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RESEARCH ARTICLE

EXPLORATION OF PROBIOTIC POTENTIAL IN INDIGENOUS LACTIC ACID BACTERIA

*Muneera Naz Baloch, Roquya Siddiqi, Humera Erum and Mariam Zia

Department of Microbiology, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

Aims: To screen for the new effective antibacterial and probiotic strain of lactic acid bacteria (LAB) from indigenous sources such as milk, yogurt, tomato and infant feces.

Methods and Results: On the basis of morphology, cultural characteristics and catalase test isolates were identified up to genus level. Stab and agar well assay was performed to investigate the antagonistic potential of isolates. In order to rule out the effect of acid neutralized cell free culture supernatants (NCFCS) were used. Of all the isolates screened for antagonistic activity a milk isolate designated as LBM-86 appeared as strong bacteriocin producer, hence it was selected for further studies to determine its acid and bile tolerance which is generally considered important property for survival of probiotic strain in small intestine. When LBM-86 was grown in different concentrations of bile, decrease in absorbance and CFUml⁻¹ was observed at high concentrations of bile, but bacteriocin activity was enhanced at these concentrations.

Conclusions: Our isolate LBM-86 displayed antibacterial activity and bile tolerance, this could emphasize its candidature as probiotic.

Significance and impact of the study: LBM-86 exhibited promising results and possess potentials for further research to explore its role as probiotic strain for nutritional and beneficial aspects.

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INTRODUCTION

Lactic acid bacteria are a heterogeneous group of bacteria that occur naturally in several raw materials (e.g. milk, flour, vegetables, meat etc.) and generally regarded as safe for use in food and food products. Various members of this group are used as 'natural' or 'selected' starters for the fermentation, preservation and manufacture of food products, including dairy products, fermented vegetables, fermented dough's, cheese, alcoholic beverages, probiotics in animal feeds, and meat products (Kunene *et al.*, 2000; Rodriguez *et al.*, 2000; Zhu *et al.* 2000). They are non-pathogenic, acid fast, bile tolerant, and exhibit resistance to lysozyme, gastric juice and duodenal fluids. LAB is an important 'biodefence factor' and act against pathogens in the gut by production of antibacterial compounds, competition for nutrition and adhesion sites, and stimulation of immunity. Therefore much interest exists in the use of LAB in food production and food supplements as probiotics. Lactic acid bacteria produces lactic acid from carbohydrates, resulting in a pH decrease that leads to proteolysis as to liberate short products peptides and free amino acids, thus affecting the flavor and texture of products (Mouley *et al.*, 2006). They exert a strong antagonistic activity against many food spoilage bacteria as a result of production of organic acids, ethanol,

Hydrogen peroxide, CO₂, inhibitory enzymes and bacteriocins (Hernadz *et al.*, 2005; Corsetti *et al.*, 2004; Neysens *et al.*, 2003). Bacteriocins (antibiotic like substances) are ribosomally synthesized antimicrobial peptide or proteins that kill mostly closely related bacteria and are produced by both gram positive and gram negative bacteria (Lewus *et al.*, 1991; Paik 1996; Navarro *et al.*, 2000; Savadgo *et al.*, 2004). Being proteinaceous in nature it can easily be digested into amino acids in human gastrointestinal tract (GIT). In recent years interest in this compound has developed significantly due to their potential usefulness as natural substitute for chemical food preservatives.

