Harnessing the power of synthetic biology to remove phosphate out of wastewater

Meet Phil!

Phil Phosphate

Harnessing the power of synthetic biology to remove phosphate out of wastewater

Team: K. Aare, L. Alexianu, A. Brain, K. Davis, C. Draper, G. Thomas, J. Jung, M. Milner, A. Rowbotham
Advisors: A. Evans, J. Chong, A. Parkin, Prof. Maggie Smith, Dr. Gavin Thomas
Supervisors: Dr. James Chong, Dr. Alison Parkin, Prof. Maggie Smith, Dr. Gavin Thomas

What’s happening with our waste?

Current phosphate removal methods require expensive and limited resources such as ferric sulfate or uncharacterised organisms.

Phosphate Uptake

Inorganic phosphate ions enter the periplasm through the PhoE phosphorylase. Phosphate can enter the cytoplasm through the PhoP-specific transporter (an ABC transporter) PhoSCAB. Phosphate ions can then be used for cellular metabolism which includes ATP formation from ADP and phosphate by the F,F,-ATPase ATP is the substrate for the Polyphosphate kinase enzyme (PPK1) which reversibly transfers the γ-phosphate to the acceptor

Phosphate Pathway in Phil

Expression vector “pAdapt” (PolyP). The cell releases orthophosphate residues one residue at a time from PolyP’s ends.

Function:
- Vector
- Double Terminators T1 from E.coli using polyphosphate as the inducer in T7 (42GK_BX_03),
- Hybrid shuttle vector (pShuttle-CMV) for self-cloning, selectable marker

Meet Phil!

Phil Phosphate

Harnessing the power of synthetic biology to remove phosphate out of wastewater

Team: K. Aare, L. Alexianu, A. Brain, K. Davis, C. Draper, G. Thomas, J. Jung, M. Milner, A. Rowbotham
Advisors: A. Evans, J. Chong, A. Parkin, Prof. Maggie Smith, Dr. Gavin Thomas
Supervisors: Dr. James Chong, Dr. Alison Parkin, Prof. Maggie Smith, Dr. Gavin Thomas

What’s happening with our waste?

Current phosphate removal methods require expensive and limited resources such as ferric sulfate or uncharacterised organisms.

Phosphate Uptake

Inorganic phosphate ions enter the periplasm through the PhoE phosphorylase. Phosphate can enter the cytoplasm through the PhoP-specific transporter (an ABC transporter) PhoSCAB. Phosphate ions can then be used for cellular metabolism which includes ATP formation from ADP and phosphate by the F,F,-ATPase ATP is the substrate for the Polyphosphate kinase enzyme (PPK1) which reversibly transfers the γ-phosphate to the acceptor

Phosphate Pathway in Phil

Expression vector “pAdapt” (PolyP). The cell releases orthophosphate residues one residue at a time from PolyP’s ends.

Function:
- Vector
- Double Terminators T1 from E.coli using polyphosphate as the inducer in T7 (42GK_BX_03),
- Hybrid shuttle vector (pShuttle-CMV) for self-cloning, selectable marker

Phosphate Assay

To measure both orthophosphate and polyphosphate concentrations in cell supernatant and within growth media, we used an abcam kit containing malachite green and ammonium molybdate as indicator components. Following preliminary experiments on E.coli lystate, the unaltered assay did not detect polyphosphate. Instead, we indirectly compared orthophosphate within the cell lystate and media to determine phosphate removal of our constructs (figures 1a and 1b respectively).

Here’s how we’ve fixed it

By developing Phil - a culturable Phosphate Accumulating Organism (PAO) with improved phosphate remediation abilities, which will allow wastewater treatment companies to extract and recycle the phosphate.

Growth Assay

Knocking out phosphate transporters exhibits a growth phenotype, and based on modelling results we expected a change in the growth phenotype if phosphate is accumulated. Growth assays were based on optical density measurements of cell cultures at 650nm over a set period of time, either 7 or 24 hours. The growth rates and curves of multiple phosphate transporter knockout strains have been compared and analysed in an attempt to find both a chassid for characterisation of genes, as well as experimentally test for a decrease in cell growth in figures 2 and 3.

