Rhodopseudomonas palustris CGA009 Lignin Catabolism and Bioplastic Production

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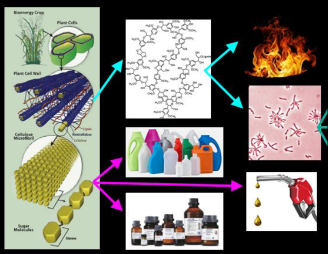
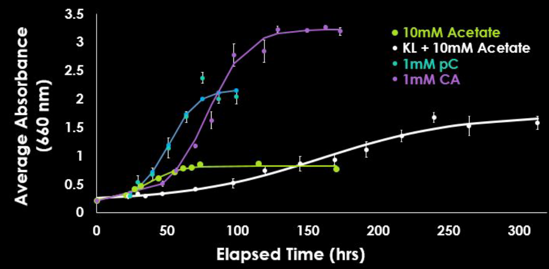
Overview

- Why?



- Growth on Kraft lignin and LBPs



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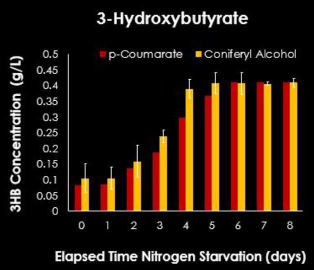
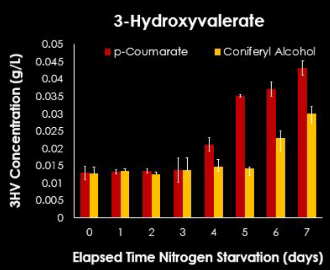
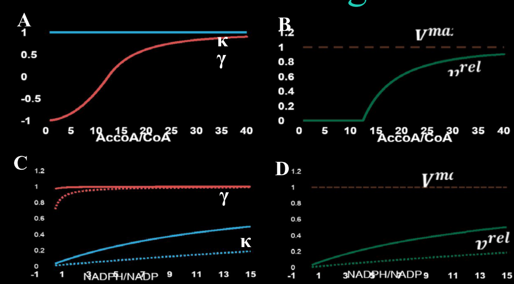
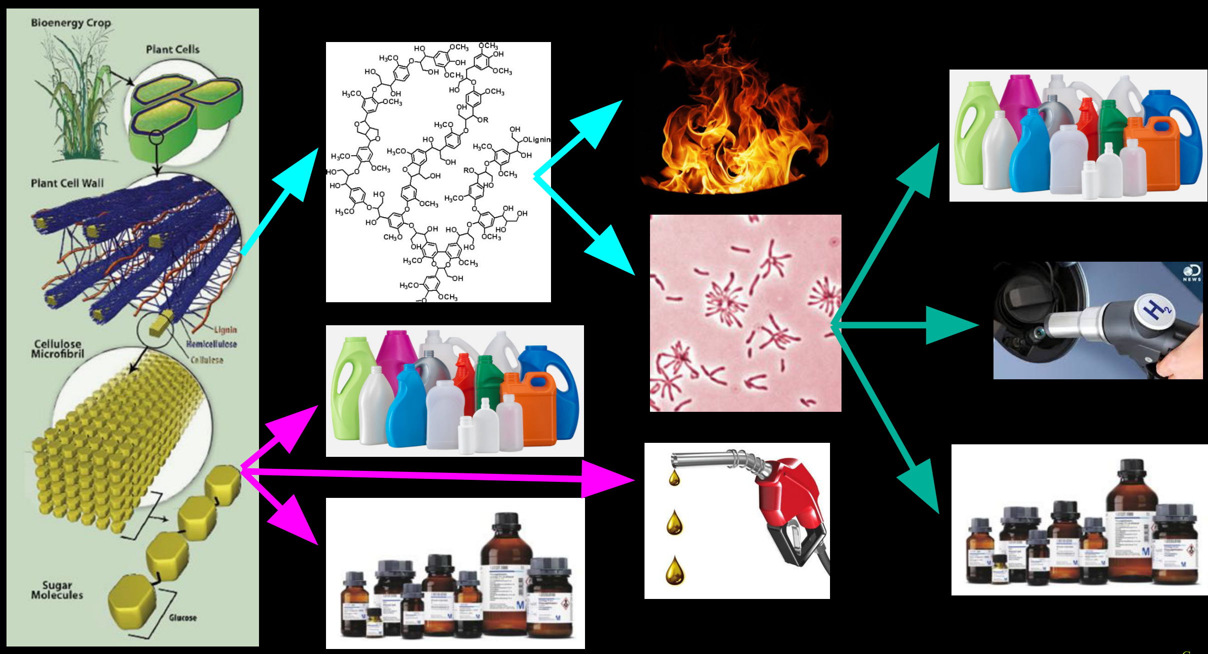




