Archiv für Lebensmittelhygiene 61, Heft 4 (2010), Seiten 125–164

Arch Lebensmittelhyg 61,
132–138 (2010)
DOI 10.2376/0003-925X-61-132

ISSN 0003-925X

Korrespondenzadresse:
kadrin.meremae@emu.ee

[1]Department of Food Hygiene, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 58A, 51014 Tartu, Estonia; [2]Rakvere Hospital, Laboratory of Microbiology, Lõuna põik 1, 44316 Rakvere, Estonia; [3]Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki, Agnes Sjöbergin katu 2 (P.O. Box 66), 00014, Finland [4]Department of Food Science and Technology, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 58, 51014 Tartu, Estonia

In vitro study of the antimicrobial effect of selected probiotics combined with prebiotics on *Campylobacter jejuni*

In-vitro-Studie über die antimikrobielle Wirkung von ausgewählten Probiotika kombiniert mit Präbiotika auf Campylobacter jejuni

Kadrin Meremäe[1], Mati Roasto[1], Terje Tamme[1], Marina Ivanova[2], Marja Liisa Hänninen[3] and Priit Elias[4]

Summary

The present study investigated the antimicrobial effect of *Lactobacillus acidophilus, Lactobacillus fermentum, Bifidobacterium bifidum* and *Bifidobacterium longum* with and without 1 % inulin or 1 % oligofructose on the survival and growth of antibiotic-resistant as well as susceptible *C. jejuni* strains (n = 6) using the co-culture experiments *in vitro*. The pH of the medium and certain organic acids produced by probiotic bacteria were also measured. High performance liquid chromatography (HPLC) was used to determine the concentrations of lactic and acetic acid. *L. acidophilus* with 1 % inulin or 1 % oligofructose and *Bifidobacteria* combined with 1 % oligofructose inhibited the growth of all the tested *C. jejuni* strains and none of the tested *C. jejuni* strains were detectable in 48 hours of coincubation. *C. jejuni* did not affect the growth of the probiotic strains. The antimicrobial activity of probiotics combined with prebiotics against *C. jejuni* was most probably associated with the reduction of pH via the production of organic acids in liquid media.

Keywords: probiotic bacteria, *Campylobacter jejuni,* growth impact, inulin, oligofructose

Zusammenfassung

In der vorliegenden Studie wurde die antimikrobielle Wirkung von *Lactobacillus acidophilus, Lactobacillus fermentum, Bifidobacterium bifidum* und *Bifidobacterium longum* mit und ohne Zusatz von Inulin oder Oligofruktose auf Überleben und Wachstum von antibiotikaresistenten und -empfindlichen *Campylobacter jejuni*-Stämmen (n = 6) untersucht, und zwar unter Verwendung von Co-Kultur-Experimenten. Die Untersuchungen umfassten auch die Bestimmung des pH-Wertes des Mediums sowie die Analyse der von den probiotischen Bakterien produzierten organischen Säuren. *L. acidophilus* mit Inulin oder Oligofruktose und beide *Bifidobacterium* spp. mit Oligofruktose hemmten das Wachstum aller getesteten *C. jejuni*-Stämme. Alle geprüften *C. jejuni*-Stämme waren nach 48-stündiger Ko-Inkubation nicht mehr nachweisbar. *C. jejuni* hatte andererseits keinen Einfluss auf das Wachstum der probiotischen Kulturen. Die antagonistische Aktivität der Kombination von Probiotika und Präbiotika gegen *C. jejuni* ist nach den Ergebnissen der Studie mit großer Wahrscheinlichkeit auf die Reduzierung des pH-Wertes durch die Produktion von organischen Säuren zurückzuführen.

Schlüsselwörter: probiotische Bakterien, *Campylobacter jejuni,* Antagonismus, Inulin, Oligofruktose

Introduction

Campylobacter jejuni is one of the most frequent causes of acute gastroenteritis in humans (Skirrow and Blaser, 2000). It is estimated that campylobacteriosis is a growing concern worldwide, as approximately 180 000 cases occur annually in the European Union (EFSA, 2009). Furthermore, an increasing number of human infections caused by *C. jejuni* have been shown to be caused by organisms resistant to antimicrobials (Engberg et al., 2001). It is well established that broiler chicken meat, which is considered to be a major source of human campylobacteriosis, is often contaminated with *C. jejuni* (Hänninen et al., 2000; Wingstrand et al., 2006). In terms of food safety and human health, lactic acid bacteria (LAB) can be used as an alternative measure to control antibiotic-resistant as well as susceptible foodborne pathogens (Brashears et al., 1998; Carson and Riley, 2003).

LAB such as *Lactobacillus* spp. and *Bifidobacterium* spp. of intestinal origin, also named probiotics, are frequently used for probiotic food production in dairy and meat industry because of health promoting effects (Collins and Gibson, 1999; Sanders, 2000). Probiotic bacteria ingested with food have capability to inhibit the growth of enteropathogens in the intestinal microbiota, to restore the balance of microbiota in the digestive tract, and to prevent foodborne diseases (Collins and Gibson, 1999; Sanders, 2000).

The antimicrobial activity of probiotic bacteria against foodborne pathogens is explained in terms of their ability to produce organic acids, such as lactic and acetic acid in the fermentation of the mixture of sugars resulting in the decrease of the growth media pH (Fooks and Gibson, 2003). Furthermore, the antagonistic properties of probiotic bacteria are also associated with the production of hydrogen peroxide, antimicrobial enzymes and specific inhibitory components, such as bacteriocins. They also have the ability to limit dramatically the availability of the necessary nutrients for pathogens in their living environment (Ouwehand and Vesterlund, 2004).

