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Inhibitory effects of *Lactobacillus casei* upon the adhesion of enterotoxigenic *Escherichia coli* K99 to the intestinal mucosa in gnotobiotic lambs

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Abstract

Observations were carried out of the interactions between Lactobacillus casei 294/89 and enterotoxigenic *Escherichia coli* CCM 612 (O101:K99) *in vivo*. In gnotobiotic lambs, inoculation with enterotoxigenic *E. coli* (ETEC) resulted in diarrhea with a typical clinical picture and patho-anatomical findings. *E. coli* adhered to the mucosa of the digestive tract at counts amounting to 10^5 per cm^2. In these lambs, disturbances of intestinal biochemical processes became evident; proteolytic enzyme activity was significantly reduced. Preventive administration of *Lactobacillus casei* inhibited the negative effects of ETEC in gnotobiotic lambs, minimized the clinical signs to those of a very moderate diarrhea in the first 12 h after inoculation and significantly reduced the patho-anatomical findings. Enterotoxigenic *E. coli* counts decreased by 99.1 and 76% on days 2 and 4 after inoculation respectively, and amounted to 10^3 per cm^2. The inhibitory effects of *L. casei* against *E. coli* were most obvious in the jejunum and ileum. The numbers of adhering *E. coli* increased from the duodenum with the length of the gut. ETEC counts in the digestive tract of lambs that had been preventively treated with *L. casei* amounted to 10^7 ml^{-1}. It can be assumed that, in addition to competitive exclusion, the inhibitory effect of *L. casei* upon ETEC adherence was also mediated by a *Lactobacillus*-produced substance that inhibited *E. coli* adhesion to the gut mucosa.

Keywords: Lactobacillus casei. ; Escherichia coli. ; Adhesion ; Inhibition; Gnotobiotic lamb

1. Introduction

When rearing young animals, diarrhoic diseases present a serious health and economic problem. These diseases may be caused by different infectious agents acting either separately or in association with other microorganisms and essential factors. *Escherichia coli*, *salmonellae*, *rota-* and *coronaviruses* as well as *cryptosporidia* play an important part in the etiology of the diarrhoic syndrome. In the young, enterotoxigenic *E. coli* appear to be the most frequent diarrhea-causing agents (Tzipori, 1981); they can be

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found in stables with a reduced level of husbandry where morbidity and mortality rates are high. *Lactobacilli* are a component of the natural microflora of the gut; they colonize the latter as early as within the first hours of life. They have rather beneficial effects upon the macroorganism and inhibit several pathogens. Raccach et al. (1989) and Sudirman et al. (1993) reported *Lactobacilli* to inhibit *Listeriae* while Watkins and Miller (1982) and Chateau et al. (1993) observed these microorganisms to inhibit *Staphylococci*. According to Watkins et al. (1982), Fourniat et al. (1992) and Lidbeck et al. (1987) *Lactobacilli* also inhibit pathogenic *E. coli*. Employing *Lactobacilli* in the form of probiotics that would be based on their inhibitory effects against pathogens seems to be a very efficacious method of preventing and treating diseases caused by pathogenic microorganisms, mainly the diarrhoeic syndrome in the young of farm animals (Gilliland et al., 1980). The administration of *Lactobacilli* containing probiotics is a biotechnological method having not only beneficial effects upon the health state of the young but also a positive impact on the environment. *Lactobacillus acidophilus, L. casei, L. lactis, L. reuteri, L. plantarum, L. fermentum, L. brevis and L. delbruecki* (Fuller, 1989; Jonsson and Conway, 1992) are those strains that are most widely incorporated in probiotic preparations.

