The Thai Journal of Veterinary Medicine

Volume 46
Issue 2 *June, 2016*

Article 12

6-1-2016

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Thomrongsuwannakij, Thotsapol; Chuanchuen, Rungtip; and Chansiripornchai, Niwat (2016) "Identification of Competitive Exclusion and Its Ability to Protect Against Campylobacter jejuni in Broilers," *The Thai Journal of Veterinary Medicine*: Vol. 46: Iss. 2, Article 12.
Available at: https://digital.car.chula.ac.th/tjvm/vol46/iss2/12

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Original Article

Identification of Competitive Exclusion and Its Ability to Protect Against *Campylobacter jejuni* in Broilers

Thotsapol Thomrongsuwannakij[1] Rungtip Chuanchuen[2] Niwat Chansiripornchai[1*]

Abstract

This study aimed to identify competitive exclusion (CE) isolated from native chickens and organic layers raised under non-antimicrobial usage farms. The protection of CE was tested against *Campylobacter jejuni* challenges in broilers. *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* were identified from 50 adult chicken feces of those farms. According to antimicrobial-resistance concerns of European Food Safety Authority (EFSA), the use of CE that is susceptible to antimicrobials is preferred. The numbers of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* equal to 195, 93 and 58 strains, respectively, were tested for their minimum inhibitory concentrations (MICs) of 10 antimicrobials. As a result, only 51 isolates passed these criteria and were further *in vitro* tested for acid and bile tolerances. *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 demonstrated their powerful activities and were, therefore, used as CE during oral gavage of 1-day-old broilers for 3 days consecutively. Then, at 14 days old the broilers were challenged with a Thai field strain, CU11 of *C. jejuni*. As a result, the treatment groups had no significant differences in *C. jejuni* re-isolations or feed conversion ratio at 41 days. However, body weight of the broilers in group 8, which were orally gavaged with a commercial CE, was significantly higher than that in groups 1 and 2, which were orally gavaged with *L. Acidophilus* 1/4 and *B. subtilis* 206/1, respectively, and group 10, which was the negative control. The results showed that these CE were not able to compete against *C. jejuni* challenges in the broilers, which might be the result of the pathogenesis of *C. jejuni*, primarily colonizing the mucosal layer and not invading the intestinal cells of chickens.

Keywords: broilers, *Campylobacter jejuni*, competitive exclusion

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Introduction

Campylobacter is the leading cause of bacterial gastroenteritis in humans worldwide. For example, this disease is the most frequently reported foodborne illness in the European Union (EU) with over 190,000 human cases annually. The loss of campylobacteriosis to public health systems and productivity in the EU may cost as much as EUR 2.4 billion a year as estimated by the European Food Safety Authority (EFSA) (EFSA, 2015). Broiler carcasses and derived food products play an important role as a source of human *Campylobacter* infection, since broiler ceca can be colonized and carry a high number of *Campylobacter* spp., mainly *Campylobacter jejuni*, until slaughter. In Thailand, the average prevalence of *C. jejuni* was 65% isolated from broiler caeca (Chansiripornchai and Sasipreeyajan, 2009). A high level of *C. jejuni* can increase the chance of meat product contamination. In addition, the major cause of campylobacteriosis in humans comes from the consumption of unsuitably prepared contaminated poultry products (Friedman et al., 2004); the control of *Campylobacter* contamination in poultry currently remains a serious challenge. Nowadays, strict hygienic standards on chicken farms are effective in reducing *Campylobacter* load in the environment, although biosecurity alone is unlikely to always protect flocks from *Campylobacter* colonization (Berndtson et al., 1996; Lin, 2009). Thus, new administration must be developed to decrease this in poultry at farm level.

