Original Paper

ISSN: 2321-1520

Efficacy and Functional Aspects of Commercially Available Probiotic Products

Singh Khushboo, Saraf Meenu*

Deapartment of Microbiology and Biotechnology,
School of Sciences, Gujarat University Ahmedabad .

sarafmeenu@gmail.com

*Corresponding author

Received Date: 23-11-2016
Published Date: 1-3-2017

Abstract

Probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of human being by improving the intestinal microbial balance. Probiotic bacteria may produce various compounds, which are inhibitory to the growth of pathogen, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. In the present study, a total of six commercial probiotic products were assessed if they contain efficient probiotic microbes which could survive in the human GIT thereby promoting positive health benefits to humans. Total four isolates were isolated and tested for their probiotic potentials. Futher all four of them were also checked for their functional aspects *in vitro.* All isolates were found to be Gram positive and were evaluated to be efficient probiotics. They were identified by Vitek 2 compact method and found to be *Saccharomyces cerevisiae*, *Lactobacillus sporogenes, Enterrococcus hirae* and *Alicyclobacillus acidocaldarius.*

Introduction

The term probiotic was defined as "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance" (Aslam and Qazi, 2010). Probiotic bacteria may produce various compounds, which are inhibitory to the pathogen's growth, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. The organic acids not only lower the pH, thereby affecting the growth of the pathogen, but they can also be toxic to

the microbes (Tambekar and Bhutada, 2010). There is increasing evidence that probiotics are beneficial in gastrointestinal disturbances, such as diarrhoea, dysentery, typhoid etc (Tambekar and Bhutada, 2010).

The most common genera *Lactobacillus* and *bifidobacterium,* because they are considered as GRAS (Generally Recognized As Safe) (Butel 2014). *Lactobacillus* and *Bifidobacterium* species are also dominant inhabitants in human intestine (Rivera Espinoza 2010). However bacterial species belonging to *Lactococcus, Enterococcus*, *Propionibacterium,* yeasts and filamentous fungi are also used as probiotics due to their health promoting nature (Tripathi and Giri 2014). However more specific and profound targeted function in the human alimentary tract is provided by dairy probiotic products supplemented with multispecies (Saxelin et al., 2010).

Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the LAB and *Bifidobacteria*. Within the group of LAB, *Lactobacillus* species are most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics. Lactic acid bacteria including *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus* and *Bifidobacterium* are found throughout the gastrointestinal tract.

Lactobacillus and *Bifidobacterium spp.* are prominent members of the intestinal flora and are the commonly studied probiotic bacteria. They cause reduced lactose intolerance alleviation of some diarrhea's, lowered blood cholesterol, increased immune response and prevention of cancer. The selection criteria for probiotic LAB include: safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue ability to colonize the gastro -intestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose activity (Savodago *et al*., 2006).

Lactic acid bacteria (LAB) play a critical role in food production and health maintenance. There is an increasing interest in these species to reveal the many possible health benefits associated with them. The actions of LAB are species and strain specific, and depend on the amount of bacteria available in the gastrointestinal tract. Consumers are very concerned of chemical preservatives and processed foods. However, products with or processed with LAB are accepted as a natural way to preserve food and promote health.

Probiotic products need to be supplemented with additional ingredients to support the viability throughout processing, storage, distribution, and gastrointestinal tract to reach the colon. Several reports have shown that survival and viability of probiotic bacteria is often low in yogurt. The efficiency of added probiotic bacteria depends on dose level and their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment.

Therefore an attempt was made to isolate probiotic bacteria from commercial products and

to evaluate their potential as probiotics.

Materials And Methods

Isolation and identification of bacteria from commercial samples

For isolation of probiotic bacteria, serial dilution agar technique was used. Serial dilution of 10^{-1} to 10^{-7}were prepared. 0.1 ml of each dilution was inoculated to MRS agar plates and incubated at 37^{0}C for 24-48h for bacterial growth. The plates were observed for appearance of colonies and each of the isolates were purified onto the slants which were maintained in refrigerator for further analysis. Cultural and morphological characteristics were noted for all the isolates. Further confirmative tests like Gram staining, 3% KOH string test, acid fast staining, capsule staining, metachromatic granule staining, catalase test, oxidase test and motility test were performed.