- Metabolic modeling



Why Lignin? And Why *Rhodopseudomonas palustris*?



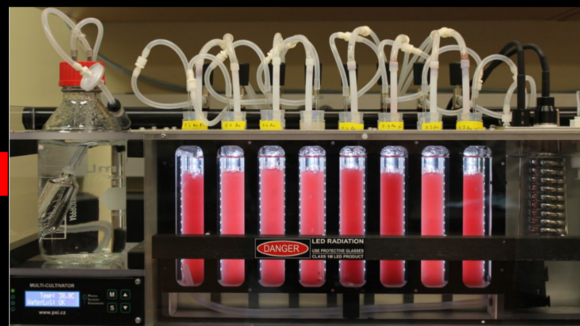
Google images

R. palustris as a Biotechnology Chassis

Robust host

Waste products

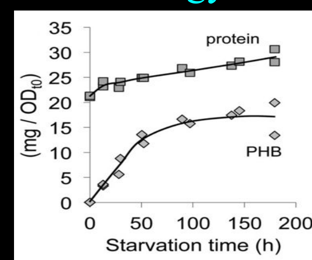
- Carbon dioxide
- Aromatic compounds
- Excess nutrients



Value-added products

- Hydrogen
- Bioplastics
- n-Butanol

Biotechnology chassis



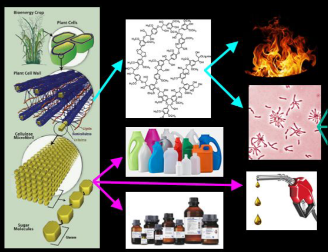
McKinlay et al., J. Biol. Chem., 2014

Systems and synthetic biology tools needed

- Genome-scale modeling
  Reconstruction of metabolic network
  Simulating metabolism
- Synthetic biology tool and chassis development

Overview

- Why?



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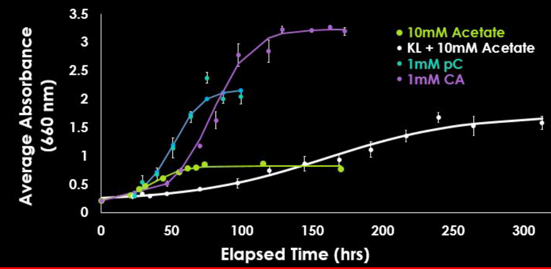

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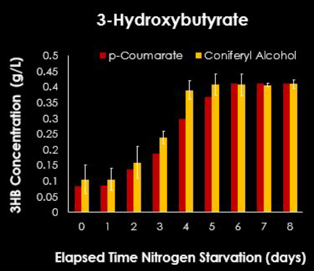

