INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 5, p. 94-99, 2015

RESEARCH PAPER OPEN ACCESS

Optimization of probiotic activity of *Lactobacillus acidophilus* characterized from commercial yoghurt

Md. Ashrafuzzaman[1], Farhana Momtaz[2], Md. Javed Foysal[1*], Md. Hazrat Ali[1], Md. Mahbubur Rahman[3]

[1]*Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh*

[2]*Laboratory of Virology, ICDDR,B, Dhaka, Bangladesh*

[3]*Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh*

Key words: Yoghurt, *Lactobacillus acidophilus*,Antibacterial activity, Probiotics.

http://dx.doi.org/10.12692/ijb/7.5.94-99 Article published on November 30, 2015

Abstract

In this study, five (5) *Lactobacillus acidophilus*isolates characterized from local yoghurt sample after enrichment in MRS broth using biochemical and morphological examination. Two of the five, isolate LA002 and LA005, posed best probiotic activity on test organisms.Al of the*L. acidophilus*isolates grew best at 37°C with an initial pH 5. However, the best activity of*L. acidophilus* was observed in pH 6. The two lactic acid bacterial isolates, LA002 and LA005 demonstrated potent antimicrobial activity against different multidrug resistant isolates of *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhimorium*. The *L. acidophilus* was found to be the most potent antagonistic microbe against *E. coli* and activity was much higher than onion and garlic. *K. pneumonia* also significantly inhibited by *L. acidophilus*isolates. However, *L. acidophilus* isolates can not considerably inhibit the growth of *S. typhimorium*.These results demonstrated that *L. acidophilus* bacteria can support a healthy digestive tract and can be used as a probiotic supplementation.

* **Corresponding Author:** Md. Javed Foysal ✉ mjfoysal-geb@sust.edu

Introduction

Curd is the regular and common food component in all major areas of Bangladesh.Lactic acid bacteria (LAB) are a group of Gram positive, non-respiring, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Lactobacilli are one of the major microbial components of commercial curd considered to be beneficial participants in human microbial ecology (Hummel *et al.,* 2007). Among LAB group, *L.acidophilus* found plentiful in commercial curd and recommended as a preventive approach to maintain the balance of intestinal microflora (Shah, 2007).

Probiotics' are live microbial food and feed supplements that are supposed to benefit health by improving the balance of the intestinal microbial community.

The term probiotics was first introduced by Parker (1974).According to him probiotics are "Organisms and substances which contribute to intestinal microbial ecological balance". Helpful bacteria, such as *L.acidophilus* have the ability to kill off other bacteria by secreting small quantities of antibiotic–like substances, including lactic-acid, acetic–acid, benzoicacid, hydrogen peroxide, acidolin, lactocidin and acidophilin. Research has shown that the benefits of the friendly bacteria are achieved without the undesirable side effects of antibiotic therapy, including diarrhea, digestive problems and vaginal yeast infections (Fuller, 1989). Lactobacilli of human intestinal origin have been shown to exhibit antagonistic activity against both Gram positive and Gram negative bacteria. Many strains belonging to the *L. acidophilus* groups have been reportedto produce antimicrobial compounds, which show a great variety as to their inhibition spectrum. Antimicrobial substance produced are smaller peptides and do not contribute to the formation of resistance to pathogens (Alla *et al.*, 2003).

Lactobacillus acidophilus is a well-known and well-studied probiotic microorganism. However, it is now clear that different strains vary in their efficiency and probiotic potentials in different conditions (Ng *et al.*, 2009). Hence the present study was conducted to determine an optimized growth condition of *L.acidophilus* in culture that showed a potent activity against human clinical samples and thus can be used as potential probiotic.

Materials and methods

Curd collection

A total of 20samples were collected aseptically from three different sources: Bogra, Sylhet and Dhaka. After collection, the samples were immediately brought to the laboratory in cold condition and processed within an hour.

Preparation of media

Curd is the best source for lactobacillus spp. among the other dairy products such as milk, buttermilk etc. Curd istaken in sterilized flask. Under the aseptic conditions curd was serially diluted from 10-1 to 10-14 from this 14dilutions 10-5 10-7 10-9 are selected. For this 3 tubes spread plate technique further with streak plate technique isdone on MRS medium. They are incubated in incubator at 37°C which is optimum temperature for lactobacillus species for 24 hours. After the period of incubation 3 isolated colonies were grown. Colony characterization is done for this 3 colonies found to be lactobacillus species. 1 colony shows 100% resemblance with lactobacillus acidophilus. Further catalase test was done. Selected colonies were purified by streak plate method. The purified bacterial isolates were stored at -80C in sterile reconstituted skim milk 12.5 (w/v) supplemented with 15% glycerol.