Evaluation of probiotic potentials of isolated bacterial cultures

pH tolerance: The isolated bacterial cultures were inoculated into sterile MRS broth tubes of varying pH, i.e. pH 2, 3, 4, 5, 6, 7 and 8 and incubated at 37^{0}C for 2-3 days. Sensitivity to low pH conditions by the acid tolerant LAB were determined according to the method followed by (Anukam 2007).

Bile salt tolerance : The isolates were inoculated onto sterile MRS agar plates with varying bile concentrations (0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 2.5%) and incubated at 37^{0}C for 48h. The growth of cultures on agar plates was used to designate isolates as bile salt tolerant (Tambekar and Bhutada, 2010).

NaCl tolerance: The test isolates were grown in MRS broth tubes for 24h at 37ºC containing variable salt concentrations of 1%, 3%, 5%, 8%, 10% and 15% . Growth of the bacterial isolates was determined spectrophotometrically at 600 nm.

Temperature sensitivity: The selected bacterial cultures were grown at varying temperatures, i.e. 37, 55, 4^{0}C and room temperature for 48-72 hrs. (Tambekar and Bhutada, 2010).

Lactose utilization: Sterilized fermentation medium was inoculated with different cultures and incubated at 35ÚC for 24-48 hrs. Change in colour from red to yellow indicates the production of acid (Ahmed and Kanwal, 2004)

Antibiotic susceptibility: The test isolates were initially grown in the MRS broth at 37ºC for 24h under microaerophilic condition for activation. 0.1ml suspension of standardized freshly grown bacterial cultures was spread on MRS agar plates. The antibiotic discs were placed on the surface of agar and the plates were incubated at 37ºC for 48 hrs. Positive results were observed as zone of inhibition surrounding the disc.

Titrametric determination of lactic acid production: MRS broth was inoculated with activated test isolate cultures. Samples were withdrawn from each flask and centrifuged at 10,000rpm for 15 minutes. Collected supernatant was titrated with 2-3 drops of phenolphthalein

against 0.1 (N) NaOH. Amount of NaOH used for titration was noted and lactic acid % was calculated (Demirci 1994).

Functional aspects of probiotic isolates

Cholesterol lowering potential: The cholesterol estimation was done by CHOD-POD method. A 9.9 ml of MRS broth containing 0.2% bile salt (w/v) was mixed with 0.05 ml of serum containing high cholesterol was inoculated separately with 0.1ml of 24h active test culture isolates. Control containing 9.9 ml of MRS broth adjusted with 0.2% bile salt and 0.05 ml of serum was prepared containing high cholesterol but without inoculation of isolates. Further initial and final cholesterol estimation for each MRS broth containing serum of high cholesterol was estimated by enzymatic method using cholesterol estimation kit.

Calculation

Amount of cholesterol present in the serum was calculated by the following equation:-

Cholesterol (mg/dl) = absorbance of test/ Absorbance of standard x 200

Exopolysaccharide production: Active inoculums of isolates were inoculated in sterilized modified MRS broth. Initial and final viscosity of broth was measured after five days and finally the broth was proceeded for EPS extraction

Antimicrobial activity: The test organisms were enriched in nutrient broth. Antimicrobial activity was determined by agar well diffusion method. Sterile Mueller Hinton agar plates were then inoculated with 0.1 ml of each standardized test cultures by spread plate method. Wells of approx 6-7 mm were prepared and loaded with 0.02 ml of isolated probiotic culture supernatents and these plates were incubated at 37ºC for 24-48h. The antimicrobial activity was determined by measuring the diameter of inhibition zone (mm) surrounding the wells.

Haemolytic activity: 24 h activated cultures were spot inoculated onto the blood agar medium and the plates were observed as clear zones surrounding the colonies.

Cell surface hydrophobicity determination (to assess adhesion property of probiotics): Cell surface hydrophobicity of isolates was determined by microbial adhesion to hydrocarbons (MATH) method described by (Geertsema-Doornbusch *et al.,* 1993) using hexadecane and toluene as solvents.

