Pak. J. Bot., 40(2): 867-875, 2008.

TECHNOLOGICAL CHARACTERIZATION OF INDIGENOUS ENTEROCOCCAL POPULATION FOR PROBIOTIC POTENTIAL

MUSHTAQ HUSSAIN[1], MUHAMMAD TANWEER KHAN[1], ABDUL WAJID[2] AND SHEIKH AJAZ RASOOL[1*]

[1]Department of Microbiology, University of Karachi, Karachi, Pakistan
[2]HEJ Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract

Probiotics are the live microbial supplements of single or mixed cultures that produce health beneficial effects when ingested. Diversity in metabolic and/or physiological attributes has made *Enterococcus* a probiotic organism and quite conversely a second or third most common agent of nosocomial infections. The present study is a technological screening for the selection of potential probiotic isolates from the indigenous enterococcal population. Over 500 enterococcal strains have been isolated from sewage samples and baby fecal material, respectively collected from all 18 towns and well recognized hospitals of Karachi. Production of several enzymes and bioactive peptides/proteins has been screened from isolated microbes for instance alkaline phosphatase, bacteriocins, β-galactosidase, urease, protease, cytolysin and lipase etc. Among the total, 95.7%, 78.2% and 3.4% of enterococci have been found as producers of β-galactosidase, bacteriocin and hemolysin (cytolysin) respectively. Other metabolites have been less frequently produced by the isolates. The high prevalence of β-galactosidase suggests the constitutive nature of gene while fluctuation in different metabolite production indicates their dispensability and concomitantly delineates the significance of selection for probiotic organisms. Moreover, far less frequency of hemolytic enterococcocci suggest low prevalence of pathogenicity island in the indigenous enterococcal population. Conclusively, the findings facilitate not only the down right selection of occult probiotic enterococci but also provide baseline information for composition of potentially probiotic and pathogenic enterococci in the local microbial population.

Introduction

Probiotics are generally defined as live microbial supplements which provide health benefits to the host by bringing balance in the host intestinal microbial flora (Fuller, 1989). Probiotics have been found as prophylactic/therapeutic agents in case of many gastrointestinal ailments like lactose maldigestion, infections, constipation, cholesterolemia, hypertension, colorectal carcinoma, ulcerative colitis, Crohn's diseases, irritable bowel syndrome (IBS), food allergies and antibiotic induced diarrhoea (Daly & Davis, 1998; Sanders, 1998; Famularo *et al*., 2005; Capurso *et al*., 2006; Fan *et al*., 2006; Parkes, 2007). Additionally, they are also known for the production of butyric acid and other cytokine inducing factors which have anti-aging and immuno-modulating effects respectively (Matsuzaki *et al*., 1998; Roy *et al*., 2006; Vizoso Pinto *et al*., 2007). Certain *In vivo* studies are concluded on the note that LAB decreases the occurrences of DNA damages, inhibit activity of cancer causing enzymes and other cancer associated changes, this implies their role against cancer (Goldin & Gorbach, 1984; Ling *et al*., 1994; Pool-Zobel *et al*., 1993; 1996). World Health Organization actuation for the use of alternative therapeutics such as probiotics as disease control measures has intensified their public and research interest to

[*]Correspondence: Email: rasoolajaz@yahoo.com
Ph: 92-21-9261300-7 Ext. 2255, 92-21-9243026; Cell: 0333-2344760; Fax: 92-21-9243206

