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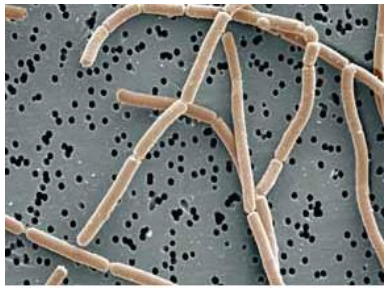
Advances in Biopreservation Technologies for Foods



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Processing controls for food quality and safety

1. Physical

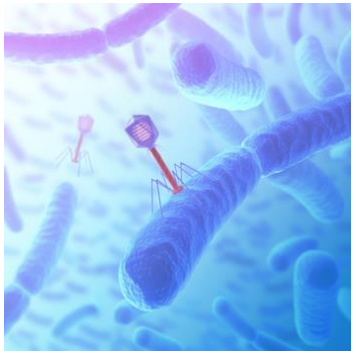
- Heat
- Refrigeration
- High pressure
- Irradiation

2. Chemical

- Sanitizers (chlorine, QACs, peracetic acid)
- Preservatives (nitrites, sulphur dioxide, benzoate)

3. Biological

- Bacterial
  - Bacteriocins
  - Protective cultures
  - Fermentates
- Bacteriophage
- Plant extracts



Challenges of biological control

Biological control (biocontrol, biopreservation) is not new

- 10,000 BC

Application to foods:

Benefits

- natural
- consumer perception of being safer

Challenges

- efficacy
- impact on shelf-life
- sensorial properties
- application of agent to food
- cost
- regulation/labelling

Biopreservatives of microbial origin

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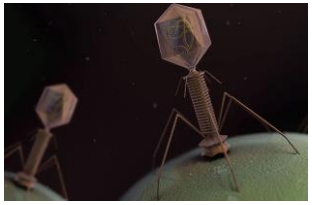
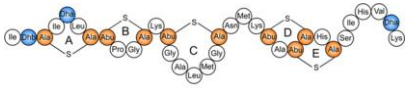
Bacteriophage

Bacteria

- Bacteriocins
- Protective cultures (cells)
- Bacterial fermentates (cells + supernatant)



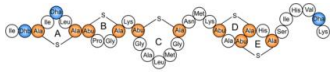
e.g. ListShield™



e.g. nisin

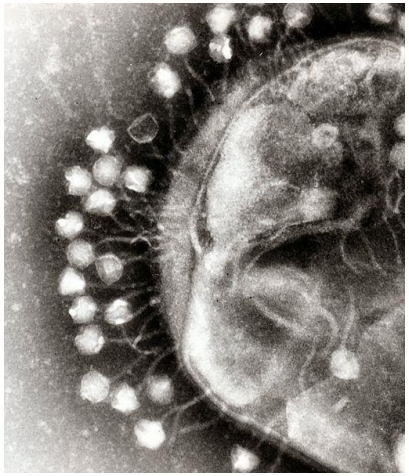
e.g. *Lactobacillus Pediococcus*

+

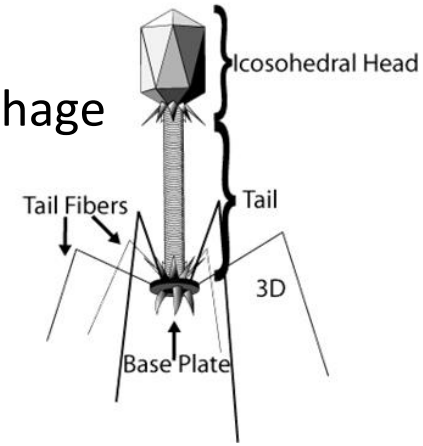


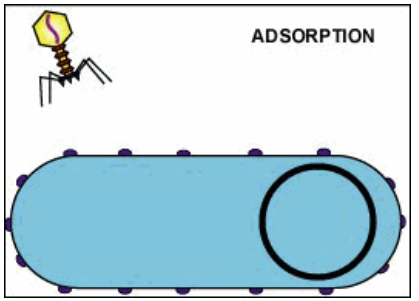
100 YEARS AHEAD

Bacteriophage (phage)



- Phages
  - most abundant microorganisms in the biosphere (10^{31} phage)
    - naturally present in food and water
    - up to ~10^{8} phage/g food
  - every bacterial species has its own phage
    - natural enemies of bacteria
    - don't attack indiscriminately
      - genus/species specific
    - do not infect humans
  - Potential for control of pathogenic bacteria
    - Medical
    - Food





Bacteriophage

- *Listeria monocytogenes* control
  - Phageguard Listex (P100)
  - Listshield™
- *Salmonella* control
  - PhageGuard S (phages S16 and FO1a)
  - SalmoFresh™
- *E. coli* O157:H7
  - PhageGuard E
  - EcoShield™

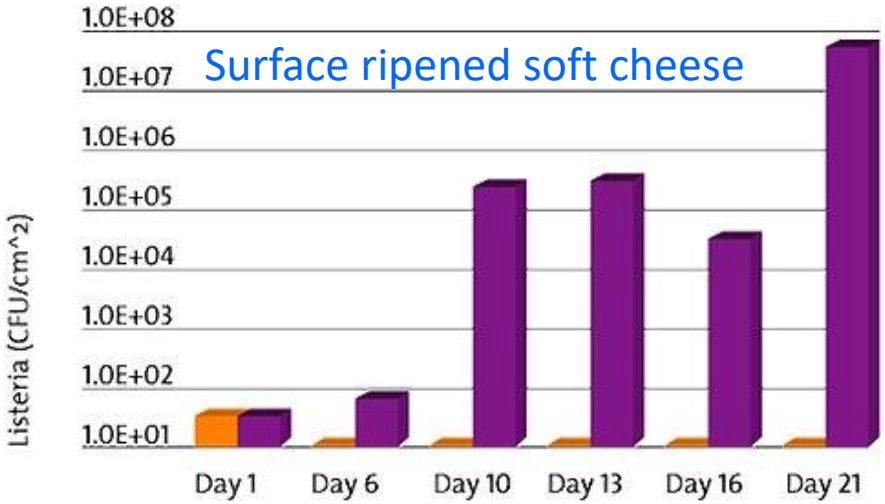
https://www.phageguard.com/
http://www.intralytix.com/