Many *in vitro* experiments have been previously performed to examine the antimicrobial activity of lactobacilli and bifidobacteria in relation to various foodborne pathogenic bacteria such as *Escherichia, Staphylococcus, Salmonella, Listeria, Campylobacter* and their antibacterial effect is manifested *in vitro* (Annuk et al., 2003; Fernández et al., 2003; Hütt et al., 2006). The antimicrobial properties of LAB have also been observed in various foods and documented by many researchers (Gusils et al., 1998; Brashears et al., 1998; Ito et al., 2003; Chaveerach et al., 2004).

Compared with numerous studies (Zdolec et al., 2009) designed to investigate the antagonistic activity of the probiotics, only a few *in vitro* studies have been published aimed to analyze the combined influence of probiotics and prebiotics on pathogenic bacteria (Fooks and Gibson 2002, 2003; Klewicki and Klewicka, 2004). However, it is well known that probiotic bacteria in combination with prebiotics have synergistic co-effects via promoting the growth and viability of potentially health-stimulating bacteria (Niness, 1999; Bosscher et al., 2006). Prebiotics, including the best-studied inulin and oligofructose, have been widely used in food technologies due to their nutritional or technological properties and they can provide selected lactobacilli and bifidobacteria a competitive advantage (Franck, 2002; Callaway et al., 2002). Still, there are limited data available on growth dynamics of probiotics combined with prebiotics and foodborne pathogens such as *C. jejuni,* and their interactions in co-cultures.

Therefore, the objective of this study was to investigate the impact of *Lactobacillus acidophilus, Lactobacillus fermentum, Bifidobacterium bifidum* and *Bifidobacterium longum,* in combination with and without 1 % inulin or 1 % oligofructose, on the survival and growth of antibiotic-resistant and susceptible *C. jejuni* strains *in vitro.* Changes in pH values of the growth media and certain organic acids produced by probiotic bacteria were also measured.

Materials and Methods

Probiotic bacteria

Probiotic bacteria such as *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus fermentum* ATCC 14931, *Bifidobacterium bifidum* Bb12 and *Bifidobacterium longum* B46 were obtained from the collection of the Institute of Microbiology, University of Tartu, Estonia. Pure cultures of probiotic bacteria were streaked on de Man Rogosa Shape (MRS) agar (Oxoid, Basingstoke, UK) and incubated at 37 ± 0.5 °C under anaerobic conditions for 48 h, except for *L. acidophilus,* which was grown under microaerobic conditions. One microliter (µl) inoculation loops were used to take one loopful of each probiotic culture into 5 ml MRS broth (Oxoid) and incubated at 37 °C ± 0.5 °C for 24 h under the conditions mentioned above. After incubation, the bacterial cells were centrifuged for 10 min at 15 000 rpm, and each strain was added into the 10 ml MRS broth to level of approximately 10^8 CFU/ml.

Prebiotic ingredients

1 % (w/v) inulin (RAFTILINE®HP) and 1 % (w/v) oligofructose (RAFTILOSE®P95), provided by ORAFTI Active Food Ingredients (Tienen, Belgium), were used in our experiments.

Campylobacter jejuni strains

The strains of *C. jejuni* L30.1, *C. jejuni* L23.1 and *C. jejuni* L06.06 were isolated from Estonian broiler chicken meat in 2006. The strains of *C. jejuni* C135 and *C. jejuni* C1055, isolated from two hospitalized patients with severe campylobacteriosis in 2007, were obtained from the Laboratory of Microbiology of Rakvere Hospital (Rakvere, Lääne-Virumaa, Estonia). The strain of *C. jejuni* ATCC 33291 as control was included in our study. Each of the *C. jejuni* strains were streaked onto charcoal cefoperazone deoxycholate (CCDA) agar (Oxoid) and incubated at 42 °C ± 0.5 °C under microaerobic conditions for 48 h. Next, one µl inoculation loops were used to take one loopful of each *C. jejuni* culture into 5 ml of Mueller-Hinton (MH) (Oxoid) broth and was incubated as described above. The cells of *Campylobacter* were concentrated separately by centrifugation for 10 min at 15 000 rpm and all strains were individually added into the tubes of 10 ml MH broths to achieve around 10^8 CFU/ml.

Characterization of *C. jejuni* strains

C. jejuni strains selected for the present investigation were both antibiotic susceptible, resistant as well as multi-resistant. The antibiotic susceptibility tests for *C. jejuni* strains, isolated from broiler chicken meat, were performed in our previous study (Roasto et al., 2007), and for *C. jejuni* strains of human origin the susceptibility patterns were determined in the Laboratory of Microbiology of Rakvere Hospital. *C. jejuni* ATCC 33291 and L30.1 were both susceptible to all the tested antibiotics. *C. jejuni* C135 was

Archiv für Lebensmittelhygiene 61, Heft 4 (2010), Seiten 125–164

resistant to erythromycin (MIC = 16 µg/ml). *C. jejuni* C1055 was resistant to erythromycin (MIC = 16 µg/ml) and enrofloxacin (MIC = 4 mg/ml). *C. jejuni* L23.1 was resistant to enrofloxacin (MIC = 4 µg/ml) and nalidixic acid (MIC = 64 µg/ml). *C. jejuni* L06.06 was resistant to enrofloxacin (MIC = 4 µg/ml), erythromycin (MIC = 16 µg/ml), nalidixic acid (MIC = 128 µg/ml), and oxytetracyclin (MIC = 32 µg/ml).

