Diversity of Lactobacilli Associated with Camel Milk in Southern Rajasthan

Final report of

UGC MAJOR RESEARCH PROJECT

Reference No.-UGC F.No.40-168/2011 (SR)

Submitted by

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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **Title of the Project**
Diversity of lactobacilli associated with camel milk in Southern Rajasthan.

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR
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11. OBJECTIVES OF THE PROJECT - Copy enclosed (Encl.-1)

12. WHETHER OBJECTIVES WERE ACHIEVED – Copy enclosed (Encl. 3a)

13. ACHIEVEMENTS FROM THE PROJECT – Copy enclosed (Encl.2)

14. SUMMARY OF THE FINDINGS – Copy enclosed (Encl. 3b)

15. CONTRIBUTION TO THE SOCIETY – Copy enclosed (Encl. 4)

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT- Encl. 4

17. NO. OF PUBLICATIONS OUT OF THE PROJECT – 4 Research papers (Copy enclosed)

(PRINCIPAL INVESTIGATOR) (REGISTRAR/PRINCIPAL)

Encl.1

Objectives of the Project:

1. Bacteriological analysis of camel milk.
2. Isolation of lactobacilli from camel milk samples.
3. Morphological, cultural and biochemical characterization of isolates.
4. Molecular typing of lactobacilli using genus specific PCR or RAPD.
5. Screening of lactobacilli isolates for bile tolerance, antibacterial activity, detection of *bsh* gene and antibiotic resistance

Encl.2

Publication

- Khandelwal, D., Joshi, H. and Chaudhary, B.L. (2012). Molecular identification of lactobacilli isolated from camel milk. *Int. J. Agri. Food sci. tech.,*: 170-172.
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- Khandelwal, D., Joshi, H. and Chaudhary, B.L. (2015). Antagonistic effect of lactobacilli of camel milk against Aeromonas veronii isolated from pichola lake Udaipur. *Res. J. Recent. Sci*., 4: 170-172.

Conferences

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- Khandelwal, D., Joshi, H. and Chaudhary, B.L. (2013). "Identification of lactobacilli isolated from camel milk",19th ISBC International Conference (ISCBC – 2013), Indian Society of Chemists & Biologists and Department of chemistry, MLSU, Udaipur, India.
- Khandelwal, D., Joshi, H. and Chaudhary, B.L. (2012). "Molecular identification of lactobacilli isolated from camel milk", International conference on agriculture, food science and environment technology for sustainable global development, Krishi Sanskriti., J.N.U New Delhi, India.

Encl.3a

1. Collection of Samples

A total of 12 samples were collected from different regions parts of Southern Rajasthan (Table 1). A total of 1 sample collected from Banswara, 1 sample from Chittorgarh, 1 sample from Dungarpur, 1 sample from Pratapgarh, 2 samples from Rajsamand, 2 samples from Sirohi and 4 samples from Udaipur. These samples were collected in a presterlized screw cap bottle.

2. Bacteriological analysis of camel milk

A total 12 sample were analysed for TVBC (Total viable bacterial count) and coliform count. The data of the TVBC and coliform count found in raw camel milk from the entire collected sample is presented in (Table 2). The TVBC value for different camel milk samples ranged from 7.03 to 8.07 log cfu/ml. The mean value of TVBC ranged between 7.03 to 7.87 log cfu/ml.

The coliform count of the camel milk samples confirmed the presence of coliform in all the samples. The coliform count of different camel milk samples ranged between 5.15 to 4.01 log cfu/ml. The mean value of coliform count ranged between 4.97 to 4.01 log cfu/ml.

3. Isolation of lactobacilli

Isolation of lactobacilli from camel milk samples was done on MRS and Elliker agar. A total of 150 isolates comprising of 4 from Banswara district, 9 from Chittorgarh district, 14 from Dungarpur district, 4 from Pratapgarh district, 12 from Rajsamand district, 40 from Sirohi district and 66 from Udaipur district were recovered (Table 3).

4. Morphological and cultural characterization of lactobacilli Isolates:

A total of 150 isolates were subjected to morphological characterization based on Gram staining. The data pertaining to same has been presented in Table 3. A total of 87 isolates comprising of 3 from Banswara, 2 from Chittorgarh, 10 from Dungarpur, 1 from Pratapgarh, 5 from Rajsamand,

16 from Sirohi and 50 from Udaipur were found to be gram-positive and rod shaped. Rest of the isolates were found to be either gram-negative or cocci. All the 87 isolates were grown on MRS agar for analyzing cultural characteristics. Colonies of all 87 isolates were appeared off white in color, smooth, shiny, opaque with entire margin and convex elevation.

4. PCR based identification of lactobacilli

A total of 87 isolates were subjected to PCR by using Lb1 and Lb2 primer (Quere *et. al*., 1997).A total of 70 isolates (10 from Dungarpur, 10 from Sirohi and 50 from Udaipur) out of 87 isolates showed 200 bp products thereby confirming that they belong to genus *Lactobacillus*. The remaining 17 isolates didn't show 200 bp product.

5. Biochemical identification of lactobacilli isolates

A total of 70 isolates were subjected to biochemical characterization. All isolates were subjected to test the catalase activity, litmus milk reaction, ability to produce gas by the fermentation of glucose, ability to grow at different temperatures (45ºC and 15ºC), growth on BCP-MRS medium, reduction of nitrate, NH_3 production from arginine and hydrolysis of esculin. A total of 70 isolates were found to be catalase negative. Among 70 isolates, 68 isolates reduced litmus milk after 48h of incubation and only 2 isolates reduced litmus milk after 24 h. A total of 43 isolate out of 70 produced gas from glucose. A total of 19 isolates were able to grow at both 15^0C and 45^0C. The remaining 43 isolates showed growth only at 45^0C and rest of 8 isolates were able to grow at 15^0C only. All isolates produced yellowish colony on MRS-BCP supplemented medium. All the isolates were found to be negative for nitrate reduction test. A total of 43 isolate out of 70 produced ammonia (NH_3) from arginine. A total of 27 isolates out of 70 hydrolyzed esculin.

A total of 70 isolates were further investigated for carbohydrate fermentation reactions. Different sugar discs of maltose, fructose, dextrose, lactose, sucrose, rhamnose, raffinose and mellibiose were used. A total of 48 isolates out of 70 were able to ferment maltose, fructose, dextrose, lactose, sucrose, raffinose and mellibiose except rhamnose . A total of 19 isolates were able to ferment maltose, fructose, dextrose, lactose, sucrose, rhamnose except raffinose and melliobiose.

Rest of 3 isolates were able to ferment maltose, fructose, dextrose, lactose except sucrose, rhamnose, raffinose and melliobiose.

6. Identification of lactobacilli using PIB Bryant software

These results obtained from biochemical tests were fed to *Lactobacillus* matrice of PIB Bryant software and the following results (Table-4) were obtained from the programme. Among 70 isolates, 43 isolates showed identification score 0.9995 for *Lactobacillus fermentum* and therefore they were identified as *Lactobacillus fermentum*. A total of 19 isolates showed identification score 0.9965 for *Lactobacillus rhamnosus* and they were identified as *Lactobacillus rhamnosus.* A total of 5 isolates showed identification score 0.9998 for *Lactobacillus plantarum*. Identification score of remaining 3 isolates did not reach to 0.95. The most likely taxa is *Lactobacillus casei.*

In previous report, the isolate CMU 1 was identified as *L. plantarum* and CMU 3 and CMU 14 were identified as *Lactobacillus lactis.* Further experiments were carried out to identify these strains. The results for the same indicated a different sugar pattern for these three isolates. On the basis of the results finally CMU 1 was identified as *L. fermentum* and CMU 3 and CMU 14 were identified as *L. rhamnosus.*

7. 16S rRNA sequencing:

A total of 70 isolates were subjected to 16S rRNA sequence analysis. The DNA of all isolates was amplified by PCR using semi universal primers (Lb1 and Lb2). All the isolates gave specific band of 200bp which is further sequenced by Bangalore genei pvt. Ltd. Sequence data obtained after partial sequencing of 16S rRNA were analysed by BLAST and were submitted to EMBL-EBI database under the accession numbers as given in Table 5.

8. Screening of potential probiotic *Lactobacillus* isolates for different activities

All *Lactobacillus* isolates were screened for potential probiotic properties. The properties taken under consideration were bile tolerance, antibacterial activity, BSH activity and antibiotic resistance.