Bacteriophage

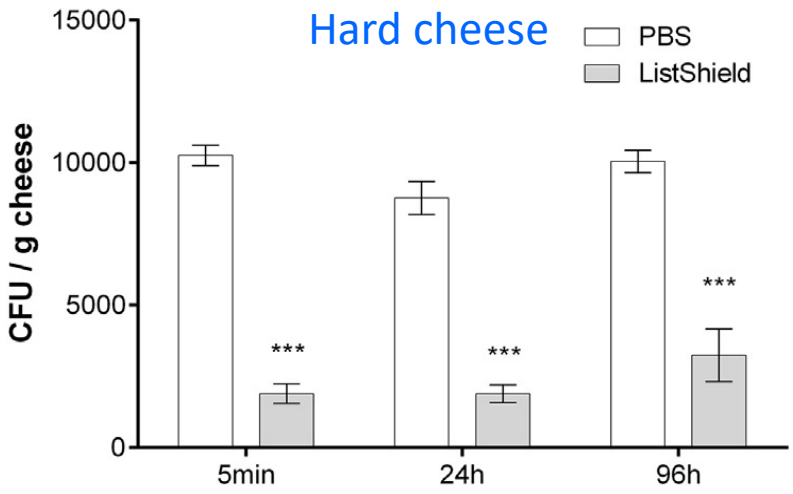
- Generally Recognized As Safe (GRAS) status
- Most approved as a processing aid (no labelling) in:
    - USA, EU, Canada, Australia, New Zealand, Switzerland and Israel
  - Use in ‘at risk‘ foods
    - Cheese, RTE meats, fish, fruit, vegetables
    - Raw red meat and poultry
    - Food contact surfaces (*L. monocytogenes*)

Bacteriophage - *Listeria*

- PhageGuard Listex
  - Kills 99% of *L. monocytogenes* strains (1000s tested)
  - Tested on surface contaminated cheese first



- *L. monocytogenes* (7 CFU/cm^2) growth at 14˚C up to day 16 and 6˚C afterwards with or without 3x10^{9}PFU/ml PhageGuard Listex in smearing solution



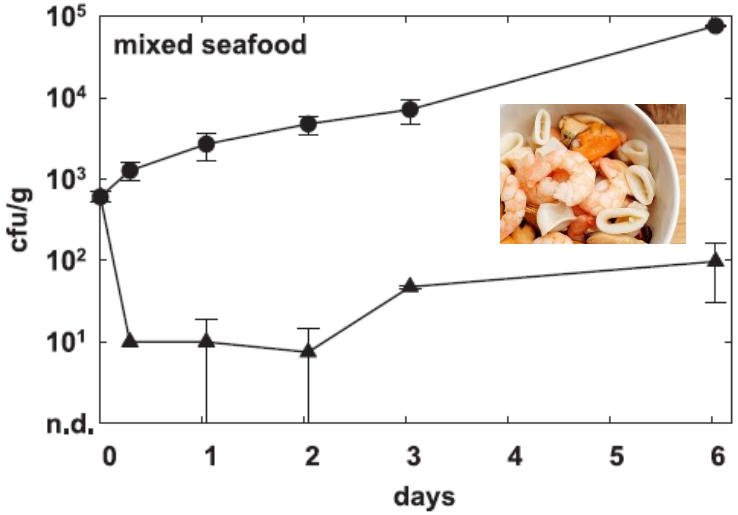
- *L. monocytogenes* survival at 4°C following treatment with ListShield™

