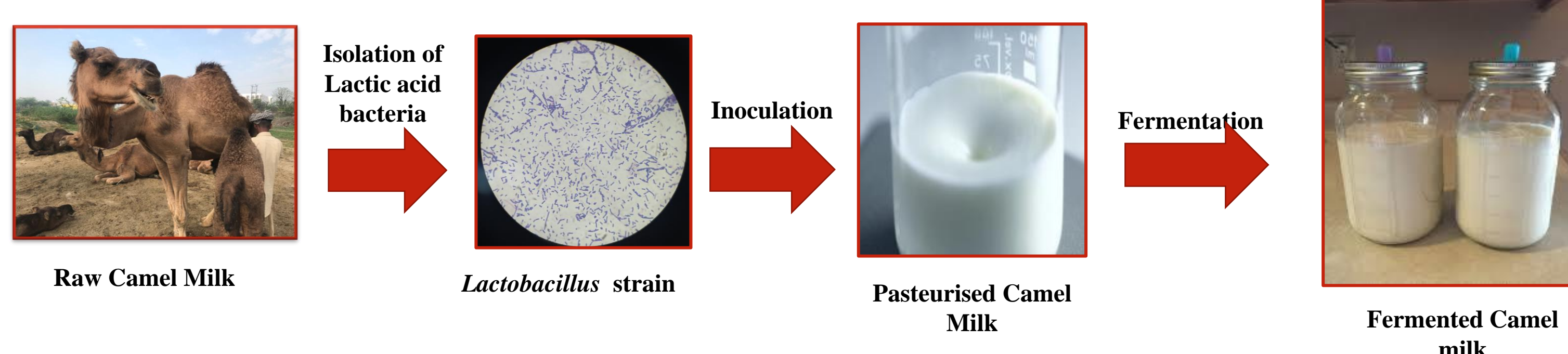


Development of anti-diabetic fermented camel milk with indigenous probiotic *Lactobacillus* cultures (containing α -glucosidase and DPP-IV inhibitory properties)

Mohammad Rizwan, Nishchal Thakur, and Shilpa Vij*
Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, 132001, India
*Email: shilpavijn@gmail.com

Introduction

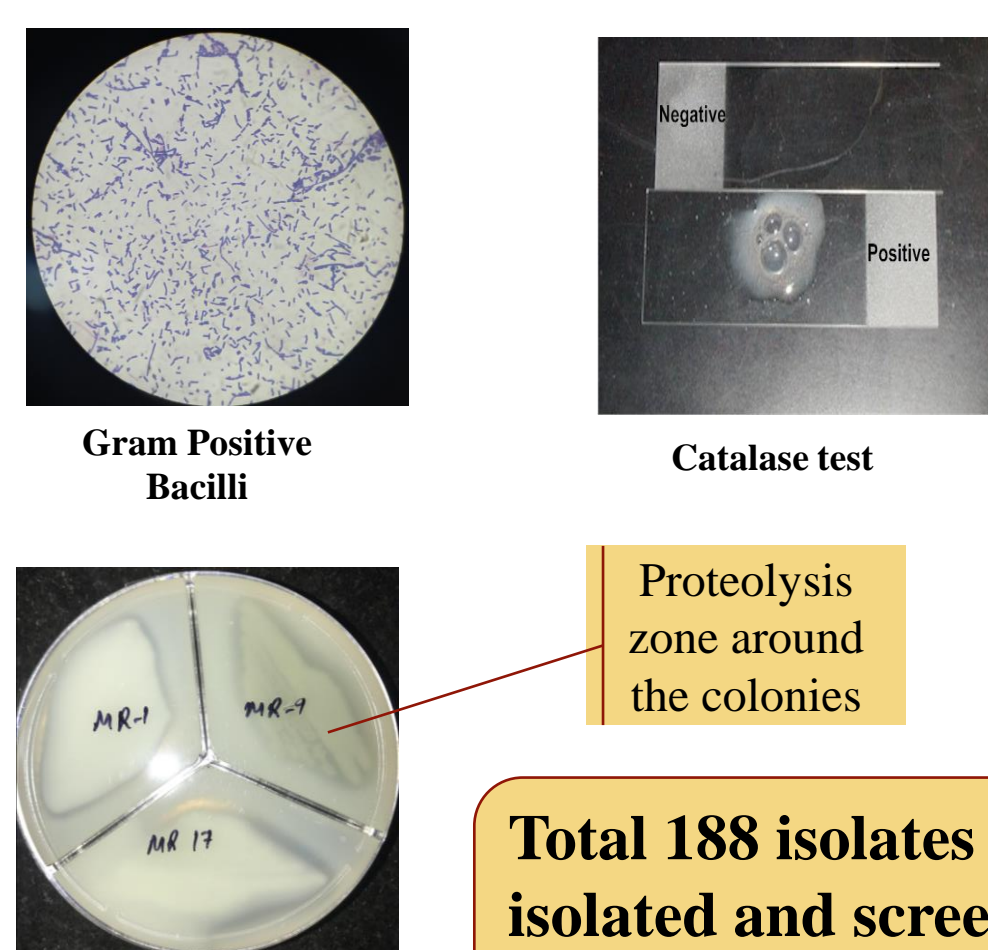
The most prevalent chronic condition, diabetes mellitus, is characterized by high blood sugar levels due to deficiencies in insulin secretion (known as Type 1 Diabetes Mellitus-T1DM) or insulin activity (known as Type 2 DM). T1DM accounts for 10% of all diabetic cases, as the body cannot generate insulin. T2DM, which affects up to 90% of diabetic cases, occurs when the body produces insulin but does not use it effectively. According to WHO (2018), camel milk has demonstrated anti-diabetic properties that can be further enhanced by utilizing specific lactobacilli. Lactobacilli extracted from camel milk that exhibit α -glucosidase and DPP-IV inhibitory activities are the most appropriate for producing anti-diabetic fermented camel milk.



