

New Probiotic Cheddar Cheese

Drs. Catherine Stanton and Paul Ross

Cheddar cheeses containing high levels of probiotic strains following ripening, were successfully produced using normal manufacturing procedures. The sensory quality of the cheeses was not adversely affected by the addition of probiotic strains and flavour was in fact enhanced by one probiotic strain. A patent has been filed on the process which has attracted intense industry interest.



Functional Foods in Relation to Health and Disease

(New Probiotic Cheddar Cheese)

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Project Team:

C. Stanton

P. Ross

G. Fitzgerald*

K. Collins*

G. Gardiner

*Dept of Microbiology, University College Cork

The Dairy Products Research Centre
Moorepark, Fermoy, Co. Cork.

This project forms part of a major collaborative research programme into probiotic micro-organisms and their application in functional food development, between University College, Cork and Teagasc Dairy Products Research Centre, Moorepark.

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Teagasc 19 Sandymount Avenue
Ballsbridge Dublin 4

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Summary and Conclusions



Growing public awareness of diet-related health benefits has fuelled the demand for probiotic foods. These foods contain probiotic bacteria which are described as live microbial supplements that improve the intestinal microbial balance and are intended

for maintenance of health and/or the prevention of disease. Probiotic bacteria for human use must be proven to be safe and beneficial, and should preferably be of human origin as evidence suggests that these bacteria are species specific and perform best in the species from which they were isolated. They must also retain both viability and efficacy in a particular food product throughout its shelf-life, and following consumption. Above all however, probiotic food products must be proved effective in controlled validated clinical trials.

Dairy foods, including in particular, fermented milks and yogurt are among the best accepted food carriers for probiotic cultures. The aim of this study was to develop new probiotic foods, particularly, the production of high quality Cheddar cheese containing high levels of probiotic bacteria.

Main Conclusions and Achievements

* Cheddar cheeses containing high levels of probiotic strains following ripening, were successfully produced using normal manufacturing procedures. The sensory quality of the cheeses was not adversely affected by the addition of probiotic strains and flavour was in fact enhanced by one probiotic strain.

A patent has been filed on the process which has attracted intense industry interest and commercial trials are being conducted in one case.

* In pig feeding trials it was shown that mature Cheddar, containing high levels of probiotic bacteria compares favourably with fresh yogurt as an effective delivery system in colonising the gastrointestinal tract.

A positive immune response (serum IgG) was found in pigs fed the probiotic strains.

* Spray dried skim milk powders containing high numbers of probiotic strains, (even after storage at 4°C for 3 months), were successfully produced for use in functional foods including Cheddar cheese, yogurt, ice cream, etc.

Research and Results

Selection of Probiotic Strains

A number of probiotic strains (Fig 1), including *Lactobacillus* and *Enterococcus* spp., were assessed for use in Cheddar cheese.

The probiotic *Lactobacillus* strains used in this study were obtained from the Microbiology Dept., University College Cork under a restricted materials transfer agreement. These strains had previously been isolated from the human gastrointestinal tract, and characterised in detail with regard to their probiotic potential at UCC. In this respect, these strains have been shown to be acid and bile tolerant, adherent to human epithelial cells, non-pathogenic and to have desirable antibiogram profiles, in addition to contributing to a reduction in disease activity in Crohn's disease patients.

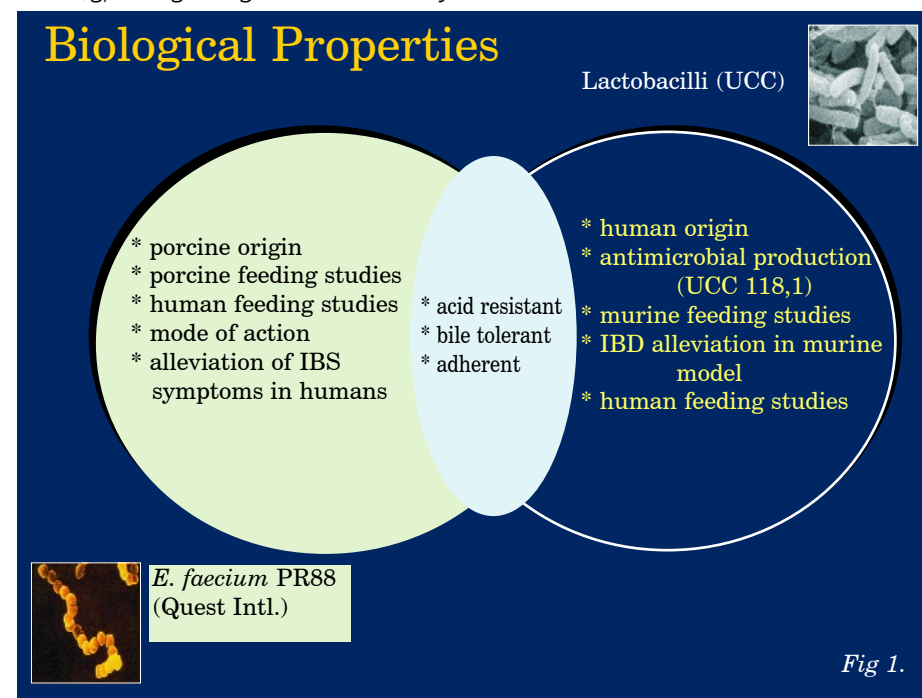
The probiotic strain *Enterococcus faecium* Fargo 688[®] was obtained from Quest International. *In a human clinical trial, it had already been demonstrated that consumption of this led to alleviation of the symptoms of irritable bowel syndrome in humans (Allen et al., 1996) and so demonstrating potential for use as a probiotic strain.*

