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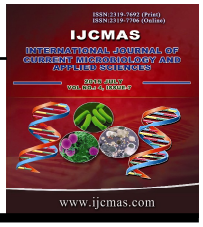

Original Research Article

Screening of Probiotic Goat Milk and Cow Milk Isolates for Acid Resistance, Antagonistic Activity and Tolerance to Antimicrobial Activity of Spices: Molecular Identification of Potential Probiotic Goat Milk Isolate, G8

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Keywords

Isolation, Probiotics, Lactic Acid Bacteria, Acid tolerance, Antagonistic activity, Tolerance to Spices, 16s rRNA gene sequencing, *Lactobacillus plantarum*

A B S T R A C T

Probiotics are live, microbial cells with several beneficial health effects on humans. The beneficial effect of probiotics mainly depends on their survival in the gastrointestinal tract. Several scientific reports confirmed that different spices possess antimicrobial activity. The consumption of spicy food may result in poor survival of probiotic bacteria in the gut. In view of this, the present study aimed at isolation of probiotic microorganisms from goat milk and cow milk, and screening of these organisms for few probiotic properties such as acid tolerance, antagonistic effect against pathogenic bacteria and also for tolerance to antimicrobial activity of spices. A total of 11 and 15 strains were isolated from goat milk and cow milk samples respectively. Four goat isolates, G4, G7, G8 and G11, and one cow isolate, C12 exhibited acid resistance. Antagonistic activity study of these potential acid resistant isolates revealed that goat isolate, G8 is the promising isolate inhibiting the growth of both gram-positive and gram-negative bacteria. Screening of probiotic isolates to antimicrobial activity of spices indicated that all the goat and cow milk isolates were tolerant to chilly, ginger, coriander and cinnamon. Except G7, all the isolates are sensitive to garlic. Two goat isolates G7 and G8 are tolerant to cloves while remaining isolates are sensitive. Among all the isolates of goat milk and cow milk, the G8 isolate of goat milk can be used as a potential probiotic as it is tolerant to acidity in the gut, majority of spices and inhibitory to pathogenic microorganisms. This strain was identified as *Lactobacillus plantarum* by 16s rRNA gene sequence analysis.

Introduction

With growing interest in self-care and health, the market for functional foods or foods that promote health beyond providing basic nutrition is flourishing and so is Probiotics.

Probiotics are "live, nonpathogenic microorganisms which when administered in adequate amounts confer a beneficial health effect on the host" (Fuller, 1989). These probiotic organisms beneficially affect the

host by improving the balance of the intestinal micro flora which is often disturbed during antibiotic treatment and are important for the improvement of the immune system. Other health-related effects include managing lactose intolerance, prevention of colon cancer, lowering blood pressure, lowering the incidence or duration of diarrhoea, reduction of cholesterol, reduction of allergic symptoms, and prevention of urogenital symptoms (Berner and O'Donnell, 1998; Saarela *et al.,* 2002; Mc Naught and Mac Fie, 2001; Rafter, 2003). Most probiotics are bacteria among which, Lactic Acid Bacteria (LAB) are more popular. Lactic acid bacteria (LAB) occur naturally as indigenous micro flora in raw milk. But the species composition of LAB is varying and inconsistent in different sources of milk, and the "wild" strains of the LAB are prospective probiotics (Rinkinen *et al.,* 2003). They can be safely used as probiotics for medical and veterinary applications and considered as generally recognized as safe (GRAS) organisms (Sieladie *et al.,* 2011)

The most important factor for probiotics to exert beneficial effects on the consumer is their viability and successful transfer through gastrointestinal tract for which the probiotic strain needs to be resistant to stressful conditions of stomach (P^H- 3.0). Probiotics have also been shown to inhibit the growth of pathogenic bacteria due to the production of inhibitory compounds such as bacteriocins, hydrogen peroxide and alteration of p^H values by the production of organic acids and competitive adhesion to the epithelium (Kolida *et al.,* 2006). So, acid tolerance and antagonistic activity of strain against pathogenic organisms are important selection criteria for probiotics (Kosin and Rakshit, 2006).

Spices have traditionally been used to enhance the flavor and aroma of foods. Several scientific studies confirm that the growth of Gram positive and Gram negative organism can be inhibited by Garlic, Cinnamon, Cloves and other spices (Bowles and Miller, 1993; Bowles *et al.,* 1995; Blaszyk and Holley, 1998). So, consumption of spicy food may lead to injury and death of a percentage of orally administered probiotics. Therefore, resistance to antimicrobial activity of various spices is another important selection criterion for potential probiotic strain. To our knowledge, studies on screening of probiotic isolates for tolerance to antimicrobial activity of spices have not been reported.

All the probiotic isolates are not alike and the individual merits are likely to vary between strains. Each should be tested for its own merits and should be identified. In recent years, the use of 16S rRNA gene has been regarded as gold standard for the identification and phylogenetic analysis of bacteria (Ludwig and Schleifer, 1999).

Hence, the focus of the present work is isolation of LAB from Goat milk and Cow milk, and screening of isolates for tolerance to acidity, antagonistic activity against pathogenic microorganisms and tolerance to antimicrobial activity of Spices, followed by identification of potential probiotic organism by 16S rRNA gene sequence analysis.

Materials and Methods

Sample source and enrichment technique

Milk samples collected from local goat and cow were used for isolating probiotic microorganisms. The unpasteurized milk samples were added separately to MRS broth and incubated at 30^oC for one week under static conditions for enrichment of microorganisms.