Oxalate degradation capacity

The isolated cultures were tested for oxalate utilization using agar well-diffusion method in calcium oxalate plate as described by Allison and Campieri (Allison 1985 and Campieri et al., 2001).

Identification of the isolates

Genetic identification of all the four isolates was done by API card system i.e. by **VITEK 2 COMPACT** method. Vitek 2 is an automated microbiology system utilizing growth based technology. This system works on colorimetric reagent cards that are incubated and are interpretated automatically.

Results And Discussions

Probiotic potential of cultures : pH and Bile salt tolerance

In the present study, total four isolates were isolated from the assessed commercial probiotic products. These isolates were tested for their probiotic potentials like pH, salt, bile salt tolerances and different temperature conditions. In the present study, all the isolates showed least growth at pH 2, hence its concluded that isolates LBC and VBT were able to grow at 3pH leaving the rest isolates that show only 50% survival in the GIT. In case of bile tolerance isolates were able to survive at 0.1%, 0.2%, 0.5%, 1% and 1.5% bile salt concentrations. In addition the LBC isolate was able to survive even at 2% bile concentration. On other hand lactic acid bacteria isolated from baobab (maari) fermented seeds were able to survive at pH 2.5 but could tolerate bile salt concentration of 0.3% only (Kabore *et al.*, 2012). Tambekar (2010) reported that the three isolated excellent probiotic acid tolerance at pH 2.0 and bile salt tolerance at 2.0%. Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host (Havenaar et al.,1992). This will help *Lactobacilli* to reach the small intestine and colon and contribute in balancing the intestinal microflora (Tambekar and Bhutada, 2010).

NaCl tolerance, Temperatures, and Lactose utilization

In case of varying salt concentrations, the isolates were able to survive at 1-8% with the isolate LBC as an exception surviving even at 10% concentration. If the lactic acid bacteria was sensitive to NaCl then it would not be able to show it's activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacterial isolates, whereas Hoque *et al*. (2010) observed the NaCl (1-9%) tolerance of their *Lactobacillus* sp. isolated from yoghurts. Whereas isolates were tested for varying temperature conditions and were found to survive at temperature 37ºC, 55ºC and room temperature. The temperature is an important factor which can dramatically affect the bacterial growth. The reason for choosing this temperature range was to detect whether the isolated cultures were able to grow within range of normal body temperature or not. As if the isolates were not able to survive within the selected temperature range then they would not have been able to survive in the human gut, which is an essential factor of probiotics to show their effectiveness. The results obtained were positive for growth at chosen temperature range. The entire selected LAB isolates were grown in fermentation medium supplemented with lactose and were observed for change in colour from red to yellow which indicates the production of lactic acid. Lactose utilisation of LAB isolated from camel milk was assessed by Ahmed and Kanwal (2004).

Antibiotic susceptibility

Further isolates were tested against twelve antibiotics and susceptibility pattern was noted down for all of them. Isolate A was sensitive to drug Ampicillin (10mm), Co-trimoxazole (21mm), Cephalexin (25mm), Tetracyclin (7mm), Ciprofloxacin (18mm), Levofloxacin (12mm), Linezoloid (14mm), Cloxacillin (16mm), Roxythromycin (12mm), Lincomycin (9mm), Gentamicin (10mm).

Isolate B was sensitive to drug Ampicillin (22mm), Co-trimoxazole (20mm), Cephalexin (24mm), Tetracyclin (7mm), Ciprofloxacin (19mm), Levofloxacin (18mm), Linezoloid (15mm), Cloxacillin (18mm), Roxythromycin (10mm), Lincomycin (8mm), Gentamicin (7mm). Isolate C was sensitive to drug Ampicillin (23mm), Co-trimoxazole (17mm), Cephalexin (20mm), Tetracyclin (14mm), Ciprofloxacin (16mm), Levofloxacin (23mm), Linezoloid (22mm), Cloxacillin (15mm), Roxythromycin (12mm), Lincomycin (13mm), Gentamicin (9mm). Isolate D was sensitive to drug Ampicillin (25mm), Co-trimoxazole (15mm), Cephalexin (10mm), Tetracyclin (22mm), Ciprofloxacin (18mm), Levofloxacin (24mm), Linezoloid (20mm), Cloxacillin (17mm), Roxythromycin (14mm), Lincomycin (12mm), Gentamicin (10mm). In addition all the isolates showed resistance to the drug cefotaxime.