Research objective: To study anti-diabetic potential of fermented camel milk with indigenous probiotic *Lactobacillus* cultures.

Methods

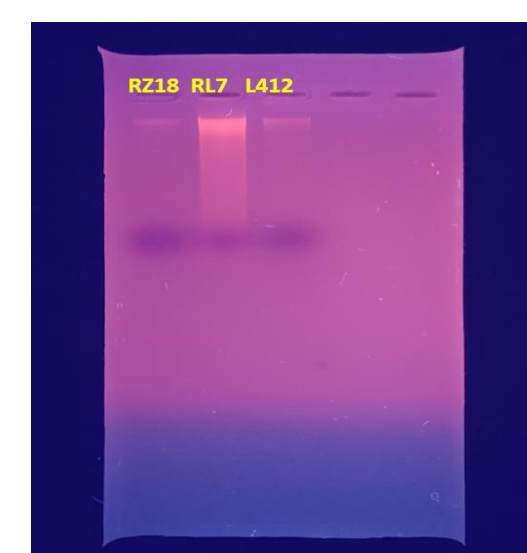
Camel milk collection	
Source	Camel Species
NRCC, Bikaner	Bikaneri
NRCC, Bikaner	Jaisalmer
NRCC, Bikaner	Kachchi
NRCC, Bikaner	Mewari
Kalayati, Haryana	Desi Breed



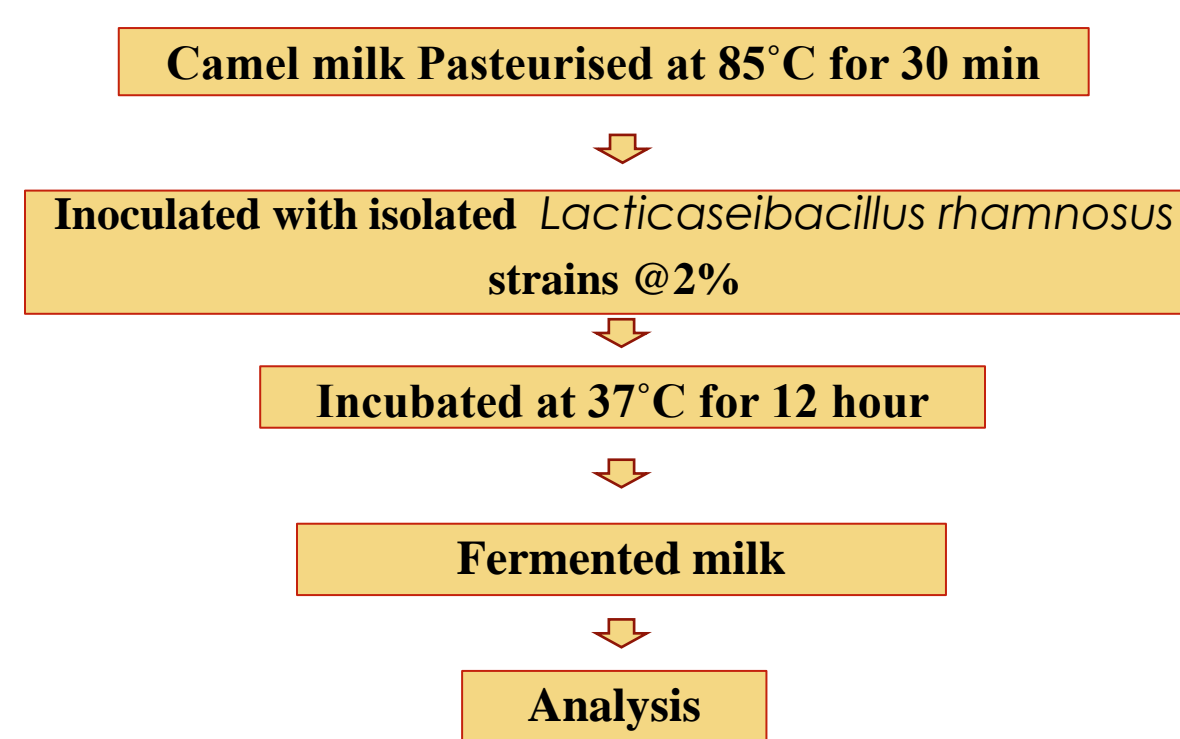
Screening of *Lactobacillus* strain from Camel milk

Identified by 16s rRNA sequencing by Sanger's Methods

S. No	Name	Site	Identified name	Match on NCBI
1.	RL4	Camel Milk	<i>Lactocaseibacillus rhamnosus</i>	100%
2.	RZ18	Camel Milk	<i>Lactiplantibacillus argentoratensis</i>	100%
3.	LG12	Camel Milk	<i>Lactocaseibacillus rhamnosus</i>	98%



