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Modification of cheese flavour through the use of surface microbiota

Key external stakeholders:

Cheese makers,
SMEs
Farmhouse cheese producers

Practical implications for stakeholders:

Smear bacteria and yeasts establish themselves successfully on the surface of young Cheddar cheese curd and produce novel surface-ripened cheeses with a range of aromas and flavour with-in a short ripening time.

- The composition of the microbiota that develops on the cheese surface influences the colour, flavour and aroma of the cheese.
- This technology can be be applied to produce novel cheeses with a diverse range of flavours and aroma within a short time frame.

Main results:

- A model system medium was developed to screen Gram positive bacteria for their ability to produce volatile compounds important in cheese flavour.
- Single strains of smear bacteria in conjunction with *Debaryomyces hansenii,* or commercial smear culture mixes established themselves successfully on the surface of young Cheddar cheese curd.
- Novel cheese varieties with a diverse range of flavour and aromas were developed using smear bacteria and yeasts applied on the surface of young Cheddar cheese curd.

Opportunity / Benefit:

This research has potential for small cheese producers/SMEs to produce novel cheeses from young Cheddar cheese possibly in conjunction with a commercial Cheddar cheese manufacturer without the need for investment in expensive cheese making equipment.

Collaborating Institutions:

UCC

Teagasc project team:	Dr. Mary Rea (PI) Dr. Kieran Kilcawley Dr. Diarmuid Sheehan Dr. Andrea Bertuzzi
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1. Project background:

With the abolition of the milk quotas within the EU in 2015 there is a renewed interest in developing novel cheeses with a range of flavours. There is a progressive increase in global cheese consumption, with an annual production in Ireland of 205,000 tons in 2016 (data from Eurostat). Therefore the aim of this work was to develop a novel cheese with diverse aromas and short ripening time using cheese curd made in a traditional Cheddar cheese plant. Ripening time for Cheddar cheese can be from a little as 3 months for mild cheese up to > 9-12 months for mature/extra mature varieties. In this study, the ability of smear bacteria and yeast to grow on the surface of young Cheddar cheese curd was investigated in order to produce cheese varieties with different flavour and appearance compared to Cheddar cheese within a short time frame of 30-35 days.

2. Questions addressed by the project:

- Can model systems be developed to assess the capability of bacteria isolated from surface-ripened cheese to synthesise volatile compounds important in the development of cheese flavour?
- Can smear bacteria and yeast become established on the surface of young Cheddar cheese?
- Can smear bacteria and yeast modify flavour of young Cheddar cheese in short short ripening time?
- Can next generation sequencing techniques be used to monitor microbial succession on the surface of smear ripened cheese?

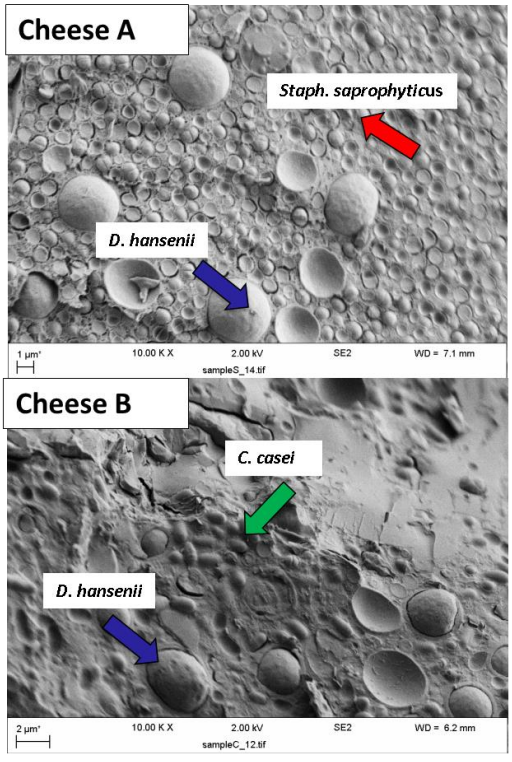
3. The experimental studies:

