



# $N_2$ GAS FLUSHING OF BOVINE RAW MILK BETTER PRESERVES BACTERIAL DIVERSITY AT LOW TEMPERATURE

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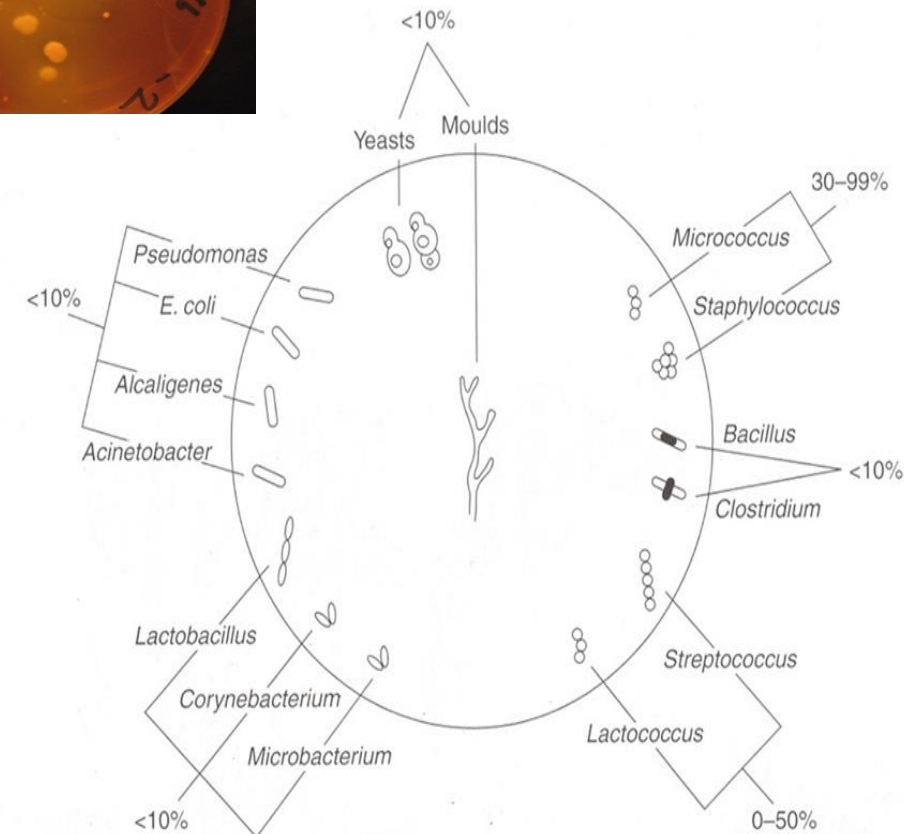
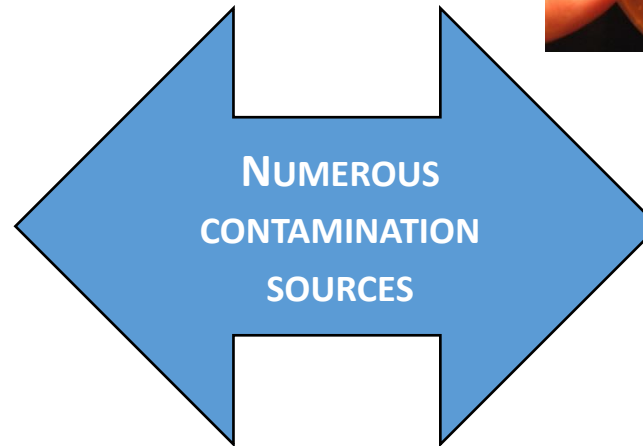
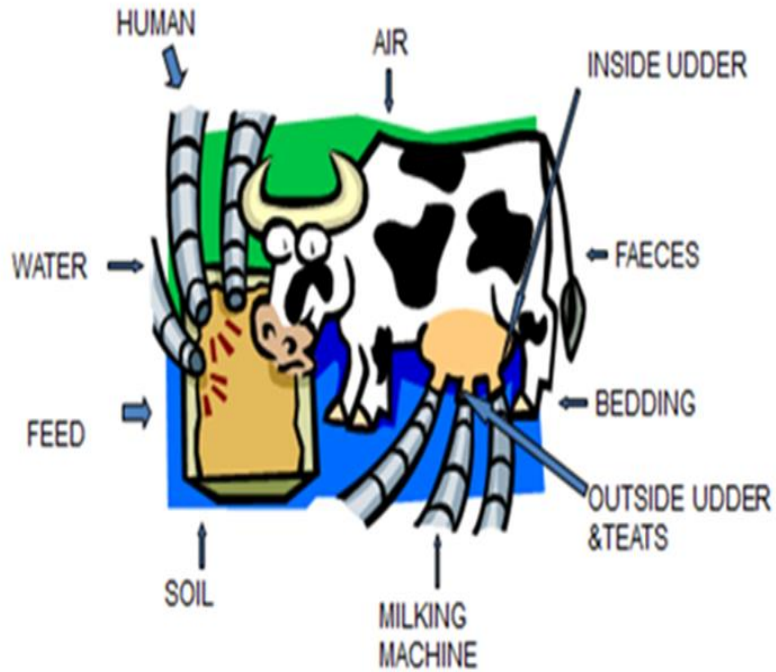
**UNIVERSITY OF HELSINKI,**  
Department of Food and Environmental Sciences,  
Division of Food Technology / Dairy Technology Group

Mikkeli, the 9<sup>th</sup> of June 2016

# IMPORTANCE OF BACTERIA

Raw milk is a highly perishable material: its composition is ideal for microbial/bacterial growth

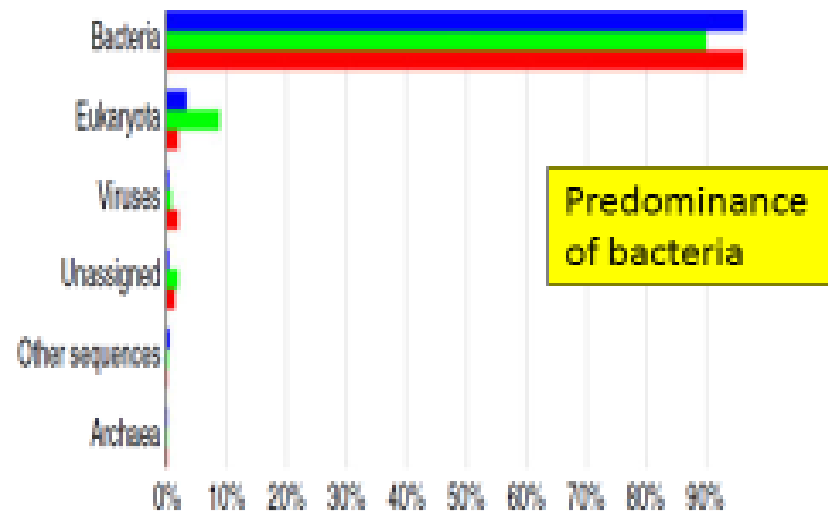
Culture dependant methods : a complex microbiota



(Encyclopedia of Dairy Sciences)

