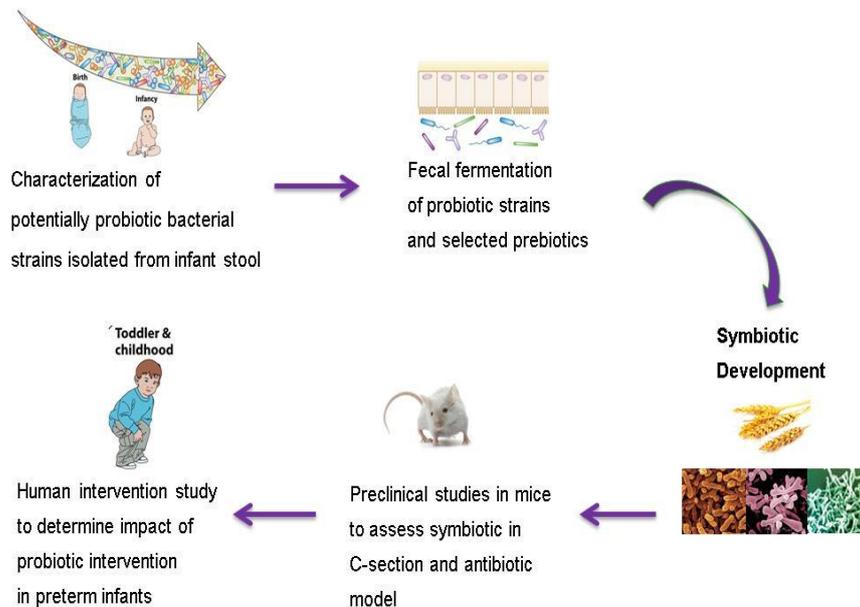


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Food solutions for replenishing disrupted microbiota in toddlers



Key external stakeholders:

DAFM, infant milk formula industry, dairy and other functional foods companies

Practical implications for stakeholders:

- Gut microbiome composition and function may be manipulated in a positive sense through nutrition for developing children.
- This work developed new "intelligent" food ingredients and infant formula for infants and toddlers and for the relevant food ingredient and dairy companies.
- TODDLERFOOD project contributed new knowledge about the 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).

Main results:

A symbiotic food solution consisting of a probiotic mixture plus a non-digestible oligosaccharide prebiotic can modulate the microbiota and can reverse CS-induced deficits in social and cognitive behaviour in mice.

Opportunity / Benefit:

This project will guide product development in both the infant milk formula and food industry in terms of the functional effects of prebiotics, probiotics and symbiotic formulations.

Collaborating Institutions:

Teagasc, UCC, Cork University Maternity Hospital (CUMH)

Teagasc project team: Prof. Catherine Stanton (Project Leader)

External collaborators: Prof. John Cryan, UCC
Prof. Tony Ryan, CUM

1. Project background:

The first two years of life represents the most critical period for dietary interventions to improve child growth/development. This is the period when intestinal microbiota, a vital asset for health and neurodevelopment is established. Nutrition influences gut microbiome evolution, with breast feeding in early life the gold standard. Malnutrition is caused not only by nutrient deprivation/imbalance, but also by disrupted gut microbiota, leading to impaired nutrient availability and energy harvesting from the diet. Perturbation of optimum microbiota development, arising from preterm birth or antibiotics has likely long-term implications for microbial diversity and consequent health and little is known about how early gut microbiome is positively manipulated through nutrition.

2. Questions addressed by the project:

1. What are the potential probiotic activities of the selected strains?
2. Does a symbiotic food solution consisting of a probiotic mixture plus a non-digestible oligosaccharide prebiotic replenish a disrupted microbiota in infants as a result of C-section delivery or early-life antibiotic administration?
3. Are the dietary interventional strategies, in form of prebiotic and symbiotic supplementation, able to reverse C-section induced deficits in cognitive, anxiety and social behavior, in adult mice?
4. Can a symbiotic treatment appear to reverse the deficits produced after antibiotic treatments (C-section) and act as an anti-depressant in the adult mouse?

3. The experimental studies:

- *In vitro* studies to identify food solutions to replenish the disrupted microbial composition associated with early antibiotic administration and/or C-section delivery.
- Development of bifidogenic probiotic mixtures and subsequent symbiotic combinations (pre and probiotics) for replenishment of early gut microbiota and their evaluation in *in vitro* studies, based on fecal fermentation of infant intestinal microbiota for boosting Bifidobacteria.
- Preclinical studies in rodents to assess selected food-based solutions (from Tasks 1&2) to replenish the disrupted microbiota composition associated with early antibiotic administration and/or C-section delivery, and evaluate health outcomes from birth to adulthood in mice.
- Symbiotic food product development incorporating optimised mixes of pre and probiotics based on Tasks 1, 2 and 3 for enhancing microbial diversity, with potential for enhancing health outcomes and development on infants and toddlers.
- Human intervention study in infants to determine impact of probiotics in newborns.

