



# BIOTECH FOR BUSINESS

## Green to Gold with CircBio

Grass is the most abundant land resource in Ireland, accounting for over 50% of total land use and 90% of agricultural land use. While forming a key part of the economy, supplying food nationally and internationally and providing rural employment, grass-based farming systems such as dairy are a major contributor to Ireland's greenhouse gas emissions. Grass biorefining, which involves the processing of grasses and other green protein-rich biomass into multiple products can offer opportunities to overcome emissions challenges in traditional agriculture, while helping to provide protein independence and offering diversification opportunities for dairy and beef farmers through extraction of multiple value added products.

Over the last two years, the Circular Bioeconomy Research Group (CIRC BIO) at Shannon ABC, MTU have been evaluating and demonstrating green biorefinery approaches alongside Carbery Group and other partners on a number of projects including Biorefinery **Glas** and **Farm Zero C**.

**Biorefinery Glas:** Through the DAFM-funded Biorefinery Glas initiative, partners produced a range of novel co-products from grass using an integrated biorefinery process and tested these co-products in a variety of applications. These include:

- Presscake – a sustainable replacement to silage in dairy cow diets, delivering comparable milk but with 20% lower nitrogen losses, and 15% less rumen methane production
- Protein concentrate – a sustainable protein co-product alternative to soybean meal in monogastric diets, which has demonstrated increased weight gain in pigs of 10%
- Fructo-oligosaccharides – a prebiotic solution extracted from grass biorefinery which has exhibited similar performance to on-the-market prebiotics
- Whey – grass biorefinery whey is the residual biorefinery stream and represents an excellent and free feedstock for anaerobic digestion, helping to meet the biorefinery energy needs and showing a clear synergy between biorefinery and biogas systems.



**Farm Zero C:** The SFI funded Farm Zero C initiative targets an economically viable, resilient, net-zero emissions model for the dairy sector through integration of diverse strategies including sustainable land management and agronomy, anti-methanogenic animal feeds, renewable energy and grass biorefining and increasing farm natural habitats. Through Farm Zero, Shinagh Farm in Cork will act as a demonstrator farm, with replication through satellite farms, achieved throughout the project. The project will also develop a carbon neutral app, integrating life cycle assessment and natural capital data which will allow farmers to understand the environmental impacts of their own farms, and the strategies best suited to mitigating these impacts. Farm Zero C is a collaboration between BiOrbic, Carbery, UCDD, Trinity College Dublin, MTU, Teagasc and GRASSA.

## WAVA team progress to Seed Phase of SFI's Future Innovator prize of €2 million

"Valorising food Waste into Value added commodities" (WAVA) is one of the four Science Foundation Ireland's (SFI) Future Innovator Prize food challenge projects shortlisted in the Seed phase for a prize fund of €2 million. "WAVA is an example of transformative applied biosciences research at Shannon Applied Biotechnology Centre in LIT. The research team will develop disruptive enabling technologies to reduce food loss and waste across the food supply chain from "farm to fork" as commented by Dr Patrick Murray, Head of Research and Technology Transfer, at LIT. This project aims to help in future global food security and reduce greenhouse gas emissions (GHG) by acting as CO<sub>2</sub> sink.

Valorisation of food waste is an important goal of sustainable development. Food waste represents a valuable resource for renewable energy, nutrients for new healthy crops, food and feed supplements, and commercially important fatty acids production to replace controversial palm oil. In total, 4.2 million tonnes of food-waste is generated annually in Ireland, of which the food sector accounts for 19%, producing 36,900 tonnes of fruit and 758,650 tonnes of vegetable waste annually.

The Shannon ABC led team, working with BiOrbic Bioeconomy SFI Research Centre, is developing an integrated technology platform to turn food waste, destined for landfill, into useful commodities. The technology will be developed at two operational scales (household to farm scale and at industrial scale) to efficiently recycle the food waste across the food chain, thus providing a unique zero-waste circular bioeconomy solution.