Fermentation of camel milk



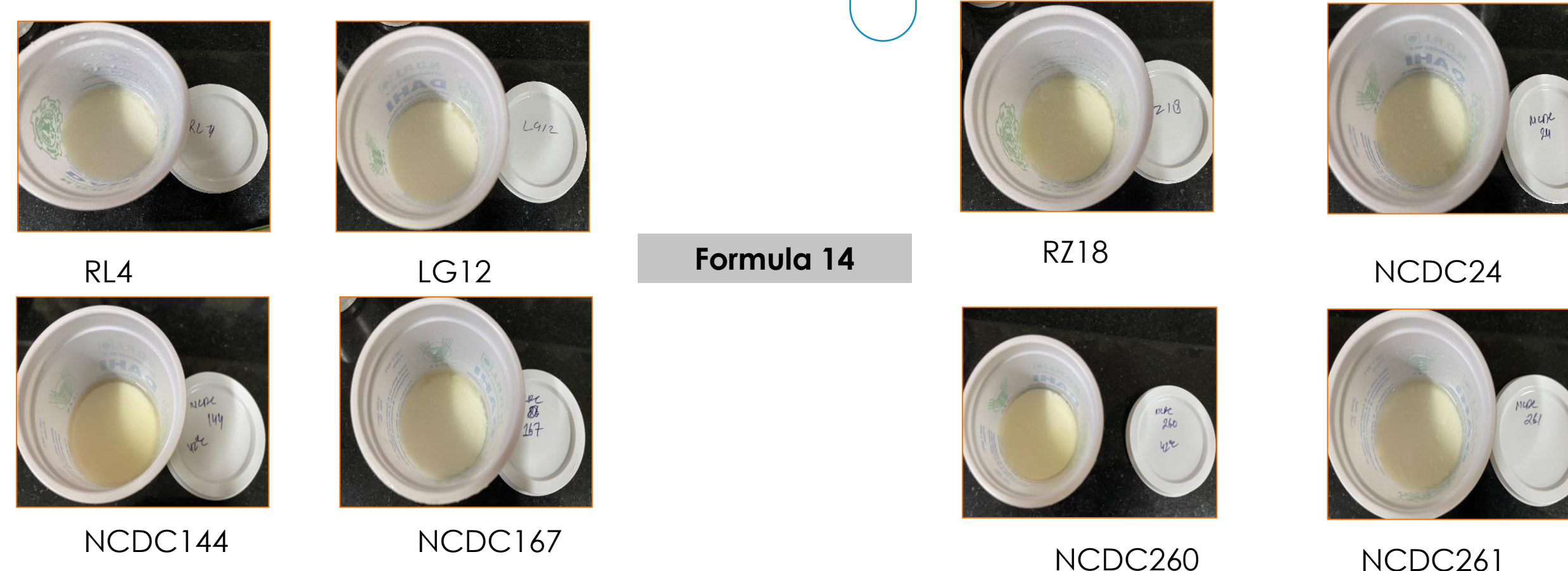
Selection of Formulation for the suitable product

CM + Whey Protein (4%), Casein (2%), Alginate (0.2%)				CM + SMP (2%), Carrageenan (0.25%)			
Strain	Texture	Taste	pH	Strain	Texture	Taste	pH
RL4	Curd	Sweet	4.12	RL4	Solid curd	Sweet	3.89
LG12	Curd	Sweet	3.85	LG12	Solid Curd Sweet		3.77
RZ18	Curd	Sweet	4.91	RZ18	Solid Curd Sweet		4.03
NCDC24	Curd	Sweet	4.36	NCDC24	Solid Curd Sweet		4.01
NCDC144	Curd	Sweet	4.06	NCDC144	Solid Curd Sweet		4.48
NCDC167	Curd	Sweet	4.19	NCDC167	Solid Curd Sweet		3.9
NCDC260	Curd	Sweet	4.09	NCDC260	Solid Curd Sweet		4.18
NCDC261	Curd	Sweet	4.01	NCDC261	Solid Curd Sweet		3.96
CONTROL	loose	Sour	5.9	CONTROL	loose	Sour	5.15

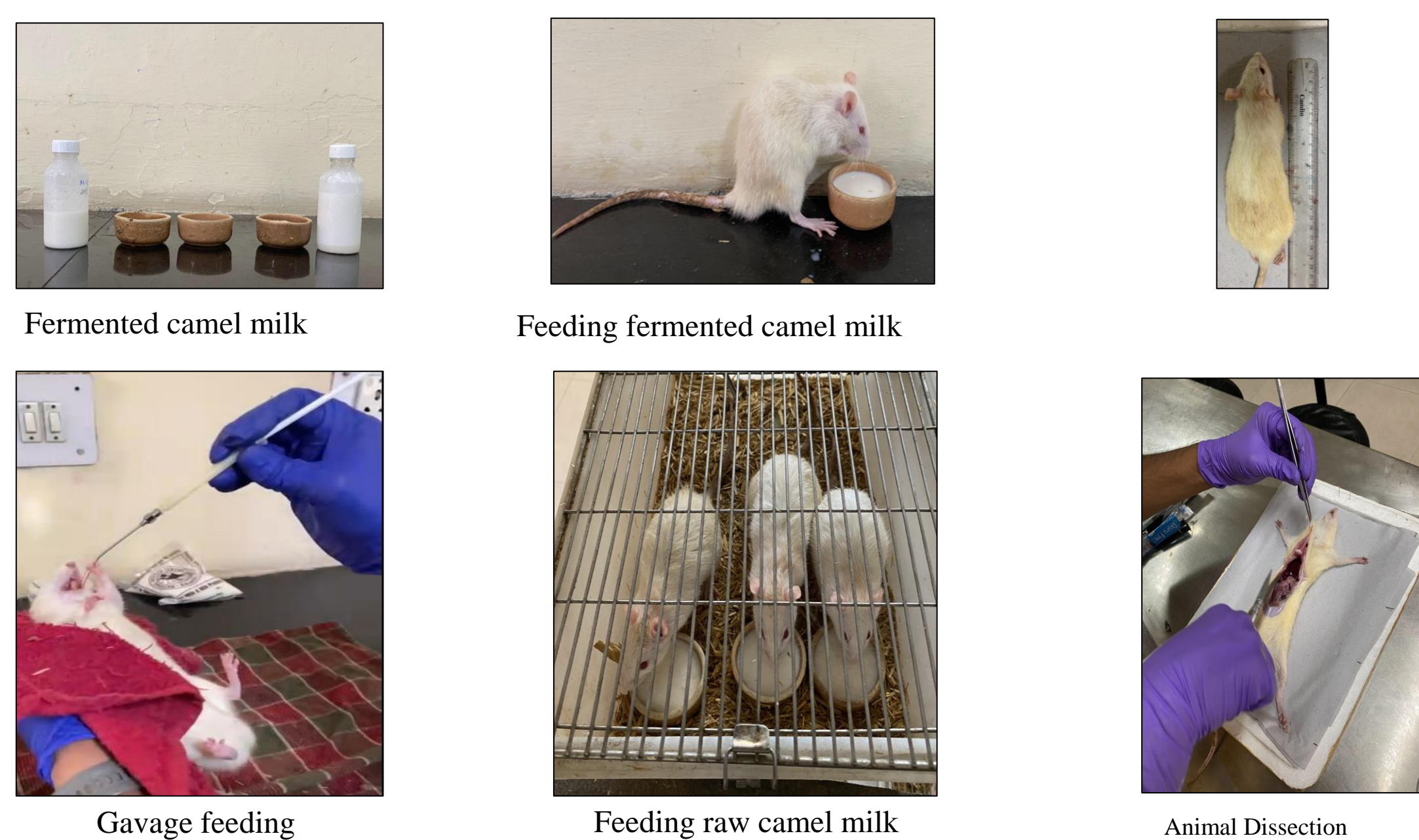
Composition for the curd formation of camel milk has been established as