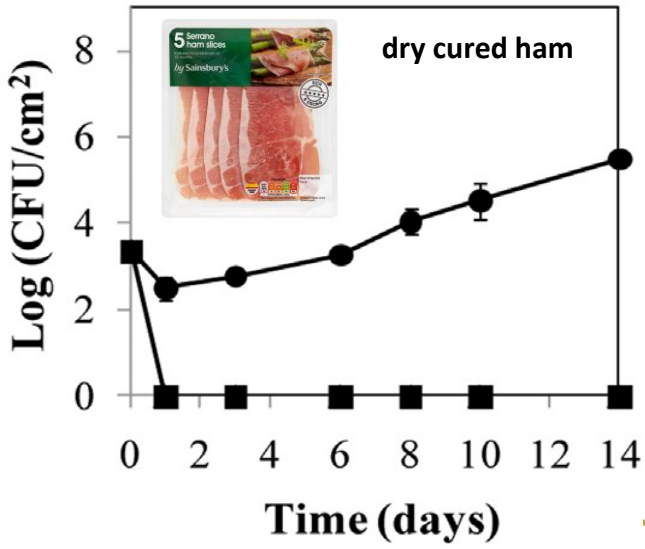
Carlton et al., Reg. Tox & Pharm, 2005

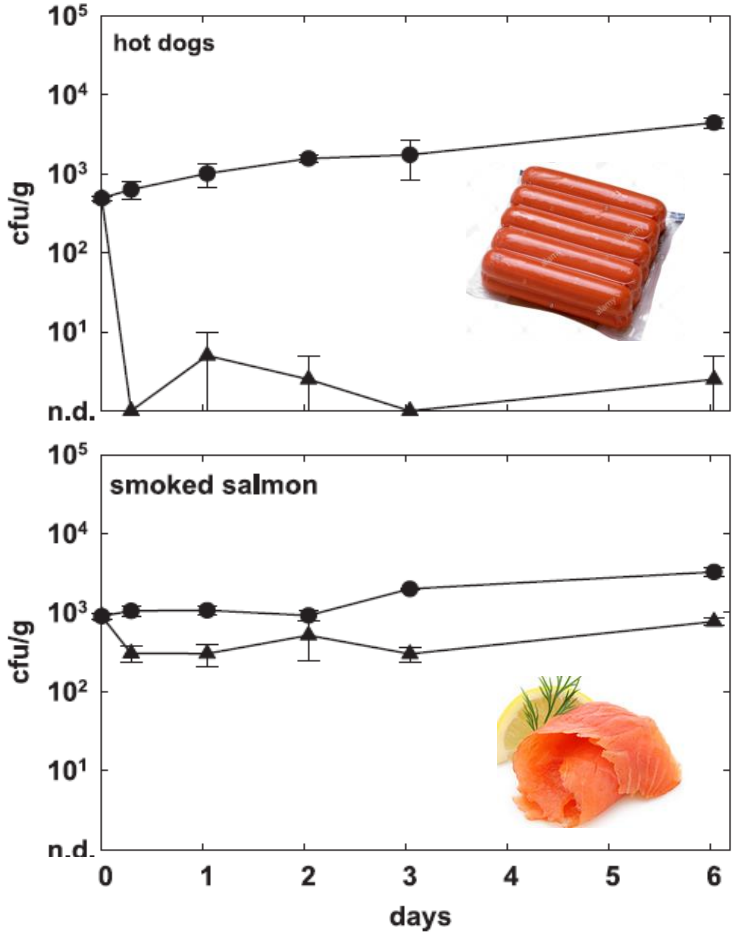
Perera et al. 2015 Food Microbiology 52 (2015) 42e48

Bacteriophage - *Listeria*

- *L. monocytogenes* growth at 6˚C with (▲) and without (●) 3x10^{8}PFU/g PhageGuard Listex







- treated with ListShield™ 10^7 PFU/cm^2 at 4˚C

Guenther et al., AEM 2009
Gutierrez et al. (2017) Food Control

Bacteriophage - *Salmonella*

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PhageGuard S

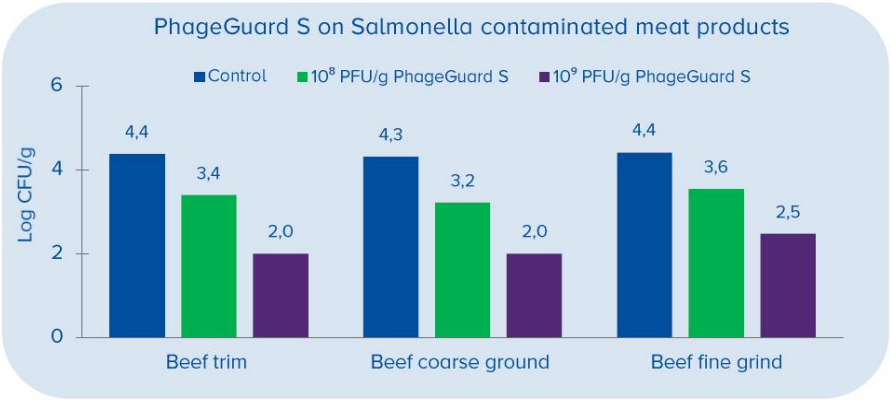

Figure 1 1.0 - 2.1 log Salmonella kill in ground beef product, 6 hours after the application of 10^8 or 10^9 PFU/g (5% or 50% dosage) on trim, coarse or fine grind.

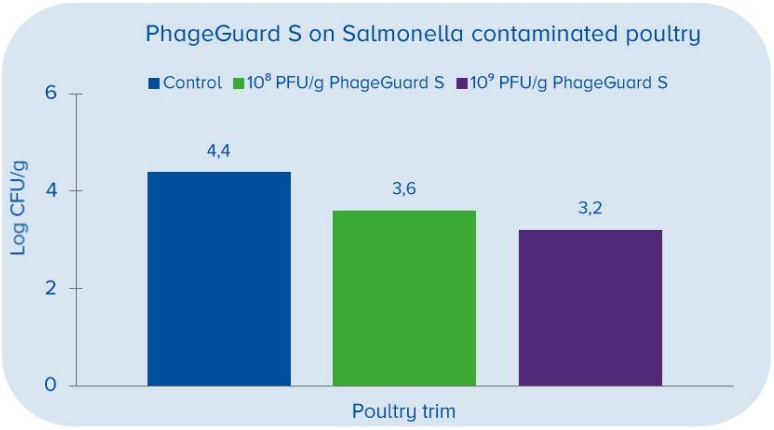

Figure 2 1 - 1.2 log Salmonella kill in ground poultry product, 24 hours after the application of 10^8 or 10^9 PFU/g (5% or 50% dosage) on poultry trim.

