Spoilage bacteria in the feed of New Zealand dairy sheep

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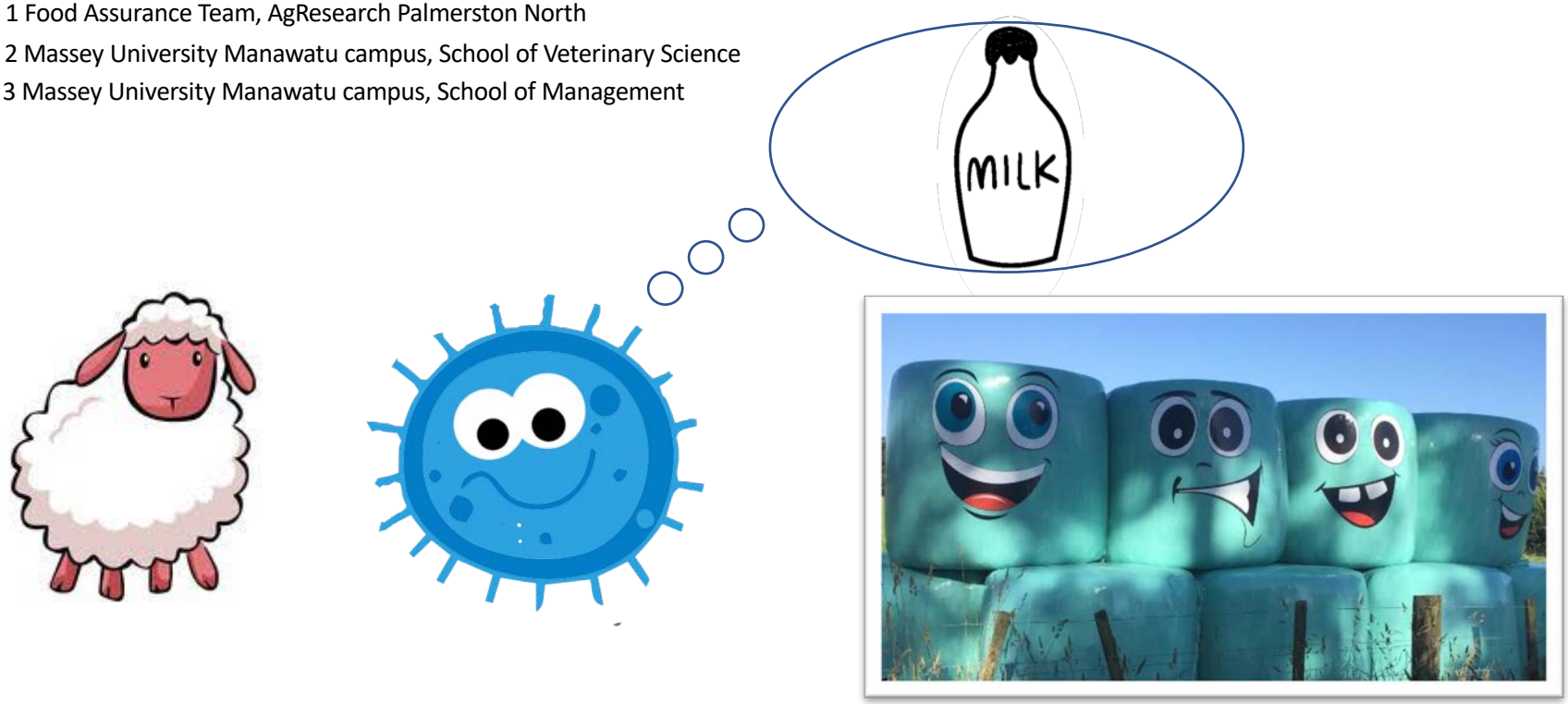

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Bacteria are friends and foes !

Pathogens

Opportunistic pathogens

Spoilage bacteria

Commensals

Dairy spoilage bacteria

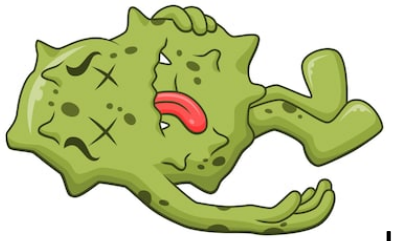
- In a healthy animal, raw milk is considered sterile.

- Bacteria coming from the farm environment may contaminate the milk during milking & storage.

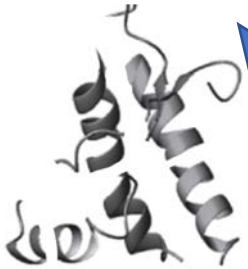
- Bacterial contamination can lead to off-odours or off-flavours and even early spoilage.

