Technology Updates **Food**

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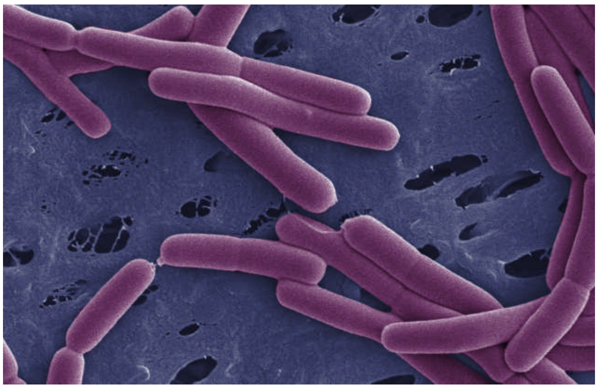
Genomic Analysis of *Lactobacillus helveticus* DPC4571



Key external stakeholders:

Dairy industry, starter supply companies, research community

Practical implications for stakeholders:

Bacteria, used in the manufacture of cheese play a major role in determining the flavour of the final product. The capacity to direct cheese flavour development to address specific consumer desires through the use of such bacteria offers the cheese manufacturer a significant advantage in the market place. Previous research at Teagasc has demonstrated that the use of *Lactobacillus helveticus* in cheese manufacture results in cheese with a very desirable sweet nutty flavour. This project sought to use molecular biological approaches to gain a greater understanding of *Lb. helveticus* and its role in cheese flavour development.

The main focus of the research was:

- Generation of the first complete genomic sequence of *Lb. helveticus*
- Exploitation of the genomic data to gain a greater understanding of the manner in which this bacterium impacts on cheese flavour development

Main results:

- The complete genomic sequence of *Lb. helveticus* DPC4571 was assembled and analyzed
- Four genes with the potential to impact on cheese flavor were examined using molecular approaches
- Molecular tools for the further genetic manipulation of *Lb. helveticus* were developed

Opportunity / Benefit:

These projects resulted in the first published genome sequence of a *Lb. helveticus* strain, thus providing a complete overview of the metabolic capabilities of what is a very successful cheese-making bacterium. The successful completion of the projects has provided an insight into how this bacterium impacts on cheese flavour development during ripening and this has enabled selection of additional strains that can impact successfully on cheese flavour development. By so doing the projects support the efforts of the Irish cheese makers to exploit markets for cheese, in particular cheeses with sweet nutty flavours that are highly prized by the modern cheese consumer. Expressions of interest from companies interested in this area are welcome.

Collaborating Institutions:

UCC

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Teagasc project team:	Dr. Tom Beresford (PI) Prof. Paul Ross Dr. John O'Callaghan
External collaborators:	Prof. Gerald Fitzgerald, UCC

1. Project background:

The contribution of microbial flora to the development of cheese flavour is a complex process which has been the subject of intensive research for many years. The main micro-organisms involved in this process are members of the lactic acid bacteria group with particular reference to *Lactococcus lactis, Streptococcus thermophilus* and various members of the genus *Lactobacillus.*

Lactobacillus helveticus are routinely added during the manufacture of a range of cheese and fermented milk products. Traditionally their use was linked to rapid acid production and to flavour development in a range of dairy products; however, they have not traditionally been used during the manufacture of Cheddar cheese. However, they have gained significant usage in recent years as many strains impart desirable sweet nutty flavours while removing undesirable bitter taste from the cheese. Developing an understanding of how *Lb. helveticus* impacts on the development of these commercially relevant traits and using this information to select new strains with such desirable characteristics was the basic focus of this project.

A large collection of *Lb. helveticus* strains was available to the project team and previous research identified a particular strain, *Lb. helveticus* DPC4571 which had a very pronounced positive impact on Cheddar cheese flavour. This strain was thoroughly investigated as a Cheddar cheese starter and was demonstrated to undergo rapid autolysis during cheese ripening which resulted in high levels of secondary proteolysis and production of a diverse range of volatile flavour compounds. This project attempted to assemble a complete genomic sequence map of this strain with a view to identifying genes that could contribute to the development of the desirable flavours observed. The availability of the genome of DPC4571 would facilitate an in depth analysis of this strain with a view to identifying the metabolic pathways and individual genes responsible for its beneficial industrial traits. In addition this information could be used to screen other strains available to the research team for traits that could be beneficial during cheese manufacture and ripening.

2. Questions addressed by the project:

- Can the complete genomic sequence of *Lb. helveticus* DPC4571 be assembled?
- Can *Lb. helveticus* genes with the potential to impact on cheese flavour be identified?
- Can molecular tools for the further genetic manipulation of *Lb. helveticus* be developed?