https://phageguard.com/salmonella-solution-2/

100 YEARS AHEAD

Bacteriophage – *E. coli* O157:H7

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EcoShield™

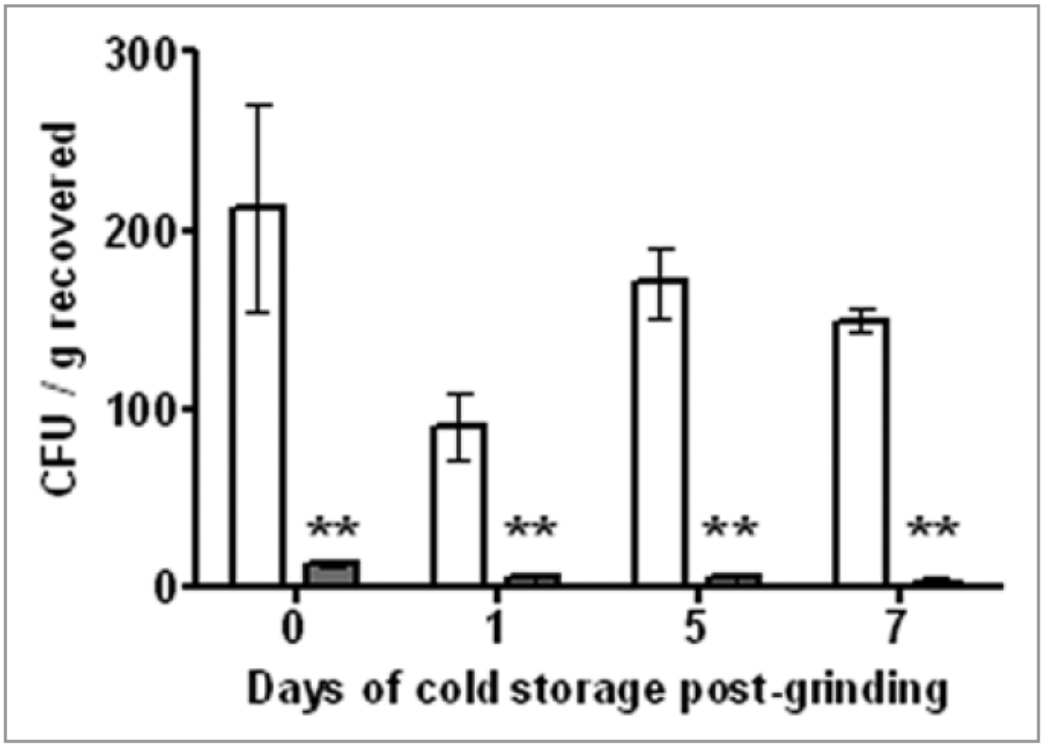

Figure 2. Impact of food storage conditions on *E. coli* O157:H7 levels in ground beef post phage treatment. White bars indicate PBS controls lacking EcoShield™, gray bars indicate EcoShield™ treated test groups. Error bars represent the SEM (n = 3). ** $p < 0.05$.

Carter, et al 2012. Bacteriophage

Protective cultures and fermentates

- Mostly lactic acid bacteria (*Lactobacillus*)
- For fermentates – cells pre-grown in food grade ingredients
  - Milk-based, dextrose, wheat

- Non-pathogenic (generally recognised as safe [GRAS])
  - Used in a variety of food fermentations and as probiotics
  - Naturally present on fresh foods (fruits, vegies, meat)
    - 1000's CFU per serving

- Mechanisms
  - competition for nutrients
  - bacteriocins
  - organic acids [lactic acid, acetic]
  - hydrogen peroxide
  - small compounds

Protective cultures and fermentates

- Pathogen control
  - *L. monocytogenes*

MicroGARD® Fermentates

- Shelf-life extention
  - Gram-negative aerobes
  - Late blowing in cheese (*Clostridium tyrobutyricum*)
  - Yeast and mould spoilage in yoghurt

Improving food & health

FreshQ®

Protective cultures - yoghurt

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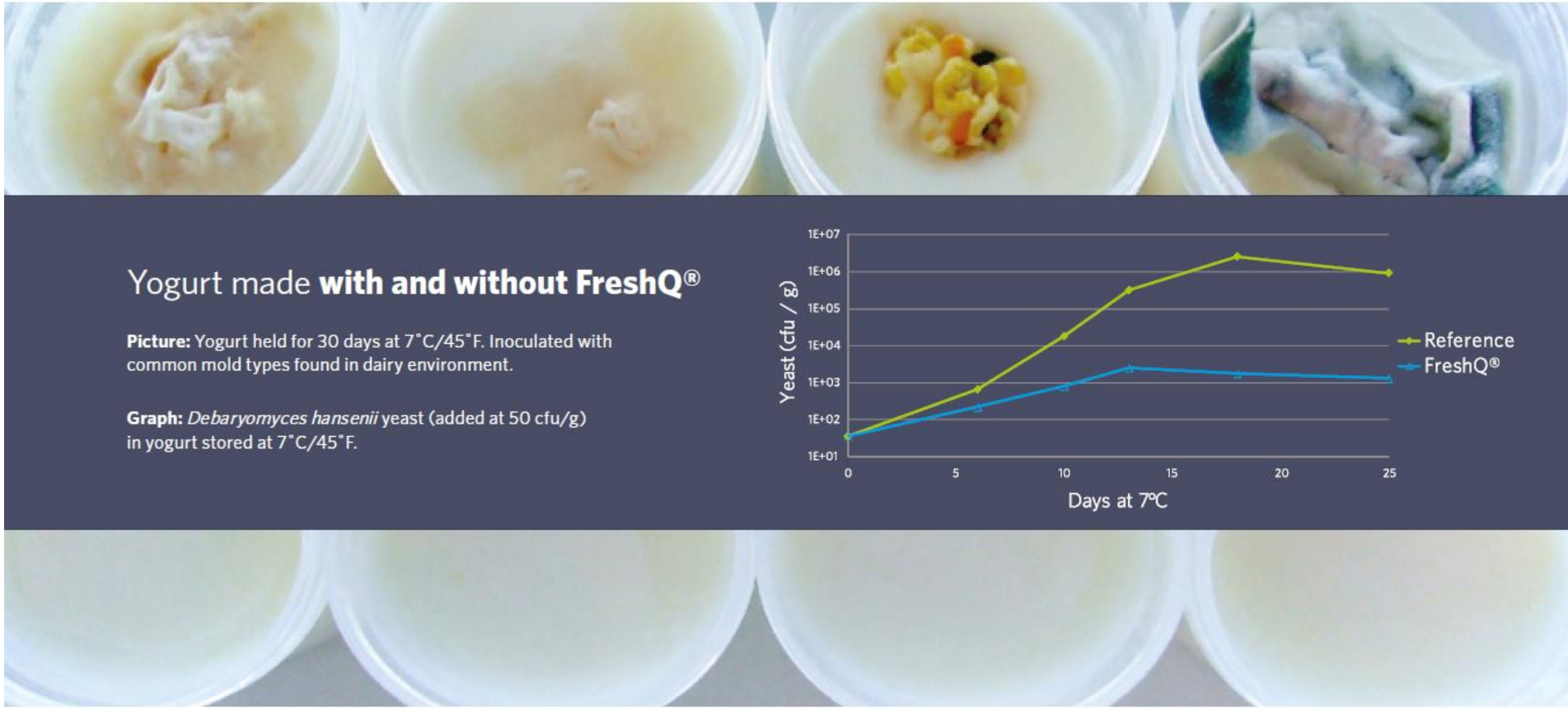
FreshQ® inhibits yeast and mold



Yogurt made **with and without FreshQ®**

Picture: Yogurt held for 30 days at 7˚C/45˚F. Inoculated with common mold types found in dairy environment.