Determination of the growth dynamics of bacteria in co-cultures

The impact of selected probiotic bacteria on the *C. jejuni* strains was determined in coculture experiments, as described by Chaveerach et al. (2004). The test scheme is shown in Figure 1. In the co-culture experiments, 100 µl of each viable lactobacilli or bifidobacteria strain from the MRS broth and 100 µl of each *C. jejuni* strain from the MH broth were both inoculated into 10 ml of MH broth, containing approximately 10^6 CFU/ml of bacterial strain at 0 h. Control cultures contained probiotic bacteria strains and *C. jejuni* strains inoculated separately in MH broths on the level of 10^6 CFU/ml at 0 h. Additionally, the MH broths' pH was adjusted to 6.8 with 0.1 N NaOH for all samples at 0 h in order to obtain similar pH values in all MH broths. All MH broths were incubated at 37 ± 0.5 °C under microaerobic conditions for 48 h. In addition, the experiments included tests with probiotic bacteria in combination with 1 % inulin and 1 % oligofructose. Cocultivation experiments with 1 % inulin or 1 % oligofructose in MH broths were carried out as described above. The bacterial counts of experimental cultures as well as control cultures were performed as follows: 0 h, 24 h and 48 h on CCDA agar for *Campylobacter* and MRS agar for probiotic bacteria using the spread technique. All bacteria were incubated under the same conditions as described above. The colonies were counted and results were expressed as $\log_{10}$ CFU/ml ± standard deviation (SD). Additionally, pH was measured in all the MH broths at 0 h, 24 h and 48 h with a pH meter (Consort C833). All experiments and measurements were carried out in triplicate.

Determination of the concentration of certain organic acids

Lactate and acetate concentrations were determined at 24 h and 48 h from MH broths with and without 1 % inulin or 1 % oligofructose, which were separately inoculated with *L. acidophilus* ATCC 4356, *L. fermentum* ATCC 14931, *B. bifidum* Bb12 and *B. longum* B46. Measurement was described by Akalin et al. (2004) with minor modifications. Briefly, high performance liquid chromatography (HPLC) analyses were performed using a liquid chromatograph Agilent 1100 (Agilent 1100 Series, Waldbronn, Germany). Two milliliters of sample was taken from incubated MH broth and centrifuged for 10 min at 4000 rpm. The sample was filtrated through a 0.20 µm filter and 10 µl of supernatant was used for the HPLC analyzes. The wavelength of detection was optimized at 210 nm for the determination of acids. Reversed-phase column Phenomenex Synergi 4 µ Hydro-TP 80A (250 x 4.6 mm) was used at ambient temperature. The mobile phase at 1 ml/min and 5 mM H_2SO_4 were used. Standard solutions of lactic and acetic acid (Sigma, USA) were dissolved in distilled water. Calibration curves and elution times were obtained, which were used as the basis for calculating the acids' content. All measurements were performed in duplicate.

Statistical analysis

Total counts of probiotic bacteria and total counts of *C. jejuni* strains in coculture experiments were statistically analyzed using Student's *t*-test to estimate the significance of changes ($P < 0.05$) in bacterial numbers.