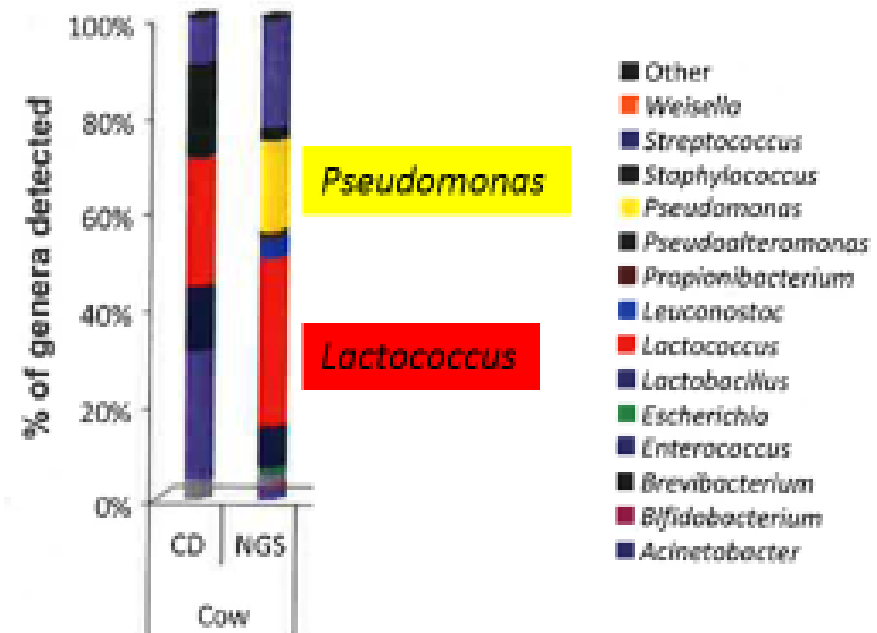
# Next Generation Sequencing (NGS): DNA-based studies

Domain-wise distribution of sequences in milk from 3 cattle breeds



(J. App. Microbiol. 2012/112/639-650)

Relative abundance of bacterial genera



More diversity as expected!

(FEMS Microbiol. Rev. 2013/37/664-698)

# MILK ASSOCIATED-BACTERIA: VARIED ROLES

## Positive effects...

Many are Lactic Acid Bacteria

- \*increase of shelf-life

- \*starter strains/fermentation  
(*Lactobacillus*, *Lactococcus*,  
*Streptococcus*...)

- \*flavour, texture

- \*Probiotics



## Spoilage...

*Pseudomonas*,  
*Acinetobacter*,  
*Clostridium*...



## Pathogens...

Severe illness (Oliver et al. 2009) , CDC/US “Raw milk is inherently dangerous”

### Common Pathogens Found in Raw Milk



*CAMPYLOBACTER*



*E. COLI*



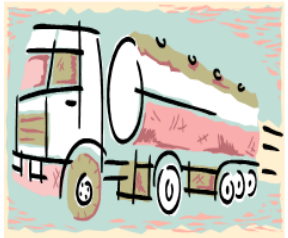
*SALMONELLA*



*LISTERIA*

# Preservation of raw milk (FAO recommends)

## DEVELOPED COUNTRIES

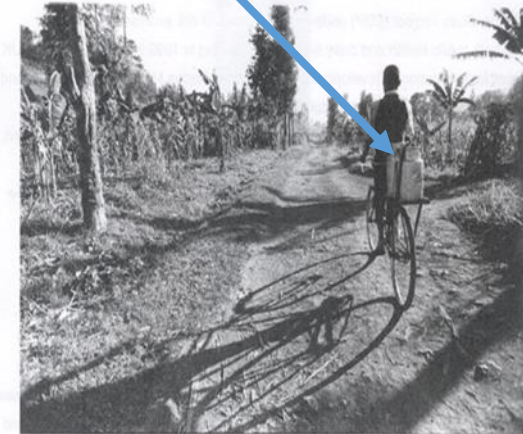


**Cold  
storage**



## DEVELOPING COUNTRIES

**H<sub>2</sub>O<sub>2</sub> and  
thiocyanate**

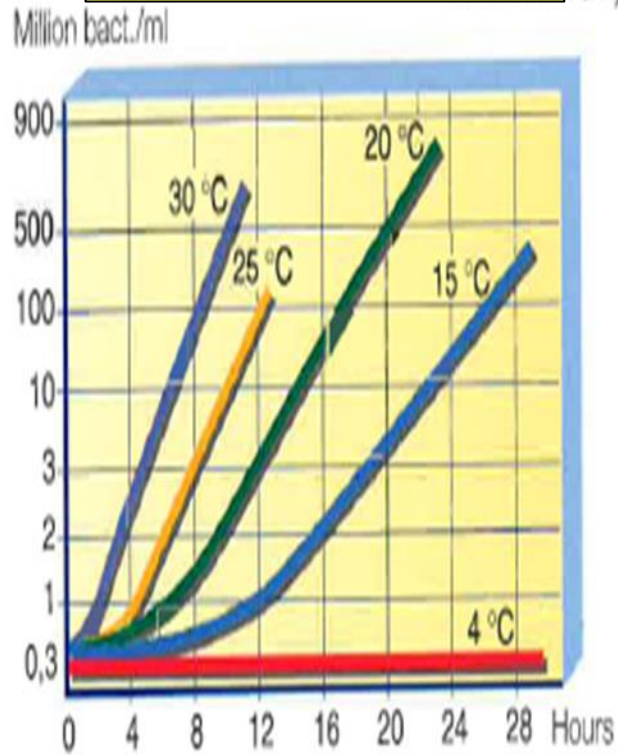


*The ubiquitous mobile milk traders in Kenya get about by bicycle, each day travelling 30–60 km and selling 50–120 litres of 'raw' milk, a main food usually drunk as boiled tea.*

