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# Inducing Increased Bioplastic Production in *R. palustris* CGA009

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# Inducing Increased Bioplastic Production in *R. palustris* CGA009


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## 1. Abstract


PHA's (polyhydroxyalkanoates) are important bio-polymers in different industries such as petroleum, medicine, and nano-technology. In the microorganisms in which they are produced, they serve as an energy storage material by storing both carbon and usable electrons. This is useful in environments where the organisms are nutrient starved. PHA's have a practical use especially in the medical field as bioplastics because they are biodegradable and biocompatible. *Rhodopseudomonas. palustris,* a common soil bacterium, is notable for its uncommon metabolic flexibility. Its diverse metabolism means that it can fix $CO_2$ and grow on many lignin-based monomers in both aerobic and anaerobic environments. Currently, *R. palustris* already produces PHB (polyhydroxybutyrate), but there are other PHA's and co-polymers that have superior processing characteristics and applications. Our research will investigate the effect of the PHA production genes from *Paraburkholderia sacchari* DSM 17165 and *Cupriavidus necator* DSM 545 when introduced into *R. palustris*, and potentially *R. palustris* strains with their native PHA production genes knocked out. Both *P. sacchari* and *C. necator* produce higher titers of PHA's as well as co-polymers with improved processing characteristics and more applications than *R. palustris*' current PHB production. Our research will work to combine the metabolic flexibility of *R. palustris* with the higher PHA and co-polymer production of *P. sacchari* and *C. necator* by introducing genes for PhaA, PhaB, and PhaC production into *R. palustris*.


