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Article

Charaterization of isolated potential lactobacilli and used as probiotic food

Monzur Morshed Ahmed[1], Md. Mizanur Rahaman[1], Md. Nur Hossain[1*], Janatul Shabnam[2], Sadia Binta Basher[2], Md. Mahfuzul Hoque[3] and Md. Zahurul Haque[1]

[1]Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh
[2]Department of Microbiology, Jagannath University, Dhaka-1000, Bangladesh
[3]Department of Microbiology, Dhaka University, Dhaka-1100, Bangladesh

*Corresponding author: Md. Nur Hossain, Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh. Phone: +8801717625372; E-mail: mnhossain84@gmail.com

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Abstract: This research was carried out to isolate and identify lactobacilli from yogurt and vegetables sample from Dhaka, Bangladesh. Twenty five *Lactobacillus* strains were isolated and identified as *Lactobacillus* species based on cultural characteristics, biochemical tests, and sugar fermentation. The results showed only four among twenty five isolates were highly responded to probiotic criteria such as low pH tolerance (2.5), bile salt tolerance (2%), gastric juice tolerance (0.5% bile salt, 0.2% NaCl, 0.32% pepsin pH 2.5) in-vitro and were identified strains as *Lactobacillus plantarum* imy-21, *Lactobacillus plantarum*imy-22, *Lactobacillus plantarum* imv-26 and *Lactobacillus acidophilus* imy-37 according to *Bergey's Manual* of Systematic Bacteriology and 16s rDNA sequencing. All of the strains were showed protease activity on skim milk agar. These strains were sensitive to ampicillin, doxycycline, erythromycin and tetracycline; also have antimicrobial activity against enteric pathogen such as ATCC of *Salmonella* Enteritidis, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shigella flexneri*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Escherichia coli* (environmental isolates). Four isolated *Lactobacilli* strains were able to maintain their probiotic properties in freeze dried condition over 90 day's storage. This study concludes that these strains may be used as potential candidate as probiotic food.

Keywords: lactobacilli; protease; probiotic food; antimicrobial activity

1. Introduction

Probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of human being and poultry by improving the intestinal microbial balance. These are microbial food supplements which, when administered in adequate amounts, confer health benefits to consumers by maintaining or improving their intestinal microbial flora (Fuller 1989; Salminen *et al*., 1998; Reid *et al*., 2003; Kabir, 2009). Probiotic microorganisms have been isolated from yogurt, raw vegetables, meat and meat products, cereals, gut of humans and other animals, and the mucosal surfaces of animals (Carr *et al*., 2002). These microorganisms produce antibacterial compounds, including lactic and acetic acid, hydrogen peroxide and bacteriocin; antibiotic-like substances which inhibit the intestinal pathogenic microorganisms (Lindgren *et al*., 1990; Hose and Sozzi, 1991). These components are not only inhibiting undesirable microflora, but also desirable for their effects on food taste, smell, color and texture.The significant roles of probiotic activities are determined in improving digestive functions, as well in immune system against infectious diseases (Pineiro and Stanton, 2007). Study suggested that use of these probiotic bacteria can be help to prevent or control the intestinal infections and contributes health benefits to individuals.

2. Materials and Methods

2.1. Sample collection

Thirty five yogurt & vegetables sample were collected from Dhaka, Bangladesh

2.2. *Lactobacillus* speciesisolation

The *Lactobacillus* strains were isolated from yogurt and vegetables sample. The sample was mixed with sterile saline buffer (0.85%, pH 7.0) and homogenized using a stomacher. Decimal dilution of these samples was inoculated on selective de Man, Rogosa and Sharpe (MRS) agar medium (HiMedia, India) at 37°C for 48 hrs under anaerobic conditions at anaerobic jar (Oxoid, UK). Then colonies with different morphology were randomly selected from the highest dilutions of each MRS agar plate and then sub cultured to acquire pure isolates.