Some authors claim the antibacterial effects of probiotics to result from the production of lactic acid and a decrease in pH, the production of hydrogen peroxide and the antibacterial properties of the latter, the production of natural antibiotic substances – bacteriocines, the antienterotoxic activity (mainly against the *E. coli* enterotoxin) and the inhibition of pathogen adherence to the wall of the intestinal tract (Vandenbergh, 1993; Chauviere et al., 1992). The pathogenicity of *E. coli* is conditional on two factors: the ability to produce enterotoxin (Smith and Gyles, 1970) and the presence of colonization factors enabling the carrier to colonize the mucosa of the small intestine (Bertschinger et al., 1972; Jones and Rutter, 1972). The ability of enterotoxigenic *E. coli* to colonize the gut presents the primary and decisive pathogenic factor since it is inevitable for the second virulence factor – the enterotoxin – to exert its effects.

It was the aim of our work to observe the effects of *L. casei* upon the adherence of enterotoxigenic *E. coli* in the digestive tract of gnotobiotic lambs and the effects of mutual interaction upon intestinal biochemistry.

2. Material and methods

2.1. Animals and nutrition

In the experiment six germ-free Improved Wallachian lambs were included. They were obtained by hysterectomy, divided into two groups (E and L–E, respectively) counting three animals each and reared in two isolators according to Bomba et al. (1993). Four times a day the animals had *ad libitum* access to a commercial milk mixture.

2.2. Inoculation of lambs

The group E lambs were inoculated at 1 day of age with enterotoxigenic *E. coli* CCM 612 (O 101: K99). The group L–E lambs were inoculated with *L. casei* 294/89 at the age of 1, 2 and 3 days. On day 4 this group was inoculated with *E. coli* similar to Group E. Each inoculum contained 1×10^8 germs in 1 ml. The inocula (2 ml) were given once a day. The 294/89 strain of *L. casei* was isolated from the rectal swab of a calf aged 2 days, chosen of a total of 324 strains of *Lactobacilli* observed and tested by routine biochemical methods.

2.3. Biological material and chemical analyses

In each of the groups one lamb was killed 2 and 4 days after inoculation, respectively. In the L–E group one lamb was killed also 7 days after inoculation, but to do the same in Group E was impossible since one lamb had died as early as 12 h after inoculation. Immediately after slaughter samples were obtained of the duodenum, jejunum, ileum and colon (10 cm^2) and their contents. Tissue samples designed for estimation of counts of microorganisms adhering to the intestinal mucosa were 3 × washed with 0.15 M PBS (pH 7.2) at moderate stirring, then the mucosa was scraped with a covering slide. The material yielded was placed into 10 ml 0.15 M PBS (pH 7.2) containing 1% Tween and decadic dilutions were

prepared. Selective Rogosa agar and MacConkey agar were used to state *Lactobacillus* and *E. coli* counts, respectively.

Lactic, acetic and propionic acids were determined using the method of capillary isotachophoresis on a capillary isotachophoresis analyzer (Radioecological Institute, Košice, Slovakia). As conductive and final electrolyte 0.01 mmol l^{-1} HCl (pH 4.25) and mmol l^{-1} capronic acid (pH 4.5) were used, respectively. For alpha-amylase determination the Spofa-test alfa-amylaza (Slovakofarma Hlohovec, Slovakia) was employed; trypsin and chymotrypsin were assayed using the test by Boehringer (Mannheim, Germany).

Statistical analysis. The results are arithmetic means. For statistical evaluation Student's t-test was used.

3. Results

3.1. Clinical observations

In the group of animals inoculated with enterotoxigenic *E. coli* CCM 612 (Group E) profuse diarrhoea occurred in two lambs as early as during the first 12 h after inoculation. Their faeces were aqueous and of a yellow-green colour. In the third lamb faeces were thinner, without apparent diarrhea. One of the lambs died 12 h following inoculation. All lambs manifested clinical signs such as dehydration, apathy, general weakness, inappetence and decreased skin turgor. In the animals who received a preventive dose of *L. casei* 294/89 and subsequently enterotoxigenic *E. coli* CCM 612 (Group L–E), a very moderate diarrhoea occurred with more watery faeces of a yellow-brown colour. Mild weakness and indistinct apathy were seen. Up to 24 h after inoculation the clinical state of all lambs apparently improved and no inappetence was present. No health disturbances could be observed later and the lambs drank milk showing good appetite. The faeces became condensed and of chocolate-brown colour.