## 2. Significance of Research

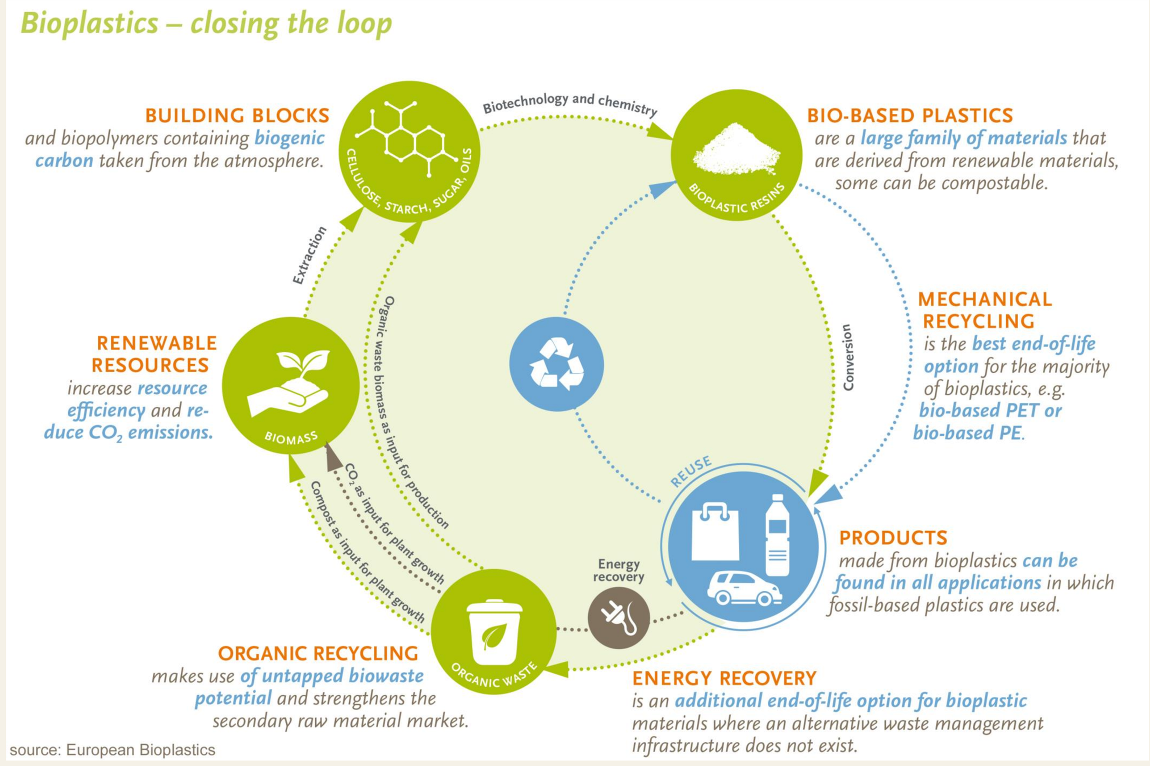
*R. palustris*

Metabolically versatile = flexibility for industry

Converts waste from ethanol and paper pulp plants into a biodegradable plastic

Alternative to burning waste / Prevents carbon from entering the atmosphere

Creates a valuable product



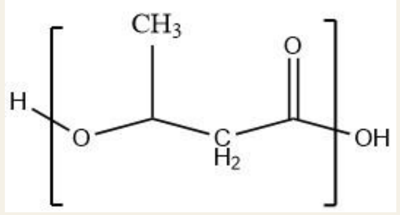

**Atkinson, Nebraska Ethanol Plant**

## 3. Research Questions

Will the introduction of *Cupriavidus necator* DSM 545 genes for PHA production increase PHA titers (mg/L) and/or increase co-polymer production?

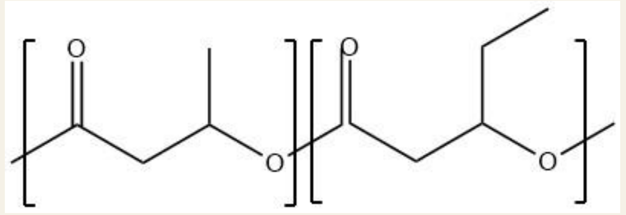
Will the introduction of *Paraburkholderia sacchari* DSM 17165 genes for PHA production increase PHA titers (mg/L) and/or increase co-polymer production?