2.3. Strain identification and preservation

Citrate utilization, indole, nitrate reduction and motility test; arginine and gelatin hydrolysis were performed. Sugar fermentation was determined in carbohydrate consumption broth (HiMedia, India) and supplemented with 1% sugar. Seventeen sugars (arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose& xylose) were subjected to a fermentation test under anaerobic condition; each tube was topped up with two drops of sterile liquid paraffin after inoculation.The isolates were identified using standard morphological, cultural and biochemical reactions (Howells, 1992). Gram positive and catalase, oxidase negative isolates were stored at -20°C in MRS broth supplemented with 20% (v/v) glycerol.

2.4. Reference microorganisms used in this study

Lactobacillus acidophilus ATCC 314, *Lactobacillus rhamnosus* ATCC 7469 and were used as reference microorganisms and as positive control. *Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 11774 were used as negative control.

2.5. Probiotic characteristic

The probiotic characteristic was tested by using the sensitivity or resistance to low pH (2.5) and bile salt (2%) (Oxgall, Oxoid, UK). Acid and bile salt resistance can be considered important properties of probiotic lactic acid bacteria (Ouwehand *et al*., 2002; Miroslava *et al*., 2009; Kabir *et al*., 2016). The physiological concentration of bile salts in the small intestine is between 0.2 and 2.0% (Gunn, 2000). The isolated lactobacilli were subjected to primary screening for acid tolerance & bile salt tolerance in MRS broth adjusted to pH 2.5, 3, 4, 5 with 1N HCl & 0.5%, 1.0%, 1.5%, 2% with bile salt respectively. The determination of growth was performed by 1.0% bacterial suspension inoculated in MRS broth and observed after 18 hrs after anaerobic incubation at 37 °C. The experiment was performed in duplicate and the mean values were calculated.

2.5.1. Gastric juice tolerance

Twenty five isolated lactobacilli were subjected to primary screening to gastric juice tolerance (0.5% Bile Salt, 0.2% NaCl, 0.32% pepsin pH 2.5) in MRS broth; control with MRS broth medium pH 6.2. The determination of tolerance was performed by 1.0% bacterial culture inoculated in MRS broth and the growth was observed after 18 hrs after anaerobic incubation at 37°C. Then absorbance was taken at 600nm of 18 hrs culture.

2.6. Molecular identification

2.6.1. DNA extraction

For the chromosomal DNA from bacterial cells inoculated to MRS broth (HiMedia, India) with 1.5% glycerol 24 hrs at 37°C in anaerobic condition. Then total chromosomal DNA was extracted by Accuprep® genomic DNA extraction kit (Cat. No.: K-3032) by supplied protocol with some modifications and DNA purity was determined spectrophotometrically.

2.6.2. Molecular detection

For molecular identification of the isolated *Lactobacillus* spp. polymerase chain reaction **(**PCR) application was performed with single primer pair. In PCR reaction *Lactobacillus acidophilus* ATCC 314 and *Lactobacillus rhamnosus*ATCC 7469 were used as positive control.

2.6.3. PCR reaction and program

The 16S rDNA gene was amplified by PCR with a thermal cycler (BIORAD, Mexico). DNA fragments of approximately 1.5 kbp were amplified using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3') (Lane, 1991; Heilig *et al*., 2002). Polymerase chain reactions were performed in 30 µl reaction volumes containing 25 µl PCR SuperMix (invitrogen, USA), 0.20 mM forward primer, 0.20 mM reveres primer and 0.4 ng of DNA. Temperature cycling conditions for PCR were as follows: an initial heating of 95^0C for 5 min, followed by 35 cycles of denaturation at 95^0C for 60s, annealing at 55^0C for 50s, extension at 72^0C for 1 min, and terminating with a 10 min final incubation of 72^0C. The PCR products were examined with electrophoresis (Gel Electrophoresis Systems. Major Science, Taiwan) on a 1% w/v agarose gel, stained by ethidium bromide, visualised and photographed on a Gel Documentation.

