



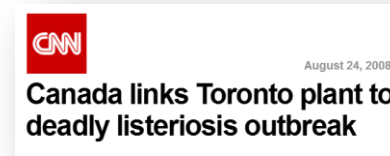
The inhibitory effect of *Carnobacterium maltaromaticum* isolated from vacuum packed meat against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium

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# INTRODUCTION

Foodborne disease outbreaks are one of the leading causes of infections, hospitalisations and deaths provoked by pathogenic bacteria.



These diseases remain a global public health challenge.

Besides the application of good hygiene practices, the development of new hurdles and processing methods could help to maintain the proper quality of food.

# INTRODUCTION

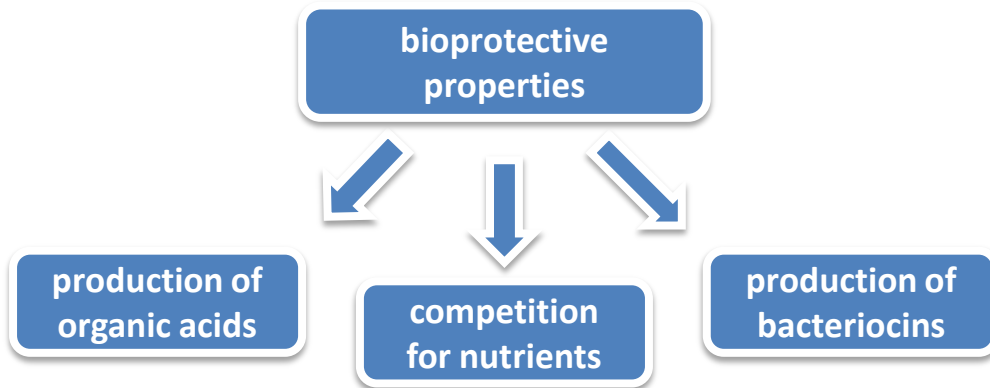
**Biopreservation:** use of controlled microorganisms or its metabolites to preserve food and extend its shelf-life

**Carnobacteria:**

- ubiquitous lactic acid bacteria
- part of the natural flora from meat
- can inhibit pathogenic and spoilage microorganisms



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Can *Carnobacterium* be a hurdle against pathogenic and spoilage bacteria in refrigerated meat and meat products?

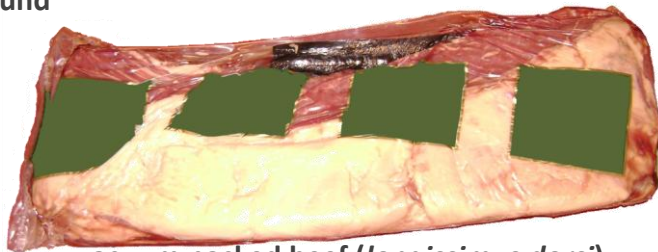


# OBJECTIVE

This study aims to evaluate *in vitro* the bioprotective potential of *Carnobacterium maltaromaticum*\* against major food pathogens:

- *Escherichia coli* O157:H7
- *Listeria monocytogenes*
- *Salmonella* Typhimurium

\* background



vacuum packed beef (*longissimus dorsi*)  
commercial shelf life = 140 days at **-1 °C**



three *C. maltaromaticum* strains

CM\_824

CM\_827  
(lab. ref.)

CM\_829

adapted to low temperatures

# EXPERIMENT 1

## Evaluation of the antimicrobial effect of *C. maltaromaticum* in cocultures

### ① Cocultures



10 mL BHI broth



*C. maltaromaticum* 10<sup>6</sup> CFU/mL  
CM\_824 or CM\_827 or CM\_829

+

*E. coli* O157:H7 or  
*L. monocytogenes* or  
*S. Typhimurium* 10<sup>3</sup> CFU/mL

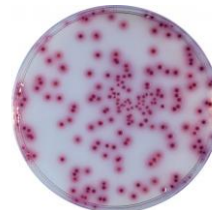
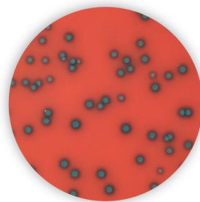
### ② Incubation



150 RPM

- 1°C	28 days
4°C	14 days
25°C	48 hours

### ③ Counting



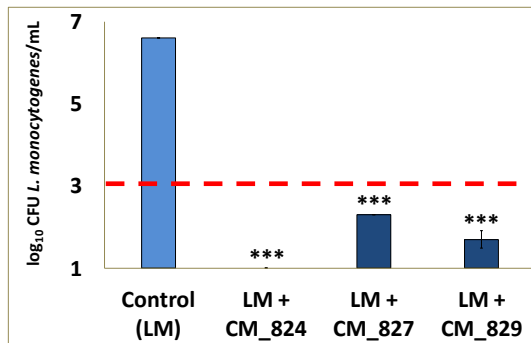
PCA  
total count

Chromogenic media  
pathogenic bacteria

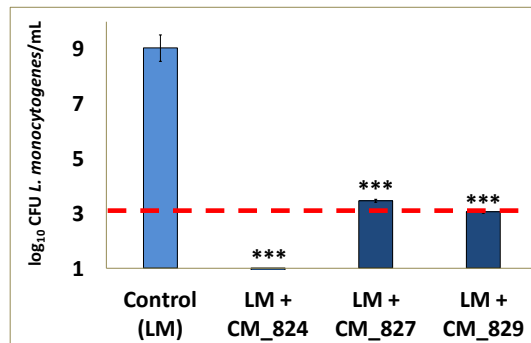
# EXPERIMENT 1

*E. coli* and *S. Typhimurium* were not inhibited when in coculture with *C. maltaromaticum* at any temperature.

