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

Campylobacter are responsible for the majority of human intestinal infectious diseases worldwide. According to Vellinga and Van Loock (2002), poultry meat would cause more than 40 percent of campylobacteriosis in our land (1). In order to control the risk of *Campylobacter* contamination at farm level, different strategies have been examined over the last decade, particularly antagonistic bacteria (2).

Our study investigated a new approach of the use of *in vitro* screening models to determine the ability of lactic acid bacteria (LAB) to alter the growth and proliferation of *C. jejuni* and *C. coli*. This research leads to improve the knowledge on antagonistic mechanisms involved during the survive competition between *Campylobacter spp.* and LAB.

Material & Methods

• **Bacteria**

➢Antagonistic bacteria
Lactobacillus plantarum CWBI-B76
Lactobacillus plantarum CWBI-B659
Pediococcus pentosaceus CWBI-B605
Weissella confusa CWBI-B902

➢Indicator bacteria
Campylobacter jejuni LMG-8841
Campylobacter coli LMG-6440

• **Antagonistic tests**

➢Kirby-Bauer disk diffusion method (3) (Figure 1)

➢Co-culture (Figure 2)

Figure 1. Diffusion tests with supernatant and supernatant adjusted at pH 6.

Figure 2. Anaerobic flask putting in direct contact the antagonistic bacteria and the indicator bacteria.

• **Evaluation of cellulase-*Weissella* synergy** in organic matter composed by chicken litter in basal medium without any other carbohydrate source.

Results & Discussion

Four strains were able to inhibit *Campylobacter spp.* by disc diffusion agar assay (Table 1).

Table 1. Diameter (mm) of inhibition zone with culture supernatant (S.) and neutralized supernatant at pH 6 (S. pH 6). Diameter of paper disk: 12,7 mm.

Strains	*C. jejuni*		*C. coli*	
	S.	S. pH 6	S.	S. pH 6
CWBI-B76	13	/	13	13
CWBI-B659	22	/	22	18
CWBI-B605	15	/	15	/
CWB-B902	18	/	27	/

In co-culture, two bacteria (*Lactobacillus ssp.* and *Weissella ssp.*) showed bactericidal activity against *C. jejuni* and *C. coli* after 48 hours of incubation (Figure 3 and 4).

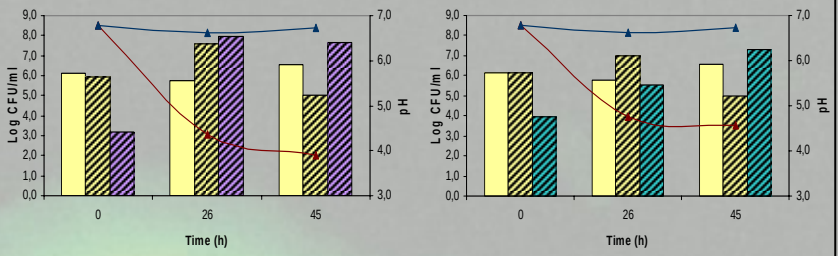

Figure 3. Evolution of microbial populations of *C. jejuni* □, *Lactobacillus plantarum* CWBI-B659 ■ and *Weissella confusa* CWBI-B902 ■ in *C. jejuni* culture □ and co-culture ▨ and the pH of these supernatants (*C. jejuni* culture ▬, co-culture ▬).

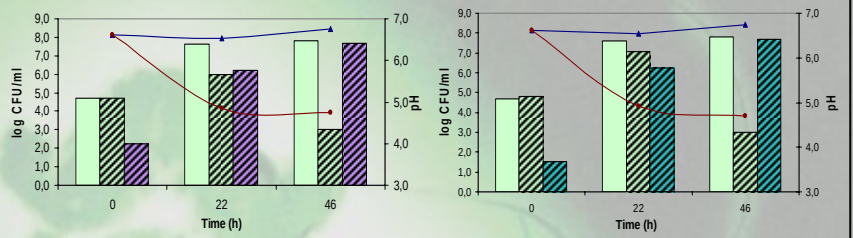

Figure 4. Evolution of microbial populations of *C. coli* □, *Lactobacillus plantarum* CWBI-B659 ■ and *Weissella confusa* CWBI-B902 ■ in *C. coli* culture □ and co-culture ▨ and the pH of these supernatants (*C. coli* culture ▬, co-culture ▬).

The *Weissella* strain showed a capacity to survive with chicken litter as unique carbon source. Moreover, the addition of cellulase with more than 150 ppm was able to increase the growth of the antagonistic strain in this medium (Figure 5).

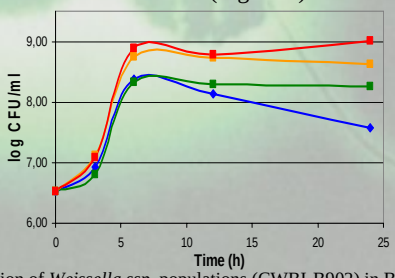

Figure 5. Evolution of *Weissella ssp.* populations (CWBI-B902) in Brucella broth (▬) and in chicken litter with several enzyme concentrations (0 ▬, 150 ▬ and 1500 ▬ ppm).

Conclusions

Results of these *in vitro* studies have revealed antagonistic effect of two strains (*Lactobacillus* and *Weissella*) against *Campylobacter*, a human entero-pathogen. Furthermore, inhibition tests have shown antagonistic potential of four strains. Co-culture assay with these strains should be promising. An *in vivo* experiment with chickens is needed to further evaluate the effect of the hopeful antagonistic bacteria on the *Campylobacter* colonisation in chickens. The enzyme-*Weissella* synergy observed *in vitro* should also be examined *in vivo*. In addition, it appears important to evaluate the ability of the antagonistic strains to resist at the technological processes (e.g. fermentation scale up, drying process, packaging process …) before their use in a broilers challenge assay.

References

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