Probiotic is a food (feed) or drug containing live microbes that when ingested, exert health benefits and confer physiologic effects to the host through microbial actions (Mishra and Lambert 1996; Gardiner *et al.*, 2000; Ohet *et al.*, 2000; Moustafa 2004; Voravuthikunchai *et al.*, 2006). Lactic acid bacteria have a number of properties which render them highly suitable to use as probiotic (Vinderola *et al.*, 2000). Established health benefits of probiotics include treatment of lactose intolerance, a decrease fecal bacterial enzyme and mutagenicity, influence on diabetic condition, osteoporosis condition control (Salminen *et al.*, 1998; Naidu *et al.*, 1999). A good probiotic strain must have potential to tolerate effect of intestinal secretions such as bile which is a complex aqueous secretion composed of 95% water and endogenous solid constituents

*Corresponding author: Muneera Naz Baloch

Department of Microbiology, University of Karachi, Karachi 75270, Pakistan

consisting of bile salts, phospholipid and cholesterol, amino acids, steroids, enzymes, porphyrins, vitamins and heavy metals as well as exogenous drugs, xenobiotics and toxins. Bile salts are the major organic solutes in bile and are necessary for emulsification and digestive absorption of dietary lipids (Michael and Peter 2006). Bile synthesized in the liver from cholesterol and secreted as amino acids conjugates into the duodenum (Ruas-Madiedo *et al.*, 2005). Although bile salts are toxic for cells but the development of tolerance in probiotic strains is an interesting feature which supported the aim of this in vitro study to evaluate the potential of Lactic acid bacteria, isolated from indigenous sources, as probiotic bacteria by characterizing them on the basis of bile salt, acid tolerance and bacteriocin production.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Lactic acid bacteria were isolated from raw milk, yogurt, tomato puree and infant feces, which were collected from different area of Karachi city. All isolates were identified up to genus level by gram reaction, morphology, cultural characteristics and catalase test. Spread plate technique was used for isolation. Samples were diluted 100 fold in PBS (pH 7.0) and 100µl of last two dilutions i.e. 10⁻⁴ and 10⁻⁶ were spread onto MRS agar (Oxoid) plates and incubated at 37°C for 24hrs. Isolated colonies were picked up with a sterile needle and transferred on MRS agar slopes and incubated at 37°C and then stored at 4°C. All cultures were sub cultured routinely. Fresh working cultures were obtained by growing them on MRS broth before their use. Isolates were preserved in the MRS broth containing 15% v/v glycerol (Scharlu) and kept in the refrigerator.

Indicator strains

Lactobacilli isolated from yogurt and milk designated as: LBY-01, LBY-03, LBM-02, LBM-13, LBM-17 and LBM-38 respectively, along with *L. casei* 25598, *B. subtilis* and *S. typhi* were used as indicator strains.

Preparation of cell free culture supernatant (CFCS)

Producer strains were grown in MRS broth at 37°C for 16-18 hrs. Culture tubes were centrifuged at 10,000 rpm for 10 min and supernatants were collected and kept in refrigerator until use.

Assay for bacteriocin activity

The antimicrobial activity of producer strain was detected by Stab assay and Agar well diffusion described by Con and Gokalp (2000). Overnight cultures of LAB were stabbed on MRS agar plates and incubated at 37°C for 24 hrs. Next day filter paper soaked in chloroform (Merck), was placed on petri plate and immediately the lid was covered and left for 20 minutes in order to kill the cells. Fifteen µl of indicator strain (A560 2.0-2.5) was inoculated into 5ml of MRS soft agar (0.85%), mixed well and then poured on to plates and incubated at 37°C for 24 hrs. Next day plates were observed

for zone of inhibition. For well diffusion assay pre poured MRS plates were overlaid with 5ml MRS soft agar containing 10µl of indicator strain (A560 2.0-2.5). Wells were cut in MRS agar plates with the help of cork borer and filled with 150 µl of culture supernatant neutralized to pH 6.5 with NaOH. These plates were kept in refrigerator for 45 min to 1hr for maximum diffusion of supernatant and then incubated at 37°C for 24hrs and examined for zone of inhibition. To demonstrate the antagonistic effect of LAB against bacteria other than LAB 10 µl of indicator strains were inoculated in Nutrient (Merck) or Muller Hinton (MH) (Oxoid) soft agar and over laid on pre poured Nutrient or MH agar plates. In case of *Bacillus subtilis* A600 was between 0.05-0.1 and in case of *S. typhi* 0.3. Rest of the procedure was the same.