8.1 Bile tolerance

To screen the bile tolerance of 70 *Lactobacillus* isolates, the MRS agar medium was supplemented with three different bile salts namely oxgall, sodium taurocholate and sodium taurodeoxycholate at different concentrations such as 0.1, 0.2, 0.3, 0.4 and 0.5% of each bile salt.

A total of 43 isolates of *Lactobacillus fermentum* showed varied degree of growth when grown in MRS medium supplemented with different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) of oxgall, sodium taurocholate and sodium taurodeoxycholate. The data for the same presented in Table 6.

All *Lactobacillus fermentum* isolates were subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of oxgall. Among 43 *Lactobacillus fermentum* strains, 100% (43/43) isolates were able to grow at 0.1% oxgall. However, 34.88 % (15/43) isolates were able to grow upto 0.2 % oxgall. Only 11.62 % (5/43) isolates were able to grow upto 0.3% oxgall. At 0.4% and 0.5% concentration of oxgall, none of the strains showed growth.

All *Lactobacillus fermentum* strains were further subjected bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of sodium taurocholate. A total of 43 strains of *Lactobacillus fermentum*, only 23.25% (10/43) strains were able to grow at 0.1% sodium taurocholate. However, 13.95% (6/43) strains were able to grow up to 0.2% sodium taurocholate. At 0.3%, 0.4% and 0.5% concentration of sodium taurocholate, none of the strain showed growth.

A total of 43 strains of *Lactobacillus fermentum* were also subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of sodium taurodeoxycholate. Among 43 *Lactobacillus fermentum* strains, 100% (43/43) isolates were able to grow at 0.1% of sodium tauroodeoxycholate. However, 97% (42/43) strains were able to grow up to 0.2%. At

0.3% and 0.4%, 81.39% (35/43) and 20.93% (9/43) strains were grown, respectively. At 0.5 % concentration of sodium taurodeoxycholate, none of the strain showed growth.

A total of 19 isolates of *Lactobacillus rhamnosus* showed varied degree of growth when grown in MRS medium supplemented with different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%) of oxgall, sodium taurocholate and sodium taurodeoxycholate. The data for the same presented in Table 7.

A total of 19 isolates of *Lactobacillus rhamnosus* were subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of oxgall. Among 19 *Lactobacillus rhamnosus* strains, 89.47% (17/19) were able to grow at 0.1% oxgall. However, 57.89% (11/19) were able to grow up to 0.2% of oxgall. Similarly, 26.31% (5/19) were able to grow up to 0.3% oxgall. At 0.4% of oxgall, 15.78% (3/19) isolates showed growth. At 0.5 % oxgall, none of the strains showed growth

All *Lactobacillus rhamnosus* strains were further subjected bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of sodium taurocholate. *Lactobacillus rhamnosus* showed less growth on MRS medium supplemented with sodium taurocholate. Among 19 isolates, only 47.38% (9/19) were able to grow at 0.1% sodium taurocholate. However, 31.57% (6/19) isolates were able to grow up to 0.2% sodium taurocholate. Only 15.78% (3/19) isolates were able to grow at 0.3 % sodium taurocholate. At 0.4 % and 0.5% sodium taurocholate, none of the strains showed growth.

All the *Lactobacills rhamnosus* strains were also subjected to test the bile tolerance on MRS agar supplemented with sodium taurodeoxycholate. At 0.1 % sodium taurodeoxycholate, 100% (19/19) strains showed growth. Among 19 *Lactobacillus rhamnosus* strains, 63.15% (12/19) strains were able to grow up to 0.2% sodium taurodeoxycholate. However, 47.36% (9/19) were able to grow up to 0.3% sodium taurodeoxycholate. At 0.4% sodium taurodeoxycholate, 26.31% (5/19) were showed growth. At 0.5% sodium taurodeoxycholate, none of the isolates showed growth.

A total of 5 isolates of *Lactobacillus plantarum* were subjected to test the bile tolerance on varied concentration (0.1%, 0.2%,0.3%, 0.4% and 0.5%) of different bile salts such as oxgall,

sodium taurocholate and sodium taurodeoxycholate supplemented in MRS agar medium. The data has been presented in Table 8.

At 0.1% oxgall, 100% (5/5) isolates showed growth. Among 5 isolates, 40% (2/5) isolates were able to grow up to 0.2% oxgall. At 0.3%, 0.4% and 0.5% concentration of oxgall, none of the isolate showed growth.

Similarly, Among 5 isolates, 20% (1/5) isolates were able to grow at 0.1 % sodium taurocholate. At 0.2 %, 0.3%, 0.4% and 0.5% sodium taurocholate, none of the isolates showed growth.

A total of 5 isolates were also subjected to test bile tolerance on sodium taurodeoxycocholate. Among 5 isolates, 100% (5/5) isolates were able to grow at 0.1 % and 0.2 % sodium taurodeoxycholate. Among 5 isolates, 40% (2/5) isolates were able to grow up to 0.3% sodium taurdeoxycholate. At 0.4% and 0.5% sodium taurodeoxycholate, none of the isolate showed growth.

A total of 3 isolates of *Lactobacillus casei* were subjected to test the bile tolerance using varied concentration (0.1%, 0.2%,0.3%, 0.4% and 0.5%) of different bile salts such as oxgall, sodium taurocholate and sodium taurodeoxycholate. The data for the same presented in Table 9.

Among 3 isolates of *Lactobacillus casei*, 100 % (3/3) isolates were able to grow at 0.1% oxgall. However, 33.33% (1/3) isolates were able to grow up to 0.2% oxgall. At 0.3%, 0.4% and 0.5% oxgall, none of the isolates showed growth.

At 0.1%, 0.2%, 0.3%, 0.4% and 0.5% sodium taurocholate, none of the isolates showed growth.

On the other hand, among 3 isolates of *Lactobacillus casei*, 100% (3/3) isolates were able to grow at 0.1% and 0.2% sodium taurodeoxycholate. However, 33.33% (1/3) isolates were grown at 0.3% and 0.4% concentration of sodium taurodeoxycholate. At 0.5% sodium taurodeoxycholate, none of the strains showed growth.

8.2 Antibacterial activity:

A total of 70 *Lactobacillus* isolates namely *Lactobacillus fermentum, Lactobacillus rhamnosus, Lactobacillus plantarum and Lactobacillus casei* were tested for antibacterial activities against

gram-negative such as *Enterobacter aerogenes, Proteus vulgaris, Serratia marcescens, Pseudomonas aeroginosa* and gram-positive bacteria such as *Micrococcus luteus.*

The antibacterial activity was determined in the two fractions of the cell free supernatants: normal cell supernatant and cell supernatant neutrilized with 1N NaOH. The normal cell supernatants of the isolates showed greater inhibition zone than the supernatants neutralized with NaOH. All the *Lactobacillus* isolates showed antibacterial activity against the test organisms included in this study though they vary in diameter of zone of inhibition.

In 43 *Lactobacillus fermentum,* the highest zone of inhibition was found against *Micrococcus luteus* (31 mm) and lowest zone of inhibition was (9 mm) against *Proteus vulgaris* in the bacterial supernatant without NaOH. The sizes of inhibition zones against rest of the indicator organisms (supernatant without NaOH) were ranged from 11 to 25 mm. Similarly, bacterial supernatant with NaOH showed highest zone of inhibition against *Micrococcus luteus* (21mm). The sizes of inhibition zone against rest of the indicator organisms (supernatent with NaOH) were ranged between 9 to 19 mm. The data are presented in Table 10.

In 19 *Lactobacillus rhamnosus* isolates, the highest zone of inhibition was found against *Micrococcus luteus* (30 mm) and lowest zone of inhibition was found against *Serretia marcescens* (10 mm) in the bacterial supernatant without NaOH. The sizes of inhibition zones against rest of the indicator organisms (supernatant without NaOH) were ranged from 12 to 29 mm. Similarly bacterial supernatant with NaOH showed highest inhibition zone against *Micrococcus luteus* (20 mm). The sizes of inhibition zones against rest of the indicator organisms (supernatant with NaOH) were ranged between 9 to 18 mm. The data are presented in Table 11.

In 5 *Lactobacillus plantarum,* the highest zone of inhibition was found against *Proteus vulgaris* (30mm) and lowest zone of inhibition against *Serretia marcescens* (12mm) in the bacterial supernatant without NaOH. The sizes of inhibition zones against rest of the indicator organisms (supernatent without NaOH) were ranged from 13 to 28 mm. Similarly, bacterial supernatant with NaOH showed highest inhibition zone against *Proteus vulgaris* (20 mm). The sizes of inhibition zone against rest of the indicator organisms (supernatant with NaOH) were ranged between 9 to 19 mm. The data are presented in Table 12.

