

Overcoming Formaldehyde Toxicity is Critical to Using Lignin-Derived Aromatics

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U.S. DEPARTMENT OF
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From Ancient Life to Fossil Fuels

Over 300 million years ago



Pressure

Fossil fuels



Coal



Oil



Natural gas

Some uses of fossil fuels



Cooking



Time



Electricity



Transportation

Depletion of Fossil Fuels Calls for the Need of Renewable Energy

The challenge(s)



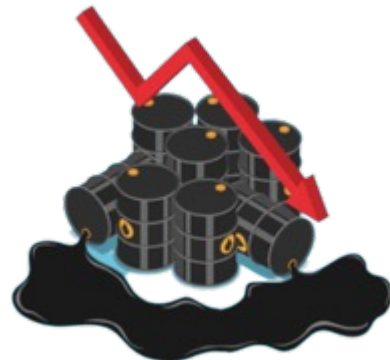
Expensive



Scarcity from geographic allocation



Emission of green house gases



Depletion & non-renewable

Proposed solution: Environmentally sustainable & renewable sources of energy



Solar



Wind

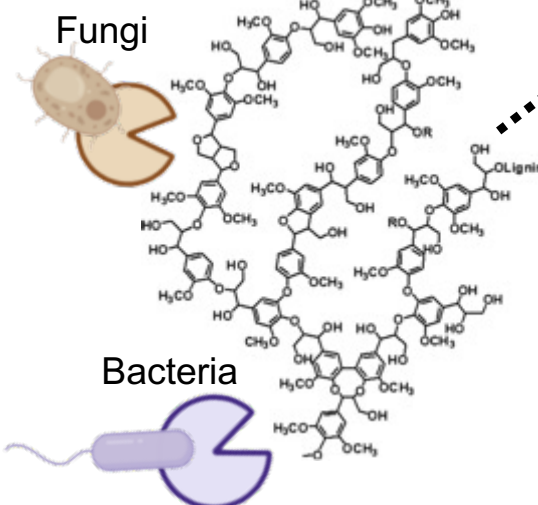
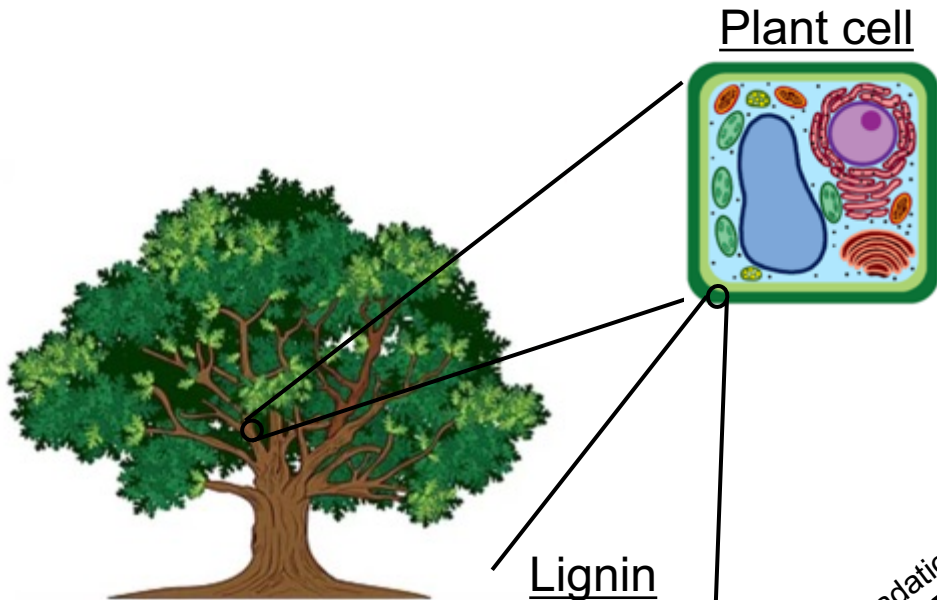


Hydro



Biofuel

Lignin is a Grossly Underutilized Source of Renewable Energy

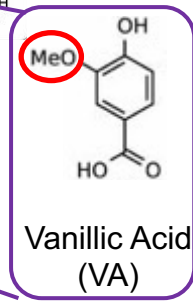


Complex mixture of aromatics

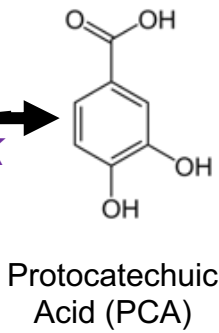
	H-lignin	G-lignin	S-lignin	C-lignin	
Phenols	Phenol	Guaiacol	Syringol	Catechol	Monolignols
Benzyl alcohols	4-Hydroxybenzyl alcohol	Vanillyl alcohol	Syringyl alcohol	3,4-Dihydroxybenzyl alcohol	
Benzaldehydes	p-Hydroxybenzaldehyde	Vanillin	Syringaldehyde	Protocatechuic aldehyde	
Benzoates	p-Hydroxybenzoate	Vanillate	Syringate	Protocatechuate	
Benzoic acids	p-Hydroxybenzoic acid	Vanillic acid	Syringic acid	Protocatechuic acid	Cinnamic acids
					Cinnamates
					Monolignolaldehydes
					Monolignolalcohols



