An Roinn Talmhaíochta, Bia agus Mara
Department of Agriculture, Food and the Marine

Food Institutional Research Measure

Final Report

'Novel prebiotics from plant-derived sugars using bifidobacterial enzymes'

Acronym: Prebiozymes

DAFM Project Reference No: **11/F/023**

Start date: **01/07/2013 (NB original project start date was 01/11/2012, this was subsequently amended and approved to 01/07/2013)**

End Date: **31/12/2017 (NB approved from original end date of 31/10/2016)**

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Collaborating Research Institutions and Researchers: Moorepark Food Research Centre (MFRC), Prof Catherine Stanton

Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental → Applied → Pre Commercial

1	2	3X	4	5	6	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report.

Priority Area (s)	**H Food for Health**

Key words: Prebiotics, bifidogenic, functional foods, probiotics

1. Rationale for Undertaking the Research

Functional foods represent a rapidly growing segment of the European food market, and in particular probiotic and prebiotic products have enjoyed increasing popularity. Both probiotics and prebiotics aim to positively influence host health through modification of the intestinal microbiota, yet each do this in a distinct manner. Although probiotic products appear to be more popular than prebiotic foods, the use of prebiotics may arguably represent a more effective manner to modulate the intestinal microbiota. Non-digestible oligosaccharides, such as fructo-oligosaccharides (FOS) and trans-galacto-oligosaccharides (TOS/GOS), are the most widely used prebiotics and are sold under various brand names such as Actilight and Oligomate 55. TOS/GOS is currently incorporated in many formula milk powders as it supplements such milk with components that stimulate growth of bacteria that are naturally present in the developing healthy infant gut. TOS has been shown to act as a growth-promoting agent of bifidobacteria in particular and for this reason is associated with various health claims. In Europe TOS is commercially produced by certain dairy companies from lactose, which is obtained from whey, using yeast or bacterial β-galactosidases.

The rationale for the Prebiozymes project was borne out of the need to develop novel prebiotics using an enzyme-based biosynthesis approach. We reasoned that the use of bifidobacterial enzymes to produce oligosaccharides (OS) would allow the development of prebiotics with enhanced specificity to selectively promote bifidobacterial growth. The starting materials used for the production of such novel oligosaccharides were common, simple and cheap sugars such as sucrose and maltose.

2. Research Approach

The research approaches taken were based on the availability of the genome sequence of *B. breve* UCC2003, which allowed the identification of various enzymes with (in some cases proven) potential for oligosaccharide (OS) production. The Prebiozymes project aimed to clone various bifidobacterial genes that are known to encode sugar-modifying enzymes. These encoded enzymes were then purified and assessed (and optimised) for their ability to produce OS of a specific composition. Such novel OSs were expected to have an enhanced selectivity to promote growth of (selected) bifidobacteria. The most promising OS (in terms of production and composition) were analysed to obtain information regarding their composition and then purified to remove any contaminating mono- and di-saccharides, and subsequently assessed for their prebiotic potential and selectivity. This latter research activity was performed first by means of *in vitro* growth experiments using various human-derived bifidobacteria. The most promising OS preparation was then tested under *ex vivo* conditions employing a miniature faecal fermentation set-up (so-called micromatrix). Three experimental modifications from the original approved project proposal were introduced during the course of the project. The first one related to the purification of the OS mixtures, where we resorted to activated carbon purification instead of chromatography methods, while the second involved the inaccessibility of an NMR facility in UCC to perform a compositional analysis of the generated OS mixtures. We solved this by performing HPAEC-PAD analysis and running these alongside known standards. Subsequently we have used an external collaborator to perform further structural analysis.

The third modification concerned the use of faecal fermentation system instead of a murine model, which due to novel EU regulations on animal trials were deemed too cumbersome in terms of obtaining regulatory approval. Despite these methodological adjustments we were still able to achieve all deliverables/milestones set out at the beginning of the project.

3. Research Achievements/Results

The main research achievements of the Prebiozymes project included the overexpression of bifidobacterial enzymes which were then successfully employed to produce novel oligosaccharide (OS) mixtures, for which structural and compositional information was obtained. Furthermore, some of these OS mixtures were successfully purified and their bifidogenic activity was then demonstrated using *in vitro* assays as well as employing a miniature faecal fermentation set-up (*ex vivo* assay). These latter assays convincingly demonstrated that the tested OS mixtures exhibit bifidogenic activity and thus

confirmed its expected prebiotic potential. Therefore, all deliverables and milestones that had originally been formulated were achieved.

