MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF *CARNOBACTERIUM MALTAROMATICUM* ISOLATED FROM VACUUM-PACKED BEEF WITH LONG SHELF LIFE

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Abstract – The aim of this study was to perform a morphological and functional characterization of a *Carnobacterium maltaromaticum* strain with a potential bioprotective effect isolated from vacuum packaged long shelf life beef. The morphological, biochemical and enzymatic profiles, the influence of different temperatures and atmospheres, and the microbial stability of fresh beef inoculated with the *C. maltaromaticum* strain were evaluated. The isolated *C. maltaromaticum* strain presented similar morphological, biochemical and enzymatic profiles as those of two reference strains (LMG 11393 and LMG 22902). The growth of *C. maltaromaticum* was slower in an atmosphere containing O_2 and CO_2. Vacuum packing is therefore suitable for this bacterium. An antimicrobial effect against *Enterobacteriaceae* was highlighted on inoculated fresh meat stored under N_2. The functional characterization of this isolate will be further pursued by a genotypic characterization to better understand its potential bioprotective effect.

I. INTRODUCTION

In order to limit chemical, enzymatic and microbial mechanisms responsible for the deterioration of meat, the use of cold chain during distribution and storage is mandatory. In practice, lower temperatures are often applied to extend the shelf life. A temperature near the freezing point of meat (~ −2 °C), associated with vacuum packaging, allows the preservation of this product up to several months (1), which makes possible the meat trade across the planet without resorting to freezing. Other the type of packaging and the storage temperature, the shelf-life of meat is directly related to its initial microbiological ecosystem (2) and its evolution.

Carnobacterium maltaromaticum is a lactic acid bacterium, and many lactic acid bacteria associated with meat are known for their bactericidal or bacteriostatic activity against other strains, species or genera of bacteria. Some *C. maltaromaticum* strains have been reported to produce class I and II bacteriocins, in addition to circular bacteriocins (3). Bacteriocin production, however, is not a pre-requisite for the biopreservative efficacy of *Carnobacterium* (4).

In this way, the presence of certain lactic acid bacteria adapted to a low temperature in fresh meat could extend the shelf life and improve the microbial stability and safety of this product. Nevertheless, undesired effects of *Carnobacterium* on food quality have been reported, e.g., the production of a malty/chocolate like aroma due to 3-methylbutanal from the catabolism of leucine (5).

The aim of the present study was to perform a morphological and functional characterization of *C. maltaromaticum* with a potential bioprotective effect isolated from vacuum packaged long shelf life beef.

II. MATERIALS AND METHODS

Sample: One strain of *C. maltaromaticum* (CFAUS2/DLC/4/E1) isolated from a vacuum packaged *longissimus dorsi*, displaying a shelf-life of 140 days, obtained from a food wholesaler located in the Walloon Region of Belgium.

Morphological, biochemical and enzymatic profiles: Macroscopic and microscopic observations, Gram staining, catalase and oxydase tests were performed. The biochemical and enzymatic profiles of the strain was evaluated using API 50CH and API ZYM galleries (bioMérieux®).

Influence of different atmospheres on growth: Minced pork meat sterilized by irradiation, used as model of sterile meat, was inoculated with a 10^5 CFU/mL suspension of *C. maltaromaticum* (1 % v/w). Eighty grams of inoculated meat were repackaged in polypropylene trays sealed with a polypropylene film (52 µm thick, oxygen permeability of 110 $cm^3/m^2 \cdot 24$ h at +23 °C and 0% RH) containing a modified atmosphere – 100 % N_2, 70 % O_2:30 % CO_2 or 30 % O_2:70 % CO_2 –, and stored up to 7 days at +4 °C, +8 °C or +12 °C. Bacterial counting was performed on PCA at +25 °C on days 0, 3 and 7.

Microbiological stability of beef inoculated with C. maltaromaticum: bovine *psoas major* samples were supplied by a food wholesaler located in the Walloon Region of Belgium 16 days after slaughter. In the lab, 3 cm thick steaks were cut and inoculated on surface with a 10^5 CFU/mL suspension of *C. maltaromaticum* (1 % v/w). They were repackaged under vacuum and stored at −1 °C during 7 days (day 7). Then, they were repackaged in polypropylene trays sealed with a polypropylene film (52 µm thick, oxygen permeability of 110 $cm^3/m^2 \cdot 24$ h at +23 °C and 0 % RH) containing a modified atmosphere – 100 % N_2 or 70 % O_2:30 % CO_2 –, and stored up to 7 days at +4 °C (day 14). Total viable count (TVC), lactic acid bacteria (LAB), *Enterobacteriaceae* (EB), *Pseudomonas* spp. (PS) and *Brochothrix thermosphacta* (BT) counts were performed on PCA (+22 °C), MRS (+22 °C), VRBG (+30 °C), CFC (+25 °C) and STAA (+22 °C), respectively.