In case of 3 *Lactobacillus casei* isolates, the highest inhibition zone was found also against *Micrococcus leuteus* (27mm) and lowest zone of inhibition was found against *Pesudomonas aeruginosa* (13 mm) in bacterial supernatant without NaOH. The sizes of inhibition zones against rest of the indicator organisms (supernatant without NaOH) were ranged from 15 to 21 mm. Similarly, bacterial supernatant with NaOH showed highest inhibition zone against *Serretia marcesens* (18 mm). The data are presented in Table 13.

8.3 Detection of BSH gene:

A total of 70 *Lactobacillus* isolates were subjected to PCR assay for detection of bile salt hydrolase activity by using bsh gene specific primer LbBSHF/R. Among 70 isolates, only 2 isolates namely *Lactobacillus fermentum* CMU 1 and *Lactobacillus fermentum* CMU 7 showed the amplification of an expected PCR product of size 231bp.These 2 isolates were found to be BSH positive strains.

8.4 Antibiotic resistance:

A total of 70 isolates were subjected to test the antibiotic resistance against 7 antibiotics namely ampicillin, tetracycline, kanamycin, streptomycin, penicillin, vancomycin and rifampicin by disc diffusion method.

All *Latobacillus fermentum* isolates (n=43) were found to be resistant to vancomycin. Among 43 isolates, only 2 isolates were sensitive to kanamycin. Rest of the isolates was resistant to kanamycin. Similarly, a total of 17 isolates out of 43 were sensitive to streptomycin and the remaining 26 isolates were resistant to streptomycin. All 43 isolates of *Lactobacillus fermentum* were sensitive against ampicillin, tetracycline, penicillin and rifampicin. The diameter of inhibition zone of *Lactobacillus fermentum* isolates against different antibiotics namely ampicillin, tetracyclin, kanamycin, streptomycin, penicillin and rifampicin were ranged from 10 mm to 55 mm. The data are presented in Table 14.

Similarly, all *Lactobacillus rhamnosus* (n=19) isolates were found to be resistant to kanamycin and vancomycin. A total of 9 isolates out of 19 were sensitive to streptomycin. The remaining 10 isolates were resistant to streptomycin. All *Lactobacillus rhamnosus* isolates were sensitive against ampicillin, tetracycline, penicillin and rifampicin except isolate CMU 50.The isolate

CMU 50 was resistant to all antibiotics which were used in this study. The inhibition zone of *Lactobacillus rhamnosus* against different antibiotics namely ampicillin, tetracycline, streptomycin, penicillin and rifampicin were ranged from 14 mm to 55mm. The data has been presented in Table 15.

A total of 5 isolates of *Lactobacillus plantarum* were strictly resistant to kanamycin and vancomycin. Among 5 isolates, 1 isolate i.e. *Lactobacillus plantarum* CMU 8 was found to be resistant to streptomycin and the remaining 4 isolates were sensitive to streptomycin. Similarly, 1 isolate i.e. *Lactobacillus plantarum* CMU 5 was found to resistant to penicillin and the remaining 4 isolates were sensitive to penicillin. All *Lactobacillus plantarum* isolates were sensitive ampicillin, tetracycline and rifampicin. The inhibiton zone of *Lactobacillus plantarum* against different antibiotics ampicillin, tetracycline, streptomycin, penicillin and rifampicin were ranged from 15mm to 55mm. The data pertaining the same presented in Table 16.

A total of 3 isolates of *Lactobacillus casei* were found to be resistant to kanamycin and vancomycin. Among 3 isolate, 2 isolates were found to be resistant to streptomycin. All 3 *Lactobacillus casei* isolates were found to be sensitive to ampicillin, tetracycline, penicillin and rifampicn. The inhibition zone of *Lactobacillus casei* against different antibiotics ampicillin, tetracycline, streptomycin, penicillin and rifampicin were ranged from 13mm to 45mm.The data has been presented in Table 17.

Table 1: Districts of sample collection and No. of sample collected

S. No.	Regions from where samples collected	No. of Samples
1.	Banswara	1
2.	Chittorgarh	1
3.	Dungarpur	1
4.	Pratapgarh	1
5.	Rajsamand	2
6.	Sirohi	2
7.	Udaipur	4

Table 2: Microbiological profile of camel milk Samples:

S.No.	District	Sample No.	TVBC (log cfu/ml)	Mean (log cfu/ml)	Coliform count (log cfu/ml)	Mean (log cfu/ml)
1.	Banswara	Banswara 1	7.80	7.80	4.28	4.28
2.	Chittorgarh	Chittorgarh 1	7.64	7.64	4.84	4.84
3.	Dungarpur	Dungarpur 1	7.03	7.03	4.01	4.01
4.	Pratapgarh	Pratapgarh 1	7.87	7.87	4.61	4.61
5.	Rajsamand	Rajsamand 1	7.76	7.82	5.15	4.97
		Rajsamand 2	7.89		4.80	
6.	Sirohi	Sirohi 1	7.28	7.19	4.80	4.55
		Sirohi 2	7.103		4.30	
7.	Udaipur	Udaipur 1	7.954	7.67	4.255	4.20
		Udaipur 2	7.29		4.096	
		Udaipur 3	8.107		4.31	
		Udaipur 4	7.348		4.146	

Table 3: Isolation and morphological characterization of lactobacilli isolates

S. no.	Sample No.	No. of isolates	No. of gram positive and rod
1.	Banswara1	4	3
2.	Chittorgarh 1	9	2
3.	Dungarpur 1	14	10
4.	Pratapgarh 1	4	1
5.	Rajsamand 1	7	3
6.	Rajsamand 2	5	2
7.	Sirohi 1	19	11
8.	Sirohi 2	21	5
9.	Udaipur 1	15	11
10.	Udaipur 2	20	17
11.	Udaipur 3	19	10
12.	Udaipur 4	12	12
	Total	**150**	**87**

Table 4: Identification of lactobacilli using PIB Bryant software:

S. No.	*Lactobacillus* isolates	Possible strains	Identification score
1.	CMU 1, CMU 6, CMU 7,CMU 17,CMU 18, CMU 19, CMU 20, CMU 25, CMU 26, CMU 27, CMU 30, CMU 33, CMU 35, CMU 38, CMU 40, CMU 46, CMU 49, CMU 55, CMU 57, CMU 60 , CMU 61, CMU 62, CMU 63 , CMU 64, CMU 65, CMU 66,CMS 1, CMS 3, CMS 4, CMS 7,CMS 13, CMS 16, CMS 21, CMS 29, CMS 38, CMD 1, CMD 2,CMD 5, CMD 7, CMD 10, CMD 11, CMD 12, CMD 13	*Lactobacillus fermentum*	0.9995
2.	CMU 3, CMU 14, CMU 15, CMU 16, CMU 24, CMU 28, CMU 29, CMU 31, CMU 32, CMU 34,CMU 37, CMU 43, CMU 44, CMU 45, CMU 50, CMU 54, CMU 56, CMU 58, CMU 59	*Lactobacillus casei* subsp. *rhamnosus*	0.9965
3.	CMU 2, CMU 4, CMU 5, CMU 8, CMU, 13	*Lactobacillus plantarum*	0.9998
4.	CMS 8, CMD 6, CMD 14	*Lactobacillus casei* subsp. *casei*	0.94381

Table 5: Identification of isolates by 16S rRNA sequencing:

S.No.	Isolate name	Species	Accession no.
1.	CMU 1	*L. fermentum*	LK985320
2.	CMU 2	*L. plantarum*	LN606815
3.	CMU 3	*L. rhamnosus*	LN613204
4.	CMU 4	*L. plantarum*	LN606816
5.	CMU 5	*L. plantarum*	LN606817
6.	CMU 6	*L. fermentum*	LM994029
7.	CMU 7	*L. fermentum*	LM994030
8.	CMU 8	*L. plantarum*	LN606818
9.	CMU 13	*L.plantarum*	LN606819
10.	CMU 14	*L. rhamnosus*	LM994031
11.	CMU 15	*L. rhamnosus*	LM994032
12.	CMU 16	*L. rhamnosus*	LN613205
13.	CMU 17	*L. fermentum*	LM994033
14.	CMU18	*L. fermentum*	LN613184
15.	CMU 19	*L. fermentum*	LN613185
16.	CMU 20	*L.fermentum*	LN613186
17.	CMU 24	*L.rhamnosus*	LN613206
18.	CMU 25	*L.fermentum*	LN613187
19.	CMU 26	*L.fermentum*	LN613188
20.	CMU27	*L. fermentum*	LM994034
21.	CMU 28	*L.rhamnosus*	LN613207