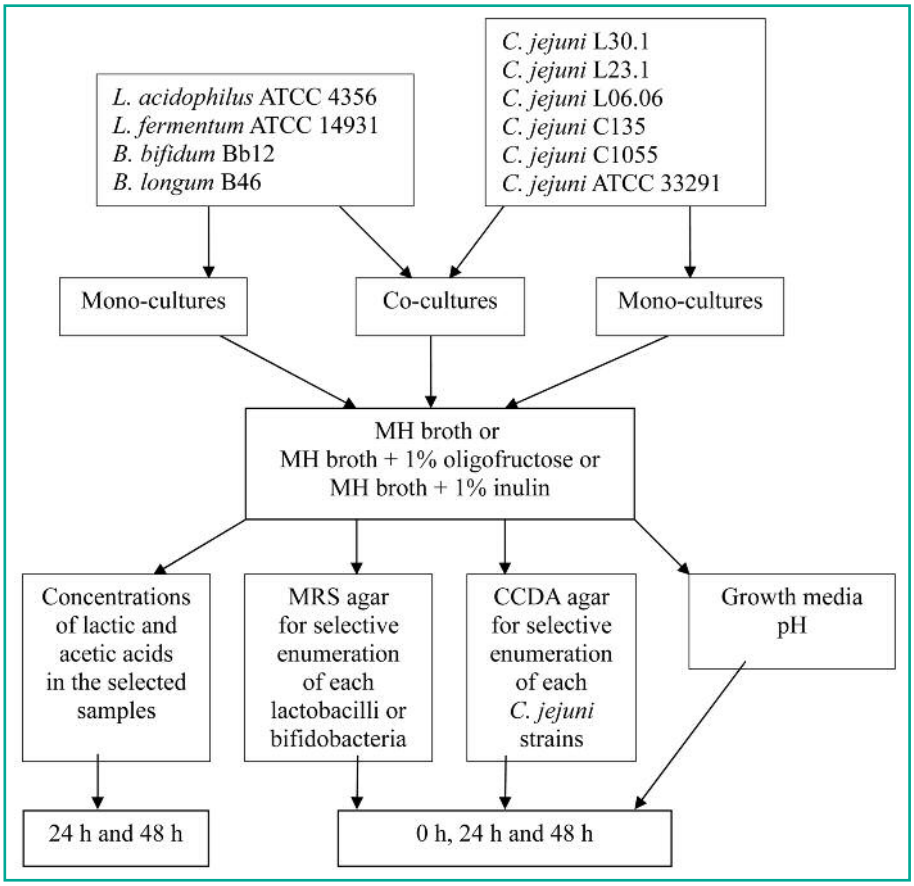

FIGURE 1: *Test scheme.*

Results

The effect of selected probiotic bacteria in combination with 1 % oligofructose on the *C. jejuni* strains in MH broths is shown in Figure 2. *L. acidophilus* ATCC 4356, *B. bifidum* Bb12 and *B. longum* B46 with 1 % oligofructose inhibited the survival and growth of all investigated *C. jejuni* strains in MH broths (Fig. 2A, C, D). After 24 h coincubation, the bacterial counts of *C. jejuni* strains in combination with *L. acidophilus* ATCC 4356, *B. bifidum* Bb12 or *B. longum* B46 rapidly decreased, on average, from 6.00±0.03 to 1.46±0.28, to 1.01±0.34, to 0.81±0.13 log CFU/ml, respectively. None of the tested *C. jejuni* strains was detectable in 48 h ($P < 0.001$). Unlike other probiotics, *L. fermentum* ATCC 14931 with 1 % oligofructose was not able to decrease the total viable counts of any of *C. jejuni* strains under the detectable limit within 48 h (Fig. 2B). The number of cam-

pylobacters slowly declined on the average from 6.09±0.02 to 4.02±0.28 log CFU/ml within 48 h. For comparison, the growth of mono-cultures of *Campylobacter* increased approximately from 6.03±0.08 to 7.01±0.12 log CFU/ml during 48 h in all experiments. No significant differences ($P > 0.05$) in the total viable count dynamics of *C. jejuni* strains as mono-cultures were observed in the MH broths. Therefore, the mean value of the total viable counts of all six investigated *C. jejuni* strains in mono-cultures represent the average total counts showen in Fig 2. The numbers of *L. acidophilus* ATCC 4356, *B. bifidum* Bb12 and *B. longum* B46 with 1 % oligofructose in both mono- and cocultures increased on average approximately up to 7.31±0.08 log CFU/ml by the end of the experiments. The CFUs of *L. fermentum* ATCC 14931 in combination with 1 % oligofructose, in both mono- and cocultures, were on average 6.88±0.19 log CFU/ml in 48 h.

The impact of selected lactobacilli and bifidobacteria in combination with 1 % inulin on the *C. jejuni* strains in MH broths is shown in Figure 3. In contrast to other tested probiotic bacteria, only *L. acidophilus* ATCC 4356 showed antagonistic activity against all *C. jejuni* strains. Bacterial counts of *C. jejuni* strains slowly decreased on average from 6.03±0.02 to 3.36±0.42 log CFU/ml in 24 h, and under the detection limit ($P < 0.01$) in 48 h (Fig. 3A). In case of the coincubation with *L. fermentum* ATCC 14931 (Fig. 3B), *B. bifidum* Bb12 (Fig. 3C) and *B. longum* B46 (Fig. 3D), the average log CFU per ml of *C. jejuni* strains in 48 h were 5.80±0.17, 6.46±0.13 and 5.94±0.22, respectively. For comparison, the total viable counts of all the tested campylobacters in control samples, which is shown as the average of total counts of six *C. jejuni* strains in mono-cultures in Figure 3, increased on average from 6.00±0.10 to 6.92±0.12 log CFU/ml within 48 h. In the presence of 1 % inulin in the growth media, the numbers of *L. acidophilus* ATCC 4356 both as mono- and cocultures increased up to 7.23±0.38 log CFU/ml by the end of the experiments. Control growths of other investigated probiotic bacteria combined with 1 % inulin in both mono- and cocultures were on average 6.60±0.19 log CFU/ml in 48 h. Probiotic bacteria without the presence