Statistical analysis: Experimental data for each response variable was analyzed by ANOVA using the GLM procedure. Whenever a *post-hoc* test was suitable, Tukey test was performed.

III. RESULTS AND DISCUSSION

Morphological, biochemical and enzymatic profiles: The colonies of *C. maltaromaticum* presented the following characteristics: circular, convex, entire, ø < 1 mm, smooth, translucent, unpigmented and odorless. Microscopic examination revealed Gram positive bacillus shaped cells arranged in pairs. The strains were catalase and oxydase negative. The API 50 CH system showed that the *C. maltaromaticum* strain could ferment the following carbohydrates and derivates: glycerol, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, gentiobiose, D-turanose and potassium gluconate. In addition, the API ZYM test revealed the activity of the following enzymes: esterase (C4), esterase lipase (C8), valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase. These profiles were similar to those of the two reference strains of *C. maltaromaticum* (LMG 11393 and LMG 22902).

Influence of different atmospheres on growth: The concentration of *C. maltaromaticum* immediately after inoculation of irradiated minced pork meat was 3.3 $\log_{10}$ CFU/g. At +4 °C a weak growth of *C. maltaromaticum* was observed. At +8 °C, only the atmosphere without oxygen (100 % N_2) allowed *C. maltaromaticum* to reach a high concentration (7.7 $\log_{10}$ CFU/g) in less than one week. At +12 °C, the 70 %-CO_2 atmosphere produced a partial bacteriostatic effect on *C. maltaromaticum*, and the 30 %-CO_2 atmosphere did not inhibit its growth. Altogether, among the studied conditions, a higher temperature (+12 °C) and an atmosphere

poor in oxygen were the optimal conditions for the growth of *C. maltaromaticum* (Figure 1). These conditions are, however, not applicable in practice.

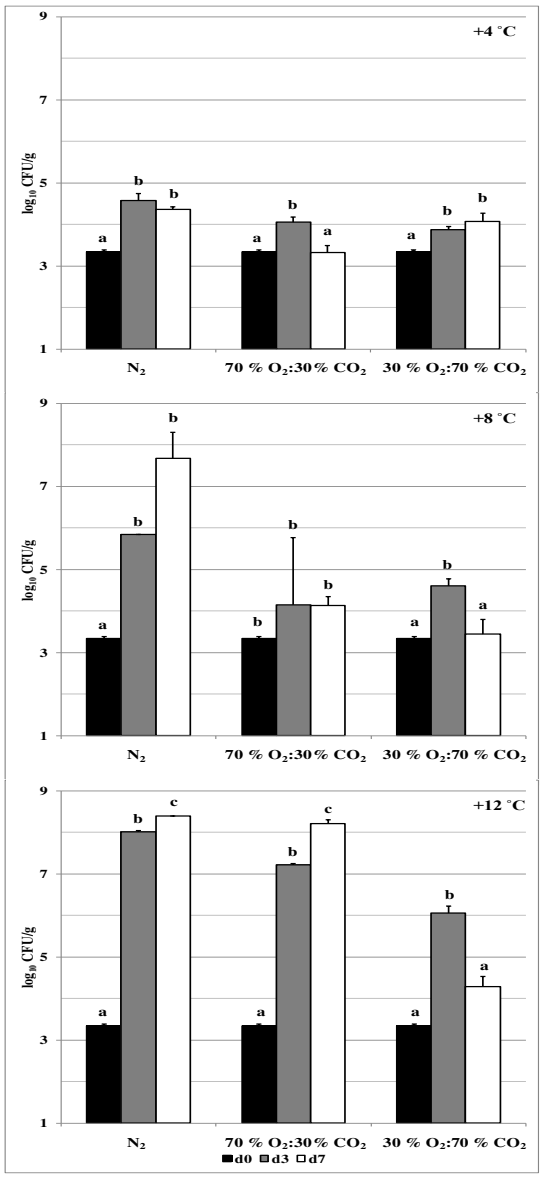

Figure 1 Growth of *Carnobacterium maltaromaticum* in sterilized minced pork meat. Different letters indicate significant differences ($P < 0.05$).