# SOME FEATURES OF SPOILAGE BACTERIA

## WHAT HAPPENS DURING COLD STORAGE ?

Bacterial growth in raw milk at different temperatures

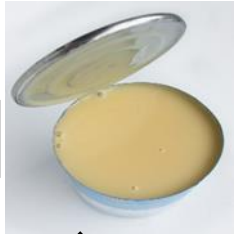


Bacterial growth in raw milk at 4°C



The critical age

Spoilage of milk

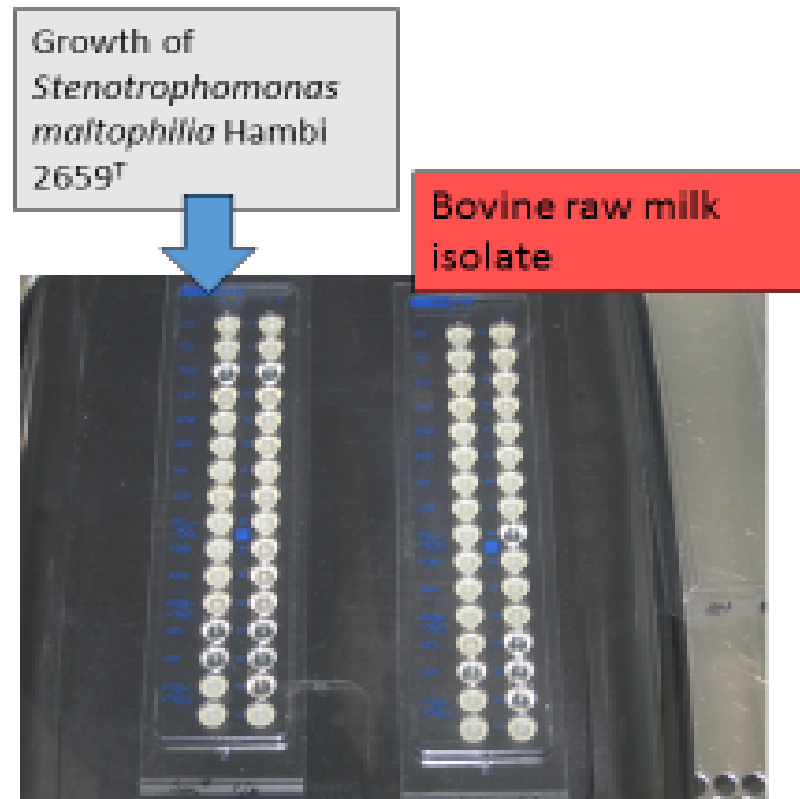


PSYCHROTROPHS

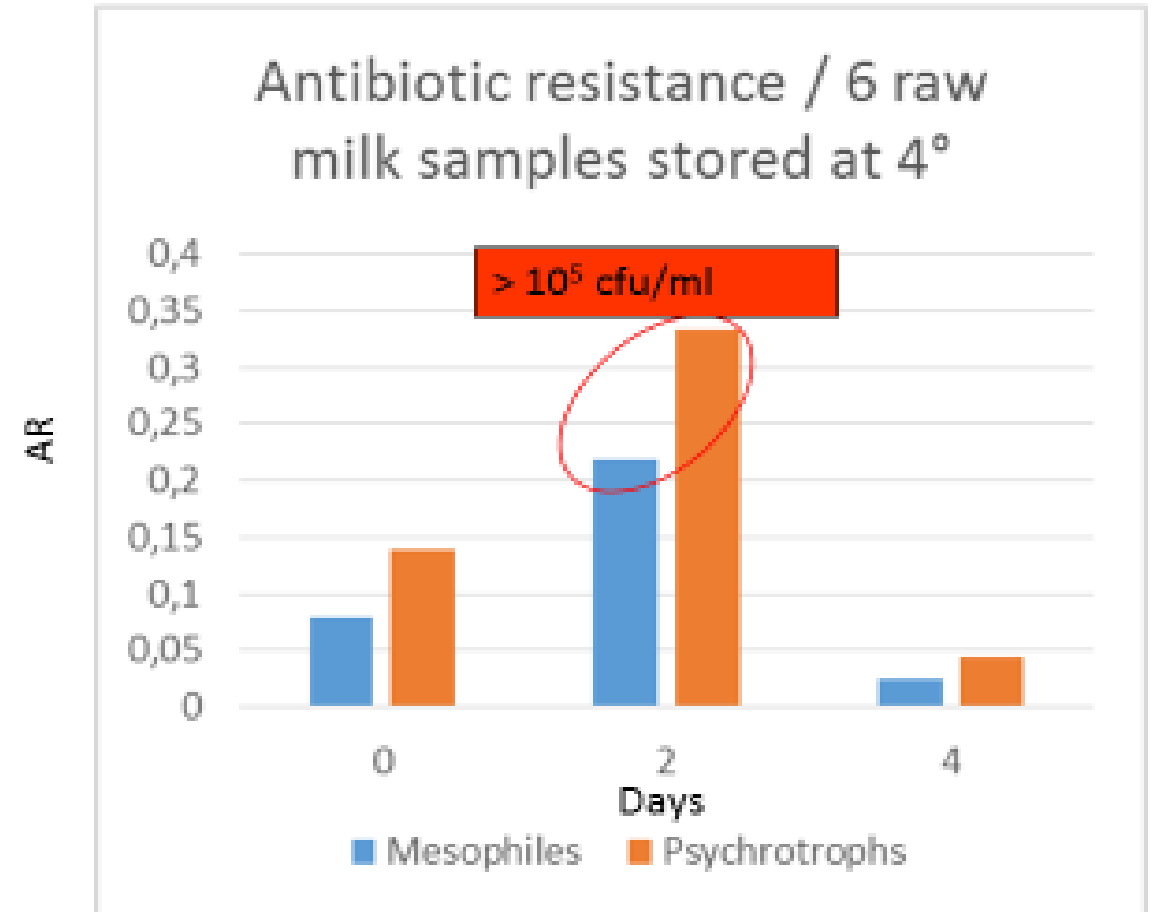
Production of heat resistant enzymes

Spoilage of UHT milk, butter, cheese

## Psychrotrophs carry antibiotic resistance features



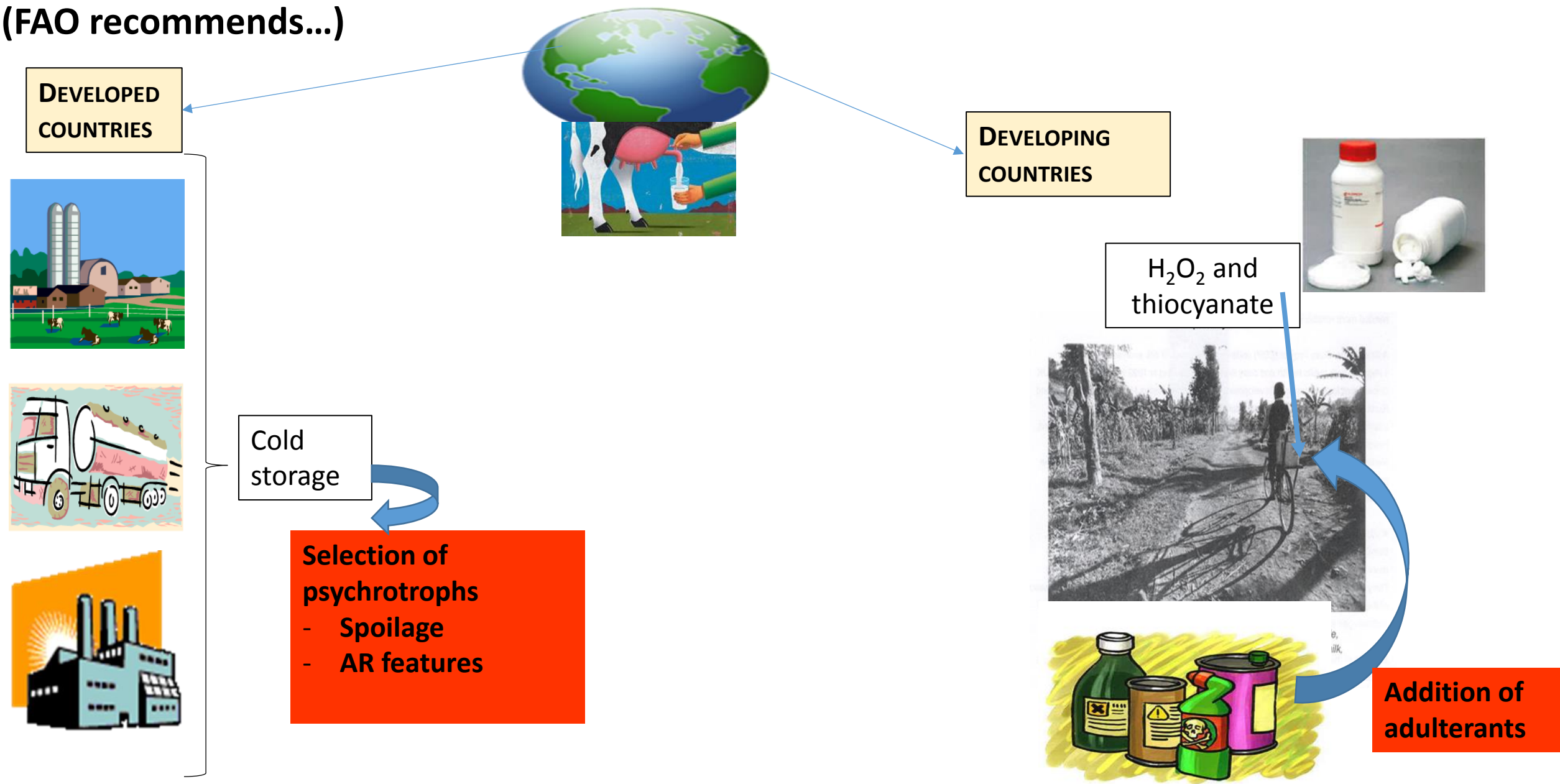
ATB®PSE strip (5 classes of antibiotics)



(Mic. Res. 2007/162/115-123; ISRN Microbiol. 2012, ID918208)