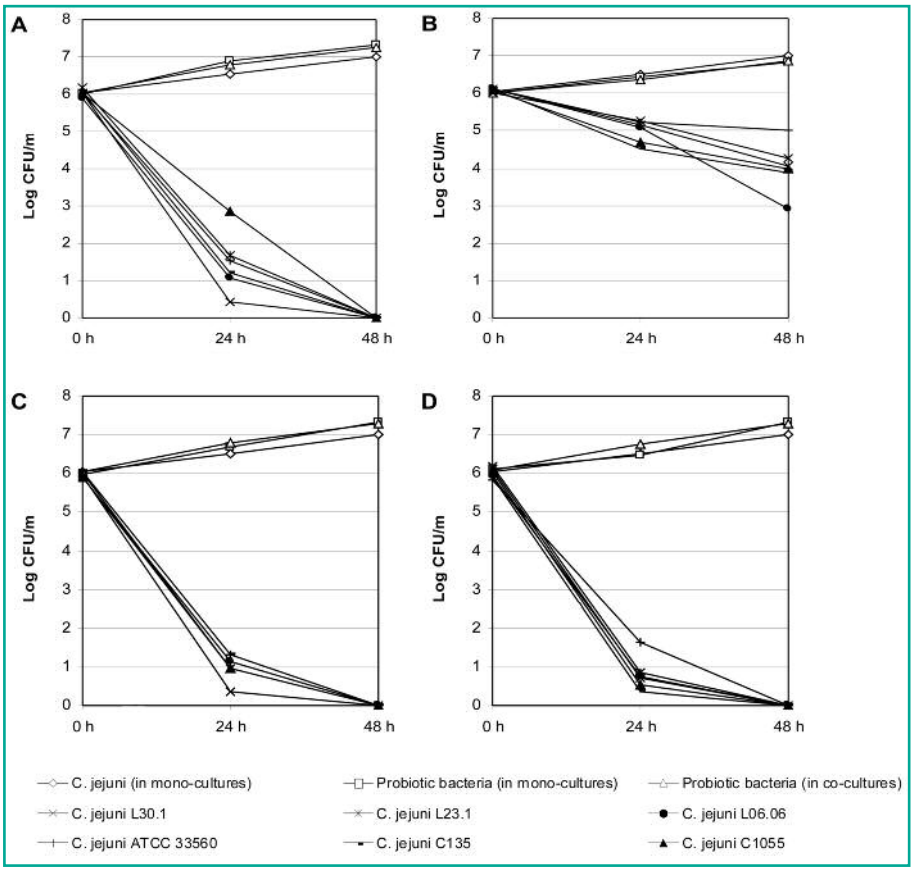

FIGURE 2: *The impact of L. acidophilus ATCC 4356 (A), L. fermentum ATCC 14931 (B), B. bifidum Bb12 (C) and B. longum B46 (D) in combination with 1 % oligofructose on the CFUs of six C. jejuni strains in co-incubation in MH broth.*

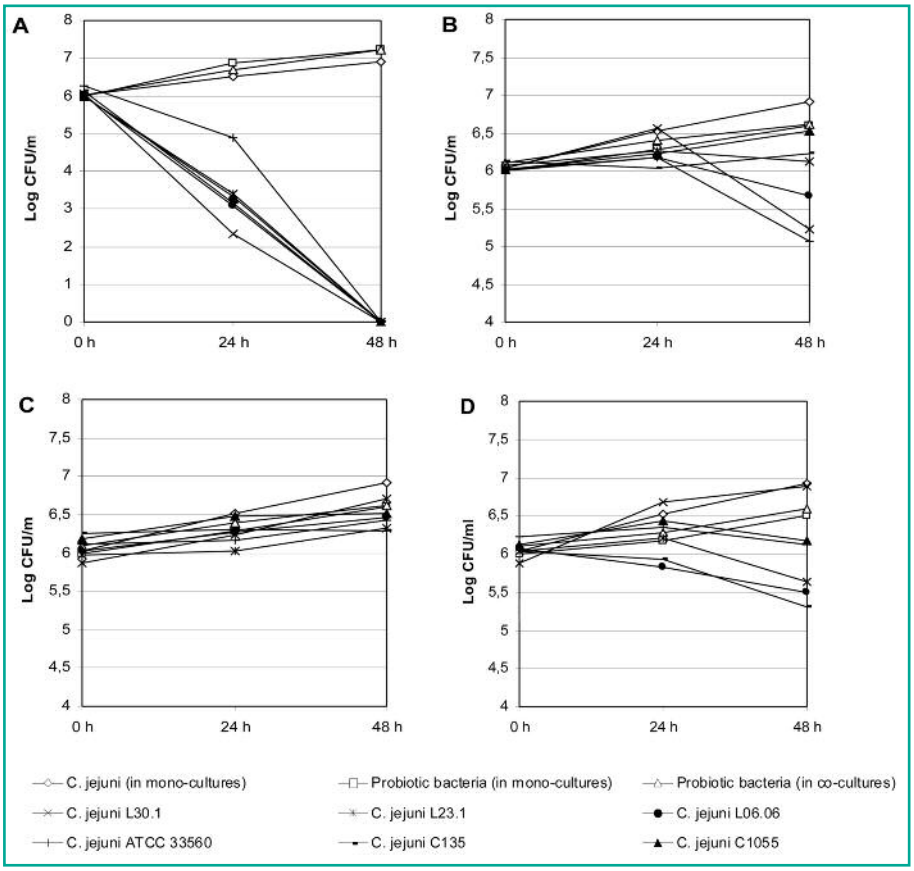

FIGURE 3: *The effect of L. acidophilus 4356 (A), L. fermentum 14931 (B), B. bifidum Bb12 (C) and B. longum B46(D) in combination with 1 % inulin on the CFUs of six C. jejuni strains in co-incubation in MH broth.*