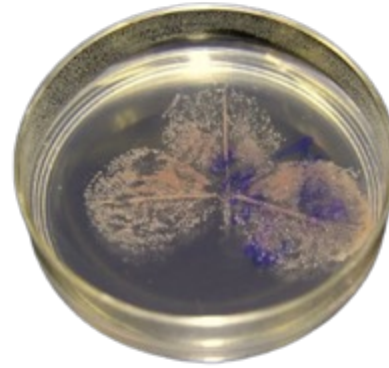
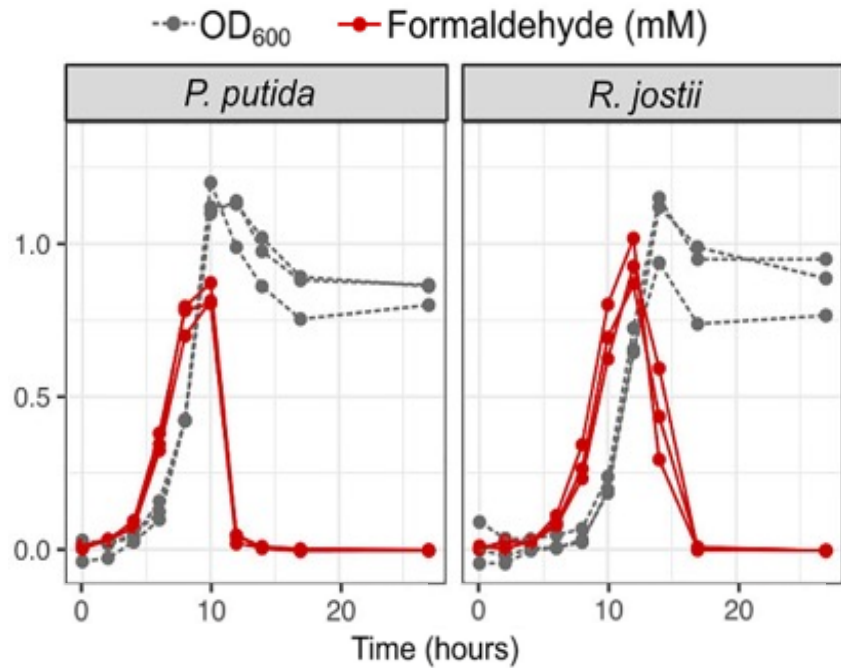
Formaldehyde
HCHO



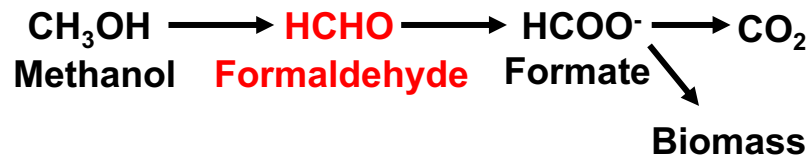
vanABK



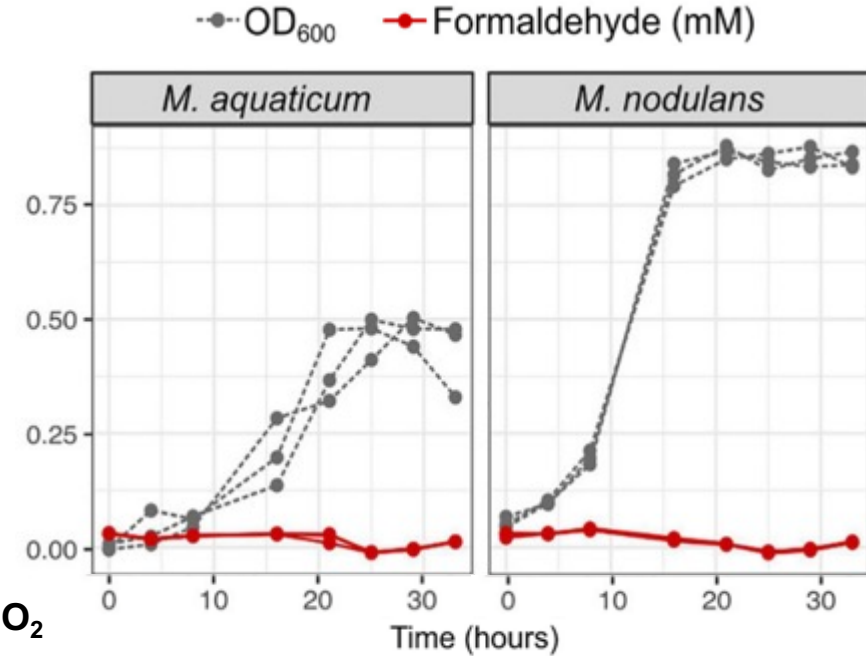
Methylobacterium Grows on VA Without Accumulating Formaldehyde