3.2. Necropsy findings

In the dead lamb of the group of animals inoculated only with enterotoxigenic *E. coli* acute diffuse haemorrhagic abomasitis and enteritis were found. In the remaining two lambs acute diffuse catarrhal abomasitis and enteritis were seen. In the lambs preventively inoculated with lactobacilli a very mild acute catarrhal enteritis was found, the sites of inflammation being circumscribed and mainly localized in the duodenum and jejunum. In the lamb killed 7 days after *E. coli* inoculation the acute catarrhal inflammation in the duodenum in part passed into a circumscribed haemorrhagic inflammation. However, the ileum and the colon did not reveal pathological changes.

3.3. Microbiological analyses

Two and four days after inoculation the numbers of enterotoxigenic *E. coli* CCM 612 (O 101: K 99) adhering to the intestinal mucosa of group E lambs counted 5.1 ± 1.0 and 5.0 ± 0.93 log 10 germs per cm^2. In the L–E group the number of *E. coli* CCM 612 ranged between 3.4 ± 0.52 and 3.6 ± 1.3 log10 germs per cm^{-2} (Fig. 1). The differences between the groups were significant 2 days after inoculation ($P < 0.05$). The counts of enteropathogenic *E. coli* CCM 612 adhering to the digestive tract mucosa of group E lambs increased from the duodenum (3.8 ± 0.32 $log10cm^{-2}$) to the colon (5.7 ± 0.73 $log10cm^{-2}$). In the digestive tract of group L–E lambs, the minimum and maximum counts of enterotoxigenic *E. coli* CCM 612 (Fig. 2) were observed in the jejunum (2.8 ± 0.15 $log10cm^{-2}$) and colon (4.7 ± 1.2 $log10cm^{-2}$), respectively. Preventive administration of *L. casei* 294/89 to lambs of the L–E group decreased the counts of enterotoxigenic *E. coli* by 99.1 and 76% on days 2 and 4 after inoculation, respectively. In animals of the L–E group the numbers of adhering enterotoxigenic *E. coli* were decreased by 23, 99.7, 99.8 and 75.8% in the duodenum, jejunum, ileum and colon, respectively.

Four and seven days after inoculation the mean counts of enterotoxigenic *E. coli* in the gut contents of the L–E lambs ranged between 7.1 ± 1.1 and 7.5 ± 0.63 $log10ml^{-1}$ reaching minimum and maximum values in the duodenum (5.5 ± 0.46 $log10ml^{-1}$) and jejunum (8.1 ± 0.24 $log10.ml^{-1}$), respectively; in the ileum and the colon 7.7 ± 0.37 and 7.5 ± 0.03 $log10ml^{-1}$ of *E. coli* were present, respectively.

The mean counts of enterotoxigenic *E. coli* in the gut contents of the E lambs were very similar.

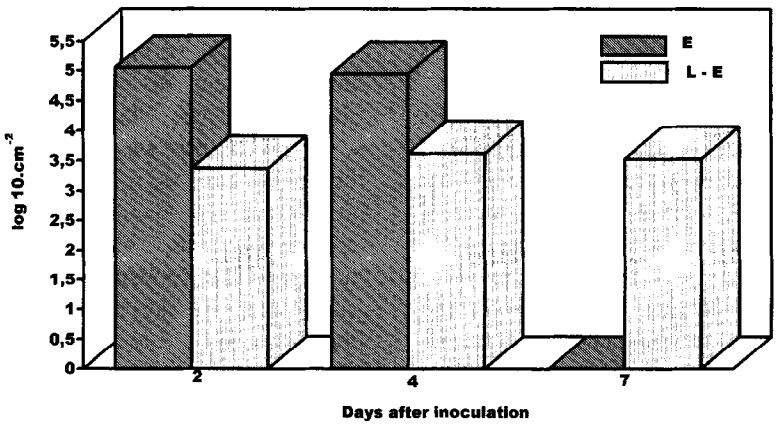

Fig. 1. Effect of *L. casei* upon the adhesion of *E. coli* to the intestinal mucosa in gnotobiotic lambs. E group of lambs($n = 3$) inoculated by *E. coli* CCM 612 (O 101:K 99), L–E group of lambs($n = 3$) inoculated by *L. casei* 294/89 and *E. coli* (O 101:K 99).