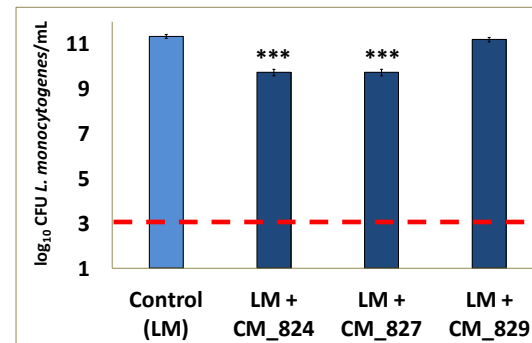
At  $-1^{\circ}\text{C}$  and  $4^{\circ}\text{C}$ , the three strains of *C. maltaromaticum* showed an inhibition effect against *L. monocytogenes*.



*L. monocytogenes* ( $-1^{\circ}\text{C}$ )



*L. monocytogenes* ( $4^{\circ}\text{C}$ )



*L. monocytogenes* ( $25^{\circ}\text{C}$ )

This experiment confirmed the antilisterial activity of the *C. maltaromaticum* strains at low temperatures. This activity might be related to competition for nutrients or to a possible production of organic acids and/or bacteriocins.

# EXPERIMENT 2

## Evaluation of the antimicrobial effect of *C. maltaromaticum* in cocultures with the addition of EDTA

### 1 Cocultures



10 mL BHI broth  
EDTA 1 mM



<i>C. maltaromaticum</i>	10 <sup>6</sup> CFU/mL
CM_824 or CM_827 or CM_829	
+	
<i>E. coli</i> O157:H7 or <i>L. monocytogenes</i> or <i>S. Typhimurium</i>	10 <sup>3</sup> CFU/mL

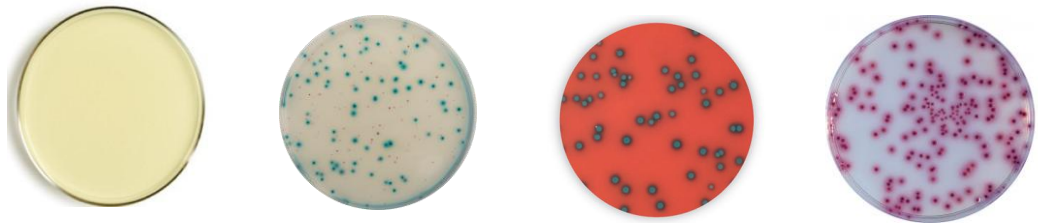
### 2 Incubation



150 RPM

25°C	48 hours
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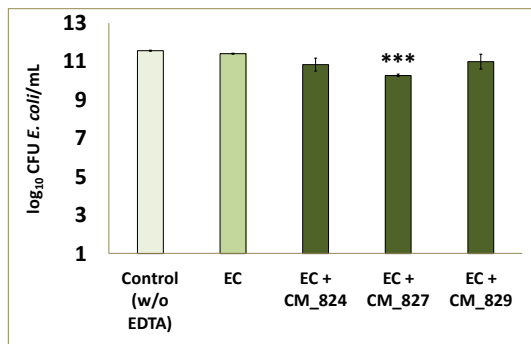
### 3 Counting



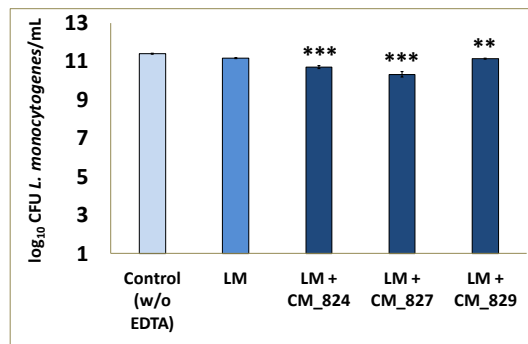
PCA
total count
Chromogenic media
pathogenic bacteria

## EXPERIMENT 2

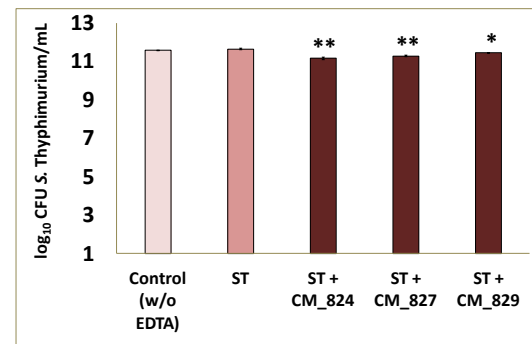
A weak, but significant, inhibition effect against all pathogenic bacteria tested was observed.