Microbiological stability of beef inoculated with C. maltaromaticum: Two different vacuum-packaged *psoas major* samples were used to evaluate the microbial stability of beef inoculated with *C. maltaromaticum* under two different modified atmospheres. An initial counting before inoculation was performed (Table 1).

Table 1 Initial microbial counts of *psoas major* samples before inoculation with *C. maltaromaticum*. Results are expressed in $\log_{10}$ CFU/cm²

	Sample 1	Sample 2
Atmosphere	100 %N_2	70 % O_2 /30 % CO_2
TVC	5.6 ± 0.0	5.7 ± 0.0
LAB	3.1 ± 0.0	3.5 ± 0.0
EB	2.5 ± 0.1	1.2 ± 0.3
PS	2.5 ± 0.1	1.3 ± 0.4
BT	2.1 ± 0.7	< 1.0

After inoculation and 7 days of storage under vacuum, no effect was observed on the total viable count and on the count of lactic acid bacteria. A reduction of *Pseudomonas* sp. and *B. thermosphacta* was observed during the first week of storage under vacuum conditions (Figures 2 and 3). *Pseudomonas* sp. counts remained lower than the counting threshold after inoculation.

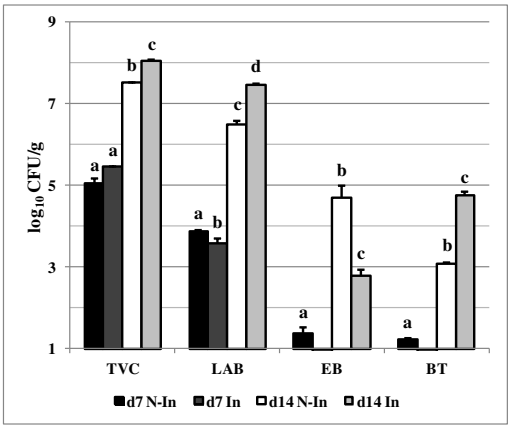

Figure 2 Microbial counts of samples after inoculation with *C. maltaromaticum* and storage under vacuum conditions at −1 °C for 7 days, and then under 100 % N_2 at +4 °C for 7 days. N-in: non inoculated, In: inoculated. Different letters indicate significant differences ($P < 0.05$).

In the samples stored under N_2, the presence of the inoculant favored the growth of *B.*

thermosphacta. On the other hand, an inhibiting effect of the inoculant on the growth of *Enterobacteriaceae* was observed.

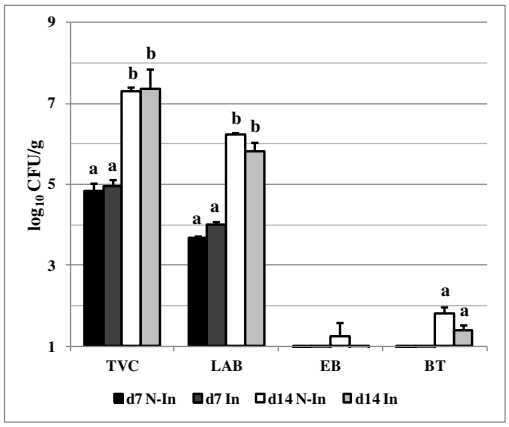

Figure 3 Microbial counting of samples after inoculation with *C. maltaromaticum* and storage under vacuum at −1 °C for 7 days, and then under 70 % O_2 / 30 % CO_2 at +4 °C for 7 days. N-in: non inoculated, In: inoculated. Different letters indicate significant differences ($P < 0.05$).

The growth of *Enterobacteriaceae* and *B. thermosphacta* was limited by the presence of CO_2. No effect of the inoculant was observed when an atmosphere 70 % O_2:30 % CO_2 was applied.

IV. CONCLUSIONS

Morphological, biochemical and enzymatic profiles of the *C. maltaromaticum* strain (CFAUS2/DLC/4/E1) isolated from vacuum packaged beef samples with extremely long shelf life were similar to those of two reference strains. The evaluation of the influence of different atmospheres showed that the growth of *C. maltaromaticum* was slower in an atmosphere containing O_2 and CO_2. Vacuum packaging and low temperatures are therefore more suitable for the growth of this bacterium. An antimicrobial effect against *Enterobacteriaceae* was highlighted on inoculated fresh meat stored under N_2.
The functional characterization of this strain will be further pursued by genotypic characterization and its potential bioprotective effect will also be studied.

ACKNOWLEDGMENTS

This study was funded by the General Operational Direction of Agriculture, Natural Resources and Environment (DGARNE) of the Walloon Region (Belgium). Project D31-1275 (CONSBBB).

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