In present study isolates were sensitive for Cephalexin but contrary to present work, the work done by Tambekar and Bhutada (2010) the isolates were resistant to Cephalexin. This may vary from strain to strain or type of strain.

Functional applications

In vitro cholesterol lowering property

Further some of the functional applications were also performed *in vitro.* Initially cholesterol lowering effect was assessed for all the isolates. All the isolates were able to reduce high serum cholesterol. The initial cholesterol levels in the MRS broth for all the isolates were found to be 252, 280, 350 and 271mg/dl respectively. And the final cholesterol levels reduced by the isolates were found to be 134, 147, 125 and 143mg/dl respectively. Since deconjugated bile acids are less soluble and are less likely to be absorbed from the intestinal lumen than are conjugated bile salts, free bile is more likely to be excreted through the intestinal tract. Therefore, with the help of BSH, deconjugation of bile salts could lead to a reduction of serum cholesterol by reducing cholesterol absorption through the intestinal lumen. Klaver and vander Meer (1993) showed that the degree of deconjugation by *L. acidophilus* RP32 was higher under more acidic conditions than if the pH was maintained at 6.0. They concluded that the removal of cholesterol was due to its coprecipitation with deconjugated bile salts in an acidic environment.

EPS production

Next assessed parametre was the EPS production activity. From the observed results all the isolates were able to produce EPS in the MRS broth supplemented with additional 5% sucrose. The obtained EPS production for all the isolates was 1.087, 1.035, 1.071 and 1.064 g/100ml respectively. Kanmani et al., 2011 determined the production of EPS from *S. phocae* PI80 and *E. faecium* MC13 which was influenced by addition of carbon sources lactose (20 g L-1) and sucrose (30 g L-1) in MRS broth. Similarly, Ismail and Nampoothiri (2010) reported that maximum EPS production by *L. plantarum* MTCC 9510 was observed in presence of lactose (40 g L-1). Arskold et al. (2007) reported that the production of EPS from *L. reuteri* ATCC 55730 was significantly influenced by sucrose (100 g L-1). Wang et al. (2010) reported that the amount of EPS production and properties are greatly dependent on the microorganisms

and their culture conditions such as temperature, pH and media composition.

Cell surface hydrophobicity determination and Antimicrobial activity

Third parameter was the cell surface hydrophobicity determination. This parameter was carried out in order to determine microbial adhesion to hydrocarbons by (MATH) method. All the isolates exhibited adhesing capacity to hydrocarbons. Hence these isolates can also be further tested on animal models or *in vivo.*The adhesion percent for all the isolates were 61.30%, 66.82%, 49.16% and 50.06% respectively. Piette and Idziak (1992) have reported that cell-surface charge and hydrophobicity can considerably influence the strength of adhesion. Jacobson *et al*., (1999) suggested adhesion scores of all the isolates except *L. delbrueckii* subsp. *bulgaricus* CH4 were more than 100 and therefore, *L. plantarum* Lp9, Lp72, Lp75, Lp77, Lp90 and Lp91 can be regarded as strongly adhesive. The fourth parameter studied was the antimicrobial activity. Maximum zone of inhibition was shown by the isolate LBS against *S.aureus* of 24mm followed by *E.coli* (20mm), *B.cereus* (18mm) and *B.megaterium* (17mm) ; isolate VBT showed maximum zone of inhibition against *B.megaterium* of 22mm followed by *E.coli* (19mm), *S.aureus* (13mm) and *B.cereus* (11mm) ; isolate AFC showed maximum zone of inhibition against *E.coli* of 23mm followed by *B.megaterium* (20mm), *S.aureus* (16mm) and *B.cereus* (14mm) and lastly the isolate LBC showed the maximum zone of inhibition against *S.aureus* by 20mm followed by *E.coli* (18mm), *B.megaterium* (15mm) and *B.cereus*(12mm). The antibacterial activity was may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compounds (Bezkorvainy 2001).