2.7. Proteolytic activity

The proteolytic activity was determined on Skim Milk Agar. The strains were inoculated by 3 mm diameter and incubated for 24 hrs at 37°C. The proteolysis activity is characterized by the observation of a clear zone surrounding the colonies. (Moulay *et al*., 2006)

2.8. Antimicrobial activity

Agar spot test method (Kilic *et al*., 1996; Nowroozi *et al*., 2004) and agar well diffusion method (Toba *et al*., 1991) were used to detect of inhibitory activity of *Lactobacillus* spp. These assays were performed in duplicate. For the agar spot test, overnight culture of *Lactobacillus* spp. were spotted (3mm) into the surface of MRS agar (HiMedia, India) plates and incubated in anaerobic jar for 48hrs at 37°C to allow colonies to develop. Approximately 5×10^7 colony forming unit (cfu) of test microorganisms was swabbed in the plate in which *Lactobacillus* spp. was grown. After incubation for 24 h at 37^0C, the radius of the clear inhibition zone around *Lactobacillus* spp. was recorded.

For agar well diffusion method 4 wells in each plate of 4 mm in diameter were cut into Tryptone soya agar (TSA) (Oxoid, UK) plate by using a sterile borer and 100 µl of the cell free supernatant (centrifugation at10,000×g for 5 min, 4ºC) of the isolates were placed into different well. The cell-free supernatant was adjusted to pH 6.2 using 1N NaOH and it was used as crude bacteriocin The plates were pre-inoculated at room temperature for the diffusion and incubated aerobically overnight at 37°C. The plates were examined for zones of inhibition.

For the detection of antimicrobial activity of *Lactobacillus* isolates, eight pathogenic microorganisms such as *Listeria monocytogenes* (2) ATCC 19112, *Staphylococcus aureus* ATCC 9144, *Shigella flexneri* (2b) ATCC 12022, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* (environmental isolate), *Vibrio parahaemolyticus* ATCC 17802 and *Bacillus cereus* ATCC 10876 were used.

2.9. Antibiotic susceptibility

Antibiotic susceptibility of strains of Lactobacilli was determined in vitro using the Kirby-Bauer agar disc diffusion method (Bauer et al., 1966). The commercial antibiotic discs used in this study were AM= ampicillin (10µg), AK= Amikacin (30µg), CIP= ciprofloxacin (5µg), DO= doxycycline (30µg), E= erythromycin (15µg), CN= gentamycin (10µg), IMP= imipenem (10µg), NA= Nalidixic acid, N= neomycin (10µg), F= nitrofurantoin (300µg), TE= tetracycline (30µg), VA= Vancomycen (Antibiotic disks were obtained from Emapol, Poland). MRS cultures were suspended at approx. 10^8cfu/ml (McFarland standard 0.5) on Mueller–Hinton agar plates incubated for 24 hours at 37 ^{0}C. For susceptibility tests, clear zone recommended for consideration of susceptible or resistance of an organism.

2.10. Cell survivality on freeze dried condition

Four probiotic *Lactobacillus* spp. were cultured in MRS broth at 37^0C for approximately 18 hrs. The 5% cultures were then incubated with pasteurized commercial milk for 4 hrs at 37^0C. Bacterial cell harvested by centrifugation at 10000×g for 5 min and were re-suspended in equal volume of sterile distilled water. Conical flash containing 100 ml of each prepared microbial mixtures frozen at -18^0 C for 24 hrs and then freeze dried for 24 hrs. The freeze dried cells were stored at room temperature up to 90 days. For enumeration of viable probiotic cells and to test their probiotic properties, samples were taken immediately after freeze drying and different time intervals (after 15 days, 30 days, 45 days, 60 days, 75 days and 90 days) during the storage. Those were re-hydrated using distilled water and used for enumeration through serial dilutions (10^{-1}–10^{-10}) in normal saline, inoculated on MRS agar medium and incubated at 37^0C for 24 hrs. Number of viable cells was determined as cfu/g.