# Preservation of raw milk (FAO recommends...)





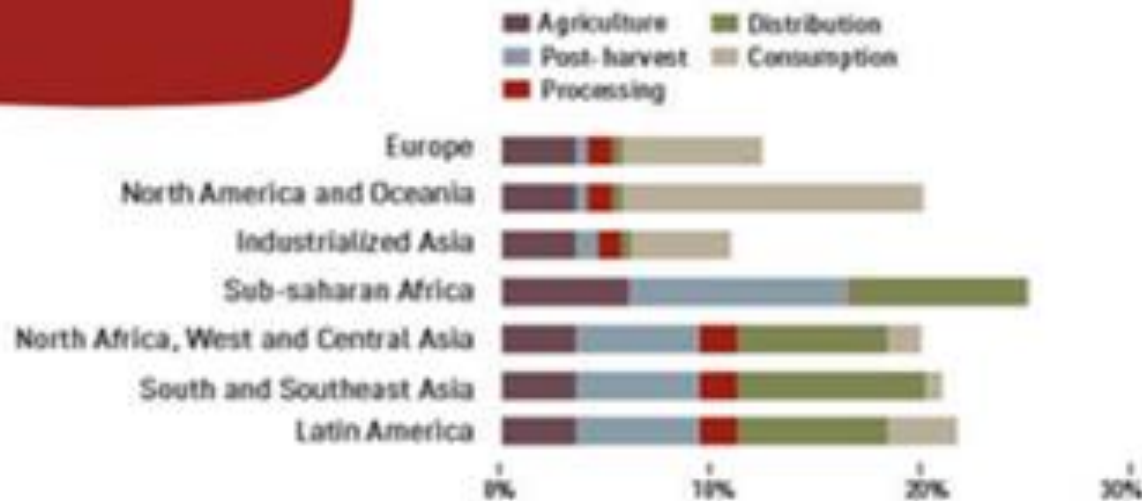


# 20% DAIRY FOOD LOSSES

In Europe alone, 29 million tonnes of dairy products are lost or wasted every year.



This is the same as  
574 billion eggs.

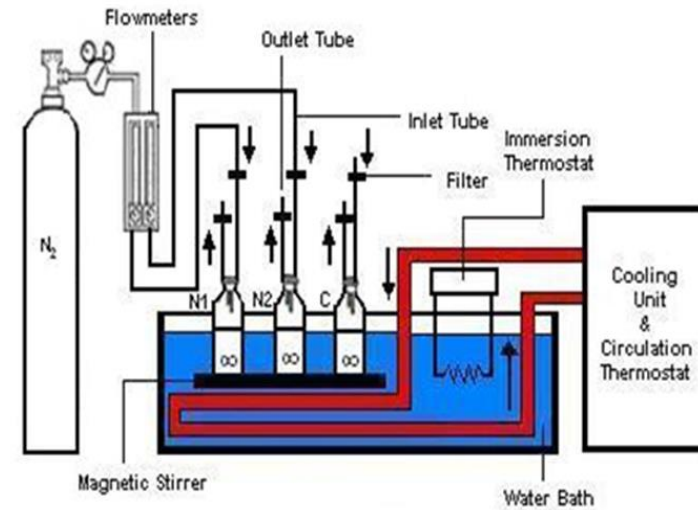


FAO (2012)

# N<sub>2</sub> GAS FLUSHING TECHNOLOGY



## Laboratory scale



(Mic. Res.  
2010/165/  
122-132)

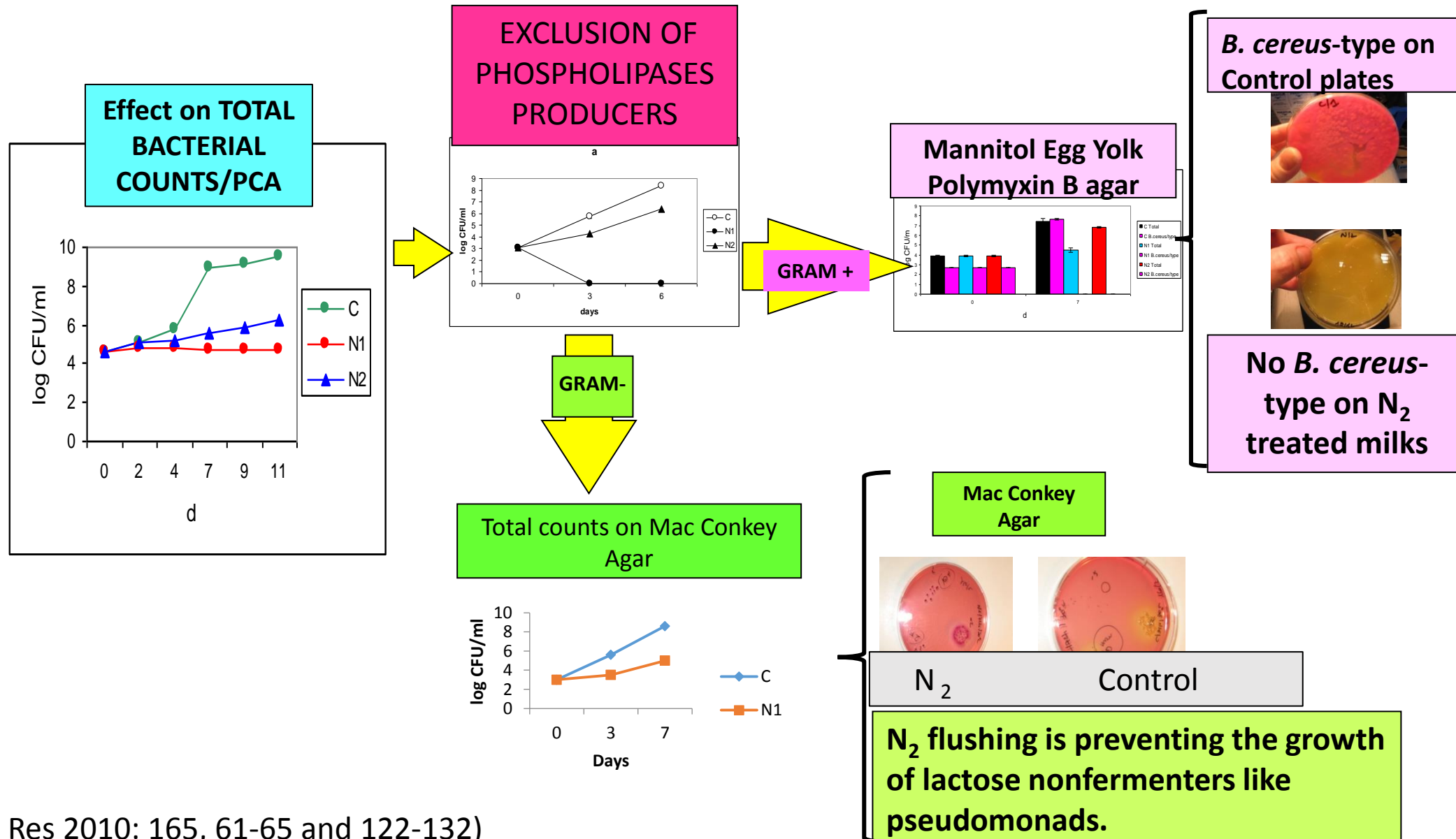
## Pilot plant scale



(Food Process.  
Technol.  
2010/10.4172)

