

Effect of bacteriocin-producing
cultures on the growth of
Listeria monocytogenes in
fresh beef sausage



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Introduction

- Lactic acid bacteria produce bacteriocins
 - Small antimicrobial peptides produced by some bacteria
 - Useful as food antimicrobials
 - considered natural
 - heat stable
 - withstand meat pH
 - Important to test in real food systems
 - e.g. Nisin is 228x more soluble at pH 2 than pH 8

Listeria monocytogenes

- Frequently isolated from ground meat
 - 52% of ground beef samples in Edmonton (Bohaychuk et al., 2006)
- Economic cost
 - Recent listeriosis outbreak cost Maple Leaf Foods
 - \$43 million in recall, restructuring, etc.
 - \$14 million in sales (estimated)
 - \$27 million in settlements (cbcnews.ca, 2009)

Biopreservation

- *Carnobacterium maltaromaticum*
UAL307
 - Produces piscicolin 126, carnocyclin, carnobacteriocin BM1 and maybe one other unknown bacteriocin
 - Activity against several *Pediococcus*, *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Bacillus*, *Clostridium* and *Listeria* spp.

Biopreservative ?

- *Enterococcus faecalis* 710C
 - Broad spectrum antimicrobial activity against gram positives
 - No growth at refrigeration temps
 - Virulence factors:
 - Cytolysin
 - Gelatinase
 - β -haemolysis

Objectives

- Determine the antimicrobial activity of the bacteriocins and bacteriocin-producing cultures on the growth of *L. monocytogenes* in fresh beef sausage
- Determine effect of *C. maltaromaticum* UAL307 on the colour of fresh beef sausage

Experimental



Meat Safety and Processing Research Unit

Methods

- Conducted inhibition assays
- Cocktail of *L. monocytogenes* used as indicator: ATCC 15313, serotype 1/2a; FS-15, serotype 1/2b and CDC 7762, serotype 4b
- *E. faecalis* 710C and *C. maltaromaticum* UAL307 had highest antimicrobial activity against cocktail of *L. monocytogenes*

Production and inoculation of sausages

L. monocytogenes and
C. maltaromaticum cultures
centrifuged and pellets collected

E. faecalis and *C. maltaromaticum*
cultures centrifuged and supernatant
collected

Cell density adjusted to
approx. 10^7 cfu/mL

Hand mixed into 6 treatments with
spices and binders, ground beef and
culture

Aerobically packaged,
stored at 4° C for
10 days

MAP (30% CO₂,
0.4% CO), stored at
4°C for 15 days

Stuffed into collagen casings

Treatments applied to sausages

1. Control (no inoculum)
2. *L. monocytogenes* alone
3. *L. monocytogenes* and *C. maltaromaticum* UAL307
4. *L. monocytogenes*, *C. maltaromaticum* UAL307 culture and *C. maltaromaticum* UAL307 supernatant
5. *L. monocytogenes* and *E. faecalis* 710C supernatant
6. *L. monocytogenes*, *C. maltaromaticum* UAL307 culture and *E. faecalis* 710C supernatant
 - *C. maltaromaticum* UAL307 10^5 cfu/g
L. monocytogenes cocktail 10^4 cfu/g

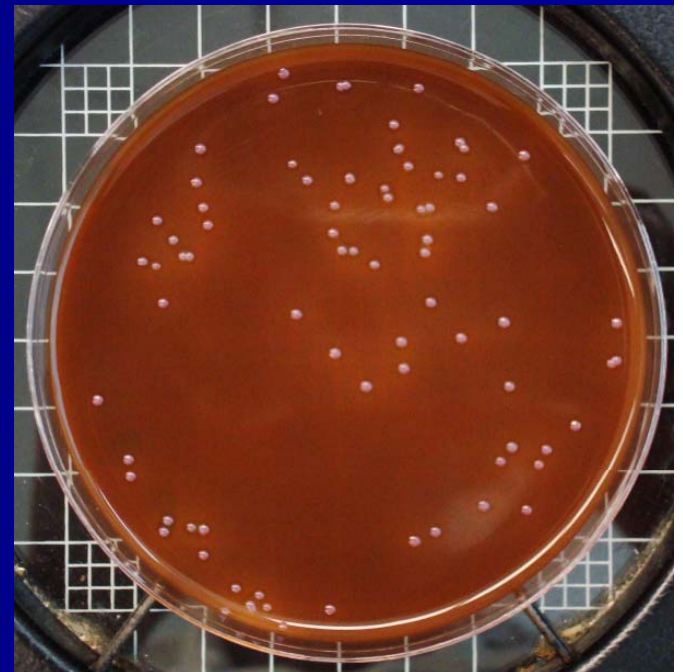
Evaluation

- *Listeria* spp.
 - PALCAM incubated at 30°C for 48 hours
 - Absence of *Listeria* in samples confirmed by enrichment in UVM (University of Vermont medium) broth
- Lactic acid bacteria
 - APT incubated at room temperature for 48 hours
- Total aerobic plate count
 - Plate count agar incubated at room temperature for 48 hours

