Joint HVB and IBioIC Call

HVB Funded Projects:

Research to create new spin-outs **Royal Holloway, University of London (RHUL) Laboratory Products; creating commercial activity from knowledge transfer**	
Lead applicant's name:	**Paul Fraser**
Co-investigator's name:	**Genny Enfissi**
Research Co-investigator's name:	**Laura Perez-Fons**
University/ research institute:	**Royal Holloway, University of London**

PROJECT ABSTRACT:

Most of the bright colours observed in nature, such as the orange and red colours witnessed in flowers are due to the presence of carotenoid pigments. Carotenoids represent the largest class of natural pigments found in nature. In addition to an intrinsic ability to confer colour, most carotenoids have health promoting benefits to humans and therefore are an important/essential component of the human diet as carotenoids cannot be made in the body. Commercially, carotenoids have widespread uses in the food, feed, cosmetic, health and pharma sectors as colourants, health supplements and phytomedicines. Annual sales of carotenoids are over $1 billion and are increasing rapidly (18%/yr.), as the demand for natural carotenoids outstrips supply. Chemical synthesis has traditionally been the production method of choice for carotenoids. However, this approach has an adverse environmental impact, due to the use of petrochemical by-products, organic solvents and metal catalysts.

The laboratory at Royal Holloway, University of London has been working on carotenoids for over fifty years and routinely make their authentic reference carotenoids in-house from biobased sources. Requests are frequently received from both the academic and industry sectors for carotenoid reference compounds. In the present proposal, knowledge transfer from our research activities will be carried out to generate a range of carotenoid reference compounds, at scale, for direct entry into the market place. The approach uses biobased waste streams as the feedstock and will generate speciality chemicals of industrial value, these characteristics are synergistic to the scope of industrial biotechnology.

RESULTS REPORTED:

The POC award has enabled the team at RHUL to build on forty years' experience in studying carotenoids. The procedures developed over many years have been used to scale-up production of carotenoids and develop stock materials for commercial activity. An important feature of the process is the use of generic horticultural by-products which would have been incinerated. The process enables us to take diverse horticultural biomass and generate multiple pure compounds.

A website (www.naturalcompoundsolutions.com) been established along with brochures, and the legal documentation in place for selling these compounds. The research staff taking on this challenge have gained valuable commerical experience first-hand and the staff member is now a HVB enterprise fellow. Upon launch at ChemUK organised by HVB and BioVale, several customers were received the following day. The enterprise fellowship will enable the group at RHUL to grow the business and expand its

product portfolio and diversify the customer base as well as incorporating more sustainability aspects to the process.

HVB products/ processes for industry

Chemoenzymatic upcycling of biocatalytic reaction waste: harnessing access to high value chiral building blocks

Lead applicant's name:	**Sebastian Cosgrove**
Co-investigator's name:	**Gavin Miller**
University/ research institute:	**Keele University**
Industrial Partner's name:	**Beatriz Dominguez**
Company:	**Johnson Matthey**

PROJECT ABSTRACT:

The mass production of waste is a pressing issue in society, with the majority of it being sent to landfill or burnt for energy. This causes many societal and environmental problems. Being able to convert waste to a high-value product can therefore offer solutions to this issue from economic and environmental perspectives. This project will use a waste material to make a chemical that can be used to produce nylon in an environmentally friendly way. Nylon is currently made from fossil-based resources in an unsustainable manner, and worldwide we produce around six million tonnes every year. It is therefore essential that we come up with new, more sustainable ways to produce the chemicals that are important in nylon production. The way this project will address the issue is by improving the way we can make a chemical called glucaric acid, which can be easily turned into one of the main components of nylon, from waste biomass resources that are made from glucose. It will use new biotechnologies to improve the way the waste glucose is converted to glucaric acid; allowing this new method to replace the old, fossil-based methods that are currently used will strengthen the UK industrial bioeconomy by making chemicals from biomass waste using bioprocesses. The benefits include a reduction in greenhouse gas emissions associated with nylon production, and the use of a free resource that would otherwise just end up in the bin, therefore reducing overall costs as well.