Formula 12 : CM + Whey Protein (4%), Casein (2%), Alginate (0.2%) and

Formula 14 : Camel milk + SMP (2%) + Carrageenan (0.25%)



Animal Study



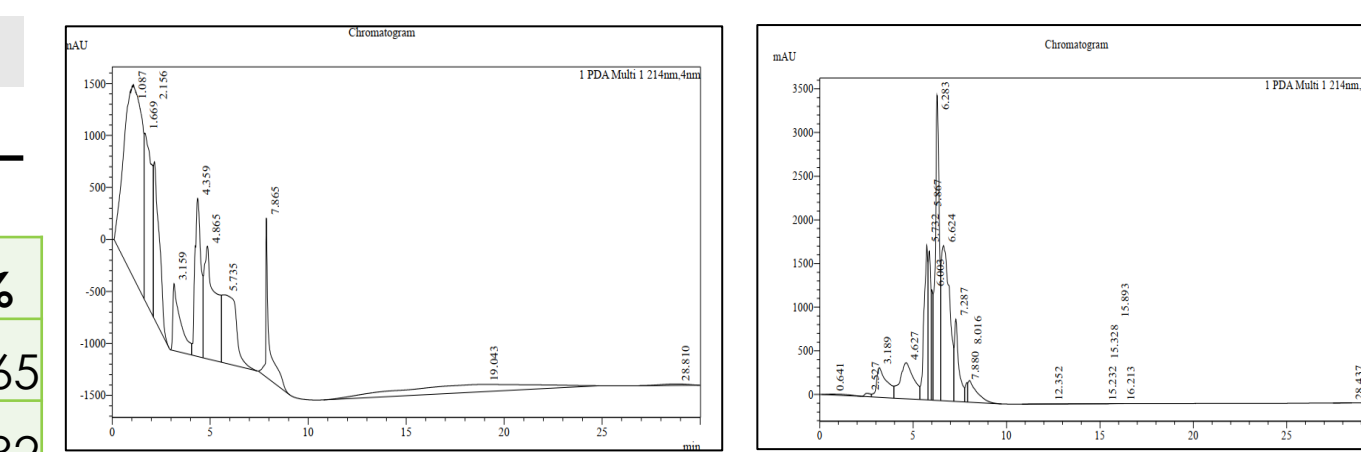
Results

Microbiological analysis of raw camel milk						
Activity	Media	Bikaneri	Jaisalmeri	Kachchi	Mewari	Desi Breed
Total count	NA	10X10 ⁶	15X10 ⁶	17X10 ⁶	13X10 ⁶	286X10 ⁶
Coliform	VRBA	Nil	Nil	Nil	Nil	46X10 ⁶
Y/M count	YPDA	Nil	Nil	Nil	Nil	Nil
Spore former	NA	15X10 ⁶	10X10 ⁶	12X10 ⁶	14X10 ⁶	12X10 ⁶

Chemical analysis of raw camel milk	
Analysis	Camel milk
pH	6.36 to 6.41
TA	0.241 to 0.243
Fat	3.45 to 3.51%
SNF	7.89 to 8.07%
Protein	2.78 to 2.81%

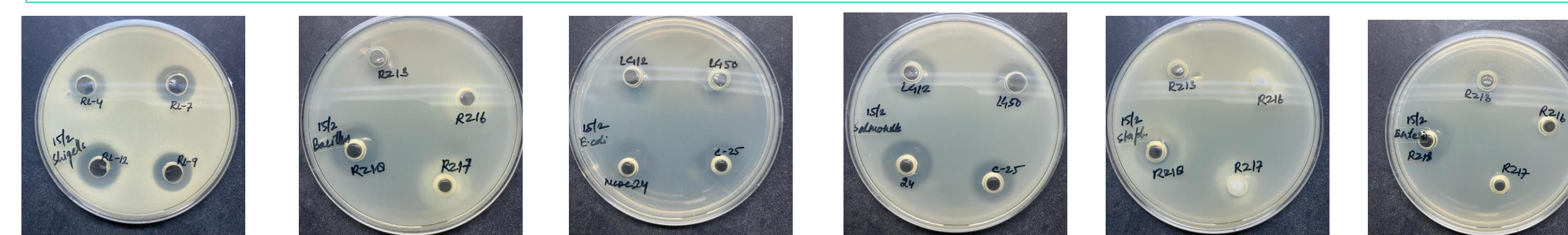


Product	Total area	RPA%
RL4	219697750	69.65
LG12	135131326	175.82
RZ18	199341450	86.97
NCDC24	90513955	311.78
RCM	372724613	0