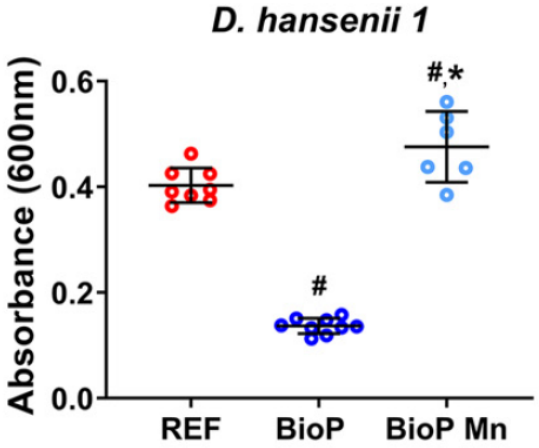
Graph: *Debaryomyces hansenii* yeast (added at 50 cfu/g) in yogurt stored at 7˚C/45˚F.

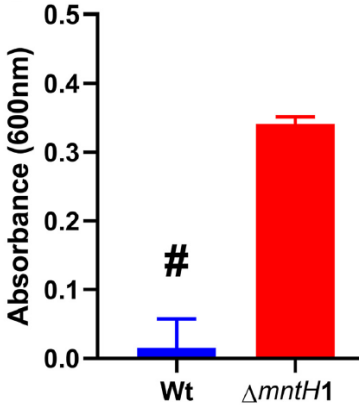
Chr. Hansen A/S, 10-12 Boege Allé,
DK-2970 Hoersholm, Denmark,

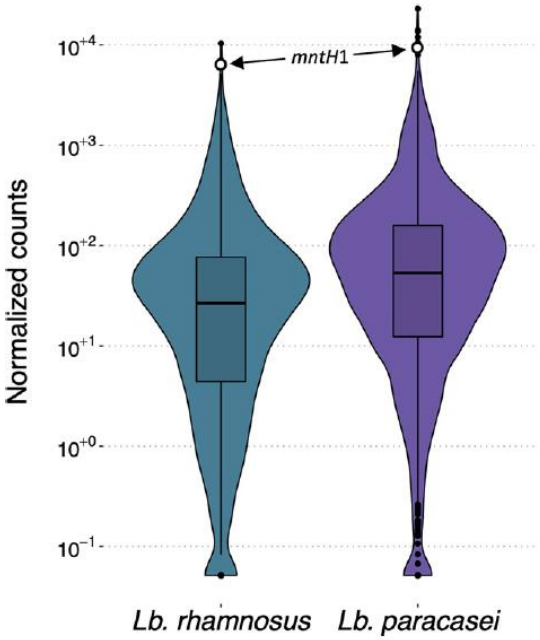
100 YEARS AHEAD

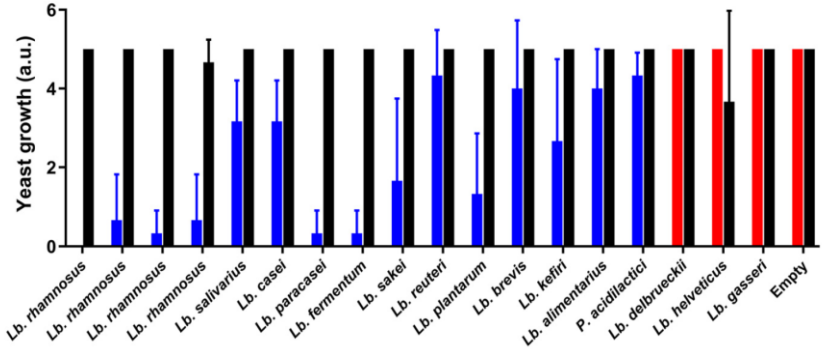
Protective cultures - mechanism

- *Lactobacillus rhamnosus* and *paracasei*
  - Manganese thieves!









Siedler et al, 2020. Appl. Environ. Micro.

Our biocontrol research

- Can LAB isolated from fruits and vegetables:
  - destroy *Listeria monocytogenes* on RTE sliced ham? (SAFETY)
  - Inhibit growth of mould on cheese? (QUALITY)

Lactic acid bacteria

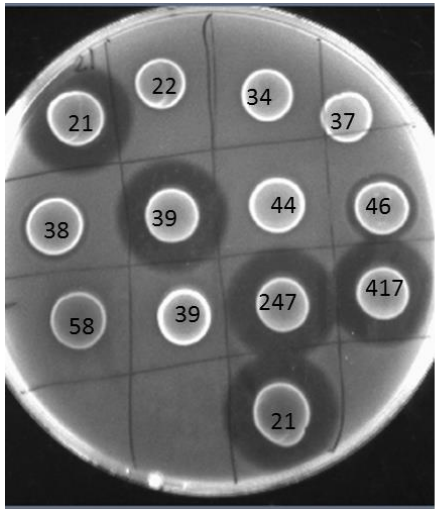
Sliced ham

Cheese

SAFETY and QUALITY

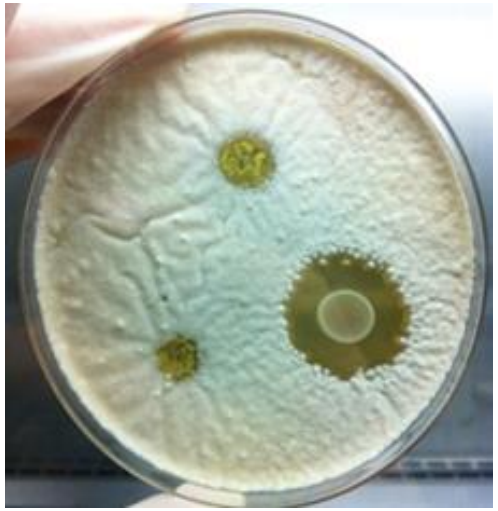
“Bioprospecting”

