HVB Funded Proof of Concept Projects

Round One 2019 **Development of a new biocatalytic process to generate high value chemicals from lignin**	
Lead applicant's name:	**Professor Neil Bruce**
University/ research institute:	**University of York/ CNAP**
Co-applicant's name:	**Professor Simon McQueen-Mason**
University/ research institute:	**University of York/CNAP**
Industrial Partner's name:	**Dr Kirk Schnorr**
Company:	**Novozymes A/S**

PROJECT ABSTRACT:

Lignin is a polyphenolic polymer that is a major structural component of plant secondary cell walls. It is the most abundant aromatic polymer in nature and has considerable potential as a sustainable source of valuable aromatic compounds that can replace fossil fuels. Recently, the flavone tricin was found to be a significant component of monocot lignin, such as that found in the secondary cell walls of wheat and rice. As tricin is a potentially valuable chemical there is significant interest in finding methods of extracting tricin from lignin. To date no efficient and economical way of isolating tricin has been found. Tricin has been reported to possess a range of pharmaceutical attributes, including anti-oxidant, anti-inflammatory and anti-aging properties. It has also been shown to have an immune-modulatory effect on macrophages and to be active against *Leishmania infantum*, the causative organism of leishmaniasis, but its current expense prevents any further in depth investigations.

Hundreds of millions of tons of agricultural residues are burnt in the field or discarded, which has negative impacts on the environment and wasting a potentially valuable resource. We are interested in investigating how microbes deconstruct these residues, with a focus on identifying new enzymes that can potentially valorise lignin. We have recently discovered a new ligninase from the soft rot fungus *Graphium* sp. that cleaves β-O-4 linkages in lignin, the aim of this project is to fully characterise the degradation products of lignin which includes tricin and establish a new method for the preparation of tricin from crop residues.

RESULTS REPORTED:

We developed an improved purification protocol for the new ligninase from *Graphium sp.* (now called *Parascedosporium putredinis* NO1) that allowed the enzyme to be highly purified in a more stable form for further characterisation. We established that the enzyme requires copper and molecular oxygen for activity and classed the enzyme as a new oxidase. The enzyme released the valuable compound tricin from wheat straw and we demonstrated by 2D heteronuclear single quantum correlation (HSQC) NMR that the enzyme was able to cleave the major structural β-ether units in lignin. We have also demonstrated that the oxidase releases p-coumaric acid and vanillin from wheat straw lignocellulose. We cloned and expressed the genes for four of the six homologues of the oxidase identified in the transcriptome of *P. putredinis* NO1 growing on wheat straw and have so far demonstrated that two of these proteins display β-etherase activity. The results from this proof-of-concept study have contributed to a publication describing the identification and characterisation of this new ligninase (Oates, N. C., Abood, A. Schirmacher, A. M., Alessi, A. M., Bird, S. M., Bennett, J. P., Leadbeater[a], D. R., Li, Y., Dowle[b], A. A., Liu, S., Tymokhin, V. I., Ralph, J., McQueen-Mason, S. J. & Bruce, N. C. 2021. A multi-

omics approach to lignocellulolytic enzyme discovery: uncovering a new ligninase activity from *Parascedosporium putredinis* NO1. Proc. Nat. Acad. Sci USA in press)

Round One 2019 **Producing Hyaluronic Acid in Acetobacter species**	
Lead applicant's name:	**Tom Ellis**
Position held:	**Professor of Synthetic Genome Engineering**
University/ research institute:	**Imperial College London**
Co-applicant's name:	**Vivianne Goosens**
Position held:	**Postdoctoral Research Associate**
University/ research institute:	**Imperial College London**
Industry partners:	**Ben Reeve**
Position held:	**CTO**
University/ research institute:	**Puraffinity**

PROJECT ABSTRACT:

Hyaluronic acid (HA) is a polysaccharide used in medical and cosmetic applications totalling a billiondollar industry. In humans, HA forms a intercellular matrix associated with protection against aging and cancer. Historically, rooster combs provided the industrial source of HA, however as pathogenic bacteria produce HA to 'hide' from the immune system, industry now uses these: with bacterial Hyaluronic Acid Synthase (HAS) systems producing animal-free HA. These *Streptococcus*-based biosynthesis methods have accelerated HA production, but manufacturing is hampered by bacterial endotoxins. High molecular weight HA is also desired but to generate longer chain lengths with *Streptococcus* requires expensive media supplementation. While most organisms do not innately secrete massive amounts of polysaccharides, *Acetobacter* species do, producing large yields of bacterial cellulose (BC). BC is produced cheaply at scale from these bacteria with low immunogenicity. Indeed, BC is routinely used in surgical implants. Importantly, due to the immense innate supply of glucose and UDP in BC-producing bacteria, these cells have the metabolic propensity to secrete long chains of sugars without supplementation. *Acetobacter* thus offer great potential as polysaccharide biosynthesis workhorses. Our team consists of a synthetic biologist with extensive tools and experience for engineering the Acetobacter *Komagataeibacter rhaeticus* and an industrial partner, Puraffinity, with a historic interest in industrial HA production. Together we are in the unique position to develop *K. rhaeticus* into a novel bacterial HA-production workhorse that, due to its native metabolism, would produce high valued long-chained HA from agroindustrial waste, in an animal free, and endotoxin-free manner.

RESULTS REPORTED:

Hyaluronic acid (HA) is a polysaccharide used in medical and cosmetic applications totalling a billion-dollar industry. In humans, HA forms an intercellular matrix associated with protection against aging and cancer. Current industrial production of HA associated with *Streptococcus*-based biosynthesis and manufacturing is hampered by bacterial endotoxins. High molecular weight HA is also desired but to generate longer chain lengths with *Streptococcus* requires expensive media supplementation. While most organisms do not innately secrete massive amounts of polysaccharides, *Acetobacter* species do, producing large yields of bacterial cellulose (BC) with low immunogenicity. Importantly, due to the immense innate supply of glucose and UDP, these bacteria have the metabolic propensity to secrete long chains of sugars without supplementation. *Acetobacter* thus offer great potential as

polysaccharide biosynthesis workhorses. Our team is in the unique position to develop the Acetobacter species *Komagataeibacter rhaeticus* into a novel bacterial HA-production workhorse that, due to its native metabolism, would produce high valued long-chained HA from agroindustrial waste, in an animal-free, and endotoxin-free manner. In this proof-of-concept study we successfully cloned and expressed the proteins required for HA synthesis in *K. rhaeticus*. Preliminary studies showed in excess of 11 mg/l amounts of HA produced. Follow on studies would focus on improving the production of HA (i.e. metabolically engineer cellulose-HA shift/media optimisation) as well as defining the quality/chain length of the produced HA.