→ Quality decrease

But, Pasteurization ?!

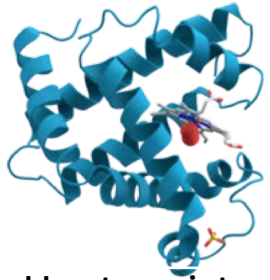


Vegetative cells



Heat-sensitive enzymes

Spores



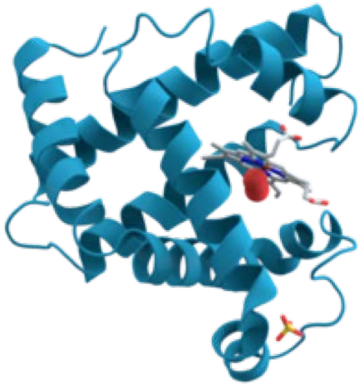
Heat-resistant enzymes

Dairy spoilage bacteria and enzymes

Spores

- Dormant bacterial state, highly resistant
- Able to grow alongside the processing chain
- Spoilage through production of enzymes after germination

Spoilage enzymes



- Mainly produced by bacteria growing during raw milk refrigeration and after pasteurization
- Able to degrade milk components (lipids, proteins)
- Only partly inactivated by heat treatments when heat-resistant

Spoilage enzymes in dairy

Lipases

- Degrade the fat molecules in milk (lipids)
- Associated with off-odours and off-flavours

Proteases

- Degrade the proteins in milk (mainly caseins)
- Associated with curdling and off-flavours

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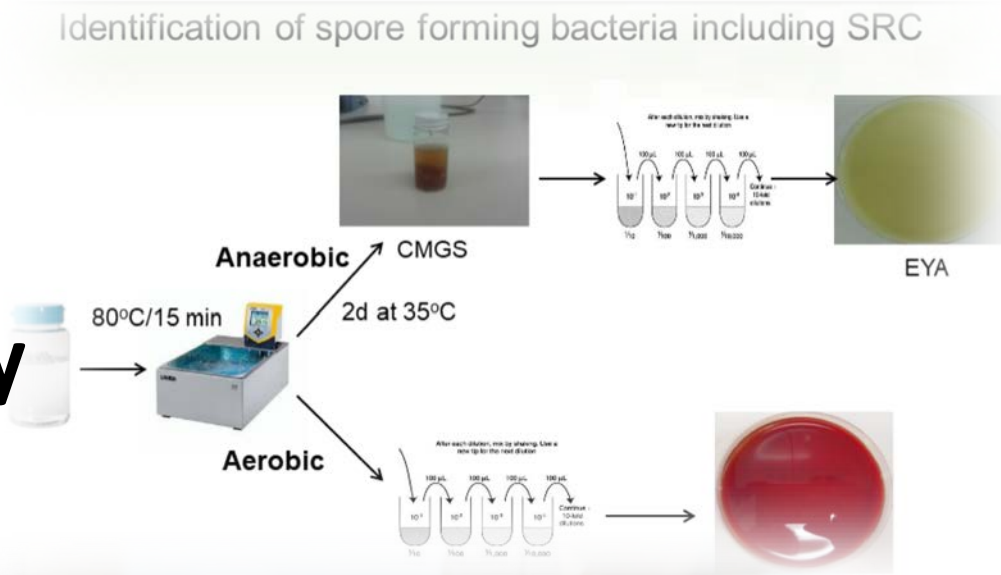
Where it started : The dairy bovine study



- AgResearch-funded project
- Early spoilage of cow dairy products
- Characterization of New Zealand on-farm spores populations
- Linking on-farm and off-farm

