# *IN VITRO* EVALUATION OF THE COMPETING EFFECT OF *CARNOBACTERIUM MALTAROMATICUM* ISOLATED FROM VACUUM PACKED MEAT AGAINST FOOD PATHOGENS

Gabriela Maia Danielski[1,2], Pedro Henrique Imazaki[1*], Georges Daube[3], Renata Ernlund Freitas de Macedo[2] and Antoine 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.
*Corresponding author email: ph.imazaki@ulg.ac.be

**Abstract – Foodborne disease outbreaks are one of the leading causes of infections, hospitalizations and deaths provoked by pathogenic bacteria including *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. *Carnobacterium maltaromaticum* is a lactic acid bacteria, which could prevent the growth of pathogens in refrigerated food. The aim of this study was to determine the bioprotective potential of three strains of *C. maltaromaticum* isolated from chilled vacuum packed beef. They were tested *in vitro* against the pathogens described above. The results indicate that the selected strains have an antilisterial activity, which is optimized at low temperatures. Moreover, when the strains were combined with EDTA it was observed a slight, but significant, inhibition of the gram-negative bacteria used in this study.**

**Key Words – antilisterial activity, biopreservation, lactic acid bacteria.**

## I. INTRODUCTION

Foodborne disease outbreaks are caused by the ingestion of contaminated food with pathogenic microorganisms, including *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*. As it is practically impossible to avoid cross-contamination during processing or after the opening of food packages, new hurdles and processing methods are being developed to maintain the proper quality of food [1].
Carnobacteria are ubiquitous lactic acid bacteria (LAB) isolated from cold and temperate environments, and it is part of the natural flora from chilled meat, fish and dairy products. In the last years, carnobacteria have been studied for their bioprotective properties, since they can inhibit pathogenic and spoilage microorganisms in chilled food [2].
Some species of the genus *Carnobacterium* are well known for their ability to produce bacteriocins. Thus, the use of bacteriocin-producing *Carnobacterium* spp. could prevent the growth of pathogens during critical phases in a variety of refrigerated foods. Nevertheless, non-bacteriocin-producing LAB may also hold great potential for bioprotection against pathogens, possibly by competition for nutrients [2,3].
In this context, this study aims to evaluate *in vitro* the bioprotective potential of *Carnobacterium maltaromaticum* isolated from vacuum-packed beef against *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* Typhimurium.

## II. MATERIALS AND METHODS

*Strains*. Three different strains of *C. maltaromaticum* (CM_824, CM_827 and CM_829) isolated from vacuum packaged beef with long shelf life at a temperature near the freezing point (−1°C) were selected for this study. Considering that these strains could be adapted to grow at low temperatures, they were then selected based on their genetic pattern. The strains of pathogenic bacteria were *E. coli* O157:H7 ATCC 35150, *L. monocytogenes* NCTC 11994 and *Salmonella* Typhimurium ATCC 14028.
*Evaluation of the antimicrobial effect of* C. maltaromaticum *in co-cultures*. The antimicrobial effect of *C maltaromaticum* on food pathogens was investigated as following: flasks with BHI broth were inoculated with $10^6$ CFU/mL of each strain of *C. maltaromaticum* and $10^3$ CFU/mL of each pathogen. The flasks were incubated at −1 °C, 4 °C and 25 °C during 28 days, 14 days and 48 hours, respectively, on a shaker at 150 rpm. The samples were plated on PCA and specific chromogenic media for the bacterial count.
*Evaluation of the antimicrobial effect of* C. maltaromaticum *in co-cultures with the addition of EDTA.* The influence of the addition of EDTA on the antimicrobial effect of *C. maltaromaticum* on pathogens was investigated. BHI broth with EDTA 1 mM was used, following the same procedure described above.
*Evaluation of the antimicrobial effect of the cell-free supernatant of* C. maltaromaticum. In order to check of the growth inhibition effect on food pathogens could be mediated through antimicrobial molecules produced in culture

supernatant, a broth containing each strain of *C. maltaromaticum* after 24 h of growth was centrifuged at 15,557 g for 5 min, treated with NaOH 1N until the pH of 6.5. Then, it was filtered through 0.2 µm sterile cellulose acetate membranes. After the treatment, the broth containing the cell-free supernatant was inserted in wells made in PCA agar previously inoculated with each of the pathogenic bacteria cited above. The halo of inhibition was measured after 48 hours of incubation at 37°C.

## III. RESULTS AND DISCUSSION

The co-culture experiments with strains CM_825 and CM_827 at 25°C showed a weak but significant ($P<0.05$) inhibition effect of *C. maltaromaticum* against *L. monocytogenes*. At −1°C and 4°C, the three strains of *C. maltaromaticum* showed an inhibition effect ($P<0.05$) against *L. monocytogenes.* The inhibition at −1°C and 4°C was higher than at 25°C ($P<0.05$). *E. coli* O157:H7 and *Salmonella* Typhimurium were not inhibited when co-cultured with *C. maltaromaticum* at any temperature. According to several authors, the genus *Carnobacterium* has an antilisterial activity, due to the competition for nutrients or by the sensitivity of *L. monocytogenes* to bacteriocins [3,4].

In the co-culture experiments with EDTA, it was observed a weak, but significant ($P<0.05$) inhibition effect of the three strains of *C. maltaromaticum* against all the pathogenic bacteria tested. In contrary to the previous co-culture experiment, the inhibition with the EDTA treatment was possibly due to the capacity of this compound to interact with the outer membrane of gram-negative bacteria and destabilise it, allowing *C. maltaromaticum* and their metabolites to act against these bacteria [3].

Finally, the evaluation of the antimicrobial activity of cell-free supernatant of *C. maltaromaticum* did not highlight any inhibition effect of the supernatants against the tested pathogens. So, the strains of this study are likely not to produce bacteriocins. A similar result was observed by Jack *et al*. [5], in which *Carnobacterium* spp. was not able to inhibit the growth of all gram-negative, such as *E. coli* and *Salmonella*, and some gram-positive bacteria (e.g., *Clostridium botulinum*).

## IV. CONCLUSION

To conclude, the three *C. maltaromaticum* strains tested showed an antilisterial potential, which was greater at −1°C and 4°C than at 25°C. This result is not surprising since *C. maltaromaticum* can better compete with *L. monocytogenes* in an environment, which is favorable to the growth of *C. maltaromaticum*. Thus, the combination of two hurdles (refrigerated storage and addition of bioprotective cultures) shows great potential to improve quality and food safety, in particular, for chilled foods such as fresh meat and processed meat products.

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