4. Main results:

- An arabinoxylan preparation was selected from a variety of non-digestible oligosaccharides for optimal prebiotic activity using batch culture fermentation. Compositional analysis from fermentations showed that the relative abundance of *Bifidobacterium* increased from 1% at the start of the fermentation (T0) to 7% after 24 hours (T24) in reactions the arabinoxylan was used as the carbohydrate source. A potential butyrogenic effect was also observed where an increase in the relative abundance of *Pseudobutyrvibrio* was observed.
- Three strains of *Bifidobacterium* strains (*B. breve*, *B. bifidum* and *B. longum*) were selected based on their potential probiotic activity: ability to utilise non-digestible oligosaccharides, Bile Salt Hydrolase (BSH) Activity, Exopolysaccharide (EPS) Production, Bacteriocin production, Antimicrobial activity (AMA) assays, Simulated gastric juice survival, percentage of adhesion to HT29 cells.
- It was demonstrated that prebiotic and symbiotic supplementation can reverse CS-induced deficits in social and cognitive behaviour. The CS-induced increase in anxiety-like behaviour was partially ameliorated following prebiotic and symbiotic administration. Changes were not observed in locomotor activity, the stress response, or intestinal motility and permeability as a result of

supplementation.

- In tests for anxiety-like behaviour, an effect of CS delivery was maintained in the elevated plus maze where a reduction in percent time spent in the open arms of the apparatus was observed. This was not ameliorated by antibiotic or symbiotic treatment and in tests for cognition, there is a trend towards cognitive impairment in the novel object recognition test ($P=0.052$), with antibiotic or symbiotic having no further impact.
- It was demonstrated that probiotic supplementation enhanced gut health and microbiome development in newborns.

5. Opportunity/Benefit:

This project will enable the end user to design and develop novel functional foods / ingredients which incorporate the optimal prebiotic to modify the infant gut in a positive manner. The identification of the optimal prebiotic for use as a food solution has innovation potential across a number of different scientific areas, from new food product development and comprehensive dietary strategies to manage host health in infants who receive early antibiotic administration and/or C-section delivery.

6. Dissemination:

Main publications:

1. Kaliannan K, Robertson RC, Murphy K et al. Estrogen-mediated gut microbiome alterations influence sexual dimorphism in metabolic syndrome in mice. *Microbiome*. 2018 Nov 13;6(1):205.
2. Watkins C, Murphy K, et al. Dose-interval study of a dual probiotic in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2018 Jun 20. pii: fetalneonatal-2017-313468.
3. Crowley EK, Long-Smith CM, Patterson E, Murphy K, et al. Dietary Supplementation with a Magnesium-Rich Marine Mineral Blend Enhances the Diversity of Gastrointestinal Microbiota. *Mar Drugs*. 2018 Jun 20; 16(6). pii: E216.
4. Watkins C, Murphy K, et al. The viability of probiotics in water, breast milk, and infant formula. *Eur J Pediatr*. 2018 Jun; 177(6):867-870.
5. Alberoni D, Baffoni L, Gaggia F, Ryan PM, Murphy K et al. Impact of beneficial bacteria supplementation on the gut microbiota, colony development and productivity of *Apis mellifera* L. *Benef Microbes*. 2018 Feb 27; 9(2):269-278.
6. Watkins C, Murphy K, et al. Effects of therapeutic hypothermia on the gut microbiota and metabolome of infants suffering hypoxic-ischemic encephalopathy at birth. *Int J Biochem Cell Biol*. 2017 Dec; 93:110-118.

Popular publications:

Irish Paediatric Association Annual Meeting, Dublin, 2016.
4th Symposium on Propionibacteria and Bifidobacteria, Cork, 2016.
INFANT Research Day, Cork, 2016.
APC Microbiome Institute Symposium, Cork, 2016
12th Annual Conference of the Metabolomics Society, Dublin, 2016.
Paediatric Academic Societies (PAS) 2016 Annual Meeting, Baltimore, USA, 2016.
Spanish Society of Probiotics and Prebiotics, IX Workshop, 2018.
Young Neuroscience Ireland Symposium, 2018.
International Symposium of Prebiotics and Probiotics in Pediatrics, 2018.

7. Compiled by: Catherine Stanton