Archiv für Lebensmittelhygiene 61, Heft 4 (2010), Seiten 125–164

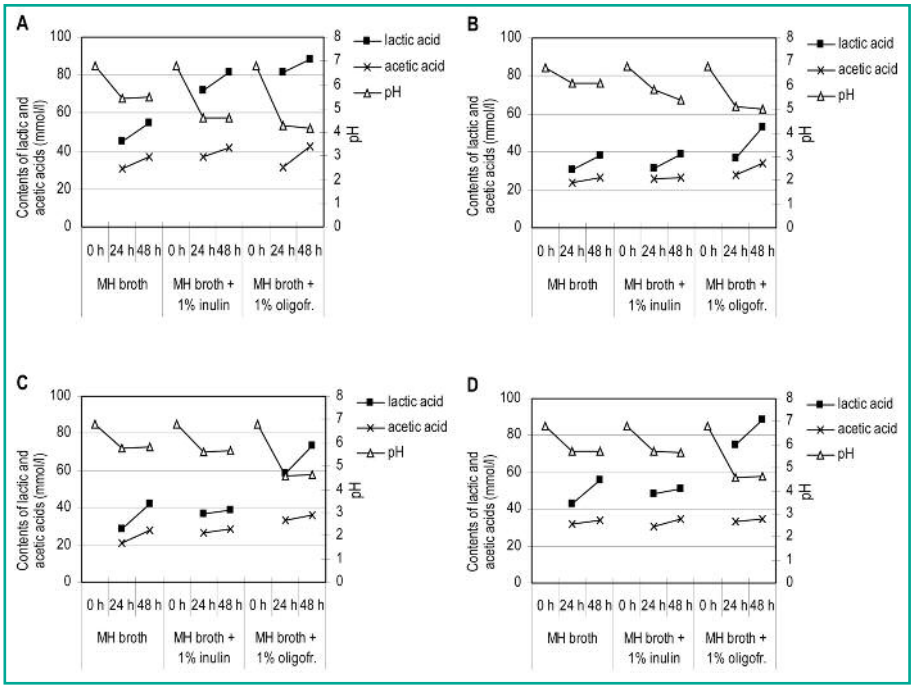

FIGURE 4: *Production of lactic and acetic acid by L. acidophilus 4356 (A), L. fermentum 14931 (B), B. bifidum Bb12 (C) and B. longum B46(D) without and with 1 % inulin and 1 % oligofructose and the dynamics of media pH in the MH broths.*

of 1 % inulin or 1 % oligofructose in MH broths had no statistically significant ($P > 0.05$) antimicrobial effect on the growth of any *C. jejuni* strains within 48 h (data not shown).

Dynamics of pH and contents of selected organic acids in different MH broths are shown in Figure 4. No significant differences ($P > 0.05$) in pH dynamics were noticed in the MH broths incubated with mono-cultures of probiotic bacteria or in combination together with campylobacters. In the presence of 1 % oligofructose in MH broths, pH declined in mono- as well as coculture with *L. acidophilus* ATCC 4356, *B. bifidum* Bb12 or *B. longum* B46 from 6.8 to 4.28, to 4.57 and to 4.52 within 48 h, respectively. In the same MH broths, the concentrations of lactic and acetic acid produced by probiotic bacteria ranged from 73.6 to 88.7 mmol/l and from 34.4 to 42.4 mmol/l in 48 h, respectively. *L. fermentum* ATCC 14931 combined with 1 % oligofructose produced lactic and acetic acids for 48 h at pH 5.03 52.1 and 33.0 mmol/l, respectively. Unlike other probiotic bacteria, the antimicrobial activity of *L. acidophilus* ATCC 4356 appeared also in combination with 1 % inulin, when the content of lactic and acetic acid in 48 h and at pH 4.69 was 81.5 mmol/l and 41.6 mmol/l, respectively. The concentrations of lactic and acetic acid in MH broths with 1 % inulin or without prebiotic compound at pH 5.50 to 6.10 were in the range from 37.9 to 55.8 mmol/l and 26.7 to 36.3 mmol/l in 48 h, respectively. The pH level in *C. jejuni* monoculture as a control sample decreased on average from 6.80 to 6.72 within 48 h.

Discussion

The antimicrobial activity of probiotic bacteria against foodborne pathogens, such as *C. jejuni,* is of great importance for public health. The antagonistic effect can be applied in food industry as well as in prevention of enteric infections. In this study, we examined antimicrobial activities of two different genera and species of LAB against *C. jejuni* strains *in vitro,* and we found that not all selected bacteria display similar intensity of antimicrobial effects under the same conditions. Of all the tested probiotic bacteria, only *L. acidophilus* ATCC 4356 in combination with 1 % inulin and 1 % oligofructose was able to totally inhibit the growth and survival of all the tested *C. jejuni* strains during co-incubation. It can be explained by the fact that *L. acidophilus* ATCC 4356 in the presence of prebiotics produced significantly more lactic and acetic acids in MH broths than any other probiotic bacteria in our study. Coconnier et al. (1997) also reported that antimicrobial activity was independent of lactic acid production. Additionally, Callaway et al. (2002) found that the benefit of prebiotics is associated with the fact that it creates a competitive advantage, which makes it possible to produce antimicrobial substances by probiotic bacteria that can directly inhibit pathogenic bacteria.

In contrast, *L. fermentum* ATCC 14931 in combinations with the same prebiotic ingredients did not sufficiently inhibit the growth of any of the *C. jejuni* strains within 48 h.

Furthermore, the availability of 1 % inulin did not significantly increase the antimicrobial activity of *B. bifidum* Bb12 or *B. longum* B46, whereas utilization of 1 % oligofructose by bifidobacteria had an antagonistic effect against target bacteria. Similarly, Fooks and Gibson (2002) reported that *C. jejuni* was totally inhibited by *B. bifidum* Bb12 combined with prebiotics such as oligofructose and xylo-oligosaccharide *in vitro.* Therefore, in our study the antimicrobial activity of the investigated lactobacilli and bifidobacteria against tested *C. jejuni* strains was dependent on the probiotic bacterial strain, their ability to use 1 % inulin or 1 % oligofructose in their metabolism and their ability to sufficiently produce organic acids. Our study demonstrated that the effect of prebiotic compound to the only selected probiotic bacteria is explicable by the higher total viable counts of their bacteria and the larger concentration of organic acids per milliliter in MH broths at the end of the experiments. Likewise, Huebner et al. (2007) found that the utilization of prebiotic by probiotics is dependent on the strain of probiotic bacteria. The efficiency of the use of prebiotic compounds by probiotic bacteria depends on the length of carbohydrate chains as described by Niness (1999). As demonstrated in our study, oligofructose which is composed of shorter-chain oligomers was preferential, and was more quickly metabolized by probiotic bacteria, compared with inulin.

