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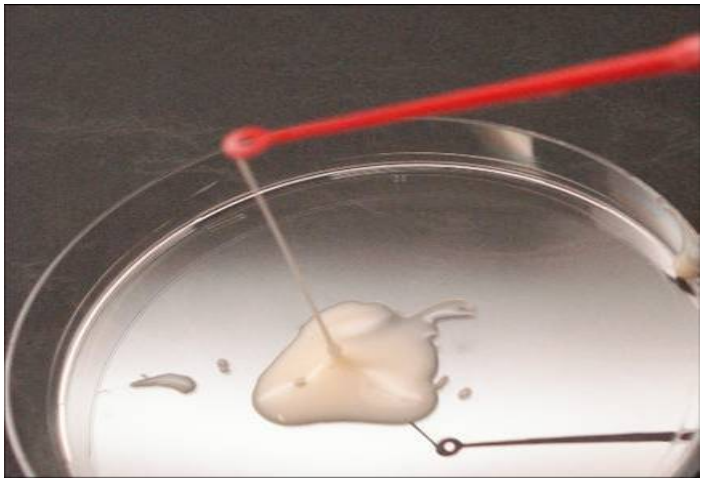
Exploitation of cheese cultures for flavour diversity and functionality



Key external stakeholders:

Dairy industry, starter supply companies, research community

Practical implications for stakeholders:

Micro-organisms are critical for cheese manufacture and ripening and are a key contributor to its flavour development. Thus, application and control of the cheese microbial flora during manufacture and ripening offers the cheese manufacturer a means to develop cheeses with flavours and functionalities targeted to specific markets. This project was sought to determine the impact of various micro-organisms on cheese flavour and functional properties with a view to identifying strains with benefit traits that could be exploited by the industry.

The main issues addressed will include investigations into:

- The potential of exopolysaccharide (EPS) producing starter to cheese manufacture and ripening
- The contribution of *Streptococcus thermophilus* to Cheddar cheese flavour
- Identification of new bacterial strains for cheese manufacture

Main results:

- A bank of 142 EPS producing lactic acid bacteria was assembled.
- It was clearly demonstrated that EPS producing strains have the capacity to improve cheese yield and enhance the texture properties of reduced-fat Cheddar cheese.
- *St. thermophilius* when used as a starter or starter adjunct impacted on flavour development in a strain specific manner.

Opportunity / Benefit:

The successful implementation of this project provides a range of options to cheesemakers to produce cheeses with improved and diverse flavours and functional properties. By so doing the project supports the efforts of Irish cheese makers to exploit markets for cheese with diverse and unique flavours, such as the speciality and extra mature Cheddar markets in the UK, to which only limited access is currently available. Expressions of interest from companies interested in this area are welcome.

Collaborating Institutions:

UCC

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Dr. John Hannon
External collaborators: Prof. Paul McSweeney, UCC

1. Project background:

Cheese is a critically important product from the Irish dairy industry and accounts for over 30% of milk utilization. Flavour, texture and cooking properties are three key criteria on which consumers base their decision on cheese purchase. A number of factors influence the development of these characteristics during ripening but micro-organisms that grow in cheese during manufacture and ripening are critical factor influencing their development. This project set out to harness various cheese flora or their enzymes to promote diversity of flavour and functionality in both Cheddar and non-Cheddar type cheese. This was achieved by investigating methods to control 'wild' NSLAB during ripening, by exploiting EPS producing cultures, by manipulating cheese flavour production through amino acid catabolism and by investigating the potential of novel food grade cultures to contribute to the ripening process. The exploitation of fermentation technology and starter production capacity to facilitate the commercial application of key outputs from the project was also investigated.

2. Questions addressed by the project:

(i) Can technologies be developed to control the development of 'wild' NSLAB in commercial Cheddar cheese?
(ii) Can EPS production by cheese flora influence on cheese yield and functionality?
(iii) Can novel strains of food grade micro-organisms be selected that will impact in a positive manner on cheese flavour development during ripening?
(iv) Does the use of *St. thermophilus* as a starter/starter adjunct culture influence flavour development in Cheddar cheese?

3. The experimental studies:

(i) Development of technologies to control the development of 'wild' NSLAB in commercial Cheddar cheese

The approach investigated was to seek strains of mesophilic lactobacilli that produce bacteriocins that inhibit typical NSLAB species. NSLAB were screened for bacteriocin production and 8 putative bacteriocin producing strains were identified. It was demonstrated that the inhibitory compound was sensitive to treatment by a number of protease enzymes suggesting that it was itself a protein, as would be expected for bacteriocins. Molecular analysis indicated that the bacteriocins produced by all of the strains was very similar and N-terminal amino acid sequencing of the bacteriocin demonstrated a high level of similarity to the previously characterized acidocin M, produced by *Lactobacillus acidophilus*. While the host range of the bacteriocin was suitable to inhibit typical NSLAB strains; however, inhibition of NSLAB was not detected in a cheese environment.