The concern about the spread of antibiotic resistance has focused on determining the elimination of antibiotics as growth promoters in livestock (Schwarz et al., 2001). This is why farms and researchers have been looking for other strategies to help maintain animal gut health to reduce the prevalence of pathogens in the food chain. An alternative and interesting approach is the use of CE, which is native bacterial flora in animal intestines that can protect their hosts by limiting the colonization of some bacterial pathogens. These CE cultures can be categorized into 2 groups: defined and undefined cultures. The defined CE cultures are microbial isolates identified and characterized for their properties such as antimicrobial sensitivity and acid and bile tolerance, while the latter are incompletely characterized (Zhang et al., 2007). According to EFSA guidance, viable microorganisms used as CE for poultry should not have antimicrobial resistance phenotypes because they can increase the risk of transferrable drug resistant genes to other gut bacteria. The development of resistance among bacteria to antimicrobials remains a significant concern. Due to the low pH of the stomach and the presence of bile acid in the intestines, a good CE must be able to defeat these obstructs to firmly adhere to the intestinal epithelium cells (Chateau et al., 1993). Hence, acid and bile tolerance tests are required for CE selection. The limitation of undefined CE cultures in poultry production is accepted in only some countries. *Lactobacillus*, *Bacillus* and *Enterococcus faecium* are found in the gastrointestinal tract of chickens, and they become attractive for selection as defined CE products. Still, there are many criteria that must be explored before establishing a new strain to be used as a defined CE. These criteria must include the non-pathogenicity of the microorganism, antimicrobial sensitivity and acid and bile tolerances. The aims of this study were to identify CE from non-antimicrobial native chicken and layer farms, and to test its protection against *Campylobacter jejuni* challenges in broilers.

Materials and Methods

Sample collection from native chickens and layers: Sample collection was conducted at 4 native chicken farms and 1 commercial layer farm in the central area of Thailand. Thirty- to forty-week-old birds from the native chicken farms and 90-week-old birds from the commercial layer farm were selected based on their non-antimicrobial history. The native chicken farms had no record of *Campylobacter* spp. prevalence while the commercial layer farm had 35% of *Campylobacter jejuni* prevalence. Feces were collected from 10, 10, 10, 10 and 20 birds at each farm, respectively. The fecal samples were kept at 4°C and transported to the laboratory, where they were then processed for bacterial isolation within 24 h.

Isolation of Lactobacillus spp., Bacillus spp. and Enterococcus faecium: Isolation of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* was performed following ISO 15214, ISO 7932 and European Community Project SMT4 CT98-2235 standards, respectively (European Commission, 2003; ISO-7932, 1993; ISO-15214, 1998). Briefly, a single 1 g from each fecal sample was dissolved in 9 ml of 0.85% normal saline. Using 1 loopful, the samples were streaked onto selective agar including de Mans Rogosa and Sharpe (MRS) agar, Manitol Egg Yolk Polymyxin-B agar and SF-streptococcus agar for *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus* spp. isolation, respectively. The inoculated plates were incubated at 37°C for 24-48 h. Suspected colonies were primarily identified by Gram's stain and biochemical tests. All bacterial isolates were kept as 20% glycerol stock at -80°C.

Identification of genus and species: DNA template was extracted by the heating method (Kwon et al., 2004). In brief, single colonies of each strain on an agar plate were suspended in distilled water and heated at 100°C for 10 min. They were then centrifuged at 12,000 g for 5 min. Supernatants were collected for use as the DNA template of polymerase chain reactions (PCRs). Multiplex PCR assay was done to verify genus and species of *Lactobacillus* and *Enterococcus faecium* (Ke et al., 1999; Dubernet et al., 2002; Jackson et al., 2004; Kwon et al., 2004). Amplified ribosomal DNA restriction analysis (ARDRA) was demonstrated for *Bacillus* identification (Wu et al., 2006). All PCRs were performed using KAPA® master mix (KapaBiosystems, Wilmington, USA) as described in the manufacturer's instructions.

Antimicrobial susceptibility test: Antimicrobial susceptibilities to ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and tylosin were evaluated by determining the minimum inhibitory concentrations (MICs). According to Clinical and

Laboratory Standards Institute guidelines (CLSI) (CLSI, 2008), MICs were done in Muller Hinton agar (MHA) using the two-fold agar dilution technique. The choice of antimicrobials and breakpoints for clarifying *Lactobacilllus, Bacillus* and *Enterococcus faecium* as resistant were suggested by EFSA (EFSA, 2012). *Escherichia coli* ATCC 25922 was used as a control organism. All antimicrobials were bought from Sigma-Aldrich (St Louis, MO).