considerable extent (Bengmark, 1998; Dunne & Shannahan, 2002). Indeed, a Leatherhead food RA's 1996 report has estimated that the probiotics share in global market is currently over US$6.6 billions (Cathro & Hilliam, 1993). The therapeutic and preventive properties of probiotics are primarily based on the metabolic potential of their constituent microorganisms. Bacteria used in probiotic supplements are found to be the producers of various metabolites including enzymes, antimicrobial peptides and/or proteins and other biologically active substances (Tuomola *et al*., 2001; Nes *et al*., 2007). Among the enzymes perhaps the most significant is the β-galactosidase which helps in lactose digestion and conversely renders oligomerization of the products with substrate, cumulatively the enzyme ameliorate the disorders associated with lactose intolerance (Kotz *et al*., 1994; Lin *et al*., 1998; Sanders, 1998). Protease and lipase have their role in maintaining microbial balance in gastrointestinal tract by facilitating formation of biofilms. Moreover, products of proteolytic enzyme may abstrusely involve in the reduction of hypertension (Bouzaine *et al*., 2005; Nallaparradsey *et al*., 2006; Tallon *et al*., 2007). Urease producing microbes may inhibit growth of various pathogenic bacteria and fungi in the gut of the host (Zwolinska-Wcislo *et al*., 2006). Bacteriocins, the antimicrobial peptides or proteins, are traditioanally defined as peptides which exhibit antimicrobial activity against closely related organisms (Jack *et al*., 1995). These are yet another arsenal of probiotic microorganisms in order to establish microbial balance in the gut and indeed included in selection criteria for the microorganisms that tend to be the part of effective probiotics. Unlike the earlier believes, their antimicrobial potential ranges from gram negative food spoilage causing bacteria (Cintas *et al*., 1997; Eijsink *et al*., 1998) to even viruses (Wachsman *et al*., 1999) and fungi (Saeed *et al*., 2006).

The foremost bacterial groups that constitute majority of the probiotic supplements include Lactic Acid Bacteria (LAB) and *Bifidobacteria* (du Toit *et al*., 1998; Sanders, 2000). However, probiotics properties for certain enterococci strains have also been stipulated (Linaje *et al*., 2004; Saavedra *et al*., 2003). *E. faecium* M-74 and *E. faecium* SF68 are two well known and commercially used probiotics strains. In particular, use of *E. faecium* SF68 has been found active in reducing the recovery period of acute diarrhoea, and decreasing blood cholesterol level (Benyacoub *et al*., 2003; Richelsen *et al*., 1996). Adhesion of enterotoxigenic strain, *Escherichia coli* K88 (an etiological agent of piglets diarrhoea) to piglet intestinal mucus was also found inhibited by *E. faecium* 18C23 by steric hindrance and alteration of pH (Jin *et al*., 2000). Additionally, *E. faecium* EK13 a bacteriocin producing strain was successfully used to alleviate the experimental contamination of gonobiotic Japnese quails by *Salmonella enteroica serovar Duesseldorf* (Laukova *et al*., 2003). Enterococci though generally considered as normal inhabitant of gastrointestinal tract but they are concomitantly the second to third most common agent of nosocomial infections (Foulquie Moreno *et al*., 2006). Considering these, excluding pathogenic enterococci from the consortium of microbes as being the candidate for probiotics holds great importance. Metabolic characterization of enterococci will strongly abet in this connection. Hence the study has been designed in order to explore the probiotic potentials residing in the local enterococcal population, which has been isolated from sewage and baby fecal material.

Materials and Methods

Isolation: During June, August and November 2006 and January 2007, sewage samples were collected from randomly selected 5 different stations of main sewage lines from all 18 towns of Karachi. Similarly 20 samples of baby (<3 months) fecal material were also collected from well recognized hospitals situated at different regions of Karachi. After serial dilutions, samples were dispensed and spread over Bile Esculin Agar (BE; Merck).

The black colonies (appeared after 24 hours of incubation) on BE plates are of enterococci, which were subsequently patched over Brain Heart Infusion agar (BHI; Merck).

Enzymatic screening: For Amylase, β-galactosidase, lipase and protease production 1% soluble starch (w/v), 0.01% X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; w/v), 0.1% Tween-20 and 0.1% Tween-80 (v/v), and 1% casein (w/v) were added in BHI agar respectively. However, simple BHI agar was used for screening alkaline phosphatase and catalase. Urease activity was checked by mixing 4M filter-sterile urea solution in Urea agar base (Oxoid). After 24 hours of incubation, lipase and protease activity was detected as precipitated zones around patches on their respective agar plates. However, pink zone over urea agar plate was reflective of urease production. On dispensing iodine, clear zones around patch over starch agar plate indicated hydrolysis of starch or amylase production by tested strain. Effervescence and yellow color production over patch present on BHI agar plate on dispensing 3% hydrogen peroxide and 5mM 4-nitrophenylphosphate/para-nitrophenylphosphate pNPP (disodium salt hexahydrate), chromogenic substrate (Sigma) indicated catalase and alakaline phosphatase production respectively (Fig. 1). Animal liver tissues and simple BHI agar were used as positive and negative control respectively.