Methylobacterium from leaf print



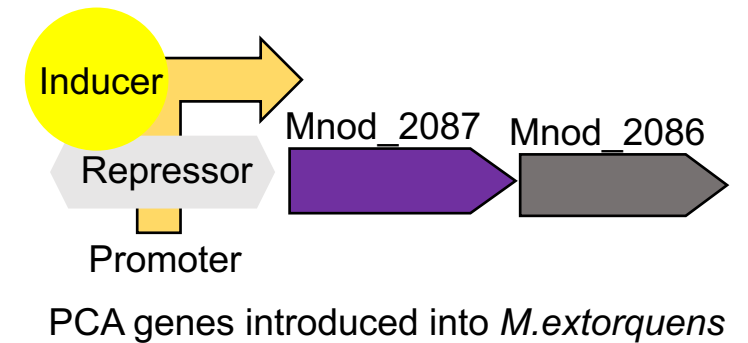
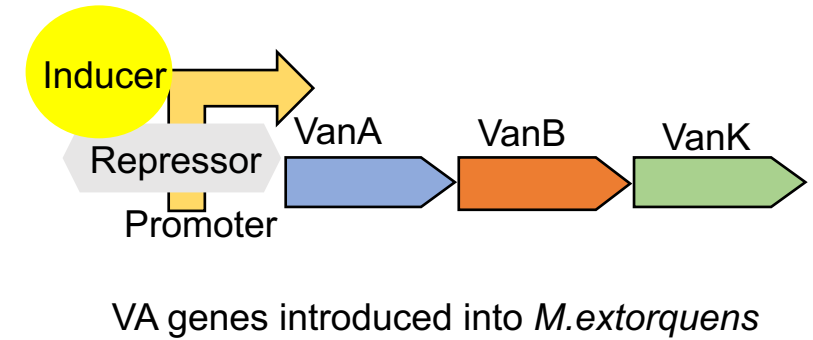
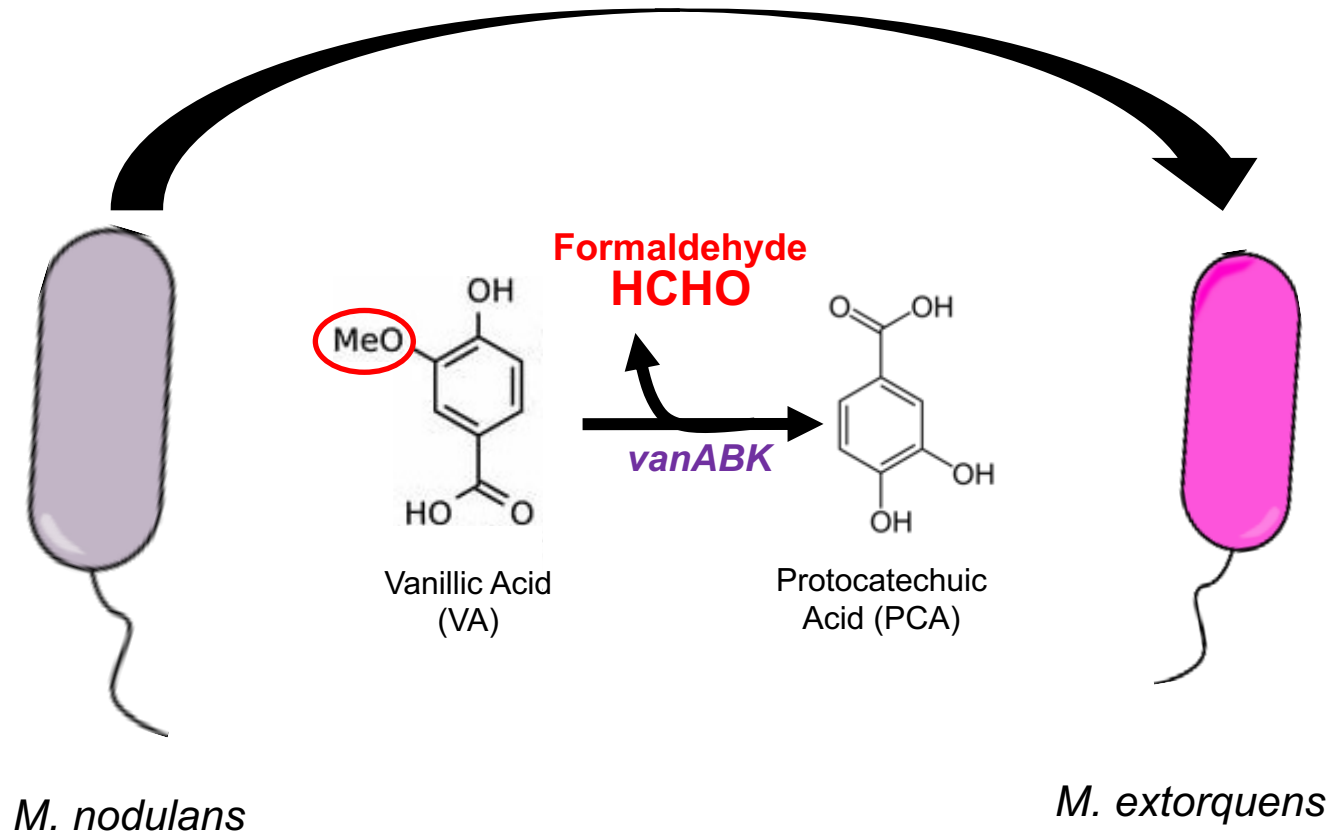
Methylotrophy



Methylobacterium grow on VA without formaldehyde release

Model lignin utilizers excrete formaldehyde into the media when grown on VA

Genes for Growth on VA/PCA were Introduced into *M. extorquens*

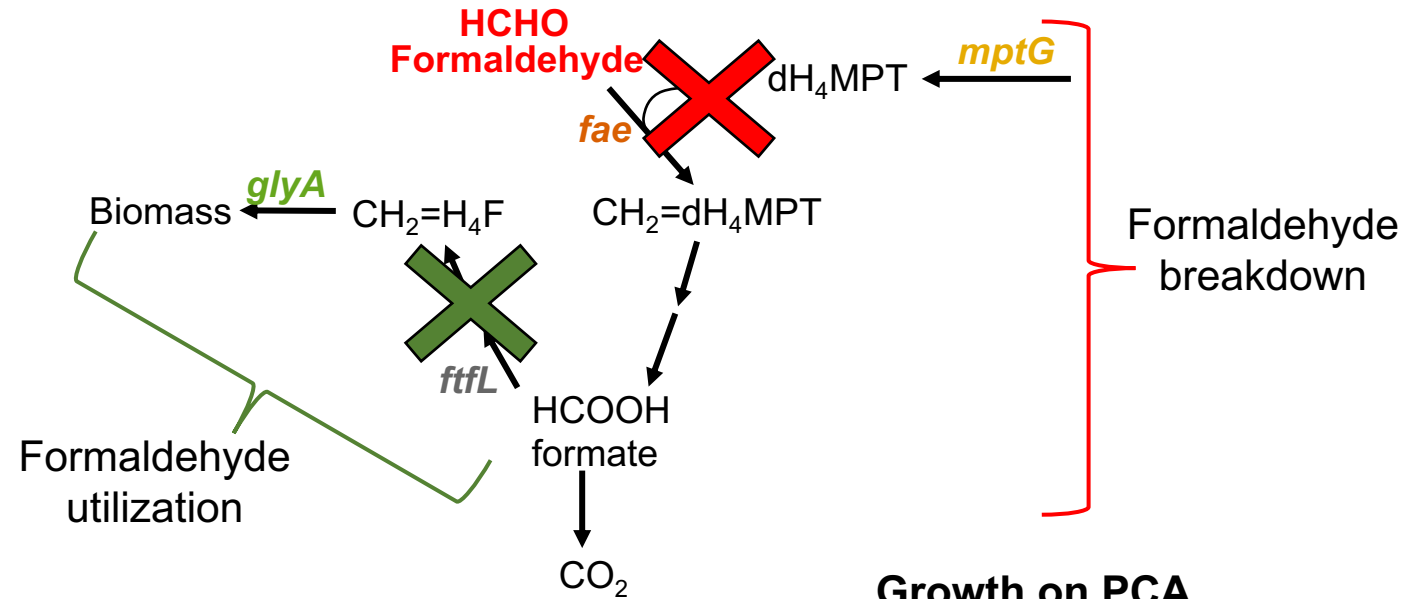
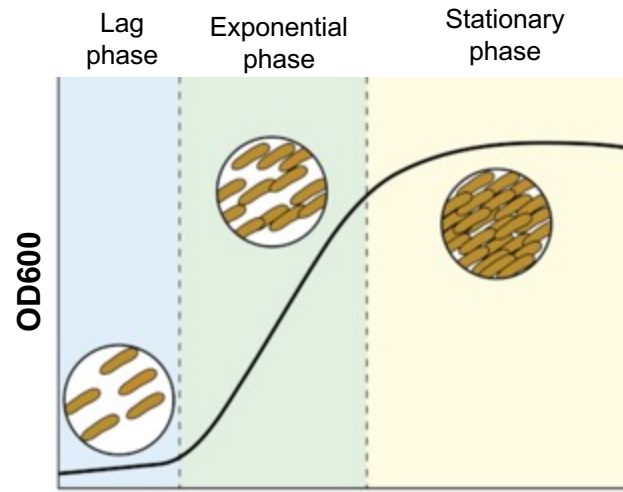


