Investigating PHA synthases for better bioplastic production

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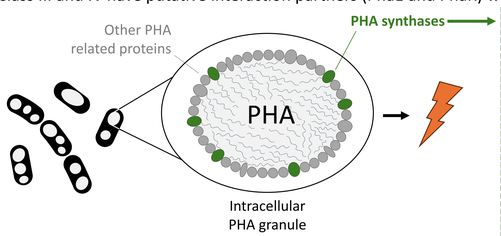
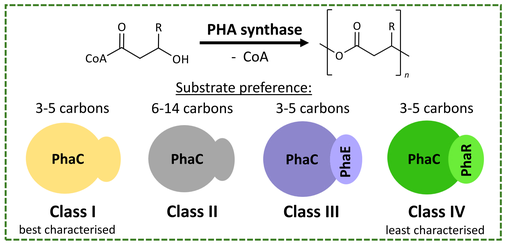
What are PHA synthases?

- Polyhydroxyalkanoates (PHAs) are biodegradable plastic-alternatives derived from renewable resources.[1,2] However, their industrial production is too inefficient to compete with conventional plastics, which damage the environment.
- PHAs are produced by different microorganisms and deposited inside granules as carbon and energy storage.[1,3,4]
- PHA synthases are the enzymes responsible for producing the PHA biopolymer chains, and can be sorted into four classes based on sequence, subunit composition and substrate specificity.[1]
- Class III and IV have putative interaction partners (PhaE and PhaR) with unclear roles.[1]





→ **Many molecular level details on PHA biosynthesis and PHA synthases are unknown, but they could provide insights required to improve industrial PHA production.**

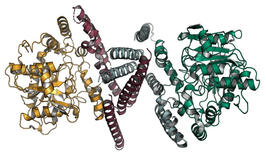
What are the roles of the different subunits and domains?

- Class I PhaCs are comprised of catalytic C- and regulatory N-terminal domains and form functional dimers.[1,5]
- For class IV, PhaRs are thought to be putative interaction partners of PhaCs, however, their structures and functions remain elusive.[1]

Class I structural information

N-terminal domain
→ dimerization
→ stabilisation
→ product egress

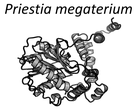
C-terminal domain
→ catalysis



Aeromonas caviae PhaC dimer (PDB: 9KNK)[5]

Class IV AlphaFold structure predictions[6]

Priestia megaterium



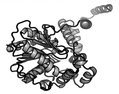
AF-A0A8D3X4F5-F1-v6

AF-D5DCX6-F1-v6

PhaC
catalysis

PhaR
putative interaction partner
no homology structures

Bacillus anthracis



AF-A0A6H3ADP5-F1-v6

AF-A0A6L7H7Y9-F1-v6

→ **Does PhaR fulfil similar roles to the class I N-terminal domain?**

→ **Is the class IV PhaC-PhaR complex formation affecting/regulating PHA synthesis?**

Aims

Investigate the enzyme complex assembly and molecular-level regulation for PhaC-PhaR from *Priestia megaterium* (*Pme*) and *Bacillus anthrax* (*Ban*) to gain a better understanding of the processes governing PHA biosynthesis is class IV microorganisms.

- Recombinantly express and purify PhaC and PhaR.
- Study PhaC-PhaR interactions and determine their impact on PHA biosynthesis.
- Probe the roles PhaR may play within PHA producing cells.

References:

[1]Neoh S. Z. *et al.*, *CRBIOT.* **2022**, *4*, 87-101.
[2]Nandakumar, A. *et al.*, *Renew Sustain Energy Rev.* **2021**, *147*, 111237.
[3]Sudesh, K. *et al.*, *Prog Polym Sci.* **2000**, *25* (10), 1503-1555.
[4]Bresan *et al.*, *Sci. Rep.* **2016**, *6* (1), 26612.
[5]Chek *et al.*, *Angew. Chem. Int Ed.* **2025**, *64*, e20250426.
[6]Jumper *et al. nature.* **2021**, *596* (7873), 583-589.

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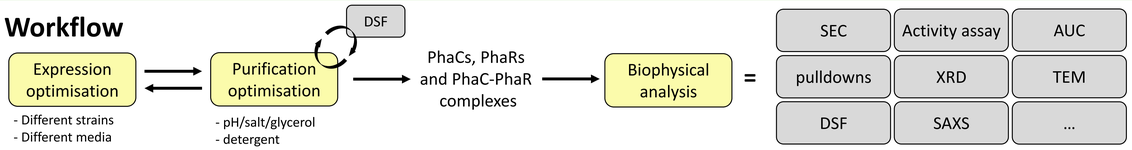
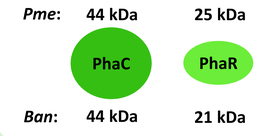
We the University of Canterbury for funding through the Aho Hinātore scholarship, and Commonwealth Chemistry for the opportunity to present this work.