Bacteriocin production: All isolated strains were stabbed on BHI agar plates and after overnight incubation were exposed to pre-autoclaved filter paper soaked with chloroform for 30minutes. Plates were then over-layered using 0.6% soft agar previously mixed with 100µl log phase (OD_{600}; 1.0) cultures of *Enterococcus* SM-18 & 43, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes* as sensitive strains (Fig. 1).

Hemolytic assay: Blood (AB+ve), drawn from a volunteer in a EDTA vacutainer was dispensed in pre-autoclaved blood agar base at 5% concentration. Isolated strains were patched over plates and incubated for 24-48 hours and hemolytic patterns were then observed as greenish (α-hemolysis) or clear zone (β-hemolysis) around the patch (Fig. 1).

Results and Discussion

Out of 535, 500 strains of enterococci were isolated from main stream sewage lines of Karachi while remaining 35 were of baby fecal origin. Isolates/strains were differentiated on the basis of their colonial morphology, pigment production, time and site of collection. All isolates were subsequently designated as SM-No.code. Enzymatic screenings revealed that out of 535 isolates, 512 (95.7%) were positive for β-galactosidase production. This high frequency of β-galactosidase producers enterococci is akin to the previous reports in this connection suggesting constitutive nature of β-galactosidase gene in the organism (Tao *et al*., 2005). As β-galactosidase is known to hydrolyze the lactose into glucose and galactose, hence the production of the enzyme by enterococci when taken as probiotic, greatly reduces the ill manifestation caused by lactose mal digestion in humans. Additionally, oligomerization of products and substrate is also mediated by β-galactosidase which subsequently stimulates cytotoxic and humoral immunity *via* activation of macrophages and T-cells (Famularo *et al*., 2005; Montalto *et al*., 2006; Mountzouris *et al*., 2007; Parkes, 2007). Hence the production of this worthy enzyme by enterococci may also beneficially influence the host immune system. Followed by β-galactosidase, around 12.7% (68), 5.2% (28), 3.9% (21), and 3.5% (19) of

the isolates showed positive activity for alkaline phosphatase, protease, lipase and urease respectively. Role of protease and lipase is well documented in biofilm formation; therefore, it seems plausible to state that the mentioned enzyme production would help the producer enterococci to inhabit GI tract and consequently inhibit colonization of various pathogenic organisms (Nallaparradey *et al*., 2006; Bourgogne *et al*., 2006). Conversely, if the protease and/or lipase activity is present in hemolytic (pathogenic) enterococci, it may aggravate the virulence of the organism (Tendolkar *et al*., 2003). Hence selection of protease and/or lipase producer organism(s) as a potential probiotic is dependent on their ability to exhibit hemolysis. Beside this other virulence factors must be taken into consideration which has been discussed later. In addition to biofilm formation, alteration of pH by the products of urease not only assists in the neutralization of acidic environment produced due to any physiological and pathological disorder, but also halts growth of any potentially pathogenic fungus in the intestine (Zwolinska-Wcislo *et al*., 2006). However, production of biogenic amines of psychopathic nature as a by product of the urease activity belied rather belittled their producer exploitation as probiotics (Bover-Cid & Holzapfel, 1999). Only 1.2% of enterococcal isolates were found as catalase and amylase producer each. As enterococci are facultative anaerobes, the absence of catalase activity is considered as a tool for enterococcal identification. As mentioned earlier that in the present study only 1.2% of enterococci were found weakly positive for catalase production, this unorthodox or so called pseudopositive catalse activity is also reported by Frankenberg *et al*., (2002). Cumulatively, both frequencies of both catalase and β-galactosidase activities increase the fidelity in the selection of enterococci during isolation (Fig. 2).

Out of 535 isolates, only 18 (3.4%) have demonstrated hemolytic activity which could be further segregated as 2.2% (12) are β-hemolytic while the remaining 6 (1.2%) are α-hemolytic (Fig. 2.). Hemolysis produced by enterococci is the function of a virulence factor concomitantly a lantibiotic (post translationally modified bacteriocins), cytolysin (Cox *et al*., 2005). As probiotics must essentially be non pathogenic neither invasive hence exclusion of pathogenic enterococci must be necessary for others to be selected as probiotics (Sanders, 2000). In this connection screening for the phenotypic expression of cytolysin (hemolysis) facilitate the initial separation of potentially pathogenic enterococci.