Determination of acid tolerance

Percent survival of the three strains wasdetermined after exposure to pH 2.5 for 2 h and 4 hat 37°C. For this, overnight grown cultures wereinoculated in MRS broth adjusted to pH 2.5. Thesamples were plated onto MRS agar at the end ofexposure time. The plates were incubated for 24 h at37°C and total viable count was determined. Uninoculatedbroth served as negative control while set of test organisms inoculated in MRS broth (pH6.2) was used as positive

control.

Determination of bile tolerance

Bile tolerance of *L. acidophilus* cultures wasexamined by inoculating them in MRS brothcontaining oxgall (Central Drug House, New Delhi,India) at the concentrations of 0.2%, 0.3% and 0.5%.The control comprised of MRS broth without bilesalt. Bacterial growth was monitored by measuringabsorbance at 600 nm after incubation for 24 h at37°C.

Antimicrobial activity

Using *in-vitro* agar well diffusion method,antimicrobial activity experiments were carried out(Zinedine and Faid 2007). The probiotic activity of*Lactobacillus* strains was tested against three common human pathogens (1) *Escherichia coli* (2) *Salmonella typhi* and (3) *Klebsiellapneumoniae* cultured earlier in nutrient agar (NA) broth. Overnight growncultures of *L. acidophilus* strains were inoculated inthe wells of nutrient agar where testmicroorganisms were inoculated by pour platetechnique. The plates were incubated at 37°C for 24h. The inhibition zones were measured at the end ofincubation period according to Foysal *et al*., 2011.

Antibiotic susceptibility assay

A disc diffusion assay was performed to studyantibiotic susceptibility of *L. acidophilus* strains. Thecultures were inoculated in MRS agar using pourplate technique. The antibiotics were supplied in theform of dodeca discs (Hi Media, India) which includednalidixic acid, norfloxacin, co-trimoxazole, gentamycin, ampicillin, cephalexin, mecillinam, chloramphenicol, tetracycline, kanamycin, amikacinand streptomycin. The zones of inhibition weremeasured after incubation at 37°C for 24 h. The strains were cultivated on control plates (MRS agarwithout antibiotic discs) under identical conditions.

Results

After primary isolation, five (5) out of twelve samples showed to be *L. acidophilus* and two (40%) of them (LA002 and LA005) showed very good probiotic properties. These two strains were come from Bogra, Bangladesh, used in regular food chain for human diet. The isolated two strains were Gram-positive, catalase negative, non-spore forming, mesophillic bacilli.

Table 1. Biochemical characteristics of *Lactobacillus acidophilus*.

Serial No.	Name of the test	*L. acidophilus*
1	Grams Reaction	Positive
2	Catalase test	Negative
3	Motility test	Non-motile
4	Gas from Glucose	Negative
5	Arginine test	Negative
6	Mannitol	Negative
7	Lactose	Positive
8	Glucose	Positive

Effect of temperature on the growth of L. acidophilus strains

In all the three experiments, effect of temperatures 28^0 C, 37^0 C and 45^0 C on the growth of two selected strains were found to be significantly different. Fig. 1 shows the effect of temperature on viability of*L. acidophilus* strains. All strains grew well at 28^0 C and 37^0 C and their growth was comparable. None of them could grow at 45^0 C. Considering the physiological temperature of human body and the results obtained, all further experiments were carried out at 37^0 C.

Growth at different pH

Table 2 shows the results of the growth of *L. acidophilus* at different pH values. The turbidity

observed for pH values in the range of 3.0 to 9.0 indicating that the bacteria preferred to grow in acidic and neutral environment, particularly at pH 5 and 6. Out of five isolates, LA002 and LA005 grow at vey low pH well bellow 3 after 1h incubation period.

Bile salt tolerance

The bacteria to be used as probiotics should be able to resist inhibitory factors in the gastrointestinal tract such as bile salts.For this purpose, the effect of different concentrations of bile salts on the growth of *L. acidophilus* bacteria in MRS broth was investigated and the results are presented in Table 2. The total viable count of *L. acidophilus* decreased with an increase in the bile salt concentration when compared with the control. *L acidophilus* showed reasonable growth at all bile salt concentrations studied. The bile tolerance results were 87.41%, 75.49%, 69.53% and 54.96 % for the 0.1%, 0.3%, 0.5% and 1.0 % of bile salt concentrations.

