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Note : Lack of influence of adherent *Lactobacillus* isolates on the attachment of *Escherichia coli* to the intestinal epithelial cells of chicken *in vitro*

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L.Z. JIN, Y.W. HO, N. ABDULLAH, M.A. ALI AND S. JALALUDIN. 1998. Two *Lactobacillus* isolates, *Lact. acidophilus* I 26 and *Lact. fermentum* I 25, were selected, based on their poor aggregation with *Escherichia coli* and strong ability to adhere to ileal epithelial cells (IEC), to study *in vitro* interactions with *E. coli* O1 : K1, O2 : K1 and O78 : K80 in an IEC radioactive-assay under the conditions of exclusion (lactobacilli and IEC, followed by the addition of *E. coli*), competition (lactobacilli, IEC and *E. coli* together) and displacement (*E. coli* and IEC, followed by the addition of lactobacilli). The results indicated that *Lact. acidophilus* I 26 and *Lact. fermentum* I 25 could not significantly reduce the attachment of *E. coli* O1 : K1, O2 : K1 and O78 : K80 to IEC under the three conditions tested *in vitro*, except that the attachment of *E. coli* O1 : K1 was slightly reduced by *Lact. fermentum* I 25 in the test for competition.

INTRODUCTION

The effects of probiotic products on animal performance have been studied, but their mode of action remains unclear. It has been suggested that probiotics serve some beneficial functions by their coaggregation with, and antagonistic action or competitive exclusion against, pathogenic bacteria including *Escherichia coli* in the animal intestine (Schleifer 1985 ; Fuller 1989).

Escherichia coli infection is one of the 'production diseases' which has become increasingly important in an intensive poultry industry. In a survey in the USA, over 40% of the loss from disease is linked directly or indirectly to *E. coli* infections (Barnes 1987). The close association between the pathogen and the host is a prerequisite for the establishment of many enterobacterial infections, including those caused by *E. coli* (Krogfelt 1991 ; Smith 1992). Any intervention at this stage in the disease process, i.e. to prevent pathogen–host association, is usually sufficient to suppress the development of clinical symptoms. It has been established that native gastrointestinal microflora in the chicken and turkey act in some ways to limit colonization by pathogenic strains of *E. coli* (Soerjadi *et al.* 1981 ; Weinack *et al.* 1981, 1982). As the use of undefined bacterial treatment preparation from a single source could result in widespread transmission of any undetected pathogens which may be present, efforts have been focused on the identification of key protective elements in undefined cultures with a view to developing a product of known bacterial composition (Mead and Impey 1987). Lactobacilli have been used as components of defined cultures for treatment bacteria, but results on the use of *Lactobacillus* cultures to prevent *E. coli* infection are inconclusive and further investigation is needed. The present investigation was carried out to study the effect of adherent *Lactobacillus* isolates on the attachment of *E. coli* to the ileal epithelial cells of chicken *in vitro*.

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MATERIALS AND METHODS

Lactobacillus cultures

Twelve *Lactobacillus* strains, isolated from the washed sections of gut tissue, which showed moderate or strong ability to adhere to the ileal epithelial cells (IEC), were used for the coaggregation test, and those strains that did not have the ability to coaggregate with *E. coli* were selected for a com-

petition assay. The 12 *Lactobacillus* strains were the same as those identified and described by Jin *et al.* (1996a,b). They were: *Lact. crispatus* I 12, *Lact. acidophilus* I 16, *Lact. acidophilus* I 26, *Lact. brevis* I 23, *Lact. fermentum* I 24, *Lact. fermentum* I 25, *Lact. brevis* I 211, *Lact. brevis* I 218, *Lact. brevis* C 1, *Lact. brevis* C 10, *Lact. fermentum* C16 and *Lact. brevis* C 17. All isolates were cultured in MRS broth incubated at 37 °C for 18 h or overnight. They were subcultured at least three times prior to the assay.

Escherichia coli cultures and radioactive labelling

There are at least 141 distinct O antigens among *E. coli* strains; O1, O2 and O78 are the most common types associated with disease in poultry. About 60% of 'coli septicaemia' strains are in fact represented by only three serotypes: O2 : K1, O1 : K1 and O78 : K80. Therefore, these three serotypes were selected for the present study. The serotypes, isolated from infected chickens, were obtained from the Veterinary Research Institute of Malaysia. The organisms were maintained by routine culture on Nutrient agar (Difco) slant. The slant cultures were stored at 4 °C between transfers. At least two additional subcultures (24 h, 37 °C) were made in fresh medium before use in the experiment.