When checked against 5 different sensitive strains (indicators) out of a total of 535 isolates, 418 (78%) were found positive with different magnitude of bacteriocin production. Some isolates for instance *Enterococcus* SM-7, SM-18, SM-19, SM-168, SM-176, SM-222, SM-312 and SM-518 had shown antagonistic activity against all the tested strains. Additionally, their zone of inhibition (diameter) against tested strains ranges from 2.7cm to 4.2cm, suggesting their strong bacteriocinogenesis ability. With reference to indicator strains 75.3% and 35.9% of the isolates produced bacteriocin active against other enterococcal strains i.e., *Enterococcus* SM-43 & SM-18 respectively. Expectedly substantial drop in bacteriocinogenic isolates percentage was noticed when they were screened against distantly related organisms like *Sterptococcus pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes*. Only 31.5%, 4.3% and 3.5% of the isolates are active against *Sterptococcus pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes* respectively (Fig. 3). It is worth mentioning here that the frequency of bacteriocin producer varies considerably with the type of indicator strains exploited to screen it. On that basis it is possible that the total frequency of the bacteriocinogenic bacteria in the population may increase or decrease based on the use of different sensitive strains. Indeed, Klaenhammer (1988) has suggested that 99% of all bacteria are the producer of at least one bacteriocin, provided the activity has to be checked against suitable indicator(s).

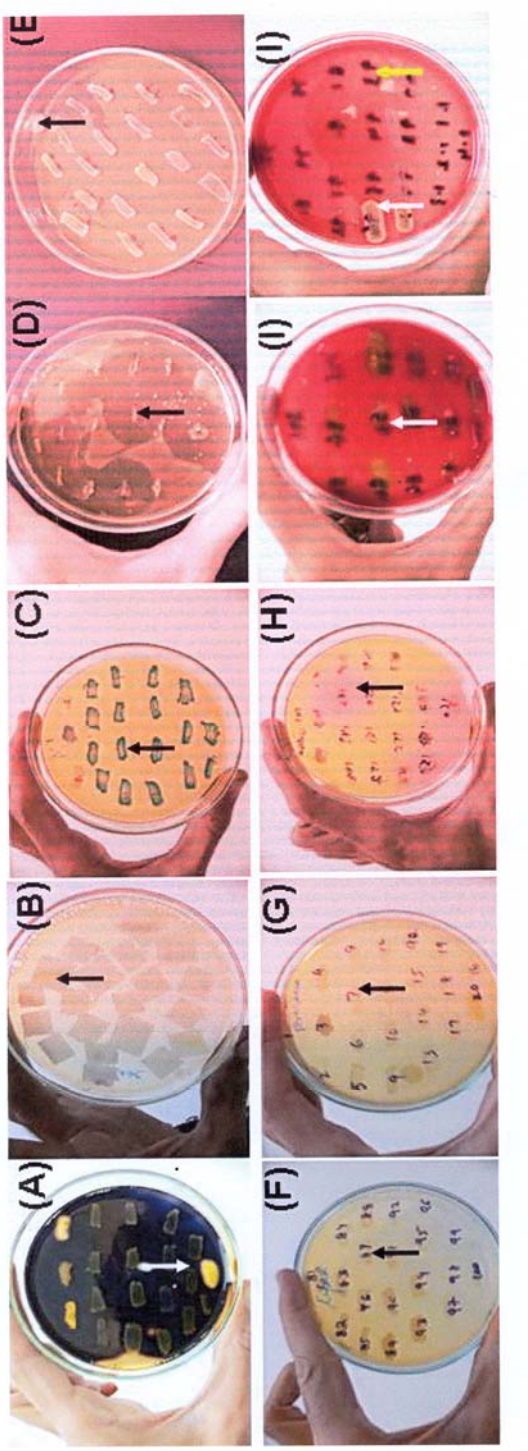

Fig. 1. Screening for probiotic associated metabolites in enterococcal population. Arrow in each inset represents positive activity of the respective metabolite as mentioned in the Key.
Key: Amylase (A), Alkaline phosphatase (B), β-galactosidase (C), Bacteriocin (D), Catalase (E), Lipase (F), Protease (G), Urease (H), hemolysis (I)