Bile tolerance

Flask containing 100 ml MRS broth supplemented with 0.2, 0.4, 0.6, 0.8 and 1 % (w/v) dehydrated bile Ox gall (Sigma) was prepared and inoculated with 1% (v/v) 16 hrs old culture of LBM-86. A control was simultaneously run without bile. Both control and test flask were incubated at 37°C and absorbance was measured at 560 nm after 30 minutes time interval, at each interval CFU/ml-1 was also determined by Miles and Misra technique and agar well diffusion assay was performed to detect the antagonistic agent.

RESULTS

Screening for antimicrobial activity

STAB ASSAY: was performed to detect the inhibitory activity of LAB, against LAB, due to release of metabolites. Twelve strains were screened as producers whereas *L. casei* 25598, LBM-03, LBM-13, LBM-17 and LBM-38 were used as indicator strains. All producers except LBM-08 exhibited zone of inhibition against different indicator strains as shown in Table 1.

Table 1. Screening of bacteriocin activity of LAB by stab assay

PRODUCERS	INDICATORS				
	<i>L. casei</i> 25598	LBM 03	LBM 17	LBM 38	LBM 13
LBT 36	-	-	+/-	+	ND
LCM 82	+	+	+	ND	+/-
LBM 32	ND	ND	+	+	ND
LBY 100	+	+	+	ND	+
LC 09	-	-	+	+	ND
<i>L. casei</i> 25598	ND	-	+	+	ND
LBM 07	ND	-	+	+	ND
LBM 86	ND	-	+	+	ND
LBM 30	ND	-	ND	ND	ND
LBY 05A	+	+	+	+	+/-
LBY 03D	+	+	+	+	+/-
LBM 08C	-	-	-	ND	-

KEYS: +: Zone of inhibition; -: No zone of inhibition; + / -: Partial zone of inhibition; ND: Not determined

AGAR WELL ASSAY: was used to detect the antagonistic activity of all isolates against Gram positive bacteria other than LAB and Gram negative bacteria. CFCS of all producers except LBY-07 was found to be active against *B.subtilis* and *S.typhi* while LBY-01, LBY-04, LBY-06, LBM-09 and LCM-95 did not show inhibitory activity against *B.subtilis*. No other strain except LBT-36 and LBM-86 demonstrated inhibitory activity in their NCFCS (data not shown). Among all the isolates LBM-86 was found to be strong bacteriocin producer strain, hence the strain was selected for further studies on bile tolerance.

Bile tolerance

Five different concentrations of bile 0.2%, 0.4%, 0.6%, 0.8% and 1% were used to investigate the bile tolerance of LBM-86 during growth cycle. As the concentrations of bile increased, there was an increase in absorbance and CFU ml⁻¹ of LBM-86 whereas control showed a normal growth pattern (Figure 1a,b and 2a,b). The zone of inhibition due to bacteriocin production was narrow in 0.2% and 0.4% bile even after 8, 10 and 24 hrs, however with the highest concentration of bile used 0.8, 1 % maximum activity was demonstrated in both CFCS and NCFCS (Figure 3).

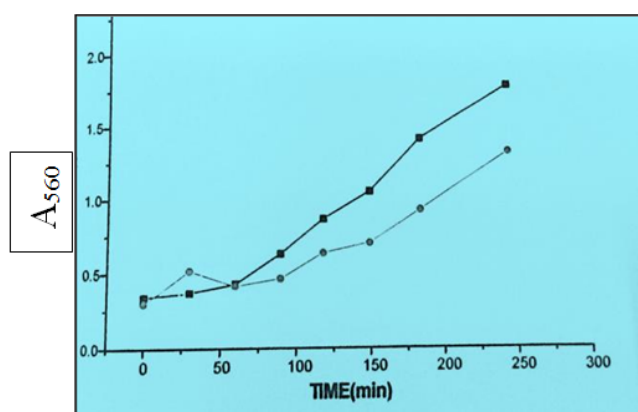


Fig. 1a. Growth kinetics [A₅₆₀] of LBM-86 in MRS broth with 0.8% bile

[—■—] growth in presence of bile; [—○—] growth in absence of bile.