Competition assay

The methods used for the isolation of IEC, the competition assays and the spectrophotometric coaggregation assay were the same as those described by Jin *et al.* (1996a), except that *Salmonella* was replaced by *E. coli* as the test pathogen.

The competition assay was carried out under three conditions: exclusion, competition and displacement, and the IEC suspension (approximately 5×10^5 ml^{-1}) was isolated from the ileum of chicken. For the exclusion test, *Lactobacillus* and IEC were incubated together for 60 min; [^{14}C]*E. coli* was then added and the mixture incubated for another 60 min. For the competition test, *Lactobacillus*, [^{14}C]*E. coli* and IEC were added simultaneously and incubated for 120 min; for the displacement test, [^{14}C]*E. coli* was mixed with IEC for 60 min, after which *Lactobacillus* was added and the mixture incubated for another 60 min.

In each test, after the incubation period (120 min), the suspensions were gently vortex-mixed and filtered through a 5 μm cellulose nitrate filter. The filter was washed with PBS and the radioactive counts, which represented the disintegration per min (dpm) retained on the filters, were determined with a liquid scintillation system (Beckman LS 6000SE, CA, USA). These counts represented the dpm retained on the filters of test samples (Test). Counts were also made on the following controls: (i) 0·5 ml of [^{14}C]*E. coli* suspension + 1·0 ml of PBS (c1); (ii) 0·5 ml of [^{14}C]*E. coli* suspension added directly to NCS (c2); and (iii) 0·5 ml of [^{14}C]*E. coli* and 0·5 ml of cells and 0·5 ml of PBS (c3). The percentage of the total added radioactivity associated with filter-entrapped IEC was calculated based on the equation of Spencer and Chesson (1994):

$$\frac{\text{Test-c1}}{\text{c2}} \times 100.$$

Statistics

The effects of the three conditions (i.e. exclusion, competition and displacement) were compared for each strain of *E. coli* using one way analysis of variance. The computation was done by using the SAS programme (SAS 1985).

RESULTS AND DISCUSSION

The results showed that there was no strong coaggregation between the 12 strains of *Lactobacillus* and the three serotypes of *E. coli*. All the eight *Lactobacillus* isolates from the ileum, which included *Lact. crispatus*, *Lact. acidophilus*, *Lact. fermentum* and *Lact. brevis*, coaggregated poorly with the three serotypes of *E. coli*; the coaggregation was less than 1–4·6%, except for *Lact. brevis* I 211 which showed a higher percentage of coaggregation with *E. coli* O78 : K80. On the other hand, lactobacilli isolated from the caecum demonstrated a better ability to coaggregate with *E. coli*, especially *E. coli* O78 : K80; the coaggregation ranged from 5·86% to 10·37%. Spencer and Chesson (1994) reported that about 40% of their *Lactobacillus* isolates demonstrated marked coaggregation with *E. coli* O149 : K88 positive. The authors also pointed out that the coaggregation was attributed to the presence of the K88 fimbrial antigen of the *E. coli* used.

Bacterial coaggregation was first recognized as highly specific between streptococci and actinomycetes in the oral cavity (Gibbons and Nygaard 1970). Unlike coaggregation in the oral cavity which leads to the formation of dental plaque, inhibitor-producing *Lactobacillus* spp., which coaggregate with pathogens of the urinary tract, probably constitute an important host defence mechanism against infection (Reid *et al.* 1988). Spencer and Chesson (1994) suggested that a similar protective mechanism involving coaggregation between strains of *Lactobacillus* and enteropathogens could also operate in the digestive tract. They further pointed out that adherent *Lactobacillus* strains might mask pathogens and toxin receptors without necessarily binding to the same epitope, and thus limit the ability of pathogens to colonize and infect. However, in this study, there is no evidence to suggest that the *Lactobacillus* spp. could provide protection by coaggregating with *E. coli*.

