Business Interaction Vouchers Round 2 (May 2020)

Development of an integrated process for the production of functional food additives from Jerusalem artichoke tubers	
Lead applicant's name	Dr Chenyu Du
University/ research institute	University of Huddersfield
Industrial partner	Michael Lewis
Company	Heugh Farm

Project abstract: Jerusalem artichoke (JA) is an underutilised crop that is currently used as cover for pheasant husbandry and is not harvested in the UK. JA has significantly higher biomass yields (~60 tonnes wet weight, 12 tonnes dry weight) per hectare than cereal crops such as wheat (~8 tonnes), and requires minimum fertiliser and maintenance. Inulin, the main carbohydrate component of JA is defined as a dietary fibre, which cannot be digested by human and can be consumed as a means of controlling blood sugar disorder. JA also contains high content of minerals and vitamins, which can be converted to a nutritional supplement.

In this project, we will explore the feasibility of extracting inulin from Jerusalem artichoke and producing a nutritional supplement by converting the remaining waste streams via yeast fermentation. A preliminary inulin extraction process has already been established at the University of Huddersfield (UoH), will be optimised in this project to increase extraction yield. The seasonable variation of inulin content will be determined. The high concentration of minerals and other bioactive compounds present in the JA hydrolysate after inulin crystallisation, will be utilised by marine yeast strains via aerobic fermentation to produce nutritional additives - specifically, spent yeast, a product similar to Marmite. A simplified techno-economic assessment of commercial production of these products from JA will be performed. The project will promote the UK Bio-economy, encourage the growth of an alternate crop for crop rotation, and generate a new market for high value products derived from Jerusalem artichokes.

Summary of project outcomes: This project demonstrated the viability of producing functional food additives from an underutilised crop, Jerusalem artichoke (JA). JA is currently used as cover for pheasant husbandry and is not harvested in the UK. It has significantly higher biomass yields of ~60 tonnes per hectare and requires minimum fertiliser and maintenance. Inulin, the main carbohydrate component of JA is defined as a dietary fibre, which cannot be digested by human and can be consumed as a means of controlling blood sugar disorder. This project analysed the inulin content in JA from ten different batches and confirmed that suitable inulin content is contained in JA tubers. The extraction of inulin from JA tuber was also tested at the lab scale. A promising extraction yield of 85% was achieved. Furthermore, conversion of inulin extraction waste into yeast biomass to produce a Marmite-style nutrient supplement was tested. Analysis demonstrated a high level of protein content accumulated in the selective yeast strain, which also contained high concentration of both iron and vitamin C, as well as significantly higher level of healthier potassium, rather than sodium, salts. A preliminary economic

assessment indicated that it is practicable to produce a healthy dietary fibre functional food and a protein/mineral rich nutrient supplement from this promising crop. In the next step, the team between the University of Huddersfield and its industrial partner Mr. Mike Lewis at Heugh Farm will study the JA based biorefining process at pilot-plant scale.

Characterising toxicity at the cell membrane during spinosyn biosynthesis	
Lead applicant's name	Dr Alan Goddard
University/ research institute	Aston University
Industrial partner	Dr E. Timothy Davies
Company	Dow Agrosciences Ltd. (Corteva Agriscience)

Project abstract: Corteva Agriscience are world-leaders in the production of spinosyns, the bioactive components of the Spinosad and Spinetoram insecticides. Spinosyns are high value, natural insecticides made from renewable resources by the bacterium *Saccharopolyspora spinosa* and, as such, have significant commercial interest. However, toxicity of specific, identified bacterial metabolites towards the producing *S. spinosa* cells limits production. The mechanism of this toxicity against *S. spinosa* is not clear.

Our initial findings suggest that these metabolites have direct effects on cell membranes – the thin barrier that separates the inside of the bacterial cells from the outside environment. Disruption of this fatty membrane kills the cells as key metabolic components leak out. In this project we will aim to characterise the interaction of the metabolites with the cell membrane to determine the mechanism of toxicity. We will achieve this using well-established model membrane systems that accurately reflect the true biological membranes of bacteria. This simplified system allows us to determine exactly how the metabolites damage the membrane; a more complete understanding of this process will allow us to define rational routes to generating more resistant *S. spinosa* and improving the efficiency of this biotechnological process.

Summary of project outcomes: Corteva Agriscience are world-leaders in the production of spinosyns, the bioactive components of the Spinosad and Spinetoram insecticides. Spinosyns are high value, natural insecticides made from renewable resources by the bacterium Saccharopolyspora spinosa and, as such, have significant commercial interest. However, toxicity of specific, identified bacterial metabolites towards the producing S. spinosa cells limits production. We have demonstrated that these specific metabolites are toxic to the cell membranes surrounding S. spinosa.

Using model cell membranes known as liposomes, and a particular dye, we have demonstrated that the metabolites generate pores within the membranes. This causes leakage of the dye and protons. Together, these data imply a model by which metabolite toxicity occurs through the generation of pores in the cell membrane and consequently a loss of cellular components, alongside disruption of the proton gradient that is essential for energy generation. This model provides a route for strain engineering to increase tolerance to these metabolites.