Evaluation (con't)

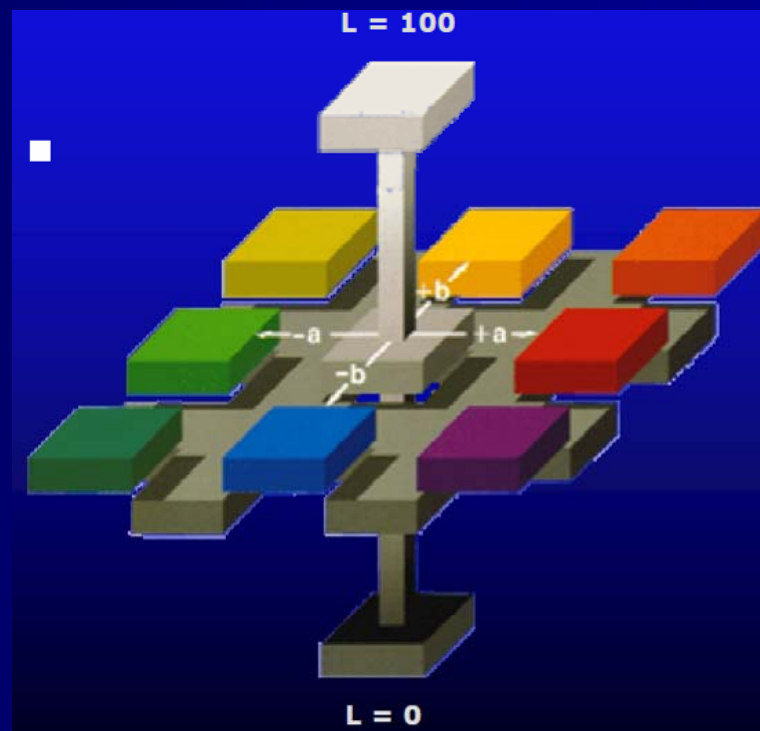
- *Carnobacterium* spp.
 - CTSI (Cresol red thallium acetate sucrose inulin)

Modified CTAS, a selective media for carnobacteria but poor recovery of some species and interference from other organisms

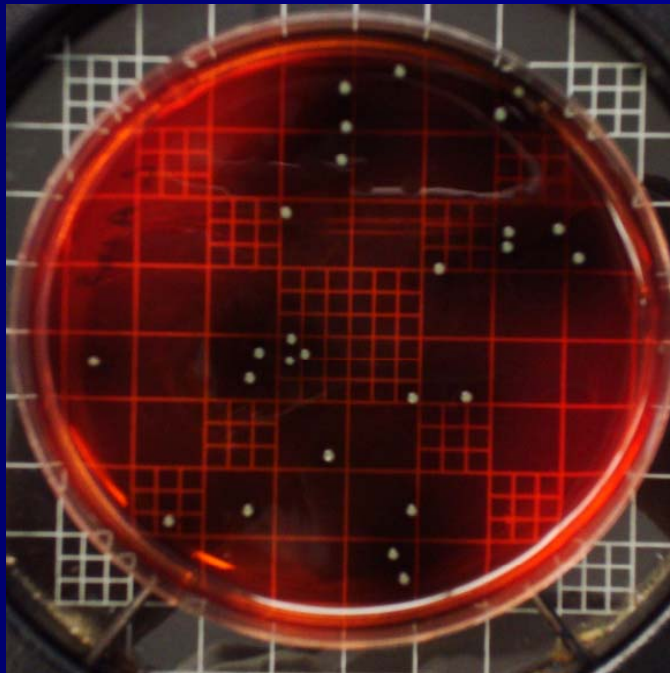


Evaluation (con't)

- pH
- Colour - Hunter Lab "a" value (redness)