Dr Tanu Gupta
Food assurance team, AgResearch

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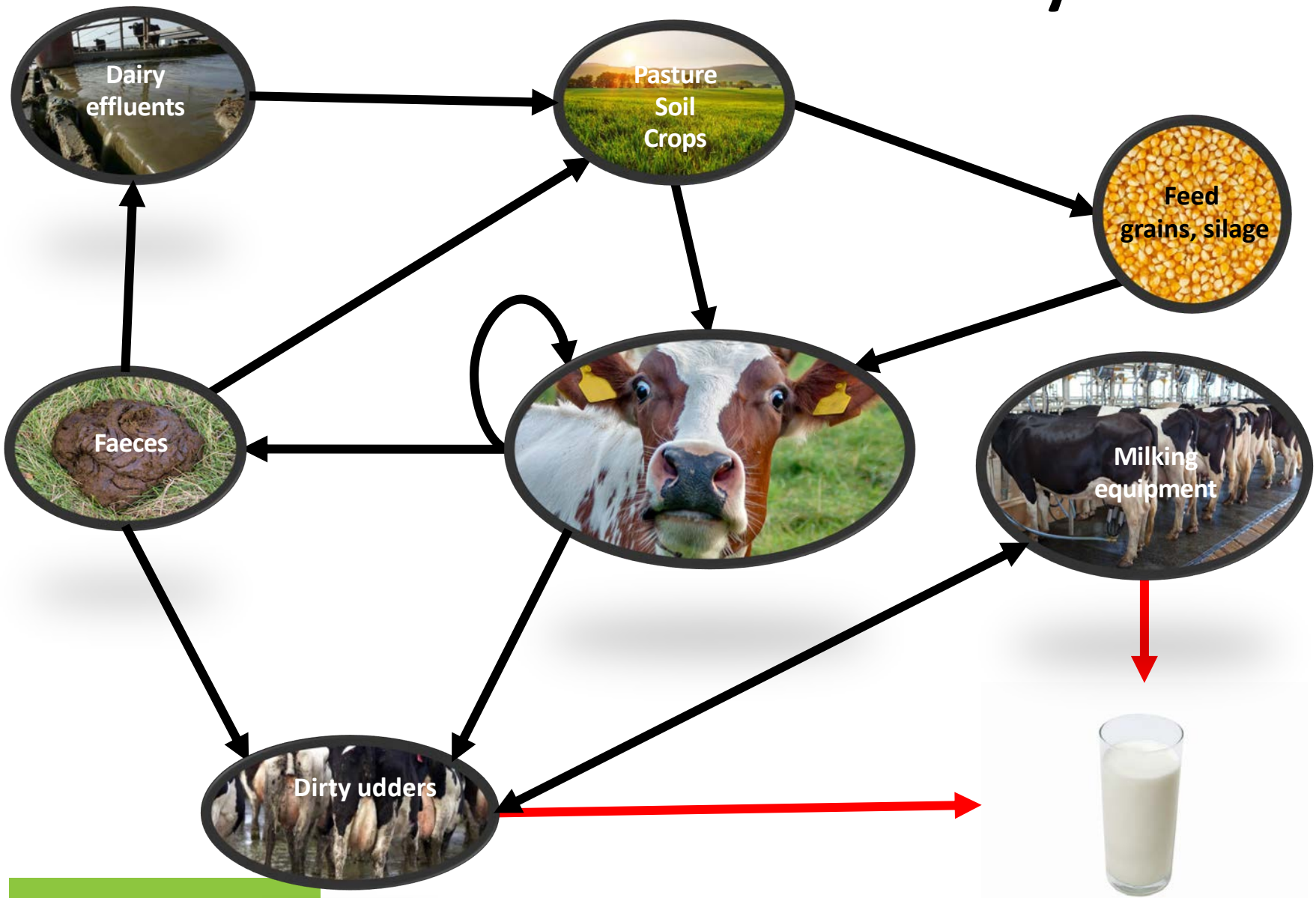
The dairy cattle farm contamination cycle



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The dairy sheep perspective

- Highly nutritious product
- No clear optimal farming guidelines
- Desire to improve and maintain product quality
- Benefit to New Zealand's sheep milk international image

The team

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AgResearch / Massey University
PhD student

PhD supervisory panel

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Scientist

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Scientist

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School of Management
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The PhD study

Goals:

- **<u>Characterize on-farm and raw milk spoilage bacteria populations</u>**

- **Investigate optimal farm practices and potential on-farm interventions to reduce raw milk's microbial load**