- Isolated ~ 900 LAB isolates from vegetables and fruits
- Screen for antimicrobial activity using agar spot assay



Inhibition of
L. monocytogenes

Lactococcus lactis
mainly

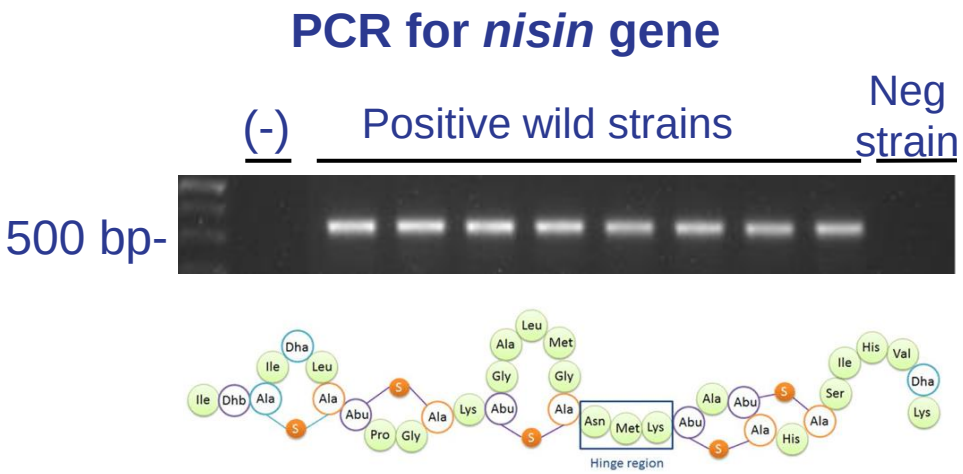


Inhibition of
Penicillum commune

Lactobacillus plantarum
only

Biocontrol *Lactococcus lactis*

- Anti-*Listeria* activity
  - Nisin production?

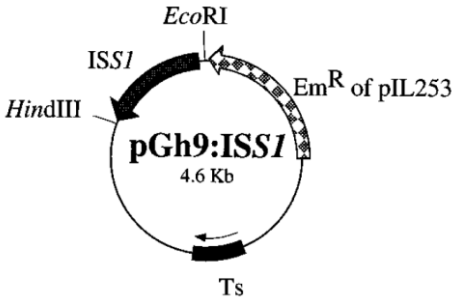


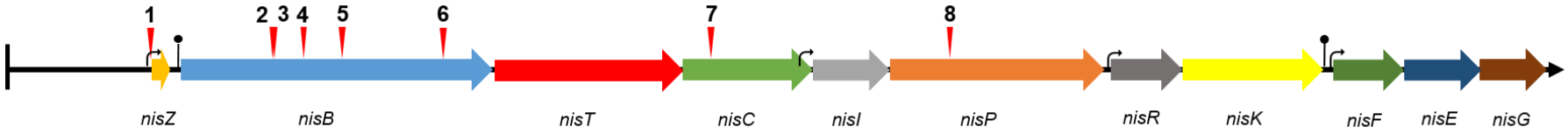
- Whole genome sequencing identified another bacteriocin gene cluster (lactococcin 972)

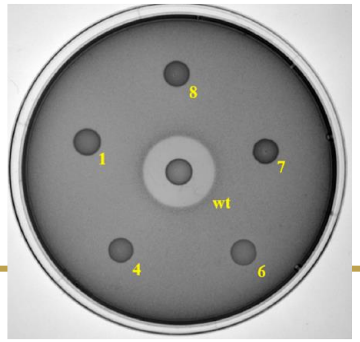
Ho et al., 2018 *Food Control*

Anti-listerial mechanism of action

- Screen 5000 *Lactococcus lactis* random transposon mutants for nisin-negative mutants



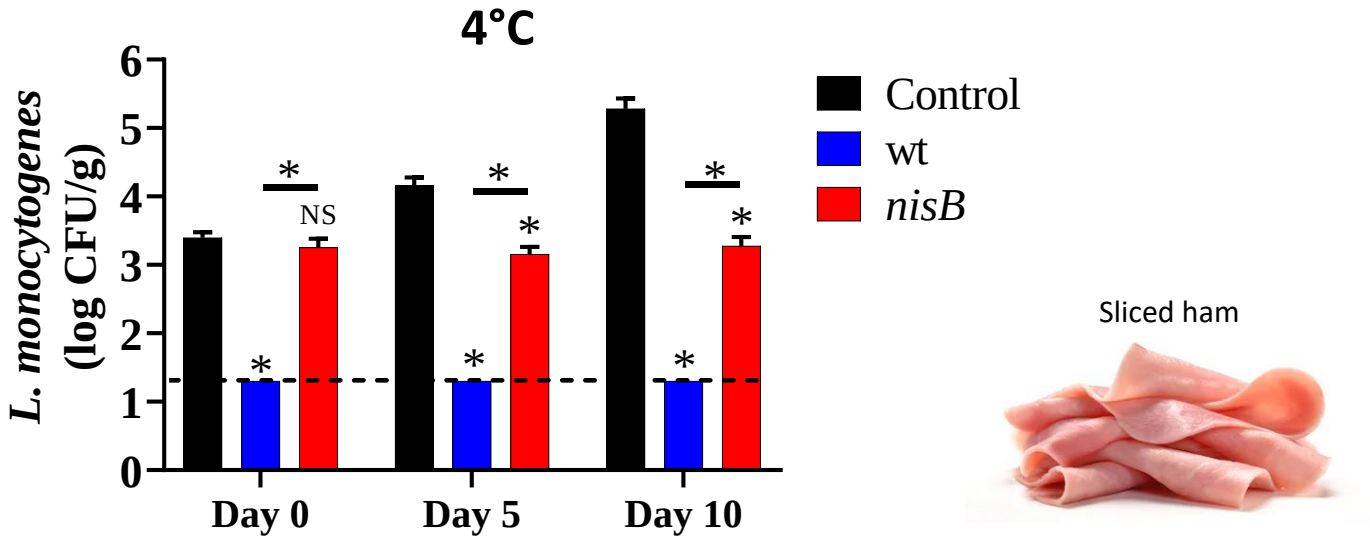




Dong et al., 2021 *Food Control*

Inhibition of *Listeria* growth on RTE sliced ham

- Minimal effects of *L. lactis* **cells only** when applied to ham
- **Cells + supernatant** (fermentate) is effective on ham



