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# 14F821 - Food solutions for replenishing disrupted microbiota in toddlers

## Final Report

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## SUMMARY

The first two years of life represents the most critical period for dietary interventions to improve child growth/development. Perturbation of optimum microbiota development, arising from preterm birth or antibiotics have likely long-term implications for microbial diversity and consequent health. In TODDLERFOOD, we have identified a synbiotic food solution consisting of a probiotic mixture plus a nondigestible oligosaccharide prebiotic for replenishing a disrupted microbiota in infants as a result of C-section delivery or early-life antibiotic administration. Faecal fermentations and the in vitro potential probiotic properties of our selected strains, such as the ability to adhere to the human intestinal mucosa, inhibition of pathogens adhesion and cytotoxicity were completed. Preclinical studies to test the early administration of selected synbiotic in the C-section delivery model revealed that adult mouse anxiety, cognitive and behavioural deficits were noted as a result of the performance of the C-section procedure. Synbiotic supplementation was able to reverse C-section induced deficits in cognitive, anxiety and social behaviour, in adult mice. Moreover, antibiotic treatment appeared to exacerbate deficits associated with C-section, and synbiotic treatment appeared to not only reverse this, but also act as an anti-depressant in the adult mouse. 16S compositional analysis revealed that administration of synbiotic appeared to alleviate the effects of antibiotics on the gut microbiota. C-section antibiotic treated mice had significantly lower alpha diversity, Bray Curtis distances and compositional differences when compared to C-section mice who did not receive antibiotic. However, when our synbiotic was administered to antibiotic treated mice these significant differences were no longer observed. A study investigating the appropriate dosing interval of a probiotic product suitable for infants at the Neonatal Unit at CUMH indicated that a daily dose is a suitable dosage for preterm infants with significantly higher levels of Bifidobacterium found in daily probiotic group.

## KEYWORDS

Infant, Nutrition, Synbiotic

## ACRONYM

TODDLERFOOD

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# Section 1 - Research Approach & Results

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## Start Date

01 December 2014

## End Date

31 May 2019

## Research Programme

Food Institutional Research Measure

## TRL Scale

TRL 1: Basic Principles Observed

## NRPE Priority area

Food for Health

## Total DAFM Award

€597,246.15

## Total Project Expenditure

€474,664.59

## Rationale for undertaking the Research

Little is known about how gut microbiome composition and function may be manipulated in a positive sense through nutrition for developing children. Moreover, following this nutritional support through toddlerhood is imperative to shape optimal health outcomes in adulthood. TODDLERFOOD took advantage of and built upon ongoing projects within our research group addressing gut microbiota in infants (INFANTMET, MYNEWGUT). This research is needed to ultimately deliver dietary ingredients for promoting a diverse gut microbiota. The global market for infant milk formula is worth approx. \$5-6 billion and Irish based companies trade approximately 15% of the infant milk formula tonnage internationally. This project will generate information for development of new “intelligent” food ingredients and infant formula for infants and toddlers and for the relevant food ingredient and dairy companies that supply them. TODDLERFOOD will contribute new knowledge about role of specific foods/food prototypes for replenishing and reshaping the disrupted early gut microbiome towards optimum development and for avoiding later diet-related lifestyle disorders (e.g. allergy, obesity, metabolic syndrome).

## Methodology

Ex vivo studies, based on faecal fermentation of infant intestinal microbiota with different dietary fibres for identification of optimal prebiotic activity towards replenishment of the disrupted microbial composition associated with early antibiotic administration and/or C-section delivery were conducted. Faecal fermentation to develop and evaluate a mix of probiotics for infant nutrition and synbiotic combinations were also completed. The gut microbiome was assessed using a metagenomic approach. This involved sequencing of DNA recovered from the fermentation using 16S rRNA amplicon sequencing on the Illumina MiSeq and identification of the taxa that are present as well as their abundance. Key questions that were asked were whether there are any detectable changes in keystone genera such as Bifidobacterium that are indicators of a healthy microbiota. Preclinical studies in rodents were used to assess whether these dietary intervention strategies can reverse C-section induced deficits in behaviour and physiology in adult mice. Mice were fed from postnatal day 21 until adulthood with the selected probiotics mixture/ prebiotic or synbiotic diet. Mice were tested at 8 weeks of age in a behavioural battery designed to assess anxiety-, depressive-, cognitive- and social-related behaviours (Tests included: Open field, marble burying, elevated plus maze, three chambers test, novel object recognition, Y-maze, forced swim test). Sensitivity to stress and gut-barrier function were also assessed (cortisol response; FITC-dextran assay). Synbiotic food product development involved testing the selected probiotic strains regarding their capability of surviving and multiplying as well as exhibiting anti-microbial activity (AMA) during growth in the presence of each other, i.e. during co-culture. Freeze drying survival and optimal storage/viability was also assessed. To determine whether probiotics administered to infants (C-section delivered) requiring antibiotic treatment for presumed sepsis improves colonization of microbiota in their gastrointestinal system we undertook a prospective cohort study at CUMH.