Unlocking Biofuel Potential: From Fossils to Lignin

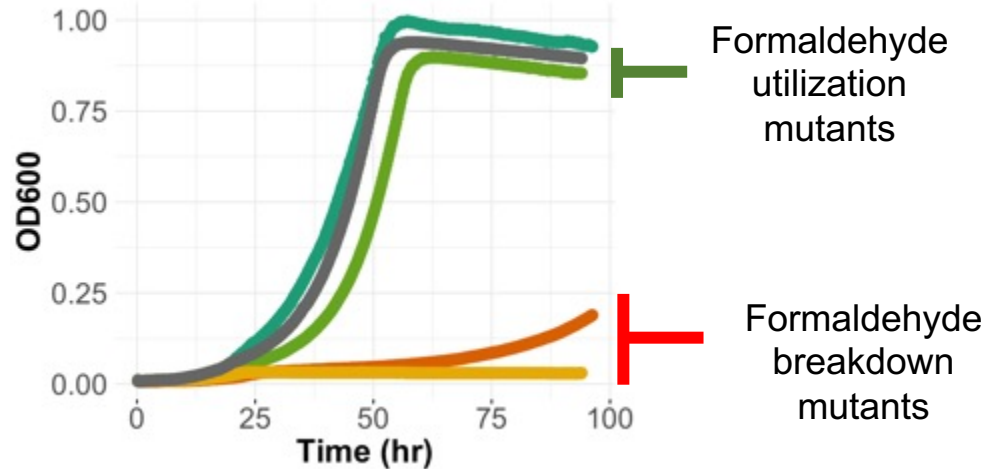
- The remains from ancient life forms gave rise to fossil fuels that are used as sources of energy
- Fossil fuels are depleting and since they are non-renewable, there is a need for renewable & sustainable energy sources such as biofuel
- A grossly underutilized source of biofuel is lignin, because of the toxicity of formaldehyde produced during its degradation
- *M. extorquens* can grow on formaldehyde and was engineered to consume some products of lignin degradation, such as VA/PCA