4. Impact of the Research

The Prebiozymes project has delivered novel oligosaccharide mixtures with prebiotic potential. This was achieved employing a new research methodology which in principle may lead to the further development and discovery of novel prebiotics with high activity and selectivity. The project therefore is expected to have considerable impact on the field of prebiotics and the associated area of functional foods. Furthermore, the research may have a far-reaching impact in terms of opportunities for industry to develop prebiotics and to consumers in terms of the choices of prebiotic products that may be suited to treat specific gastro intestinal diseases or discomforts. The main benefit of the research approaches and scientific outcomes of this project are that the newly developed prebiotics (i.e. the ones developed in this project but also other ones that may be developed using a similar strategy) are expected to be more specific in targeting very particular (beneficial) components of the human gut microbiota.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

This research has allowed interactive scientific links with various industries (such as FrieslandCampina, Tate & Lyle, Pure Fibre and General Mills) as well as with several academic institutions (in particular with Dr Irina Sadovskaya, University of Lille, France, Dr Jose Munoz, Northumbria University in Newcastle, UK, and Dr Javier Moreno, University of Madrid, Spain). All these collaborations relate to prebiotics research.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

The project has allowed the development of an enzymatic approach to produce oligosaccharides with prebiotic potential.

(iii) Outcomes with economic potential

The new oligosaccharides have shown to possess prebiotic potential under in vitro and in a fecal fermentation system, thereby clearly indicating economic potential. However, further testing and ultimately a human clinical trial will be required to generate definite proof of prebiotic activity and therefore its true economic value.

(iv) Outcomes with national/ policy/social/environmental potential

Currently it is hard to say if the project may have such potential. Nonetheless, the newly developed prebiotic may possess health benefits that may target particular gastro intestinal diseases or discomforts and as such the outcomes of the Prebiozymes project may in time generate impacts of such nature.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Peer-reviewed publications:

Kelly, E.D. , Bottacini, F., O'Calllaghan, J., O'Connell Motherway, M., O'Connell, K.J., Stanton, C., Van Sinderen, D. (2016). Glycoside hydrolase family 13 α-glucosidases encoded by *Bifidobacterium breve* UCC2003; A comparative analysis of function, structure and phylogeny. International Journal of Food Microbiology 224, 55-65.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

Not applicable

(iii) National Report

Not applicable

(iv) Workshops/seminars at which results were presented

Emer Kelly, the postgraduate student funded by this project attended the Glycosciences Summer School in Wageningen, The Netherlands (http://www.vlaggraduateschool.nl/glycosciences/) in April 2014. In order to attend this Glycosciences Summer School a research abstract was submitted and a poster was presented on the characterisation of Agl3 and Agl4.

Emer Kelly has in 2015 and 2016 been on the organising commitee for the annual joint Imperial College London and UCC postgraduate student led symposium, where her research results have been presented in poster and oral formats.

The postgraduate student has also presenting her work, both through oral and poster presentations, at the Microbiology Society Irish Division Coference 2015 & 2016; the 4th International Propio Bifido 2016 symposium; at the 1st, 2nd and 3rd Annual Imperial/UCC Postgraduate Symposium; and at the 12th International Symposium on Lactic Acid Bacteria.

(v) Intellectual Property applications/licences/patents

Not applicable, though an invention disclosure form is currently being prepared to be submitted to the Technology Transfer Office to determine if the oligosaccharide mixes and the method to produce them constitute intellectual property that will need to be patented.

(vi) Other

Not applicable

5. Scientists trained by Project

Total Number of PhD theses: 1

The PhD student funded through the Prebiozymes project, Emer Kelly, has finalized all her practical/experimental work and has written several parts of her thesis. However, she has not finalized this yet, due to her (extended) maternity leave. The anticipated thesis submission date is currently Spring 2019.

Total Number of Masters theses: NA

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
UCC	1	0.225
MFRC	2	0.029
Total	**3**	**0.254**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	0	
PhD students	1	4
Masters students	0	
Temporary researchers	1	0.5
Other		
Total	**2**	**4.5**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
Not applicable	

9. Project Expenditure

Total expenditure of the project: € 223,437

Total Award by DAFM: €234,255

Other sources of funding including benefit in kind and/or cash contribution(specify): €0

Breakdown of Total Expenditure

Category	Name Instit UCC	Name Instit MFRC	Name Institution 3	Name Institution 4	Total
Contract staff	13,867				13,867
Temporary staff					
Post doctorates					
Postgraduates	87,079				87,079
Consumables	33,960	24,165			58,125
Travel and subsistence	2,625	21			26,46
Sub total	137,531	24,186			161,717
Durable equipment	1,247				1,247
Other		11,958			11,958
Overheads	41,259	7,256			48,515
Total	**180,037**	**43,400**			**223,437**

10. Leveraging

N/A

11. Future Strategies

Outline development plans for the results of the research.

We would like to think that the area of development of novel prebiotics through a reverse enzyme strategy has just started and that this scientific approach will gain further scientific traction and associated industry interest. We therefore aim to further develop our abilities to produce different oligosaccharides, to determine their structure and composition, and to assess their prebiotic potential. The latter will involve a range in vitro, ex vivo and in vivo technologies to allow analysis of health-promoting abilities of such prebiotics.