In vitro evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum packed meat against food pathogens

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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.
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**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

Could *Carnobacterium* be a hurdle against pathogenic and spoilage bacteria in refrigerated meat and meat products?
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**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
adapted to low temperatures

* three *C. maltaromaticum* strains
  - CM_824
  - CM_827 (lab. ref.)
  - CM_829
**EXPERIMENT 1**

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

1. **Cocultures**
   - 10 mL BHI broth
   - C. maltaromaticum \(10^6\) CFU/mL
   - CM_824 or CM_827 or CM_829
   - E. coli O157:H7 or L. monocytogenes or 10³ CFU/mL S. Typhimurium

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

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**EXPERIMENT 1**

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

At −1°C and 4°C, the three strains of *C. maltaromaticum* showed an inhibition effect against *L. monocytogenes*.

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
   - C. maltaromaticum 10⁶ CFU/mL
     - CM_824 or CM_827 or CM_829
   - +
   - E. coli O157:H7 or L. monocytogenes or 10³ CFU/mL
   - S. Typhimurium

2. **Incubation**
   - 25°C
   - 48 hours
   - 150 RPM

3. **Counting**
   - PCA total count
   - Chromogenic media pathogenic bacteria

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**EXPERIMENT 2**

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

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.
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**EXPERIMENT 3**

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

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

   - CM_824 or CM_827 or CM_829
   - BHI broth 24 h at 25°C
   - 15,557 g 5 minutes
   - pH = 6.5
   - 0.2 µm

2. **Supernatant inoculation**

   - pathogenic bacteria
     - *E. coli* O157:H7 or *L. monocytogenes* or *S. Typhimurium*
   - cell-free supernatant
   - PCA 48 h at 37°C

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

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°C and 4°C than at 25°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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THANKS FOR YOUR ATTENTION

QUESTIONS?

ULiège – Faculty of Veterinary Medicine

PUCPR – Campus Curitiba