In L–E group, the numbers of mucosa-adherent *L. casei* 294/89 following *E. coli* inoculation varied between 1.9 and 2.7 log10.cm^{-2}.

Comparable numbers of mucosa-adherent *L. casei* (2.2 ± 0.86 and 2.5 ± 0.15 log10.cm^{-2}) were counted in the individual gut segments of L–E lambs, revealing a minimum increase between the duodenum and the colon whereas in the digesta an increase could be seen between the duodenum (4.9 ± 0.92 log10ml^{-1}) and the ileum (6.6 ± 0.1 log10ml^{-1}). The difference between the counts of mucosa-adherent Lactobacilli and the counts of *Lactobacilli* in the digesta in the duodenum, ileum and colon showed to be significant at the level of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

3.4. Biochemical analyses

Lactic acid (LA) levels in the duodenal and ileal digesta of L–E lambs were twice those of the E lambs (Table 1). In both groups, maximum LA levels were observed in the duodenum (3.6 and 6.7 mmol.l^{-1}, respectively). Acetic acid levels were prevailingly increased in the digesta of the L–E group, but without marked differences. In comparison to group E, propionic acid levels in the small intestine of L–E lambs were 2–4-times increased.

Inoculation of enterotoxigenic *E. coli* markedly reduced the enzyme activity of the digesta, mainly that of the proteolytic enzymes. Preventive administration of *L. casei* inhibited the abovementioned

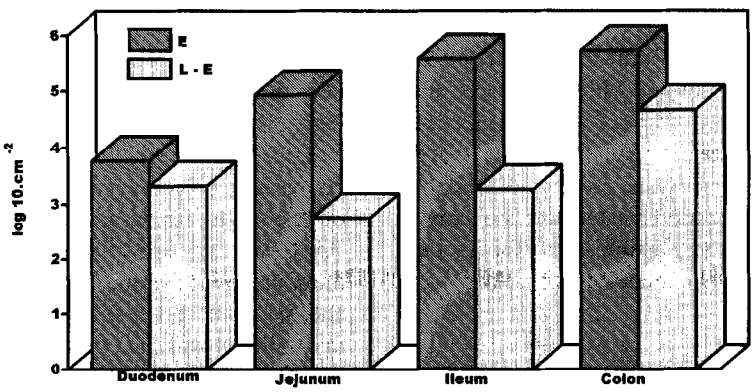

Fig. 2. Effect of *L. casei* upon the adhesion of *E. coli* to the mucosa of intestinal segments in gnotobiotic lambs. E group of lambs($n = 3$) inoculated by *E. coli* CCM 612 (O 101:K 99), L–E group of lambs($n = 3$) inoculated by *L. casei* 294/89 and *E. coli* (O 101:K 99).

Table 1
Effect of *L. casei* upon the biochemical parameters of intestinal content after inoculation with *E. coli* in gnotobiotic lambs

Intestine	Group	Lactic acid (mmol l^{-1})	Acetic acid (mmol l^{-1})	Propionic acid (mmol l^{-1})	Amylasc (ukat l^{-1})	Trypsin (U l^{-1})	Chymotrypsin (U g^{-1})
Duodenum	E	3.62 ± 1.00	1.81 ± 0.17	2.16 ± 1.56	2.82 ± 2.13	0.003 ± 0.002	0.015 ± 0.008
	L–E	6.74 ± 1.85	2.66 ± 0.68	7.78 ± 4.06	5.98 ± 0.82	0.21 ± 0.08	0.88 ± 0.67
Jejunum	E	2.40 ± 0.20	7.06 ± 1.00	2.47 ± 1.35	5.69 ± 0.65	0.13 ± 0.06	0.17 ± 0.13
	L–E	2.77 ± 0.43	4.35 ± 2.37	8.09 ± 4.99	6.84 ± 0.14	0.46 ± 0.21	0.42 ± 0.12
Ileum	E	1.20 ± 0.20	7.06 ± 3.73	1.53 ± 0.94	6.31 ± 0.91	0.12 ± 0.07	0.27 ± 0.04
	L–E	2.03 ± 0.32	9.60 ± 2.89	3.72 ± 1.25	6.28 ± 0.53	0.58 ± 0.27	0.50 ± 0.22
Colon	E	4.89 ± 0.40 **	15.54 ± 11.53	8.25 ± 7.65	5.84 ± 0.76	0.003 ± 0.001	0.10 ± 0.09
	L–E	0.46 ± 0.11	17.74 ± 0.85	6.22 ± 0.63	6.68 ± 0.27	0.07 ± 0.03	0.05 ± 0.03