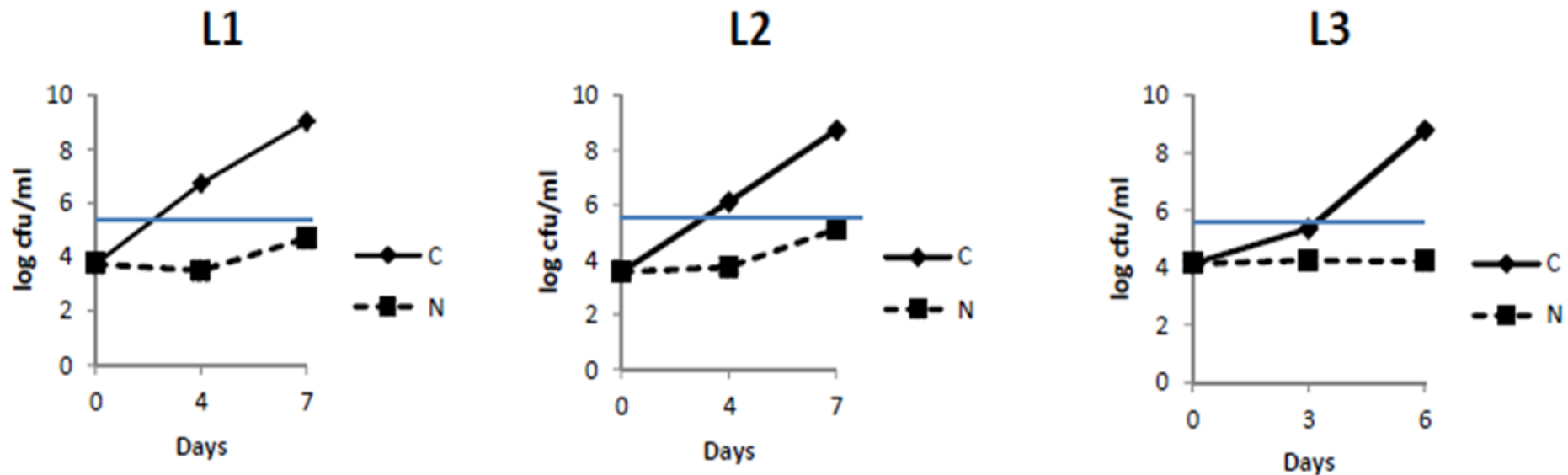
# N<sub>2</sub> APPLIED TO RAW MILK /LAB SCALE (100 ML MILK): STORAGE AT 6°C

## 1. CULTURE DEPENDENT METHODS



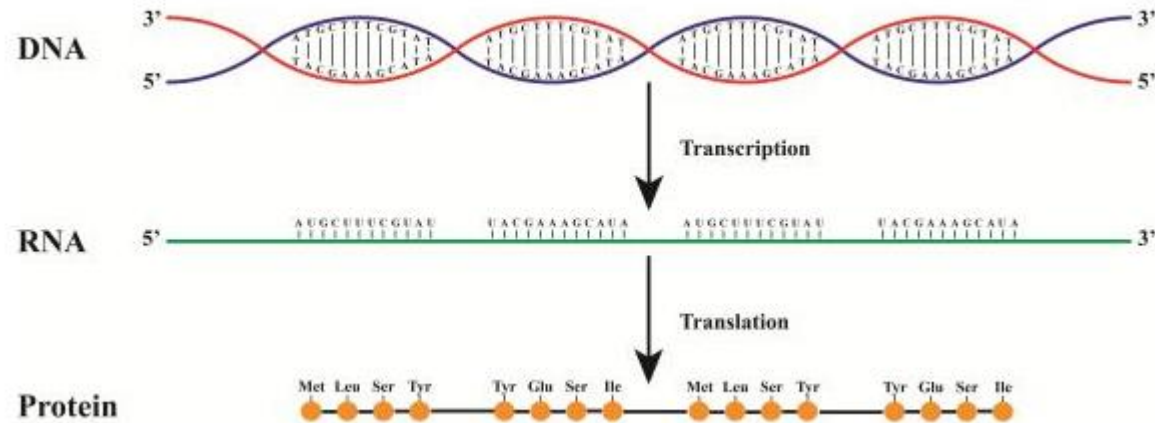
## 2. N<sub>2</sub> flushing compared to the only cold storage at 6°C / Next Generation Sequencing (NGS)

Bacterial counts from 3 raw milk samples stored at 6°C



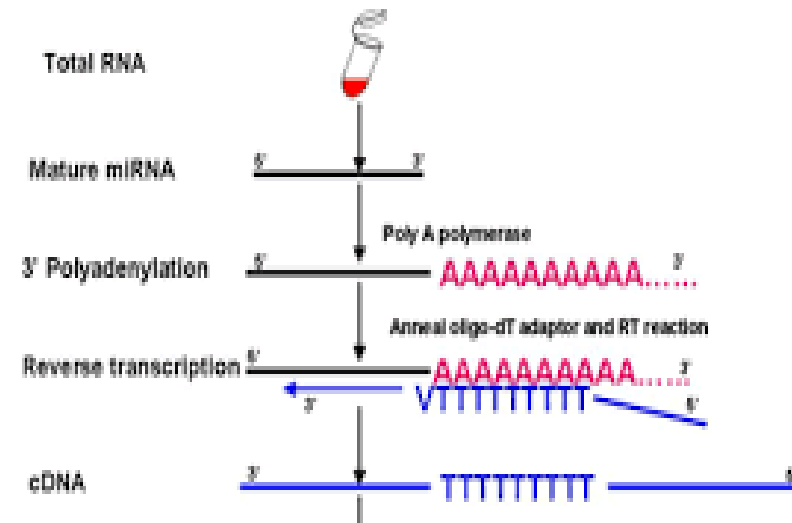
(PLoS ONE 2016:e0146025)

# RNA based approach: Central Life Dogma (science- explained. Com)



RNA

## Synthesis of cDNA



We focused on the  
active bacterial  
population in raw  
milk samples

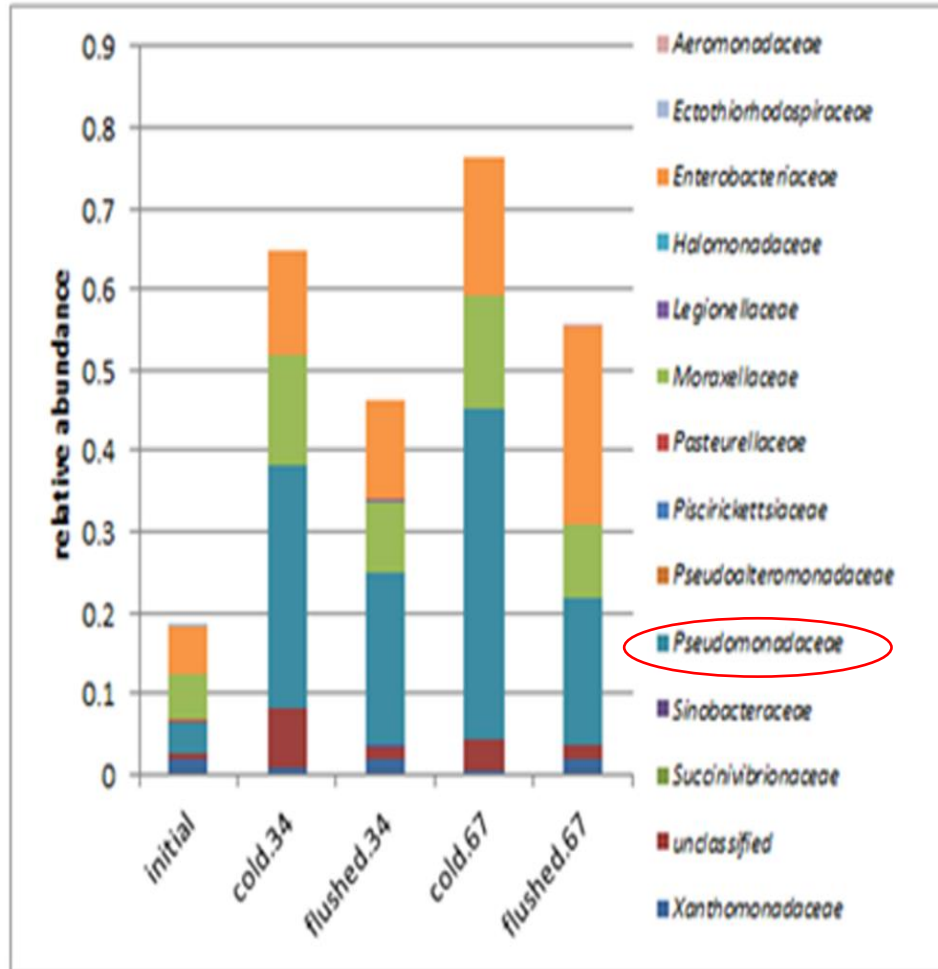
How many OTUs<sup>a</sup> in initial (=lorry) raw milk?