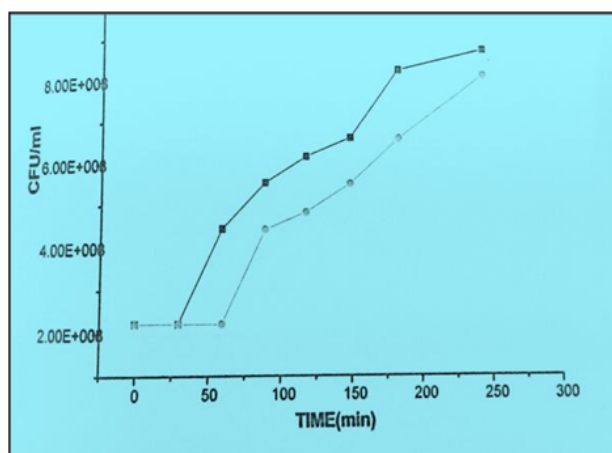


Fig. 1b. Growth kinetics [cfu/ml] of LBM-86 in MRS broth with 0.8% bile

[—■—] growth in presence of bile; [—○—] growth in absence of bile.

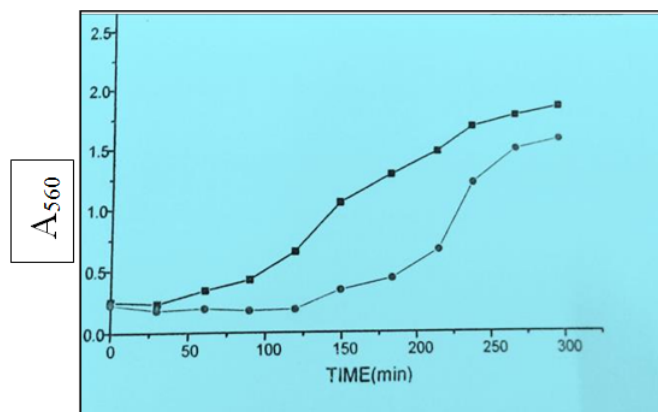


Fig. 2a. Growth kinetics [A₅₆₀] of LBM-86 in MRS broth with 0.1% bile

[—■—] growth in presence of bile; [—○—] growth in absence of bile.

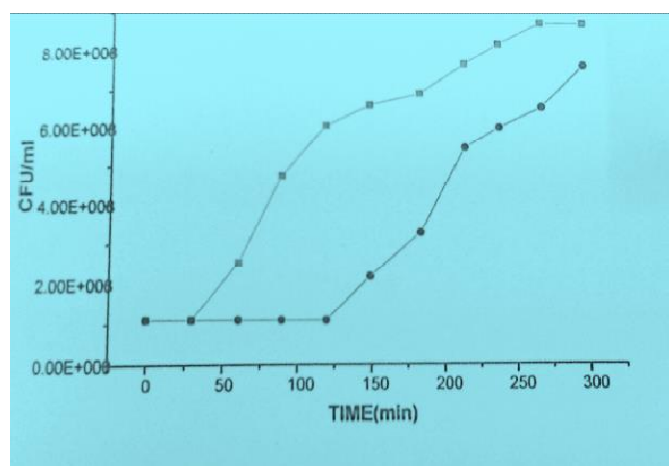


Fig. 2b. Growth kinetics [cfu/ml] of LBM-86 in MRS broth with 0.1% bile

[—■—] growth in presence of bile; [—○—] growth in absence of bile.