3. Results and Discussion

3.1. *Lactobacillus* species isolation and identification

A total of 35 samples (yogurt & vegetables) were analyzed and twenty five (25) isolates were identified as *Lactobacillus* species. Most of them were shiny creamy color, circular, smooth and convex; some of them were found to be irregularin MRS agar (HiMedia, India). Bacillary and cocci forms were positive to Gram reactions under a light microscope and most of them were rod or short rod chain and coccoid rods. Some *Lactobacillus* spp. was found to be irregular, short, even coccoid rods with round tapered ends, sometimes longer also (Kandler*et al*., 1983). Most of the isolates were found to be non-motile; oxidase, catalase, arginine hydrolysis, indole negative; nitrates were not reduced and gelatine was not liquefied. The results of selected isolates of *Lactobacilli* were shown in Table 1. Four isolates (imy-21, imy-22, imv-26 & imy-37) were able to grow at 15°C and37°C and cannot grow at 8 % NaCl (w/v). Kandler and Weiss (1986) have classified *Lactobacillus* isolates according to their morphology, physiology and molecular characteristics. These isolates were also able to ferment sugars at different percentages which were much significant for identification of the species. Among seventeen different sugars; fructose, glucose, lactose, maltose and sucrose was fermented by those 4 isolates. Galactose, melibiose, ribose and salicin was utilized by 75% of the isolates; 50% of the isolated isolates were able to ferment trehalose and mannitol sugars; arabinose, raffinose, rhamnose and xylose was not fermented by any one of the 4 isolates.

3.2. Probiotic characteristics

3.2.1. Acid & bile tolerance test

Probiotic potential of lactobacilli had ability to resist bile salts and acidic pH (Lee and Salminen, 1995).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). The effect of pH (2.5, 3, 4 & 5) and bile salts (0.5%, 1%, 1.5% & 2.0%) and NaCl (1% to 2%) of 31 isolates was studied. It was found that most isolates might grow at above criteria except pH 2.5, 3 & bile concentration 1.5%, 2%. But most of them were able to survive that low pH & bile salt. Jacobsen *et al*., (1999) suggested that the pH of 2.5 seemed to be damaging to the bacteria. The isolates from our experiment showed stronger bile tolerance than those reported by other investigators (Papamanoli *et al*., 2003). In this study, four probiotic isolates ((imy-21, imy-22, imv-26 & imy-37) showed acid tolerance at pH 2.5 and bile salt tolerance at 2% (w/v). On the basis of low pH tolerance & high bile salt tolerance test twelve (12) presumptive *Lactobacillus* spp. were selected for gastric juice tolerance test.

3.2.2. Gastric juice tolerance

Among 12 only four *Lactobacillus* isolates (imy-21, imy-22, imv-26 & imy-37) had excellent growth with the gastric juice composition (0.5% bile salt, 0.2% NaCl, 0.32% pepsin) in MRS broth up to pH 4 & significantly survive at pH 2.5. These were excellent probiotics. The survival of *L. plantarum, L. acidophilus L. rhamnosus* strains in simulated gastric juice, pH 2.0, for 90 min (Corcoran *et al*., 2005).

3.3. Molecular identification

Genetic identification of presumptive *Lactobacillus* spp. was carried out by molecular techniques based on the amplification and sequencing of the 16S rDNA gene. PCR amplification of isolates was done by 27F/1492R primer pair. Results of the PCR detection methods, using primers reported as specific for *Lactobacillus* spp. were summarized in Table 2. Desirable PCR product (1500 bp) was obtained in four isolates (Figure 1). In this study we identified our probiotic isolates as *Lactobacillus plantarum* imy-21, *Lactobacillus plantarum* imy-22, *Lactobacillus plantarum* imv-26 and *Lactobacillus acidophilus* imy-37 through 16s rDNA sequencing and phylogeny analysis (data not shown).

Table 2. Results of *Lactobacillus* genus specific PCR product.

Isolates ID	Source	Primer pairs used
		27F&1492R
LY-21, LY-22 & L 37	Yogurt	+
LV-26	Vegetable	+

Table 1. Phenotypic and biochemical characteristics of selected lactobacilli.