22.	CMU 29	*L.rhamnosus*	LN613208
23.	CMU 30	*L.fermentum*	LN613189
24.	CMU31	*L.rhamnosus*	LN613209
25.	CMU 32	*L.rhamnosus*	LN558831
26.	CMU 33	*L. fermentum*	LN613190
27.	CMU 34	*L. rhamnosus*	LN558832
28.	CMU 35	*L. fermentum*	LN613191
29.	CMU 37	*L.rhamnosus*	LN613210
30.	CMU 38	*L. fermentum*	LN613192
31.	CMU 40	*L. fermentum*	LN613193
32.	CMU 43	*L. rhamnosus*	LN613211
33.	CMU 44	*L. rhamnosus*	LM994035
34.	CMU 45	*L.rhamnosus*	LN613212
35.	CMU 46	*L. fermentum*	LN613194
36.	CMU 49	*L. fermentum*	LN613195
37.	CMU 50	*L. rhamnosus*	LM994037
38.	CMU 54	*L. rhamnosus*	LN613213
39.	CMU 55	*L. fermentum*	LN613196
40.	CMU 56	*L. rhamnosus*	LM994036
41.	CMU 57	*L. fermentum*	LN558823
42.	CMU 58	*L. rhamnosus*	LN613214
43.	CMU 59	*L. rhamnosus*	LN613215
44.	CMU 60	*L. fermentum*	LN558824

45.	CMU 61	*L. fermentum*	LN558825
46.	CMU 62	*L. fermentum*	LN558826
47.	CMU 63	*L. fermentum*	LN558827
48.	CMU 64	*L.fermentum*	LN558828
49.	CMU 65	*L.fermentum*	LN558829
50.	CMU 66	*L.fermentum*	LN558830
51.	CMS1	*L. fermentum*	LM994038
52.	CMS 3	*L. fermentum*	LM994039
53.	CMS 4	*L. fermentum*	LN558815
54.	CMS 7	*L. fermentum*	LN558816
55.	CMS 8	*L. casei*	LN626980
56.	CMS 13	*L. fermentum*	LN558817
57.	CMS 16	*L. fermentum*	LN558818
58.	CMS 21	*L. fermentum*	LN613197
59.	CMS 29	*L. fermentum*	LN613198
60.	CMS 38	*L. fermentum*	LN613199
61.	CMD 1	*L. fermentum*	LN558819
62.	CMD 2	*L. fermentum*	LN558820
63.	CMD 5	*L. fermentum*	LN558821
64.	CMD 6	*L.casei*	LN626981
65.	CMD 7	*L. fermentum*	LN558822
66.	CMD 10	*L. fermentum*	LN613200
67.	CMD 11	*L. fermentum*	LN613201

68.	CMD 12	*L. fermentum*	LN613202
69.	CMD 13	*L. fermentum*	LN613203
70.	CMD 14	*L. casei*	LN626982

Table 6: Tolerance of *Lactobacillus fermentum* isolates to different concentrations of Oxgall, Sodium taurocholate and Sodium taurodeoxycholate

S. No.	Name of isolates	Oxgall (%)					Sodium taurocholate (%)					Sodium taurodeoxycholate (%)				
		0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
1.	*L. fermentum* CMU 1	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
2.	*L. fermentum* CMU 6	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
3.	*L. fermentum* CMU 7	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
4.	*L. fermentum* CMU 17	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_
5.	*L.fermentum* CMU 18	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_
6.	*L.fermentum* CMU 19	+	+	_	_	_	_	_	_	_	_	+	+	+	+	_
7.	*L.fermentum* CMU 20	+	+	_	_	_	_	_	_	_	_	+	+	+	+	_
8.	*L.fermentum* CMU 25	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
9.	*L.fermentum* CMU 26	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
10.	*L. fermentum* CMU 27	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_

11.	*L.fermentum* CMU 30	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
12.	*L. fermentum* CMU 33	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
13.	*L. fermentum* CMU 35	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
14.	*L. fermentum* CMU 38	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
15.	*L. fermentum* CMU 40	+	+	_	_	_	_	_	_	_	_	+	+	+	+	_
16.	*L. fermentum* CMU 46	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
17.	*L. fermentum* CMU 49	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
18.	*L.fermentum* CMU 55	+	+	_	_	_	_	_	_	_	_	+	+	+	_	_
19.	*L. fermentum* CMU 57	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
20.	*L. fermentum* CMU 60	+	+	_	_	_	+	+	_	_	_	+	+	+	_	_
21.	*L. fermentum* CMU 61	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
22.	*L. fermentum* CMU 62	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_
23.	*L. fermentum* CMU 63	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
24.	*L. fermentum* CMU 64	+	+	_	_	_	_	_	_	_	_	+	+	+	_	_
25.	*L.fermentum* CMU 65	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
26.	*L.fermentum* CMU 66	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
27.	*L. fermentum* CMS 1	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_

28.	*L. fermentum* CMS 3	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
29.	*L. fermentum* CMS 4	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
30.	*L. fermentum* CMS 7	+	+	_	_	_	+	_	_	_	_	+	+	+	+	_
31.	*L. fermentum* CMS 13	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
32.	*L. fermentum* CMS 16	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
33.	*L. fermentum* CMS 21	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
34.	*L. fermentum* CMS 29	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
35.	*L. fermentum* CMS 38	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
36.	*L. fermentum* CMD 1	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
37.	*L. fermentum* CMD 2	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
38.	*L. fermentum* CMD 5	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
39.	*L. fermentum* CMD 7	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
40.	*L. fermentum* CMD 10	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
41.	*L. fermentum* CMD 11	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
42.	*L. fermentum* CMD 12	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_
43.	*L. fermentum* CMD 13	+	_	_	_	_	_	_	_	_	_	+	+	+	_	_

Table: 7- Tolerance of *Lactobacillus rhamnosus* isolates to different concentrations of Oxgall, Sodium taurocholate and Sodium taurodeoxycholate:

S.No.	Name of isolate	Oxgall (%)					Sodium taurocholate (%)					Sodium taurodeoxycholate (%)				
		0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
1.	*L.rhamnosus* CMU 3	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
2.	*L.rhamnosus* CMU 14	+	+	_	_	_	_	_	_	_	_	+	+	_	_	_
3.	*L. rhamnosus* CMU 15	+	+	+	_	_	+	+	_	_	_	+	+	+	+	_
4.	*L.rhamnosus* CMU 16	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
5.	*L.rhamnosus* CMU 24	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_
6.	*L. rhamnosus* CMU 28	+	+	+	+	_	+	+	+	_	_	+	+	+	+	_
7.	*L.rhamnosus* CMU 29	+	+	+	+	_	+	+	+	_	_	+	+	+	+	_
8.	*L.rhamnosus* CMU 31	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_
9.	*L.rhamnosus* CMU 32	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_
10.	*L.rhamnosus* CMU 34	+	+	_	_	_	+	_	_	_	_	+	+	_	_	_
11.	*L.rhamnosus* CMU 37	_	_	_	_	_	_	_	_	_	_	+	_	_	_	_

12.	*L. rhamnosus* CMU 43	+	+	+	+	_	+	+	+	_	_	+	+	+	+	_
13.	*L. rhamnosus* CMU 44	+	+	_	_	_	_	_	_	_	_	+	+	+	_	_
14.	*L.rhamnosus* CMU 45	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_
15.	*L. rhamnosus* CMU 50	_	_	_	_	_	_	_	_	_	_	+	_	_	_	_
16.	*L. rhamnosus* CMU 54	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_
17.	*L. rhamnosus* CMU 56	+	+	_	_	_	+	+	_	_	_	+	+	+	_	_
18.	*L. rhamnosus* CMU 58	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_
19.	*L. rhamnosus* CMU 59	+	_	_	_	_	_	_	_	_	_	+	_	_	_	_

Table 8: Tolerance of *Lactobacillus plantarum* isolates to different concentrations of Oxgall, Sodium taurocholate and Sodium taurodeoxycholate:

S.No.	Name of isolate	Oxgall (%)					Sodium taurocholate (%)					Sodium taurodeoxycholate (%)				
		0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
1.	*L.plantarum* CMU 2	+	+	_	_	_	+	_	_	_	_	+	+	+	_	_
2.	*L.plantarum* CMU 4	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
3.	*L.plantarum* CMU 5	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
4.	*L.plantarum* CMU 8	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
5.	*L.plantarum* CMU 13	+	+	_	_	_	_	_	_	_	_	+	+	+	_	_