Haemolytic activity and oxalate degradation potential

The next assessed was the virulence factor of probiotics i.e., haemolytic activity. From the observed results no isolate showed clear zone of hydrolysis surrounding the colony on the blood agar plates.

Lastly oxalate degradation potential was determined for all the isolates. And here also no zone of oxalate hydrolysis was observed on the MRS medium supplemented with 1% calcium oxalate.

Turroni found that *Lactobacillus acidophilus* and *Lactobacillus gasseri* showed significant oxalate degradation in 5mM oxalate whereas other strains showed less oxalate consumption especially; *Lactobacillus salivarius* which showed 20% oxalate degrading ability (Turroni et al., 2007). Murphy also reported that oxalate utilization among probiotics *in vitro* was interspecies dependent (Murphy et al., 2009)

Identification of the isolates

Identification of the isolates was done by Vitek 2 compact system. Isolate LBCwas identified as *Saccharomyces cervisiae* with 99% probability. Isolate VBT was identified as *Enterococcus hirae* with 99% probability. Isolate LBS was identified as *Bacillus coagulans/ Lactobacillus sporogenes* with 91% probability. Isolate AFC was identified as *Alicyclobacillus*

acidocaldarius/Alicyclobacillus acidoterrestris with 92% probability.

Results And Discussions

Table 1. Showing bile salt tolerance of isolates

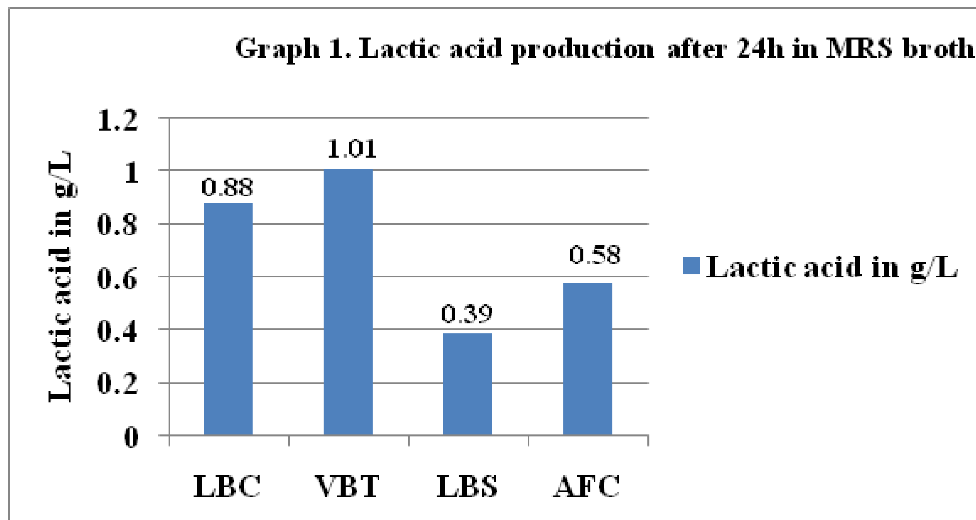
Isolates	0.1%	0.2%	0.5%	1%	1.5%	2%	2.5%
LBC	++++	+++	+++	++	++	+	-
VBT	++	+	-	-	-	-	-
LBS	+++	++	+	+	-	-	-
AFC	++	+	+	-	-	-	-