**Polyhydroxybutyrate (PHB) Structure**



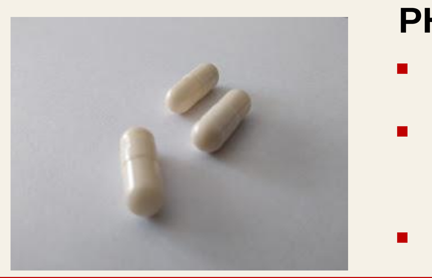
Initially produced

**Polyhydroxybutyrate-polyhydroxyvalerate (PHBV) Structure**



Goal production

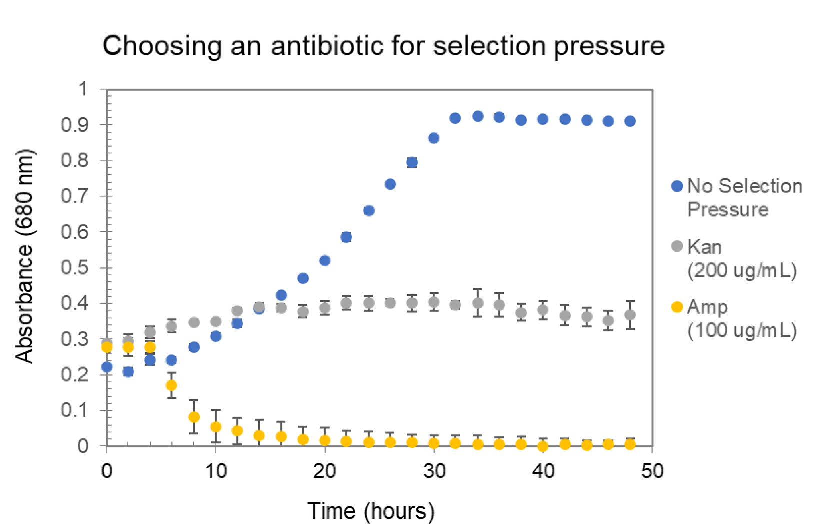
Hydroxybutyrate Hydroxyvalerate



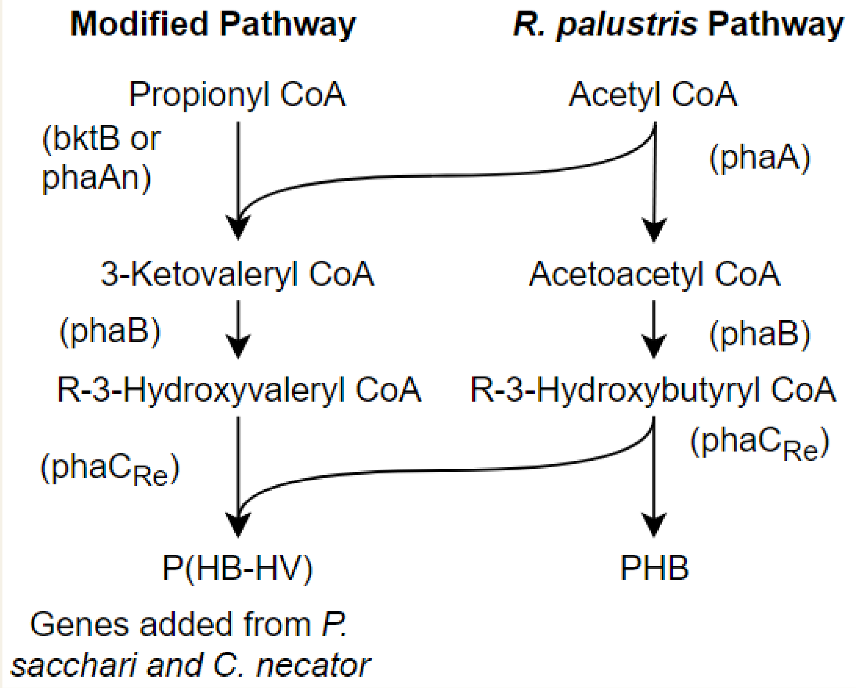
**PHBV is:**

- biodegradable.
- useful in single use and medical applications.
- easier to process than PHB.

