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

G. M. Danielski[1,2], P. H. Imazaki[1], G. Daube[3], R. E. F. de Macedo[2] & A. Clinquart[1]

[1]Fundamental and Applied Research for Animal & Health (FARAH), Laboratory of Food Technology, University of Liège, Belgium
[2]Graduate Program in Animal Science, School of Life Sciences, Pontifical Catholic University of Paraná, Brazil
[3]Fundamental and Applied Research for Animal & Health (FARAH), Laboratory of Food Microbiology, University of Liège, Belgium

INTRODUCTION

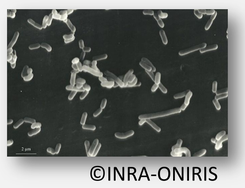
AIM

To evaluate the bioprotective potential *in vitro* of *Carnobacterium maltaromaticum* against: *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium.

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

The development of new hurdles and processing methods could help to maintain the proper quality of food.

<u>Carnobacteria</u>: - ubiquitous lactic acid bacteria
- part of the natural flora from chilled meat
- can inhibit pathogenic and spoilage microorganisms



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bioprotective properties

- **production of organic acids**
- **competition for nutrients**
- **production of bacteriocins**

Could *Carnobacterium* be a hurdle against pathogenic bacteria?

MATERIALS AND METHODS

<u>Experiment 1</u> Evaluation of the antimicrobial effect of *C. maltaromaticum* in co-cultures

❶ **Co-cultures**

10 mL BHI broth

+ *C. maltaromaticum* (10^6 CFU/mL)
CM_824 or CM_827 or CM 829 (lab. ref.)

+ pathogen bacteria (10^3 CFU/mL)
E. coli O157:H7 ATCC 35150 or
L. monocytogenes NCTC 11994 or
S. Typhimurium ATCC 14028

❷ **Incubation**

150 rpm

− 1°C → 28 days
4°C → 14 days
25°C → 48 hours

❸ **Counting**

PCA → total count

chromogenic media → pathogen bacteria

For <u>Experiment 2</u> EDTA 1 mM was added to the co-cultures and only the incubation at 25°C for 48 hours was studied.

<u>Experiment 3</u> Evaluation of the antimicrobial effect of the cell-free supernatant of *C. maltaromaticum*

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

BHI broth

+ *C. maltaromaticum*
CM_824 or CM_827 or CM 829

24 h

25°C

15,557 g
5 min

+ NaOH

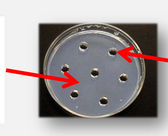
pH 6.5

0.2 µm

❷ **Supernatant inoculation**

pathogenic bacteria

E. coli O157:H7
L. monocytogenes
Salmonella Typhimurium



cell-free supernatant

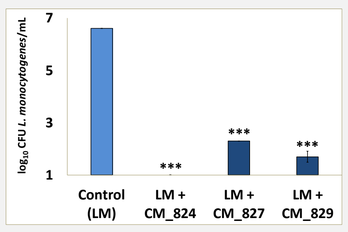
PCA
48 h
37°C

RESULTS

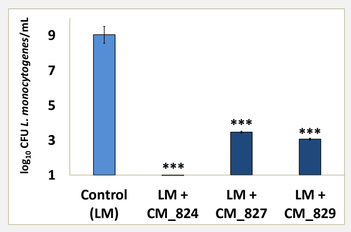
<u>Experiment 1</u> Evaluation of the antimicrobial effect of *C. maltaromaticum* in co-cultures

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

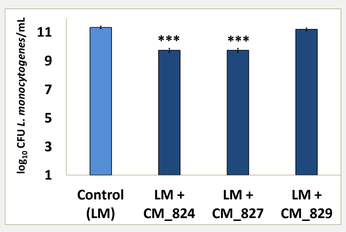
At −1°C and 4°C, the three strains of *C. maltaromaticum* showed an inhibition effect against *L. monocytogenes*.



L. monocytogenes (−1°C)



L. monocytogenes (4°C)

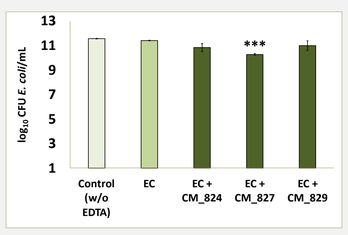


L. monocytogenes (25°C)

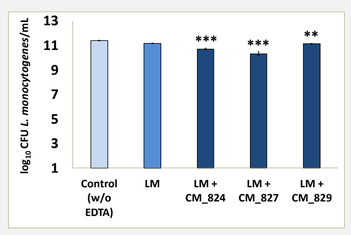
This experiment confirmed the antilisterial activity of the *C. maltaromatium* strains. This activity might be related to competition for nutrients or to a possible production of bacteriocins.

<u>Experiment 2</u> Evaluation of the antimicrobial effect of *C. maltaromaticum* in co-cultures with the addition of EDTA

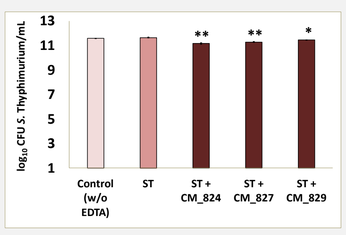
A weak, but significant, inhibition effect against all pathogenic bacteria tested was observed.



E. coli (25°C)



L. monocytogenes (25°C)



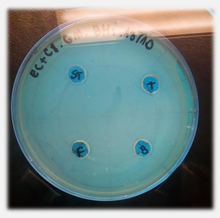
S. Typhimurium (25°C)

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

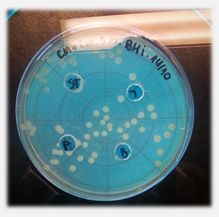
Tests in lower temperatures could have produced higher inhibition effects.

<u>Experiment 3</u> Evaluation of the antimicrobial effect of the cell-free supernatant of *C. maltaromaticum*

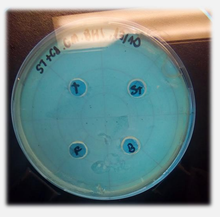
No inhibition effect of the supernatant (pH = 6.5) against the pathogenic bacteria tested was observed.



E. coli (37°C)



L. monocytogenes (37°C)



S. Typhimurium (37°C)

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.