Formaldehyde Breakdown & Utilization Genes are Helpful for Growth on VA

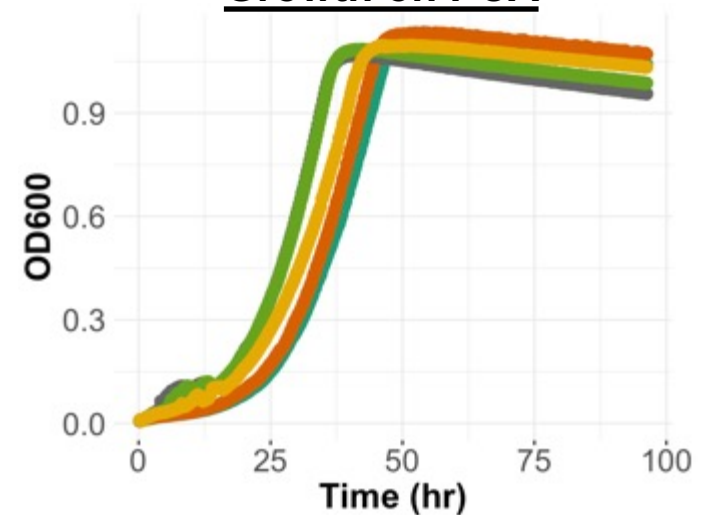
Bacteria Growth Curve



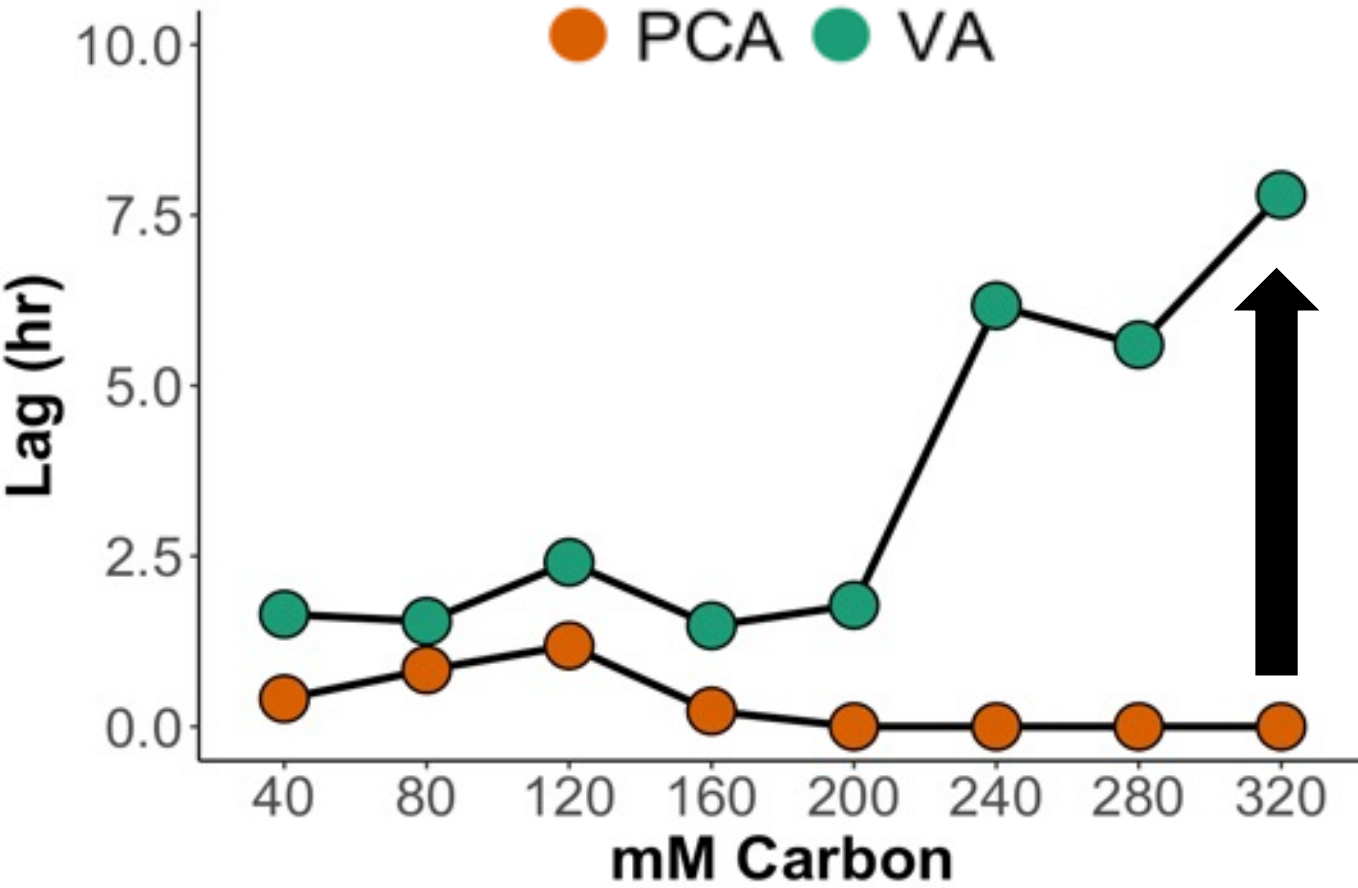
Growth on VA



Growth on PCA

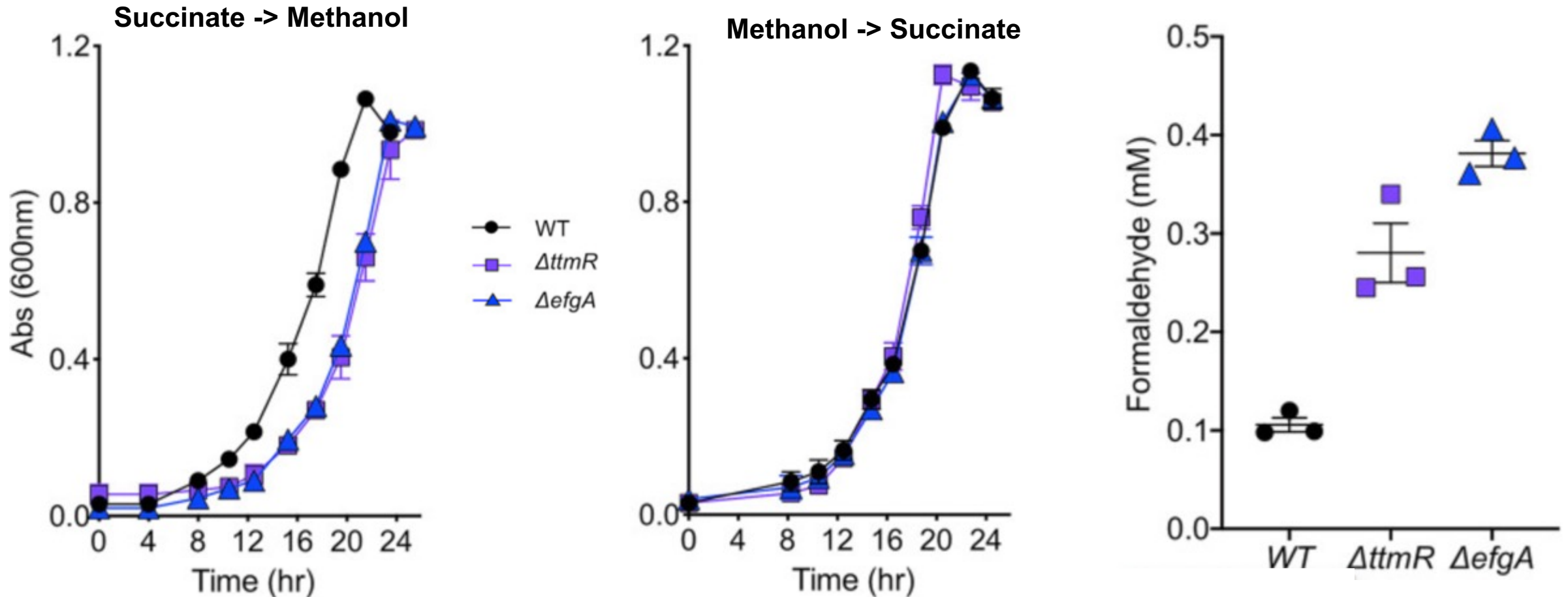


Long Lag Makes the Difference in Growth on VA vs PCA at High Carbon Concentrations



