Secretion of Polyhydroxyalkanoates from *Escherichia coli*

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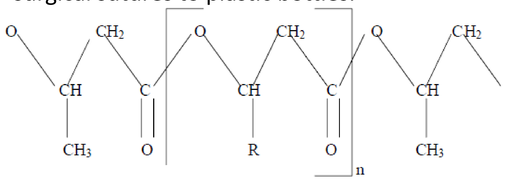
Abstract

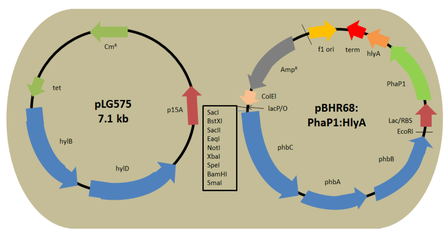
Traditional plastics are derived from petroleum and are non-biodegradable. Currently, there is pressure to reduce the dependence on petroleum derived products and a move towards biodegradable products. Polyhydroxyalkanoates (PHAs) are a group of biodegradable bioplastics that are produced by a wide variety of microorganisms, as a storage medium for energy and carbon. Studies have suggested that PHA based materials (and its co-polymers, e.g. polyhydroxyvalerate) have suitable properties (comparable to polypropylene and polystyrene) for a variety of applications, from surgical sutures to plastic bottles.



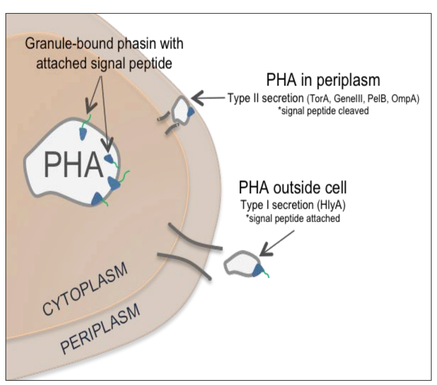
Typical PHA structure (PHB has R= CH_3)

Clearly there are many applications for PHAs. However, microbial based production is currently not economically viable as bacteria have to be killed in order to harvest the PHA. The extraction methods account for over 70% of the bioplastic costs. In order to save on downstream processing, a method of secreting PHA from bacteria has been developed.

Development of secretion system

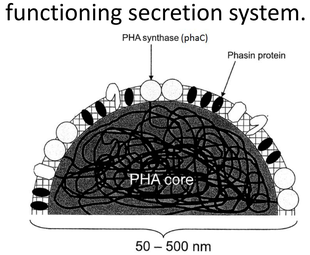


PHB production and secretion system



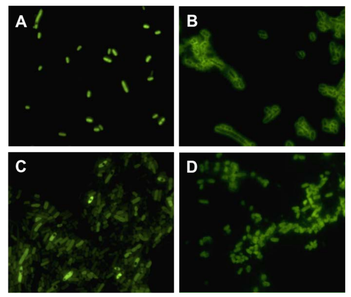
Schematic of bioplastic secretion

Phasin, a low molecular weight protein, which specifically binds to PHA, was targeted for secretion. A PHA secretion system was constructed by combining the plasmid pBHR68 (carrying PHA producing genes) with phasin:HlyA. *E. coli* cells containing this construct were co-transformed with pLG575 carrying HlyBD proteins necessary for HlyA secretion. Initial studies were carried out with GFP to demonstrate a functioning secretion system.

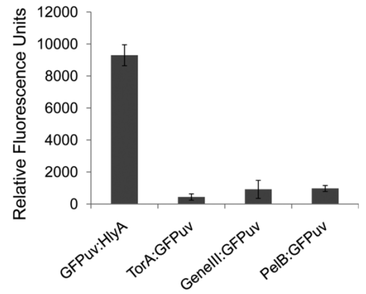


PHA granule with associated proteins

Fluorescence analysis

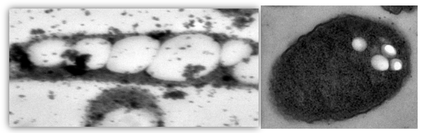


Fluorescence microscopy images for: A)GFPuv, B)TorA:GFPuv, C)GFPuv:HlyA, and D)GeneIII:GFPuv

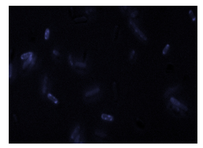


Measurement of fluorescence in concentrated extracellular media. GFPuv:HlyA gave highest fluorescence