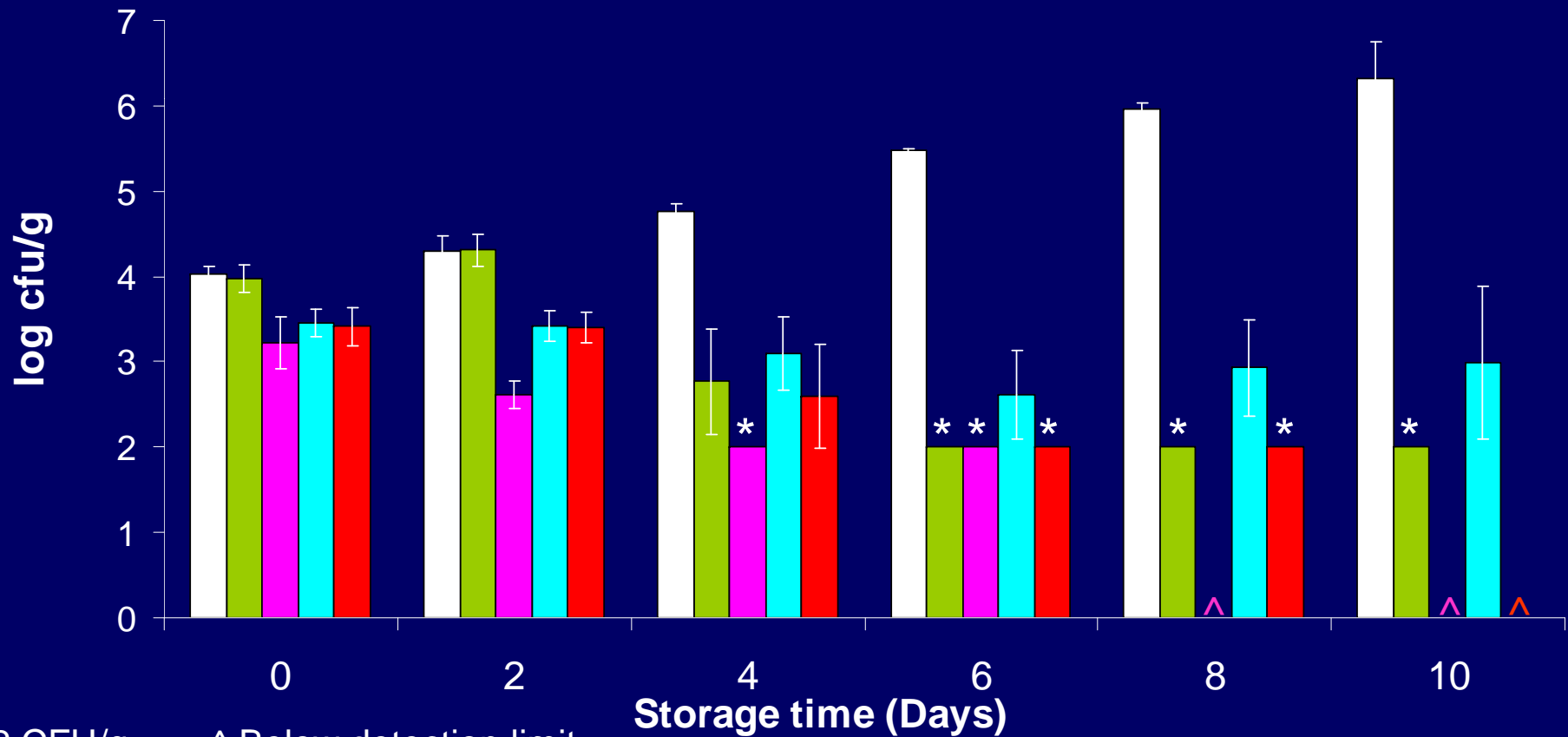


Results



Enumeration of *Listeria* spp. on aerobically stored fresh beef sausage

- *Listeria* alone
- *C. maltaromaticum* UAL307
- *C. maltaromaticum* UAL307 culture + supernatant
- *E. faecalis* 710C supernatant
- *C. maltaromaticum* UAL307 + *E. faecalis* 710C supernatant