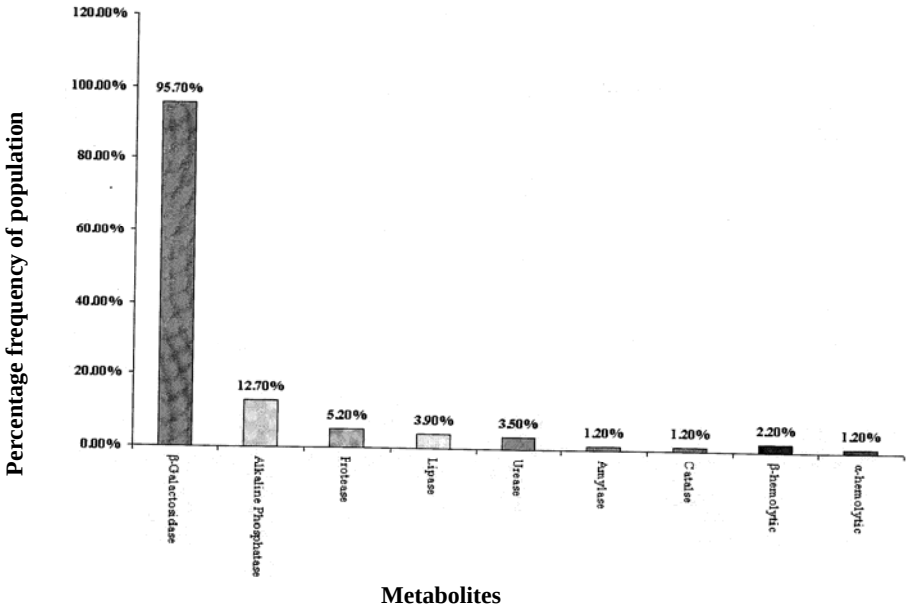

Fig. 2. Metabolic profile of Indigenous enterococcal population: Bar graph suggest that most enterococci are positive for β-galactosidase activity while pathogenic enterococci are relatively less dense in the total population. (For details please see Result and Discussion in text).

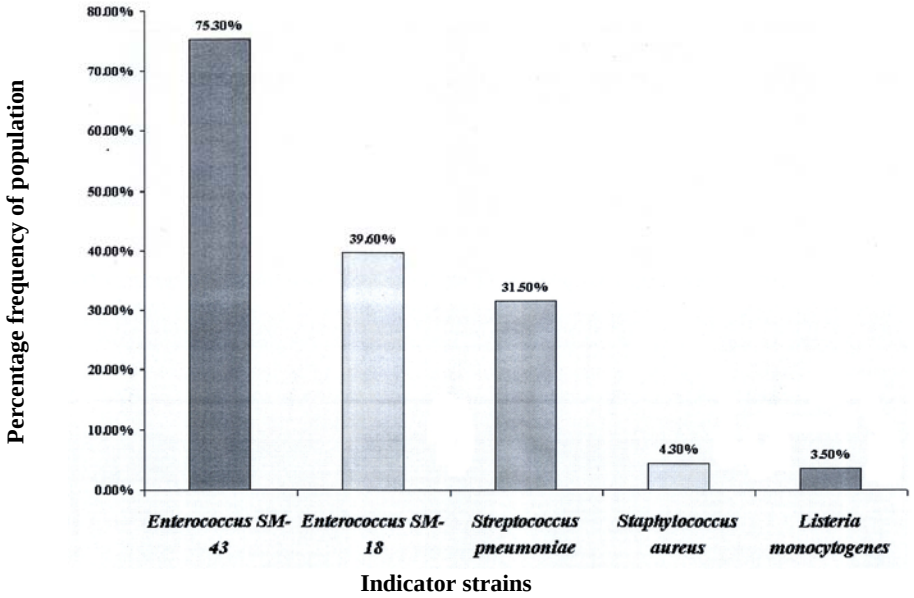

Fig. 3. Bacteriocinogenesis in indigenous enterococcal population: Note the gradual descend in number of bacteriocin producer enterococci in local enterococcal population when screened against evolutionary distant organims (see text for details).