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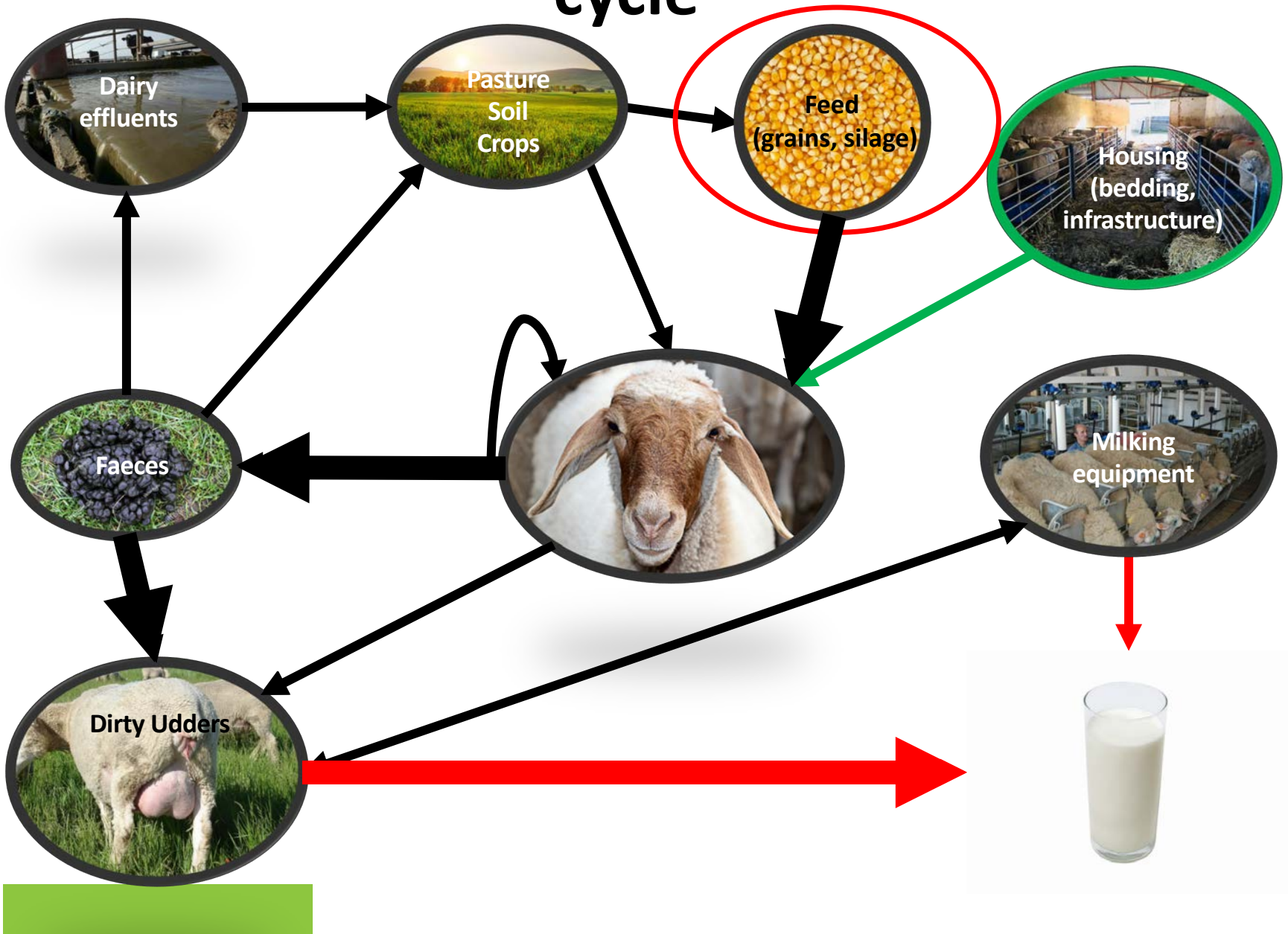
The PhD study research plan: How?

- Bi-annual sampling of dairy sheep farms
  → Ecological profiles of spoilage bacteria
  → Identify route of entry in the raw milk

- In-depths characterization of key spoilage bacteria
  → Resistance, biofilm formation, spoilage activity

- Preliminary studies to mitigate the impact of spoilage bacteria on milk quality

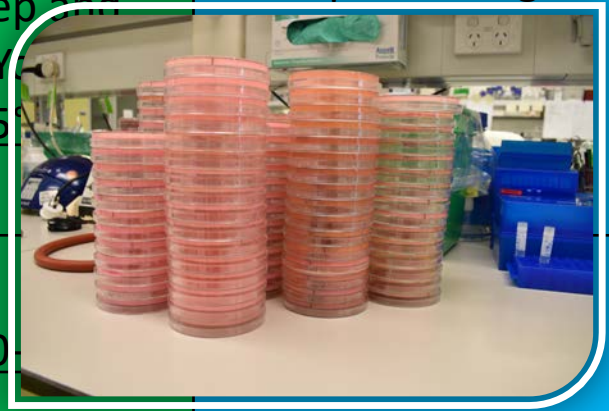
The dairy sheep farm contamination cycle



Sample processing: methodology

- Mixing of 20 silage samples into an individual composite
- Selective culture-based processing
  - Spore selection: 80 °C 10min
- Retrieving of unique isolates

	Spore-forming				Non-spore-forming
	Cold temperature growing	Moderate temperature growing	Hot temperature growing	Anaerobically growing	Cold temperature growing
Lab growth	Sheep Blood Agar (SBA) plate	Sheep Blood Agar (SBA) plate	Sheep Blood Agar (SBA) plate	Enrichment step and Egg Y…	Sheep Blood Agar
Temperature range	25…			30…	



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How many bacteria in the feed ?

