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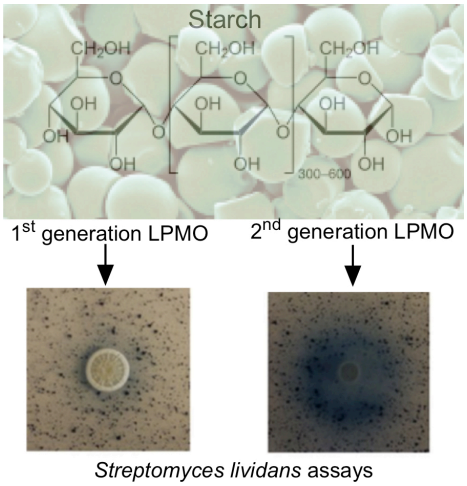
Creating new starch-active copper LPMOs through the generation of loop libraries

"We are pleased to be part of this research project as we believe that LPMOs will have significant role in starch hydrolysis and starch modification. So far the development of the LPMOs has progressed very swiftly and we are excited to see the next steps in this project." Johannes de Bie, WeissBioTech

Jonathan Worrall, University of Essex; Johannes de Bie, WeissBioTech

OUTCOMES: To create second-generation starch-active lytic polysaccharide monooxygenases (LPMOs), loops that form the active surface surrounding the catalytic copper ion were targeted. In total, there are five active surface loops that may be considered important for interaction and specificity with starch. Active surface loop libraries have been designed *in silico* with saturating mutations in selected amino acid positions in each loop based on sequence variability within the starch-active LPMO family. This business interaction voucher allowed for the synthesis — by combining site evaluation library and combinatorial library technology — of two out of the five loop libraries in a starch-active LPMO. These two loop libraries will be screened in *Streptomyces lividans* for enhanced activity relative to the wild-type LPMO. Following this, we will conduct *in vitro* characterisation of recombinantly produced proteins and then test the activity of the selected variants under industrial conditions.



Improving the degradation efficiency of insoluble starch granules through the creation of 2nd generation LPMOs.

INITIAL AIMS: The efficient deconstruction of plant biomass into biofuels and other chemicals is a key challenge to secure a low carbon economy. In nature, many microorganisms secrete enzymes that can break down recalcitrant biomass that is composed mostly of lignocellulose into soluble substrates. Harnessing the catalytic power of these enzymes to treat biomass outside of their natural habitats is challenging and a major goal of industrial biotechnology. Recently, a new class of enzyme that drastically increases the efficiency of biomass conversion has been identified. These enzymes contain a copper ion and are called lytic polysaccharide monooxygenases (LPMOs). The aim of this project is to assess whether second-generation LPMOs with enhanced substrate activities can be created. As a proof of principle, we will use a starch-degrading LPMO as a template to design and synthesize DNA libraries that will then be screened for substrate activity.

- Two loop libraries from a starch-active LPMO were synthesised
- Further project work between the academic and industrial partners is ongoing