Bacteriocins are generally referred as ribosomally synthesized antimicrobial peptides or proteins of bacterial origin, which inhibit growth of closely related organisms. However, in light of past decade it is now increasing evident that their antibacterial activity span is well beyond to what is earlier stipulated. Reportedly, bacteriocins from enterococci are also found active against even viruses and fungi. Bacteriocin production certainly helps enterococci to colonize in the GI tract triumphantly and this also renders to the inhibition of pathogenic organisms in the intestine (Wachsman *et al*., 1999; Saeed *et al*., 2006). In the study enterococcal isolates exhibiting antibacterial activity against *Sterptococcus pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes* provides additional advantage to them to be exploited as probiotics. Moreover, the difference in antibacterial spectrum of bactericinogenic enterococci plausibly implies the diversity of the bacteriocins produced by enterococcal population. These bacteriocins in addition to be an essential characteristic of probiotic may have many other industrial and therapeutic values.

Laconically, the present study suggests that preliminary screening of locally isolated enterococci has greater probiotic potentials primarily because of high frequency of β-galactosidase production and bacteriocinogenesis and less intense pathogenecity. On that ground further intense screening with reference to antibiotic resistance and bile salt tolerance etc., and *In vivo* animal model studies will certainly help in exploring more probiotic attributes in the isolated organisms. In addition to this molecular identification of other virulence factors like aggregation substance, membrane surface adhesion molecules etc among the organism population might also be helpful in order to develop a more conspicuous epidemiological picture of the indigenous enterococcal population. Studies in this regard are in process and will be reported in future.

Acknowledgement

We are indebted to Dr. Mujtaba Hasan Naqvi, Ex-Director General of Institute of Biotechnology and Genetic Engineering (KIBGE) for financial and technical support in order to conduct this work.

References

Bengmark, S. 1998. Ecological control of the gastrointestinal tract. The role of probiotic bacteria. *Gut.*, 42: 2-7.

Benyacoub, J., G.L. Czarnecki-Maulden, C. Cavadini, T. Sauthier, R.E. Anderson, E.J. Schiffrin and T. von der Weid. 2003. Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. *J. Nutr*., 133: 1158-1162.

Bourgogne, A., S.G. Hilsenbeck, G.M. Dunny and B.E. Murray. 2006. Comparison of OG1RF and an isogenic fsrB deletion mutant by transcriptional analysis: the Fsr system of *Enterococcus faecalis* is more than the activator of gelatinase and serine protease. *J. Bacteriol.*, 188: 2875-2884.

Bouzaine, T., R.D. Dauphin, P. Thonart, M.C. Urdaci and M. Hamdi. 2005. Adherence and colonization properties of *Lactobacillus rhamnosus* TB1, a broiler chicken isolate. *Lett. Appl. Microbiol.*, 40: 391-396.

Bover-Cid, S. and W.H. Holzapfel. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int. J. Food Microbiol.,* 53: 33-41.

Capurso, G., M. Marignani and G.D. Fave. 2006. Probiotics and the incidence of colorectal cancer: when evidence is not evident. *Dig. Liver Dis*., 38: 277-282.

Cathro, J.S. and M.A. Hilliam. 1993. Future opportunities for functional and healthy foods in Europe. An in-depth consumer and market analysis. *Leatherhead Food RA special report. Surrey, United Kingdom: Leatherhead*, 1993.

Cintas, L.M., P. Casaus, L.S. Havarstein, P.E. Hernandez and I.F. Nes. 1997. Biochemical and genetic characterization of enterocin P, a novel *sec*-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. *Appl. Environ. Microbiol.,* 63: 4321-4330.

Cox, C.R., P.S. Coburn and M.S. Gilmore. 2005. Enterococcal cytolysin: a novel two component peptide system that serves as a bacterial defense against eukaryotic and prokaryotic cells. *Curr. Protein Pept. Sci*., 6: 77-84.

Daly, C. and R. Davis. 1998. The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. *Agri. Food Sci. Finland.*, 7: 251-265.

du Toit, M., C.M.A.P. Franz, L.M.T. Dicks, U. Schillinger, P. Haberer, B. Warlies, F. Ahrens and W.H. Holzapfel. 1998. Characterization and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *Int. J. Food Microbiol*., 40: 93-104.