Table 9: Tolerance of *Lactobacillus casei* isolates to different concentrations of Oxgall, Sodium taurocholate and Sodium taurodeoxycholate

S. No.	Name of isolate	Oxgall (%)					Sodium taurocholate (%)					Sodium taurodeoxycholate (%)				
		0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	0.5
1.	*L. casei* CMS 8	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_
2.	*L. casei* CMD 6	+	+	_	_	_	_	_	_	_	_	+	+	+	+	_
3.	*L. casei* CMD 14	+	_	_	_	_	_	_	_	_	_	+	+	_	_	_

Table 10: Antibacterial activity of *Lactobacillus fermentum*:

S. No.	**Identified species**	***Enterobacter aerogenes***		***Proteus vulgaris***		***Serretia marcesens***		***Micrococcus luteus***		***Pseudomonas aeruginosa***	
		With NaOH	**without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**without NaOH**
1.	*L. fermentum* CMU 1	19 mm	25 mm	15mm	23 mm	14mm	25mm	15mm	25mm	_	13mm
2.	*L. fermentum* CMU 6	_	15mm	_	9mm	13mm	15mm	11mm	25mm	11mm	15mm
3.	*L. fermentum* CMU 7	_	14mm	16mm	24mm	16 mm	24mm	14mm	24mm	12mm	15mm
4.	*L. fermentum* CMU 17	14mm	21mm	13mm	21mm	_	21mm	15mm	26mm	12mm	18mm
5.	*L. fermentum* CMU 18	15 mm	20 mm	14mm	22mm	15mm	25mm	12mm	24mm	11mm	14mm
6.	*L. fermentum* CMU 19	12mm	22 mm	11mm	15mm	_	20mm	15mm	25mm	15mm	21mm
7.	*L. fermentum* CMU 20	_	18mm	11mm	15mm	_	15mm	12mm	20mm	_	18mm
8.	*L. fermentum* CMU 25	14mm	21mm	15mm	22mm	13mm	23mm	13mm	20mm	12mm	15mm
9.	*L. fermentum* CMU 26	13mm	20mm	14mm	21mm	12mm	22mm	12mm	20mm	11mm	14mm
10.	*L. fermentum* CMU 27	12mm	21mm	11mm	17mm	15mm	24mm	12mm	21mm	12mm	14mm
11.	*L. fermentum* CMU 30	14mm	23mm	12mm	19mm	13mm	20mm	16mm	30mm	11mm	15mm
12.	*L. fermentum* CMU 33	_	15mm	_	21mm	_	11mm	21mm	31mm	11mm	17mm

13.	*L. fermentum* CMU 35	_	_	12mm	20mm	_	_	12mm	21mm	11mm	20mm
14.	*L. fermentum* CMU 38	_	12mm	_	14mm	_	15mm	14mm	26mm	_	11mm
15.	*L. fermentum* CMU 40	_	13mm	_	13mm	_	13mm	13mm	25mm	_	12mm
16.	*L. fermentum* CMU 46	_	13mm	12mm	20mm	_	15mm	15mm	25mm	11mm	16mm
17.	*L. fermentum* CMU 49	_	14mm	_	23mm	_	11mm	15mm	24mm	11mm	16mm
18.	*L.fermentum* CMU 55	10mm	16mm	14mm	17mm	11mm	15mm	15mm	30mm	10mm	14mm
19.	*L. fermentum* CMU 57	12mm	22mm	11mm	15mm	_	20mm	15mm	25mm	15mm	21mm
20.	*L. fermentum* CMU 60	_	18mm	11mm	15mm	_	15mm	13mm	23mm	_	18mm
21.	*L. fermentum* CMU 61	_	19mm	11mm	16mm	_	15mm	14mm	26mm	11mm	18mm
22.	*L. fermentum* CMU 62	_	19mm	_	15mm	11mm	20mm	14mm	23mm	9mm	16mm
23.	*L. fermentum* CMU 63	_	20mm	_	14mm	10mm	18mm	12mm	20mm	_	12mm
24.	*L. fermentum* CMU 64	13mm	21mm	10mm	14mm	14mm	24mm	13mm	22mm	11mm	19mm
25.	*L. fermentum* CMU 65	12mm	21mm	9mm	14mm	12mm	20mm	11mm	21mm	12mm	18mm
26.	*L. fermentum* CMU 66	_	15mm	_	16mm	11mm	20mm	12mm	23mm	10mm	15mm
27.	*L. fermentum* CMS 1	13mm	20mm	10mm	15mm	18mm	21mm	15mm	26mm	_	13mm
28.	*L. fermentum* CMS 3	17mm	21mm	10mm	15mm	16mm	22mm	11mm	20mm	11mm	16mm
29.	*L. fermentum* CMS 4	19 mm	25mm	15mm	23mm	14mm	25mm	12mm	21mm	_	13mm

30.	*L. fermentum* CMS 7	15mm	20mm	12mm	20mm	15mm	21mm	14mm	24mm	10mm	14mm
31.	*L. fermentum* CMS 13	_	19mm	_	14mm	11mm	18mm	12mm	28mm	12mm	16mm
32.	*L. fermentum* CMS 16	13mm	20mm	10mm	18mm	14mm	21mm	15mm	30mm	_	12mm
33.	*L. fermentum* CMS 21	12mm	21mm	11mm	17mm	15mm	24mm	14mm	26mm	12mm	14mm
34.	*L. fermentum* CMS 29	14mm	23mm	12mm	19mm	13mm	20mm	16mm	30mm	11mm	15mm
35.	*L. fermentum* CMS 38	13mm	22mm	10mm	13mm	12mm	23mm	12mm	26mm	12mm	14mm
36.	*L. fermentum* CMD 1	14mm	21mm	9mm	13mm	13mm	23mm	11mm	20mm	13mm	15mm
37.	*L. fermentum* CMD2	13mm	21mm	10mm	14mm	14mm	24mm	12mm	24mm	11mm	16mm
38.	*L. fermentum* CMD 5	12mm	21mm	9mm	14mm	12mm	20mm	13mm	26mm	9mm	16mm
39.	*L. fermentum* CMD 7	11mm	20mm	10mm	15mm	12mm	21mm	13mm	25mm	_	15mm
40.	*L. fermentum* CMD 10	_	15mm	_	15mm	13mm	20mm	15mm	28mm	_	13mm
41.	*L. fermentum* CMD 11	_	16mm	_	16mm	13mm	20mm	16mm	25mm	_	12mm
42.	*L. fermentum* CMD 12	_	12 mm	_	11mm	_	13mm	12mm	23mm	_	_
43.	*L. fermentum* CMD 13	_	20mm	_	15mm	12mm	21mm	11mm	20mm	_	12mm

Table 11: Antibacterial activity of *Lactobacillus rhamnosus:*

S. No.	**Name of isolates**	***Enterobacter aerogenes***		***Proteus vulgaris***		***Serretia marcesens***		***Micrococcus luteus***		***Pseudomonas aeruginosa***	
		With NaOH	**Without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**Without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**without NaOH**
1.	*L. rhamnosus* CMU 3	_	15mm	18 mm	29 mm	_	13mm	14mm	25mm	13mm	17mm
2.	*L. rhamnosus* CMU 14	10mm	15mm	11mm	13mm	_	14mm	16mm	24mm	12mm	16mm
3.	*L. rhamnosus* CMU 15	_	15mm	_	22mm	_	11mm	20 mm	30mm	9mm	17mm
4.	*L. rhamnosus* CMU 16	9mm	16mm	10mm	20mm	11mm	15mm	14mm	28mm	10mm	16mm
5.	*L. rhamnosus* CMU 24	11mm	20mm	12mm	21mm	13mm	22mm	14mm	22mm	9mm	14mm
6.	*L. rhamnosus* CMU 28	14mm	20mm	12mm	18mm	14mm	21mm	13mm	24mm	13mm	16mm
7.	*L. rhamnosus* CMU 29	12mm	16mm	10mm	16mm	10mm	17mm	14mm	28mm	13mm	15mm
8.	*L. rhamnosus* CMU 31	11mm	20mm	10mm	17mm	12mm	21mm	11mm	20mm	10mm	16mm
9.	*L. rhamnosus* CMU 32	_	14mm	16mm	24mm	_	10mm	11mm	18mm	12mm	15mm
10.	*L. rhamnosus* CMU 34	_	_	15mm	22mm	_	_	15mm	30mm	10mm	20mm
11.	*L. rhamnosus* CMU 37	_	12mm	_	17mm	_	15mm	15mm	30mm	12mm	18mm