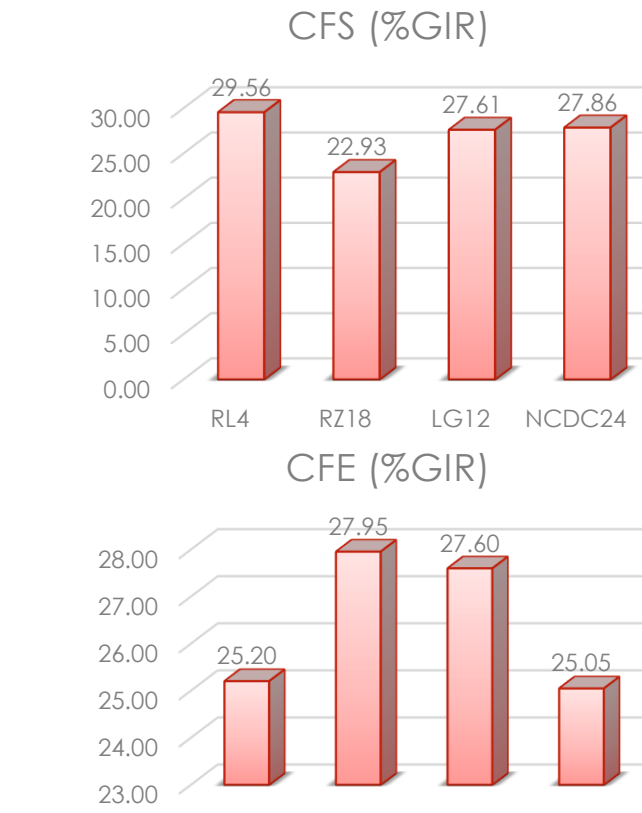
Antimicrobial activity of fermented camel milk

RZ18,20,21,22,23,26,RL4,7,9,12,16, NCDC17,24,195, DC,FM, LG12 give good antimicrobial activity against *E. coli*, *S. typhi*, *S. aureus*, *B. cereus*, *E. faecalis* and *shigella*.



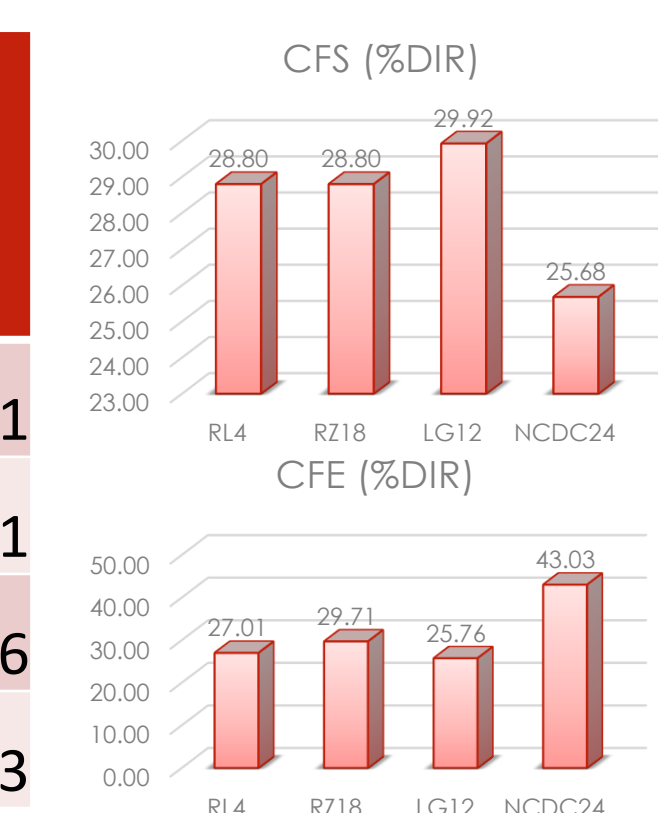
Alpha- glucosidase inhibitory activity of selected strains

Isolates	CFS (GIR %)	CFE (GIR %)
RL4	29.56	25.20
RZ18	22.93	27.95
LG12	27.61	27.60
NCDC24	27.86	25.05