Acid and Bile tolerance tests: Acid and bile tolerance tests were performed according to the protocols of Hyronimus et al. (2000) with some modifications. For the acid tolerance test, the stock bacteria kept at -80°C were grown in MRS broth at 37°C for 24 h. They were then pipetted into another MRS broth with pH value adjusted to 2.5 using 5M HCl (Merck) and sampled for colony count at 0 and 3 h of incubation time onto MRS agar by the pour plate technique. Survival rates were calculated by using the formula below. For the bile tolerance test, the protocol was similar to the acid test, but the MRS broth (pH 2.5) was replaced by oxgall bile 0.3% (Difco) (Gilliland et al., 1984). A count of colony numbers for the bile tolerance test was conducted at 0 and 24 h of incubation time. Survival rates were calculated by using the formula below, where Log N is the log number of colony present at the end of the test and Log N_0 is the log number of colony present at the start of the test.

$$\text{Survival rates (\%)} = \frac{\log N}{\log No} \times 100$$

Campylobacter jejuni challenges: Two hundred and ten 1-day-old non-vaccinated female Cobb broilers from a commercial hatchery were divided into ten groups. As shown in Table 1, groups 1-7 were orally gavaged with 0.5 ml of the top-three qualified CE bacteria which passed MICs and acid and bile tolerance criteria as a single, double or triple strains. Group 8 was gavaged with a commercial product (AVIPROB™, Diasham Resources, Singapore). Group 9 and 10 served as positive control and negative control groups, respectively. At 11 days of age, feces of all broilers were collected to produce cultures to confirm *Campylobacter* spp.-free status before challenges. All *Campylobacter*-negative broilers except the negative control group were orally inoculated with Thai field strain number CU11 of *Campylobacter jejuni* with an approximate concentration of 10^6 CFU/ml, 1 ml/broiler at 14 days. Fifteen fecal samples of each group were collected for *Campylobacter* colony count at 17, 21, 28 and 35 days of age, respectively. At 41 days of age, all broilers were euthanized and ceca were collected for *Campylobacter* colony count. All broilers were weighed at 1, 14 and 41 days to calculate their feed conversion ratio (FCR) and body weight. The birds were provided with feed and water *ad lib* and raised under an ethical approval for animal experimentation approved by Chulalongkorn University Animal Care and Use Committee no. 13310021.

Table 1 CE application programs in broilers during 1-3 days of age

Group	Selected CE or products	Total Conc. (CFU/ml)	Challenge *C. jejuni* at 14 days
1	*Lactobacillus acidophilus* 1/4	2 x 10^8	+
2	*Bacillus subtilis* 206/1	2 x 10^8	+
3	*Enterococcus faecium* 122	2 x 10^8	+
4	*Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1	4 x 10^8	+
5	*Lactobacillus acidophilus* 1/4 + *Enterococcus faecium* 122	4 x 10^8	+
6	*Bacillus subtilis* 206/1 + *Enterococcus faecium* 122	4 x 10^8	+
7	*Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1 + *Enterococcus faecium* 122	6 x 10^8	+
8	Commercial product (AVIPROB™)	2 x 10^8	+
9	Positive control	0.85% NSS	+
10	Negative control	0.85% NSS	-

Campylobacter isolation, identification and enumeration: One-gram fecal samples were added to 9 ml of 0.85% normal saline. The suspension was decimally diluted, and 0.1 ml of each diluted suspension was spread onto modified charcoal cefoperazone deoxycholate (mCCD) agar (Oxoid, France) in duplicate for *Campylobacter* enumeration. The inoculated plates were incubated at 42°C for 48-72 h under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) using a gas pack jar system (Mitsubishi Chemicals, Tokyo, Japan). One typical *Campylobacter* colony was selected for further identification by gram's staining and biochemical tests using hydrolysis of hippurate and Indoxyl acetate (Sigma-Aldrich, USA) which were performed according to the manufacturer's instructions. Moreover, multiplex PCR was performed to verify genus and species and confirm the biochemical results (Wang, 2002).

Statistical analysis: Differences in *C. jejuni* numbers at 17, 21, 28, 35 and 41 days of age and in body weight at 41 days of age were calculated by One-way Analysis of Variance (ANOVA) and Duncan's new multiple range tests. Significance was tested at a probability level of 0.05.

Results

Antimicrobial resistance phenotypes: The numbers of all isolates, totally 346 strains, identified as *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* equaled 195, 93 and 58 strains, respectively. All strains were tested for antimicrobial susceptibility. The frequencies of antimicrobial resistance and their MIC ranges are shown in Table 2. Those strains which had lowered or equal cut-off MIC values proposed by EFSA totally equaled 51 strains, which included 27, 15 and 9 strains of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*, respectively. Most qualified strains had quite low MIC data compared to the breakpoints in each recommended antimicrobial agent.