* <2 CFU/g

^ Below detection limit

Enumeration of *Carnobacterium* spp. on aerobically stored fresh beef sausage

Control

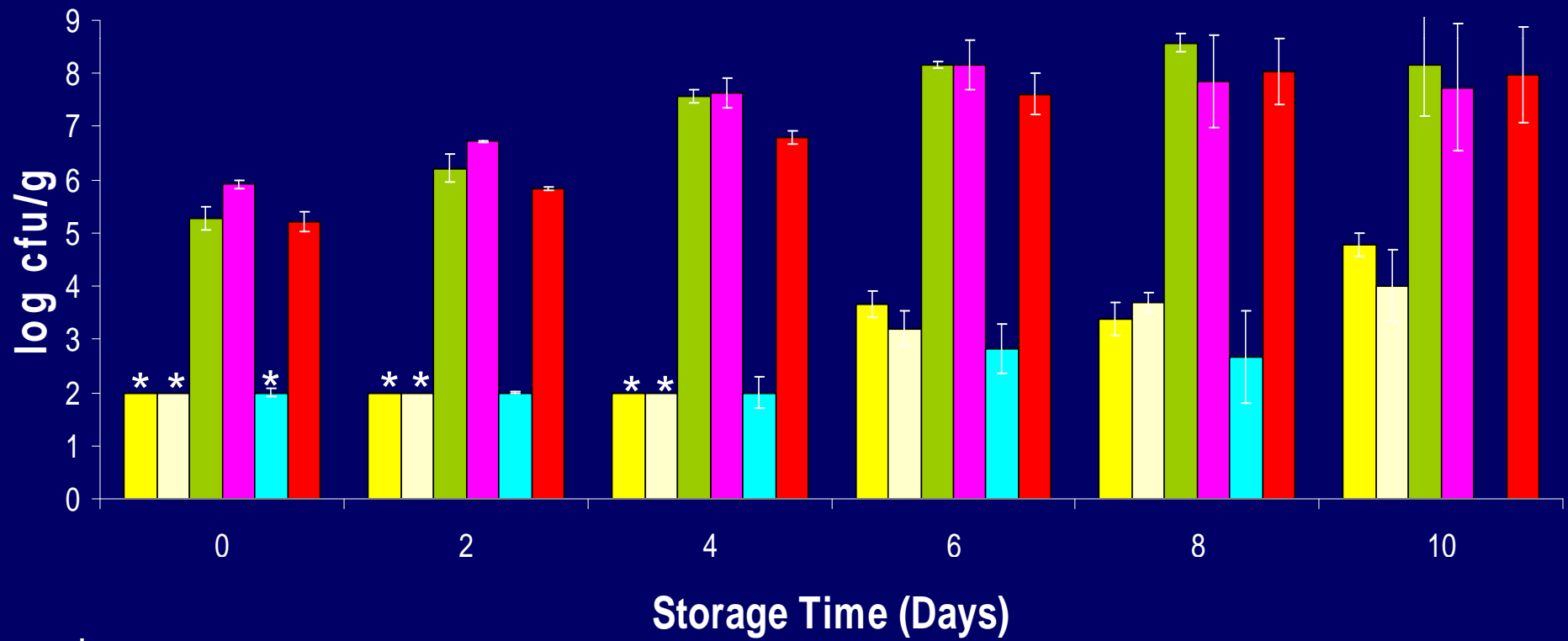
Listeria alone

C. maltaromaticum UAL307

C. maltaromaticum UAL307
culture + supernatant

E. faecalis 710C supernatant

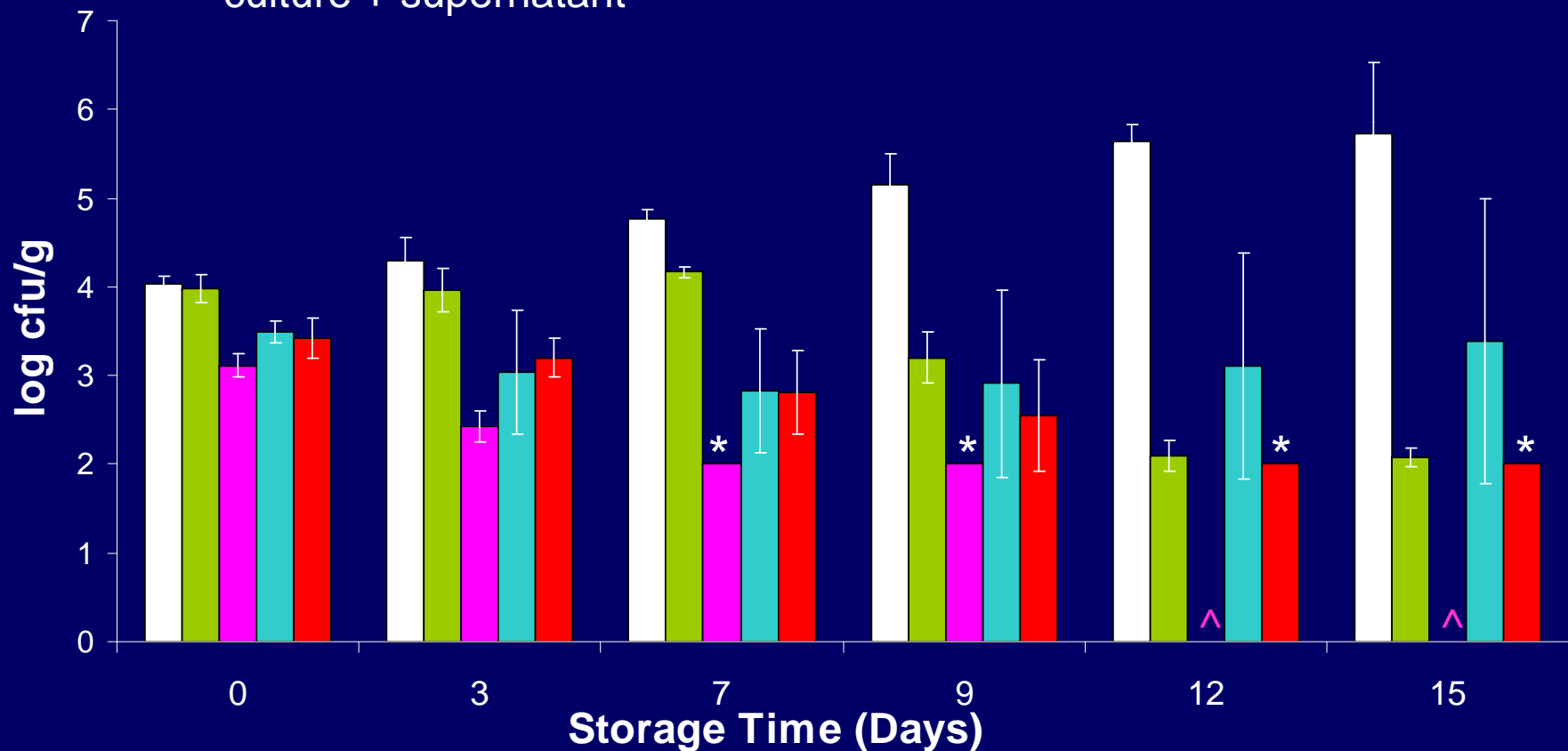
C. maltaromaticum UAL307 +
E. faecalis 710C supernatant



* < 2 log cfu/g

Enumeration of *Listeria* spp. on MAP stored fresh beef sausage

- *Listeria* alone
- *C. maltaromaticum* UAL307
- *C. maltaromaticum* UAL307 culture + supernatant
- *E. faecalis* 710C supernatant
- *C. maltaromaticum* UAL307 + *E. faecalis* 710C supernatant