- Two model systems were formulated to screen bacteria isolated from surface ripened cheese for their ability to synthesise volatile flavour compounds.
- The genome of *Staph.saphrophyticus* DPC5671was sequenced and analysed for genes potentially involved in flavour development.
- Two approached were taken to develop cheese with novel flavours using Cheddar cheese curd, namely inoculating single bacteria either *Staphylococcus saprophyticus* DPC5671 or *Corynebacterium casei* DPC5298 combined with *Debaryomyces hansenii* DPC6258, or applying commercial smear culture mixes containing a mixture of bacterial genera and yeasts.
- High throughput sequencing combined with gas chromatography mass spectrometry (GCMS) was used to correlate the individual microbial components and volatile compounds detected on the cheese surface during ripening.

4. Main results:

- Two model systems were developed containing a) free amino acids and salt using stationary phase cells (MS1) and b) cheese curd, lactose, tryptone and salt using log phase cells (MS2). A representative number of genera commonly isolated from surface ripened cheese were tested in both model systems. The results obtained with GCMS analysis showed that MS1 was a better screening tool for evaluating the capability of smear bacteria to produce volatile compounds as there were more compounds detected and significantly more compounds were associated with particular strains compared to MS2.

- The surface of young Cheddar cheese curd was inoculated with *D. hansenii* DPC6258 at day 1 and, after 5 days incubation at 16°C, was re-inoculated with with *Staph. saprophyticus* DPC5671, or *C. casei* DPC5298. *Staph.saprophyticus* DPC5671 and *C. casei* DPC5298 established themselves successfully on the cheese surface during ripening and were the dominant bacteria on the cheese surface at



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the end of ripening (Fig. 1). Proteolysis, lipolysis, and colour development were influenced by the growth of the smear cultures on the cheese surface. These bacteria, in combination with *D. hansenii* DPC6258, were able to diversify the appearance, aroma and flavour of the cheese in a short ripening time (35 days).

Fig 1. Cryogenic scanning electron micrographs of the cheese surface after 35 days of ripening showing the predominance of *Staph. saprophyticus* DPC5671 (cheese A) or C. casei DPC5298 (cheese B).

- Two commercial smear culture mixes (D4, S5) containing different combinations of yeast and bacteria (Fig 2) were selected and inoculated onto the surface of young Cheddar cheese curd. The cheese was incubated at 16°C at 97% relative humidity for 30 days. Microbial succession and flavour development were followed throughout ripening using a combination of whole metagenomic shotgun sequencing and volatile analysis (GCMS).

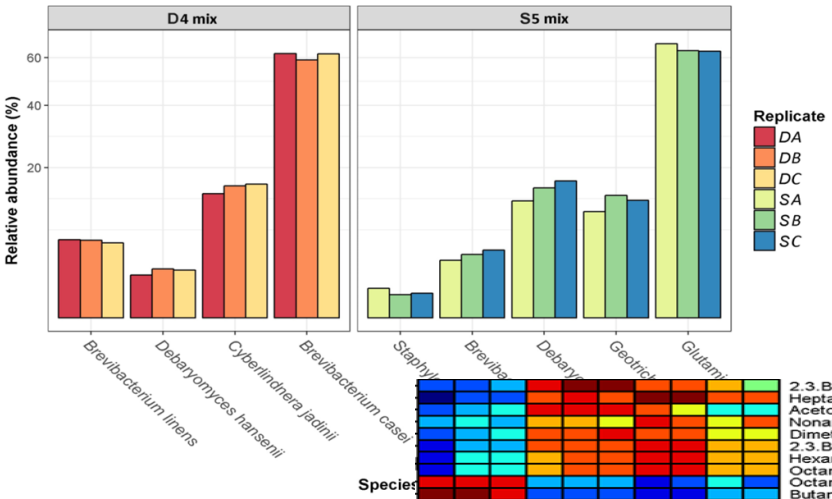

Fig 2. Relative abundance of yeasts and bacteria within the smear culture mixes D4 and S5

- Whole metagenomic shotgun sequencing demonstrated the microbial succession over the ripening period and showed that the yeasts were present at the highest abundance in the early stages of ripening with bacteria being more abundant at the later stages of ripening. In some cases not all the yeasts or bacteria present in the culture mixes established themselves on the surface of the cheese.
- Volatile analysis (GCMS) in combination with sequencing data allowed for the correlation between microbial species and flavour compounds produced during ripening (Fig 3).