Business Plan

We developed a bioreactor (fig. 7) to replace the current inefficient method. Using Phil would reduce wastewater companies’ operation costs significantly as well as reducing their environmental impact. The wastewater industry is one of the fastest growing sectors, with global projections of worth rising past £61 billion (£94.6 billion) by 2019! We estimated that if Yorkshire Water used Phil we could recycle 10,010 kg of phosphate per day (annually worth £365,000) For more details, check out our wiki

Human Practices

Our human practices focused on the public’s opinion and education relating to GMOs. Main public outreach events include:

- A GM knowledge survey answered by 1000 people
- Strawberry DNA extraction demonstration (28-29/07/15)
- Strawberry DNA extraction demonstration (28-29/07/15)
- Educational Workshop at Badger Hill Primary School (14/07/15)
- Demonstrating Genetic Engineering with high school students (15-17/07/15)

Parts Submitted

<table>
<thead>
<tr>
<th>Source Organism</th>
<th>Gene</th>
<th>Part name</th>
<th>Function:</th>
<th>Characterised</th>
<th>Sequenced</th>
<th>Submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>E-PKK</td>
<td>BiBBa_3107007</td>
<td>PolyPhosphate Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>E-PKK</td>
<td>BiBBa_3107001</td>
<td>ExopPphosphatase</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kingella</td>
<td>K-PKK</td>
<td>BiBBa_3107001</td>
<td>PolyPhosphate Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Sinorhizobium</td>
<td>lacZ</td>
<td>BiBBa_3107007</td>
<td>Phosphate specific transporter</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candidatus Accumulibacter phosphatis</td>
<td>ApPSCAB</td>
<td>BiBBa_3107007</td>
<td>Phosphate specific transporter</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Candidatus Accumulibacter phosphatis</td>
<td>ApPSCAB</td>
<td>BiBBa_3107001</td>
<td>PolyPhosphate Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Candidatus Accumulibacter phosphatis</td>
<td>ApPSCAB</td>
<td>BiBBa_3107003</td>
<td>PolyPhosphate Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Candidatus Accumulibacter phosphatis</td>
<td>ApPSCAB</td>
<td>BiBBa_3107002</td>
<td>PolyPhosphate Kinase</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Candidatus Accumulibacter phosphatis</td>
<td>ApPSCAB</td>
<td>BiBBa_3107001</td>
<td>Phosphate specific transporter</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Expression vector</td>
<td>&quot;PhaP&quot; in pBB1C3</td>
<td>pSB1C3</td>
<td>used as a cloning vector</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>


Figure 1: Comparison of parts using the abcam phosphate assay, constructs were tested after 6 hours of growth. Ac SK12, ApP SK11, and ApP SK11 represent Candidatus Accumulibacter phosphatis pppk gene from the strain SK12, JU1 and BA91 respectively. Expression vector BiBBa_3107013 from the RIDD collection (ApP). BiBBa_31013 is used as a wild type E.coli strain for the experiment. Figure 1(a) shows the amount of intracellular orthophosphate within T7 plate. Figure 1(b) shows the amount of orthophosphate accumulated within the media of our parts standardized by cell growth. A one-way ANOVA was conducted and post-hoc LSD was used, *NS signifies no statistical significance, * signifies p<0.001 ** signifies p<0.001 *** signifies p<0.001.

Figure 2a and b: A growth assay over 24 hours to compare KEIO knockouts: ApP SK11 and ApP SK11, and parent strain BiBBa 31013. Cells were grown in MOPS media with phosphate levels of (a): 1.32mM (b): 0.05 mM. Figure 2(b) shows a clear phenotype of diminished growth in ΔpstC

Figure 3a shows a clear phenotype of diminished growth in ΔpstC strain BW25113. Cells were grown in MOPS media with phosphate levels of (a): 1.32mM, (b): 0.05 mM.

Figure 3b shows a clear phenotype of diminished growth in ΔpKK.

Figure 4a (Additional material): We have replaced this organism’s high-density high-affinity phosphate transporter as a replacement for E.coli’s high-affinity low-velocity phosphatase

Figure 4b (Additional material): We have replaced this organism’s PPK from 3 different strains that might turn-out to be different species in the future) and Psh.

Figure 5: A schematic of our vector is seen in figure 5, and proof of its functionality in figure 6.

Figure 6: Blue-white screening using X-Gal/IPTG, proof of functionality of our BiBBa_3187000 construct - the expression vector we made and used.

Figure 7: A step by step depiction of how Phil would be used - 1. Phosphate in wastewater enters the reaction chamber. 2. Phil is in the reactor uptaking phosphate. 3. A stimulus makes Phil stop to the surface. 4. Phosphate is removed from Phil in a new bioreactor, and 5. Phosphate can be used for fertilizer or recycled otherwise.