*E. coli* (25°C)



*L. monocytogenes* (25°C)



*S. Typhimurium* (25°C)

EDTA possibly interacted with the outer membrane of gram-negative bacteria, allowing *C. maltaromaticum* and its metabolites to act against these bacteria.

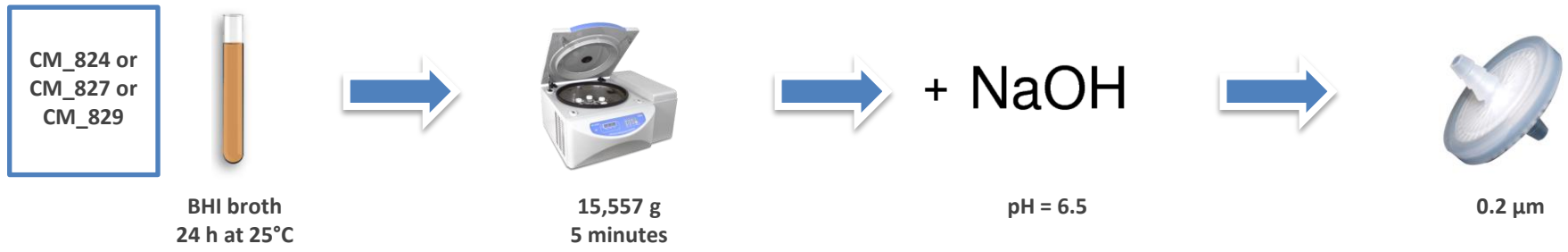
Tests in lower temperatures could have produced higher inhibition effects.



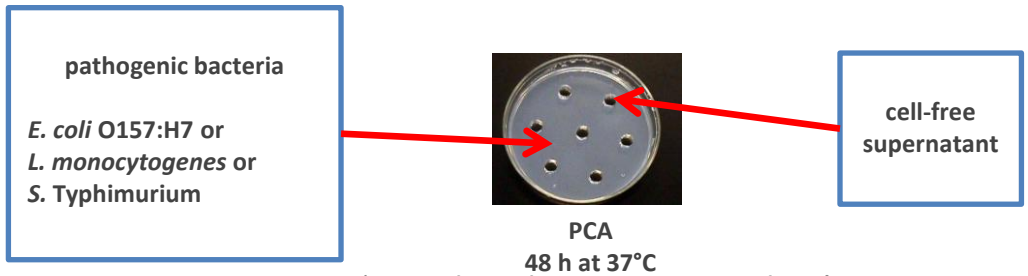
# EXPERIMENT 3

## Evaluation of the antimicrobial effect of the cell-free supernatant of *C. maltaromaticum*

### ① *C. maltaromaticum* culture and cell-free supernatant preparation

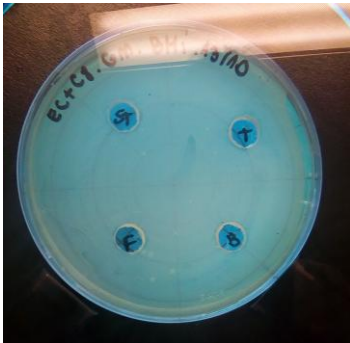


### ② Supernatant inoculation



## EXPERIMENT 3

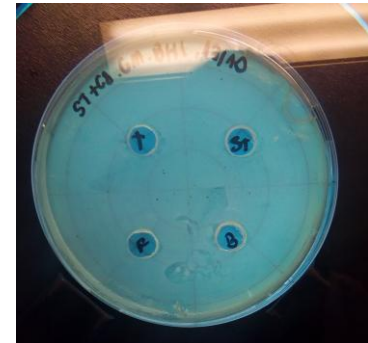
No inhibition effect of the supernatant against the pathogenic bacteria tested was observed.



*E. coli*



*L. monocytogenes*



*S. Typhimurium*

The three *C. maltaromaticum* strains are likely not to produce bacteriocins under the studied conditions.

## CONCLUSIONS

The three *C. maltaromaticum* strains tested showed an antilisterial potential, which was greater at  $-1^{\circ}\text{C}$  and  $4^{\circ}\text{C}$  than at  $25^{\circ}\text{C}$ .

The combination of two hurdles (refrigerated storage and bioprotective cultures) shows great potential to improve quality and food safety.

The behaviour of these strains, as well as their effect against pathogenic and spoilage bacteria, will be studied in meat products.

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Georges Daube



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Renata E. F. de Macedo

# THANKS FOR YOUR ATTENTION QUESTIONS?



ULiège – Faculty of Veterinary Medicine



PUCPR – Campus Curitiba