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Isolation and identification of probiotic bacteria from home made and commercial curd

***[1]Pavani Sure**
[1]Asst. Professor, Department of Pharmaceutics,
Vignan Institute of Pharmaceutical Sciences, Hyderabad
Pavani20684@gmail.com

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Abstract
Bacteria have always been the most abundant living creatures on earth which may be either helpful to humans in the form of single cell proteins, probiotics, natural gut flora or many a times be harmful and cause several infections becoming fatal. Application of these bacteria and their natural process for the development and well being of humans is the major focus of several researchers. Importance of probiotic bacteria has long been known to the mankind and has been the study subject. The current work involves isolation and identification of different bacteria from selected probiotic food. Since curd is the most natural and probiotic rich food consumed most commonly it has been selected for the study. This study included selective study of microbes in commercial and home made curd. The focus is to summarize the common microbes in both commercial and home made curd and to identify if any special organisms are included in the commercial curd. The bacterial identification after isolation is mainly based on molecular techniques like sequencing and BLAST assay for high end accuracy.

Keywords: Probiotics, Curd, Bacteria, Molecular Analysis, BLAST

Introduction
Dairy and the related products have long been the most abundantly used food by common man. Additionally it is one of the foods that show the availability of complete nutrition high minerals. Apart from its nutritional quality one of the very good quality of milk based products especially curd and cheese is their microbial content which are highly beneficial to the human digestive system [5]. These dairy products are included as value added products in the human diet due to their positive influence on human health and well being. According to the research study of 2002 which was published in European Journal of Clinical Nutrition, the total number and type of friendly bacteria in curd vary from place to place and show a large diversity [9]. It has been revealed that even Lactobacillus present in curd show as many as 250 different species and have different importance [10].
Many of the studies state that cheese and curd are not true probiotics [2]. According to the standard rule of WHO/FAO a probiotic should contain live microbes which should be 1 billion on an average. Further they must show resistance to gastric bile, pH and pancreatic juice in order to travel to the target site which may be either small intestine or large intestine and produce its beneficial effect on the consumer. Further it should be certified as a probiotic only after a well controlled and scientifically validated clinical trials[3].
However such probiotics are not consumed by all category of people as normal diet. But curd being most commonly consumed by every individual, it is considered for the study.

Materials and Methods

Collection of samples
The study included both home made curd from natural milk and store brought curd which are 2 in number of different brands. The names of the brands are not disclosed in the work. Natural curd was prepared by regular procedure from the buffalo milk obtained freshly. The milk was pasteurized on low flame and allowed to cool until lukewarm. A little pre made curd was added to the warm milk as an inoculum to produce curd. This was kept overnight under warm conditions for obtaining curd. Other two curd samples were directly brought from the nearby store.

Isolation of Bacteria
Initially all the bacteria were obtained by dilution. A standard serial dilution technique was used to obtain a most dilute inoculum to seed the plates. 10 fold dilution was performed using the sterile distilled water with all the three curd samples. The inoculum from the last 3 $10^{-8,}$ 10^{-9} and 10^{-10} tubes were used for inoculation. The plates were incubated overnight at 37^0C in an incubator.

Composition of nutrient agar media used for the study [6]
0.5% Peptone
0.5% Beef Extract/ Yeast Extract
0.5% Sodium chloride
1.5% Agar agar
Sterile distilled water
pH of the medium should be 6.8 to 7

All the contents are dissolved in water and heated until complete dissolution. The media along with the glassware is sterilized using autoclave for 120lb pressure for 15 minutes. Once the media and glassware is sterilized they can be used.

The sterile media is poured into the petriplates and allowed to solidify. The complete procedure is performed in a laminar airflow chamber. Once the media is solidified 0.1 ml of the inoculum is added to the plate and spread using a sterile spreader. All the plates are prepared in replicas to obtain accurate result. The plates are incubated in an incubator.

Subculture of bacteria on to MRS media:
The isolated bacterial colonies were subjected for pure culture preparation by streaking onto MRS media plates. The reason for electing MRS media [8] for pure culturing is its high speciality in encouraging the growth of lactobacillus group which are predominant in curd. Apart from its special focus on lactobacillus group this media also allows the growth of other bacterial species also.
MRS media was prepared, Sterilized and poured onto plates and solidified. The plates were used for pure culture preparation by streaking. Each colony in the master plate was used to streak onto the MRS plate under laminar air flow conditions.