Survival rate of acid and bile tolerance tests: A total of 27 *Lactobacillus*, 15 *Bacillus* and 9 *Enterococcus faecium* were tested for acid and bile tolerance. The survival rates of those strains are summarized in Table 3. For acid tolerance, 3 *Bacillus* strains had the highest survival rates, with a range of 100.77-101.57%. All strains had a wide range in survival rates for the bile tolerance test. *Enterococcus faecium* showed quite low ability to tolerate bile acid compared to *Lactobacillus* spp. and *Bacillus* spp. According to the results of MIC values as well as acid and bile tolerance, the best *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* were selected, and their species level was identified using the PCR method. The selected CE were *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122, which had 96.85, 101.47 and 99.39% survival rates of acid tolerance and 113.93, 130.97 and 90.10% survival rates of bile tolerance, respectively. These CE were used for the challenge experiment in broilers.

Table 2 MIC data of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*

Strain (n)	MIC range (µg/ml)									
	AMP ()[a]	CHP	CLI	ERY	GEN	KAN	STR	TET	TYL	VAN
Lactobacillus spp. (27)	0.125-1 (4)	0.5-2 (8)	<0.125-0.5 (2)	<0.125-0.5 (1)	0.5-4 (32)	1-8 (64)	1-8 (64)	0.5-2 (32)	n.r.[b]	0.25-1 (2)
Bacillus spp. (15)	n.r.	<1-4 (8)	0.5-2 (4)	<0.5-2 (4)	0.25-1 (4)	<0.5-4 (8)	<2-4 (8)	0.5-2 (8)	n.r.	0.5-2 (4)
E. faecium (9)	0.5-2 (2)	<1-4 (16)	0.5-2 (4)	<0.5-2 (4)	4-16 (32)	64-256 (1024)	32-64 (128)	0.5-2 (4)	0.5-1 (4)	0.5-4 (4)

n = number of isolates in each row; [a]microbiological cut-off values (µg/ml) is indicated in brackets; AMP, ampicillin; CHP, chloramphenicol; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; TET, tetracycline; TYL, tylosin; VAN, vancomycin; [b]n.r. = not required

Table 3 Survival rate of acid and bile tolerance in each bacterial strain

Genus	Strains	Survival rates (%)		Strains	Survival rates (%)	
		Acid tolerance	Bile tolerance		Acid tolerance	Bile tolerance
Lactobacillus spp.	L 22/2	98.74	95.95	L 58/3	93.19	90.28
	L 31/3	97.13	86.03	L 44/4	93.04	95.25
	L 1/4	96.85	113.93	L 44/1	91.41	101.61
	L 31/4	96.78	95.87	L 28/1	91.4	96.28
	L 48/1	95.96	89.87	L 27/2	91.16	98.74
	L 23/1	95.41	88.48	L 27/1	90.87	92.78
	L 1/3	94.6	94.45	L 49/4	90.46	86.22
	L 40/1	94.27	95.31	L 55/4	90.38	99.36
	L 10/2	93.98	102.08	L 38/1	90.02	92.82
	L 8/1	93.82	87.12	L 19/2	89.69	105.69
	L 5/3	93.58	93.91	L 8/2	88.11	88.54
	L 35/2	93.58	101.56	L 17/1	84.37	89.36
	L 50/1	93.39	88.25	L 19/1	82.27	78.55
	L 14/4	93.36	98.15			
Bacillus spp.	B 201/1	101.57	94.72	B 205/2	92.19	89.34
	B 206/1	101.47	130.97	B 227/2	92.15	102.57
	B 214/2	100.77	93.9	B 220/1	91.43	123.13
	B 230/1	97.3	118.16	B 206/2	90.04	91.3
	B 235/1	95.77	99.18	B 224/1	89.23	103.32
	B 239/1	95.67	102.75	B 230/2	86.16	96.47
	B 204/1	94.36	103.21	B 210/1	84.99	99.27
	B 217/2	93.54	100.87			
Enterococcus faecium	E 122	99.39	90.1	E 172	88.61	75.07
	E 135	98.45	75.06	E 144	88.34	61.09
	E 110	95.36	71.66	E 107	86.74	82.63
	E 130	92.25	82.58	E 118	77.03	78.07
	E 114	91.69	76.72			