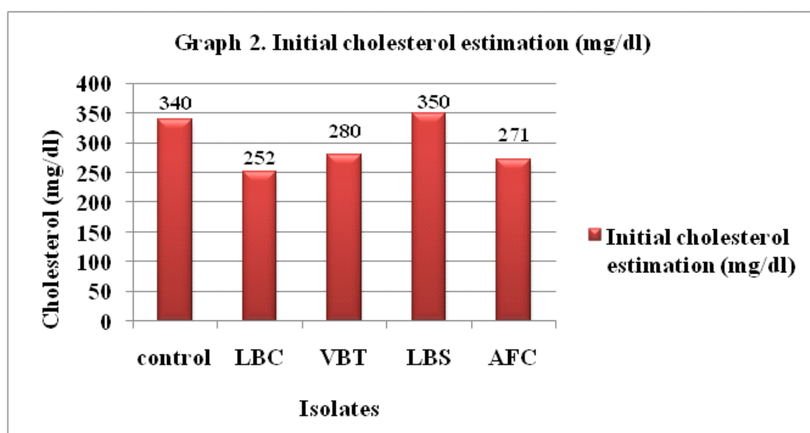
Table 2 Showing NaCL tolerance of isolates

Isolates	NaCL concentrations					
	1%	3%	5%	8%	10%	15%
LBC	1.437	1.288	1.265	0.987	0.486	-
VBT	1.165	1.118	0.900	1.407	-	-
LBS	1.207	1.308	1.367	1.104	-	-
AFC	1.242	1.207	0.712	0.102	-	-

Table 3. Showing results for antibiotic susceptibilty

Antibiotics (mcg)	Diameter in millimeter(mm)			
	Isolate LBC	Isolate VBT	Isolate LBS	Isolate AFC
Ampicillin sulbactum (AS-20)	10	22	23	25
Co-trimoxazole (BA-25)	21	20	17	15
Cephalexin (PR-30)	25	24	20	10
Tetracyclin (TE-30)	7	7	14	22
Cefotaxime (CF-30)	00	00	00	00
Ciproofloxacin (RC-5)	18	19	16	18
Levofloxacin (QB-5)	12	18	23	24
Linezoloid (LZ-30)	14	15	22	20
Cloxacillin (CX-1)	16	18	15	17
Roxythromycin (AT-15)	12	10	12	14
Lincomycin (LM-2)	9	8	13	12
Gentamicin (GM-10)	10	7	9	10





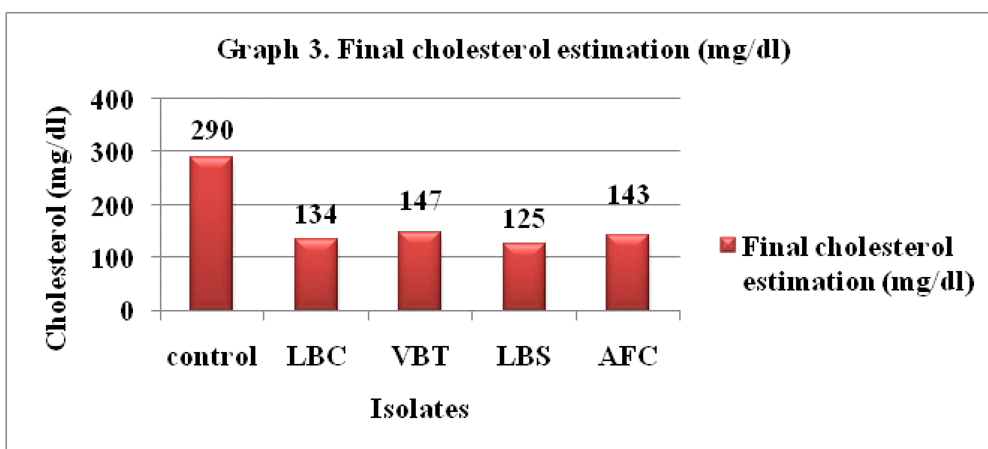
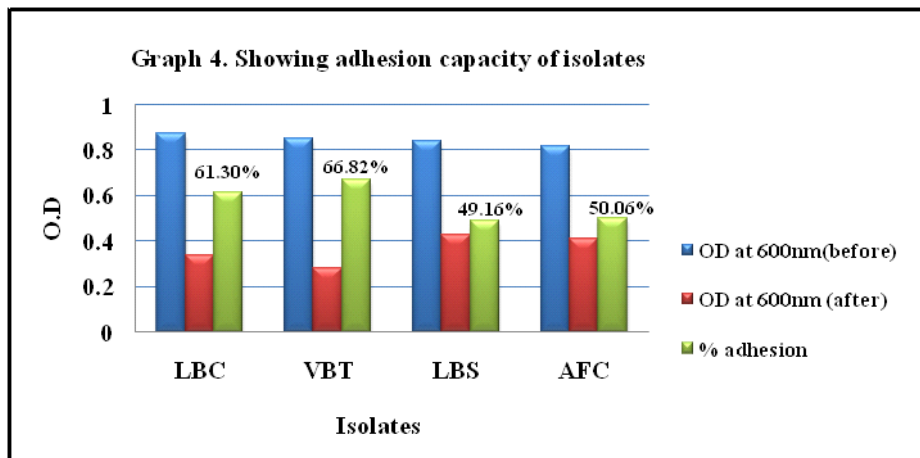


