POC SUMMARY

A special bacterial chassis with the potential to capture carbon from air.

The Challenge - Some biological systems can efficiently carry out Hydrogen-Dependent Carbon Dioxide Reduction (HDCR) to capture CO_2 and store it as aqueous formic acid. But the enzymes are sensitive to attack by oxygen. Can we design a cellular system that can grow in air but contains air-sensitive enzymes to help us capture CO_2?

Aims - Nature might provide the answer – but can we harness nature to our own ends? *Azotobacter vinelandii* is a soil bacterium that requires oxygen for growth – but has a special anaerobic cytoplasm. It has its own air-sensitive enzymes (e.g. nitrogenase) but this project is to introduce an alien Hydrogen-Dependent CO_2 Reductase (*Pectobacterium atrosepticum* FHL-2) in to this potentially exciting new chassis.

Key Findings -

The organism could be transformed with standard vectors and was found to be sensitive to β-lactam antibiotics, unless transformed. Evidence for heterologous production of enzymes was obtained (Fig. 1)

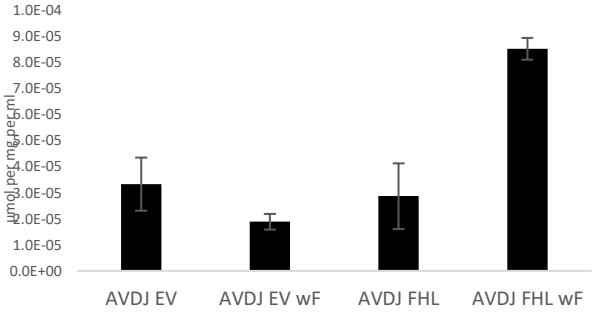

Figure 1: Evidence for FHL-2 activity in *A. vinelandii*.
The *A. vinelandii* DJ strain ('AVDJ') was transformed with the empty vector 'EV' or a vector encoding the entire FHL-2 ('FHL'). Cells were grown in 'M2' medium containing mannitol and suspended in 20 mM MOPS (pH 7.2) and 0.2% (w/v) sodium formate. H_2 production was assayed by GC.

Outcomes – We now have experience, know-how and procotols to allow us to grow and transform *Azotobacter vinelandii* in the laboratory. We have preliminary data on heterologous expression of an air-sensitive hydrogenase. We have trained a researcher in techniques relevant to the bioenergy and industrial biotechnology community, including Gas Chromatography for bio-H_2 quantification and uHPLC for quantification of organic acids and alcohols.

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