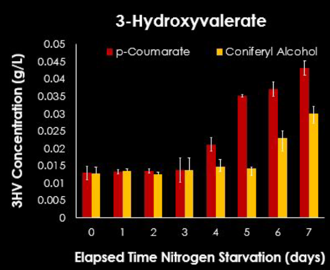

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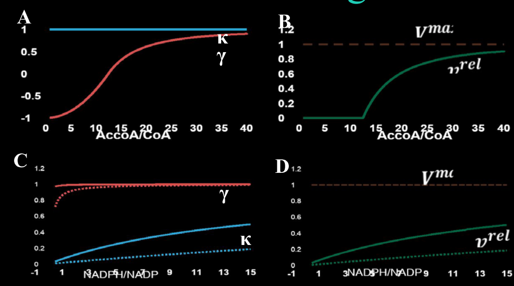
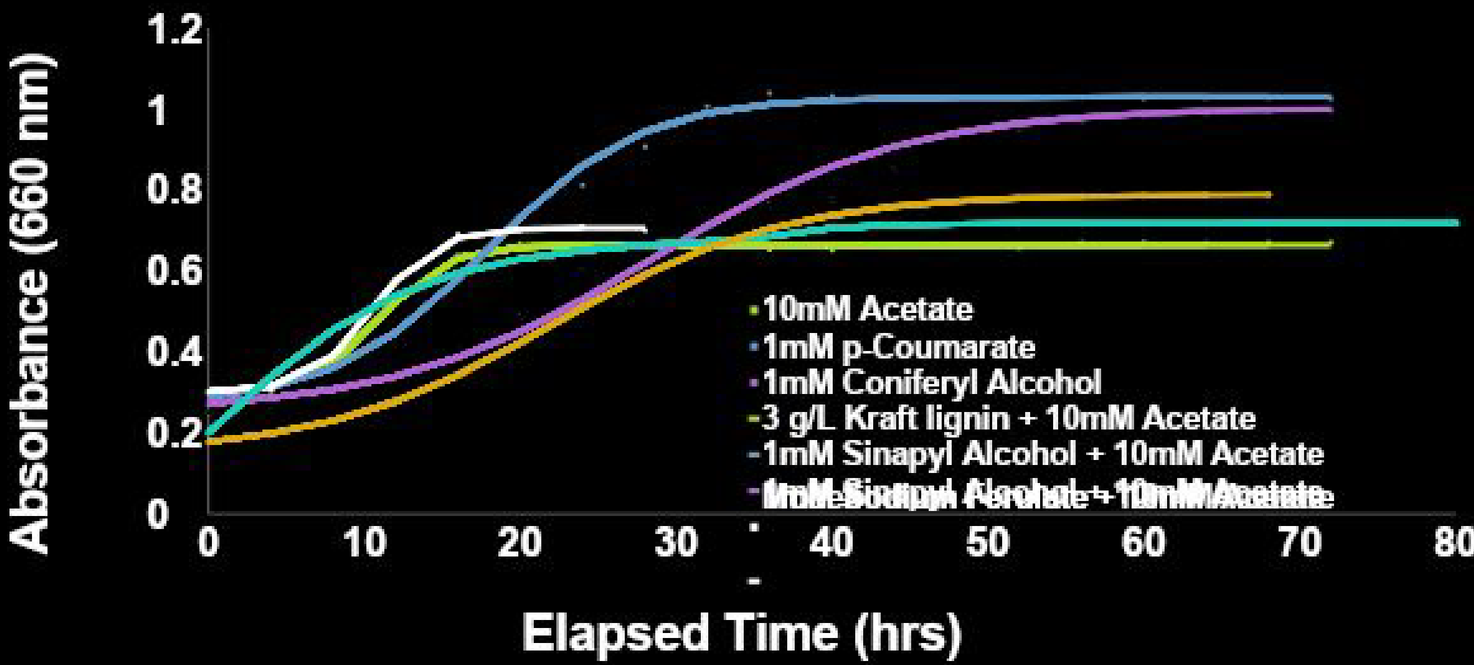
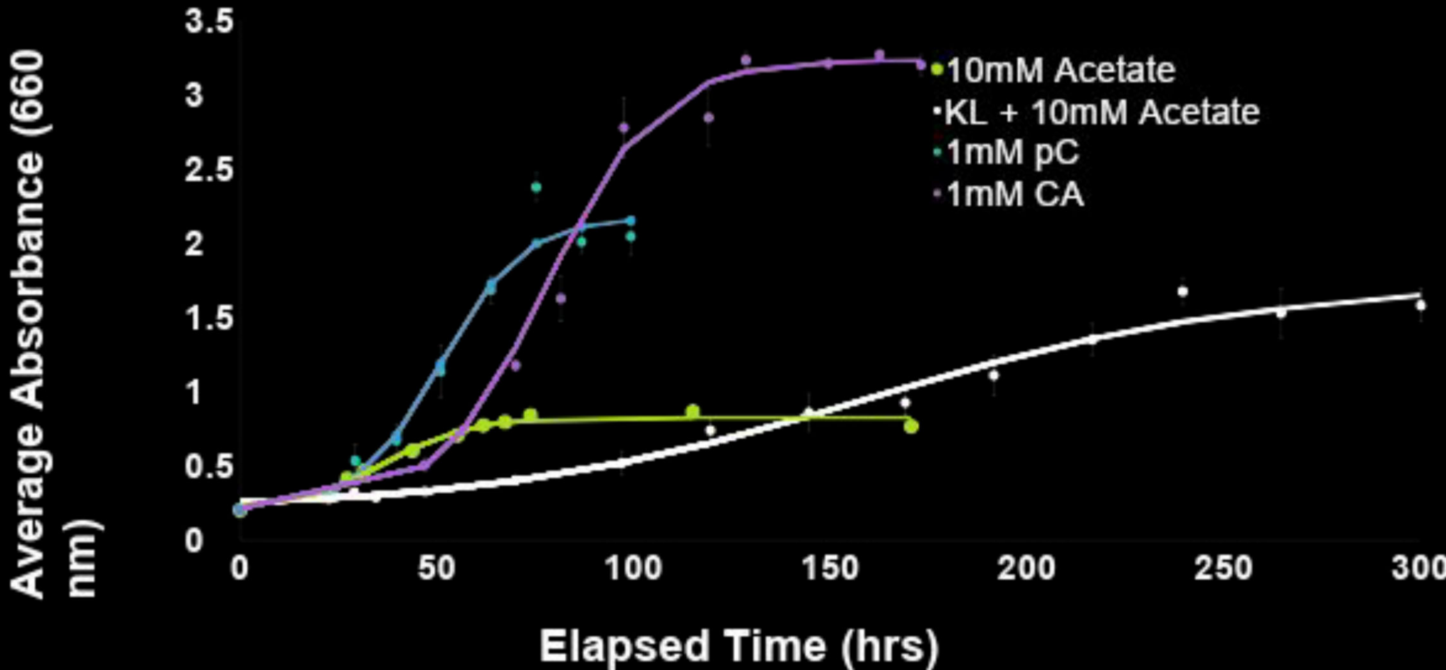