Isolation and characterization of LAB

Probiotic microorganisms were isolated in MRS Agar medium by Pour plate technique. Enriched samples were serially diluted from 10^{-1} to 10^{-10} and 1 mL of aliquot of the dilutions was pour plated. The plates were incubated at 37°C for 24–48 h under anaerobic conditions. After incubation, individual colonies were selected and streaked on MRS agar until pure isolates were obtained. The isolates were examined according to their colony morphology, gram staining and catalase reaction.

Tolerance of isolated LAB to Acidic pH

Resistance to pH 3 is often used in *in vitro* assays to determine the resistance to stomach pH. In stomach, the food stays for 3h. So, 3h time limit was taken into account. The tolerance of isolated LAB to acidic pH was performed as described by Gotcheva *et al.* (2002).

Pure isolates were inoculated in MRS broth and incubated at 37°C overnight. The 24h bacterial cultures were centrifuged at 5000 rpm for 10 min at 4°C and the pellets were washed in sterile phosphate-buffered saline (PBS, 0.1M phosphate buffer, 0.8% NaCl, pH-7.2) and resuspended in 1ml of PBS (pH7.2). From this, 0.1mlwas added to10ml of PBS at pH3.0 (test) and pH 7.2 (control), and incubated at 37°C for 3 h. After incubation, 1ml is added to 25ml of MRS broth, incubated at 37°C for 24 h and O.D at 620 nm was taken using spectrophotometer.

Antagonistic activity of isolated LAB

The antagonistic activity of isolated LAB was tested against two gram-positive bacteria, *Bacillus subtilis, Staphylococcus aureus,* and two gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* by agar spot test (Jacobsen *et al.*, 1999).

Prior to conducting the test, the potential probiotic isolates were propagated in MRS broth medium and incubated anaerobically at 37°C for 24 hrs. For agar spot test, 4µL of propagated LAB bacterial isolates were spotted on the center of the surface of MRS agar medium containing only 0.2% glucose and 1.2% agar, and incubated anaerobically for 24 h at 37°C to allow colonies to develop. Approximately 10^7 cells of test pathogens (i.e., heavy growth) in 15ml of nutrient agar were poured on plate in which LAB were grown. After incubation for 24hat 37°C, the inhibition zone around the LAB spot was observed. This clear zone was used as an indicator of the ability of isolated LAB to antagonize the tested pathogen.

Tolerance of isolated LAB to antimicrobial activity of various spices

The tolerance of acid resistant isolates to various spices such as chilly, ginger, garlic, coriander, cinnamon and cloves was performed in two ways, by turbidimetric method and agar well diffusion method. In order to mimic the conditions of the diet, aqueous extracts of spices were used.2%, 4%,6% and 8% concentrations of spices were used for turbidimetric method and 4% and 8%concentrations were used for well diffusion method.

Turbidimetric method

Each bacterial isolates was grown in MRS broth and incubated at 37°C overnight under static conditions. The bacterial cultures were then centrifuged at 5000rpm for 10 min at 4°C and the pellets were re-suspended in 5ml of MRS broth.1ml of bacterial suspension is added to the broth containing 2%, 4%, 6% and 8% of various spices and incubated for 3h at 37°C.After incubation,

0.1ml each of the above samples were inoculated in MRS broth, incubated at 37^{o}C overnight and OD at 620 nm was taken using spectrophotometer.

Well diffusion method

For well diffusion method, 1ml of bacterial culture grown in MRS broth was added to 25ml of MRS agar and pour plated. After the solidification of the agar, wells were prepared and 0.2ml of different spices aqueous extract was added into the wells. Controls were also prepared by adding distilled water into the wells instead of spices extract.

Identification of the potential probiotic bacteria

Among all the isolates screened, the promising probiotic isolate was identified using 16SrRNA gene sequencing (Bioserve India, Hyderabad). The 16S rRNA gene fragments were amplified by PCR using universal primers: 27F (5'-AGAGTTTGA TCMTGGCTCAG-3' and 1525 R (5'-CGYTAMCTTWTTACGRCT-3)' in an automated thermo cycler (Eppendorf mastercycler). The aliquots of the amplified products were subjected to 1% Agarose gels in TAE buffer. Gels were stained with Ethidium bromide and visualized under UV light. PCR amplicon with the expected size (1.5 kb) was excised from agarose gel and purified using Gel elution kit. 16S rRNA gene sequencing was performed in an automated gene sequencer.

The 16s rRNA gene sequence obtained was compared to those available in NCBI using BLAST (Altschul *et al.*, 1990) to determine sequence similarity. The phylogenetic relatedness of the promising probiotic isolate with other closely related bacterial strains of the genus *Lactobacillus* was examined. The reference sequences required for comparison were downloaded from NCBI. All the sequences were aligned using the multiple sequence alignment programme, CLUSTALW. The phylogenetic tree was constructed based on Unweighed Pair Group Method with Arithmetic Mean (UPGMA) using Molecular Evolution Genetic Analysis MEGA 6.0.

Results and Discussion

Isolation and characterization of LAB

A total of eleven and fifteen pure cultures were isolated from goat milk and cow milk respectively. All the isolated pure cultures were characterized by morphology, gram staining and catalase test.

Colony morphology

All the LAB isolates were observed as cream colonies on MRS medium. Size of the colonies ranges from 0.5mm to 10mm.

Gram staining

On gram staining, violet colored, gram positive cocci and rods were observed in cultures isolated from both goat and cow milk. The different sizes and shapes were reported in tables 1 and 2.

Catalase test

All the isolates did not show any effervescence on the addition of hydrogen peroxide and were considered as catalase negative (Tables 1 and 2).