(ii) EPS production by cheese flora and its influence on cheese nutrition, yield and functionality

A bank of 142 EPS producing strains of LAB was assembled consisting of ropey, capsular and ropey plus capsular phenotypes. The genotypes of the strains were also determined using PCR based methods. Methods for the isolation, purification and compositional analysis of EPS were developed.

An isogenic strain set of EPS and non-EPS producing *Lactococcus lactis* was developed by means of plasmid curing. This strain set was used to manufacture cheese in an effort to determine definitively the impact of EPS on cheese manufacture and ripening. The data obtained clearly demonstrated that the EPS producing strain increases cheese yield and moisture without affecting coagulation, survival of starter and growth of NSLAB. At the same time, the presence of EPS in half-fat Cheddar cheese had little impact on flavour but improves the texture and flowability to a level similar to a full-fat cheese, making the EPS-producing starter an attractive tool to overcome the undesirable texture proprieties of reduced fat cheeses.

Trials on the influence of EPS on coagulation and syneresis suggest that EPS has no significant effect on coagulation but has some effects on syneresis, as the cheeses manufactured with the EPS-producing culture retained more moisture than the cheeses made with the non-EPS-producing starter culture, and therefore had less syneresis during manufacture.

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(iii) Selection of novel strains of food grade micro-organisms to impact in a positive manner on flavor formation in cheese during ripening

A number of approaches were used to identify such strains including investigating the contribution of starter autolysis and application of different strains as starter adjuncts during cheese manufacture.

Experiments designed to investigate the relationship between autolysis and sensory characteristics of cheese, using isogenic strains of *L. lactis* AM2 clearly demonstrated a relationship between autolysis and the sensory characteristics. In addition, analysis of the volatile compounds identified in the cheese demonstrated that many of the compounds were the result of amino acid catabolism.

The capacity of a range of food grade cheese micro-organisms to catabolise amino acids was determined. These strains potentially could impact on cheese flavour development during ripening and thus constitute a bank of strains was that will form the basis for future studies in this area.

(iv) Contribution of *Streptococcus thermophilus* to cheese manufacture and ripening

The genetic diversity of both commercial strains and those in the Moorepark Culture Collection was determined. The data accumulated demonstrated that the diversity available from various commercial supply companies was quiet similar. A significant level of biodiversity, in excess of that demonstrated by the commercial cultures analysed, was identified within the Moorepark Culture Collection. Access to this biodiversity could enable the identification of new strains with commercial potential.

A major body of data on the growth profiles of *St. thermophilus* strains during cheese manufacture, their behaviour during ripening and their contribution to cheese ripening was established. The key observation throughout this research was that *St. thermophilus* when added as a starter or starter adjunct during Cheddar cheese manufacture does have the capacity to influence flavour development. There are strain specific differences in this capacity and also it was observed for some strains that their influence increased as the cheeses matured. The practical outcome of this research is that we have clearly demonstrated that use of *St. thermophilus* impacts on cheese flavour and quality and thus commercial cheese manufacturers need to consider such impacts when selecting such strains. However, use of such strain also offers industry an approach to flavour diversification.

4. Main results:

- A bank of 142 EPS producing lactic acid bacteria was assembled
- It was clearly demonstrated that EPS producing strains have the capacity to improve cheese yield and enhance the texture properties of reduced-fat Cheddar cheese
- A bank of food grade organisms with differing amino acid catabolising capability and thus the potential to influence cheese flavour development was assembled
- *St. thermophilius* when used as a starter or started adjunct impacted on flavour development in a strain specific manner

5. Opportunity/Benefit:

The successful implementation of this project has resulted in Teagasc undertaking follow on research projects with eight companies, seven of who are major international food companies. Two companies have licensed strains coming from the research. A number of additional opportunities for follow on commercial research are still available on discussions with a number of companies are ongoing.

6. Dissemination:

Research preformed in this project was presented to the Irish industry at the 49th Relay Workshop covering Cheese Research Highlights: 2000-2010 held in Moorepark, November 2007 and to international audiences at a number of international scientific conferences. As a consequence, two Irish companies and eight international companies expressed interest in the research. Follow on projects have been established with eight companies and two companies have licensed strains emanating from the research.

Main publications:

Costa, N.A., Hannon, J.A., Guinee, T.P., Auty, M.A.E., McSweeney, P.L.H. and Beresford, T.P. (2010). Effect of Exopolysaccharide Produced by Isogenic Strains of *Lactococcus lactis* on Half-Fat Cheddar

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Cheese. *Journal of Dairy Science* 93, 3469-3486

Beresford, T.P. (2011) Citrate fermentation by lactic acid bacteria. *Encyclopedia of Dairy Sciences*, second edition, **3**, 166-172

Costa, N.E., Wang, L., Auty, M.E., Hannon, J.A., Sweeney, P.L.H., Beresford, T.P. (2012). Rheological, microscopic and primary chemical characterization of the exopolysaccharide produced by *Lactococcus lactis* subsp. *cremoris* DPC 6532. *Dairy Science & Technology* DOI 10. 1007/s13594-012-0059-4

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