12.	*L. rhamnosus* CMU 43	_	14mm	10mm	15mm	_	15mm	14mm	28mm	_	13mm
13.	*L. rhamnosus* CMU 44	10mm	15mm	11mm	13mm	_	14mm	12mm	30mm	12mm	16mm
14.	*L. rhamnosus* CMU 45	_	14mm	12mm	18mm	_	16mm	12mm	30mm	12mm	15mm
15.	*L. rhamnosus* CMU 50	_	_	_	15mm	_	_	16mm	25mm	12mm	15mm
16.	*L. rhamnosus* CMU 54	_	15mm	_	22mm	_	11mm	16mm	24mm	9mm	17mm
17.	*L. rhamnosus* CMU 56	_	16mm	_	22mm	_	13mm	13mm	26mm	12mm	18mm
18.	*L. rhamnosus* CMU 58	11mm	20mm	_	14mm	12mm	18mm	11mm	25mm	15mm	20mm
19.	*L. rhamnosus* CMU 59	_	16mm	10mm	15mm	_	15mm	12mm	21mm	14mm	16mm

Table 12: Antibacterial activity of *Lactobacillus plantarum*

S. No.	**Name of isolates**	***Enterobacter aerogenes***		***Proteus vulgaris***		***Serretia marcesens***		***Micrococcus luteus***		***Pseudomonas aeruginosa***	
		With NaOH	**Without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**without NaOH**	**With NaOH**	**With NaOH**	**Without NaOH**	**With NaOH**
1.	*L. plantarum* CMU 2	11mm	14mm	10mm	15mm	10mm	16mm	15mm	19mm	12mm	16mm
2.	*L. plantarum* CMU 4	15mm	18mm	20mm	30mm	_	14mm	15mm	22mm	10mm	15mm
3.	*L. plantarum* CMU 5	11mm	14mm	13mm	19mm	10mm	12mm	19 mm	26mm	11mm	18mm
4.	*L. plantarum* CMU 8	_	16mm	14mm	22mm	_	15mm	12mm	26mm	9mm	13mm
5.	*L. plantarum* CMU 13	_	14mm	12mm	20mm	11mm	22mm	13mm	28mm	11mm	17mm

Table 13: Antibacterial activity of *Lactobacillus casei*:

S. No.	**Name of isolates**	***Enterobacter aerogenes***		***Proteus vulgaris***		***Serretia marcesens***		***Micrococcus luteus***		***Pseudomonas aeruginosa***	
		With NaOH	**Without NaOH**	**With NaOH**	**Without NaOH**	**With NaOH**	**Without NaOH**	**With NaOH**	**With NaOH**	**Without NaOH**	**With NaOH**
1.	*L. casei* CMS 8	14mm	20mm	10mm	15mm	18mm	21mm	11mm	25mm	11mm	15mm
2.	*L. casei* CMD 6	_	15mm	_	16mm	11mm	20mm	14mm	27mm	_	14mm
3.	*L. casei* CMD14	12mm	19mm	11mm	15mm	11mm	19mm	12mm	20mm	_	13mm

Table 14: Antibiotic resistance pattern of *Lactobacillus fermentum* isolates:

S. No.	Name of isolates	Amp	Tet	Kan	Stp	Pen	Van	Rif
1.	*L. fermentum* CMU 1	42mm	18mm	12mm	17mm	27mm	R	25mm
2.	*L. fermentum* CMU 6	23mm	20mm	R	R	20mm	R	27mm
3.	*L. fermentum* CMU 7	32mm	28mm	R	R	35mm	R	30mm
4.	*L. fermentum* CMU 17	30mm	30mm	R	20mm	36mm	R	40mm
5.	*L.fermentum* CMU 18	30mm	22mm	R	R	35mm	R	30mm
6.	*L.fermentum* CMU 19	40mm	33mm	R	R	35mm	R	40mm
7.	*L.fermentum* CMU 20	30mm	29mm	R	R	30mm	R	32mm
8.	*L.fermentum* CMU 25	48mm	35mm	R	12mm	45mm	R	40mm
9.	*L.fermentum* CMU 26	34mm	13mm	R	12mm	30mm	R	28mm
10.	*L. fermentum* CMU 27	32mm	30mm	R	R	31mm	R	31mm
11.	*L.fermentum* CMU 30	23mm	20mm	R	R	20mm	R	27mm
12.	*L. fermentum* CMU 33	34mm	30mm	R	R	30mm	R	27mm
13.	*L. fermentum* CMU 35	35mm	25mm	R	20mm	32mm	R	25mm
14.	*L. fermentum* CMU 38	30mm	23mm	R	10mm	30mm	R	35mm

15.	*L. fermentum* CMU 40	20mm	15mm	R	R	13mm	R	20mm
16.	*L. fermentum* CMU 46	45mm	51mm	R	20mm	48mm	R	55mm
17.	*L. fermentum* CMU 49	16mm	20mm	R	R	14mm	R	15mm
18.	*L.fermentum* CMU 55	20mm	22mm	R	R	20mm	R	25mm
19.	*L. fermentum* CMU 57	40mm	33mm	R	R	35mm	R	40mm
20.	*L. fermentum* CMU 60	30mm	29mm	R	R	30mm	R	32mm
21.	*L. fermentum* CMU 61	35mm	25mm	R	R	25mm	R	15mm
22.	*L. fermentum* CMU 62	35mm	30mm	R	R	32mm	R	32mm
23.	*L. fermentum* CMU 63	33mm	25mm	R	R	27mm	R	30mm
24.	*L.fermentum* CMU 64	45mm	35mm	R	13mm	42 mm	R	45 mm
25.	*L.fermentum* CMU 65	25mm	31mm	R	R	32mm	R	35mm
26.	*L.fermentum* CMU 66	35mm	20mm	R	18mm	25mm	R	25mm
27.	*L. fermentum* CMS 1	35mm	30mm	R	R	34mm	R	33mm
28.	*L. fermentum* CMS 3	40mm	30mm	R	R	29mm	R	35mm
29.	*L. fermentum* CMS 4	42mm	18mm	12mm	17mm	27mm	R	25mm
30.	*L. fermentum* CMS 7	30mm	22mm	R	R	35mm	R	30mm

31.	*L. fermentum* CMS 13	48mm	35mm	R	12mm	45mm	R	40mm
32.	*L. fermentum* CMS 16	34mm	13mm	R	12mm	30mm	R	28mm
33.	*L. fermentum* CMS 21	32mm	30mm	R	R	31mm	R	31mm
34.	*L. fermentum* CMS 29	40mm	30mm	R	R	33mm	R	40mm
35.	*L. fermentum* CMS 38	42mm	34mm	R	16mm	35mm	R	40mm
36.	*L. fermentum* CMD 1	32mm	31mm	R	12mm	30mm	R	32mm
37.	*L. fermentum* CMD 2	45mm	35mm	R	13mm	42mm	R	45mm
38.	*L. fermentum* CMD 5	25mm	31mm	R	R	32mm	R	35mm
39.	*L. fermentum* CMD 7	25mm	15mm	R	R	23mm	R	30mm
40.	*L. fermentum* CMD 10	35mm	22mm	R	R	18mm	R	40mm
41.	*L. fermentum* CMD 11	20mm	25mm	R	15mm	15mm	R	25mm
42.	*L. fermentum* CMD 12	35mm	25mm	R	15mm	18mm	R	25mm
43.	*L. fermentum* CMD 13	20mm	20mm	R	R	20mm	R	26mm

Table 15: Antibiotic resistance pattern of *Lactobacillus rhamnosus*:

S.No.	Name of isolates	Amp	Tet	Kan	Stp	Pen	Van	Rif
1.	*L. rhamnosus* CMU 3	30mm	25mm	R	15mm	45mm	R	40mm
2.	*L. rhamnosus* CMU 14	28mm	30mm	R	20mm	20mm	R	25mm
3.	*L. rhamnosus* CMU 15	25mm	35mm	R	20mm	20mm	R	30mm
4.	*L. rhamnosus* CMU 16	30mm	30mm	R	R	30mm	R	35mm
5.	*L. rhamnosus* CMU 24	35mm	20mm	R	25mm	40mm	R	35mm
6.	*L. rhamnosus* CMU 28	25mm	30mm	R	R	40mm	R	30mm
7.	*L. rhamnosus* CMU 29	38mm	28mm	R	R	42mm	R	29mm
8.	*L. rhamnosus* CMU 31	22mm	21mm	R	R	24mm	R	25mm
9.	*L. rhamnosus* CMU 32	32mm	28mm	R	R	35mm	R	30mm
10.	*L. rhamnosus* CMU 34	40mm	29mm	R	R	36mm	R	32mm
11.	*L. rhamnosus* CMU 37	23mm	20mm	R	R	24mm	R	23mm
12.	*L. rhamnosus* CMU 43	44mm	54mm	R	15mm	45mm	R	50mm
13.	*L. rhamnosus* CMU 44	40mm	24mm	R	R	35mm	R	42mm
14.	*L.rhamnosus* CMU 45	42mm	53mm	R	14mm	50mm	R	53mm