Tolerance of Isolated LAB to acidic pH

All the LAB isolates survived an incubation period of 3h at pH 3.0, but exhibited

variation in tolerance to acidic pH. The isolates that exhibited >80% growth when compared to controls were grouped as highly resistant (R), 60–79% as moderately resistant (MR), 30–59 are sensitive(S) and<30% as highly sensitive (HS).

Tolerance of isolated LAB from goat milk to acidic pH

Among 11 goat milk LAB isolates tested for tolerance to acidic pH, three isolates G4, G7 and G8 showed growth as 83%, 98%and 99% of controls, respectively and were considered resistant. G11 was considered as moderately resistant as it showed growth as 66% of controls. Remaining 7 isolates were considered sensitive (Table 3).

Tolerance of LAB cow milk isolates to acidic pH

15 isolates obtained from cow milk were screened for tolerance to acidic pH. One isolate, C12 showed growth as 90% of control and was considered as highly resistant. Seven isolates, C1, C2, C3, C4, C5, C6, and C14 were considered as sensitive as they exhibited growth of30% to60% of controls. The remaining 7 isolates were considered highly sensitive (Table 4).

In vitro antagonistic effect of isolated LAB

The isolates (4 goat isolates, G4,G7, G8, G11and one cow isolate, C12) which exhibited growth > 60% of controls were used for antagonism test against indicator organisms, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

The LAB isolates exhibited significant differences in the antagonistic effect. Among 4 goat milk isolates tested, G4 and G11 showed antagonistic activity against gram positive organisms, *Staphylococcus aureus* and *Bacillus subtilis* did not produce antagonistic effect against gram negative organisms, *Escherichia coli* and *pseudomonas aeruginosa.* G7 isolate did not show inhibitory effect against all the tested bacteria. G8 isolate exhibited interesting results, showing inhibitory effect against both gram positive and gram negative bacteria. Cow isolate, C12 did not showed antagonistic effect against *Staphylococcus aureus* and P*seudomonas aeruginosa* and demonstrated inhibitory effect against *Bacillus subtilis, Escherichia coli* (Table 5).

Tolerance of isolated LAB to antimicrobial activity of various Spices

Screening is done by two methods, turbidimetric method and well diffusion method.

Effect of chilly

All the goat milk LAB isolates survived and exhibited almost equal growth as controls in the presence of 2%, 4%, 6%, & 8% concentrations of chilly while Cow isolate, C12 showed slight decrease in the growth at 6% & 8% concentration but exhibited growth as 90% of control (Figure 1). In well diffusion method, zone of inhibition was not observed for all isolates tested (Table 6 and Figure 7a). So, all the isolated LAB were considered resistant to chilly powder.

Effect of ginger

All the tested goat milk and cow milk isolates did not show reduction in growth from 2% to 8% concentration of ginger (Figure 2). Zone of inhibition was not observed for both 4% & 8% studied in well diffusion method (Table 6 and Figure 7b). So, all goat milk and cow milk LAB isolates were considered resistant to ginger.

Effect of garlic

Among 4 goat milk LAB isolates studied, G4, G8 and G11 showed reduction in growth at 2% itself and there was no further reduction with increasing concentrations.G7 isolate did not show reduction in growth and was considered as resistant (Figure 3). The 3 goat isolates, G4, G8, G11 and cow isolate, C12 showed zone of inhibition at both 4% & 8% concentrations and were considered sensitive to garlic (Table 6).

Effect of coriander

Coriander powder did not show inhibitory effect on all 4 goat milk LAB isolates studied (Figure 4 and Table 7). Moreover the isolate G8 showed stimulating growth. Zone of inhibition was not observed for both 4% and 8% concentrations of coriander (Figure 7c). Similar results were also observed for cow milk isolate, C12. So, G4, G8, G7, G11 and C12 were considered resistant to coriander.

Effect of cinnamon

All the tested goat milk and cow milk isolates were resistant to cinnamon concentrations from 2% to 8%. Moreover, addition of cinnamon to broth medium even stimulated growth (Figure 5). Zone of inhibition was not observed for all the LAB isolates studied at both 4% and 8% concentrations (Table 7).

Effect of cloves

Among 4 goat milk LAB isolates, G7 and G8 were found to be resistant to all the concentrations studied from 2% to 4% while G4 and G_{11} isolates showed reduction in growth from 2% to 8% concentrations.C12 isolate showed reduction in growth and no difference in growth was observed with increasing concentrations of cloves (Figure 6).

In well diffusion method, G4, G11 and C12 isolates showed zone of inhibition and were considered sensitive. G7 and G8 isolates did not show zone of inhibition for both 4% and 8% concentrations and were considered resistant to cloves (Table 7).

Identification of the potential probiotic bacteria

Among all the goat milk and cow milk isolates, G8 isolate of goat milk exhibited acid resistance, antimicrobial activity against all the tested bacteria and tolerance to majority of spices. Hence the potential probiotic isolate, G8 was identified by 16S rRNA gene sequence analysis.

PCR amplification of 16S rRNA gene from G8 isolate yielded PCR product of approximately 1.5 kb (Figure 8). The 16S rRNA gene was sequenced and the sequence similarity search revealed 100% similarity with *Lactobacillus plantarum*. Nucleotide sequence for 16S rRNA gene of this isolate has been submitted to GenBank databases under accession number JX183220. The phylogenetic tree analysis revealed that G8 is closely related to *Lactobacillus plantarum* on evolutionary basis (Figure 9). Hence, the potential probiotic goat isolate, G8 was identified as *Lactobacillus plantarum.*

Most commonly used probiotic bacteria are different species of *Lactobacillus.* A total of 11 and 15 isolates were isolated from Goat and Cow milks respectively. Since LAB species are known to be Gram positive rods, cocci and catalase negative (Holt *et al*, 1994), the cultures isolated from Goat milk and Cow milk may be considered as LAB species.