Survival of probiotic strains in Cheddar cheese

A method of distinguishing the added probiotic strains from the normal non-starter lactic acid bacteria (NSLAB) in mature cheese was successfully developed. It involved the use of arbitrarily selected oligonucleotides in polymerase chain reactions (PCR) to generate discrete DNA fingerprints of the strains. This method was successfully employed for identification of the probiotic strains following isolation from Cheddar cheese.

In these studies it was demonstrated that two strains of *Lb. paracasei* grow and sustain high viability in cheese (Fig 2) while *Lb. salivarius* strains die during the ripening period. Growth of these strains in milk was found to be relatively slow, but MRS medium was capable of supporting the growth of high cell numbers of these strains (~2 x 10⁹ cfu/ml) with corresponding rapid growth rates.

Enterococcus faecium Fargo 688[®] was also found to survive to high numbers (4 x 10⁸ cfu/g) in Cheddar cheese during ripening at 8°C for 15 months (Fig 3) and in yogurt (4 x 10⁷ cfu/g) during storage at 4°C for 21 days.

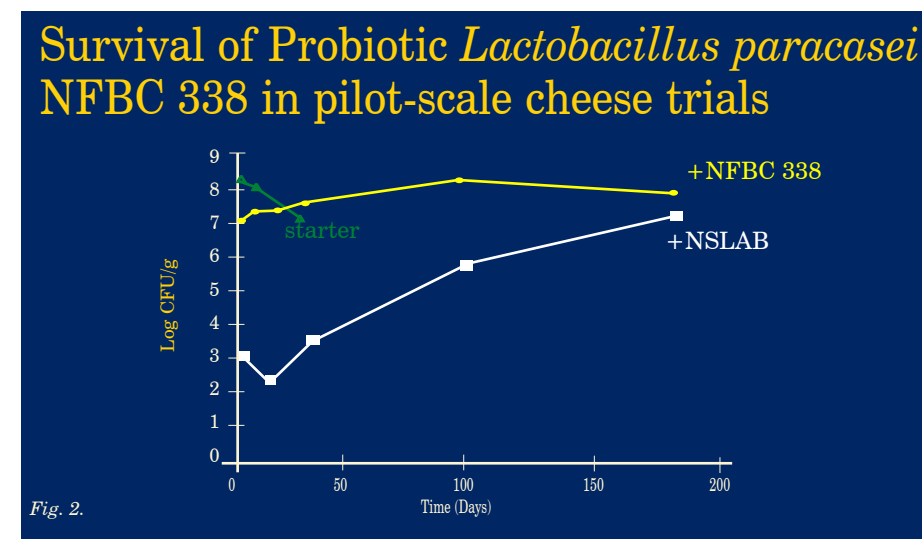


The incorporation of these strains did not adversely effect cheese quality (aroma, flavour and texture) while the incorporation of the *Enterococcal* strain actually improved Cheddar flavour. This probiotic cheese contained higher levels of odour-active volatile compounds compared with the control, as quantified by GC-MS head-space analysis, as well as increased levels of free amino acids, quantified by HPLC.

These Cheddar cheeses can be manufactured to contain high levels of probiotic strains (10⁸ cfu/g cheese) at a relatively low cost to the manufacturer using normal make procedures.

A patent was filed on the process for the manufacture of probiotic cheese and the project has attracted intense industry interest.

