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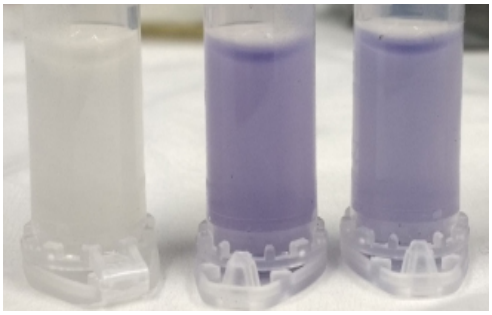
Cloning and metal analysis of recombinant aldehyde ferredoxin oxidoreductase

"We showed that a novel compartmentalisation strategy can be used to produce a biotechnologically important enzyme in bacteria. Our results are being used to help acquire further strategic investment in the partner company." Martin Warren, University of Kent

Martin Warren and Stephanie Frank, University of Kent; Michelle Gradley, ZuvaSyntha Ltd.



The activity of recombinant AOR in *E. coli*, as shown by an increase in the violet colour.

OUTCOMES: We first cloned two genes encoding for aldehyde ferredoxin oxidoreductase (AOR) from *Clostridium ljungdahlii* into *E. coli*. The purified proteins, termed AOR1 and AOR2, contained the predicted Fe-S centre and and a tungsten-pterin cofactor, which was produced by the *E.coli*. We next showed that the enzymes were active (but only when produced under strictly anaerobic conditions) in a whole cell assay (figure), with AOR2 displaying more activity than AOR1. We then investigated the targeting of AOR proteins to a bacterial microcompartment (BMC), by expressing the enzymes with a tag that directs them to the BMC. When the enzymes were co-produced with BMC shell-proteins, the AORs were indeed targeted to the BMC. Our work with the expression of AOR from the thermophile *Pyrococcus furiosus* proved more problematic, and is still under investigation. Our results demonstrate that recombinant AORs are active in *E. coli* if grown and kept under anaerobic conditions, and can be targeted to BMC. Our next steps are to see if the activity of AOR can be enhanced in acteogens, since this provides a powerful way in which acetate can be redirected for the production of commodity chemicals.

INITIAL AIMS: The aim of the project is to enhance the recombinant production of a key enzyme of biotechnological importance: aldehyde ferredoxin oxidoreductase (AOR). This enzyme allows the transformation of carboxylic acids into aldehydes, which could have use in the sustainable production of 1,3-butadiene – a key commodity chemical for the rubber and tyre market. We intend to explore the recombinant production of AORs in *E. coli* and to determine conditions that allow for the successful incorporation of its unusual metal complement, which includes a tungsten-molybdopterin cofactor and a 4Fe-4S centre. Moreover, we also want to see if this protein can be targeted to bacterial microcompartments (BMC) — proteinaceous organelles that can be used to encase metabolic process to protect a cell from toxic products — and to determine if AORs retain their metal complement once inside the BMC.

- The results this project have secured two further grants: Early Stage Catalyst Funding from BBSRC and Proof of Concept Funding from C1Net
- ZuvaSyntha and the University of Kent are seeking IP projection for aspects of this work

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