As *Lact. acidophilus* I 26 and *Lact. fermentum* I 25 were found to have poor coaggregation ability ($<$1–4·6% and $<$1–1·07%, respectively) and also a strong ability to adhere to

Table 1 The effects of *Lactobacillus acidophilus* I 26 and *Lact. fermentum* I 25 on the attachment of [^{14}C]*Escherichia coli* to ileal epithelial cells under the conditions of exclusion, competition and displacement*†

E. coli serotype	Total dpm of [^{14}C]*E. coli*	[^{14}C]*E. coli* attached to cell (%) (c3)	Competition (%)	Exclusion (%)	Displacement (%)
		Lactobacillus fermentum I25			
O1 : K1	19280·5	52·62	45·04 ± 2·31‡	49·63 ± 2·25	49·17 ± 2·51
O2 : K1	19596·2	68·59	64·01 ± 1·21	64·50 ± 1·68	64·56 ± 1·96
O78 : K80	23257·0	44·89	42·76 ± 1·13	41·90 ± 1·87	43·27 ± 1·88
		Lactobacillus acidophilus I 26			
O1 : K1	19280·5	52·62	51·83 ± 2·81	49·98 ± 2·17	53·08 ± 1·98
O2 : K1	19596·2	68·59	65·23 ± 1·20	67·39 ± 1·34	67·28 ± 1·51
O78 : K80	23257·0	44·89	48·13 ± 1·67	43·62 ± 1·43	46·97 ± 1·73

* Values are expressed as the percentage of the added radioactivity retained on filters.
† All values are mean of five observations with a duplicate each. c3 represents the dpm retained on the filters of 0·5 ml of [^{14}C]*E. coli* and 0·5 ml of cells and 0·5 ml PBS.
‡ $P < 0{\cdot}10$.

IEC (Jin *et al*. 1996b), they were selected for subsequent competitive assays. Table 1 shows the effects of *Lact. acidophilus* I 26 and *Lact. fermentum* I 25 on the attachment of *E. coli* to IEC under the conditions of exclusion, competition and displacement. There was no significant reduction in the attachment of *E. coli* O1 : K1, O2 : K1 and O78 : K80 to IEC with either *Lact. acidophilus* I 26 or *Lact. fermentum* I 25 under the three conditions tested. The attachment of the three *E. coli* serotypes was, however, slightly reduced by *Lact. fermentum* I 25 in the tests for competition, exclusion and displacement, particularly the attachment of *E. coli* O1 : K1 in the test for competition ($P < 0{\cdot}10$). All the three serotypes of *E. coli* showed a good ability to adhere to IEC. Their attachment to IEC varied from 44·89 to 68·59% (c3, Table 1). This is similar to the finding of Spencer and Chesson (1994) in which strongly adherent *Lactobacillus* strains did not affect the attachment of enterotoxigenic *E. coli* to porcine jejunal enterocytes *in vitro*. Recently, Bomba *et al*. (1996) also reported that adherent *Lactobacillus* spp. could not prevent the adhesion of enteropathogenic *E. coli* O8 : K88(+) Ent(+) to the mucosa of the jejunum and ileum of gnotobiotic pigs. In contrast, Chan *et al*. (1984) found that the normal urethral, vaginal and cervical microflora of healthy women could competitively block the attachment of uropathogenic bacteria to the surfaces of uroepithelial cells of women with or without a history of urinary tract infections. The competitive exclusion of uropathogens by cell wall fragments from a single strain of *Lactobacillus* has also been demonstrated (Chan *et al*. 1985), and a heat-destroyed *Lactobacillus* has been shown to exclude enterotoxigenic *E. coli* from Caco-2 cells of human (Chauviere *et al*. 1992; Coconnier *et al*. 1992). It has been suggested that adherent *Lactobacillus* strains could mask the receptors on the intestinal cells and effectively block the attachment of pathogens by conditions of competitive exclusion (Spencer and Chesson 1994). Unfortunately, the results from the present study showed that the two *Lactobacillus* isolates (i.e. *Lact. acidophilus* I 26 and *Lact. fermentum* I 25) did not have such an ability to exclude the attachment of *E. coli*, although they have been found to reduce the attachment of *Salmonella pullorum* and *Salm. typhimurium* to IEC under conditions of exclusion and competition (Jin *et al*. 1996a). It is probable that the *Lactobacillus* isolates have no effect on *E. coli* binding to IEC *in vitro* except that the attachment of *E. coli* O1 : K1 is slightly reduced by *Lact. fermentum* I 25 in the test for competition.

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