

# POSITIVE MODULATION OF MEAT MICROBIAL ECOLOGY BY FEEDING STRATEGIES

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**Abstract –** The objective of this work was to determine if it is possible to modulate carcass/meat contamination by feeding farm animals with a diet supplemented with a protective culture, here *Carnobacterium maltaromaticum* CB1/UAL307 producing three bacteriocins, including carnocyclin A. A total of 144 weaned Grimaud female rabbits (a commercial meat breed) were divided into two feeding groups: 1) a control commercial diet and 2) the same diet supplemented with Micocin®, a commercial preparation of *C. maltaromaticum* CB1, at a final concentration of 8-log CFU (Colony Forming Unit) per kg of feed. Rabbits were fed their respective diet until they reached a commercial slaughter weight of 2.2 kg. Presence of *C. maltaromaticum* producing carnocyclin A was confirmed by PCR amplification for three specific genes. Its prevalence was greater in faeces, on thighs and in ground meat from rabbits fed the ration supplemented with Micocin®. These results demonstrate that the microflora of the feed can influence the organisms contaminating the end products and emphasized the importance of providing farm animals with high microbial quality feed and hygienic conditions to grow.

**Key Words -** *Carnobacterium maltaromaticum* CB1, Micocin, rabbit.

## I. INTRODUCTION

It is paramount to slaughter healthy animal since their muscles contain little to no microorganisms, with the exception of lymph nodes [1]. Even when meat is produced under strict hygienic conditions, surface contamination by spoilage and pathogenic microorganisms is to be expected. Meat gets contaminated by microorganisms from hide/skin of the animal, gut content, workers' hands and the slaughter

environment [2]. Pre and probiotic have been extensively studied for their health benefit (e.g., improved digestibility, diarrhea prevention) and more recently as a complementary treatment for metabolic disorders in humans [3]. Commercial preparations are available for farm animals but their impact, with respect to meat hygiene, remains to be established. In this study, weaned Grimaud female rabbits were fed a commercial ration enriched with Micocin®, a protective culture approved for the control of *Listeria monocytogenes* in luncheon meats. Our hypothesis is that by enriching the diet of farm animals with a positive microflora, it will improve the microbial quality of the end products, notably carcass and meat primal cuts. Contrary to other antimicrobial systems that wipe out microorganisms (e.g., heat treatment), the idea here is rather to modulate the microflora in order to have the microorganisms that we want, at the level that we want and at the timing that we want, a concept described as "Microflora management" [2].

## II. MATERIALS AND METHODS

A total of 144, 35-day-old weaned female Grimaud breed rabbits were obtained from a commercial farm (Laprodéo, Saint-Tite, Quebec, Canada). They were raised in conventional commercial cages, with six rabbits per cage; the cage constituted the experimental unit. Twelve cages each were analyzed, respectively, for the control and the experimental group. The latter had its ration supplemented with the protective culture Micocin® (Griffith Laboratories, Toronto, Canada) containing *C. maltaromaticum* CB1 at a final concentration of 8-log CFU (Colony Forming Unit) per kg of feed. The

animals were housed in two different but similar rooms. On a daily basis, control group were always visited first and the personnel changed clothes, mask, hair net and gloves between each group. If control group needed to be revisited, personnel had to shower first. Animals were slaughtered at a commercial target weight of 2200 g in a provincially inspected establishment according with the rules and regulations in the province of Quebec, Canada, after a total feed withdrawal of 15 h. The two groups were slaughtered on two different days to avoid cross contamination, beginning with the control group.

Feces were collected from the pan underneath the 12 cages and were analysed once a week for the presence of *C. maltaromaticum* CB1 producing carnocyclin A and the enumeration of presumptive Lactic Acid Bacteria (LAB) on All Purpose Tween media (APT; incubated anaerobically for 48 h at 25 °C). APT was used instead of deMan, Rogosa and Sharp (MRS) media because *Carnobacterium* is unable to metabolize acetate and grow poorly on MRS. A 25-g feces sample was homogenized in 225 ml of peptone water (0.1% wt/vol) for 2 min, serially diluted and 100 µl of the appropriate dilution was spread plated in duplicate. On thighs, a sampling procedure, similar to the one described by Brichta-Harhay et al. [4] for whole poultry carcasses, was used. Thigh was sealed in a sterile Stomacher bag (Stomacher® 400C, Seward Laboratory Systems Inc., London, UK) after addition of 300 ml of peptone water. The bag was placed on a rotary shaker (Boekel Scientific Orbitron Rotator II, model 260250, New York, USA) for one minute on each side. The samples were then manually massaged for 30-sec to remove microorganisms from the surface.

To determine the presence and the prevalence of *C. maltaromaticum* CB1 on feaces and thighs, characteristic colonies from the APT enumeration plates were subcultured in 1 ml of APT broth and incubated as described above. A 100 µl aliquot of each of those cultures were placed in U-bottom 96-well microtiter plates (Greiner bio-one CELLSTAR® 96 Well plate, VWR International, Alberta, CA). Using a 48-pin Microplate Replicator (2.54cm Pin Length,

V&P Scientific, San Diego, CA) aliquots were transferred onto APT plates and were let to dry under a biosafety cabinet. For early detection of bacteriocin production by *C. maltaromaticum*, a soft agar (7.5 ml, 7.5% agar) inoculated (1%) with the indicator organisms (*Pediococcus acidilactici* UL5 and *C. divergens*) was poured on the replicated plates [5]. Plates were incubated at 25 °C under anaerobiosis as described for presumptive LAB enumeration. Cultures with zone of inhibition were further characterized for the detection of the carnocyclin A gene. With ground meat, analysis was performed directly on cell pellet collected by centrifugation from a meat homogenate in peptone water.