- 594 ± 143 OTUs (n=3)
- Rare taxa (= OTUs < 1%): over 20% of the numbers of initial OTUs

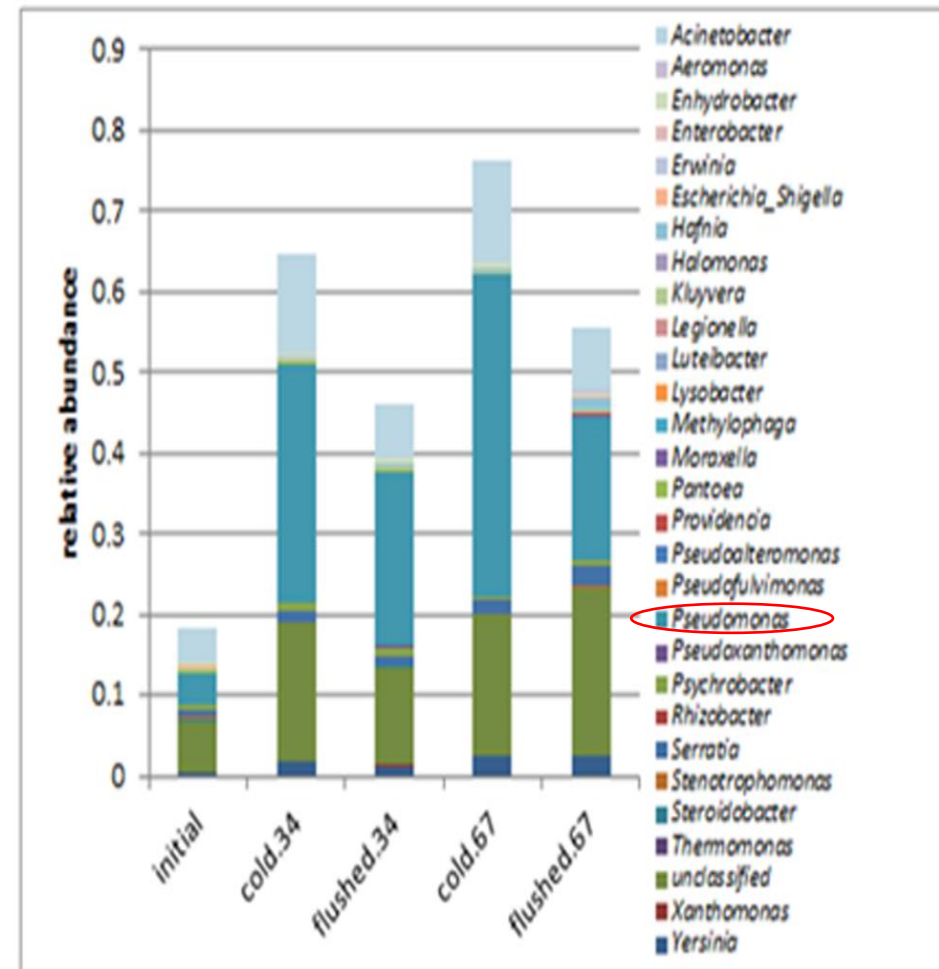
<sup>a</sup>OTU = operational taxonomic unit



(i)



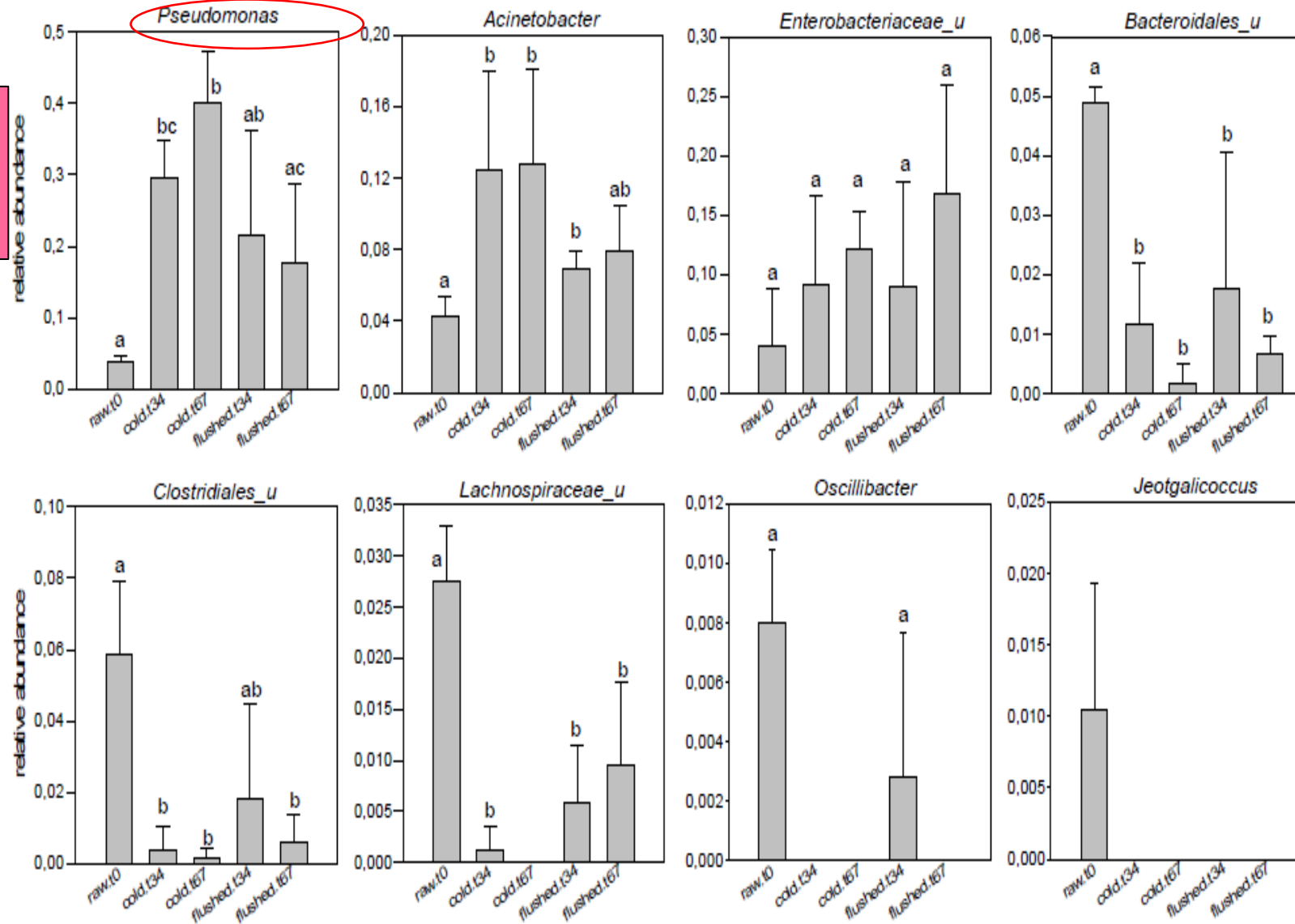
(j)



