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

Food-borne diseases are an important cause of morbidity and mortality worldwide (WHO, 2015). In 2014, the largest number of food-borne disease outbreaks of bacterial origin in the European Union was caused by *Salmonella*, and the two most commonly reported *Salmonella* serovars in confirmed human cases were *S.* Enteritidis and *S.* Typhimurium (ST) (EFSA and ECDC, 2015). The types, severity and impacts of food-borne diseases have changed through the ages and are still diverse across regions, countries and communities (WHO, 2015). For instance, many of the pathogens of greatest concern today, including *Escherichia coli* O157:H7 (EC) and *Listeria monocytogenes* (LM), were not recognised as causes of food-borne illness a few decades ago (Mead *et al.*, 1999). Therefore, the development of new hurdles and processing methods able to inhibit these pathogens could help to maintain the proper safety of food.
Carnobacteria are ubiquitous lactic acid bacteria isolated from cold and temperate environments and are part of the natural flora from chilled meat, fish and dairy products (Leisner *et al.*, 2007). In the last years, carnobacteria have been studied for their bioprotective properties, since they can inhibit pathogenic and spoilage microorganisms, thus acting as a hurdle in chilled food. Some species of the genus *Carnobacterium* are known for their ability to produce bacteriocins. Hence, 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 (Martin-Visscher *et al.*, 2011).
In this context, this study aims to evaluate in vitro the bioprotective potential of *Carnobacterium maltaromaticum* (CM) isolated from vacuum-packed beef against EC, LM and ST.

Material and methods

Strains. Three strains of CM (CM_824, CM_827 and CM_829) isolated from vacuum-packaged beef with long shelf life at −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 in order to obtain a large genetic variability. The strains of pathogenic bacteria were EC ATCC 35150, LM NCTC 11994 and ST ATCC 14028.
Evaluation of the antimicrobial effect of CM in co-cultures. The antimicrobial effect of CM on food pathogens was investigated as follows: flasks with BHI broth were inoculated with each strain of CM (10^6 CFU/mL) and each pathogen (10^3 CFU/mL). 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 CM in co-cultures with the addition of EDTA. The influence of the addition of EDTA on the antimicrobial effect of CM 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 CM. In order to check if the growth inhibition effect on food pathogens could be mediated through antimicrobial molecules produced in the culture supernatant, a broth containing each strain of CM after 24 h of growth was centrifuged at 15,557 g for 5 min, treated with NaOH 1 N until the pH of 6.5 in order to neutralise the

organic acids eventually produced by the CM strains. 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 h of incubation at 37°C.

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 CM against LM. At −1°C and 4°C, the three strains of CM showed an inhibition effect ($P < 0.05$) against LM. The inhibition at −1°C and 4°C was higher than at 25°C ($P < 0.05$). EC and ST were not inhibited when co-cultured with CM 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 LM to bacteriocins (Jack *et al.*, 1996).
In the co-culture experiments with EDTA, a weak, but significant ($P<0.05$) inhibition effect was observed of the three strains of CM 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 CM and its metabolites to act against these bacteria (Martin-Visscher *et al.*, 2011).
Finally, the evaluation of the antimicrobial activity of cell-free supernatant of CM did not highlight any inhibition effect of the supernatants against the tested pathogens. So, the strains of this study are not likely to produce bacteriocins. A similar result was observed by Jack *et al.* (1996), 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 including *Clostridium botulinum*.

Conclusion

To conclude, the three CM 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 CM can better compete with LM in an environment which is favourable to the growth of CM. Thus, the combination of two hurdles (refrigerated storage and addition of bioprotective cultures) shows great potential to improve the safety of, in particular, chilled foods such as fresh meat and processed meat products.

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