All the streaked plates were incubated in an incubator for further growth.

MRS Media composition:

- 1.0% peptone
- 1.0% beef extract
- 0.4% yeast extract
- 2.0% glucose
- 0.5% sodium acetate trihydrate
- 0.1% polysorbate 80 (also known as Tween 80)
- 0.2% dipotassium hydrogen phosphate
- 0.2% triammonium citrate
- 0.02% magnesium sulfate heptahydrate
- 0.005% manganese sulfate tetrahydrate
- 1.0% agar
- pH adjusted to 6.2 at 25 °C

Identification of Bacterial isolates
There are 3 major steps in the identification of bacteria. Which include
1) Gram's staining and microscopy
2) Biochemical tests
3) Molecular methods like 16s RNA sequencing and analysis

Gram's staining [7]
It is one of the most standard and predominantly used method for basic level categorization of bacteria into gram positive and negative group. This method is based on cell wall composition of bacteria. Bacteria rich in peptidoglycan layer with low lipid content are identified to be gram positive and the one with very thin peptidoglycan layer and high lipid content are classified to be gram negative group.

All the bacterial cultures streaked are subjected for smear preparation on slides. These slides are subjected for gram's staining which involves the use of crystal violet, gram's iodine, 70% ethanol and saffronin in series with intermittent washing in water. After the addition of final stain the slides are allowed for air dry and finally observed under 100X oil immersion objective. The color and shape of the cells is noted further analysis.

Biochemical tests for the identification of bacteria
In order to identify an unknown bacteria the use of its biochemical properties and enzymatic activities is vital. Bergey's manual [1] is a systemic chart showing the list of biochemical tests in a specific series that are to be performed to identify an unknown bacteria. This chart begins with the details about the gram's nature and share of bacteria. Based on grams staining result these biochemical tests have to be performed that can yield the identity of the bacteria.

Bergey's manual can be accessed from the link:
https://web.archive.org/web/20081217040626/http://www.uiweb.uidaho.edu/micro_biology/250/IDFlowcharts.pdf

Molecular methods for the identification of bacteria
The main steps in molecular identification include:
DNA extraction from bacterial cells, amplification using 16s RNA primers, sequencing, BLAST analysis.
Isolation of bacterial genomic DNA is performed using biopure DNA extraction kits of BioAxis DNA Research centre. The kit includes a buffer, lysis solution, water etc. It includes a manual for performing the exaction. Once the extraction is performed the extracted DNA is precipitated using chilled ethanol. The extracted pellet is air dried and stored in TE buffer and dissolved until next use.

Amplification of DNA using 16s RNA primers
In order to use the extracted DNA in molecular identification, it must be amplified to increase the no of copies. The amplification is targeted to the region 16s RNA [4] as it is the one that helps in identification of bacteria.

PCR based amplification was performed using the 16S rRNA primers. 30 cycles of PCR were run. The final amplified products are subjected for sequencing using ABI 3730 sequencer that runs on the principle of capillary electrophoresis. The final amplicon sequence obtained was than subjected for BLASTN, which confirms the identity of the organism.

BLASTN analysis for identification of the organism
The sequences obtained from sequencer are subjected for BLASTN analysis to identify the organisms. BLASTN analysis performs pairwise sequence alignment and similarity search based on the alignment performed. BLASTN can be used from the site :