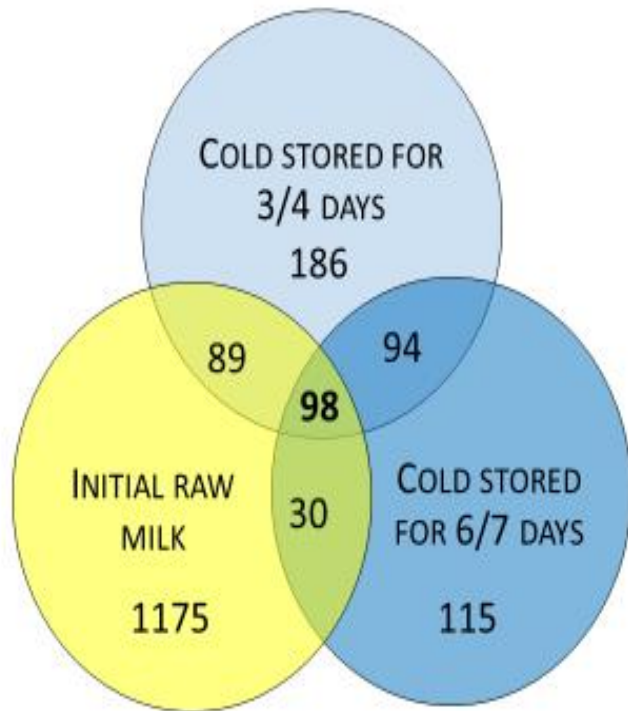
Pseudomonadaceae and *Pseudomonas* are key players

# Groups of interest:

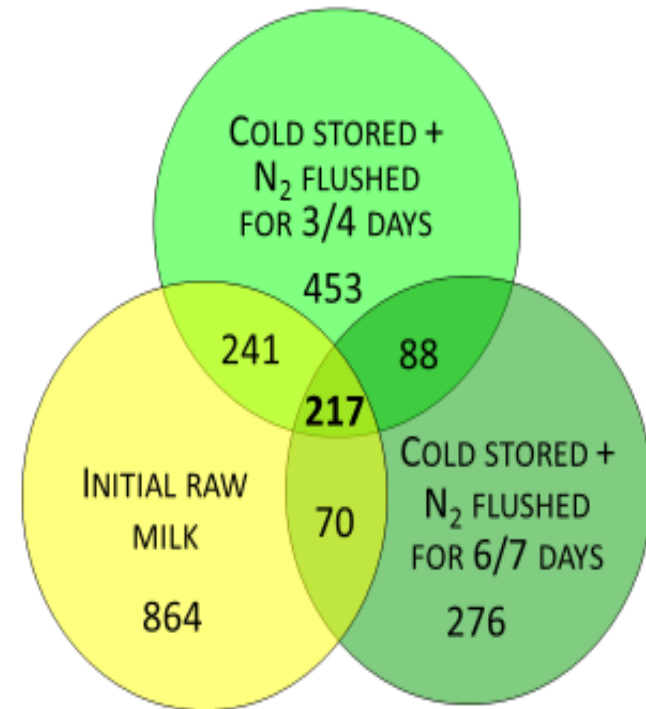
The treatment targets  
Pseudomonas !



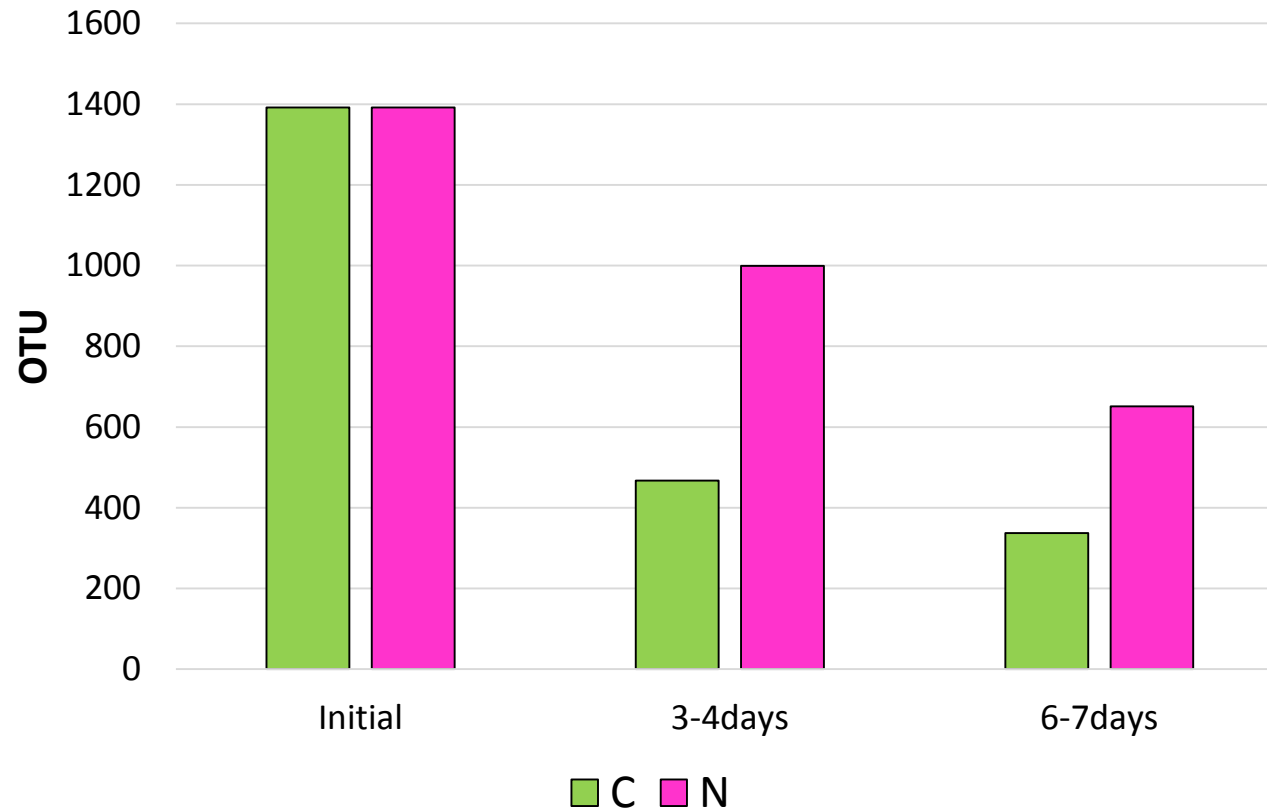
## COLD STORAGE



## COLD STORAGE+N<sub>2</sub> FLUSHING

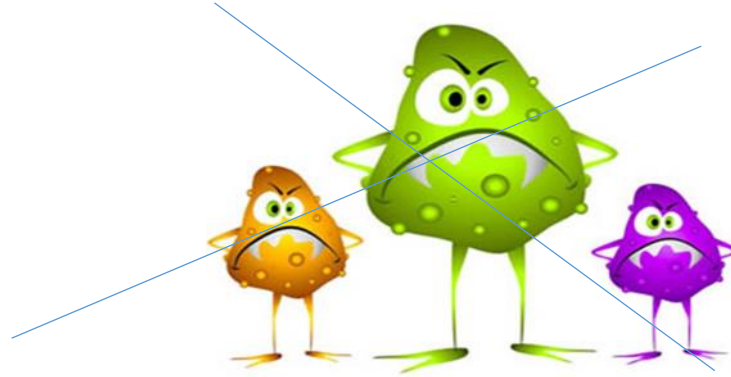


Number of OTUs from 3 raw milk samples during cold storage at 6°



\*During cold storage there is a loss of bacterial diversity  
\*The N<sub>2</sub>-treatment reduces the loss