Our study revealed that suppression of bacterial counts of all the tested *C. jejuni* strains can be explained by the low pH in the growth media. It is also one explanation, why our study results showed that no significant differences ($P > 0.05$) in the effect of probiotic bacteria were observed on the CFUs of *C. jejuni* strains that originated from broiler chicken meat in comparison to *C. jejuni* isolates that origi-

nated from human faeces. In contrast, Chaveerach et al. (2004) found that the antagonistic activity of potentially useful bacteria is most effective when isolated from the same environment from which the pathogens originate. Moreover, the impact of selected probiotic bacteria on the antibiotic resistant as well as susceptible *C. jejuni* isolates was also similar in this study.

The antibacterial activity of probiotic bacteria combined with suitable prebiotic against target bacteria increased significantly ($P < 0.001$) if the pH of the MH media was in the range from 4.28 to 4.69 in 48 h in our study. On the contrary, the findings of Drago et al. (1997) demonstrated that the activity of pathogens was suppressed by probiotics without the decrease of pH. We suppose that lowering of media pH via production of organic acids probably leads to the strengthening of the antibacterial effect of selected probiotics against the antibiotic resistant as well as susceptible campylobacters in the presence of 1 % prebiotic. Similarly, Hütt et al. (2006), Fooks and Gibson (2003) and Yusof et al. (2000) showed that the production of the lactate and acetate by the probiotics is associated with the decrease of pH in the growth media and plays an important role in the antagonistic activity of probiotic bacteria against pathogens. It can be explained by the fact that at low pH values the organic acids are in the undissociated form, which can harm all campylobacters (Chaveerach et al., 2002). Consequently, the death of target bacteria is caused by the changes in the internal pH and in gene expression, resulting in the termination of protein synthesis and other living processes (Olson, 1993). However, antimicrobial effects of probiotic bacteria are not exactly known as unidentified antimicrobial factors may occur.

Our co-culture experiments made possible to monitor the growth dynamics of both probiotic and target bacteria together in MH media. In accordance with Drago et al. (1997), the target bacteria did not affect the growth of probiotic bacteria in all investigated MH broth samples in our study. It indicates that tested probiotic bacteria could survive and propagate in the MH media, which is usually used only for *Campylobacter* cultivation in many studies (Chaveerach et al., 2004; Roasto et al., 2007).

Conclusion

The results of our *in vitro* study revealed that due to coapplication, selected probiotic bacteria and suitable prebiotic ingredients have the ability to inhibit survival and growth of susceptible, resistant or multiresistant *C. jejuni* strains. The antagonistic activity of probiotics against *C. jejuni* is most probably related with the reduction of pH via the production of organic acids in liquid media. Further experiments are needed to study the various antimicrobial mechanisms of probiotic bacteria against campylobacters.

Acknowledgements

This study was supported by: The financing project of Estonian University of Life Sciences “Minimizing of biological and chemical hazards in Estonian food chains”, P 5081 VLVL05. Graduate School in Biomedicine and Biotechnology of the Estonian University of Life Sciences.

References

Akalin AS, Fenderya S, Akbut N (2004): Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. Int J Food Sci Tech 39: 613–621.

Annuk H, Shchepetova J, Kullisaar T, Songisepp E, Zilmer M, Mikelsaar M (2003): Characterization of intestinal lactobacilli as putative probiotic camdidates. J Appl Microbiol 94: 403–412.

Bosscher D, Van Loo J, Franck A (2006): Inulin and oligofructose as prebiotics in the prevention of intestinal infections and diseases. Nutr Res Rev 19: 216–226.

Brashears MM, Reilly SS, Gilliland SE (1998): Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. J Food Prot 61: 166–170.

Callaway TR, Anderson RC, Edrington TS, Elder RO, Genovese KJ, Bischoff KM, Poole TL, Jung YS, Harvey RB, Nisbet DJ (2002): Preslaughter intervention strategies to reduce foodborne pathogens in food animals. J Anim Sci 81: 17–23.

Carson CF, Riley TV (2003): Non-antibiotic therapies for infectious diseases. Commun Dis Intell 27: 144–147.

Chaveerach P, Lipman LJA, Knapen F (2004): Antagonistic activities of several bacteria on in vitro growth of 10 strains of *Campylobacter jejuni/coli.* Int J Food Microbiol 90: 43–50.

Chaveerach P, Keuzenkamp DA, Urlings HA, Lipman LJ, van Knapen F (2002): In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. Poult Sci 81: 621–628.

Coconnier MH, Liévin V, Bernet-Camard MF, Hudault S, Servin AL (1997): Antibacterial effect of the adhering human *Lactobacillus acidophilus* strain LB. Antimicrob Agents Chemother 41: 1046–1052.

Collins MD, Gibson GR (1999): Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr 69: 1052–1057.

Drago L, Gismondo MR, Lombardi A, Haën C, Gozzini L (1997): Inhibition of *in vitro* growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. FEMS Microbiol Let 153: 455–463.

EFSA (2009): The Community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2007. Information on specific zoonoses. *Campylobacter.* The European Food Safety Authority (EFSA) Journal 2009, 95–116.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I (2001): Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* resistance mechanisms and trends in human isolates. Emerg Infect Dis 7: 24–34.

Fernández MF, Boris S, Barbés C (2003): Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol 94: 449–455.

Fooks LJ, Gibson GR (2003): Mixed culture fermentation studies on the effects of synbiotics on the human intestinal pathogens *Campylobacter jejuni* and *Escherichia coli.* Anaerobe 9: 231–242.

Fooks LJ, Gibson GR (2002): *In vitro* investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. FEMS Microbiol Ecol 39: 67–75.