Since most microorganisms are destroyed by low p^H and HCl in the stomach, good probiotic strain should withstand at least pH 3.0 (Fernandez *et al.,* 2003). In our study 4 isolates from Goat milk and 1 isolate from Cow milk demonstrated significant tolerance to acidic pH (Tables 3 and 4). These results revealed that G4, G7, G8, G11 and C12strains can tolerate low pH and survive during passage through gastrointestinal tract.

Lactic acid bacteria have shown to possess inhibitory activities mostly towards Gram positive pathogens and closely related bacteria due to the bacteriocidal effect of bacteriocins (Jack *et al.,* 1995; Klayruang *et al.,* 2008). LAB were also able to control the growth of Gram negative pathogens including food borne pathogens by the production of organic acids and hydrogen peroxide (Lu and Walker, 2001; Ito *et al.,* 2003).

The two goat milk LAB isolates, G4 and G11 demonstrated a clear bacteriocidal effect against gram positive organisms *Staphylococcus aureus* and *Bacillus subtilis.* However, no antagonistic effect against gram negative organisms was detected (Table 5). From this preliminary test, it was concluded that G4 and G11 isolates controlled gram positive organisms using bacteriocin production rather than organic acids and hydrogen peroxide. G8 isolate was inhibitory for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa.* This inhibitory effect towards both gram positive and gram negative organisms may be due to production of both bacteriocins and other antibacterial compounds such as organic acids and hydrogen peroxide (Table 5). Growth of both Gram positive and Gram negative bacteria could be inhibited by garlic, cinnamon, cloves and other spices. The antimicrobial activity varies depending on the type of the spice, test medium and microorganisms. In the present study chilly, ginger, coriander and cinnamon did not have inhibitory effect against isolated LAB from both Goat and Cow milk (Figures 1, 2, 4 & 5). This suggests that LAB are quite resistant to antimicrobial activity of spices (Rees *et al.,* 1993; Gonzalez-Fandos *et al.,* 1996; Dorman *et al.,* 2000). This resistance to spices could be due to other reasons also. The spice extract may not be extracted into the aqueous solution or essential active ingredients may be destroyed during autoclaving. But all these conditions mimic the normal food preparations. Many of the LAB isolates exhibited stimulated growth in the presence of cinnamon which might be due to the presence of carbohydrates and nutrients in the cinnamon. Garlic has been reported to possess a broad spectrum antimicrobial activity (Kumar and Berwal, 1998). Among the four Goat isolates tested, G4, G8 and G11 were found to be sensitive and G7 isolate was resistant to garlic (Figure 3). The results revealed that the isolated LAB differ in their resistance to garlic (Zaika, 1988). Inhibitory action of garlic towards LAB isolates is due to the presence of Allicin, which is an inhibitor of respiratory SH-group enzyme (Beuchat and Golden, 1989) and of acetyl co A synthatase (Focke *et al.,* 1990).

Two Goat isolates, G7 and G8 showed resistance to cloves. The remaining two Goat isolates, G4 and G11 and Cow isolate, C12 were found to be sensitive (Figure 6). Inhibitory action of cloves on the growth of isolated LAB may be due to bacteriocidal action of Eugenol by the inhibition of energy generation (Gill and Holley, 2004).

Table.1 Gram staining, shape and catalase characteristics of isolated LAB from Goat milk

Goat isolates	Gram Staining	Shape	Catalase Test
G1	+ve	Rods	-ve
G2	+ve	Short rods	-ve
G3	+ve	Short rods	-ve
G4	+ve	Large cocci	-ve
G5	+ve	Short rods	-ve
G6	+ve	Short rods	-ve
G7	+ve	Large cocci	-ve
G8	+ve	Very short rods	-ve
G9	+ve	Short rods in pairs	-ve
G10	+ve	Long rods	-ve
G11	+ve	Long rods	-ve

Table.2 Gram staining, shape and catalase characteristics of isolated LAB from Cow milk

Cow isolate	Gram Staining	Shape	Catalase Test
C1	+ve	Short rods	-ve
C2	+ve	Short rods	-ve
C3	+ve	short rods	-ve
C4	+ve	Very Short rods	-ve
C5	+ve	short rods	-ve
C6	+ve	Very Short rods	-ve
C7	+ve	Short rods	-ve
C8	+ve	Short rods in pairs	-ve
C9	+ve	Thin short rods	-ve
C10	+ve	Short rods	-ve
C11	+ve	Short rods	-ve
C12	+ve	Short rods	-ve
C13	+ve	Small cocci	-ve
C14	+ve	Small cocci	-ve
C15	+ve	rods	-ve

Table.3 Tolerance of isolated LAB from goat milk to acidic pH

Isolate	Absorbance at 620 nm		Growth as percentage of control	Acid resistance
	Control	Test		
G1	0.655	0.215	32	S
G2	0.625	0.233	38	S
G3	0.605	0.225	37	S
G4	0.772	0.641	83	**R**
G5	0.697	0.210	30	S
G6	0.686	0.210	31	S
G7	0.597	0.587	98	**R**
G8	0.689	0.682	99	**R**
G9	0.661	0.207	31	S
G10	0.668	0.316	48	S
G11	0.662	0.440	66.46	**MR**