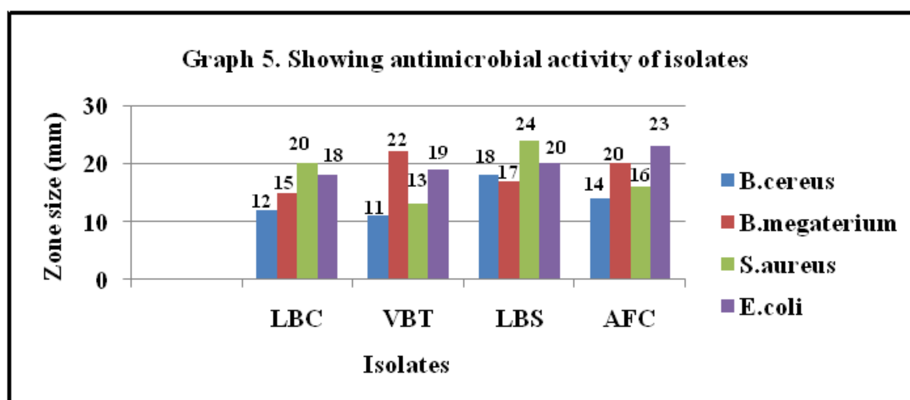
Table 4. Viscosity estimation of isolates

Isolates	Viscosity of fermentation broth in m.pa.sec^{-1}		
	Initial viscosity	Viscosity after 3 days	Viscosity after 5 days
LBC	0.158	1.364	0.382
VBT	0.137	1.280	0.360
LBS	0.161	1.563	1.212
AFC	0.127	1.418	0.371

Table 5. Showing results for EPS production after incubation

Isolates	EPS wet wt g/100ml		EPS dry wt g/100ml	
	After 3 days	After 5 days	After 3 days	After 5 days
LBC	1.367	1.154	1.101	1.087
VBT	1.121	1.078	1.042	1.035
LBS	1.428	1.125	1.115	1.071
AFC	1.169	1.132	1.090	1.064





Identification of the isolates by Vitek 2 compact instrumentation

All the isolates were identified Vitek 2 compact method. The identified isolates were ***Saccharomyces cerevisiae, Enterococcus hirae, Lactobacillus sporogenes/Bacillus coagulans and Alicyclobacillus acidoterrestris/acidocaldarius.***

Conclusion

In the present study, the probiotic isolates were obtained from six different commercial probiotic products. Total four isolates were obtained all of which were gram positive and were carried further for the entire study. The basic aim for the present investigation was to test the commercial probiotic products that claim to contain the efficient probiotics that could confer potential health benefits to humans. The obtained isolates were initially evaluated for their probiotic capabilities and were then further tested for their functional aspects. Hence the commercial products assessed for their probiotic criterias do contain good probiotic organisms that can confer potential health benefits to humans. In addition all the isolates could also be implemented for the animal studies or on animal models for further assessment.

References

Aslam, S. and Qazi, J.I. Isolation of acidophilic lactic acid bacteria antagonistic to microbial contaminants. Pakistan Journal of Zoology, 2010; 42(5): 567-573.

Anukam KC, Koyama TE (2007) Bile and Acid Tolerance of Lactobacillus plantarum KCA-1: A potential probiotic agent. International Journal of dairy Science 2:275-280.