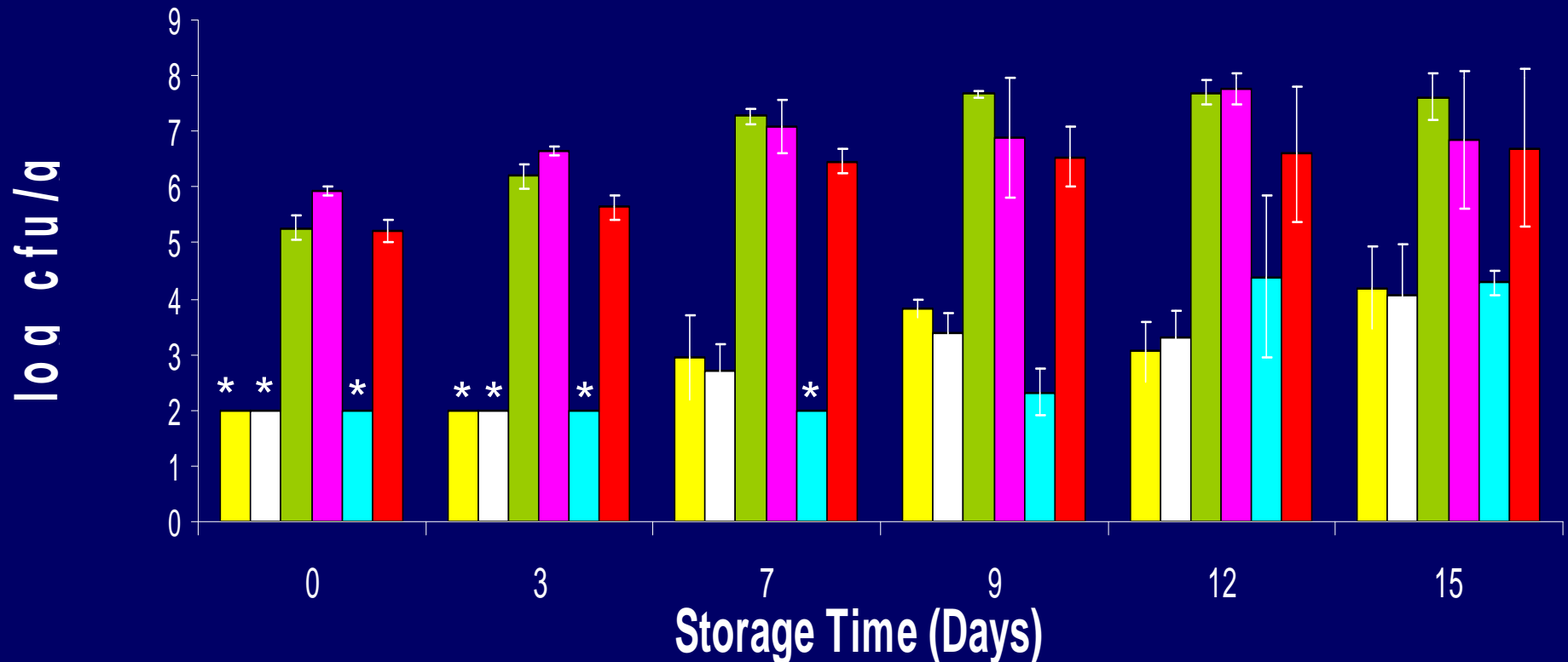


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Enumeration of *Carnobacterium* spp. on MAP stored fresh beef sausage

- Control
- Listeria* alone
- C. maltaromaticum* UAL307
- C. maltaromaticum* UAL307 culture + supernatant
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* < 2 log cfu/g

Effect of inocula on sausage colour during refrigerated storage



Day 0 Aerobically stored

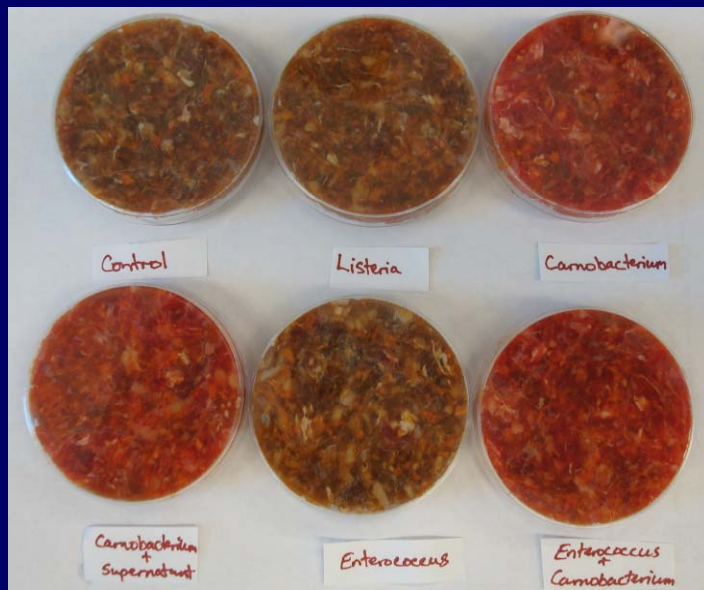


Day 10 Aerobically stored



Day 15
Anaerobically
stored

Effect of inocula on meat colour during refrigerated storage



Meat from aerobically stored sausage at Day 10



Vacuum packaged ground beef inoculated with *C. maltaromaticum* UAL307 after 6 weeks storage at 4°C

(Photo supplied by Kamila Moquin)

Conclusions

- *C. maltaromaticum* UAL307 inhibits the growth of *L. monocytogenes* in fresh beef sausage
 - + Supernatant of *C. maltaromaticum* UAL307 or *E. faecalis* 710C eliminated *L. monocytogenes*
- *C. maltaromaticum* UAL307 antimicrobial activity still effective in 30% CO₂ atmosphere
- *C. maltaromaticum* has colour stabilizing properties in sausage and negligible effect on meat pH

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