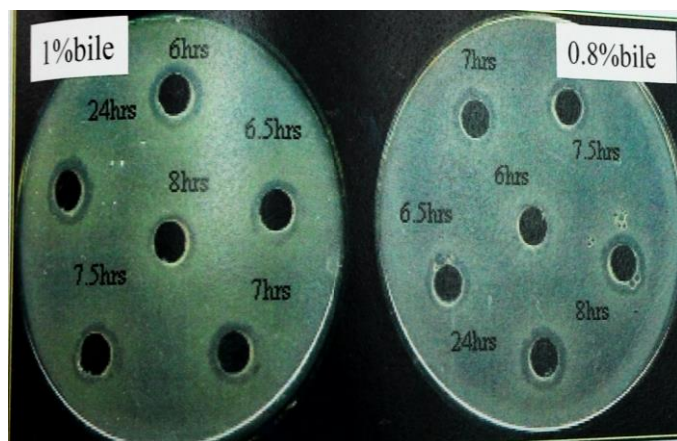


Fig.3. Bacteriocin production by LBM-86 during the growth cycle

DISCUSSION

Thirty two strains of LAB were isolated from yogurt and milk samples whereas one was from tomato and one from infant feces. Although tomato is not a very common source for the isolation, species of *Leuconostoc* as previously reported by

Sajur *et al.* (2007) as dominant LAB species from tomato surface and were considered to reduce the proliferation of contaminant during storage of tomato puree at ambient temperature. In our study milk and yogurt appeared as a good source for the isolation of LAB. Milk was found to be the main source for the isolation of Lactococci and Lactobacilli. The predominance of Lactococci in milk sample implies its proteolytic activity, which provides amino acids for high cell density growth (Moulay *et al.*, 2005). In the present study stab and agar well diffusion assay were used to detect the inhibitory activity. Previously Nowrozi *et al.* (2004) have used these methods for the detection of the antimicrobial activity and reported that 14.3 % (4 out of 28) Lactobacilli were positive for bacteriocin like substances with these assays. The antagonistic activity of LAB is due to variety of metabolites, these substances are cationic peptides and reported to have a hydrophobic properties and the bacterial membranes in the most cases are the target for their activity Savadago *et al.* (2004). If the antimicrobial metabolites produced by LAB are extra cellular and released into the growth medium, inhibition takes place by diffusing through the layer of agar Tadesse *et al.* (2006).

These observations correspond well with our results where the CFCS of all the isolates produced zone of inhibition, but the activity of remaining isolates were lost in NCFCS except LBT-36 and LBM-86 showed the inhibitory activity which may possibly be due to bacteriocin production. McLean & McGroarty (1996), while studying the NCFCS of *L. Acidophilus* and *L. Fermentum* noticed approximately 60% and 95% inhibitory activity was lost from CFCS respectively. Sensitivity of Gram positive bacteria by bacteriocins of LAB has previously been reported by many workers (McAuliffe *et al.*, 1998; Daeschel *et al.*, 1986). Reports on antagonistic activity of LAB bacteriocin against Gram positive bacteria are also available e.g. bacteriocin of *Lactobacillus* sp 100-37 (McCormick and Savage, 1983). Bacteriocin produced by our strain can be categorized as a broad spectrum, because it was found to be active against not only closely related species but also inhibited *B. subtilis* and *S. typhi*.

The most significant role of lactic acid bacteria is their use as a probiotic because of their useful metabolic and potential health associated properties. A commercially accepted probiotic must have the following properties: to bring about desired health benefits i.e. adherence to the host epithelial tissue, acid and bile tolerance, pathogen exclusion by occupying the adhesion sites, acids, hydrogen peroxide, bacteriocin production safe for use, show improvement for intestinal microform. Bile tolerance is considered to be prerequisite for probiotic LAB for colonization in the GIT. Therefore we investigated this aspect of LAB-86, recording its antagonistic activity at different concentrations of bile. The extreme concentration obtained in animal or human intestine during the first hour of digestion is 2% whereas, the concentration of bile in intestine is 0.3% (Mourad and NourEddine, 2006). It is quite difficult to suggest a precise concentration to which the selected strain should be tolerant Havenaar *et al.* (1992). However LBM-86 displayed resistance to even 1% bile which is quite promising and higher than those reported by Fernandez *et al.* (2003) where (*L. acidophilus* and *L. Gasseri*) were resistant to 0.15%