Table 2. Effects of pH and different concentrations of bile salts on the growth of *L. acidophilus*.

Temperature	Result
25°C	++
37°C	+++
45°C	–
pH	
3.0	+
4.0	+ +
5.0	+++
6.0	+++
7.0	++
8.0	-
9.0	-
Bile salt concentration (%)	Bile tolerance (%)
0.0	100.0
0.1	87.4
0.3	75.5
0.5	69.5
1.0	55.0

Antimicrobial activity of the isolates

All the five strains were screened initially for antimicrobial activity using pate assay method using MRS media against the three pathogenic organisms, *E.coli, Salmonella typhimurium* and *Klebsiellapneumonia*. All three bacterial isolates were resistant to sulphamethaxazole (SXT), gentamycin (GM) and cefradine (CF). Preliminary screening of bacterial isolates showed that LA002 and LA005 had excellent antibacterial activity against *E. coli* and *K.pneumonia*but had low (very low for LA005) inhibitory activity against*S. typhimurium* compared with other two bacteria. Both of the two isolates (LA002 and LA005)exhibited greater antibacterial activity against *E. coli* than onion and garlic on culture plate.

Discussion

Bangladesh is agriculture based country since civilization; cow milk contributes a major part of economy. Thousands of milk products like curd, butter, cream, cheese, lacci, rassogolla regularly used in Bengali community in different occasions and festivals all over the year. Especially, yogurt is very essential and regular food diet in Bangladesh. In general, yogurt contains good intestinal flora thatincrease digestion of food and ensured balanced health of human.

Table 3. Inhibitory effect of *L. acidophilus* against opportunistic human pathogen.

Isolates	*E. coli, E17*	*K.pneumonia, K14*	*S. typhimurium, ST2*
LA002	20.5mm	12.2mm	4.2mm
LA005	18.6mm	11.8mm	2.8mm
Onion	16.8mm	15.5mm	14.4mm
Garlic	18.5mm	17.2mm	16.6mm

In general, *Lactobacillus acidophilus* grows best at human body temperature. In this study, after 48 hours post incubation, majority of the isolates yielded best growth at 37°C under low acidic environment (pH 4 and 5). Similar study conducted by Aslam and Qazi in 2010 and found exactly same optimum growth temperature. Probiotic bacteria are mostly delivered in a food system and must be acid and bile tolerant to survive in the human gastrointestinal tract.Survival at low pH 3.0 for 2h is considered optimal acid tolerance for probiotic strains (Usman, 1999). In this study,*Lactobacillus* species produced yogurt and tolerated highly acidic conditions *i.e.*, upto pH 3 after 40 minutes incubation period, while *L. acidophilus* strain LA002tolerated well up to pH 1. Comparable results have been reported by Liong and Shah (2005), indicated that *L. acidophilus* survived best under the acidic conditions.These characteristics together with their antagonistic effects against frequently food contaminating microorganisms render them good probiotics candidates. Yogurt has long been supplemented with *Lactobacillus acidophilus* to enhance mucosal and systemic immune responses to cholera (Aslam and Qazi, 2010).

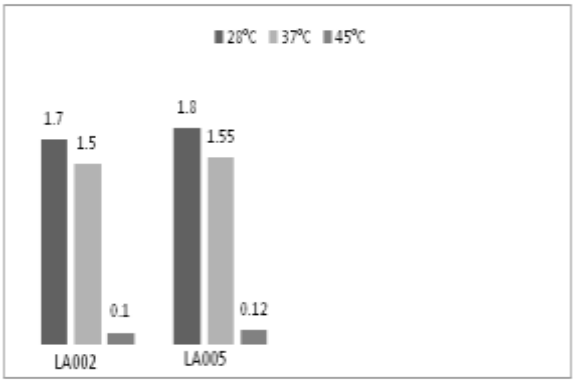

Fig. 1. Effect of temperature on *L. acidophilus* isolates.

Antimicrobial effect exerted by the *L. acidophilus* is mainly due to acid production, hydrogen peroxide, fatty acids, aldehydes and other compounds (Daeschel, 1989). Goderska and Czarnecki (2007) reported*Lactobacillus acidophilus* as a strong inhibitor (26mm zone of inhibition) of*E. coli*. Present data also support previous finding where both two isolates posed strong antibacterial activity against *E. coli,* better than onion and garlic. Very low inhibitory effect observed against *Salmonella typhi*for LA002 and almost no inhibition was observed for LA005. Millette *et al.* (2007) reported no inhibition against gram negative bacteria after 8 h of fermentation by a mixture of *Lactobacillus acidophilus* and *Lactobacillus casei*. The varying results from different locations may be due to large number of differences inherent to the microbes evaluated as probiotics, test organisms and the physiochemical environments of assays procedures.