Industrial trials have been undertaken to examine the feasibility of scale-up to commercial scale while parallel clinical trials have recently been completed to confirm the health-promoting properties of this new functional cheese product.



Feeding trials

*In an in vitro model system, Cheddar cheese was found to have a greater protective effect than yogurt upon exposure of probiotic, *Lb. paracasei*, NFBC 338 culture to porcine gastric juice pH 2.*

In vivo follow-up trials were undertaken to evaluate Cheddar cheese as a food delivery vehicle for probiotic bacteria by supplementing the diet of pigs with probiotic Cheddar and yogurt. The probiotic strain - antibiotic (rifampicin) resistant variant of *Lb. paracasei* NFBC 338 was recovered from the porcine small intestine in similar numbers (10⁴ - 10⁵ cfu/ml) whether ingested in Cheddar cheese (total ingested = 10⁸ - 10⁹ cfu) or yogurt (total ingested = 10¹¹ cfu) (Fig 4).

Survival of Fargo 688® during cheese ripening

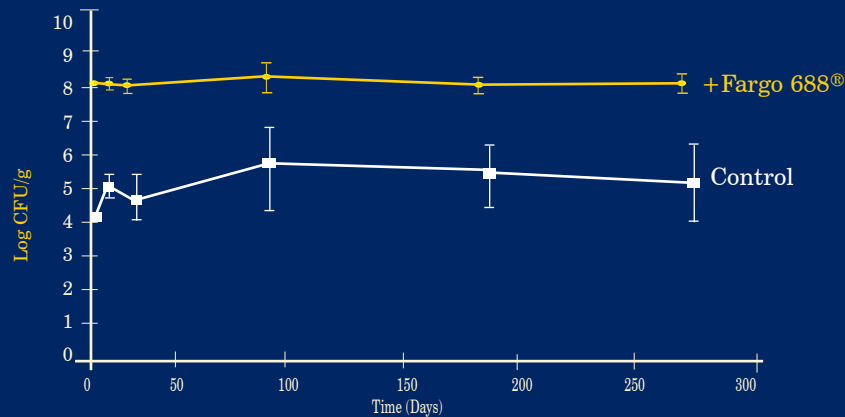


Fig. 3.

In a further feeding trial involving 8 pigs per group, a rifampicin resistant variant of the probiotic strain *Enterococcus faecium* Fargo 688® was fed for 21 days at a mean daily intake of 1.3×10^{10} cfu per day from Cheddar cheese or 3.7×10^9 cfu per day from yogurt. During the feeding period, Cheddar cheese yielded a significantly ($P < 0.05$) higher mean faecal probiotic count (2×10^6 cfu/g faeces) than yogurt (5.2×10^5 cfu/g faeces) (Fig 5).

These data indicate that mature Cheddar cheese compares very favourably with fresh yogurt as a delivery system for viable probiotic micro-organisms to the gut. However, there was no effect on body weight gain or feed conversion efficiency (FCE) in pigs receiving the enterococcal probiotic strain in either Cheddar cheese or yogurt compared with control animals.

Since probiotic micro-organisms have previously been shown to stimulate/modulate the immune system, the serum IgG response to the administered probiotic strain was measured in a subset of the animal population by flow cytometry. A positive response was found in pigs fed the probiotic strain, while no response was detected in the control animals. However, the mechanisms involved are, as yet, not fully understood.

Production of probiotic ingredient

In order to expand the probiotic product portfolio, spray-dried skim-milk powders, containing high numbers of probiotic strains were prepared for subsequent use in the preparation of probiotic functional foods, such as Cheddar cheese, yogurt and ice cream. Typical counts obtained in these powders at manufacture were 10^9 - 10^{10} cfu/g of the probiotic strain *Lb. paracasei* NFBC 338 and these levels were maintained following

storage of the powders at 4°C for 2 - 3 months.