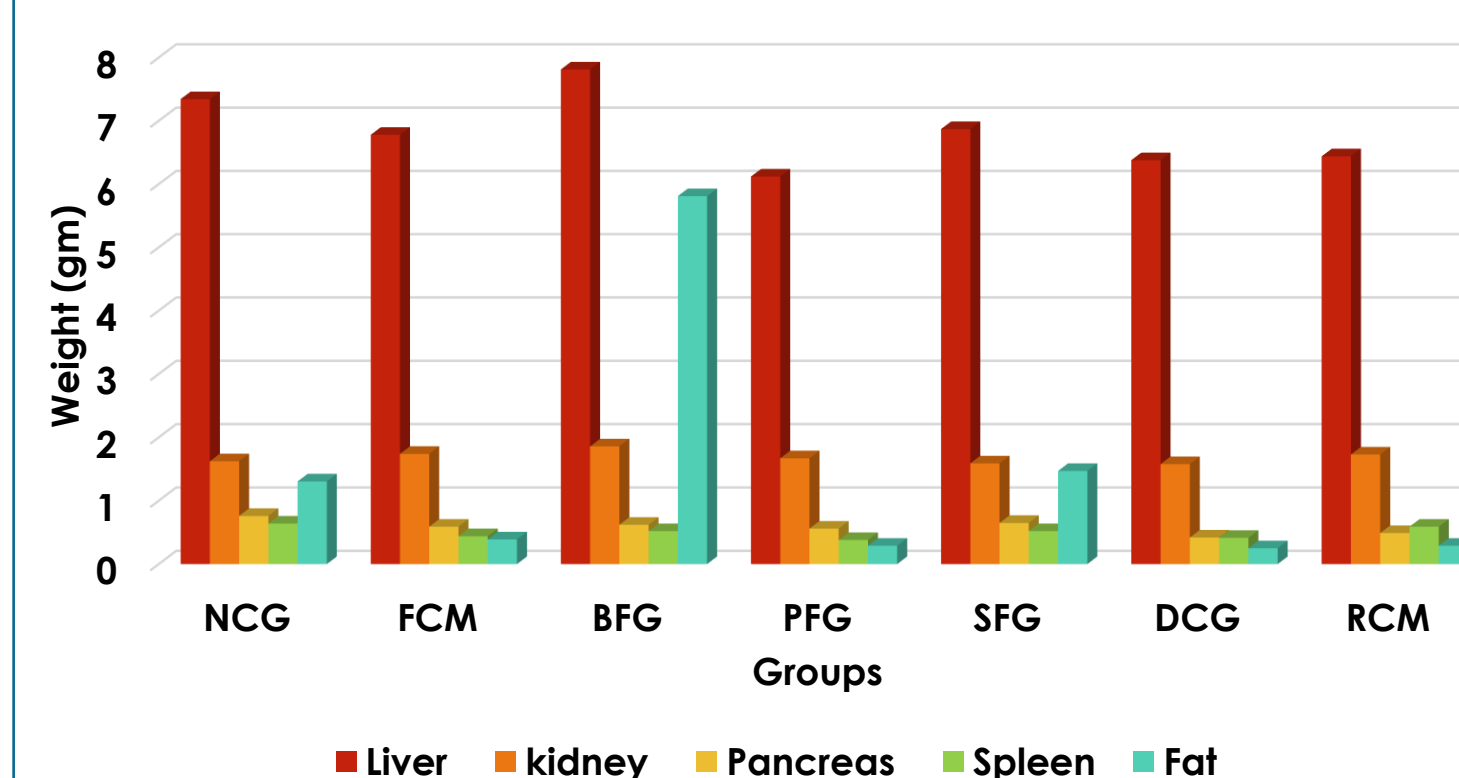
DPP-IV inhibitory activity of selected strains

Isolates	CFS (GIR %)	CFE (GIR %)
RL4	28.80	27.01
RZ18	28.80	29.71
LG12	29.92	25.76
NCDC24	25.68	43.03

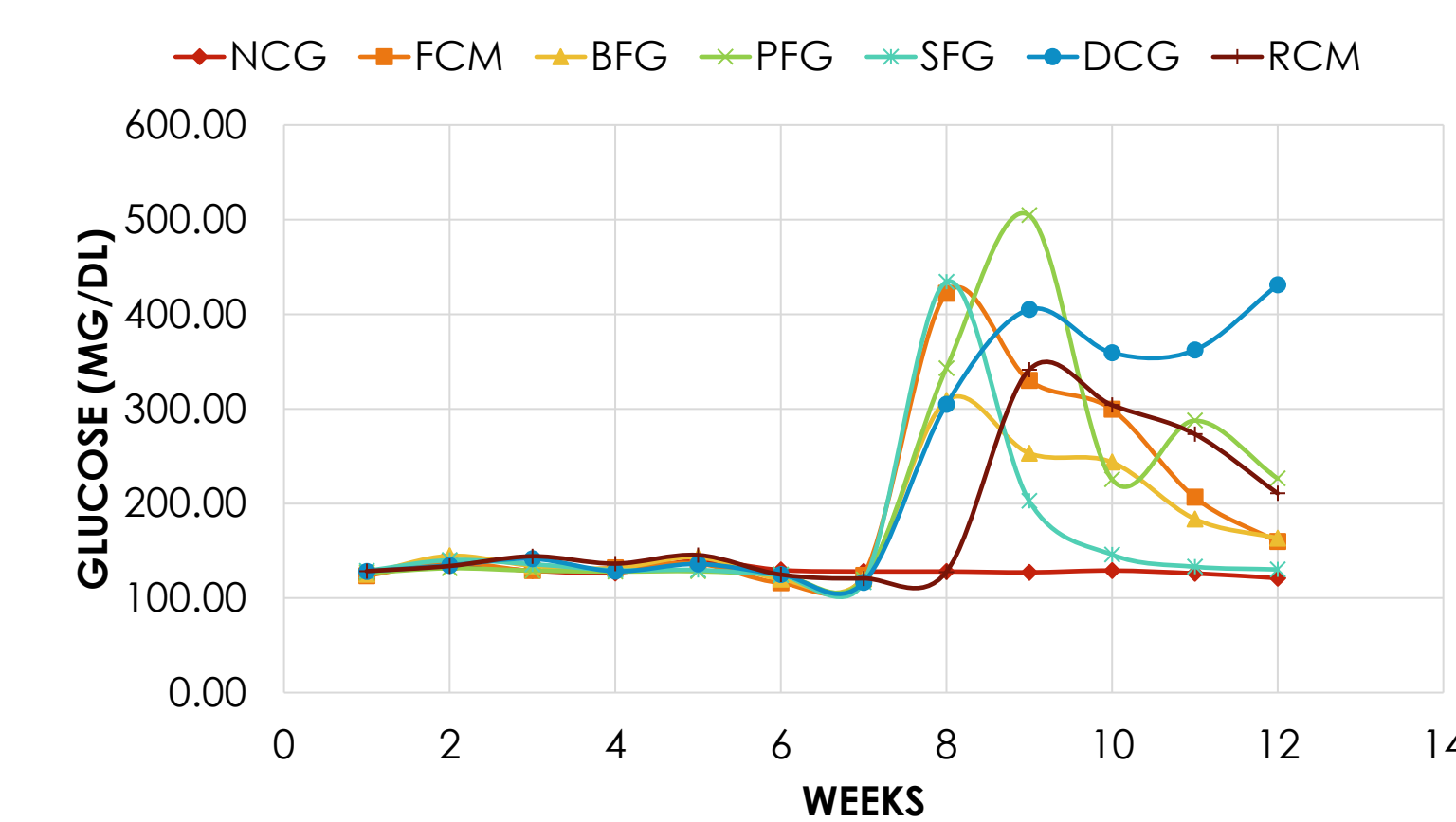


(Method: *Lacvix* and *Li-chan* 2013)