Franck A (2002): Technological functionality of inulin and oligofructose. Br J Nutr 87: 287–291.

Gusils C, Perez Chaia A, Apella M, Amoroso MJ, Oliver G (1998): Antibacterial activity of *Lactobacillus animalis* isolated from chicken against *Salmonella gallinarum.* Microbiol Aliment Nutr 16: 265–273.

Huebner J, Wehling RL, Hutkins RW (2007): Functional activity of commercial prebiotics. Int J Dairy Technol 17: 770–775.

Hänninen ML, Perko-Mäkelä P, Pitkälä A, Rautelin H (2000): A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J Clinical Microbiol 38: 1998–2000.

Hütt P, Shchepetova J, Lõivukene K, Kullisaar T, Mikelsaar M (2006): Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol 100: 1324–1332.

Archiv für Lebensmittelhygiene 61, Heft 4 (2010), Seiten 125–164

Ito A, Sato Y, Kudo S, Sato S, Nakajima H, Toba T (2003): The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychrotrophic food-borne pathogens. Curr Microbiol 47: 231–236.

Klewicki R, Klewicka E (2004): Antagonistic activity of lactic acid bacteria as probiotics against selected bacteria of the *Enterobaceriacae* family in the presence of polyols and their galactosyl derivatives. Biotechnol Lett 26: 317–320.

Niness KR (1999): Inulin and oligofructose: what are they? J Nutr 129: 1402–1406.

Olson R (1993): Influence of pH on bacterial gene expression. Mol Microbiol 8: 5–14.

Ouwehand A, Vesterlund S (2004): Antimicrobial components from lactic acid bacteria. In: Salminen S, von Wright A and Ouwehand A (ed.), Lactic Acid Bacteria Microbiological and Functional Aspects. Marcel Dekker, New York, 375–389.

Roasto M, Juhkam K, Tamme T, Hörman A, Häkkinen L, Reinik M, Karus A, Hänninen ML (2007): High level of antimicrobial resistance in *Campylobacter jejuni* isolated from broiler chickens in Estonia in 2005 and 2006. J Food Protect 70: 1940–1944.

Sanders ME (2000): Considerations for use of probiotic bacteria to modulate human health. J Nutr 130: 384–390.

Skirrow MB, Blaser MJ (2000): Clinical aspects of *Campylobacter* infection. In: Nachamkin I and Blaser MJ (ed.), *Campylobacter.* American Society for Microbiology Press, Washington, DC, 69–88.

Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC, Molbak K (2006): Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg Infect Dis 12: 280–284.

Yusof RM, Haque F, Ismail M, Hassan Z (2000): Isolation of *Bifidobacterium infantis* and its antagonistic activity against ETEC 0157 and *Salmonella typhimurium* S-285 in weaning foods. Asia Pac J Clin Nutr 9: 130–135.

Zdolec N, Kozacinski L, Njari B, Filipovic I, Hadziosmanovic M, Kuzmanovic Z, Mitak M,Samac D (2009): The antimicrobial effect of lactobacilli on some foodborne bacteria. Arch. Lebensmittelhyg. 60: 115–119.

Address for correspondence:
Kadrin Meremäe, MSc
Department of Food Hygiene
Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences
Kreutzwaldi 58A
51014 Tartu, Estonia
Email: kadrin.meremae@emu.ee

Berichtigung

In dem Artikel **„Identification of tropical shrimp species by RFLP and SSCP analysis of mitochondrial genes“** von Karin Schiefenhövel und Hartmut Rehbein (Archiv für Lebensmittelhygiene, Heft 2/2010, ab Seite 50) sind leider einige Druckfehler veröffentlicht worden. Diese sollen hiermit korrigiert werden.

1 **Fehler in „Table 1A“, Zeile 3 (Seite 51). Richtig ist:**

TABLE 1A: *List of authentic samples*

Code	Origin	Product	Latin name	English name	Authentication
3	Wholesale trader	r	*(Fennero)penaeus indicus*	Indian white prawn	vi, BLAST: 100 % id to FJ002574.1

*r = raw, c = cooked, p = peeled, ** vi = visual inspection of a whole animal.

2 **Fehler in den Absätzen „PCR conditions“ und „Single strand conformation...“ (Seite 52). Richtig ist:**

PCR conditions

...

For all amplifications, DNA concentration in the PCR assay was adjusted to 1 ng DNA/µl; the primer concentration was 0.5 µM. PCR was performed with reagents from Solis BioDyne (Tartu, Estonia) using HotFirePol DNA polymerase I (final activity 2.5 units), BD Buffer (5 µl/assay), dNTP mix (200 µM final concentration), $MgCl_2$ (2.5 mM final concentration); the assay volume was 50 µl.

Final solutions:
Fixing solution: 40 ml fixing concentrate + 160 ml fixing diluter.
Washing solution: 100 ml washing concentrate + 500 ml distilled water.
Silvering solution: 40 ml silvering concentrate + 160 ml distilled water + 260 µl formaldehyde (37 %, w/v).
Developing solution: 40 ml developing concentrate + 160 ml distilled water + 260 µl formaldehyde (37 %, w/v) + 200 µl thiosulfate concentrate.

3 **Es wurde eine falsche E-Mail-Adresse unter „Address for correspondence“ angegeben (Seite 56). Richtig ist:**

Address for correspondence:
Dr. Hartmut Rehbein
Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish Products
Palmaille 9, 22767 Hamburg, Germany
Hartmut.rehbein@mri.bund.de