Ahmed, T. and Kanwal, R. Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture. Pakistan Vet. J. 2004; 24:87-91.

A. Hodgkinson and A. Williams, "An improved colorimetric procedure for urine oxalate," *Clinica Chimica Acta*, vol. 36, no. 1, pp. 127–132, 1972.

Butel MJ (2014) Probiotics, gut microbiota and health. Med Mal infect 44:1-8.

Bezkorovainy A. Probiotics: determinants of survival and growth in the gut. Am J Clin Nutre 2001; 73:399S-405S.

C. Campieri, M. Campieri, V. Bertuzzi et al., "Reduction of oxaluria after an oral course of lactic acid bacteria at high concentration," *Kidney International*, vol. 60, no. 3, pp. 1097– 1105, 2001.

C. Murphy, S. Murphy, F. O'Brien et al., "Metabolic activity of probiotics—oxalate degradation," *Veterinary Microbiology*, vol. 136, no. 1, pp. 100–107, 2009.

Demirci M, Gunduz H. Dairy technology handbook. Hasad Press. Turkey; 1994.

F. A. M. Klaver and R. van derMeer, "The assumed assimilation of cholesterol by lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity," *Applied and Environmental Microbiology*, vol. 59, no. 4, pp. 1120–1124, 1993.

Havenaar, R., Brink, B.T., Huis in't Veld, J.H.J. (1992). Probiotics: Selection of strains for Probiotic use. The Scientific Basis. R. Fuller (Ed) Chapman and Hall. London. 209:221.

Hoque, M. Z., Akter, F., Hossain, K. M., Rahman, M. S. M., Billah, M. M. and Islam, K. M. D. Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. World Journal of Dairy & Food Sciences. 2010; 5 (1): 39-46.

Jacobsen CN, Rosenfeldt Nielsen V, Hayford AE, Moller PL, Michaelsen KF, Paerregaard A, *et al*. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Appl Environ Microbiol* 1999; 65: 4949-56.

Kabore, D., Sawadogo-Lingani, H., Dicko, M.H., Diawara, B. and Jakobsen, M. Acid resistance, bile tolerance and antimicrobial properties of dominant lactic acid bacteria isolated from traditional "maari" baobab seeds fermented condiment. African Journal of Biotechnology. 2012; 11:1197-1206.

M. J. Allison, K. A. Dawson, W. R. Mayberry, and J. G. Foss, "*Oxalobacter formigenes* gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract," *Archives of Microbiology*, vol. 141, no. 1, pp. 1–7, 1985.

Piette JPG, Idziak ES. A model study of factors involved in adhesion of *Pseudomonas fluorescens. Appl Environ Microbiol* 1992; *58* : 2783-91

Rivera-Espinoza Y1, Gallardo Navarro Y (2010) Non-dairy probiotic products. Food Microbiol 27:1-1.

Saxelin M, Tynkkynen S, Salusjarvi T et al., (2010) Developing a Multispecies Probiotic Combination. Int J Probiotics Prebiotics 5:169-181.

Savadago, A., Ouattara C. A. T., Bassole I. H. N. and Traore, S. A. Bacteriocins and lactic acid bacteria – a minireview.African journal of biotechnology. 2006: 5(9): 678-683.

S. Turroni, B. Vitali, C. Bendazzoli et al., "Oxalate consumption by *lactobacilli*: evaluation of oxalyl-CoA decarboxylase and formyl-CoA transferase activity in *Lactobacillus acidophilus*," *Journal of Applied Microbiology*, vol. 103, no. 5, pp. 1600–1609, 2007.

Tambekar, D.H. and Bhutada, S.A. An evaluation of probiotic potential of *lactobacillus* sp. from milk of domestic animals and commercial available probiotic preparations in prevention of enteric bacterial infections. Recent Research in Science and Technology.2010; 2: 82-88.

Tatnime, A. Y., and H. C. Deeth. 1980. Yogurt: technology and biochemistry. J. Food Prot. 43:939.

Tripathi MK, Giri SK (2014) Probiotic functional foods: Survival of probiotics during processing and storage. J Func Foods 9:225-241