Aerobic Growth on Lignin Breakdown Products (LBPs)



First time *R. palustris* grown aerobically on many LBPs

Anaerobic Growth on LBPs



Kraft lignin doubled the growth compared to acetate alone

Growth on LBPs

Carbon Source	Oxygen	Max Absorbance	Growth Rate (hrs^{-1})	Lag Phase (hrs)	Reported Previously
p-Coumarate	+	1.03	0.020	7.9	No
	-	2.17	0.047	30.0	Yes[1]
Coniferyl alcohol	+	1.00	0.013	12.0	No
	-	3.22	0.054	50.0	No
Kraft lignin (3 g/L) + acetate	+	0.71	0.051	6.5	No
	-	1.50	0.010	80.0	No
Sinapyl alcohol + acetate (1st phase*)	+	0.68	0.041	1.8	No
Sinapyl alcohol + acetate (2nd phase*)	+	0.77	0.008	12.0	No
Sinapyl alcohol + acetate	-	1.09	0.023	12.0	No
p-Coumaryl alcohol + acetate	+	0.71	0.047	2.0	No
	-	1.05	0.019	12.0	No
Sodium ferulate + acetate	+	0.79	0.022	7.4	Yes[1-2]
	-	1.14	0.032	17.7	Yes[1,2,3-4,5,6-7]
Acetate	+	0.62	0.028	3.8	Yes[5]
	-	0.08	0.014	14.2	Yes[6-7]