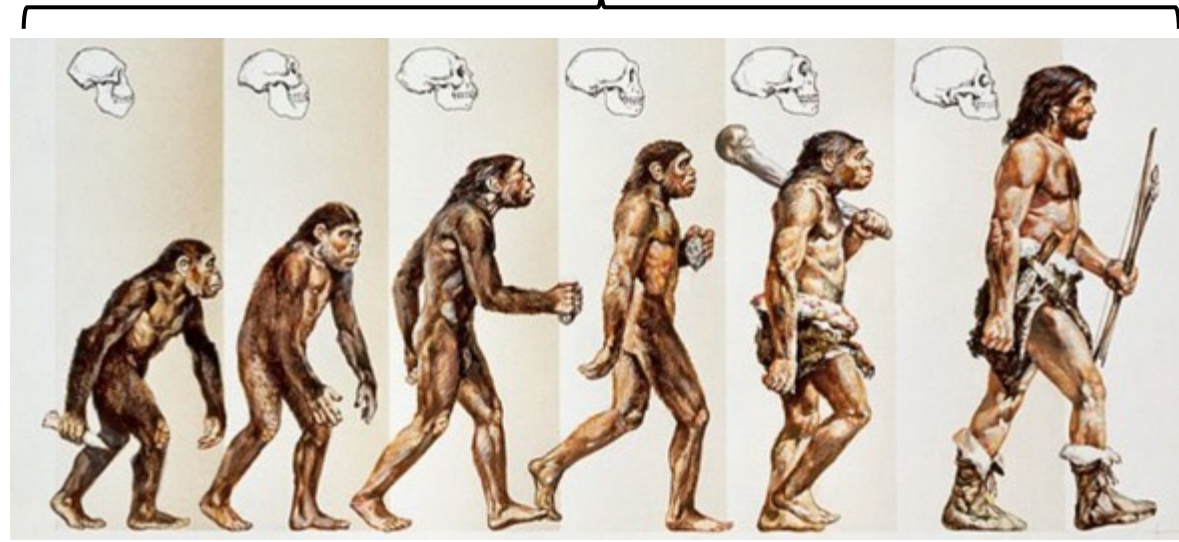
Lag increases on high amounts of VA but not PCA

Formaldehyde Accumulation Accounts for Long Lag in Transition to Methylotrophy



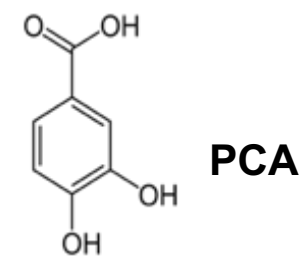
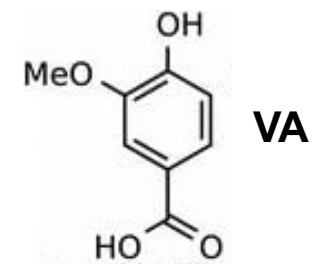
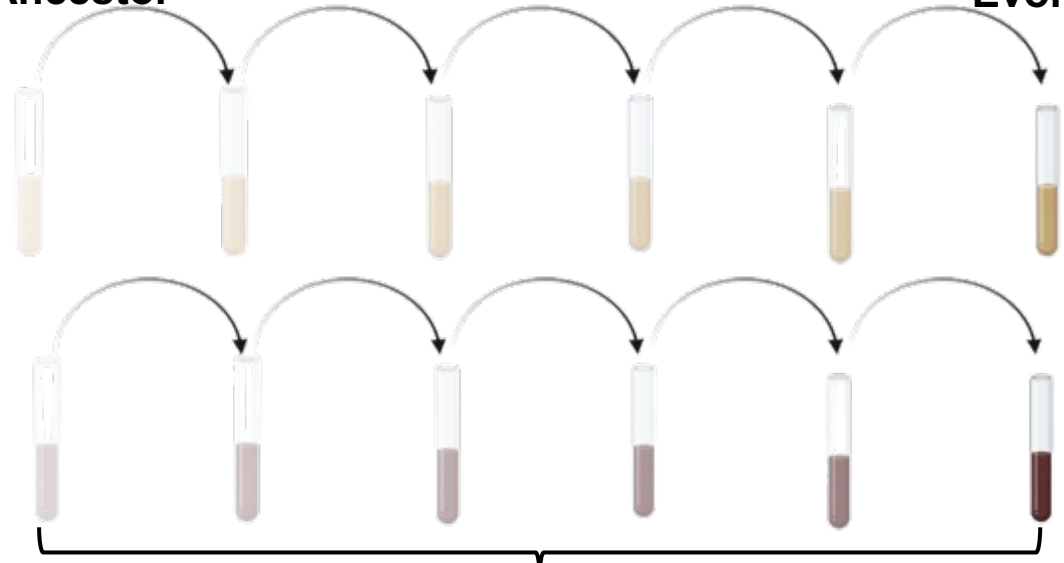
Laboratory Evolution Toward Improved Growth on VA/PCA