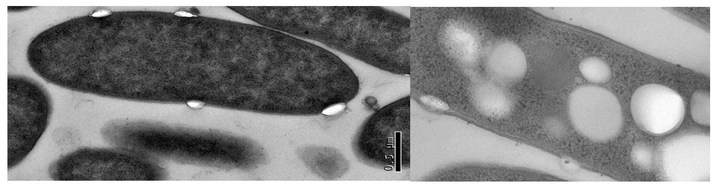
Images of PHA production



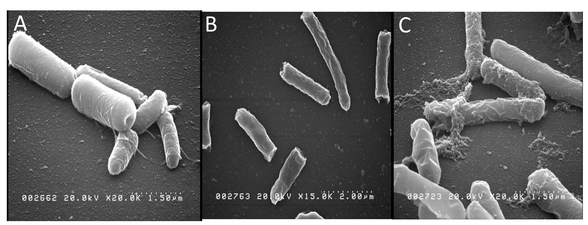
TEM images of non-secreting PHA inside *E.coli*



Nile blue staining of *E.coli* containing PHA

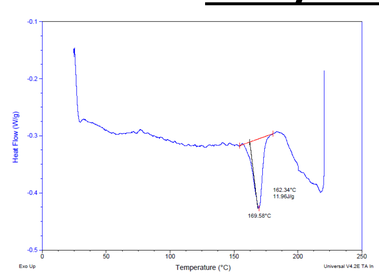


TEM images of secreting PHA *E.coli*

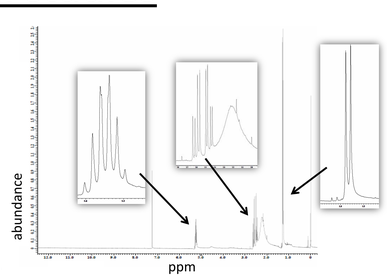


SEM images: A) non-secreting bacteria, B) non-secreting bacteria with Phasin, and C) complete PHA production and secretion system

Analytical studies

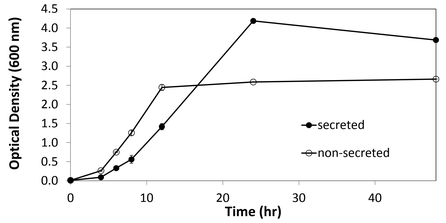


DSC thermograph for PHA melting at 170°C

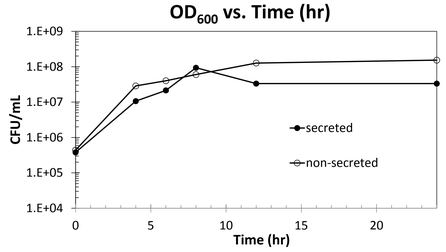


Typical NMR spectra for PHA

Growth studies



OD_{600} vs. Time (hr)



CFU/mL vs. Time (hr)

- Growth studies were conducted in shaker flasks in an orbital shaker.
- The secreting strain had higher turbidity. This is likely due to secreted PHA in the media.
- No difference was observed in the CFU/mL between the secreting and non-secreting strains.
- The non-secreting strain reached stationary phase before the secreting strain.

PHA secretion analysis

Secreted vs. non-secreted PHA 8-48 hrs

Strain	Time (hrs)	%mass of PHA in Secreted	%mass of PHA in Not secreted	Dry mass
Secreting	8	0.00	4.98	
	12	1.60	8.96	
	24	10.34	39.85	
	48	31.33	48.00	
Non-secreting	8	0.00	0.00	
	12	6.13	5.60	
	24	2.09	7.79	
	48	5.80	13.09	

E.coli secreting strain accumulated PHA as 39% of the cell dry mass after 24hrs and 48% after 48 hrs. The secreting strain secreted 10% and 31% of the cell dry weight after 24 and 48 hrs respectively. *E.coli* cells containing just the plasmid for PHA production (pBHR68) accumulated 7% and 13% PHB of the cell dry mass after 24 and 48 hrs respectively.

Conclusions and future studies

- A secretion based method for PHA production and recovery has been developed.
- Studies comparing physical properties of secreted PHA vs. internal PHA will be carried out in the future.
- Efficient downstream processing will be conducted in the future to more efficiently separate secreted and non-secreted PHA.

References/Acknowledgements

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- Miller, C.D., Linton, E. and Sims, R.C. 2009. A novel use of the phasin protein for purification of Polyhydroxyalkanoates (PHAs). Patent pending. United States Patent Application 20110159555.
- Rehm, B. H. A., Bacterial polymers: Biosynthesis, modifications and applications. *Nature Reviews Microbiology* **2010,** *8* (8), 578-592.

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