C. jejuni challenged against CE application: At 11 days of age, all birds were tested negative against *Campylobacter* spp. contamination. At 14 days of age, the birds in all groups except for the negative control group were challenged with the Thai field strain number CU11 of *C. jejuni*. At 17, 21, 28 and 35 days of age, the fecal samples of 15 birds were collected for *C. jejuni* count; and at 41 days of age, the cecal content of all broilers was counted for *C. jejuni* colonies. No statistically significant difference in the *C. jejuni* numbers from both fecal and cecal samples was observed between the positive control and treatment groups (Table 4). FCR was recorded at 14 and 41 days and body weight was calculated at 41 days. At 41 days of age, the body weight of the broilers in group 8, which was orally gavaged with the commercial CE, was significantly higher than that of the broilers in groups 1 and 2, which were orally gavaged with *Lactobacillus acidophilus* 1/4 and *Bacillus subtilis* 206/1, respectively, and group 10, which was the negative control.

Table 4 Average number of *C. jejuni*, FCR and body weight

Group	Average number of *C. jejuni* (log CFU/g) (Mean±SD)					FCR at 14 days	FCR at 41 days	Body weight at 41 days (Mean±SD)
	17 days	21 days	28 days	35 days	41 days			
1	5.80 ± 0.86	6.17 ± 0.64	6.18 ± 0.88	6.24 ± 0.55	6.48 ± 0.99	1.14	1.82	1843.50 ± 348.96[a]
2	5.94 ± 0.89	6.23 ± 0.66	6.38 ± 0.51	6.53 ± 0.71	6.75 ± 1.00	1.19	1.84	1856.32 ± 323.86[a]
3	6.29 ± 0.81	6.39 ± 0.73	6.39 ± 0.59	6.09 ± 0.64	6.97 ± 1.03	1.16	1.77	1937.25 ± 240.43[ab]
4	6.43 ± 0.63	6.53 ± 0.41	6.31 ± 0.67	5.59 ± 0.45	6.01 ± 0.89	1.13	1.82	1894.75 ± 204.07[ab]
5	6.53 ± 0.59	6.50 ± 0.57	6.56 ± 0.53	6.10 ± 0.80	6.15 ± 1.19	1.17	1.74	1935.00 ± 217.64[ab]
6	6.22 ± 0.61	6.46 ± 0.94	6.26 ± 0.71	5.54 ± 0.37	6.02 ± 0.89	1.15	1.78	1896.90 ± 137.67[ab]
7	5.92 ± 0.47	6.55 ± 0.62	5.81 ± 0.47	5.82 ± 0.64	5.81 ± 0.65	1.14	1.68	1992.62 ± 106.79[ab]
8	6.78 ± 0.45	6.93 ± 0.76	6.74 ± 0.40	6.20 ± 1.02	6.52 ± 0.90	1.15	1.72	2061.94 ± 149.02[b]
9	6.46 ± 0.52	6.86 ± 0.40	6.74 ± 0.82	6.79 ± 0.49	6.74 ± 1.03	1.14	1.72	1991.05 ± 178.60[ab]
10	n.d.	n.d.	n.d.	n.d.	n.d.	1.18	1.79	1820.52 ± 307.63[a]

n.d = not detected (detection limit = 2 log CFU/g), [a,b]The different superscript in each column means statistically significant difference (p<0.05). Broilers in different groups received different CE application program during 1-3 days of age. Gr. 1: *Lactobacillus acidophilus* 1/4, 2 x 10^8 CFU/ml; Gr. 2: *Bacillus subtilis* 206/1, 2 x 10^8 CFU/ml; Gr. 3: *Enterococcus faecium* 122, 2 x 10^8 CFU/ml; Gr. 4: *Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1, 4 x 10^8 CFU/ml; Gr. 5: *Lactobacillus acidophilus* 1/4 + *Enterococcus faecium* 122, 4 x 10^8 CFU/ml; Gr. 6: *Bacillus subtilis* 206/1 + *Enterococcus faecium* 122, 4 x 10^8 CFU/ml; Gr. 7: *Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1 + *Enterococcus faecium* 122, 6 x 10^8 CFU/ml; Gr. 8: Commercial product (AVIPROB™) 2 x 10^8 CFU/ml; Gr. 9: Positive control; and Gr. 10: Negative control.