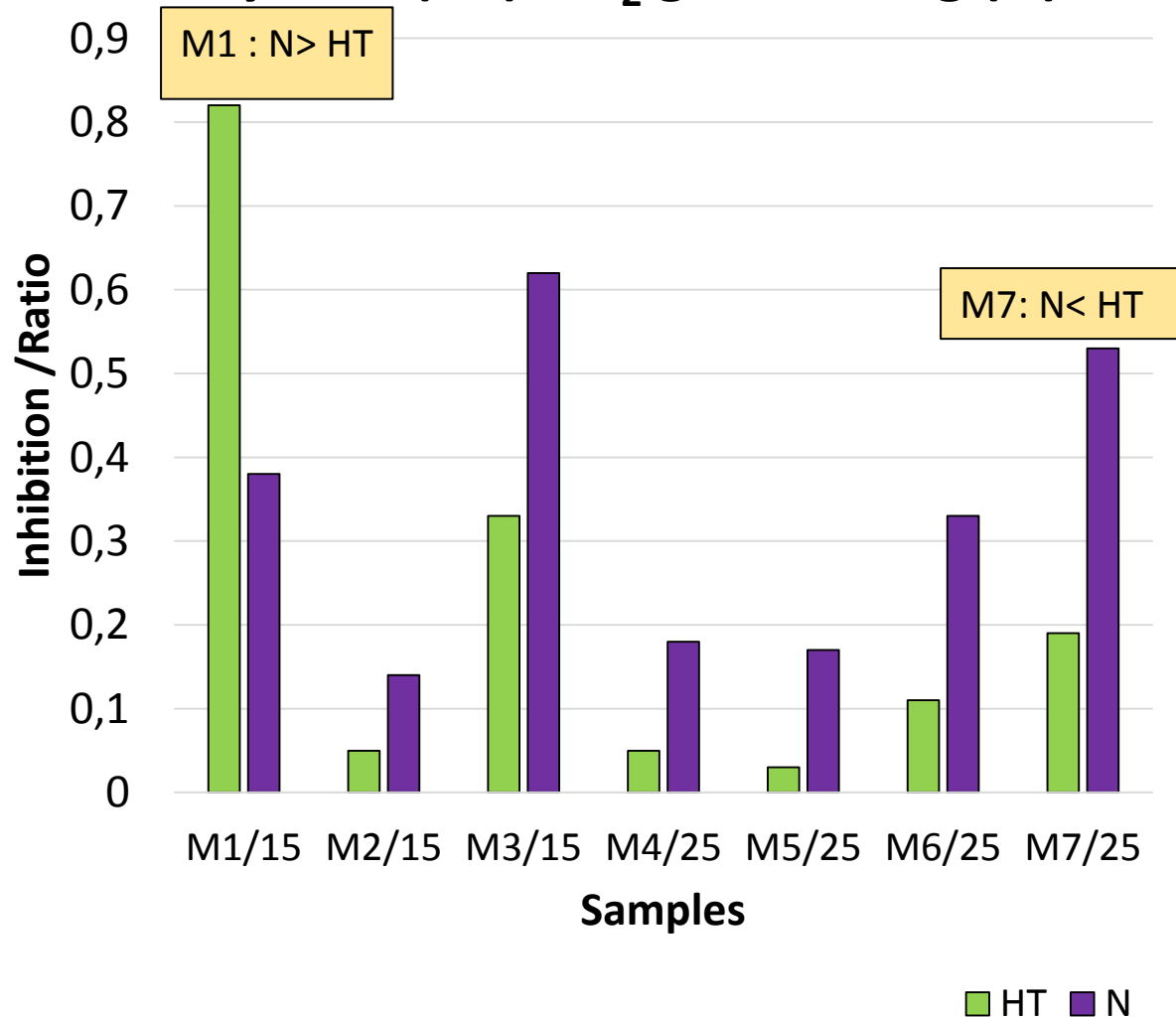
## ***MAIN POINT:***



No bacteria causing milk spoilage, not any well known human pathogen, no anaerobe, no spore former benefitted from the N<sub>2</sub> gas flushing treatment.

## Raw milk stored at 15 and 25° C

### Comparison Lactoperoxidase system (HT) / N<sub>2</sub> gas flushing (N)



Seven experiments:

- 1 case where N > HT (M1)
- 1 case where N < HT (M7)
- 5 cases where N and HT were equivalent

(Frontiers in Microbiol. 2016)



# How to apply this study approach to the organic milk research?

- Differences of bacterial population between raw milks from organic farming and conventional farming in Finland?
- Sources of variation in bacterial population of raw milks (organic and conventional farming) in Finland:
  - \* Effects of period of year?
  - \* Effects of cow breedings, age, lactation time, feeding?
  - \* Effects of farm and milking practices etc.?
- Differences of bacterial population between raw milks (organic and conventional) from Finland and other countries?
- Etc...

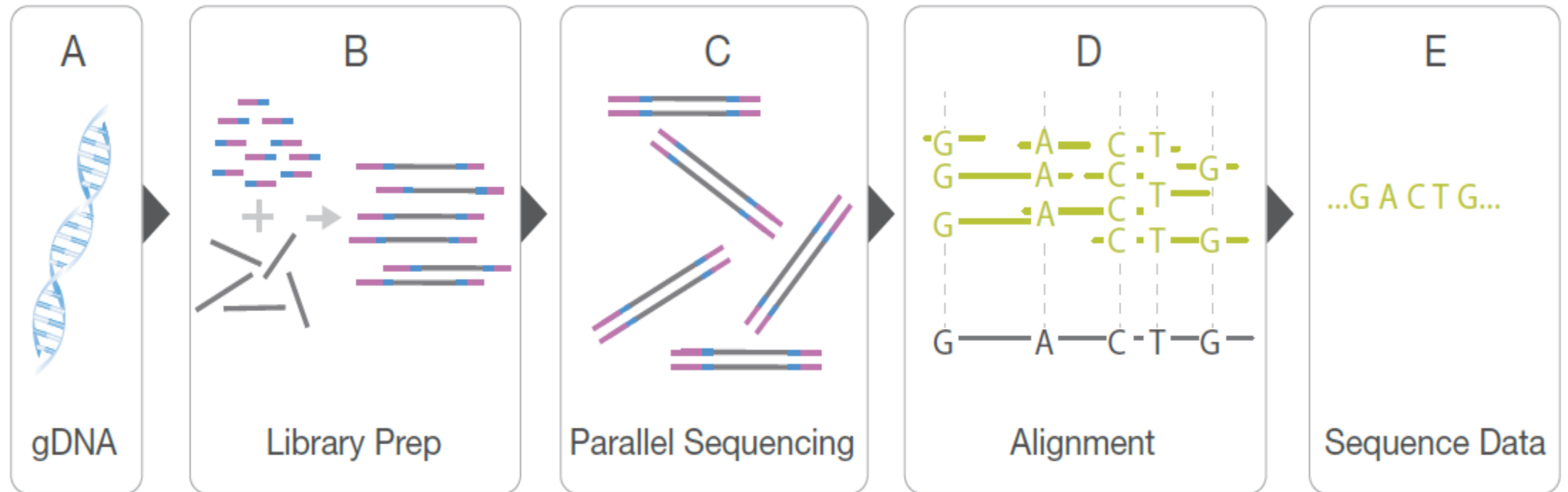
## Acknowledgments:

- Helsingin Dairy Ltd : Antti Alavuotunki (raw milk samples)
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- Dr. Ingrid De Man and Romanie Quintyn, Vives University College Campus Roeselare, Belgium

**THANK YOU FOR YOUR ATTENTION AND FOR YOUR INVITATION!!**



Figure 1: Concepts of Next-Generation Sequencing



- A. Extracted gDNA.
- B. Sample preparation fragments genomic DNA and adds adapters to generate a library.
- C. DNA fragments within the library are each sequenced in parallel.
- D. Individual sequence reads are reassembled by aligning to a reference genome.
- E. The whole genome sequence is derived from the consensus of aligned reads.

(Manufacture's document)