1. Harwood, C. S.; Gibson, J., Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium Rhodopseudomonas palustris. Appl Environ Microbiol 1988, 54 (3), 712-7.
2. Puskas, L. G.; Inui, M.; Kele, Z.; Yukawa, H., Cloning of genes participating in aerobic biodegradation of p-coumarate from Rhodopseudomonas palustris. DNA Seq 2000, 11 (1-2), 9-20.
3. Elder, D. J.; Morgan, P.; Kelly, D. J., Anaerobic degradation of trans-cinnamate and omega-phenylalkane carboxylic acids by the photosynthetic bacterium Rhodopseudomonas palustris: evidence for a beta-oxidation mechanism. Arch Microbiol 1992, 157 (2)
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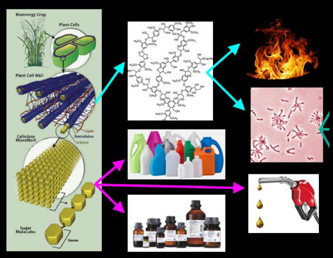
5. Butow, B.; Dan, T. B., Effects of growth conditions of acetate utilization byRhodopseudomonas palustris isolated from a freshwater lake. Microb Ecol 1991, 22 (1), 317-28.
6. McKinlay, J. B.; Harwood, C. S., Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proc Natl Acad Sci U S A 2010, 107 (26), 11669-75.
7. McKinlay, J. B.; Harwood, C. S., Calvin cycle flux, pathway constraints, and substrate oxidation state together determine the H2 biofuel yield in photoheterotrophic bacteria. MBio 2011, 2 (2).

Overview

- Why?



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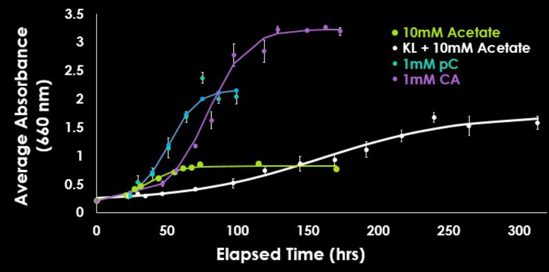

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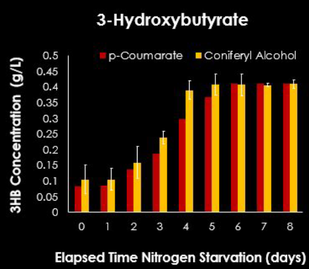

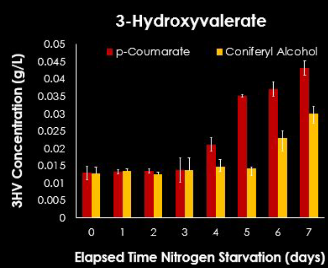

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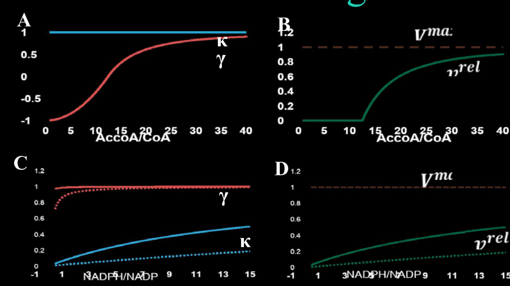
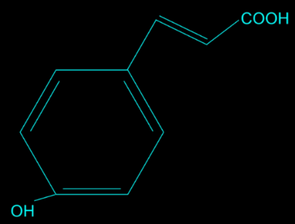
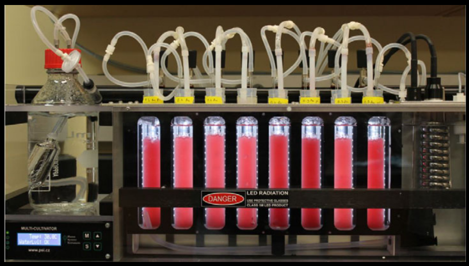


