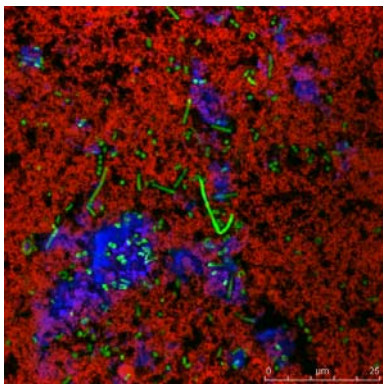
Project number: 5647
Funding source: FIRM (06RDTMFRC450)
Date: November, 2012
Project dates: Jan 2007 – Dec 2010

Heart friendly foods



Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies.

Practical implications for stakeholders:

- Dairy products enriched in soluble dietary fiber and beta-glucan, based on use of novel adjunct food-grade cultures with soluble fiber producing capacity during milk fermentation were developed in this project. These cultures were also used as dietary adjuncts for *in situ* production of beta glucan in the gut, and shown to exhibit cardioprotective properties.
- As a cardioprotective diet enriched in dietary fiber, is recommended to protect against the development of cardiovascular disease, and dairy products are poor sources of soluble dietary fiber and beta glucan, this represents an opportunity for dairy industry to produce functional foods and dried dairy ingredients for protection against development of cardiovascular disease, for functional and medical food markets.
- With cardiovascular disease being the leading cause of death and morbidity in the EU, and on the increase among the Irish population, the availability of such functional foods within the market would be of significant benefit to consumers and food producers alike.

Main results:

- Soluble fiber-producing food-grade cultures, including beta-glucan producing cultures from culture collections and novel sources were identified and characterised.
- *In situ* production of beta glucan by food-grade cultures resulted in increased survival of the beneficial strain in conditions of elevated heat, simulated gastric juice, acid, bile and antibiotic stress.
- The low-fat yoghurt developed with these adjunct strains exhibited superior functional properties compared to product manufactured without the cultures.
- Development of dried dairy ingredients and Functional dairy foods enriched with soluble fiber and beta glucan producing cultures with excellent rheological properties were developed.
- Efficacy was demonstrated against atherosclerosis development of selected soluble fiber and beta glucan producing cultures in an animal model of lipid-driven atherosclerosis.

Opportunity / Benefit:

The opportunity exists to further investigate the potential of microbially produced soluble fiber as a potent bioactive food ingredient and potential pharmaceutical product for human health benefit with a view to commercialisation. A patent application is in the process of being filed. Expressions of interest from relevant companies are welcome and opportunities to collaborate and license this technology can be discussed.

Collaborating Institutions:

University College Cork

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1. Project background:

Cardiovascular disease is the leading cause of death and morbidity in the EU. There is an increasing prevalence of hear disease among Irish population (Bennett et al., 2005). Prevention is better than cure, and among the recommendations to avoid heart disease is to ingest a cardioprotective diet (Lancet, 2005). Heart health protective bioactive components exist in food, including phytochemicals and antioxidants within fruits and vegetables, and soluble dietary fiber. Dairy products are poor sources of soluble dietary fiber. In this project, the objective is to develop fermented dairy products enriched in soluble dietary fiber and beta glucan. These beneficial compounds will be enhanced during milk fermentation with novel beta glucan-producing food-grade strains isolated and characterised as part of this project, and these will also be used as dietary adjuncts for *in situ* production of soluble fiber and beta glucan in the gut, and evaluated in an animal model of lipid-driven atherosclerosis.

2. Questions addressed by the project:

- Are food-grade beta glucan producing bacteria available in the culture collection bank at TFRC and UCC, and what are their characteristics?
- What quality of yoghurt can be expected from use of most promising strains for development of product enriched in beta glucan?
- Is the beta glucan rich dairy product effective for prevention of cardiovascular disease in animal model of lipid-driven atherosclerosis?
- Is the administration of live beta glucan producing probiotic strains useful as an alternative dietary approach for prevention of cardiovascular disease in animal model of lipid-driven atherosclerosis?

3. The experimental studies:

- Identification and characterisation of soluble fiber-producing food-grade cultures from culture collections and novel sources
- Genetic characterisation of specific gene clusters responsible for production of soluble fibers in producing strains
- Development of recombinant beta glucan producing *Lb. paracasei* NFBC 338 for proof-of-concept studies
- Exploition of soluble fiber and beta glucan producing strains for production of fermented milk rich in beta glucan, with a view to development of beta glucan rich foods aimed at reducing risk of cardiovascular disease
- Development of technologies for the optimised large scale production of the stable bioactive food components
- An investigation of the technological performance of a beta glucan producing strain, relative to control non-producing strain
- An investigation of the functional properties and rheology of low fat yoghurt manufactured with adjunct beta glucan producing probioitcs compared to control product made without beta glucan producing cultures.
- An investigation of the potential of soluble fiber/beta glucan producing cultures for prevention of cardiovascular disease in an animal model of lipid-driven atherosclerosis.

