Proceedings of the Eleventh Conference on Food Microbiology

Faculty of Veterinary Medicine, University of Liege

14 & 15/09/2006, University of Ghent, Belgium

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Selection of bacteria to decrease *in vitro* growth of *Campylobacter jejuni* and *Campylobacter coli* and characterization of their antagonistic activities

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Introduction

Chicken meat contaminated with *Campylobacter spp*. represents an important source of human gastroenteritis worldwide. According to Vellinga and Van Loock (2002), poultry meat would cause more than 40 percents of campylobacteriosis in our land (1). Particularly free-range chicken has been proved as more frequently contaminated (2). In order to control the risk of *Campylobacter* contamination at farm level, different strategies have been examined over the last decade, particularly antagonistic bacteria (3). The aim of this study was to investigate the effect of twelve bacteria against *C. jejuni and C. coli* growth *in vitro*. This antagonistic effect consists in providing a natural antimicrobial compound (organic acids, bacteriocins …)

Material and methods

Among bacteria from the CWBI collection, twelve bacteria were selected on the basis of their growth kinetics and their ability to acidify the broth. Several inhibition tests were realized by the Kirby-Bauer disk diffusion method (4).
Two lactic bacterial strains (*Lactobacillus ssp*. CWBI-B659 and *Weissella ssp*. CWBI-B902) were studied in co-culture (at 37°C under micro-aerophilic atmosphere) in Brucella broth with a supplement of lysis horse blood.
The *Weissella* strain was also evaluated for its ability to grow in organic matter composed by chicken litter. This growth was evaluated in a basal medium without any carbohydrate source. An eventual synergy with this cellulase enzyme (cellulose A, Beldem s.a., Andenne, Belgium) was also evaluated by adding different concentrations of the cellulase to the culture medium. Several sampling were made on erlen flasks during 24 hours to estimate *Weissella* growth, pH, and dextrose and lactate concentrations.

Results and discussion

Ten strains were able to inhibit *Campylobacter spp*. by disc diffusion agar assay. The production in fermenter has been carried out for bacteria which presented good antagonistic performances against *Campylobacter*. The conservation by spray-drying and freeze-drying has already shown good results for two strains (CWBI-B76 and CWBI-B1070).
In co-culture, both bacteria showed bactericidal activity against *C. jejuni* and *C. coli* after 48 hours of incubation. This antagonistic effect was expressed by a *Campylobacter* reduction comprised between 2 and 5 log cfu ml^{-1}.
The pH of supernatants after 48 hours of co-culture with *Lactobacillus* and *Weissella* were respectively 4,67 ± 0,39 and 4,68 ± 0,06. On the other hand, the pH of the supernatant of *Campylobacter spp*. culture was 6,79 ± 0,06.
The *Weissella* strain showed a capacity to survive with chicken litter as unique carbon source. Moreover, the addition of cellulase at 1500 ppm was able to increase the growth of the antagonistic strain in this medium.

Conclusion and perspectives

Results of these *in vitro* studies revealed antagonistic effect of two strains (*Lactobacillus* and *Weissella*) against *Campylobacter*, a human entero-pathogen. Furthermore, inhibition tests have shown antagonistic potential of eight other strains. Co-culture assay with these strains should be promising.
An *in vivo* experiment with chicken is needed to further evaluate the effect of the hopeful antagonistic bacteria on the *Campylobacter* colonisation in chicken. The enzyme-*Weissella* synergy observed *in vitro* should also be examined *in vivo*. An optimisation of production of the micro organism and conservation is necessary.

Acknowledgement

The financial support of the D.G.A. (Direction Générale de l'Agriculture; project D31-1093) of the Walloon Region is kindly acknowledged.

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