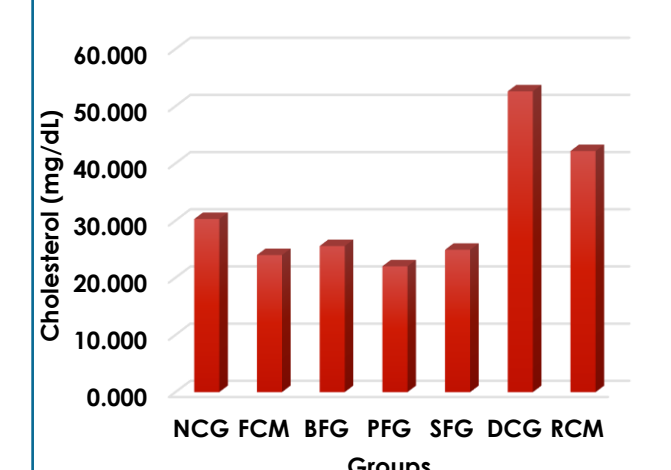
Organ weight of Rats



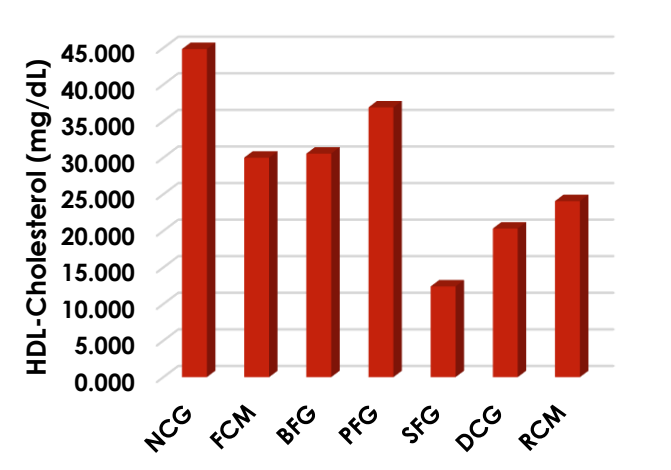
BLOOD GLUCOSE OF RATS



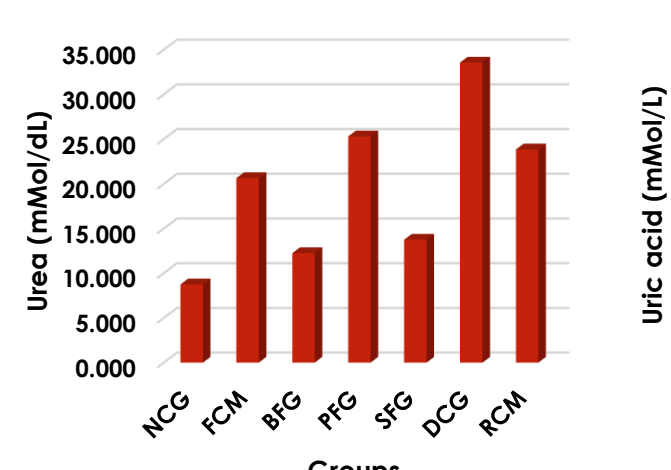
Total Cholesterol



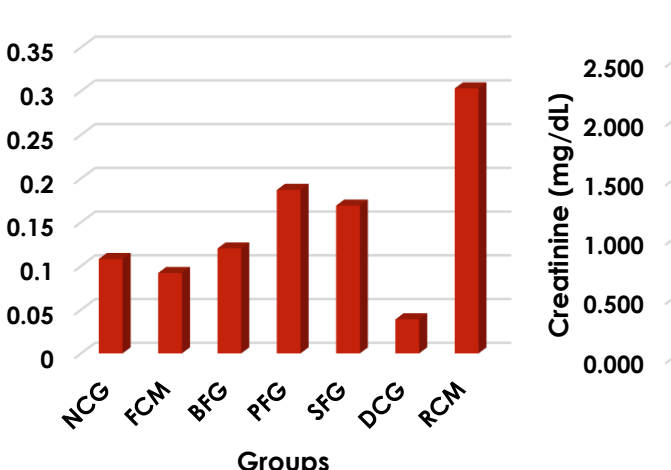
HDL-Cholesterol



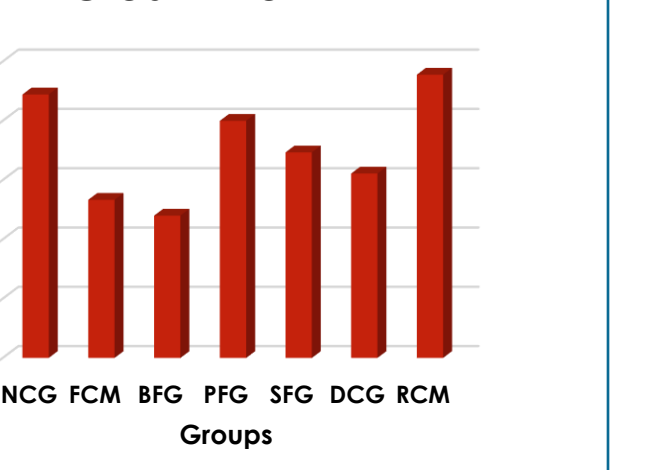
Urea



Uric acid



Creatinine



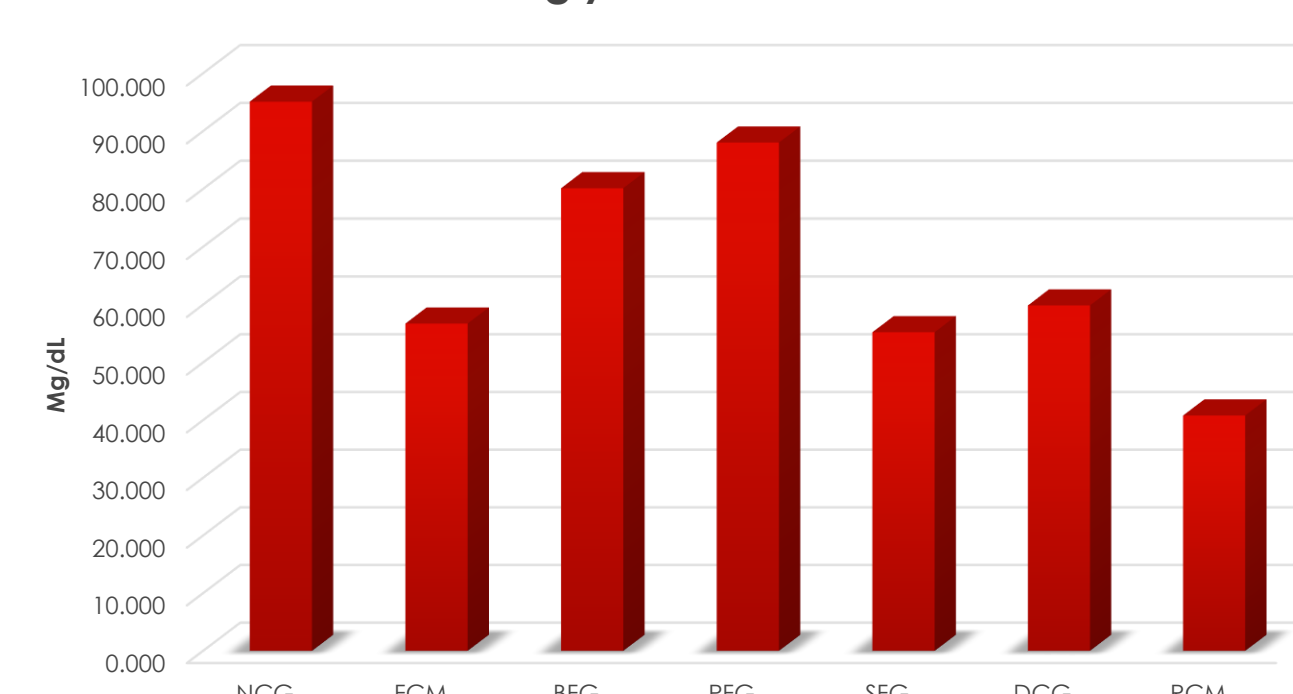
NCG= Negative control Group, FCM= Fermented camel milk, BFG= Bacteria Fed group, PFG= Peptide Fed group, SFG= Sitagliptin Fed group, DCG= Diabetic control group, RCM= Raw camel milk

Bioactivities of ultrafiltered fractions of fermented milk

Fractions	Peptide Content	Bioactivity in IC50 (mg/mL)		
		DPP-IV inhibition *	α -glucosidase inhibition ** ABTS	Anti-oxidant activity (EC50 mg/mL)*** DPPH
>10kDa	1.11±0.056	1.19±0.060	2.21±0.111	1.01±0.051
10kDa	0.423±0.021	0.46±0.023	0.78±0.039	0.45±0.023
5kDa	1.021±0.51	0.35±0.018	0.62±0.031	0.35±0.018
3kDa	0.466±0.023	0.31±0.016	0.51±0.026	0.21±0.011
Diprotein - A*	-	0.0032±0.000	-	-
Acarbose**	-	-	0.0012±0.0001	-
Trolox***	-	-	-	0.0024±0.001

* For DPP-IV inhibition
** α -glucosidase inhibition
*** Anti-oxidant activity

Triglyceride value



Conclusion