15.	*L. rhamnosus* CMU 50	R	R	R	R	R	R	R
16.	*L. rhamnosus* CMU 54	45mm	55mm	R	25mm	48mm	R	55mm
17.	*L. rhamnosus* CMU 56	43mm	52mm	R	15mm	47mm	R	50mm
18.	*L.rhamnosus* CMU 58	30mm	40mm	R	15mm	32mm	R	35mm
19.	*L.rhamnosus* CMU 59	36mm	20mm	R	R	24mm	R	27mm

Table 16: Antibiotic resistance pattern of *Lactobacillus plantarum*:

S. No.	**Name of isolates**	**Amp**	**Tet**	**Kan**	**Stp**	**Pen**	**Van**	**Rif**
1.	*L. plantarum* CMU 2	25mm	21mm	R	15mm	15mm	R	25mm
2.	*L. plantarum* CMU 4	32mm	35mm	R	18mm	40mm	R	40mm
3.	*L. plantarum* CMU 5	24mm	25mm	R	15mm	R	R	28mm
4.	*L. plantarum* CMU 8	40mm	25mm	R	R	30mm	R	32mm
5.	*L.plantarum* CMU 13	25mm	55mm	R	20mm	15mm	R	50mm

Table 17: Antibiotic resistance pattern of *Lactobacillus casei*:

S. No.	Name of isolates	Amp	Tet	Kan	Stp	Pen	Van	Rif
1.	*L. casei* CMS 8	13mm	28mm	R	R	27mm	R	33mm
2.	*L.casei* CMD 6	35mm	20mm	R	18mm	25mm	R	25mm
3.	*L. casei* CMD 14	45mm	26mm	R	R	25mm	R	30mm

Encl.3b

Summary

The salient features of present investigation on the bacteriological analysis of camel milk, diversity of *Lactobacillus* isolated from camel milk and screening for probiotic potential such as bile tolerance, antibacterial activity, BSH activity and antibiotic susceptibility /resistance are summarized as follows:

1. A total of 12 samples were collected from different regions parts of Southern Rajasthan which includes Banswara, Chittorhgarh, Dungarpur, Pratapgarh, Rajsamand, Sirohi and Udaipur.

2. A total 12 sample were analysed for TVBC (Total viable bacterial count) and coliform count. The TVBC value for different camel milk samples ranged from 7.03 to 8.07 log cfu/ml. The coliform count of different camel milk samples ranged between 5.15 to 4.01 log cfu/ml.

3. A total of 150 isolates comprising of 4 from Banswara district, 9 from Chittorgarh district, 14 from Dungarpur district, 4 from Pratapgarh district, 12 from Rajsamand district, 40 from Sirohi district and 66 from Udaipur district were recovered.

4. A total of 87 isolates out of 150 were found to be gram-positive and rod shaped. Colonies of all 87 isolates were appeared off white in color, smooth, shiny, opaque with entire margin and convex elevation.

5. A total of 70 isolates (10 from Dungarpur, 10 from Sirohi and 50 from Udaipur) out of 87 isolates showed 200 bp products thereby confirming that they belong to genus *Lactobacillus.*

6. Among 70 isolates, 43 isolates were identified as *Lactobacillus fermentum*. A total of 19 isolates were identified as *Lactobacillus rhamnosus.* A total of 5 were identified as *Lactobacillus plantarum*. A total of 3 isolates were identified as *Lactobacillus casei.*

7. A total of 70 isolates were subjected to 16S rRNA sequence analysis. Among 70 isolates, 43 isolates showed sequence similarity to *Lactobacillus fermentum.* A total of 19 isolates showed sequence similarity to *Lactobacillus rhamnosus.* A total of 5 isolates showed sequence similarity to *Lactobacillus plantarum.* A total of 3 isolates were found to be greater 97 % sequence similarity to *Lactobacillus casei.*

8. A total of 70 *Lactobacillus* isolates were subjected to bile tolerance on MRS agar supplemented with various concentrations (0.1% to 0.5%) of oxgall, sodium taurocholate and sodium taurodeoxycholate.

9. Among 43 isolates, 11.62 % (5/43) isolates were able to grow upto 0.3% oxgall. None of the isolates showed growth up to 0.3% sodium taurocholate. At 0.3% and 0.4% sodium tarodeoxycholate, 81.39% (35/43) and 20.93% (9/43) strains were grown, respectively.

10. Among 19 *Lactobacillus rhamnosus* strains, 26.31% (5/19) and 15.78% (3/19) were able to grow up to 0.3% and 0.4% of oxgall, respectively. Only 15.78% (3/19) isolates were able to grow at 0.3 % sodium taurocholate. . However, 47.36% (9/19) were able to grow up to 0.3% sodium taurodeoxycholate. At 0.4% sodium taurodeoxycholate, 26.31% (5/19) were showed growth.

11. Among 5 isolates of *Lactobacillus plantarum*, at 0.3%, 0.4% and 0.5% concentration of oxgall, none of the isolate showed growth. Similarly none of the isolates showed growth at 0.2 %, 0.3%, 0.4% and 0.5% sodium taurocholate. Among 5 isolates, 40% (2/5) isolates were able to grow up to 0.3% sodium taurdeoxycholate.

12. Among 3 isolates of *Lactobacillus casei,* none of the isolates showed growth at 0.3%, 0.4% and 0.5% oxgall. Similarly, none of the isolates showed growth at 0.1%, 0.2%, 0.3%, 0.4% and 0.5% sodium taurocholate. However, 33.33% (1/3) isolates were grown at 0.3% and 0.4% concentration of sodium taurodeoxycholate.

13. Among three bile salts (Oxgall, sodium taurocholate, sodium taurodeoxycholate) tested more tolerance of 70 *Lactobacillus* isolates was observed in the presence of Sodium taurodeoxycholate.

14. A total of 70 *Lactobacillus* isolates namely *Lactobacillus fermentum, Lactobacillus rhamnosus, Lactobacillus plantarum and Lactobacillus casei* were tested for antibacterial activities against gram-negative such as *Enterobacter aerogenes, Proteus vulgaris, Serratia marcescens, Pseudomonas aeroginosa* and gram-positive bacteria such as *Micrococcus luteus.*

15. In 43 *Lactobacillus fermentum,* the highest zone of inhibition was found against *Micrococcus luteus* (31 mm) and lowest zone of inhibition was (9 mm) against *Proteus vulgaris.* In 19 *Lactobacillus rhamnosus* isolates, the highest zone of inhibition was found against *Micrococcus luteus* (30 mm) and lowest zone of inhibition was found against *Serretia marcescens* (10 mm) in the bacterial supernatant. In 5 *Lactobacillus plantarum*, the highest zone of inhibition was found against *Proteus vulgaris* (30mm) and lowest zone of inhibition against *Serretia marcescens* (12mm) in the bacterial supernatant without NaOH. In case of 3 *Lactobacillus casei* isolates, the highest inhibition zone was found also against *Micrococcus leuteus* (27mm) and lowest zone of inhibition was found against *Pesudomonas aeruginosa* (13 mm) in bacterial supernatant without NaOH.

16. A total of 70 *Lactobacillus* isolates were subjected to PCR assay for detection of bile salt hydrolase activity. Among 70 isolates, only 2 isolates namely *Lactobacillus fermentum* CMU 1 and *Lactobacillus fermentum* CMU 7 showed the amplification of an expected PCR product of size 231bp.These 2 isolates were found to be BSH positive strains.

17. A total of 70 isolates were subjected to test the antibiotic resistance against 7 antibiotics namely ampicillin, tetracycline, kanamycin, streptomycin, penicillin, vancomycin and rifampicin.