Discussion

In this study, *in vitro* and *in vivo* experiments of CE were performed, as CE has been known to prevent pathogenic bacteria in poultry for decades. Nurmi and Rantala (1973) showed how newly hatched chickens treated with intestinal contents from adult chickens had increased resistance to infection by *Salmonella* spp. CE bacteria are composed of 2 groups, defined and undefined CE. The defined CE cultures are more acceptable because the microbial isolates are identified and characterized for their properties such as antimicrobial susceptibility and acid and bile tolerance (Zhang et al., 2007). Normally, CE bacteria should be isolated and used in the same hosts because of their host specificity (Fuller, 1975). In this study, the samples were collected from feces, different from previous studies that used samples isolated from intestinal organs (Garriga et al., 1998; Ehrmann et al., 2002). Although *Lactobacillus acidophilus*, *Bacillus subtilis* and *Enterococcus faecium* are considered Generally Recognize as Safe (GRAS), their antimicrobial susceptibility needs to be clarified. CE bacteria may serve as hosts for antibiotic resistance genes that are probably transferred to commensal and pathogenic bacteria in the gut, leading to a concern of antimicrobial resistance in humans. All selected strains were sensitive to several antimicrobials, including some of the many drugs used in poultry farms such as amoxicillin, tylosin and erythromycin, none of which will lead to the spread of resistant properties against these antimicrobials to bacterial hosts (Schwarz et al., 2001).

CE has to survive passage through the gastrointestinal tracts of broilers. From *in vitro* experiments, *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 demonstrated great survival rates after 3 and 24 h incubation time for acid and bile tolerance tests, respectively. These results indicated that the 3 CE bacteria might be able to survive the transit and reach the broiler ceca environment since the total movement through the broiler gastrointestinal tract takes around 4 to 9 h, depending on the feed and age of the broilers (Sundu, 2009). *Bacillus* spp. was quite more tolerant of acid and bile compared *Lactobacillus* and *Enterococcus* spp. because *Bacillus* spp. can produce endospores structured by a complex protein coat under stressful environmental conditions (McPherson et al., 2005). Timmerman et al. (2004) revealed that a mixture of different strains, rather than only one strain, would be successful for use as CE bacteria. However, the results of this study showed no significant difference in *C. jejuni* numbers between the treatment and control groups, which is in agreement with the study of Robyn et al. (2013). Although some CE bacteria preparations can decrease the level of colonization in chickens (Mead et al., 1996; Zhang et al., 2007), other studies did not observe the protective effect of CE (Shanker et al., 1988; Stern et al., 2001). The reason why the results were inconsistent remains unclear, but it might reflect the variable nature of CE agent and the susceptibility

of *Campylobacter* strain. This study showed that these CE were not able to compete against *C. jejuni* challenges in broilers, which might be the result of the pathogenesis of *C. jejuni*, primarily colonizing the mucosal layer in the gastrointestinal tract of chickens (Young et al., 2007). This is different from *C. jejuni* pathogenesis in humans, which can move into the intestinal epithelial layer, leading to inflammation and diarrhea.

In conclusion, the CE isolated from fecal samples exhibited non-resistant antibiotic profiles and great survival rates for acid and bile tolerance. Although they could not significantly reduce *C. jejuni* when compared to the positive control broilers, these CE bacteria should be further evaluated as protection against other foodborne bacteria found in broilers such as *Salmonella* and *E. coli* in further studies.

Acknowledgements

This study was supported by the Graduate School and Faculty of Veterinary Science, Chulalongkorn University, National Research Council of Thailand with grant no. GAB-APS-29-56-31-02 and the Thailand Research Fund through the Royal Golden Jubilee Ph.D. program (Grant No. PHD/0215/25/52). The authors would like to thank Prof. Dr. Jiroj Sasipreeyajan and Dr. Wechsiri Wannaprasat for their crucial suggestions and laboratory trainings, and all farm owners for their cooperations.

