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 survival 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)

Results & Discussion

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

<table>
<thead>
<tr>
<th>Strains</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWBI-B76</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>CWBI-B659</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>CWBI-B605</td>
<td>15</td>
<td>18</td>
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<tr>
<td>CWBI-B902</td>
<td>18</td>
<td>27</td>
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</tbody>
</table>

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

**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.**

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<td>CWBI-B76</td>
<td>S.</td>
<td>S. pH 6</td>
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<tr>
<td>CWBI-B659</td>
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<td>S.</td>
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<td>CWBI-B605</td>
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Figure 3. Evolution of microbial populations of C. jejuni and C. coli after 48 hours of incubation (Figure 3 and 4).

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**Figure 4. Evolution of microbial populations of C. coli and Weissella spp. and Campylobacter jejuni C. jejuni culture and co-culture in Brucella broth ( ) and the pH of these supernatants ( ).**

**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 spp. populations (CWBI-B902) in Brucella broth ( ) and in chicken litter with several enzyme concentrations (0 ppm, 150 ppm and 1500 ppm).**

Conclusions

Results of these in vitro studies have revealed antagonistic effect of two strains (Lactobacillus and Weissella) against Campylobacter, a human enteropathogen. 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 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