Publications

Cheese delivers high numbers of probiotic lactobacilli to the porcine GIT

*A) Probiotic lactobacilli fed in either 5-mo old Cheddar cheese or fresh yogurt

*B) Administered strain recovered from small intestinal contents

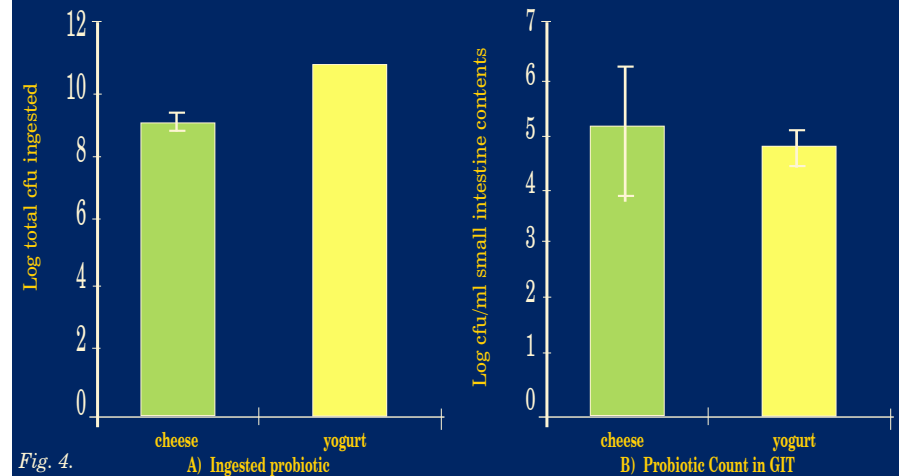


Fig. 4.

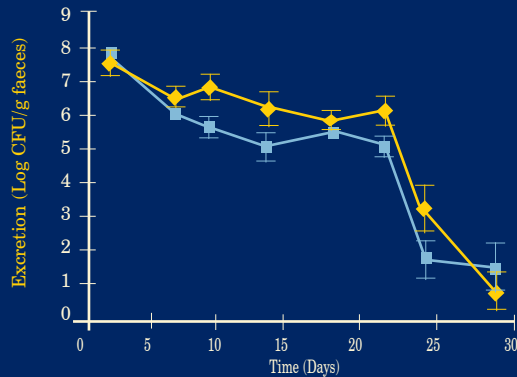
Allen, W.D., Linggood, M.A. and Porter, P. (July 1996). *European patent 0508701B1*.

Gardiner, G., Ross, R.P., Collins, J.K., Fitzgerald, G. and Stanton, C. (1998). Development of a probiotic Cheddar cheese containing human-derived *Lactobacillus paracasei* strains. *Applied and Environmental Microbiology*, 64, 2192-2199.

Stanton, C., Gardiner, G., Lynch, P. B., Collins, J.K., Fitzgerald, G. and Ross, R.P. (1998). Probiotic Cheese. *International Dairy Journal*, Vol. 8, Issue 5-6, pages 491-496.

Delivery of viable Fargo 688® to the porcine GIT in cheese or yogurt

■ Excretion - Cheddar cheese (15 months old)
◆ Excretion - yogurt (1 week old)



Cheese-fed pigs yielded significantly ($P < 0.05$) higher mean daily probiotic excretion than yogurt-fed pigs

Fig. 5.

Gardiner, G., Stanton, C., Lynch, P.B., Collins, J.K., Fitzgerald, G. and Ross, R.P. (1999). Evaluation of Cheddar Cheese as a Food Carrier for Delivery of a Probiotic Strain to the Gastrointestinal Tract. *Journal of Dairy Science*, 82: 1379-1387.

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Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P.B. and Ross, R.P. (2000). Market potential for probiotics. *American Journal of Clinical Nutrition* (In press).

Gardiner, G.E., O'Sullivan, E., Kelly, J., Auty, M.A.E., Fitzgerald, G.F., Collins, J.K., Ross, R.P. and Stanton, C. (2000). Comparative Survival Rates of Human-Derived Probiotic *Lactobacillus paracasei* and *L. salivarius* Strains During Heat Treatment and Spray Drying. *Applied and Environmental Microbiology* 66: (6) 2605-2612.

Patent

Process for the manufacture of probiotic cheese. BioResearch Ireland and Forbairt, Patent Application No. 980408.

Media Uptake

A measure of the interest generated by this project can be gauged by uptake in the media. The following are 3 examples:

'Trials show how Probiotic Cheese can Boost Immunity to Lots of Nasty Bugs', **Irish Times**, October 1997.

'Good Bugs - Cheddar cheese soon might join the ranks of yogurt and some other dairy products as food that can deliver a good dose of healthy bacteria', **La Crosse Tribune**, USA, 9th Sept., 1998

'Say Cheese', **Bioscience**, USA, Vol. 48, No. 8, p. 664, August 1998.

For further information, please contact Dr. Catherine Stanton or Dr. Paul Ross





Dr. Catherine Stanton



Dr. Paul Ross



DAIRY PRODUCTS RESEARCH CENTRE

Moorepark, Fermoy, Co. Cork, Ireland

Tel: +353 (0) 25 42222 - Fax: +353 (0) 42340

E-mail: reception@moorepark.teagasc.ie