Workflow



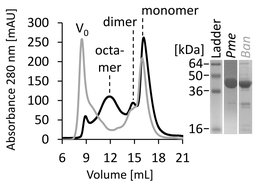


Results

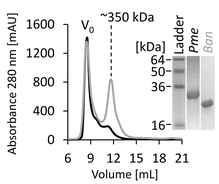
A) PhaCs and PhaRs can be recombinantly produced

(size exclusion chromatography (SEC): Superdex 200 Increase 10/300 GL)

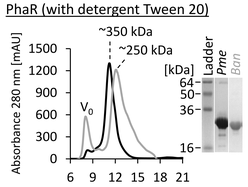
PhaC



PhaR



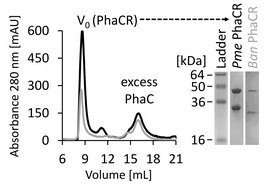
PhaR (with detergent Tween 20)



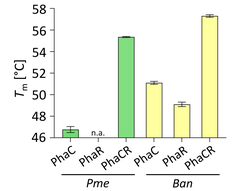
- **PhaCs can oligomerise on their own, unlike the class I C-terminal domains.**
- **PhaRs elute at volumes associated with high MW species. Detergent causes elution at comparatively lower MW.**

B) PhaCs and PhaRs form complexes

Pulldown
(SEC: Superdex 200 Increase 10/300 GL; 1:1 mix of PhaCs and PhaRs (Tween 20))



PhaC and PhaR DSF
(standard error bars)

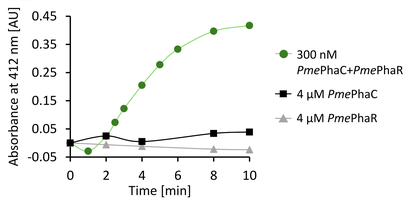


- **PhaCs and PhaRs interact and form a large MW species.**
- **PhaC-PhaR complexes are more thermally stable than PhaCs and PhaRs on their own.**
- **Further details regarding stoichiometry and strength of the protein-protein interactions remain currently unknown.**

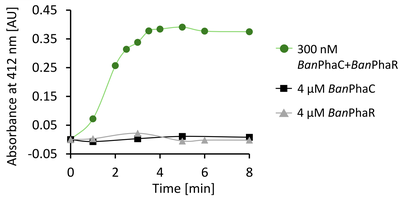
C) PhaC requires PhaR for PHA synthase activity

(quantification of CoA release; 750 µM 3-hydroxybutanoyl-CoA; 25 °C; Only results using PhaRs (Tween 20) shown)

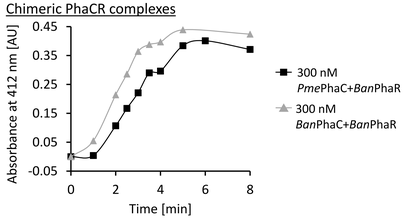
Pme



Ban



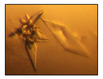
Chimeric PhaCR complexes



- ***Pme* and *Ban* PhaC-PhaR complexes are active.**
- **Chimeric complexes also show catalytic activity.**
- **PhaRs are required for activity, as PhaCs on their own don't perform catalysis.**
- **It remains unclear how exactly PhaRs are involved in catalysis.**

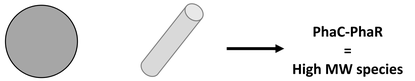
D) Structuaral details on class IV PHA synthases remain elusive

- X-ray crystallography efforts to date remain unsuccessful.



Few crystals
Non-diffracting crystals
Salt crystals

- Preliminary AUC, SAXS and TEM data suggest:



- **First low-resolution information on class IV PHA synthases was obtained.**
- **PhaRs were identified to occur in elongated structures with high anisotropy, matching observations made during size exclusion chromatography.**
- **Our results support the structural predictions made by AlphaFold, which previously lacked experimental evidence, and homology structure insights.**

***P. megaterium* and *B. anthracis* PhaCs and PhaRs were expressed, purified and identified to form stable and active protein-protein complexes *in vitro*. This confirms PhaRs as important interaction partners for the catalytic PhaCs of class IV PHA synthases. Parallels can be drawn between PhaRs and regulatory class I N-terminal domains, however, our results suggest that they do not operate equivalently. Future work aims to further investigate the nature of PhaC-PhaR interactions to disseminate their potential relevance in the regulation of PHA biosynthesis, which may help aid efforts to improve industrial PHA production.**