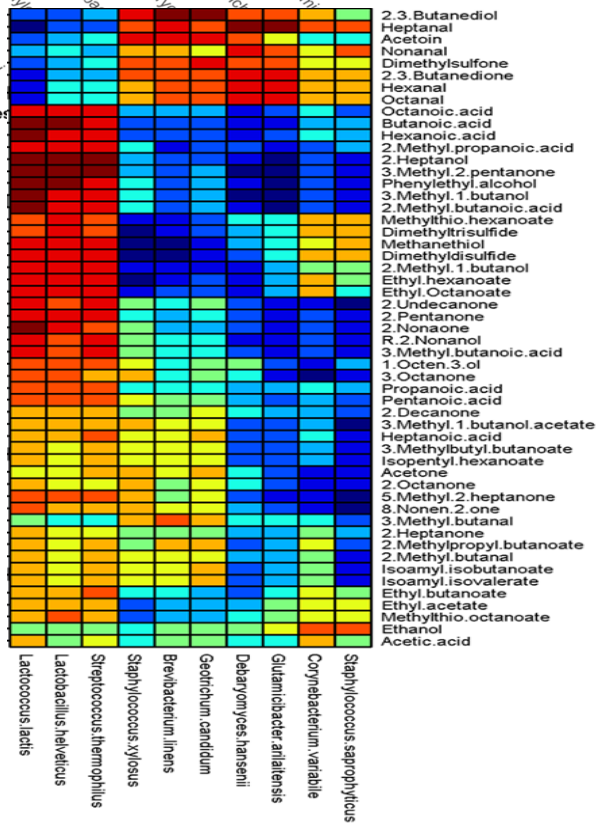

Fig. 3 Spearman correlation analysis revealed correlations between the microbial population and the volatiles detected. The red tiles indicate a low correlation, while the blue tiles a high correlation.

5. Opportunity/Benefit:

The model system developed will be used in the future to screen isolates in the DPC Culture Collection for their potential to produce volatile flavour compounds for food fermentation applications.

Inoculation of surface bacteria and yeasts onto the surface of young Cheddar cheese could potentially represent a low-cost solution for medium-small cheese industries to produce cheese with a variety of flavours and aroma within a short ripening time.

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6. Dissemination:

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2. Bertuzzi, AS ., McSweeney, PLH., McSweeney, PLH., Rea, MC., Kilcawley,KN. (2018). Detection of Volatile Compounds of Cheese and Their Contribution to the Flavour Profile of Surface-Ripened Cheese. Comprehensive Reviews in Food Science and Food Safety. On line January doi: 10.1111/1541-4337.12332
3. Bertuzzi, A.S., Guinane, C.M., Crispie, F., Kilcawley, K.N., McSweeney,P.L.H. and. Rea, M.C. (2017). Genome Sequence of *Staphylococcus saprophyticus* DPC5671, Strain Isolated from Cheddar Cheese. Genome Announc. Apr; 5(16): e00193-17
4. Bertuzzi, AS., Kilcawley, KN., Sheehan, JJ., O'Sullivan, M.G., Kennedy, D., McSweeney, PLH.and Rea. M.C. (2017). Use of smear bacteria and yeasts to modify flavour and appearance of Cheddar cheese. International Dairy Journal 72: 44-54
5. Stevanovic, E., Maillard, MB., Bertuzzi, A., Rea, MC., Fitzgerald, G., Mc Auliffe, O., Kilcawley, KN (2017) Strains of the *Lactobacillus casei* group show diverse abilities for the production of flavor compounds in 2 model systems. Journal. Dairy Science 100; 6918–6929

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