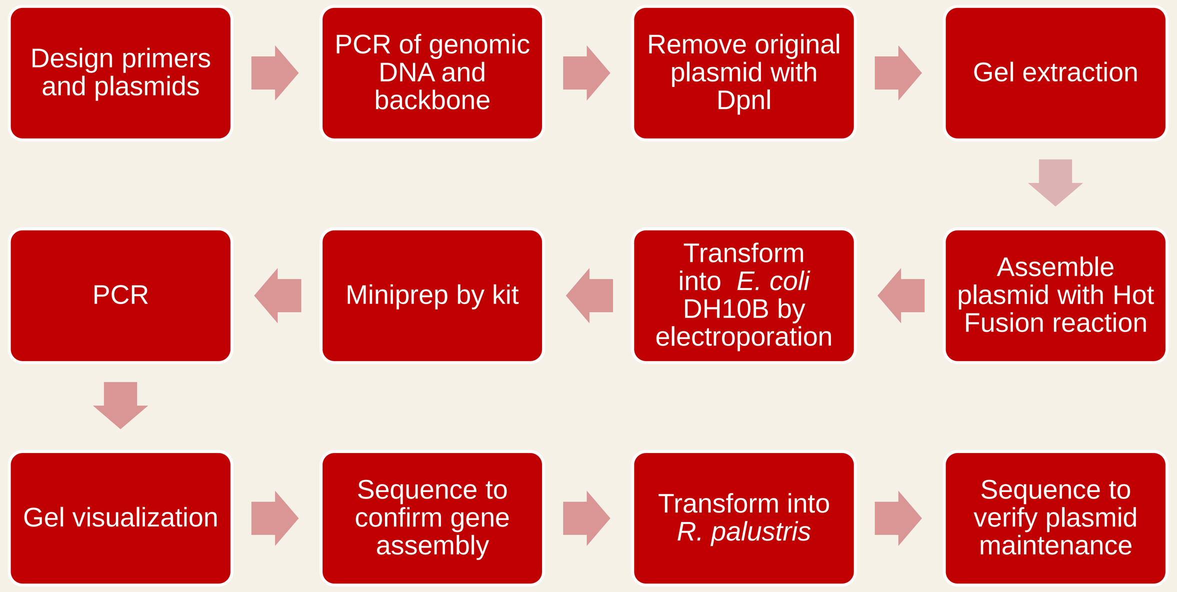
## 4. Experimental Plan

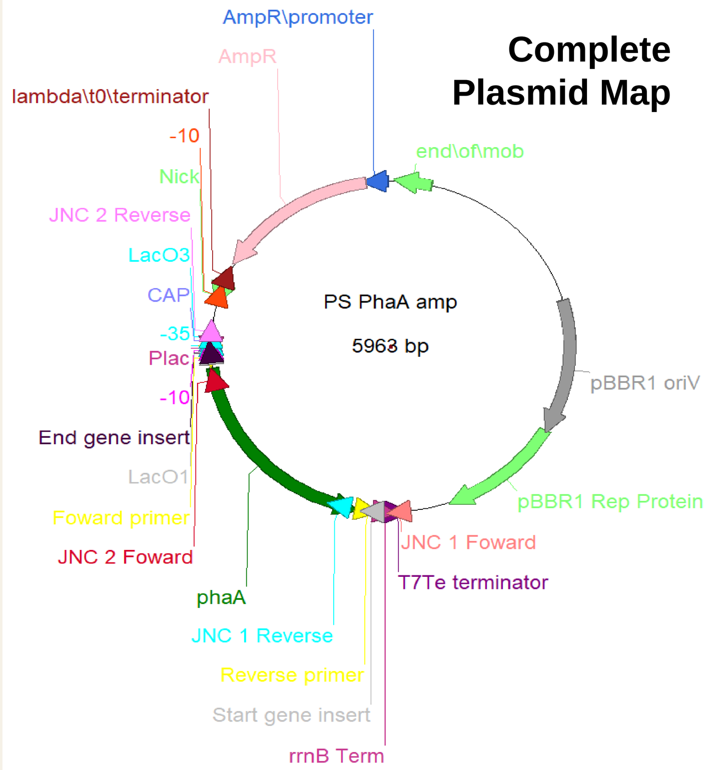



- For the *P. sacchari* genes, ampicillin worked to maintain the plasmid in *R. palustris*.
- For the *C. necator* genes, ampicillin did not reliably maintain the plasmid.