	Spore-forming			Non-spore-forming
	Cold temperature-growing	Moderate temperature-growing	Hot temperature-growing	Cold-temperature growing
Bacteria/g of feed	**35,000**	**1,500,000**	**25,000**	**650,000**

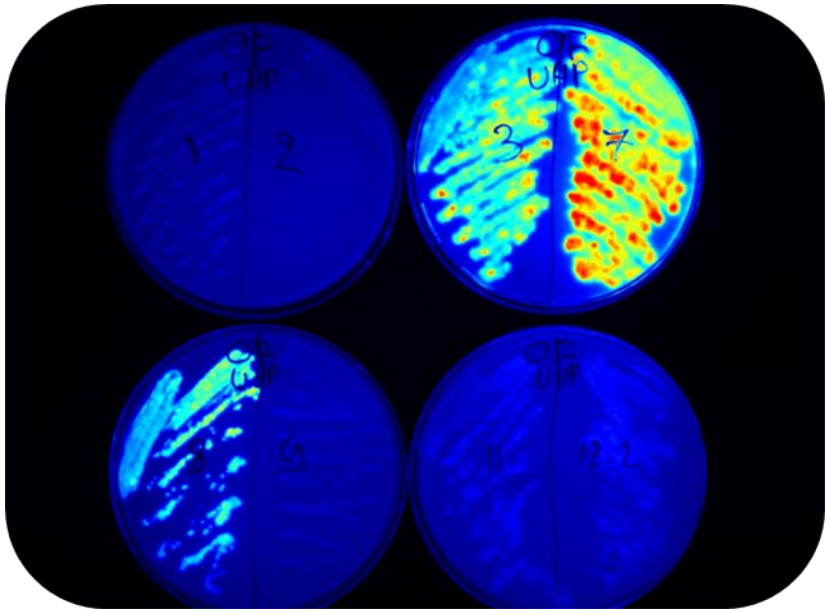
-Moderate temperature-growing spore and cold growing non-spores had the highest numbers

So who's there?

Cold temperature growing	*Psychrobacillus psychrodurans* *Bacillus psychrosaccharolyticus* *Paenibacillus xylanexedens* *Bacillus gibsonii* *Paenibacillus borealis*
Moderate temperature growing	*Bacillus licheniformis* *Bacillus pumilus* *Bacillus aerophilus* *Solibacillus silvestris*
Hot temp	
Anaero	

- A total of 213 isolates
- Selection of relevant isolates
- Identification using 16S rRNA sequences of bacteria: a unique barcode

Spoilage potential



Lipase testing:
Olive Oil -Rhodamine B

Protease testing: Milk Agar

- 36% positive for protein-degrading activity
- 24% positive for lipid-degrading activity

→ Mostly **moderate- and cold-temperature growing bacteria**

Protein-degrading
Lipid-degrading

Identification & spoilage potential

Cold temperature growing	*Psychrobacillus psychrodurans* *Bacillus psychrosaccharolyticus* *Paenibacillus xylanexedens* *Bacillus gibsonii* *Paenibacillus borealis*
Moderate temperature growing	*Bacillus licheniformis* *Bacillus pumilus* *Bacillus aerophilus* *Solibacillus silvestris*
Hot temperature growing	*Thermoactinomyces vulgaris* *Geobacillus thermodenitrificans* *Geobacillus thermoglucosidasius*
Anaerobically growing	*Clostridium sporogenes* *Clostridium bifermentans* *Clostridium butyricum*

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Summary

- **Identification of spoilage bacteria present in the feed**

  Most common:

  Bacillus licheniformis, Thermoactinomyces vulgaris,

  Bacillus psychrosaccharolyticus, Bacillus pumilus,

  Clostrium sporogenes, Clostridium bifermentans

- **A variety of different temperature-growing spores were isolated**

- **Spoilage potential**: Lipid-degrading 26%

  Protein-degrading 32%

- **Feed could be introducing spoilage bacteria with potential to contaminate the raw milk and decrease its quality**

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What's coming next?

- **Process the remaining samples**: Bedding, Dairy effluents, Faeces, Pasture, Water, Milking cups & Raw milk: 50 samples/season

  → Funding for a helper

- **Trace back raw milk contaminants** to the farm environment using DNA finger-printing techniques

- **In-depths characterization** of key spoilage bacteria

Acknowledgments

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- AgResearch: Internal core funding for the PhD and infrastructures
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