bile salt. On the other hand Olejnik *et al.* (2005) reported that *L. acidophilus*, *L. Helveticus* and *L. Casei* were resistant to high concentration of bile as high as 3 % whereas in other report *L. lactis*, *L. helveticus* and *L. mesenteroides* showed maximum tolerance 2.1% of bile salts (Bunthof *et al.*, 2001). The resistance to bile salts varies a lot among LAB species and even between the strains themselves (Xanthopoulos 1997) and those having high resistance to bile salts can be considered as probiotic strains, for example different strains of *L. acidophilus* (Chou and Weimer 1999; Suskobvic *et al.*, 1997). Bile resistance of some strains is associated to specific enzyme activity bile salt hydrolase (BSH), which helps to hydrolyse conjugated bile, thus reducing its toxic effect (Du Toit *et al.*, 1998). BSH activity in LAB is strongly correlated with natural habitat, those species that live in mammalian intestines have 100% BSH activity such as *L. acidophilus*, *L. gasseri* and *L. johnsonii*, while strains of typical dairy species like *L. delbrueckii* and *L. helveticus* have either no or a very low BSH activity (Tanaka *et al.*, 1999). On determining bacteriocin production during the growth cycle of LBM-86, it was observed that bacteriocin production started in late exponential phase. Aly *et al.* (2006) reported that bacteriocin production in LAB is growth dependent. It usually occurs throughout the growth phase and ceases at the end of the exponential phase (or sometimes before the end of the growth phase). It is known that bacterial cells can rely on mechanisms for survival and resistance against stress condition.

For instance, exposure to various concentration of bile results in the expression of genes, which leads to increased resistance. On the contrary some reports that bacteriocin production is stimulated by stress conditions, resulting in lower growth rates, lower cell yields and relatively high bacteriocin activity (Vuyst *et al.*, 1996). Our findings correlate with this and as we noticed an enhanced bacteriocin production at high concentration of bile even with no cell growth. Reports indicating decrease in bacteriocin production in the presence of bile salts also available as Leroy and Vuyst (1999) suggested that decrease is due to interference of salt molecules with binding of induction factor, which is essential for bacteriocin production to its receptor. In another statement of Fernandes and Shahani (1988) reported the effect of bile salts on bacteriocin production, as the concentration of bile salt in the broth increase, antimicrobial activity decreased. In conclusion, since LBM-86 exhibited promising results regarding bile tolerance along with showing strong antagonism possess great potential for further research to explore its role as probiotic strain. Although further studies are desirably required to investigate all the essential probiotic properties until which it could be known as an ideal probiotic candidate and accepted for commercial use.

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REFERENCES

- Aly, S., Cheik, O., Imael, A.T.B. and Alfred, T.S. 2006. Bacteriocins and lactic acid bacteria - a mini review. *Afr.J.Biotechnol.*, 5(9): 678-683.