4. Main results:

The main findings of the project were as follows:

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- A number of strains, from within the DPC and UCC culture collections and from food grade sources, including Kefir grains and malt were found to produce microbial fibers including beta glucan. These were all lactic acid bacteria and members of the species *Lactobacillus, Pediococcus, Lactococcus* and *Leuconostoc. Bifidobacterium longum* derived from the human intestine was also positive for the production of beta glucan.
- The membrane associated glycosyltransferase enzyme (encoded by *gtf* gene) from *Pediococcus parvulus 2.6* responsible for microbial beta glucan production, which catalyses the conversion of sugar nucleotides to beta glucan was heterologously expressed in *Lactobacillus paracasei* NFBC 338. Phenotypic and microscopic analyses indicated beta glucan biosynthesis by the recombinant strain, while agglutination tests carried out with *Streptococcus pneumoniae* type 37-specific antibodies, which specifically detect glucan-producing cells confirmed production. Ability of *Lactobacillus paracasei* NFBC 338 strain to produce the polysacharide was associated with significantly increased protection during heat stress (60 fold), acid stress (20 fold) and simulated gastric juice (15 fold). Bile stress assays revealed a more modest but significant 5.5 fold increase in survival for the beta glucan producing strain when compared to the control strain. These results suggest that production of a beta glucan exopolysaccharide by strains destined for use as probiotics may afford them greater performance/protection during cultivation, processing and ingestion.
- The benefits of using a beta glucan producer as adjunct strain during yoghurt manufacture were demonstrated. Use of the recombinant probiotic *Lactobacillus paracasei* NFBC 338 strain during yoghurt manufacture resulted in a yoghurt product with improved textural characteristics, displaying a smooth, shiny appearance and ropy phenotype compared to a control (non-exopolysaccharide-producing) yoghurt which had a more granular appearance and did not display the ropy phenotype. Furthermore shelf-life studies demonstrated that the beta glucan containing yoghurt exhibited superior techno-functional properties during storage with reduced syneresis over 28 days at 4°C, compared to control yoghurt. The performance of beta glucan producing and non-producing strains was also evaluated during spray drying, with no differences found between the strains.
- A *Lactobacillus* strain derived from the mammalian gastrointestinal tract was shown to be a potent microbial polysaccharide producer. The molecular basis of EPS production by this strain was characterised. Yoghurt was manufactured using the *Lactobacillus* strain as adjunct culture which exhibited improved sensory and textural properties compared to product made without the strain, and throughout storage, the yoghurt exhibited higher water holding capacity and consequently less syneresis than control.
- The apoE deficient mouse model, prone to development of lipid rich proinflammatory atherosclerotic plaque was used to test the efficacy of soluble fiber and beta glucan producing bacteria for cardioprotective effects. High fat, high cholesterol chow-fed animals (n=9) received either recombinant probiotic *Lactobacillus paracasei* NFBC 338 versus isogenic control strain (pNZ44 in *L. paracasei* NFBC 338), or food-grade producer daily for 12 weeks. Following the intervention for 12 weeks, total cholesterol and triglycerides in serum, risk markers for atherosclerosis development, were significantly reduced for test groups compared with respective controls. This study demonstrated the potential of microbial beta glucan production, and fermented functional foods derived from use of such cultures for prevention of cardiovascular disease.

5. Opportunity/Benefit:

The opportunity is the availability of polysaccharide-producing probiotic strains which can improve yoghurt quality and have a positive impact on the human heart health. Some of the results are considered intellectual property and are being patented.

6. Dissemination:

Main publications:

Meng, X. C., Stanton, C., Fitzgerald, G. F., Daly, C. and Ross, R. P. (2008). Anhydrobiotics: The challenges of drying probiotic cultures. Food Chemistry, 106: 1406-1416.

Mills, S., Stanton, C., Ross, R.P. (2009). Microbial production of Bioactives: from Fermented Functional Foods to Probiotic Mechanisms. Australian Journal of Dairy Technology, 64: 41-49.

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Kearney, N., Meng, X.C., Stanton, C. Kelly, J. Fitzgerald, G. F. Ross, R.P. (2009). Development of a spray dried probiotic yoghurt containing *Lactobacillus paracasei* NFBC 338. International Dairy Journal, 19: 684-689.

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