It is concluded from the present study that the *Lactobacillus* species isolated from the yogurt appeared strong antagonistic against *E. coli* isolates while inhibiting, *Klebsiella pneumoniae* too. However, *Lactobacillus acidophilus* can not strongly inhibit *Salmonella typhi* in culture media. Thus, *Lactobacillus acidophilus* contain yogurt can be used a probiotics in regular healthy diet.

References

Alla A, Aroutcheva J, Simoe A, Faro S. 2003. Antimicrobial protein produced by vaginal*Lactobacillusacidophilus* that inhibits Gardenella vaginalis. Parthenon Publishing**9**, 33-39. http://dx.doi.org/10.1155/S1064744901000060

Aslam S, Qazi JI. 2010. Isolation of acidophilic lactic acid bacteria antagonistic to microbial contaminants.Pakistan Journal of Zoology **42(5),** 567-573.

Corzo G, Gilliland S. 1999. Bile salt hydrolase activity of three strains of *Lactobacill acidophilus*. Journal of Dairy Science **82**, 472-480. http://dx.doi.org/10.3168/jds.S0022030299752562

Daeschel MA. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technology **43**, 164.

Foysal MJ, Rahman MM, Alam M. 2011. Antibiotic sensitivity and in-vitro antimicrobial activity of plant extracts to *Pseudomonas fluorescens* collected from diseased fish. International Journal of Natural Sciences **1**, 82-88. http://dx.doi.org/10.3329/ijns.v1i4.9733

Foysal MJ, Rahman MM, Hossain MN, Yeasmin FM, Miyan SH. 2012. Antibiotic susceptibility profiling and in-vitro antibacterial activity of some plant extracts to *Escherichia coli* characterized from spoiled rice and egg. International Journal of Biosciences **2,** 40-46.

Foysal MJ, Rahman MM, Prodhan SH. 2013. PCR based molecular detection of gyrB2 gene from *Klebsiella* sp. isolates from patients who were suffering with pneumonia and urinary tract infections (UTI). Journal of Clinical and Diagnostic Research**7**, 23-25. http://dx.doi.org/10.7860/JCDR/2012/4661.2661

Fuller R. 1989. Probiotics in man and animals. Journal ofApplied Bacteriology **66,**365-378.

Goderska K, Czarncki Z. 2007. Characterization of selected strains from *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. African Journal of microbiology Research **1,** 65-78.

Hassan P, Peh KK. 2014.Characterization and identification of *lactobacillus acidophilus* using biolog rapid identification system**.** International Journal of Pharmacy and Pharmaceutical Sciences **6,** 189-193.

Hummel AS, Hertel C, Holzapfel WH, Franz C. 2007. Antibiotic resistance of starter and probiotic strains of Lactic acid bacteria. Applied and Environmental Microbiology **73,** 730-739. http://dx.doi.org/10.1128/AEM.02105-06

Liong MT, Shah MT. 2005. Acid and bile tolerance and cholesterol removal ability of *lactobacilli* strains. Journal of Dairy Science**88,** 55-66. http://dx.doi.org/10.3168/jds.S0022030205726622

Millette M, Luquet FM, Lacroix M. 2007.*In vitro* growth control of selected pathogens by *Lactobacillus acidophilus* and *Lactobacillus casei*-fermented milk. Letter in Applied Microbiology **44,** 314-319. http://dx.doi.org/10.1111/j.1472-765X.2006.02060.x

Ng SC, Hart AL, Stagg AJ, Knight SC. 2009. Mechanisms of action of probiotics: recent advances. Inflammatory Bowel Diseases **15,** 300-310. http://dx.doi.org/10.1002/ibd.20602

Parker B. 1974.Probiotics, the other half of the antibiotics story. Animal Nutrition and Health **29,** 4-8.

Shah NP. 2007. Functional cultures and health benefits. International Dairy Journal **17(11),** 1262-1277. http://dx.doi.org/10.1016/j.idairyj.2007.01.014

Usman HA. 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* strains. Journal of Dairy *Science* **82,** 243-248. http://dx.doi.org/10.3168/jds.S0022030299752295

Zinedine A, Faid M. 2007. Isolation and characterization of strains of *Bifidobacteria* with probiotic properties in vitro.World Journal of Dairy and Food Sciences **2**, 28-34.