~ 6-7 million years



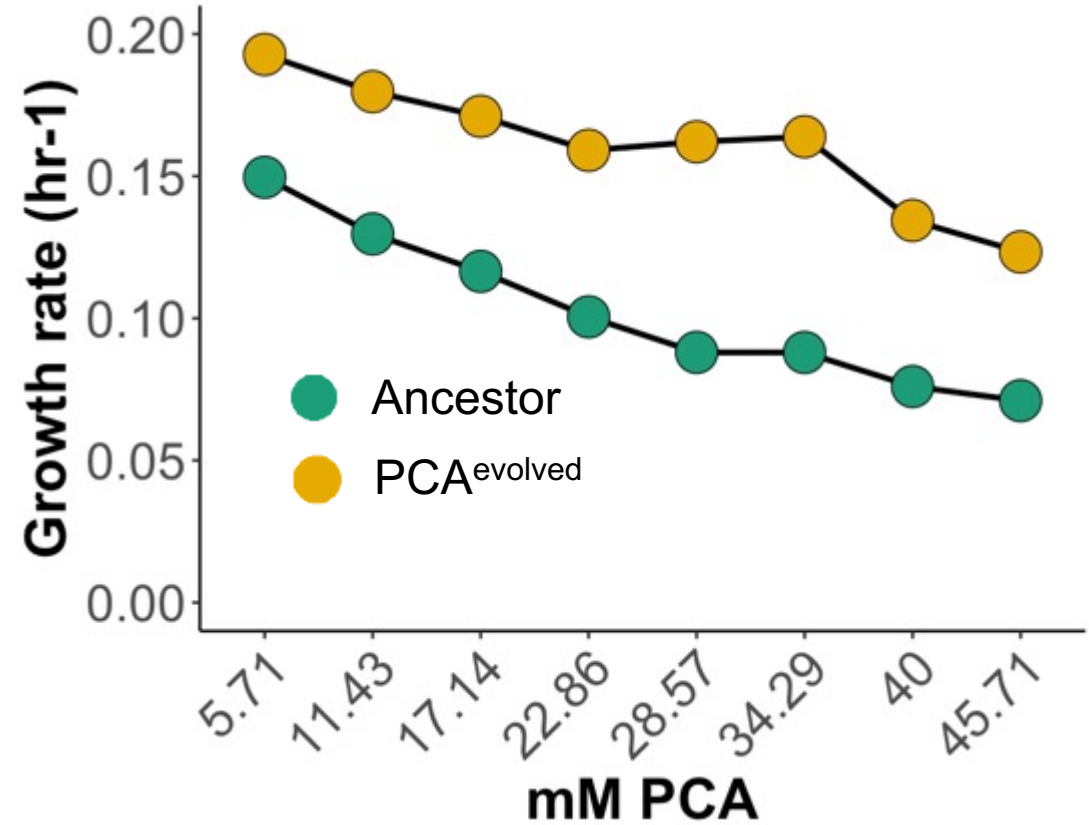
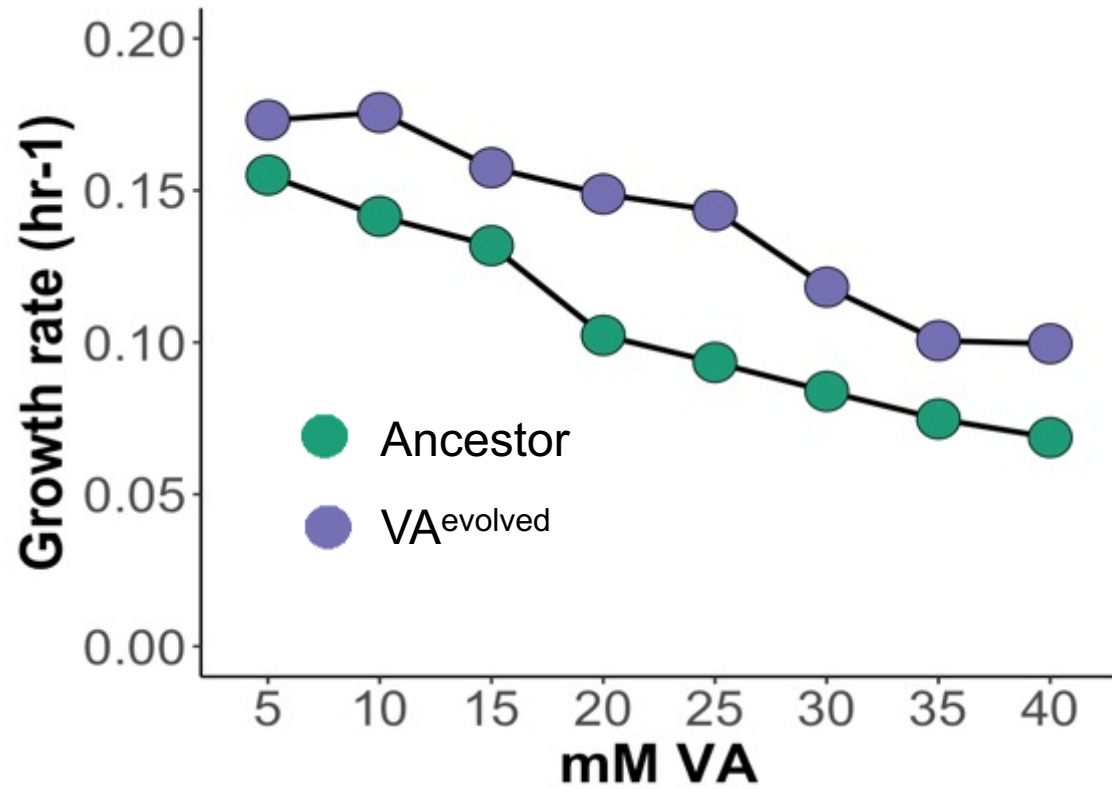
Ancestor

Evolved

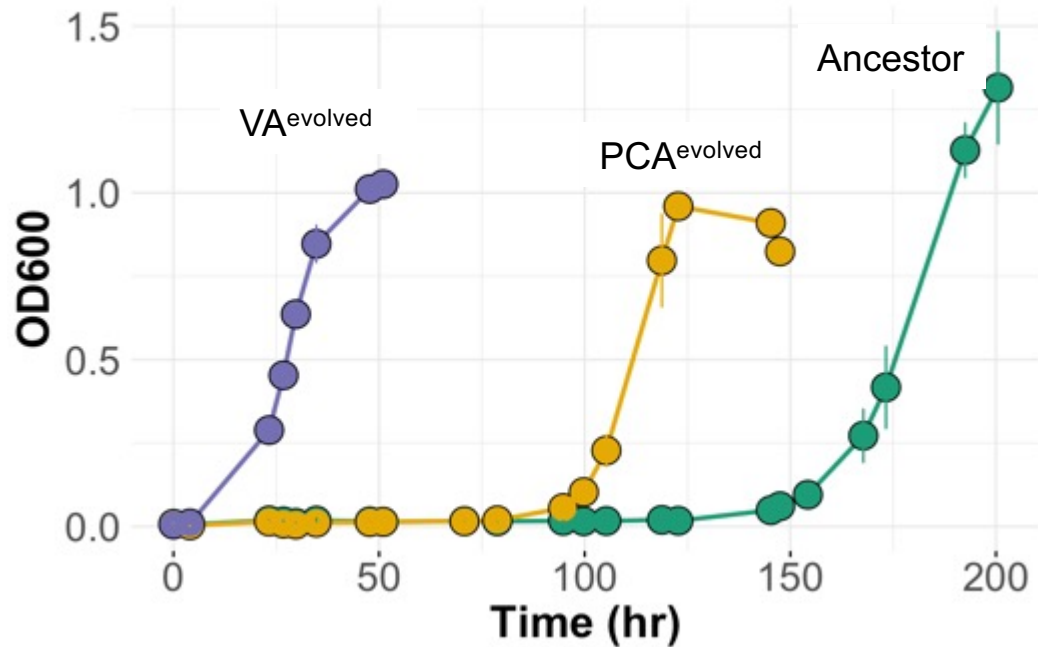


120 generations (20 weeks)

