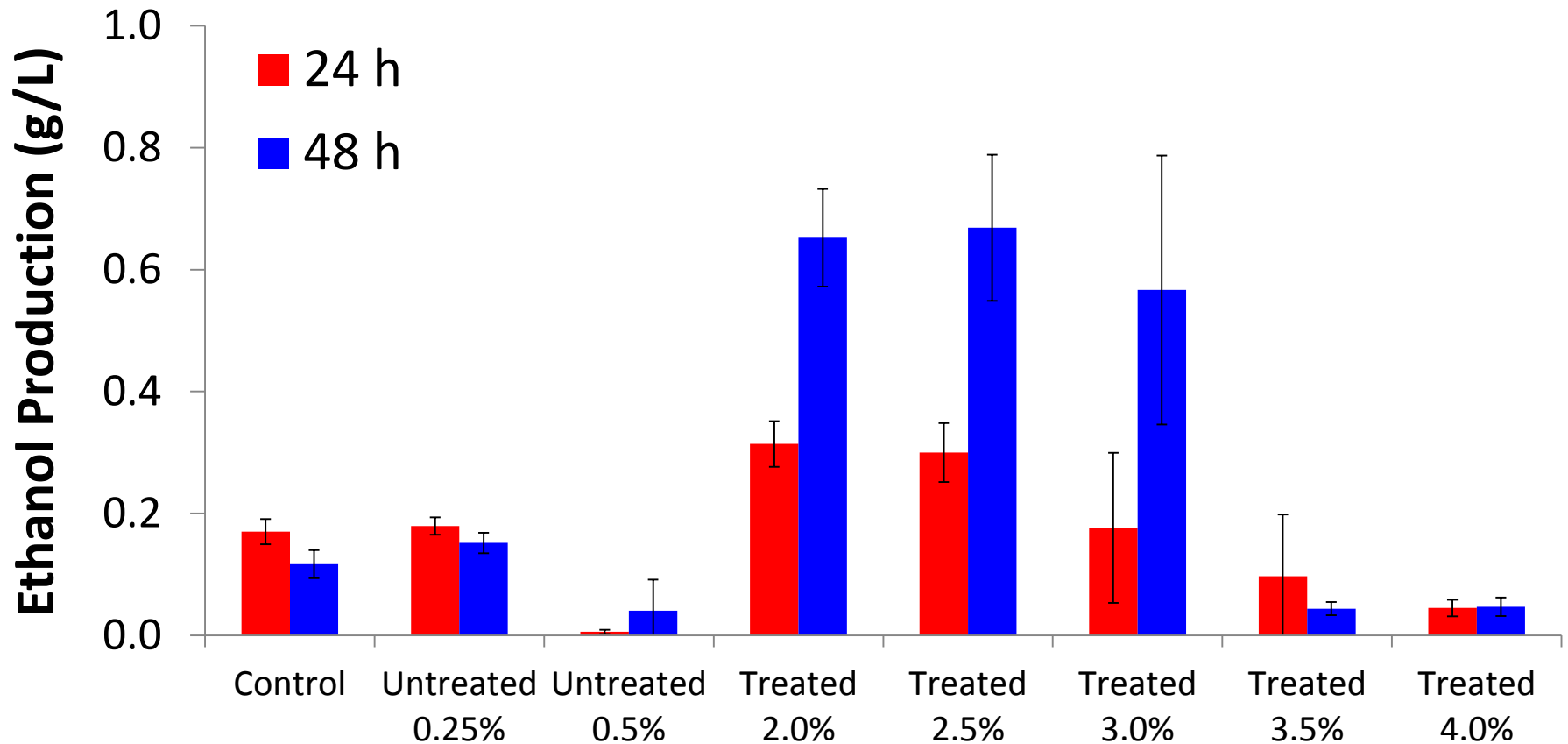
Detoxification of Pyrolytic Acetate



Detoxification of Pyrolytic Acetate

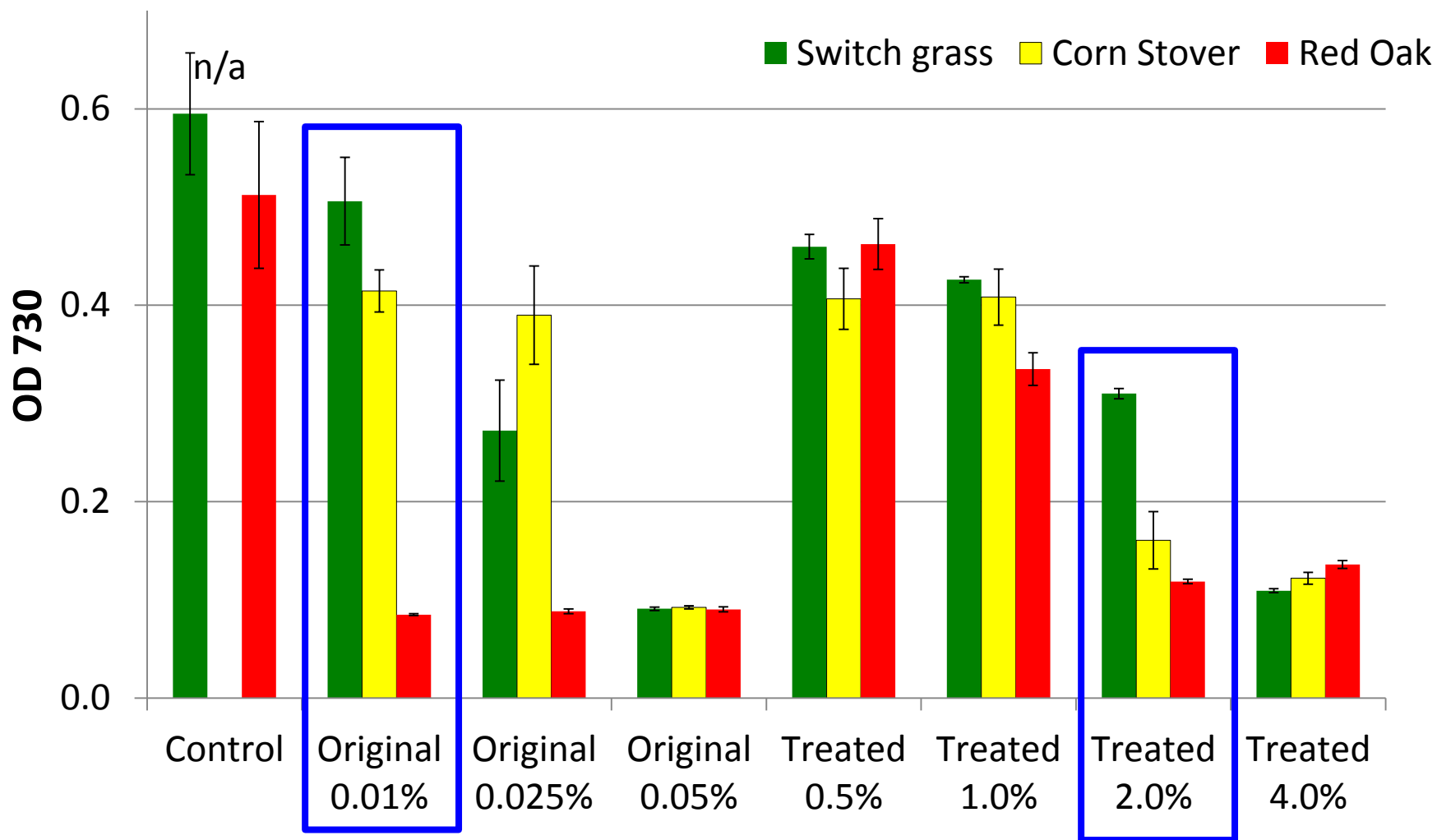


Detoxification of Pyrolytic Sugars by Overliming



“Overliming” treatment with $\text{Ca}(\text{OH})_2$ is effective in improving the fermentability of pyrolytic sugars, though sugar levels and product titers are still far below the ability of this strain using pure sugars. Identification of the removed (and remaining) inhibitors is informative.

Relationship to Biomass Type - Acetate



Biomass identity impacts the fermentability of the pyrolytic sugars (not shown) and acetate. This provides insight into process design and mechanisms.

Treatment = "overliming" with NaOH

Conclusions and Acknowledgements

- Biocatalyst robustness towards pyrolytic substrates can be improved by evolution; reverse engineering of evolved strains is key
- Detoxification, even if not economically viable, can provide insight into the nature of the inhibitory compounds (*data not shown*)
- Variation in biomass composition and pyrolytic product fermentability can guide future efforts

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Collaborators: Zhiyou Wen, Robert C. Brown, D.W. Choi

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