Table.4 Tolerance of isolated LAB from Cow milk to acidic pH

Isolate	Absorbance at 620 nm		Growth as Percentage of control	Acid resistance
	Control	Test		
C 1	0.520	0.256	49	S
C2	0.455	0.207	46	S
C3	0.571	0.2	35	S
C4	0.432	0.245	57	S
C5	0.891	0.401	45	S
C6	0.381	0.161	42	S
C7	0.931	0.001	NIL	HS
C8	0.063	0.001	NIL	HS
C9	0.042	0.001	NIL	HS
C10	0.072	0.001	NIL	HS
C11	0.012	0.001	NIL	HS
C12	0.200	0.24	90	**R**
C13	0.200	0.001	NIL	HS
C14	0.355	0.182	51	S
C15	0.395	0.063	16	HS

Table.5 Antogonistic activity of acid resistant goat milk and cow milk isolates against indicator micro organisms (Zone of inhibition in mm)

Isolate	Indicator micro organisms			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
G4	17	12	Nil	Nil
G7	Nil	Nil	Nil	Nil
G8	20	12	12	28
G11	12.5	10	Nil	Nil
C12	Nil	17	18	Nil

Table.6 Antimicrobial activity of Chilly, Ginger and Garlic on acid resistant goat milk and cow milk isolates (Zone of Inhibition in mm)

Isolate	4% Chilly	8% Chilly	4% Ginger	8% Ginger	4% Garlic	8% Garlic
G4	Nil	Nil	Nil	Nil	15	16
G7	Nil	Nil	Nil	Nil	Nil	Nil
G8	Nil	Nil	Nil	Nil	14	16
G11	Nil	Nil	Nil	Nil	14	16
C12	Nil	Nil	Nil	Nil	13	15

Table.7 Antimicrobial activity of coriander, cinnamon and cloves on acid resistant goat milk and cow milk isolates (Zone of Inhibition in mm)

Isolate	4% Coriander	8% Coriander	4% Cinnamon	8% Cinnamon	4% Cloves	8% Cloves
G4	Nil	Nil	Nil	Nil	14	15
G7	Nil	Nil	Nil	Nil	Nil	Nil
G8	Nil	Nil	Nil	Nil	Nil	Nil
G11	Nil	Nil	Nil	Nil	15	15
C12	Nil	Nil	Nil	Nil	16	16

Figure.1 Growth of isolated LAB from goat milk and cow milk in the presence of different concentrations of chilly

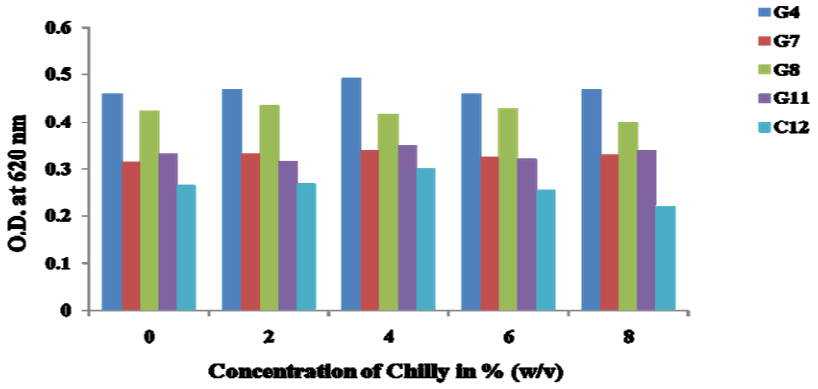


Figure.2 Growth of isolated LAB from goat milk and cow milk in the presence of different concentrations of ginger

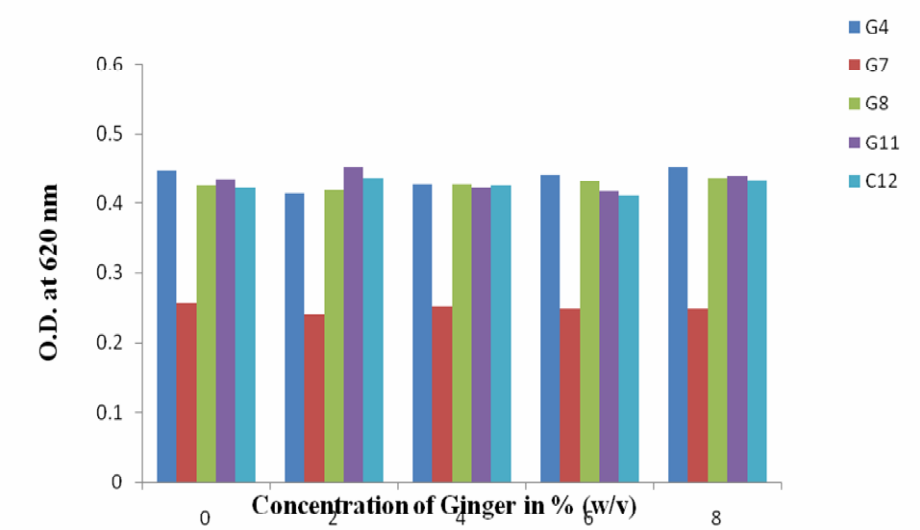


Figure.3 Growth of isolated LAB from goat milk and cow milk isolates in the presence of different concentrations of garlic