** $P < 0.01$ Statistical differences between groups E and L–E.

effect of *E. coli*. The activity of alpha-amylase (E.C.3.2.1.1) in the digesta of L–E lambs was increased, however, the differences in comparison to E lambs (except of the duodenum) were negligible. Trypsin activity of the digesta in the jejunum and ileum of L–E lambs revealed a 4- and 5-fold increase, respectively, when compared to that of the E group. Chymotrypsin activity in the jejunum and ileum of the L–E lambs was twice that of the E group.

4. Discussion

Inoculation of ETEC CCM 612 (O101:K99) to lambs induced diarrhoea with complex symptoms, a typical clinical picture and patho-anatomical findings. *E. coli* colonized the mucosa of the digestive tract in numbers approximating 100000 germs per cm^2. The biochemistry of the gut was disturbed, the activity of the proteolytic enzymes was markedly reduced. Preventive administration of *L. casei* 294/89 inhibited the negative effects of ETEC upon germ-free lambs, minimizing the clinical symtpoms to a very mild diarrhea in the first 12 h after inoculation and markedly reducing the patho-anatomical findings. Two and four days after ETEC inoculation the numbers of ETEC adhering to the mucosa of the digestive tract decreased by 99.1 and 76%, respectively, and counted 1000 germs per cm^2. The inhibitory effects of *L. casei* upon the addherence of ETEC were most obvious in the jejunum and ileum.

The results obtained show that *L. casei* 294/89, though it could not fully inhibit the adherence of ETEC to the mucosa of the digestive tract, remarkably reduced the number of adhering *E. coli*, thus preventing the development of diarrhoea with its clinical symptoms and patho-anatomical picture. A similar effect was observed by Underdahl et al. (1982) who preventively administered *Streptococcus faecium* C-68 to germ-free suckling piglets in order to inhibit the adherence of ETEC.

Several authors have reported *Lactobacilli* to have inhibitory effects upon the adherence of *E. coli* in the digestive tract.

Muralidhara et al. (1973) observed the administration of *Lactobacilli* to fully inhibit the adherence of ETEC in the first three segments of the small intestine that had been divided into nine segments. Fourniat et al. (1992) described inhibition of enterotoxigenic *E. coli* B 41 (O10:K99:F41:ST^+) to HeLa cells by heat-killed (100–105°C) *L. acidophilus*. Watkins et al. (1982) also point to the marked inhibitory effects of *L. acidophilus* upon pathogenic *E. coli* in germ-free fowl.

The mechanism of the effects of probiotics is widely discussed. It is very important that an essential stage in the pathogenesis of intestinal infections, especially diarrhoea of bacterial origin, involves the adhesion of the microorganisms concerned to the intestinal epithelial cells. In principle, bacterial diarrhoea could be treated by preventing adhesion of pathogenic bacteria (Fourniat et al., 1992). The following methods are in use:

1. Vaccination against bacterial adhesion factors (Levine et al., 1983; Runnels et al., 1987).
2. Administration of antibiotics which inhibit the expression of adhesion factors (Deneke et al., 1985; Chopra and Hacker, 1986).
3. Oral administration of substances containing structures similar to those of the adhesion factors of pathogens (Neeser et al., 1988) or structures that mimic receptors of the intestinal mucosa (Mouricout et al., 1986). Oral administration of certain strains of *Lactobacilli* has also been found to inhibit implantation of various pathogens in the digestive tract. This action may be due to non-specific competitive inhibition of the adhesion of pathogenic strains to epithelial cells of the digestive tract (Barrow et al., 1980). As can be seen from the results obtained, it was not only competitive inhibition that caused the inhibitory effect of *L. casei*. This is testified by the low counts of *L. casei* adhering to the epithelium of the digestive tract and the unreduced counts of *E. coli* in the gut contents. It can be concluded that *L. casei* 294/89 produced unknown substance which inhibited the adhesion of ETEC either through blockage of receptors in the intestinal mucosa of lambs, or through disturbance of the adhesion factors or inhibition of their expression in *E. coli*. Similarly Blomberg et al. (1993) observed *L. fermentum* 104R to produce a proteinaceous component detectable in spent culture fluid during growth in both complex and defined media; this component inhibited the adhesion of K88ab and

K88ac fimbriae to ileal mucus by interacting with mucus components. Keeping in mind the prevention of clinical manifestation of the disease an anti-enterotoxic effect may also be considered. The anti-enterotoxic activity of *Lactobacilli* was also reported by Mitchell and Kenworthy (1976). The administration of *L. casei* 294/89 proved to have rather beneficial effects upon gut biochemistry and to prevent a decrease in proteolytic enzyme activity observed after the inoculation of ETEC CCM 612.

Further experiments will be conducted in order to study factors stimulating the inhibitory effects of *Lactobacilli* against ETEC and factors selectively supporting the adhesion of *Lactobacilli*. This may lead to the development of potentiated probiotics – new preparations for prevention and treatment.

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References

Barrow, P.A., Brooker, B.E., Fuller, R. and Newport, M.J., 1980. The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. J. Appl. Bacteriol., 48: 147–154.

Bertschinger, H.U., Moon, H.W. and Whipp, S.C., 1972. Association of *Escherichia coli* with the small intestinal epithelium. I. Comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. Infect. Immun., 5: 595–605.

Blomberg, L., Henrikson, A. and Conway, P.L., 1993. Inhibition of adhesion of *Escherichia coli* K 88 to piglet ileal mucus by *Lactobacillus spp.* Appl. Environ. Microbiol., 59: 34–39.

Bomba, A., Králičeк, L', Koniarová, I., Lešník, F., Pošivák, J., Bučko, V. and Žitňan, R., 1993. The recovery and rearing of gnotobiotic lambs and their use in veterinary medicine. Vet. Med. -Czech, 38: 403–411.

Chateau, N., Castellanos, I. and Deschamps, A.M., 1993. Distribution of pathogen inhibition in the *Lactobacillus* isolates of a commercial probiotic consortium. J. Appl. Bact., 74: 36–40.

Chauviere, G., Coconnier, M.H., Kerneis, S., Darfenille Michaud, A., Joly, B. and Servin, A.L., 1992. Competetive exclusion of diarrheagenic *Escherichia coli* (ETEC) by heat killed *Lactobacillus*. FEMS Microbiol. Letters, 91: 213–218.

Chopra, I. and Hacker, K., 1986. Inhibition of K 88 – mediated adhesion of *Escherichia coli* to mammalian receptors by antibiotics that affect bacterial protein synthesis. J. Antimicrob. Chemother., 18: 441–451.

Deneke, C.F., Thorne, G.M., Larson, A.D. and Gorbach, S.L., 1985. Effect of tetracycline on the attachment of K 88^+ enterotoxigenic *Escherichia coli* to porcine small – intestinal cells. J. Infect. Dis., 152: 1032–1036.

Fourniat, J., Colomban, C., Linxe, C. and Karam, D.:1992 Heat killed *Lactobacillus acidophilus* inhibits adhesion of *Escherichia coli* B 41 to HeLa cells. Ann. Rech. Vet., 23: 361–370.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365–378.