This technology at small scale will consist of a plug and play integrated anaerobic digester (AD) for complete recycling of all types of food waste generated at household, restaurant or farm scale. The AD will provide biogas for user's energy requirements while CO<sub>2</sub> and liquid-digestate from the AD will be used for microalgae cultivation in an integrated reactor. The resulting microalgal biomass will be used for feed and other applications. At industrial scale, this integrated system will have additional features of novel pre-treatment of AD feedstock and post-treatment of digestate. The AD process will be modified to produce high value fatty acids as a substitute for palm oil. The treated AD effluent and CO<sub>2</sub> from the AD reactor will be used as nutrients for microalgae cultivation and will be harvested and sold for extraction of nutraceuticals or used as animal feed.

Overall, this complete robust and circular system, will be able to fully valorise food waste using a modified AD process to produce food crops, nutritional ingredients from microalgae, palm-oil alternatives, balanced environmentally safe fertiliser and sustainable energy while acting as a CO<sub>2</sub> sink.

The core team includes Dr Sushanta Kumar Saha (Shannon ABC, Limerick Institute of Technology (LIT)); Dr Ajay Menon (BiOrbic, University College Dublin (UCDD)); Dr Lena Madden, (Shannon ABC, LIT), Prof James Lyng (BiOrbic, UCDD) and Mr. Adam Lord of Food Surplus Management Ltd. (FSM).

## SHANNON ABC

# How can High Performance Thin Layer Chromatography (HPTLC) help your company?

In Enterprise Ireland's Institutes of Technology Capital Call 2019, Shannon ABC were successfully awarded a HPTLC suite to the value of €105,000. This suite is the first of its kind in Ireland available for commercial and research projects, and can be used for a range of applications:

- Pharmaceutical - Quality Control; Content Uniformity Test; Identity and purity checks; stability tests
- Herbals – Identification; Stability tests; Detection of adulteration; Assay of marker compounds
- Clinical - Lipids; metabolism studies; drug screening; doping control
- Food and Feed - Quality Control; Analysis of additives e.g. vitamins; pesticides; stability tests
- Cosmetics - Identity of raw material; Analysis of preservatives, colouring materials etc; screening of illegal ingredients
- Biotechnology - Characterization of enzymes (product profiles); Proteomics (coupling HPTLC to Mass Spectrometry); Process development and optimization;
- Process monitoring - Cleaning validation,
- Environment - Water; soil; residue analysis
- Forensics - Molecule investigation; dyestuff analyses

High Performance Thin Layer Chromatography (HPTLC) gives much greater resolution and separation of components than normal TLC. It uses chromatographic stationary phases with excellent separation efficiency and employs state of the art instrumentation for all steps in the procedure. This includes precise sample application, standardized reproducible chromatogram development and software-controlled evaluation. HPTLC shows at a glance the similarities and differences between samples and references. Therefore, it can be used for analysis of raw materials & finished products, for the determination of purity (adulteration/fraud) and stability studies (shelf life). It can also be used for process development i.e. samples can be analysed at different stages of a process. By using reference standards, compounds can be quantified precisely.

HPTLC is a rapid cost-effective method in comparison to other separation methods and analysis of many samples in parallel takes typically less than an hour to run. As HPTLC has a non-destructive nature, analytes can be eluted after separation and identified using our existing equipment in laboratory e.g. Mass Spectrometry.



## Shannon ABC Cell Culture Suite

**Cell culture is the process by which cells can be grown under controlled conditions and can be an excellent way to determine the mode of action and efficacy for a wide range of compounds.**

Cell culture is a strategic starting point for testing ingredients in a relevant cell line to assess their potential as a novel ingredient or formulation. This provides a valuable screening tool to enable companies to make informed decisions regarding the performance and impact of their ingredients or products.

Shannon ABC has capabilities within cell culture to examine many areas, such as toxicity, cell proliferation, inflammation, immune response, antioxidant activity and production of a range of biological end-points. Shannon ABC has two independent cell culture suites providing the necessary conditions for maintaining healthy and contamination-free cell lines.