- Bunthof, J. C., Bloemen, K. P., Breeuwer, M.F., Rombouts. And Abee, T. 2001. Flow cytometric assessment of viability of lactic acid bacteria. *Appl. Environ. Microbiol.*, 67: 2326-2335.
- Chou, L.S. and Weimer, B. 1999. Isolation and characterization of acid and bile tolerant isolated from strains of *L.acidophilus*. *J. Dairy. Sci.*, 82: 23-31.
- Con, A. H. and Gokalp, H. Y. 2000. Production of Bacteriocin-like metabolites by lactic acid cultures isolated from sucuk samples. *Meat Sci.* 55: 89-96.
- Corsetti, A., L. Settanni and Sinderen, V. 2004. Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their in vitro and in situ activity. *J.Appl. Microbiol.* 96:521-534.
- Daeschel, M. A., Mckenney, M.C. and McDonald, L.C. 1990. Bacteriocidal activity of *Lactobacillus plantarum* C-11. *Food microbial.*, 7: 91-98
- De Vuyst, L., Callewaert, R. and Crabbe, K. 1996. Primary metabolite kinetics of bacteriocin biosynthesis by *Lactobacillus amylovorus* and evidence for stimulation of bacteriocin production under unfavorable growth conditions. *Microbiology*, 142:817-827.
- Du Toit, M., Schillinger, C.U., Warles, B. and Holzappfel, W. 1998. Characterization and selection of probiotic lactobacilli for a preliminary minipig-feeding trial and their effect on serum cholesterol level, feces pH and feces moisture contents. *Int. Food. Microbiol.*, 40: 93-104.
- Effect of dominant specie of lactic acid bacteria from tomato on natural microflora development in tomato puree. *Food control.* 18:594-600.
- Fernandes, C.F. and Shahani, K.M. 1988. Effect of nutrient media and bile salts on growth and antimicrobial activity of *Lactobacillus acidophilus*. *J. Dairy Sci.*, 71:3222-3229.
- Fernandez, F.M., Boris, S. and Barbes, C. 2003. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J.Appl.Microbiol.*, 94: 1-9.
- Gardiner, G. E., O'Sullivan, E. Kelly, J., Auty, M.A. E., Fitzgerald, G. F., Collins, J. K., Ross, R.P. and Stanton, C. 2000. Comparative survival rates of human-derived probiotic *L. paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Appl. Environ. Microbiol.*, 66(6):2605-2612.
- Havenaar, R., Ten Brink, B. and Huis, J.H.J. 1992. Selection of strains for probiotic use. In: Probiotics. The Scientific basis ed. Fuller, R. 209-224. London: Chapman and Hall.
- Hernandez, D., Cardell, E. and Zarate, V. 2005. Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, bacteriocin-like substances produced by *Lactobacillus plantarum* TF711. *J. Appl. Microbiol.*, 99:77-84.
- Kunene, N. F., Geornaras, I., von Holy, A. and Hastings, J.W. 2000. Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented weaning food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. *Appl. Environ. Microbiol.*, 66(3):1084-1092.
- Leroy, F. and De Vuyst, L. 1999. The Presence of salt and a curing agent reduce bacteriocin Production by *Lactobacillus sakei* CTC 494, a Potential starter culture for sausage fermentation. *Appl. Environ.Microbial.*, 65(12): 5350-5356.
- Lewus, C.B., Kaiser, A. and Montiville, T.J. 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.*, 57(6):1683-1688.
- McAuliffe, O., Ryan, M.P., Ross, R. P., Hill, C., Breeuwer, P. and Abee, T.1998. Lacticin 3147, a Broad-Spectrum Bacteriocin Which Selectively Dissipates the Membrane Potential. *Appl. Environ. Microbiol.*, 64(2):439-445.
- McLean, N.W. and McGroarty, J.A. 1996. Growth inhibition of metronidazole susceptible and metronidazole resistant strains of *Gardnerella vaginalis* by lactobacilli in vitro. *Appl. Environ. Microbiol.*, 62:1089-1092.
- Michael, T. and Jansen, P. 2006. Mechanisms of bile formation- an introduction. Chapter category: Channels and Transporters. Molecular pathogenesis of Cholestasis. Bioscience chapter database, 3387.
- Moulay, M., Aggad, H., Benmecherrhene, Z., Guessas, B., Henni, D.E. and Kihal, M. 2005. Cultivable Lactic acid bacteria isolated from Algerian raw goat's milk and their proteolytic activity. 2006. *W.J. Dairy & Food. Sci.*, 1(1): 12-18.
- Mourad, K. and Nour-Eddine, K. 2006. In vitro preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. *Int. Probiotics and Prebiotics.* 1(1):27-23.
- Moustafa Y.M. El-Naggar. 2004. Comparative study of probiotic cultures to control the growth of *Escherichia coli* 0157: H7 and *Salmonella typhimurium*. *Biotechnology*, 3(2):173-180.
- Naidu, A. S., Bidlack, W.R. and Clemens, R. A. 1999. Probiotic spectra of lactic acid bacteria (LAB). *Critical rev.in Food Sci.Nutr.*, 39: 13-126.
- Navarro, L., Zarazaga, M., Aenz, S.J., Ruiz-Larrea, F. and Torres, C. 2000 Bacteriocin production by 6 lactic acid bacteria isolated from Rioja red wines. *J. Appl. Microbiol.*, 88:1-44.
- Nowroozi, J., Mirzaii, M. and Norouzi, M. 2004. Study of *Lactobacillus* as probiotic bacteria. *Iranian. J. Publ Health*, 33(2): 1-7.
- Oh S., Kim, S.H. and Worobo, R.W. 2000. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. *J Dairy Sci.*, 83:2747-2752.
- Olejnik, A., Lewandowska, M., Obarska, M. and Grajek, W. 2005. Tolerance of *Lactobacillus* and *Bifidobacterium* strains to low pH, bile salts and digestive enzymes. *Food. Sci. Technol.*, 8: 1505-0297.
- Paik, Hyung-Dong and Doo-whan OH. 1996. Purification, characterization and comparison of bacteriocins. *J. Microbiol. Biotechnol.*, 6:151-161.
- Rodriguez, E., Gonzales, B., Gaya, P., Nunez, M. and Medina, M.2000. Diversity of bacteriocin produced by lactic acid bacteria isolated from raw milk. *Int. Dairy J.*, 10, 7-15.
- Ruas –Maldeido, P., Hernandez-Barranco, A., Margolles, A. and de los Reyes-Gavila, C.G.2005. A bile salt-resistant derivative of *Bifidobacterium animalis* has an altered fermentation pattern when grown on glucose and maltose. *Appl. Environ. Microbiol.*, 71(11): 6564-6570.
- Sajur, S.A., Saguir, F.M. and Manca de Nadra, M. C. 2007.