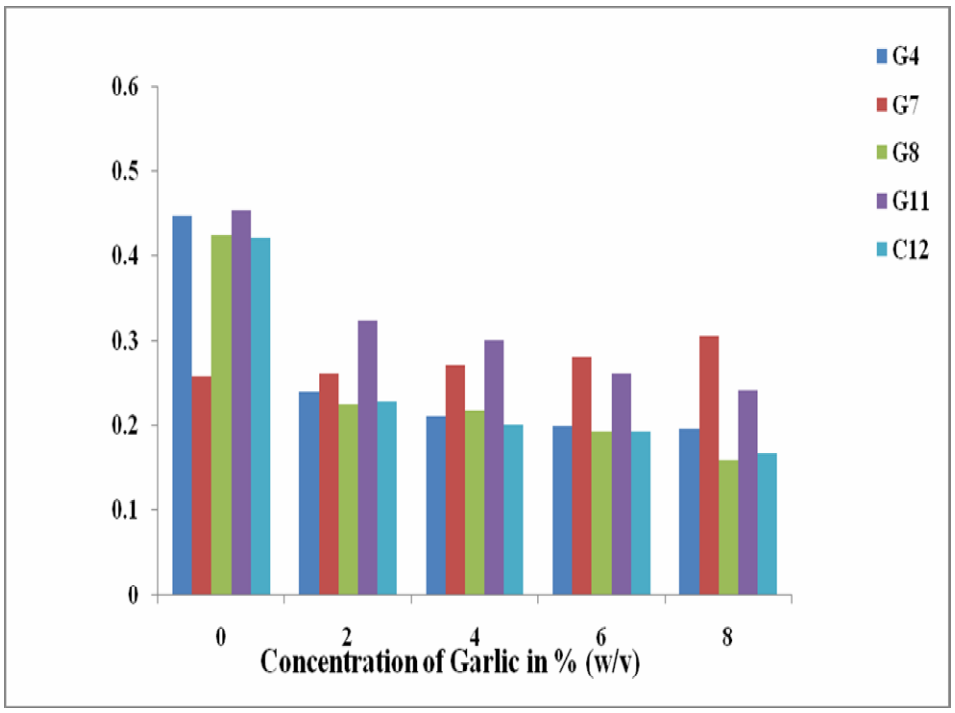


Figure.4 Growth of isolated LAB from goat milk and cow milk in the presence of different concentrations of coriander

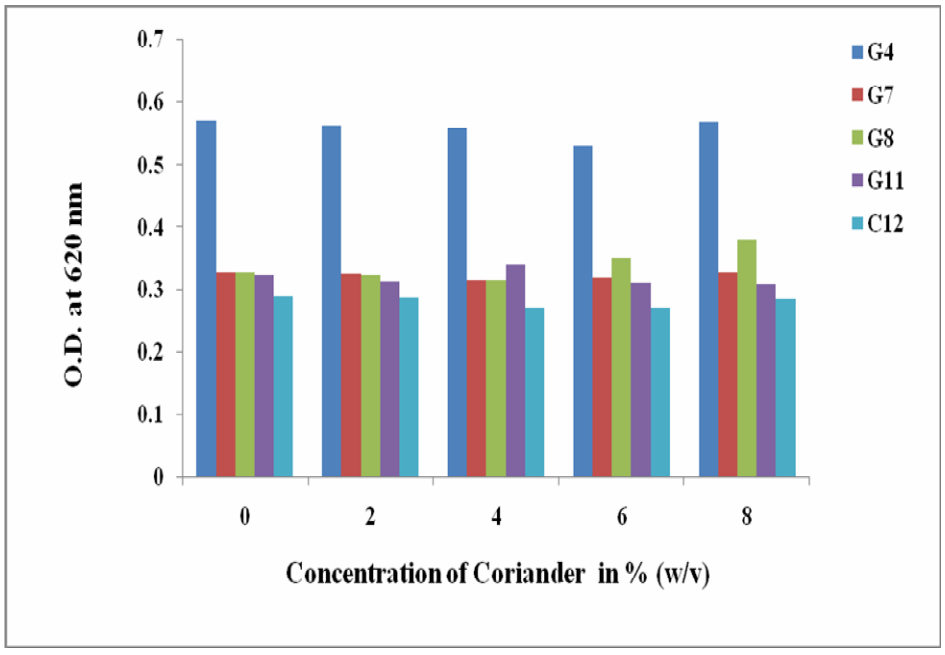


Figure.5 Growth of isolated LAB from goat milk and cow milk in the presence of different concentrations of cinnamon

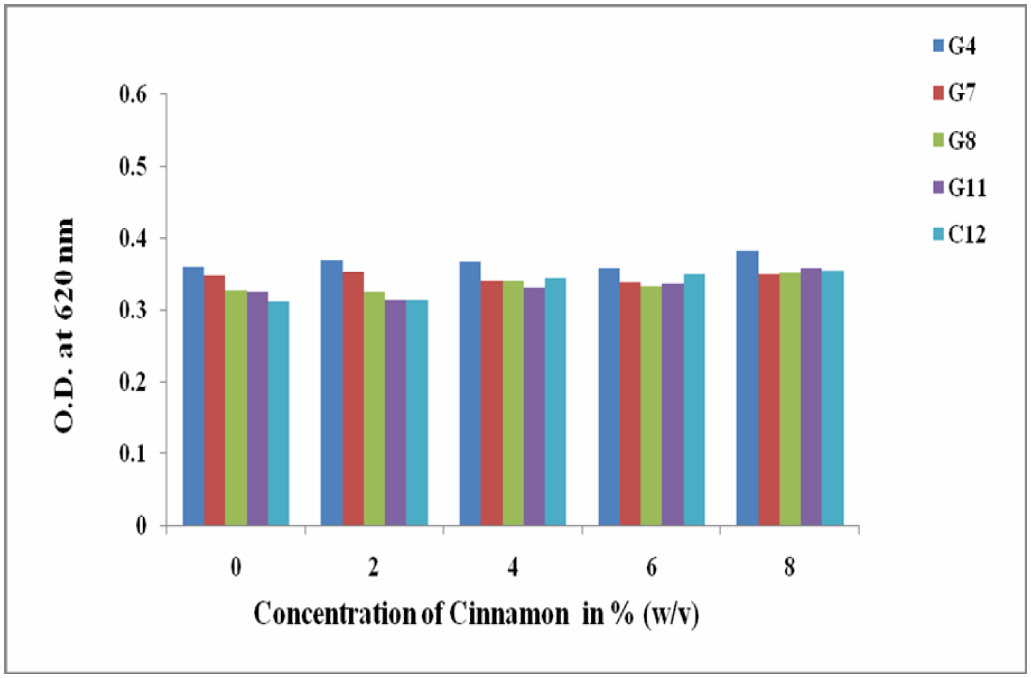


Figure.6 Growth of isolated LAB from goat milk and cow milk in the presence of different concentrations of cloves