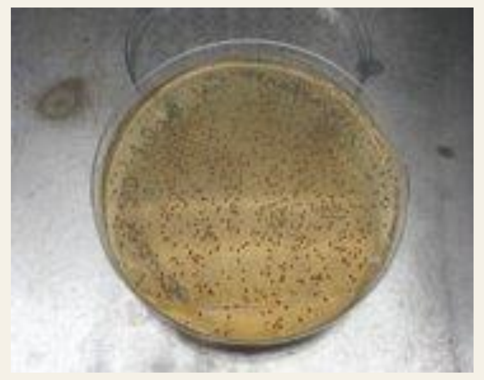



## 5. Strain Construction

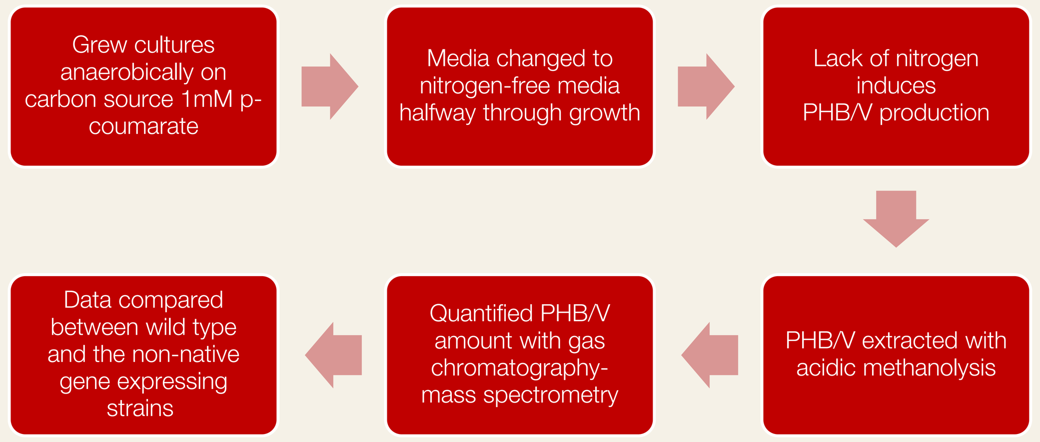



***R. palustris* BktB colonies**



## 6. PHBV Testing

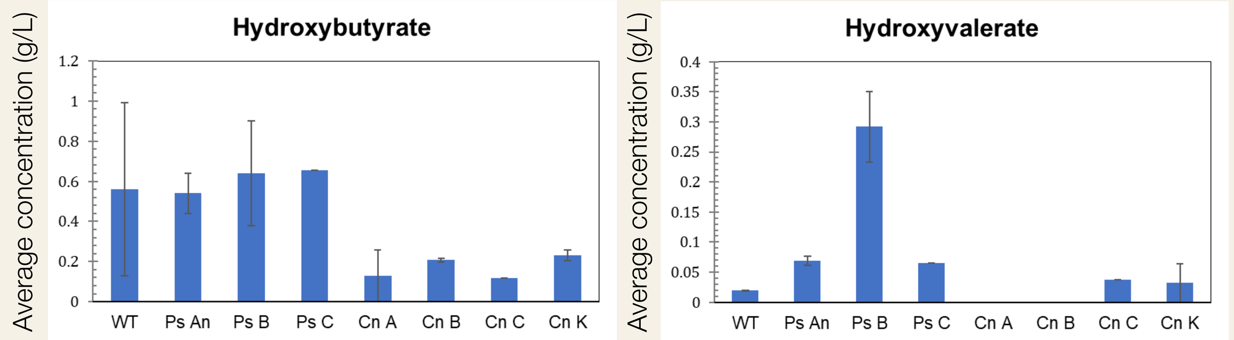



1mM *p*-coumarate is a lignin breakdown product.

$CO_2$ and light are also used by the cultures.

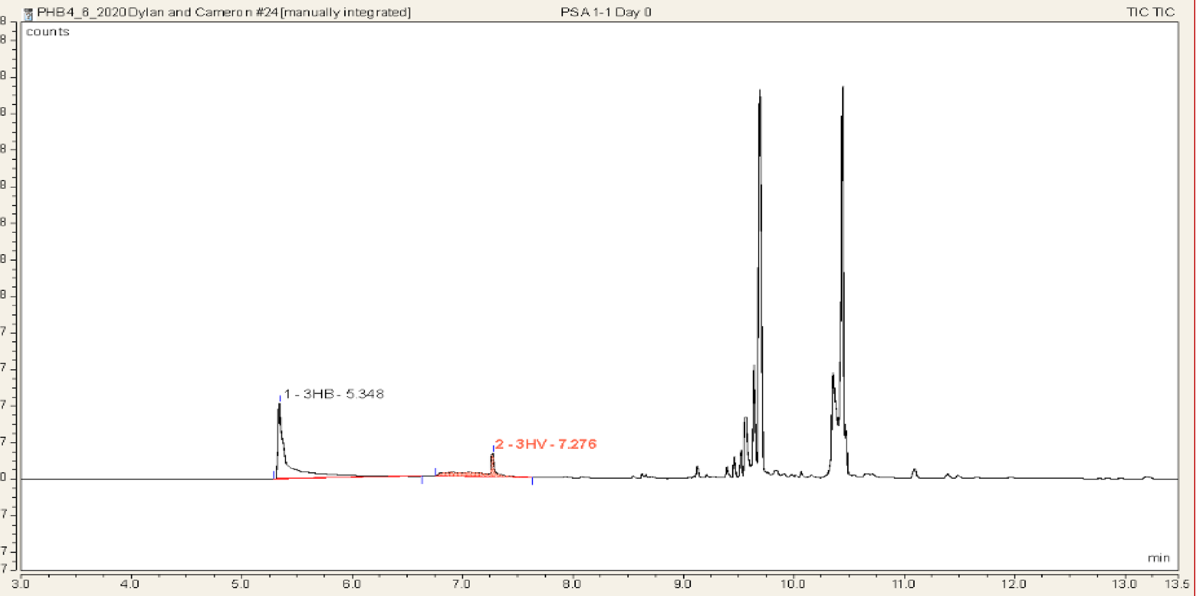
**Seed cultures 5 days after nitrogen starvation**