Test Parameter	imy-21	imy-22	imv-26	imy-37
Growth at 15°C	+	+	+	+
Growth at 37°C	+	+	+	+
Growth at 45°C	+	+	+	+
Growth at 8 % NaCl	-	-	-	-
Hydrolysis of arginine, gelatine	-	-	-	-
Citrate utilization	-	-	-	-
Indole, Motility, Nitrate reduction	-	-	-	-
Survival at 60°C for 30 min	-	-	-	+
Arabinose	-	-	-	-
Fructose	+	+	+	+
Galactose	+	+	-	+
Glucose(acid)	+	+	+	+
Glucose(gas)	-	-	-	-
Lactose(acid)	+	+	+	+
Maltose	+	+	+	+
Mannitol	-	-	+	+
Mannose	+	+	-	+
Melibiose	+	+	-	+
Raffinose	-	-	-	-
Rhamnose	-	-	-	-
Ribose	+	+	+	-
Salicin	+	+	+	-
Sorbitol	-	-	-	-
Sucrose	+	+	+	+
Trehalose	-	+	+	-
Xylose	-	-	-	-

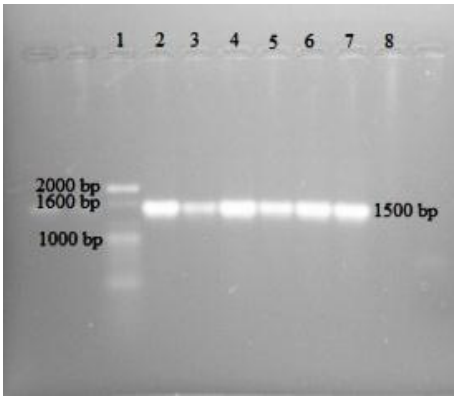

Figure 1. PCR products showing specific band (1500 bp) in agarose gel electrophoresis.

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		27F&1492R
LY-21, LY-22 & L 37	Yogurt	+
LV-26	Vegetable	+

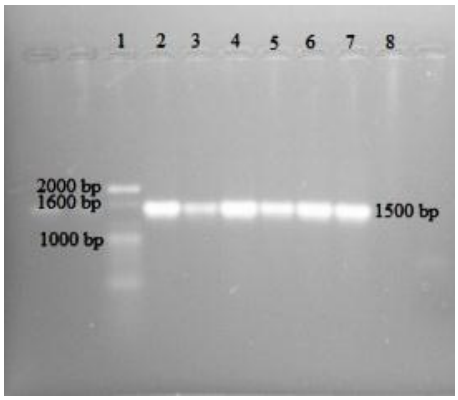

Figure 1. PCR products showing specific band (1500 bp) in agarose gel electrophoresis.

Lane 1=100 bp Marker, Lane 2= *Lactobacillus acidophilus* ATCC 314, Lane 3= *Lactobacillus rhamnosus* ATCC 7469, Lane 4 = imy-21, Lane 5=imy-22, Lane 6= imv-26 & Lane 7=imy-37, Lane 8= Blank (master mix without template)

3.4. Proteolytic activity

Selected four isolates were proteolytic lactobacilli. The proteolytic activity of these bacteria is benefit for host, by the liberation of the amino acids from feed or endogenous proteins. Isolated *L. acidophilus* from chicken gut was proteolytic probiotic microorganisms (Behira *et al*., 2009).