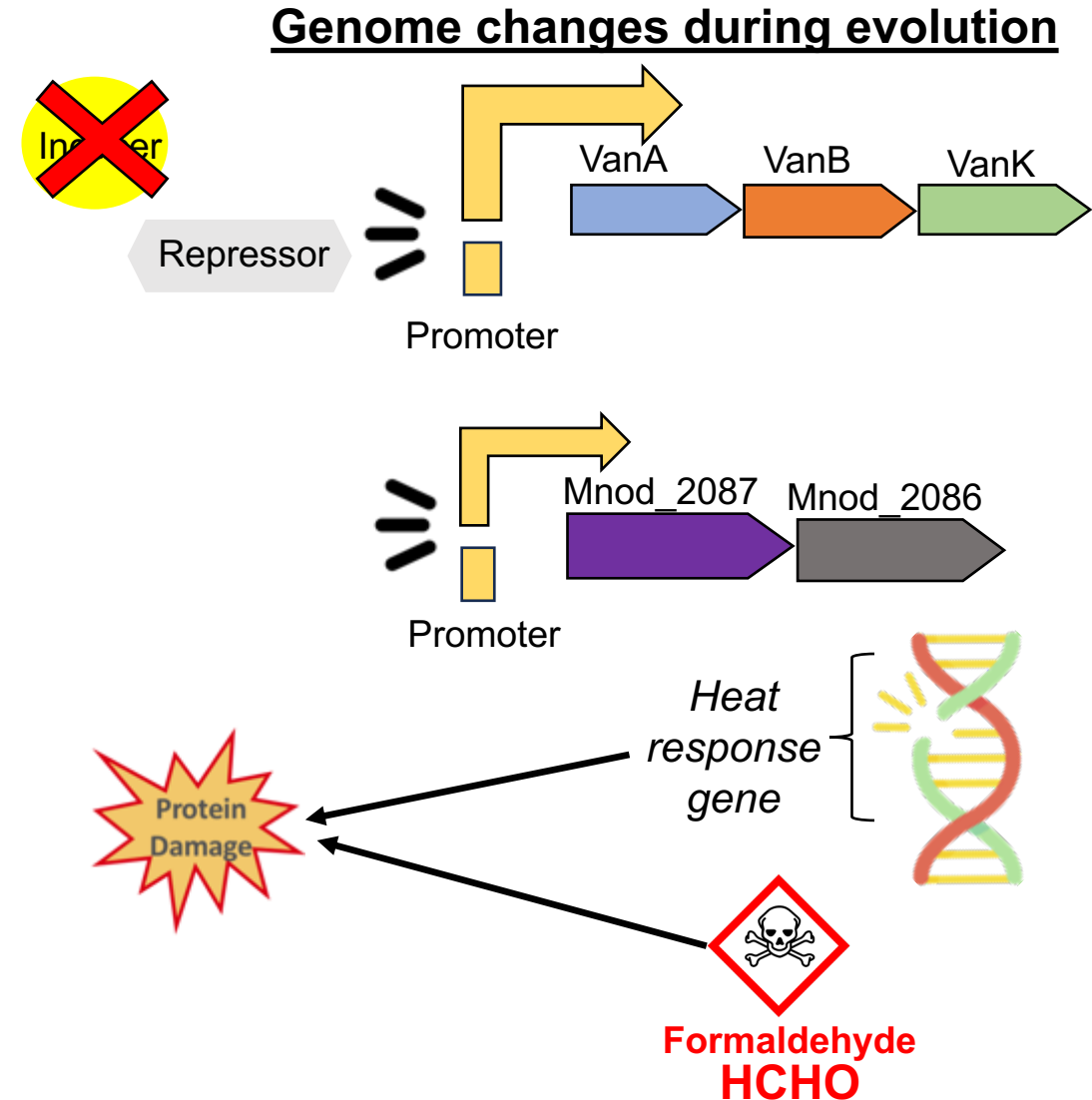
Laboratory Evolution Improved Growth on VA and PCA



Evolution Shortens Lag During Growth Transition From Succinate to VA



Hypothesis: The mutation in the heat response gene suppresses the costs of protein damage caused by formaldehyde.



Evolutionary Adaptation and Enhanced Growth on VA

- Genes for formaldehyde breakdown & utilization enable growth on VA – a product of lignin degradation.
- Formaldehyde accumulation in transition to growth on VA leads to a long lag, limiting the efficiency of conversion to biofuels.
- Evolutionary adaptation removes the growth lag caused by formaldehyde accumulation.
- *M. extorquens* has evolved to better utilize VA, harnessing its remarkable capabilities.

From the Past to the Future: Paving the Way for a Greener Tomorrow

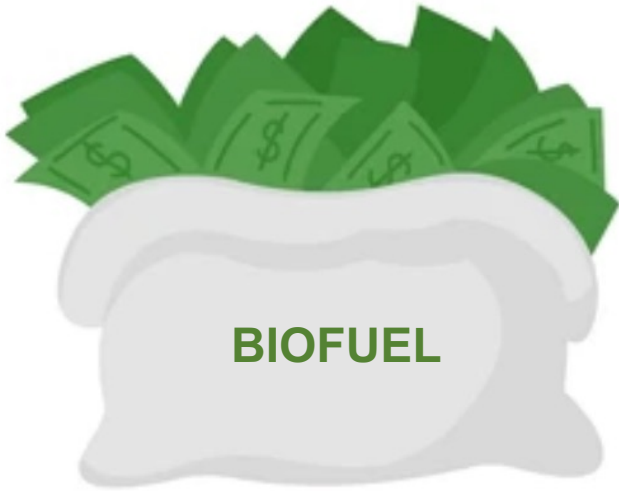


Life from over 300 million years ago



Harnessing the untapped potential of lignin

A Green & Sustainable Future is Within Reach



Biofuels are cost-efficient & economical



Sustaining Earth's future with renewable energy



From polluted skies to a healthier environment



Cleaner Transportation via biofuels

Acknowledgment



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Lab members

- Chris Marx (PI)

Post-Doc

- Alexander Alleman

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- Ellie Grant
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- Spencer Sinay

Former members

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- Jessica Lee (NASA AMES)
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- Tomislav Ticak (P&G)

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