18. All *Lactobacillus* isolates were resistant to kanamycin and vancomycin except 2 isolates *Lactobacillus fermentum* which were sensitive to kanamycin. All Lactobacillus isolates were sensitive to ampicillin, tetracycline, penicillin and rifampicin except 1 isolate of *Lactobacillus plantarum* were resistant to penicillin. Among 43 isolates of *Lactobacillus fermentum*, 26 isolates were resistant to streptomycin. A total of 10 isolates of *Lactobacillus rhamnosus* out of 19 were resistant to streptomycin. *Lactobacillus rhamnosus* CMU 50 was resistant to all antibiotics which were used in this study. Among 5 isolates of *Lactobacillus plantarum*, 1 isolate was found to be resistant to streptomycin. Among 3 isolates of *Lactobacillus casei*, 2 isolates were found to be resistant to streptomycin.

Encl.4

Ph.D. enrolled

Yes, the Project Fellow, Ms. Deepti Khandelwal, was also enrolled as a Ph.D. student. She has worked on the objectives that have been studied under this project and has submitted the Ph.D. thesis during April 2015.

Contribution to the society

The study provides data on microflora of camel milk and probiotic properties of the isolates. BSH activity of isolates can be explored as a functional probiotic biomarker for the selection of probiotic adjunct to manage hypercholesterolaemia. Antibiotic resistance of isolates can be used as recovering agent in helping to restore the damage intestinal microflora after antibiotic treatment. It may be also used for antibiotic and probiotic combination therapies for the disease such as diarrohea, female urogenital infection and infective endocarditis. A broad spectrum antibacterial property of these isolates can be used in enhancing the shelf life of fermented food products. It may also used in probiotic product for the prevention of food borne diseases. The degree of bile tolerance demonstrated by these isolates can be an important feature for growing them effectively in the upper intestinal tract. Therefore, it can concluded that the study provides data on microflora of camel milk and probiotic properties of the isolates Further exploration of these isolates for good starter activity and flavour production for industrial use as a novel starter culture for the preparation of camel cheese and other fermented dairy products can be done. Hence, some future studies such as adhesion to mucosal surface and clinical studies for human health should be performed to use these isolates reliably.

Consolidated Statement of Expenditure
(Date of Start from 1 July 2011 to 15.12.2014)

S. No.	Sanctioned Heads	Funds Allocated from UGC	Funds Actually Released	Expenditure Incurred				Actual Expenditure Incurred (Rs.)	Available Balance
				1st year (1 July 2011 to 31 March 2012)	2nd year (1 April 2012 to 31 March 2013)	3rd year (1 April 2013 to 31st March 2014)	4th year (1 April 2014 to 15 December 2014)		(III-VIII)
(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)		(VIII)	(IX)
A.	Non-Recurring								
1.	Books & Journals	30,000/-	Rs.5,98,800/- (Sanction No. F. No. 40-168/2011 (SR) dated 01.07.2011	-	30,000/-	-	-	30,000/-	-
2.	Equipment	1,25,000/-		-	98,282/-	-	-	98282/-	26718/-
B.	Recurring								
3.	Project fellow	3,29,342/-		-	78,710/-	64,000/-	1,84,484/-	3,27,194/-	2,148/-
4.	HRA	37,255/-	Rs. 4,03,397/- (Sanction No. F. No. 40-168/2011 (SR) dated 02.09.2014	-	-	-	32719/-	32719/-	4,536/-
5.	Travel	1800/-		-	1000/-	-	800/-	1800/-	-
6.	Chemicals	2,70,000/-		-	1,50000/-	-	1,20,000/-	2,70,000/-	-
7.	Contingencies	1,35,000/-		10,800/-	64,200/-	-	60,000/-	1,35,000/-	
8.	Overhead expenses	73,800/-		-	73,800/-	-	-	73,800/-	-
	Total		10,02,197/-	10,800/-	4,95,992/-	64,000/-	3,98,003/-	9,68,795/-	33,402/-

Name and Signature of Principal Investigator

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Principal Investigator, UGC Project
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Signature of Head of Department

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Signature of Comptroller/Competent financial authority

SATYAM SVG & CO.
Chartered Accountants
CA. Yogesh Chandra Pokharna
(Partner)
Membership No.-071503

COMPTROLLER
Mohanlal Sukhadia University
UDAIPUR

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. **9, 68,795/-** (Rupees Nine lakh sixty eight thousand seven hundred ninety five) out of Rs. **10, 02,197/-** (Rupees Ten lakh two thousand one hundred ninety seven) sanctioned to Dr. Harshada Joshi by the University Grants Commission under the scheme of support for Major Research Project entitled "**Diversity of lactobacilli associated with camel milk in Southern Rajasthan**" vide UGC letter no. F.40-168/2011(SR) dated 1/5.7.2011 and dated 2.9.2014 utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants. The balance of Rs.33, 402/-remain unutilized at the end of the project has been refunded / returned to UGC (vide D.D. No.011244.....dated.10/3/15.).

Principal investigator

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BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. 5, 70,792/- (Rupees Five lakh seventy thousand seven hundred ninety two) out of Rs. 5, 98,800/- (Rupees Five lakh ninety eight thousand eight hundred) sanctioned to Dr. Harshada Joshi by the University Grants Commission under the scheme of support for Major Research Project entitled "**Diversity of lactobacilli associated with camel milk in Southern Rajasthan**" vide UGC letter no. F.40-168/2011(SR) dated 1/5.7.2011 utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance of Rs.28008/-remain unutilized at the end of the financial year 2013-14.

Principal investigator

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Registrar

REGISTRAR
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UNIVERSITY GRANTS COMMISSION
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NEW DELHI – 110 002

Utilization certificate

Certified that the grant of Rs. **3, 98,003/-** (Rupees Three lakh ninety eight thousand three) out of Rs. **4, 03,397**/- (Rupees Four lakh three thousand three hundred ninety seven) sanctioned to Dr. Harshada Joshi by the University Grants Commission under the scheme of support for Major Research Project entitled "**Diversity of lactobacilli associated with camel milk in Southern Rajasthan**" vide UGC letter no. F.40-168/2011(SR) dated 2.9.2014 utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission. The balance of Rs.5394/-remain unutilized.

Principal investigator

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Installment wise bifurcation of statement of expenditure
Time period-1/7/2011 to 31/12/2014
(UGC Major Research Project: Sanction no F. 40-168/2011(SR) dated 1.7.2011)

Name of the item	Total grant allocated	Grant released in 1st installment (in Rs.)	Expenditure incurred from1st installment (in Rs.)	Grant released in 2nd installment (in Rs.)	Expenditure incurred from 2nd installment (in Rs.)	Grant yet to be released 3rd installment (in Rs.)	Expenditure incurred on loss basis (to be paid)	Total expenditure (in Rs.)
Equipment	1,25,000	1,25,000	98,282	-	-	-	-	98,282
Books	30,000	30,000	30,000	-	-	-	-	30,000
Project fellow	3,65,935	1,44,000	1,42,710	1,85,342	1,84,484	36,593	36,593	3,63,787
HRA	41,394	-	-	37,255	32,719	4,139	3,874	36,593
Travel	2,000	1,000	1,000	800	800	200	-	1,800
Chemicals	3,00,000	1,50,000	1,50,000	1,20,000	1,20,000	30,000	30,000	3,00,000
Contingency	1,50,000	75,000	75,000	60,000	60,000	15,000	15,000	1,50,000
Overhead charges	73,800	73,800	73,800	-	-	-	-	73,800
Total	**10,88,129/-**	**5,98,800/-**	**5,70,792/-**	**4,03,397/-**	**3,98,003/-**	**85,932/-**	**85,467/-**	**10,54,262/-**

Certified that the grant of Rs. **10,54,262/-(Ten lakh fifty four thousand two hundred sixty two)** out of **Rs. 10,88,129/-(Ten lakh eighty eight thousand one hundred twenty nine)** sanctioned to Dr. Harshada Joshi by the University Grants Commissions under the scheme of support for major Research Project entitled "Diversity of lactobacilli associated with camel milk in southern Rajasthan" vide UGC letter no **F. 40-168/2011(SR) dated 1.7.2011** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commissions and that the balance of Rs. **33,867/-** remaining unutilized at the end of the completion of the project has been refunded/returned to UGC [vide DD.No.**011244** dated 10.3.15 of Rs.**33,402/-** and DD.No.**503765** dated 10.3.17 of Rs. **465/-(Rs.** 200 from travel grant + Rs.265 from fellowship grant)]

Signature of PI

Harshada Joshi
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INCHARGE
Department of Biotechnology
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Chartered Accountants
CA. Yogesh Chandra Pokharna
(Partner)
Membership No.-071503

Signature of Registrar/Comptroller

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