## 7. Results




WT – Wild type *R. palustris,* Ps - Strains with a *P. sacchari* gene (*phaAn, phaB, phaC*), Cn – Strains with a *C. necator* gene (*phaA, phaB, phaC, bktB*)

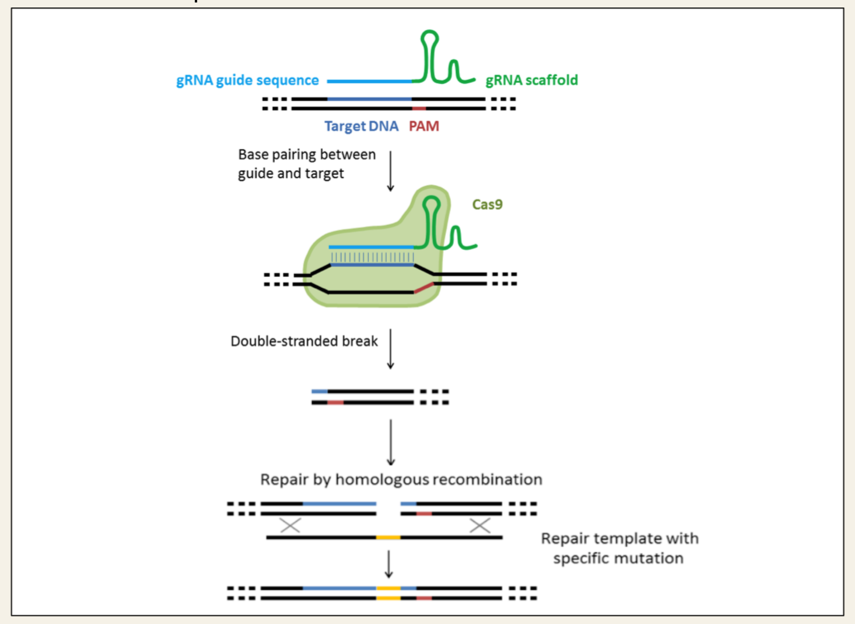
- All strains show PHB production.
- Ps B strain produces more hydroxyvalerate than any other strain.
- Ps B strain also had low growth, suggesting stress caused by hydroxyvalerate production from *P. sacchari*'s *phaB*.
- Data shown is for the strains BEFORE nitrogen starvation.



Sample mass spectrometry data

## 8. Conclusions and scope of future work

- Expression of non-native genes can increase the hydroxyvalerate concentration of the bioplastic produced by *R. palustris*
- Use of antibiotic selection to stably maintain the expression plasmid was not consistent.
- PHBV from cultures nitrogen starved for 5 days was not extracted due to the campus shutdown.




**Future work**

- Use CRISPR-Cas9, to insert the non-native genes into *R. palustris*' genome without the need for antibiotic selection pressure
- Knock out *R. palustris* genes *rpa3175, rpa2394*, and *rpa4567* to decrease propanoyl-CoA consumption
- Express different combinations of genes
- These projects have been approved as UCARE 2020-2021 proposals.

References


1. European Bioplastics, Nova-Institute (2019)
2. https://www.acnr.co.uk/2017/02/crispr-for-gene-editing-in-neuroscience-and-neurological-disease/