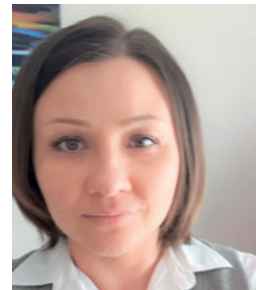
Cell culture suites are fitted with state-of-the-art equipment required for the routine culture of cells. The latest addition to the cell culture laboratory is the multiplex ELISA instrument funded from the Enterprise Ireland 2020 Capital Equipment Call. Enzyme-linked immunosorbent assay (ELISA) is an extremely sensitive technique designed for detecting and quantifying peptides, proteins, antibodies, and hormones. ELISA is one of the most commonly used methods in the Shannon ABC laboratory. It allows detection of specific protein marker secreted by cultured cells and quantifies the effect of different products on various mammalian cells. The multiplex ELISA equipment enables detection of up to 50 targets in a single reaction volume while dramatically reducing sample input and time needed for the analysis. Our new multiplex ELISA equipment can be used by multiple industries that seek detection of a variety of agents by ELISA and include biopharmaceutical media development, feed, food, cosmetic, and nutraceutical industries which seek testing performed in the cell culture laboratories.



## MEET THE TEAM

Shannon ABC is a collaboration between Limerick Institute of Technology and Munster Technological University and the Centre brings together a multidisciplinary team of researchers with commercial specialists so as to provide a centre of excellence in applied research, capable of exploiting opportunities in science and technology to the benefit of the Regional and National economy. Each quarter we will introduce you to some of our team.

**DR LENA MADDEN** is a Business Development Scientists in Shannon ABC. Lena holds a Degree in Pharmaceutical and Forensic Analysis and a PhD in Bio-nano Material Science, both completed at Limerick Institute of Technology. Lena has completed two industrial postdoctoral research projects in the area of environmental engineering and is a Principal Investigator in the area of biotechnology, analytical chemistry, environmental and materials engineering. Lena supports the management of all technical aspects of laboratory operation, infrastructure, instrumentation performance and training, project and product development for industrial clients in biotechnology and environmental engineering. Lena has a wide range of research interests that include circular bio refineries, with a focus on anaerobic digestion coupled to algal purification; waste valorisation; and hemp and cannabinoids research.



**DR AOIFE CURRAN** received her BSc (Human Nutrition) and PhD from University College Dublin (UCD). Aoife currently holds the position of Research Scientist with Shannon ABC and is responsible for the design and execution of biotechnology and cell culture based projects, recruitment of volunteer panel for skin probe testing and proactive delivery of projects for companies in the biotechnology, food and cosmetic sectors. Aoife's PhD research focused on pancreatic beta cell function- where she investigated factors related to beta cell function and determined nutrition strategies to improve it. Her research was part of the Food for Health Ireland program, where she performed a human intervention study to establish the bioavailability of milk bioactives and their effect on glycemic control. In vitro experiments were also an important aspect of her research, to support findings of human studies and to determine mechanisms of action. Dr Curran is first-author on four peer-reviewed publications. More recently, Aoife held a Scientific Writer position with Nuritas, where she gained invaluable experience across several research areas.

**CIARA DAVIS** is a postgraduate student with the CELLS group at Limerick Institute of Technology. Ciara's project will aim to increase the nutritional profile of plants using hydroponic methods under the supervision of Dr. Peter Downey and Dr. Siobhan Moane. Ciara finished her degree in Pharmaceutical and Industrial Chemistry at the University of Limerick with first class honours. As part of her degree, Ciara undertook her final year project on synthesising nanoparticles through redox reactions. Ciara has also undertaken a 7 month placement at the Institute for Molecular Bioscience, Brisbane, where she worked on synthesising antibiotic peptides.



**OLUSOJI DEMEHIN** obtained a B. Tech (Hons) in microbiology from Ondo state university of science and technology, Nigeria in 2016. As part of his degree, he studied the microbial and biochemical aspects of "Ogiri", an Africa food condiment in the southern region of Ondo state, Nigeria. He also undertook six months industrial training in the federal institute of industrial research, Oshodi (FIIRO), Lagos, Nigeria. During this period, he participated in various projects such as the production of phytase enzyme from soil microbial isolates, utilization of agro-industrial waste as substrates in the production of the mushroom *Plerotus pulmonarius*, production and analysis of fermented melon. Upon the completion of his undergraduate degree, Olusoji did a year national service with the federal government of Nigeria during which he worked as a peer educator trainer under the national youth service corps reproductive health and HIV/AIDS prevention, he also taught chemistry and biology in classes.