RESULTS REPORTED:

The development of environmentally benign chemical processes is essential as the chemical industry is one of the most polluting in the world. This aim of this project was to design a bolt-on system to capture a waste product of many industrial enzyme processes. This waste product would ordinarily be disposed of down the drain and then convert it to a key precursor for Nylon. The precursor, namely glucaric acid, is currently produced from fossil sources in an energy intensive process. Our bolt-on system is able to completely recover the precursor to glucaric acid and therefore not only reduce the waste significantly, but also provide a free resource that could be used to make green Nylon.

This project has also optimised a biocatalytic process using enzymes provided by the industrial collaborator, Johnson Matthey. The process that has been developed is coupled to the waste recovery system so produces no waste, but will also be able to replace current chemical methods that use expensive precious-metal catalysts and require high pressures of flammable hydrogen gas to work.

HVB products/ processes for industry

Commercial validation of a *Saccharomyces cerevisiae* production platform for bioactive diterpenes

Lead applicant's name:	**Ian Graham**
Co-investigator's name:	**Katherine Denby**
Research Co-investigator's name:	**Tomasz Czechowski**
University/ research institute:	**University of York**
Industrial Partner's name:	**Timothy A Miller**
Company:	**Croda Europe Ltd**

PROJECT ABSTRACT:

Diterpenoids are a chemically diverse group of natural products that have the potential to replace petrochemical derived feedstocks and deliver new functionality for various industrial applications. However, the typically low natural abundance and high structural complexity limits the development of industrial applications for these compounds. Many different classes of diterpenoids have reported bioactivity but their industrial utility has not been explored. Developing new production platforms by engineering microorganisms such as brewers yeast (*Saccharomyces cerevisiae)* is now recognised as a valid route to commercial production of these small molecule natural products.

The academic partner has already demonstrated that they can produce a relatively simple bioactive diterpene molecule, casbene, by engineering a novel strain of brewers yeast with genes taken from plants using a strategy that does not rely on existing intellectual property from other sources. The industrial partner, Croda, will evaluate performance of this strain under conditions that relate to their production facilities and will also evaluate the commercial relevance of casbene for industrial application across their agri-tech and personal care sectors.

Typically diterpene molecules become more bio-active and useful when the 20-carbon backbone is decorated with additional chemical groups. Oxidation is the main type of modification. Using gene tools already at hand the academic partner will engineer bioactive diterpenes reported to protect plants from fungal pathogens, perform laboratory bioassays using two fungal pathogens that cause disease in UK crops and transfer the resulting yeast strains to the industrial partner to validate the production platform and commercial relevance.

RESULTS REPORTED:

Engineering of the yeast strain led to enhanced casbene production, as did optimisation of growth conditions in state-of-the-art bioreactors prior to transfer of strains to the industrial partner.

Typically, diterpene molecules become more bio-active and useful when the 20-carbon backbone is decorated with additional chemical groups. Oxidation is the main type of modification. The academic partner engineered production of a number of oxidised diterpenes in brewer's yeast and performed laboratory bioassays using two fungal pathogens that cause disease in UK crops.

The casbene and oxidised casbene compounds were transferred to the industrial partner and bioassayed under industrial conditions and evaluated against commercial compounds. Two or possibly three new leads have been identified for further evaluation and product development for application in the agri-tech sector.

Results Reported:

- Strains producing high titres of precursor bioactive molecules were developed through metabolic engineering.
- Bioactivities for several molecules were identified in collaboration with the industrial partner and these are undergoing further evaluation as lead candidates for product development for application in the agri-tech sector.

HVB products/ processes for industry

Application of high-throughput enzyme evolution using artificial selection pressure to real pharmaceutical biocatalysis challenges

Lead applicant's name:	**John Heap**
Co-investigator's name:	
Research Co-investigator's name:	
University/ research institute:	**University of Nottingham**
Industrial Partner's name:	**Francesco Falcioni**
Company:	**AstraZeneca**

PROJECT ABSTRACT:

Catalysts are essential to increase chemical reaction rates in a wide range of industries, and biocatalysts (enzymes) have various advantages. The non-natural needs of industrial applications often require enzymes with new or improved properties, but these are very difficult to design rationally. Instead, approaches mimicking evolution generate huge numbers of variants of existing enzymes. Improvements are very rare, but usually variants must be tested individually, a process like searching for a needle in a haystack. We have recently developed a new artificial selection pressure (evolution's 'survival of the fittest' principle) technology, involving genetically modified cells and special incubation conditions. It is broadly applicable, unlike other such systems. The system has been successfully used in our laboratory to evolve multiple types of new enzyme variants, and metabolic pathway variants, with desired improvements. This system is industrially relevant, and several companies are interested.