- Nisin is the major fermentate antimicrobial (but other *L. lactis* factors contribute)

Dong et al., 2021 *Food Control*

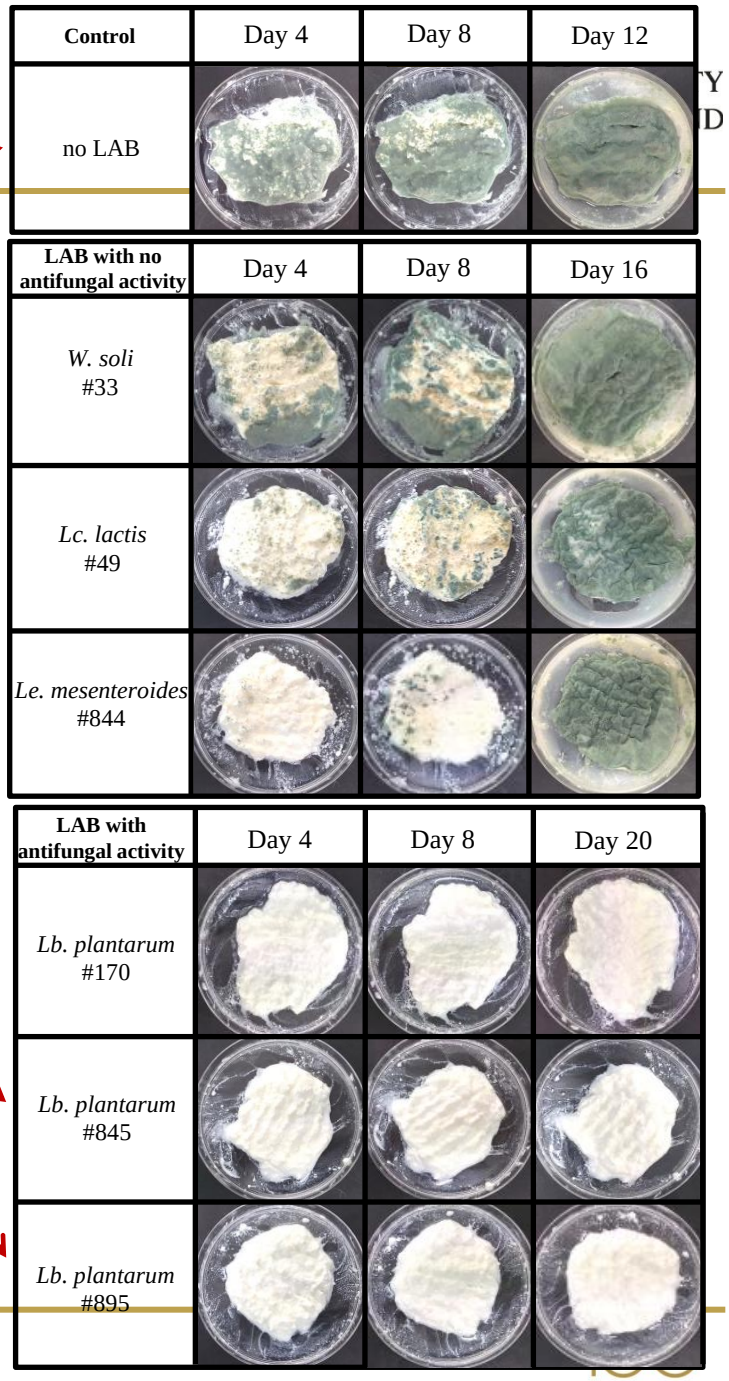
Antifungal activity of LAB – on cottage cheese

- Inoculate cheese with LAB (10^8 CFU/g)
- Incubate for 2 days
- Inoculate *Penicillium commune* (10^4 CFU/g)
- Store for 20 days at room temperature