In 2018, he received a Master by Research scholarship from MTU, Kerry to work on a project entitled "bioconversion of mushroom wastes into value added plant biostimulants" under the supervision of Dr. Maha Attjioui. The project involves the production and characterization of plant biostimulants from mushroom waste and their application on different plant systems to improve plant growth and yield and enhance their tolerance to abiotic stresses.



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**TECHNOLOGY GATEWAYS**  
 delivering solutions for industry  
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### In June 2021 Shannon ABC hosted an Online Funding Innovation Industry Webinar for current and prospective clients.

We were delighted to have some of our industry partners present on the day, on how they have engaged with Shannon ABC through a range of funding mechanisms. The presenters were John Deaton, VP Technology, presenting for Deerland Probiotics & Enzymes; Michael O'Neill, MD, for Allihies Seafood and Stephen Costello, Director, SPV Healthcare. We also exhibited a virtual tour of our laboratories and we spoke about how Shannon ABC provides help to support Irish companies. We also launched our promotional video at the webinar which can be viewed on our Shannon ABC YouTube channel.

## Case Studies

### THE COMPANY

Deerland Ireland R&D Limited

### FUNDING SOURCE & VALUE:

Enterprise Ireland Innovation Voucher €5,000

### PROJECT TITLE

Investigation of probiotic bioactivity of Bacillus species product

### PROFILE OF COMPANY

Deerland Ireland R&D Limited (part of Deerland Probiotics & Enzymes) specialize in developing science-backed solutions that benefit microbiome health, using probiotic, prebiotic and enzyme technology. With a global team of industry experts working in state-of-the-art facilities, they are positioned to serve customers from the initial stages of product conceptualization, through the design phase, final formulation and contract manufacturing.

### PROBLEM TO BE SOLVED

The purpose of the study was to investigate the efficacy and activity of one of Deerland's novel probiotic strains of Bacillus. The company have a number of clinical trials showing this strain of probiotic is effective in supporting digestive and immune health in humans. However, little is known around the actual mechanism of action responsible for the efficacy of this unique strain. Gaining insights into the mechanisms helps support development of the product to allow further innovation beyond the currently available products on the market. Understanding these details in human subjects is complicated and Deerland does not support animal testing, therefore these studies were conducted in cell culture models in Shannon ABC using intestinal epithelial cells (Caco-2 cells).

### HOW GATEWAY DELIVERED SOLUTION FOR INDUSTRY:

Two in vitro experiments were performed to investigate the antimicrobial activity of the Bacillus strain against two common pathogenic bacteria strains, and to assess the adhesion ability of the strain to the mammalian colorectal Caco-2 cell line (a key probiotic trait). The obtained results in this study showed a growth inhibitory effect of the Bacillus strain against one of the pathogenic bacteria strains and confirmed its ability to adhere to Caco-2 cells. Both findings highlight desirable traits of the product for Deerland, encouraging them to proceed to the next steps in their product development with this particular strain. The findings have given the company an understanding of the adhesion and antagonistic activity of their unique strain and this developed model can be a base for investigations of next generation strains in the company's R&D pipeline.

### IMPACT FOR THE COMPANY

The findings have given the company a scientific understanding of the probiotic activity (i.e. adhesion and antagonistic activity) of one their unique strains, helping to enhance their marketing potential for this product, particularly in the European market where they are looking to strengthen their presence. Additionally, the techniques and protocols developed in this project will be able to be utilised in mechanistic studies of additional probiotic strains coming through their R&D pipeline. Having a scientific understanding of the mechanisms behind how their strain confers digestive and immune health in the gut adds further credibility to their product and company as a whole, opening up marketing avenues and gives an opportunity for them to apply for health claims in the EU.

### COMPANY TESTIMONIAL:

*"Working with the experienced team at Shannon ABC, we have been able to investigate crucial properties of our probiotic strain. The results obtained through this study has allowed us to strengthen our sales and marketing position for the strain as well as providing support to our R&D pipeline. Following on from this project, we have carried out a number of other research projects with the team which has allowed us to remain a strong scientifically-driven company in the area of probiotics. We would highly recommend partnering with Shannon ABC to any company looking for high quality scientific research."*

**Alison Winger, Director, Deerland Ireland R&D**