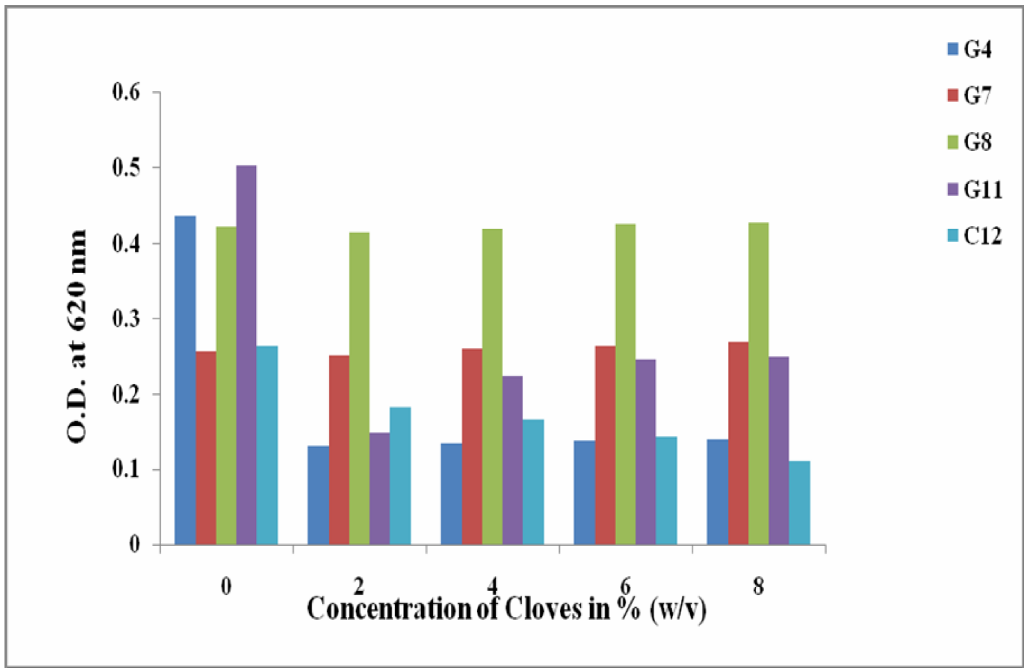
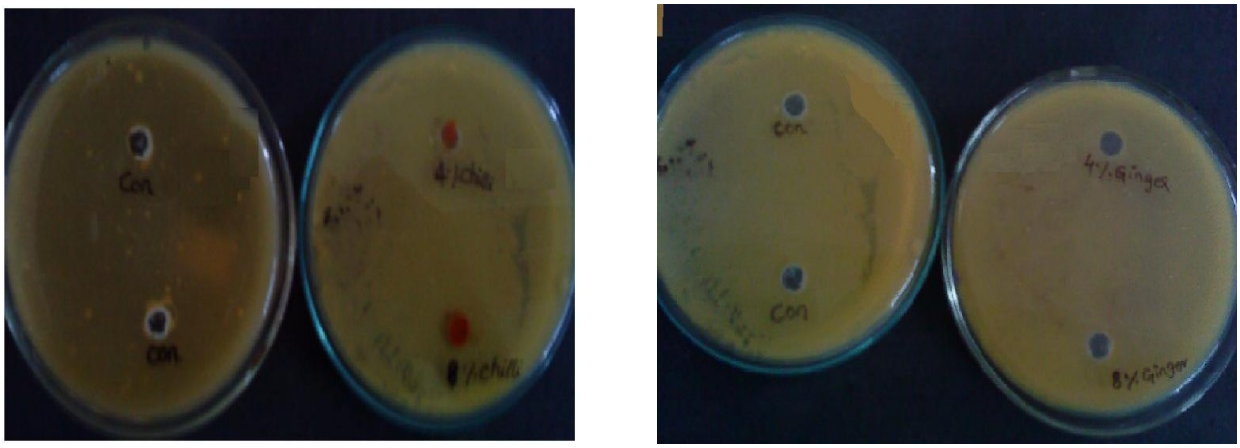
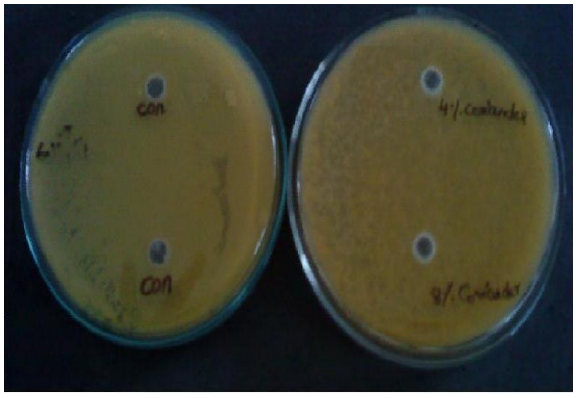