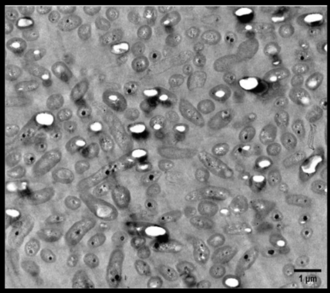
Anaerobic PHBV Production



LBPs



Nitrogen starvation

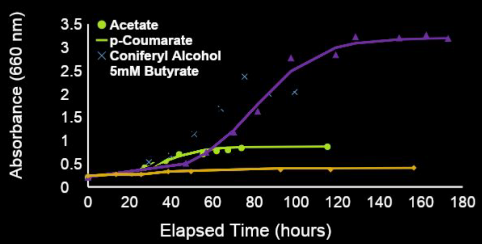


PHBV granules

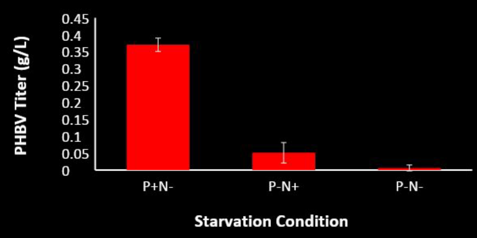
Extraction

Quantification

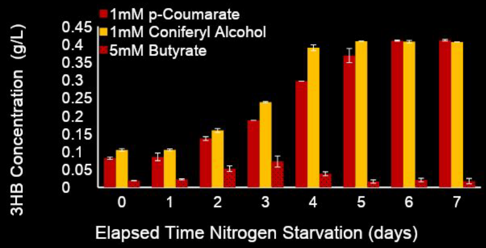
LBPs yield more bioplastic….but why?



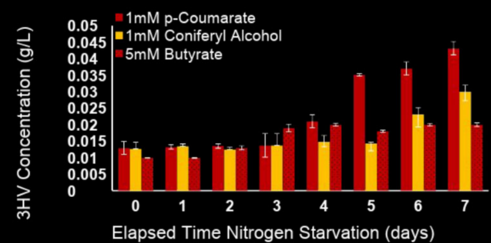
R. palustris grows to higher biomass on LBPS

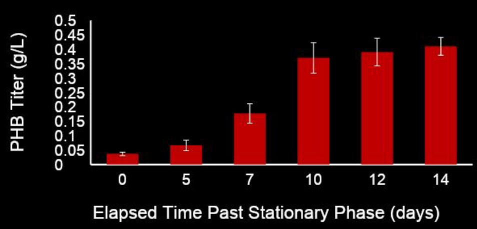


Best starvation condition is nitrogen limitation

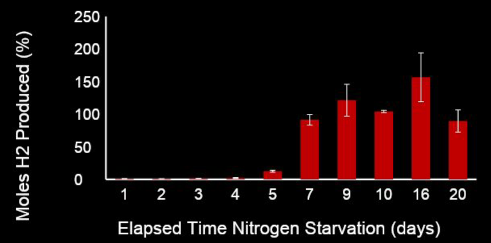


LBPs yield more PHB than butyrate. Why?