No LAB

Negative control LAB

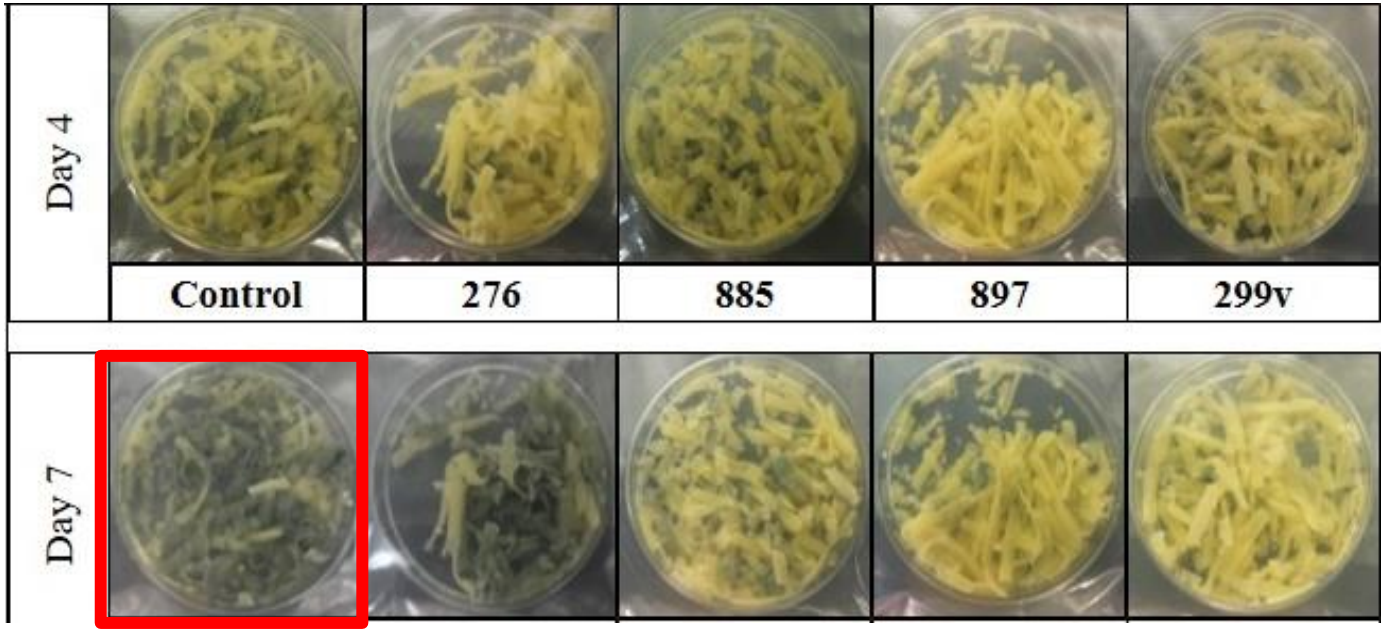
Antifungal LAB



Cheong et al., 2014 *Food Control*

Antifungal activity of LAB – in Cheddar cheese

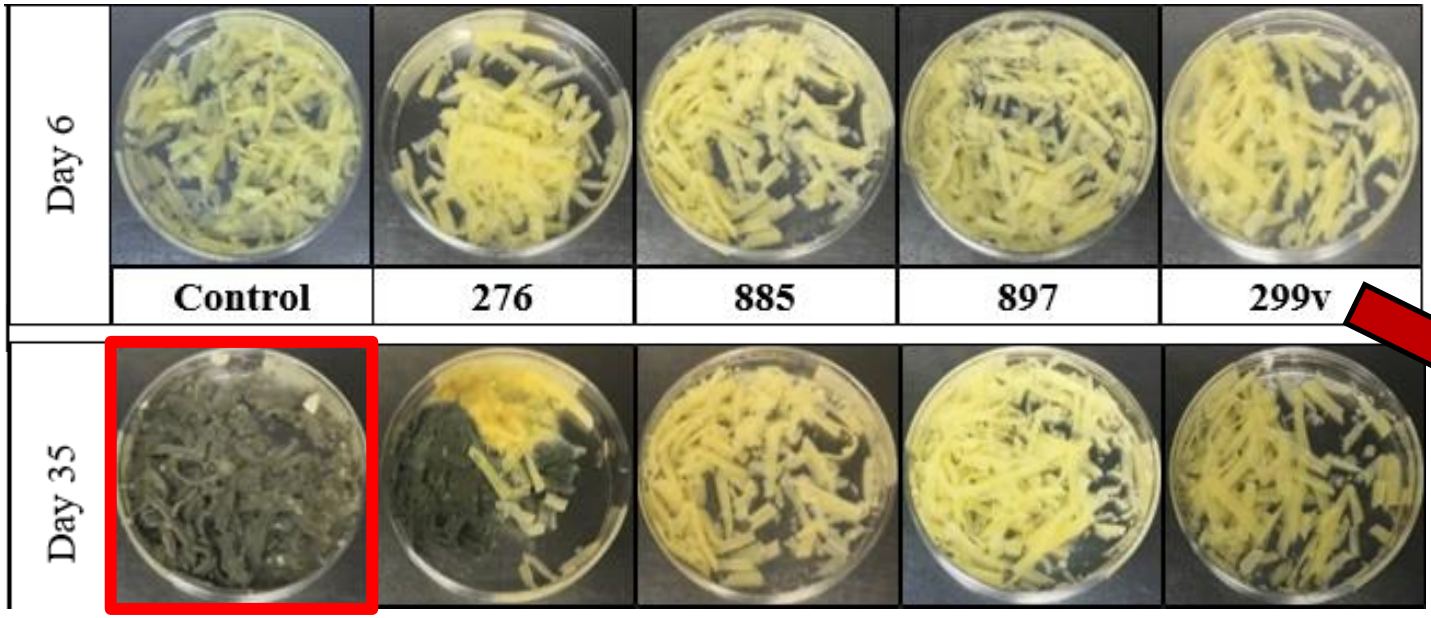
- Trial 1
- Cheddar cheese ripened for 24 weeks (+/– *Lb. plantarum*)
  - shredded
  - Inoculated with ***Penicillium commune***
  - observed on days 4 and 7



Prabawati, Turner & Bansal. *Unpublished results*

Antifungal activity of LAB – in Cheddar cheese

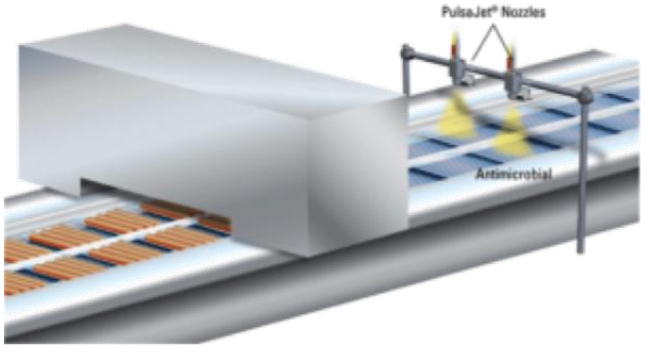
- Trial 2
- Cheddar cheese ripened for 24 weeks (+/– *Lb. plantarum*)
  - shredded
  - exposed to **airborne contamination**
  - observed on days 6 and 35



Prabawati, Turner & Bansal. *Unpublished results*

CONCLUSIONS

- Phage products for various pathogens are now approved for use in relevant foods



- Protective cultures and fermentates for quality and safety applications are in the market
  - Reduce food waste

- Useful as part of hurdle technology to extend shelf-life and improve safety

- Challenges around application method, cost and regulation/labelling likely limit their application

Acknowledgements

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Yoon Kok
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