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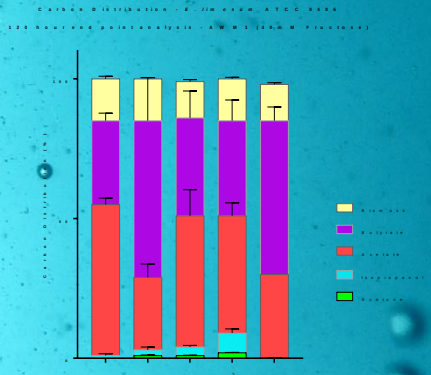
Maximising reaction productivity through protein scaffolding with cohesion-dockerin domains

AIMS: The worlds chemical and energy requirements rely on finite, fossil fuels resources. This causes environmental pollution, Greenhouse Gas (GHG) emissions and as a consequence global warming. A solution is to use GHG as the feedstock for chemical and fuel manufacture and gas eating bacteria. Here we set out to: (i) derive the gene tools needed to exploit one such bacterium, *Eubacterium limosum*, which produces both acetate (C2) and butyrate (C4) products, and; (ii) to use protein scaffolding to increase the yields of the industrially important chemicals acetone and isopropanol.

OUTCOMES: The project was able to successful design, build and test a comprehensive suite of gene tools for the chosen chassis. Transformation frequency was significantly improved, the utility of a vector based on a novel replicon from *Clostridium carboxidivorans* was demonstrated and genome editing exemplified by both *pyrE*-based alleleic exchange and CRISPR/Cas9. The effectiveness of a range of constitutive promoters needed for the proposed strain engineering was evaluated, together with our orthogonal inducible promoter, and a rank order generated based on expression of a *catP* reporter. Three different synthetic operons were assembled, comprising various combinations of genes encoding the requisite enzymes for isopropanol (IPA) production via acetone. One variation included cellulosomal dockerin and cohesion domains, and another included a thiolase gene in addition to acetyl-CoA acetyl-transferase, acetaldehyde decarboxylase and alcohol dehydrogenase. Successful production of IPA and acetone was achieved in both *E. limosum* and *Clostridium autoethanogenum*, although protein scaffolding had no effect on productivity.



OUTPUT: basis of BBSRC, BB/R021503/1: ERA-CoBioTech - BIOMETCHEM: "*Sustainable Production of Added Value Chemicals from SynGas-derived Methanol Through Systems and Synthetic Biology Approaches*". May 2018 – April 2021. Total award 1.8M €.

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