The project industrial partner is the pharmaceutical company AstraZeneca. This project will apply the new enzyme evolution technology to biocatalysis challenges of direct relevance to pharmaceutical research and manufacturing, by seeking new enzymes or variants (1) as 'greener', sustainable replacements for an existing step in the production of a candidate drug, and (2) to overcome difficulties in production of a class of chemicals which show promise as possible future medicines. Significantly, success of either aim would achieve 'Proof of Concept' for industrial application and increased 'technology readiness level', warranting further public and private investment, and opening the way towards new medicines and environmental and economic benefits.

RESULTS REPORTED:

We applied the new enzyme evolution technology to the intended biocatalysis challenges of direct relevance to pharmaceutical research and manufacturing: (1) We generated new DNA sequences to produce two enzymes thought to have a low level of the desired ability to perform the target chemical reaction step. These enzymes serve as starting points for the project and improvements. We inserted these DNA sequences into cells. Unexpectedly, we learned that the candidate drug precursor had certain properties which made it difficult to successfully work with under the relevant laboratory conditions. Therefore, substantial time and effort was spent exploring a range of strategies to tackle this challenge. The results and approaches developed are useful to guide future work with this candidate drug precursor and other chemicals which might have similar properties. (2) We performed experiments to test the compatibility of an implementation of our technology with a type of enzyme activity potentially important for the production of a class of chemicals which show promise as possible future medicines. The industrial partner proposed a list of many suitable chemicals, we assessed the chemicals on this list based on several criteria, then tested many combinations of the chemicals with the technology. We obtained a promising result which seemed to indicate that this implementation of the technology is indeed compatible with this important class of enzymes. This represents successful proof of concept. Next, this result needs to be repeated and then built upon. Potentially commercially sensitive details are omitted from this public summary.

IBioIC Funded Projects

2020 HVB products/ processes for industry (IBioIC funded)

Title: Bioactivity of Compounds Derived from *Amaryllidaceae* (Daffodil)

Lead applicant's name:	**Cherry Wainwright**
Co-investigator's name:	**Sarah Walsh, Giovanna Bermano**
Research Co-investigator's name:	
University/ research institute:	**Robert Gordon University**
Industrial Partner's name:	**Kevin Stephens**
Company:	**Agroceutical Products Ltd**

PROJECT ABSTRACT:

Heart Failure (HF) is a long-term complication of cardiovascular (high blood pressure and heart attack) and cardiovascular-related (obesity and metabolic syndrome) disorders and of drug-induced cardiotoxicity. HF is characterised by thickening and stiffening of the walls of the heart, leading to reduced cardiac function and increased morbidity. Agroceutical Products Ltd (APL) produces sustainable quantities of the naturally derived alkaloid galanthamine from daffodils, which is a key active pharmaceutical used for the treatment of Alzheimer's disease. Daffodils contain an abundance of other alkaloid compounds, some of which APL can extract alongside galanthamine. There is emerging evidence that some alkaloids may target cellular events in the heart to interrupt the HF-associated structural changes, and thus have the potential for development as novel therapeutics in the prevention of HF. This project will involve testing several alkaloids extracted from daffodils in cell-

based models that mimic the different stimuli for cardiac hypertrophy, to determine their effectiveness, and gain insight into cellular mechanism of action. This will provide essential “proof of concept” data that will identify the most efficacious compounds, inform the refinement of extraction methods to isolate different alkaloids, and potentially identify what structure-activity relationships may exist between alkaloids. This will inform APL as to which alkaloids have the potential to bring added value to their current production stream which will, in turn maximise the commercial potential for their products.