## Project Results

In Task 1 we sought to identify prebiotic ingredients to replenish the microbial composition associated with early antibiotic administration and/or C-section. A variety of non-digestible oligosaccharides were assessed for optimal prebiotic activity using batch culture fermentation. Compositional analysis revealed abundance of Bifidobacterium increased from 1% initially to 7% after 24 hours when Nexus was used as carbohydrate. Nexus also resulted in an increase in the abundance of Bacteroides. Interestingly potentially pathogenic genera Enterococcus and Clostridium increased when Synergy was used as carbohydrate source but not when Nexus was utilised. Therefore, Nexus, was selected as having greatest potential. The INFANTMET Culture Collection of over 7,400 putative Lactobacillus and Bifidobacterium spp was screened for a number of potentially probiotic traits. Faecal fermentations were performed on 11 Bifidobacterium strains with the greatest potential. Three strains, B. breve, B. bifidum and B. longum were selected for preclinical studies along with the prebiotic, Nexus. Preclinical studies in rodents to test the early administration of selected probiotics mixture/synbiotics in the C-section delivery model revealed that adult mouse anxiety, cognitive and behavioural deficits were noted as a result of the performance of the C-section procedure. Dietary interventional strategies were able to reverse C-section induced deficits in cognitive, anxiety and social behaviour, in adult mice. Moreover, antibiotic treatment appeared to exacerbate deficits associated with C-section, and synbiotic treatment appeared to not only reverse this, but also act as an anti-depressant in the adult mouse. C-section antibiotic treated mice had significantly lower alpha diversity, Bray Curtis distances and compositional differences when compared to C-section mice who did

not receive antibiotic. Upon synbiotic administration these significant differences were no longer observed. Task 4 focused on the development of a synbiotic food product and selected strains were tested regarding their capability of surviving and multiplying as well as exhibiting anti-microbial activity. All strains survived co-culture well and did not produce bacteriocin like substances against each other. Further analysis was carried out by means of growth curves on bacterial viability. The viable count of the cultures decreases in all after 24 h. The viability of the strains appeared to be unaffected by freeze drying. Task 5 evaluated the dosing interval of a probiotic suitable for infants containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus* given daily, biweekly and weekly in preterm infants. Following administration of probiotic at 31 weeks post menstrual age, *Bifidobacterium* were significantly higher in daily group (45%) in comparison to biweekly (17%) and weekly (9%) groups. At 34 weeks PMA, *Bifidobacterium* were significantly higher in daily (60%) group in comparison to biweekly (21%), weekly (23%) and control (15%) groups. At 41 weeks PMA a decrease in *Streptococcaceae* and *Enterococcaceae* were found in all three probiotic groups and by 44 weeks PMA significantly higher levels of *Lactobacillus* were found in biweekly group (16.5%) in comparison to weekly group (2.1%). Results indicate that a daily dose of probiotic mix is a suitable dosage for preterm infants, with significantly higher levels of *Bifidobacterium* found in daily probiotic group up to 44 weeks PMA.

## Section 2 – Research Outputs

### Summary of Benefits / Improvements of Project Findings

The research undertaken in Toddlerfood will guide product development in both the infant milk formula and food industry for optimization of new food ingredients for infant formula in addition to dietary advice. The development of synbiotic food products, i.e. mixtures of prebiotics and probiotics, in which the prebiotic fibre sustains the growth of the probiotic microorganism(s) supplied or of other beneficial host bacteria, constitutes an important growing market for the food industry. The infant/toddler market is of great relevance, as the health status of the gut in early life is extremely important for the well-being in later stages of life. The outcomes from this task can assist the infant formula sector in the development of intelligent infant formulas and post-weaning follow on formulas to positively influence gut microbiota composition/function in a manner more similar to that associated with breast feeding from birth. Development of 'intelligent infant formulas' with added nutritional benefits will therefore enhance the competitiveness of the Dairy Industry supplying the infant milk formula sector. The findings from Toddlerfood will also be of relevance to the healthcare industry and clinicians with regards treatment of C-section infants receiving antibiotics.

### Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	0	3	3

### Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	18	These publications include both original research and literature reviews published in a wide range of scientific journals including <i>Microbiome</i> (IF 11.607), <i>Biological psychiatry</i> (IF 12.095), <i>EBioMedicine</i> (IF 5.736), <i>Scientific Reports</i> (IF 3.998) and <i>British Journal of Nutrition</i> (IF 3.334). SEE SECTION 3, 'Project Publications', for full details.

### Intellectual Property

An Invention Disclosure Form (IDF2018599) has been documented entitled "Probiotics for infants post C-section or antibiotic treatment" with included the strains *Bifidobacterium longum*, *Bifidobacterium breve* and *Bifidobacterium bifidum*.

### Summary of other Project Outputs

Project Outputs	Details	Total Number
New Industry Collaborations Developed	An Evaluation and Option Agreement has been signed with DuPont Nutrition and Biosciences (July 2019). The strains have been sent to DuPont for evaluation with potential for licencing in the future. This was led by Teagasc TTO to ensure adherence to the National IP Protocol.	1

### Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	Toddlerfood research could aid development of 'intelligent infant formulas' with added nutritional benefits will therefore enhance the competitiveness of the Dairy Industry supplying the infant milk formula sector. In addition, the outcomes of task 5 are of particular relevance to policy makers in the healthcare industry and clinicians, as microbial therapies are now being used in the hospital environment as prophylactics. To recommend an appropriate dosage the study was carried out with a hypothesis: the optimal probiotic dosage, as assessed by faecal 16S compositional sequencing, would benefit the infant gut by reducing the colonisation of potentially pathogenic microorganisms in the intestine.

## Dissemination Activities

Activity	Details
Seminars at which results were presented	Kiera Murphy and Kizkitza Busca presented Toddlerfood research at the Postdoctoral Research Seminars at Teagasc Moorepark.
Other	Catherine Stanton gave plenary lecture outlining toddlerfood research outputs at Gut Microbiota and Health, University Messina, Sicily. Also, speaker discussing Toddlerfood research at Early Life Microbiota Development, Wageningen University, Danone Symposium. Poster presentation at Young Neuroscience Ireland Symposium. Oral presentation by Emanuella Morelli at International Symposium of Prebiotics and Probiotics in Paediatrics. Poster presentation at Society of Neuroscience Conference, Washington.
Workshops at which results were presented	Poster presentation at Spanish Society of Probiotics and Prebiotics (SEPyP), 9th Workshop.

## Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	The outcomes of task 5 generated knowledge of particular relevance to clinicians, as microbial therapies are now being used in the hospital environment as prophylactics. The optimal probiotic dosage that would benefit the infant gut by reducing the colonisation of potentially pathogenic microorganisms in the intestine was assessed.
Identify any knowledge transfer activities executed within the project.	The findings regarding appropriate probiotic dosage were published in the medical journal Archives of Disease in Childhood and therefore this knowledge is now available to clinicians.
List any impacts resulting from the knowledge transferred during the project.	An Evaluation and Option Agreement has been signed with DuPont Nutrition and Biosciences (July 2019). The strains have been sent to DuPont for evaluation with potential for licencing in the future. This was led by Teagasc TTO to ensure adherence to the National IP Protocol.

## Section 3 – Leveraging, Future Strategies & Reference

### Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Other	€6,330,991.00	This project along with DAFM founded INFANTMET have allowed the team to successfully gain further funding for infant microbiome research. Prof Stanton as of 01 Jan 2020, has been successful in achieving funding (€6,330,991) for a SFI SPOKE entitled (MiMIC - MIssing Microbes in Infants born through C-Section). The total funding is made up on contributions from both the industry partner and SFI.

### Future Strategies

The synbiotic developed in Toddlerfood proved to be successful in preclinical trials and going forward we would like to move to clinical trials. An Invention Disclosure Form (IDF2018599) has been documented entitled "Probiotics for infants post C-section or antibiotic treatment" with included the Toddlerfood strains Bifidobacterium longum, Bifidobacterium breve and Bifidobacterium bifidum. Furthermore, an Evaluation and Option Agreement has been signed with DuPont Nutrition and Biosciences (July 2019). The strains have been sent to DuPont for evaluation with potential for licencing in the future.

### Project Publications

1. Egerton S, Wan A, Murphy K, Collins F, Ahern G, Sugrue I, et al. Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. *Sci Rep.* 2020; Accepted Jan 21, 2020.
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