188 lactic acid bacteria were isolated from camel milk, with 52 being proteolytic. 23 strains were chosen for their smooth curdling, lack of whey, sweet flavor, pH range (3.50-3.75) & titrable acidity range (0.711-1.224). Out of these, 16 showed DPP-IV inhibitory activity & 12 displayed α -glucosidase activity & probiotic properties. RL4, RZ18, & LG12 were selected as *Lactocaseibacillus rhamnosus*, *Lactiplantibacillus argentoratensis*, & *Lactocaseibacillus rhamnosus* via 16s rRNA.

There is significant decrease in Blood glucose level in rat after giving the formulas such as NCG (Normal Control Group), RCM (Raw camel milk), FCM (Fermented camel milk), BFG (Bacteria Fed group), SFG (Sitagliptin fed group) and DCG (Direct control group), 121±6.05, 210.67±10.53, 159.67±7.98, 163.33±8.17, 130.33±6.52, and 431±21.55mg/dL respectively.

Other factors reported to be significant in rat serum were α -glucosidase and DPP-IV inhibitory activity, cholesterol, urea, creatinine, GLP-1, GIP, rat insulin, HDL, LDL, and VLDL, as well as triglycerides, SGOT, and SGPT.

Significance

Indigenous *Lactocaseibacillus* isolates from camel milk found to have DPP-IV & α -glucosidase inhibitory activity. Fermented camel milk with lactic acid bacteria shown effective in treating diabetes.

Investigation found proteolytic Lactobacilli with α -glucosidase & DPP-IV inhibitory activity in exotic sources like camel milk. Anti-diabetic potential of camel milk improved by using these isolates in fermented camel milk preparation