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บทคัดย่อ

การพิสูจน์ยืนยันคอมเพทิทิฟเอกซ์คลูชันและความสามารถในการป้องกันเชื้อ*แคมไพโลแบคเตอร์ เจจูไน*ในไก่เนื้อ

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การศึกษานี้มีวัตถุประสงค์เพื่อค้นหา competitive exclusion (CE) ที่แยกได้จากไก่พื้นเมือง และไก่ไข่ที่เลี้ยงในระบบที่ไม่ใช้ ยาต้านจุลชีพ และทดสอบการป้องกันการติดเชื้อแคมไพโลแบคเตอร์ เจจูไนในไก่เนื้อ โดยทำการแยกเชื้อ*แลคโตบาซิลลัส บาซิลลัส* และ *เอนเทอโรคอคคัส ฟีเซียม* จากมูลไก่ที่โตเต็มวัยจำนวน 50 ตัวอย่าง เนื่องด้วยองค์การความปลอดภัยด้านอาหารของสหภาพยุโรปให้ความสำคัญกับเรื่องการดื้อยาต้านจุลชีพ ดังนั้น CE ที่จะนำมาใช้ในสัตว์ควรมีความไวรับต่อยาต้านจุลชีพ โดยเชื้อที่แยกได้ประกอบด้วย*แลคโตบาซิลลัส บาซิลลัส* และ*เอนเทอโรคอคคัส ฟีเซียม* จำนวน 195 93 และ 58 สเตรน ตามลำดับ นำมาทดสอบหาค่าความเข้มข้นที่ต่ำที่สุดที่สามารถยับยั้งเชื้อแบคทีเรียได้จากยาปฏิชีวนะ 10 ชนิด พบว่ามีเชื้อที่แยกได้จำนวน 51 ไอโซเลตที่ผ่านเกณฑ์ดังกล่าว จากนั้นนำเชื้อเหล่านั้นมาทดสอบความทนกรดและน้ำดีในหลอดทดลอง พบว่าเชื้อ*แลคโตบาซิลลัส แอซิโดฟิลัส* 1/4, *บาซิลลัส ซับทิลิส* 206/1 และ*เอนเทอโรคอคคัส ฟีเซียม* 122 ให้ผลการทดสอบดังกล่าวในเกณฑ์ดีเยี่ยม จึงคัดเลือกเชื้อทั้ง 3 ชนิดมาใช้เป็น CE โดยทำการทดลองป้อน CE ทางปากให้ไก่เนื้ออายุ 1 วัน เป็นเวลา 3 วันต่อเนื่อง จากนั้นทำการป้อนเชื้อ*แคมไพโลแบคเตอร์ เจจูไน*ที่แยกได้ในประเทศไทย สเตรน CU11 ให้ไก่เนื้ออายุ 14 วัน การทดลองพบว่า กลุ่มที่ได้รับ CE ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในแง่ของปริมาณเชื้อแคมไพโลแบคเตอร์ที่แยกได้และอัตราการแลกเนื้อที่อายุ 41 วัน อย่างไรก็ตามพบว่าไก่เนื้อในกลุ่มที่ 8 ที่ได้รับการป้อน CE ในรูปแบบเชิงการค้า มีค่าน้ำหนักตัวสูงกว่าอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับไก่เนื้อในกลุ่มที่ 1 และ 2 ที่ได้รับการป้อนเชื้อ*แลคโตบาซิลลัส แอซิโดฟิลัส* 1/4 และ *บาซิลลัส ซับทิลิส* 206/1 ตามลำดับ และกลุ่มที่ 10 ซึ่งเป็นกลุ่มควบคุมลบ จากผลการทดลองสรุปได้ว่า CE ที่ใช้ในการทดลองนี้ไม่สามารถป้องกันการป้อนเชื้อพิษทับของ*เชื้อ แคมไพโลแบคเตอร์ เจจูไน*ในไก่เนื้อ ซึ่งอาจมีสาเหตุมาจากกระบวนการทางพยาธิกำเนิดของเชื้อ*แคมไพโลแบคเตอร์ เจจูไน*ซึ่งมักอาศัยที่ชั้นผิวของเยื่อบุเซลล์ลำไส้ในไก่ และไม่บุกรุกเข้าสู่เซลล์แต่อย่างใด

คำสำคัญ: ไก่เนื้อ *แคมไพโลแบคเตอร์ เจจูไน* คอมเพททิทีฟเอกซ์คลูชัน

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