Isolation of total DNA was performed using a QIAGEN DNeasy Tissue kit (Louisville, Kentucky, USA) following the protocol for Gram-positive bacteria according to the manufacturer's instructions. DNA purity and quantity were verified by a Nanodrop 2000 (Thermo Scientific, Wilmington, USA). The oligonucleotide primers used for in the Polymerase Chain Reaction (PCR) were obtained from IDT (Integrated DNA Technologies, Iowa, USA). Presence of *C. maltaromaticum* producing carnocyclin A was determined by using three genes: the 16S DNA region specific for *C. maltaromaticum* and *C. gallinarum* was amplified with the primer set 27F and 16-cpg [6]. Interspacer region (ISR) is a specific region of *C. maltaromaticum* located between the 16S rDNA and 23S rDNA [7, 8]. Finally, the amplification of carnocyclin A, (CclA; circular bacteriocin produced by *C. maltaromaticum*) was performed using the primers CclA-F and CclA-R [9]. All PCR reactions were performed in 25 µL reaction using a maximum of 8 µL of DNA sample. PCR products were analyzed by electrophoresis in a 2% (wt/vol) agarose gel (Life Technologies, Waltham, MA, USA).

### III. RESULTS AND DISCUSSION

*Carnobacterium* are lactic acid bacteria often isolated from meat, including vacuum packaged fresh and processed meats [10]. *C. maltaromaticum* CB1 (also known as UAL307)

was isolated from fresh pork and produces three bacteriocins, notably carnocyclin A [11], which was used to tack the organism in the faeces, on the thighs and in ground meat of rabbits fed a ration supplemented with the organism. It is not expected to be particularly resistant to the acidic environment of the stomach unless protected by the feed or food matrix. It is active against a number of gram-positive organisms including *L. monocytogenes* [12]. It is available for use as a protective culture in luncheon meat under the commercial name Micocin® (Griffith Laboratories) and it is approved in several countries including Canada and the US (GRAS status).

*C. maltaromaticum* producing carnocyccin A was identified in the faeces of the control group on week one only and throughout the feeding period for the group fed the ration containing strain CB1 at 8-log CFU/kg of feed. Rabbits were eating 0.78 to 1.2 kg/week from the beginning to the end of the feeding period (4 weeks). It was identified on the thighs from the control group stored under anaerobic conditions at 4 °C after 5 and 20, but not 15 days and for thighs stored under aerobic conditions. For the thighs coming from the animal fed with the ration supplemented with Micocin®, it was identified on those stored under aerobic conditions at day 0, 3 and 6 but not 8 days. For meat stored under anaerobic conditions, it was isolated at all sampling times up to 20 days.

For the faeces and the thighs, identification of *C. maltaromaticum* producing carnocyccin A was done on colonies grown onto APT plates where as for ground meat, it was performed on the pallet coming from all cells contaminating the meat after it was homogenized in peptone water. Here again, prevalence of *C. maltaromaticum* producing carnocyccin A was greater in the ground meat coming from the rabbits fed the ration supplemented with Micocin® (Table 1).

The greater incidence of *C. maltaromaticum* producing carnocyccin A on thighs and ground meat stored under anaerobic conditions is to be expected since lactic acid bacteria are the prevailing microflora in meat stored under such conditions [13]. Because *C. maltaromaticum* CB1 was originally isolated from the environment

(fresh pork meat), it could be widely distributed in nature, including various meat related facilities. Therefore, isolates found in the control group might have come from the raising facility, the slaughterhouse environment, etc. Stringent biosecurity measures were followed in order to limit cross contamination. Nevertheless, a higher incidence in the faeces, on the thighs and ground meat was observed in the experimental group receiving Micocin® in their diet compared to the control group raised in a separate but similar room. Also, because Micocin® was delivered by means of the feed, it probably contaminated the whole environment where the rabbits were raised. So we cannot assume that the strains isolated on the meat came only through the ones actually ingested. The hide and fur could also constitute a source of contamination of the thigh and ground meat with Micocin®.

Table 1. Presence of *C. maltaromaticum* CB1 (Micocin®) in rabbit ground meat stored at 4 and 10 °C under aerobic and anaerobic conditions (0, 3, 6, 9, 12, 15 days) as determined by PCR analysis of three specific genes: 16S-cpg, ISR and CclA<sup>a</sup>.

Treatments	Aerobic	Anaerobic
<b>Control</b>		
16S-cpg	7	6
ISR	3	3
CclA	0	1
<b>Micocin®</b>		
16S-cpg	11	11
ISR	10	10
CclA	8	8

<sup>a</sup> Number of positive gene identification out of 11 sample of ground meat for each storage conditions (n=11; one sample per temperature and storage time).

#### IV. CONCLUSION

By feeding a ration supplemented with *C. maltaromaticum* CB1 (Micocin®) we were able to modulate its presence in the faeces, on the thighs and in ground meat. Experiments are currently underway to validate if its presence provide a better control of *L. monocytogenes* on fresh ground meat.

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