Development of high-value functional food supplements from an algal biorefinery	
Lead applicant's name	Professor Jeffrey Pearson
University/ research institute	Newcastle University
Industrial partner	Dr Donal McGee
Company	AlgaeCytes Ltd.

Project abstract: AlgaeCytes Ltd (AC) is a UK SME that has developed an innovative and sustainable process for the biorefinery of high-value metabolites from microalgae. AC has developed a diverse product pipeline including omega-3's (EPA), carotenoids and peptides with novel bioactivity. Omega-3 EPA from AC microalgae strain ALG01 has been demonstrated to stimulate fibroblast generation *in-vitro* opening up new opportunities in the nutritional and skincare markets. Carotenoids and hydrolysed peptides are two value-added co-products generated from AC's algal biorefinery process. Algal carotenoids possess a higher bioavailability compared to their synthetic counterparts, therefore there is a consumer preference for these natural antioxidants. Algal derived peptides have been demonstrated to possess diverse bioactivity including; antioxidant, antimicrobial, antihypertensive, antithrombotic, anti-proliferative and anti-inflammatory activities [1–4].

There is an increasing market demand for natural and vegan algal-based functional foods and nutraceutical supplements. However, the majority of these microalgal metabolites are yet to be fully evaluated for their efficacy, bioavailability and toxicology. AC seeks to build a research collaboration with Prof. Pearson to determine the bioavailability, bioactivity and safety of AC's bioactive algal products in a novel, patented model gut system that simulates the human digestive tract.

The Pearson lab has a track record of using *in-vitro* simulations of the digestive tract to accelerate the commercialisation of bioactives and functional food products having worked with industry partners including; Britvic, Suntory, Capsugel and DuPont on pre-clinical product development.

The outputs of this HVB BIV is within scope of the BBSRC's Industrial Biotechnology strategy, as it will inform AC's product development strategy, provide crucial data for regulatory approvals and aid in defining key process performance indicators (KPIs) for the commercial production of these bioactives form AC microalgae strains.

Summary of project outcomes: The main objectives of the project were to undertake *in vitro* testing to gain data on bioavailability, bioactivity and safety of AlgaeCytes Ltd bioactive products in a model gut system and cell lines for use in product formulation and development. This goal has been met with a comprehensive data report giving Bioactive effects including enzyme inhibition and promotion of anti-inflammatory cytokines were observed, suggesting that these compounds could be used for weight management and control of inflammation.

COVID-19: Investigating the viral inhibitory effects of sulphated, polysaccharide heparin-analogues with the SARS-CoV-2 Spike RBD protein	
Lead applicant's name	Dr Mark Skidmore
University/ research institute	Keele University
Industrial partner	Ruth Yates
Company	Anglo-Italian Chemometrics Limited

Project abstract: Pharmaceutical heparin, the approved anticoagulant, inhibits infection by SARS-CoV-2 of host cells and has been used to treat COVID-19 patients with promising results.

Heparin, an established and safe pharmaceutical nevertheless suffers from a global supply problem: It is in demand in developed and, increasingly, also in developing countries but, is almost entirely sourced from Chinese pig intestines. Around 1 billion animals are required per year and pig populations themselves are threatened, having been reduced 30% by recent swine fever infections.

Working with our biotechnology industry partner, we propose several alternative routes to generate analogous compounds to heparin that are sustainable, renewable and of high value; first, by modifying existing plant and algal polysaccharides; second, using an established synthetic method starting from simple sugars to build polysaccharides rapidly. The raw materials originate from plants/algae or as by-products/waste from bioethanol and other industrial production processes. The final step introduces charged sulfate groups chemically or enzymatically.

Products will be tested in established assays of coagulation, inflammation and virus receptor binding (as a proxy for virus attachment to cells); the 4 most active being structurally characterised. These will be used to produce a discovery platform of tailored-made, heparin-analogue polysaccharides.

The project will provide our biotechnology partner with a blueprint for the modification of polysaccharides from renewable sources which can be fed directly into our in-house activity assays relating to viral activity to tackle this and future viral threats.

Summary of project outcomes: Pharmaceutical heparin inhibits infection by SARS-CoV-2 of host cells and has been used to treat COVID-19 patients with promising results. Heparin, an established and safe pharmaceutical, nevertheless suffers from a global supply problem and potential, future anti-viral use of this approved anticoagulant would place significant pressures on an already saturated supply chain.

Working with our biotechnology industry partner, we have generated analogous compounds to heparin that are sustainable, renewable and of high value. The raw materials originate from plants and algae, or as by-products/waste from bioethanol and other industrial production processes. These compounds have provided a discovery platform of tailored-made, heparin analogue polysaccharides with highly favourable antiviral bioactivities.

This novel resource of heparin analogues has been screened for biological activity against SARS-CoV-2, the causative agent of COVID-19, and potent inhibitors of viral attachment have been identified for further research. This data will feed directly into our additional in-house bioactivity assays and studies to tackle this, and future, viral threats.