Figure.7 Tolerance of goat milk isolate, G8 to antimicrobial activity of spices
(a) Chilly (b) Ginger (c) Coriander



Control Test
(a)

Control Test
(b)



Control Test
(c)

Figure.8 Agarose gel showing amplified 16s rRNA gene of goat milk isolate, G8

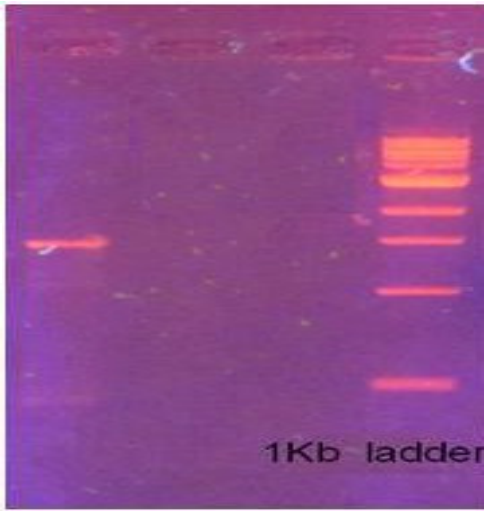
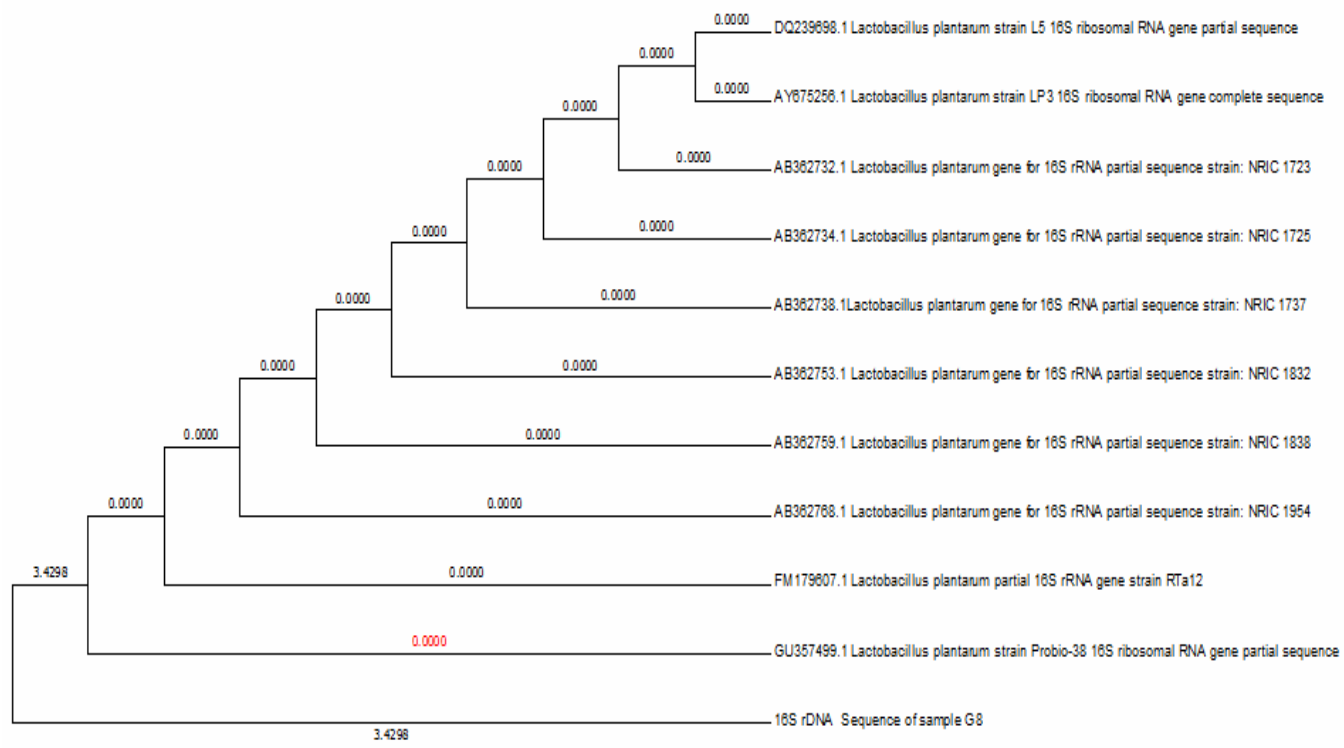


Figure.9 Dendrogram showing phylogenetic relationship of goat milk isolate, G8 with *Lactobacillus plantarum*



In conclusion, Lactic acid bacteria with probiotic potential were isolated from Goat milk and Cow milk. A total of 11 and 15 LAB strains were isolated from Goat and Cow milk samples respectively. Three Goat isolates, G4, G7, G8 and one Cow isolate, C12 were considered highly tolerant to acidic pH. Acid tolerant Cow and Goat milk isolates showed notable differences in antagonistic activity against indicator organisms.G7 isolate did not inhibit the growth of all the tested bacteria while G8 isolate exhibited promising antagonistic activity inhibiting both gram positive and gram negative organisms. Except cloves and garlic, remaining spices did not have any effect on our probiotic isolates.

G8 isolate of Goat milk was considered as potential probiotic as it exhibited acid resistance, antagonistic activity against all the tested bacteria and tolerance to antimicrobial activity of majority of spices, and was identified as *Lactobacillus plantarum* by16s rRNA gene sequencing. *Lactobacillus plantarum* G8 culture would be evaluated for other probiotic properties and stability in future studies, so that this indigenous probiotic could be used for commercialization.

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