Dunne, C. and F. Shanahan. 2002. Role of probiotics in the treatment of intestinal infections and inflammation. *Curr. Opin. Gastroenterol.*, 18: 40-45.

Eijsink, V.G., M. Skeie, P.H. Middelhoven, M.B. Brurberg, and I.F. Nes. 1998. Comparative studies of class IIa bacteriocins of lactic acid bacteria. *Appl. Environ. Microbiol.,* 64: 3275-3281.

Famularo, G., C. De Simone, V. Pandey, A.R. Sahu and G. Minisola. 2005. Probiotic lactobacilli: an innovative tool to correct the mal absorption syndrome of vegetarians? *Med. Hypotheses*., 65: 132-1135.

Fan, Y.J., S. Chen, Y. Yu, J. Si and B. Liu. 2006. A probiotic treatment containing *Lactobacillus*, *Bifidobacterium* and *Enterococcus* improves IBS symptoms in an open label trial. *J. Zhejiang Univ. Sci. B*., 7: 987-991.

Foulquie Moreno, M.R., P. Sarantinopoulos, E. Tasakalidou and L. De Vuyst. 2006. The role and application of enterococci in food and health. *Int J. Food Microbiol.,* 106: 1-24.

Frankenberg, L., M. Brugna and L. Hederstedt. 2002. An *Enterococcus faecalis* heme-dependent catalase. *J. Bacteriol.*, 184: 6351-6356.

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

Goldin, B.R. and S.L. Gorbach. 1984. The effect of milk and Lactobacillus feeding on human intestinal bacterial enzyme activity. *Am. J. Clin. Nutr*., 39: 756-761.

Jack, R.W., J.R. Tagg and B. Ray. 1995. Bacteriocins of gram-positive bacteria. *Microbiol. Rev*., 59: 171-200.

Jin, L.Z., R.R. Marquardt and X. Zhao. 2000. A Strain of *Enterococcus faecium* (18C23) Inhibits Adhesion of Enterotoxigenic *Escherichia coli* K88 to Porcine Small Intestine Mucus. *Appl. Environ. Microbiol.*, 66: 4200-4204.

Klaenhammer, T. R. 1988. Bacteriocins of lactic acid bacteria. *Biochimie*., 70: 337-349.

Kotz, C.M., J.K. Furne, D.A. Savaiano and M.D. Levitt. 1994. Factors affecting the ability of a high ß-galactosidase yogurt to enhance lactose absorption. *J. Dairy Sci.,* 77: 3538-3544.

Laukova, A., P. Guba, R. Nemcova and Z. Vasilkova. 2003. Reduction of salmonella in gnobiotic Japanese quails caused by enterocin A producing EK13 strain of *Enterococcus faecium*. *Vet. Res. Commun*., 27: 275-280.

Lin, MY., C.L. Yen and S.S. Chen. 1998. Management of lactose maldigestion by consuming milk containing lactobacilli. *Dig Dis Sci*., 43: 133-137.

Linaje, R., M.D. Coloma, P. Ge and M. Zuniga. 2004. Characterization of fecal enterococci from rabbits for the selection of probiotic strains. *J. Appl. Microbiol.,* 96: 761-771.

Ling, W.H., R. Korpela, H. Mykkänen, S. Salminen and O. Hänninen. 1994. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J. Nutr.*, 124: 18-23.

Matsuzaki, T., R. Yamazaki, S. Hashimoto and T. Yokokura. 1998. The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J. Dairy Sci*., 81: 48-53.

Montalto, M., V. Curigliano, L. Santoro, M. Vastola, G. Cammarota, R. Manna, A. Gasbarrini, and G. Gasbarrini. 2006. Management and treatment of lactose mal absorption. *World J. Gastroenterol.,* 14: 187-191.

Mountzouris, K.C., P. Tsirtsikos, E. Kalamara, S. Nitsch, G. Schatzmayr and K. Fegeros. 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium, Enterococcus* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Sci.*, 86: 309-317.

Nallaparradey, S.R., K.V. Singh, J. Sillanpaa, D.A. Garsin, M. Hook, S.L. Erlandsen and B.E. Murray. 2006. Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *J. Clin. Investigation*., 116: 2799-2807.