3. The experimental studies:

Assembly of the complete genomic sequence of *Lb. helveticus* DPC4571:

The major outcome of this task was the sequencing, assembly, annotation and publication of the genome sequence of DPC4571, the first *Lactobacillus helveticus* sequence to be published. A genomic microarray was designed and manufactured and the genome sequence was inputted into the ERGO bioinformatics suite for analysis. Two successful Comparative Genome Hybridisation experiments were completed and a rapid and reproducible strain typing method was developed and published. Analysis of the genome has provided some remarkable insights into the evolution of *the Lactobacillus helveticus/acidophilus* group of species, the role of Insertion Sequence Elements in genome plasticity, the gene sets required for niche adaptation by different *Lactobacillus* species and the evolutionary changes within DPC4571 during the time it has been in use as a starter culture.

Production of strategically important enzymes using recombinant DNA technologies:

This research represented the first attempt by the research team to identify useful genes in the entire *Lb. helveticus* genome and to clone and heterologously express these genes to yield functional proteins. The starting point was to use the bioinformatic date resulting from the genome assembly to undertake a comprehensive survey of the potential flavour producing enzymes of DPC4571. This resulted in the identification of a range of potential genes involved in protein hydrolysis, amino acid catabolism and lipolysis, all of which are known to contribute to cheese flavour development. Arising from this analysis, two aminotransferases and two peptidases (pcp and pepN) were successfully cloned into and expressed in *Lactococcus lactis* where the enzymes produced were biochemically characterised.

Development of molecular tools for the genetic manipulation of *Lb. helveticus* strains:
The two main objectives in this work were (a) the development of a transformation protocol for DPC4571 and (b) a method of targeted gene inactivation. Successful transformation of DPC4571 was achieved albeit at an extremely low frequency. The low frequency of transformation meant that the more common gene inactivation approaches were not feasible, for this reason the use of antisense RNA was investigated and successfully applied to the inactivation of the genes involved in cell lysis, an important technological trait.

In addition a number of plasmids from a range of *Lb. helveticus* strains were isolated and fully sequenced. This data has demonstrated that plasmids of *Lb. helveticus* do not carry many genes of technological significance; however, some of the plasmids studied could form the core of new cloning vectors for this species.

4. Main results:

- The complete genome sequence of *Lb. helveticus* DPC4571 was assembled
- Genes involved in cheese flavour development were identified and over-expressed in *Lc. lactis.*
- Molecular tools for the manipulation of *Lb. helveticus* were developed

5. Opportunity/Benefit:

The successful completion of these projects has put Teagasc to the forefront of international research on *Lb. helveticus,* an organism of increasing importance to the cheese industry. Arising from the projects Teagasc interacted with three companies involving follow on research and undertaking of commercial trials. Further commercial opportunities exist in this area and the research team is available for discussions with interested companies.

6. Dissemination:

Research preformed in these projects 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, three international companies expressed an interest in the research. A Canadian based company undertook commercial trials with the strain with a view to licensing the strain for use in cheese manufacture in North America, a Japanese company is undertaking collaborative research with the Moorepark team on the strain and a European based company placed a student at Moorepark to work on this and related aspects of starter culture research.

Main publications:

Callanan, M.J., Kaleta, P., O’Callaghan, J., O’Sullivan, O., Jordan, K.N., McAuliffe, O., Sangrador-Vegas, A., Slattery, L., Fitzgerald, G. F., Beresford, T.P., Ross, R.P. (2008) “Genome Sequence of *Lactobacillus helveticus*, an Organism Distinguished by Selective Gene Loss and Insertion Sequence Element Expansion” *Journal of Bacteriology,* 190, 2, 727-735.

Kaleta, P., Callanan, M.J, O'Callaghan, J., Fitzgerald, G.F., Beresford, T.P. and Ross, R.P. (2010) “Crucial role for insertion sequence elements in *Lactobacillus helveticus* evolution as revealed by interstrain genomic comparison” *Applied and Environmental Microbiology* 76, 212-220

Slattery, L., O'Callaghan, J., Fitzgerald, G.F., Beresford, T.P. and Ross, R.P. (2010) “Invited Review: *Lactobacillus helveticus* – A thermophilic dairy starter related to gut bacteria” *Journal of Dairy Science* 93, 4435 - 4454

Popular publications:

Teagasc goes genomic to boost flavour in dairy. Article published on www.foodnagivator.com

7. Compiled by: Tom Beresford