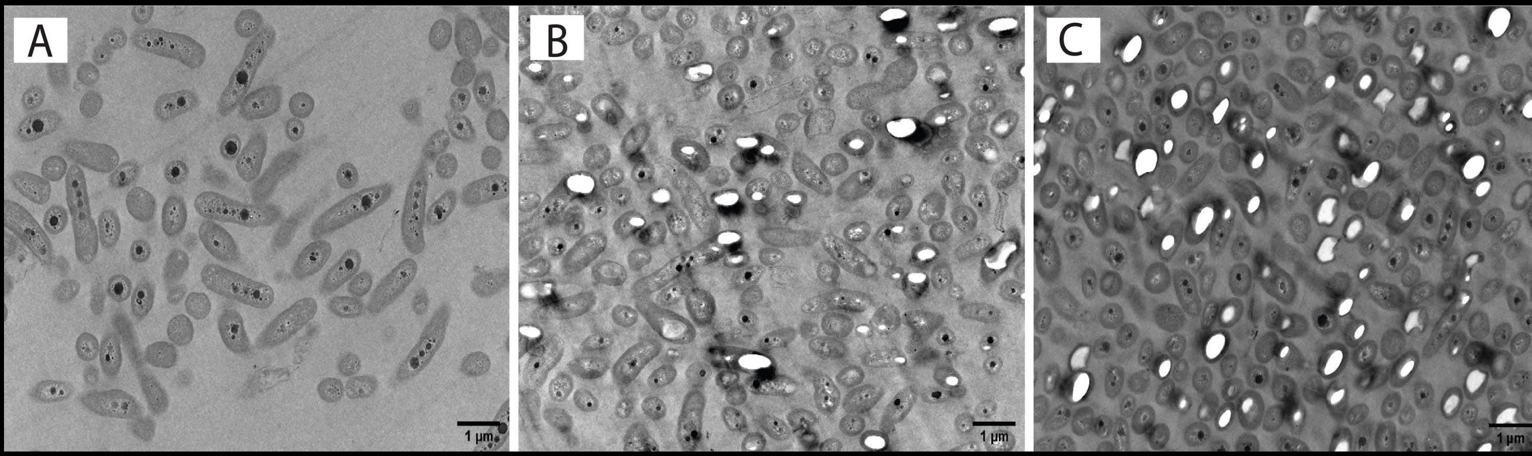


R. palustris can produce PHB without having to nitrogen starve



H_2 production increases when PHB peaks

Transmission Electron Microscopy



(A) 10mM acetate with 0.06 g/L PHBV

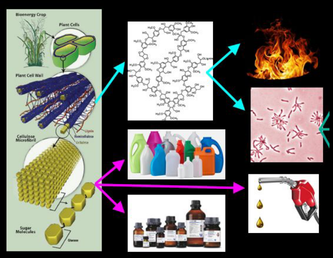
(B) 1mM *p*-coumarate with 0.41 g/L PHBV

(C) 1mM coniferyl alcohol with 0.41 g/L PHBV

Cytoplasmic space may be a major limiting factor for PHB production

Overview

- Why?



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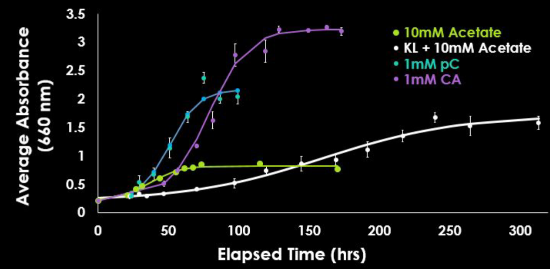

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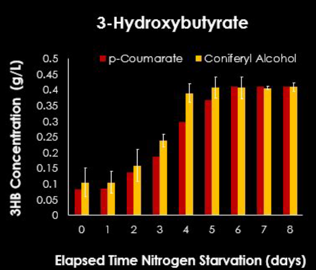