Nes, I.F., D.B. Diep and H. Holo. 2007. Bacteriocin Diversity in *Streptococcus* and *Enterococcus. J. Bacteriol.,* 189: 1189-1198.

Parkes, G.C. 2007. An overview of probiotics and prebiotics. *Nurs. Stand*., 21: 43-47.

Pool Zobel, B.L., B. Bertram, M. Knoll, R. Lambertz, C. Neudecker, U. Schillinger, P. Schmezer and W.H. Holzapfel. 1993. Antigenotoxic properties of lactic acid bacteria in vivo in the gastrointestinal tract of rats. *Nutr Cancer*., 20: 271-282.

Pool Zobel, B.L., C. Neudecker, I. Domizlaff, S. Ji, U. Schillinger, C. Rumney, M. Moretti, I. Vilarini, R. Scassellati Sforzolini and I. Rowland. 1996. *Lactobacillus*- and *Bifidobacterium*-mediated antigenotoxicity in the colon of rats. *Nutr. Cancer*., 26: 365-80.

Richelsen, B., K. Kristensen and S.B. Pedersen. 1996. Long term (6 months) effect of a new fermented milk product on the level of plasma lipoproteins a placebo controlled and double blind study. *Eur. J. Clin. Nutr.,* 50: 811-815.

Roy, C.C., C.L. Kien, L. Bouthillier and E. Levy. 2006. Short-chain fatty acids: ready for prime time? *Nutr. Clin. Pract*., 21: 351-366.

Saavedra, L., M.P. Taranto, F. Sesma and G.F. de Valdez. 2003. Home made traditional chesses for the isolation of probiotic *Enterococcus faecium* strains. *Int J. Food Microbiol*., 88: 241-245.

Saeed, S., S.A. Rasool, S. Ahmed, T. Khanum, B.H. Khan, A. Abbasi and S.A. Ali. 2006. New insight in staphylococcin research: bacteriocin and/or bacteriocin-like inhibitory substance(s) produced by *Staphyloccus aureus* AB188. *World J. Microbiol. Biotechnol.,* 713-722.

Sanders, M.E. 1998. Overview on functional foods: emphasis on probiotic bacteria. *Int. Dairy J.,* 8: 341-347.

Sanders, M.E. 2000. Considerations for use of probiotic bacteria to modulate human health. *J. Nutr.,* 130: 384S-390S.

Tallon, R., S. Arias, P. Bressollier and M.C. Urdaci. 2007. Strain- and matrix-dependent adhesion of *Lactobacillus plantarum* is mediated by proteinaceous bacterial compounds. *J. Appl. Microbiol.,* 102: 442-445.

Tao, H., M.G. Priebe, R.J. Vonk and G.W. Welling. 2005. Identification of bacteria with β-galactosidase activity in faeces from lactase non-persistent subjects. *FEMS Microbiol. Ecol.,* 54: 463-469.

Tendolkar, P.M., A.S. Baghdayan and N. Shankar. 2003. Pathogenic enterococci: new developments in the 21st century. *Cell Mol. Life Sci*., 60: 2622-2636.

Tuomola, E., R. Crittenden, M. Playne, E. Isolauri and S. Salminen. 2001. Quality assurance criteria for probiotic bacteria. *Am. J. Clin. Nutr.,* 73: 393S-398S.

Vizoso Pinto, M.G., T. Schuster, K. Briviba, B. Watzl, W.H. Holzapfel and C.M. Franz. 2007. Adhesive and chemokine stimulatory properties of potentially probiotic Lactobacillus strains. *J. Food Prot.*, 70: 125-134.

Wachsman, M.B., M.E. Farias, E. Takeda, F. Sesma, A.P. De Ruiz Holgado, R.A. De Torres and C.E. Coto. 1999. Antiviral activity of enterocin CRL35 against herpesviruses. *Int. J. Antimicrob. Agents.,* 12: 293-299.

Zwolinska-Wcislo, M., T. Brzozowski, T. Mach, A. Budak, D. Trojanowska, P.C. Konturek, R. Pajdo, D. Drozdowicz and S. Kwiecien. 2006. Are probiotics effective in the treatment of fungal colonization of the gastrointestinal tract? Experimental and clinical studies. *J. Physiol. Pharmacol.,* 57: 35-49.

(Received for publication 26 November 2007)