3.5. Antimicrobial activity

3.5.1. Antimicrobial activity of whole cell

Lactic acid bacteria pose a strong antagonistic activity against foodborne pathogenic microorganisms as a result of the production of organic acids, hydrogen peroxide, inhibitory enzymes and bacteriocins (Piard and Desmazeaud, 1991; Juven *et al*., 1992). Antimicrobial activity of probiotic microorganisms may contribute to an improvement in the quality of fermented foods. This may result from control of spoilage and pathogenic bacteria, extension of shelf life, and improvement of sensory quality (Wei *et al*., 2006; Siripatrawan and Harte, 2007). The results (Table 3) showed that all of the four strains were able to inhibit the growth of eight pathogenic microorganisms. Two strains (*L. plantarum* imy-21 & *L. plantarum* imy-22) among 4were excellent to inhibit the most pathogenic microorganisms except *Escherichia coli.* This indicates that inhibitory factors secreted into environment. This result is in concordance with the results of Kos *et al*. (2008) who was demonstrated anti-*Salmonella* activity of probiotic strains *L. acidophilus* M92 and *L. plantarum* L4; *L. acidophilus* M92 was also shown to have antilisterial activity. The study of Tambekarand Bhutada (2010) showed that *L. plantarum* had strongest antagonistic potential against *Salmonella typhi* and *Klebsiella pneumonia*. Obadina *et al*. (2006) also reported that *L. plantarum*, had a broad antimicrobial inhibitory spectrum, against *Salmonella typhi, E. coli, S. aureus* and *B. cereus*.

3.5.2. Antimicrobial activity of cell-free supernatant

Crude bacteriocins (100 µl) were placed in 4 mm in diameter well on unsupplemented Mueller-Hinton agar (Lab, Uk) to determine the antimicrobial spectrum of bacteriocin. After 18 hrs of incubation results were recorded. Mohankumar and Murugalatha (2011) observed that bacteriocins had an inhibitory effect on *Klebsiella pnuemoniae, Staphylococcus aureus* were inhibited moderately. We found that secreted bacteriocin of strains imy-21 & imy-22 was inhibited enteric pathogenic microorganism (Table 3). In vitro assay was carried to characterize the antimicrobial potential of the culture supernatant to inhibit most of the test pathogenic bacteria.

3.6. Antibiotic susceptibility

The safety of probiotic bacteria must be carefully assessed, with particular attention to transferable antibiotic resistance (Mathur and Singh, 2005). Antibiotic resistance pattern should be tested for each particular probiotic strain (Charteris *et al*., 1998). Our strains were sensitive todoxycycline, erythromycin and tetracycline; resistant to ciprofloxacin, gentamicin and nitrofurantoin (Table 4). This result was similar with the report of Danielsen and Wind (2003). They found lactobacilli have a high natural resistance to ciprofloxacin, kanamycin, gentamicin, nitrofurantoin, and vancomycin. The resistance of the probiotic strains to antibiotics used for both preventive and therapeutic purposes in controlling intestinal infections and faster recovery of the patients due to

rapid establishment of desirable microbial flora (EI-Naggar, 2004). Ahmed, 2003 suggested uses probiotic as alternatives to antibiotics due to rising antibiotic resistant bacteria. Resistance to ampicillin, ciprofloxacin, and vancomycin are commonly found in the genus *Lactobacillus*. Future experiments should investigate whether this resistance is carried by a resistance plasmid and can be transmitted to other bacteria.

Table 3. Antibacterial activity.

Name of the test microorganism	Diameter of zone of inhibition in mm									
	*Cont.		imy-21		imy-22		imv-26		imy-37	
	*a	*b	*a	*b	*a	*b	*a	*b	*a	*b
Listeria monocytogenes (2) ATCC 19112	5	5	30	15	30	15	5	5	5	5
Staphylococcus aureus ATCC 9144	10	10	30	15	25	15	10	10	5	5
Shigella flexneri (2b) ATCC 12022	8	8	30	15	32	15	10	10	5	5
Klebsiella pneumoniae ATCC 13883	5	5	40	20	40	20	10	5	10	5
Escherichia coli (environmental isolate)	5	5	5	5	5	5	15	10	15	10
Salmonella enteritidis ATCC 13076	10	10	30	20	30	15	15	10	10	5
Vibrio parahaemolyticus ATCC 17802	5	5	40	18	40	15	20	15	20	10
Bacillus cereus ATCC 10876	5	5	40	25	40	25	20	15	15	10

*Cont = *Lactobacillus rhamnosus* ATCC 7469, *a= zone of inhibition of whole cell, *b= zone of inhibition of cell-free supernatant

Table 4. Antibiotic susceptibility against antibiotic.