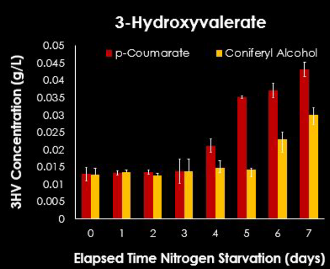

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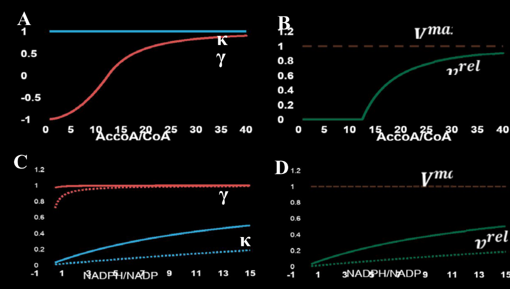
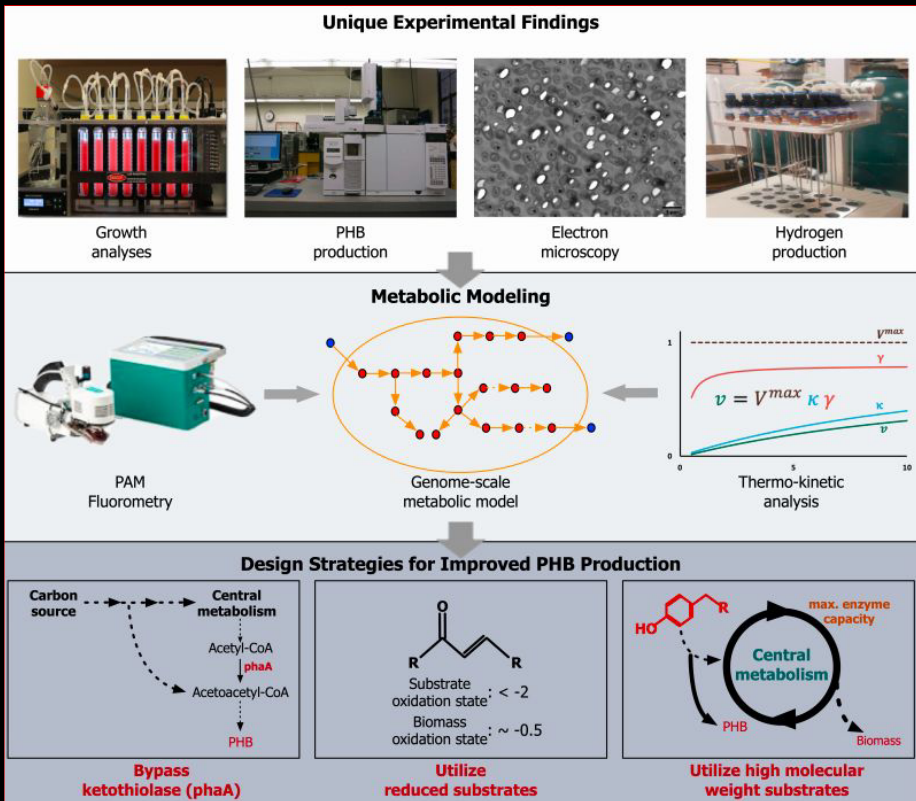


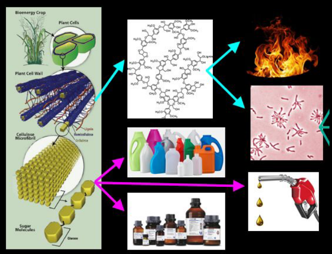
Metabolic Modeling



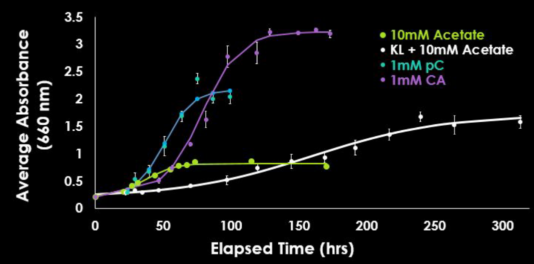
Three design strategies for PHB overproduction that can be expanded to all PHB-producing bacteria

Summary

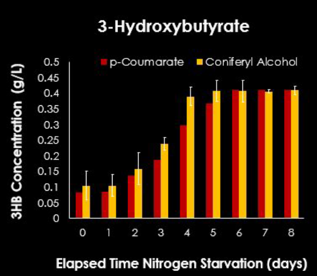
- **Why?**

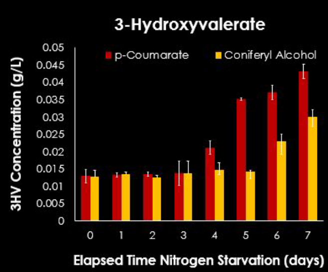


- **1st time:** growth on Kraft lignin and most LBPs

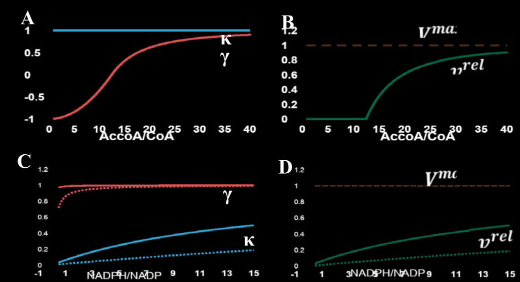


- **LBPs produce more PHB**





- **Design strategies** for overproduction



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Funding Sources

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Thank You!

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