- Salimen, S; Bouley, C.M., Boutron-Ruault, C., Cummings, J.H., Franck A., Gibson, G.R., Isolauri, E., Moreau, M. C., Roberfroid, M. B. and Rowland, I. 1998. Functional food science and gastrointestinal physiology and function. *British J. Nutr.*, 80: 147-171.
- Savado, A., Ouattara, C. A. T., Bassole, I. H. N. and Traore, A.S. 2004. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pak. J. Nutr.*, 3 (3): 174-179.
- Suskovic, J., Brkic, B., Matosic, S. and Maric, V. 1997. *L. acidophilus* M92 as potential probiotic strains. *Milchwissenschaft*, 52: 430-435.
- Tadesse, G., Ephraim, E. and Ashenafi, M. 2006. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. *Int. J. Food Safety*, 5: 13-20.
- Tanaka, H., Doesburg, K., Iwasaki, T. and Mierau, I. 1999. Screening of lactic acid bacteria for bile salt hydrolase activity. *J. Dairy Sci.*, 82: 2530-2535.
- Vinderola, C. G., Mocchiutti, P. and Reinheimer, J. A. 2002. Interaction among lactic acid starter and probiotic bacteria used for fermented dairy products. *J. Dairy Sci.*, 85:721-729.
- Voravuthikunchai, S. P., Bilasoi, S. and Supamala, O. 2006. Antagonistic activity against pathogenic bacteria by human vaginal *Lactobacilli*. *Anaerobe*, 12: 221-226.
- Xanthopoulos, V., Litopoulou- Tzanetaki, E. and Tzanetakis, N. 1997. In vitro study of *Lactobacillus* species strains on bile tolerance and cholesterol removal. In: *Lactic Acid Bacteria-Lactic*. 97. Caen: Presses Universitaires de Caen.
- Zhu, W. M; Liu, W. and Wu, D. Q. 2000. Isolation and characterization of a new bacteriocin from *Lactobacillus gasser* KT7. *J. Appl. Microbiol.*, 88:877-886.