Species	AM	AK	CIP	DO	E	CN	IMP	NA	N	F	TE	VA
*Cont	0	0	R	S	S	R	R	R	0	R	S	S
imy-21	S	I	R	S	S	R	0	0	R	S	S	0
imy-22	S	I	R	S	S	R	0	0	R	S	S	0
imv-26	R	S	S	S	S	R	R	R	R	I	S	0
imy-37	S	I	I	S	S	S	I	0	R	R	S	0

*Cont = *Lactobacillus rhamnosus* ATCC 7469, 0= not tested.

3.7. Cell survivalityof probiotic culture on freeze dried condition

Probiotic should be viable as well as functionally active during long term storage to have their expected benefits (Ouwehand and Salminen, 1998). Georgieva *et al*. (2009) studied eight candidate probiotic *L. plantarum* strains isolated from cheeses and their capacity to survive over extended shelf-times at refrigerated temperatures. Our our strains (imy-21, imy-22, imv-26 & imy-37) were able to survive and colony count was satisfactory during 90 days storage period (Figure 2). These results were agreed with some previous studies. In one of the study reported the ability of different strains of Lactobacilli to maintain their probiotic properties during storage at 4^0C after freeze drying condition (Tomas *et al*., 2009). Probiotics need to contain at least 10^6 -10^7cfu/g of live microorganism (Ouwehand and Salminen, 1998). This study showed that $\geq 10^9$cfu/g colony count was found in three probiotic strains (imy-21, imy-22, & imy-37) after 90 days storage at freeze dried condition.

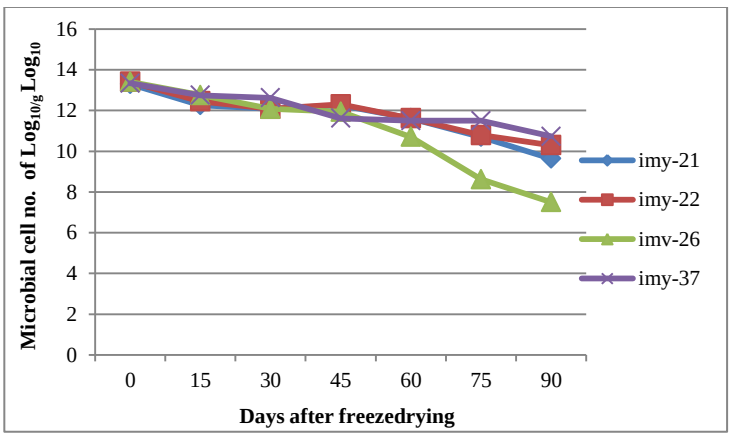

Figure 2. Microbial cell no. of Log_{10}/g against days.

In the present study, antimicrobial assay against *Salmonella* Enteritidis was used to test the antimicrobial properties of freeze dried cultures during storage at room temperature. According to the results isolated *Lactobacilli* were able to maintain their probiotic properties survive at low pH 2.5 for 2 hrs& 2% bile salts during the 90 days storage period. Two strains (imy-21 & imy-22) had excellent inhibitory capability to the test microorganisms. A gradual reduction of antimicrobial activity was observed with the increase of storage time of *Lactobacilli* at freeze dried condition.

3.8. Conclusions

Probiotic microorganisms and their products give distinctive flavors, textures, and aromas fermented foods while preventing spoilage, extending shelf-life, and inhibiting pathogenic microorganisms. The present study revealed that isolated strains *Lactobacillus plantarum* imy-21, *Lactobacillus plantarum* imy-22, *Lactobacillus plantarum* imv-26 & *Lactobacillus acodophilus* imy-37 were acid tolerant at pH 2.5, bile tolerant at 2% bile salt, antibacterial activity against enteric pathogens and able to produce the antimicrobial substances like bacteriocin which suggest their possible use as probiotic yogurt, probiotic milk and other milk products. These products provide restoration and maintenance of normal microbial flora of intestine; inhibit or reduction the pathogenic microbial contamination and prevention of side effect of antibiotics.

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