Gilliland, S.E., Bruce, B.B., Bush, L.J. and Staley, T.E., 1980. Comparison of two strains of *Lactobacillus acidophilus* as dietary adjuncts for yong calves. J. Dairy Sci., 63: 946–972.

Jones, G.W. and Rutter, J.M., 1972. Role of the K 88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun., 6: 918–927.

Jonsson, E. and Conway, P., 1992. Probiotics for pigs. In: R. Fuller (Editor), Probiotics the scientific basis, Chapman and Hall London, AS 3098, pp. 260–316.

Levine, M.M., Kaper, J.B., Black, R.E. and Clements, M.L., 1983. New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. Microbiol. Rev., 47: 510–550.

Lidbeck, A., Gustafsson, J.A. and Nord, C.E., 1987. Impact of *Lactobacillus acidophilus* supplements on the human oropharyngeal and intestinal microflora. Scand. J. Infect. Dis., 19: 531–537.

Mitchell, I. DeG. and Kenworthy, R. 1976. Investigations on a metabolite form Lactobacillus bulgaricus which neutralizes the effect of enterotoxin from Escherichia coli pathogenic for pigs. J. Appl. Bacteriol., 41: 163–174.

Mouricout, M., Petit, J.M. and Julien, R., 1986. Mode d'action d'agenst inhibititeurs de l'adhésion de Escherichia coli entérotoxigenes (ECET) aux glycoprotéines de la mugueuse intestinale bovine. Mode of action of inhibitors upon the adhesion of enterotoxigenic *Escherichia coli* (ETEC) to the glycoproteins of intestinal bovine mucosa. Rev Inst. Pasteur Lyon, 19: 161–168.

Muralidhara, K.S., Sandine, W.E., England, D.C. and Elliker, P.R., 1973. Colonization of Escherichia coli and Lactobacillus in intestines of pigs. J. Dairy Sci., 56: 635.

Neeser, J.R., Chambaz, A., Hoang, K.Y. and Link Amster, H., 1988. Screening for complex carbohydrates inhibiting hemagglutinations by CFA/I- and CFA/II- expressing enterotoxigenic Escherichia coli strains. FEMS Microbiol. Letters, 49: 301–307.

Raccach, M., McGrath, R. and Daftarin, H., 1989. Inhibiosis of some lactic acid bacteria including Lactobacillus acidophilus toward Listeria monocytogenes. Int. J. Food. Microbiol., 9: 25–32.

Runnels, P.L., Moseley, S.L. and Moon, H.W., 1987. F 41 pili as protective antigens of enterotoxigenic Escherichia coli that produce F 41, K 99, or both pilus antigens. Infect. Immun., 55: 555–558.

Smith, H.W. and Gyles, C.L., 1970. The relationship between two

apparently different enterotoxins produced by enteropathogenic strains of Escherichia coli of porcine origin. J. Med. Microbiol., 3: 387–401.

Sudirman, I., Mathieu, F., Michel, M. and Lefebvere, G. 1993. Detection and properties of curvaticin 13, a bacteriocin-like substance produced by Lactobacillus curvatus SB 13. Current Microbiol., 27: 35–40.

Tzipori, S., 1981. The aetiology and diagnosis of calf diarrhoea. Vet. Rec., 108: 510–514.

Underdahl, N.R., Torres-Medina, A. and Doster, A.R., 1982. Effect of Streptococcus faecium C 68 in control of Escherichia coli induced diarrhea in gnotobiotic pigs. Am. J. Vet. Res., 43: 2227–2232.

Vandenbergh, P.A., 1993. Lactic acid bacteria, their metabolic products and interference with microbial growth. FEMS Microbiol. Rev., 12: 221–238.

Watkins, B.A., Miller, B.F. and Neil, D.H., 1982. In vivo inhibitory effect of Lactobacillus acidophilus against pathogenic Escherichia coli in gnotobiotic chicks. Poult. Sci., 61: 1298–1308.

Watkins, B.A. and Miller, B.F., 1982. Competetive gut exclusion of avian pathogens by Lactobacillus acidophilus in gnotobiotic chicks. Poult. Sci., 62: 1772–1779.