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch

Results and Discussion

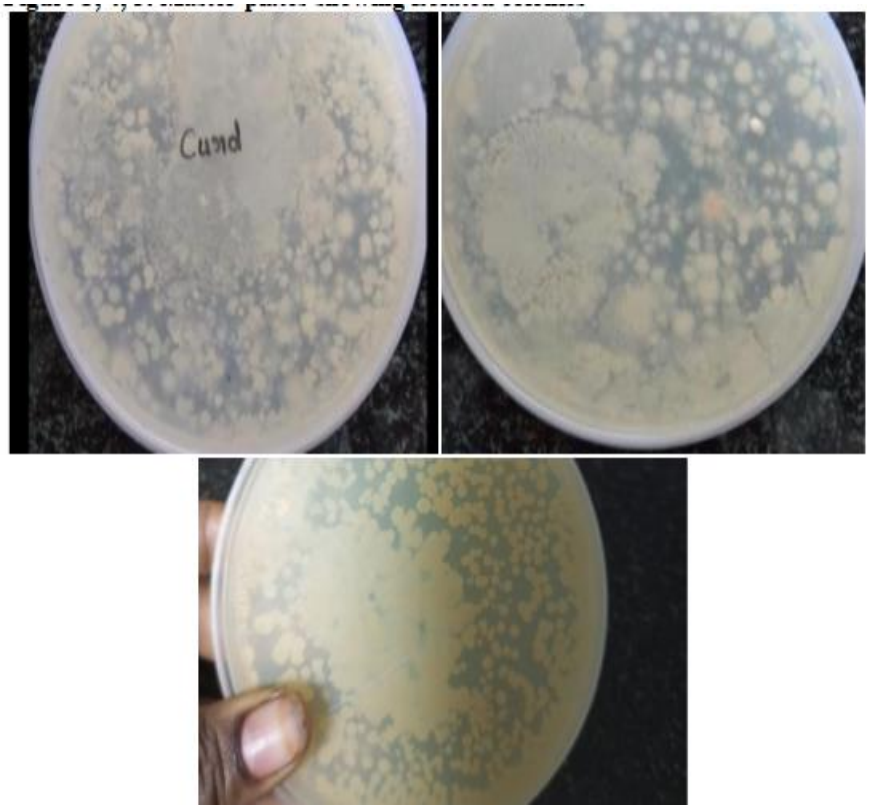
Fig1,2 showing home made curd and store brought curd collected in a beaker

Inference: The above figure shows two curd samples, 1 is the curd made from fresh milk, 2 is the curd obtained from store. Initially these 2 samples wee used later an additional store brought curd was also used.

Isolation of Microbes from original sample

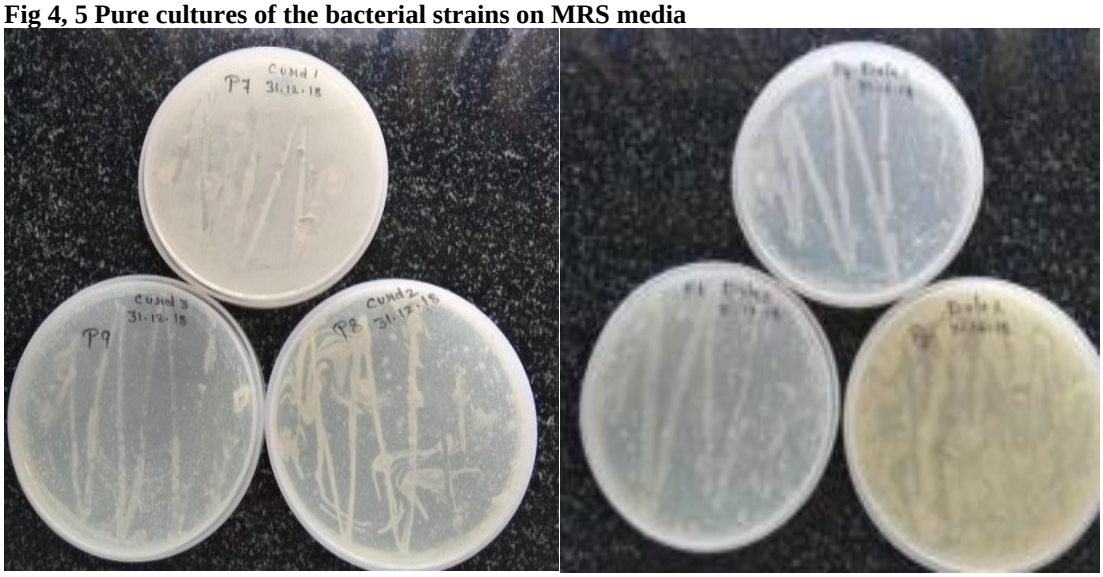
The bacterial colonies were separated on nutrient agar media and pure cultured on MRS media. The plates are shown under

Fig 3: Master plates showing isolated colonies



Inference: The above picture shows the initials isolates from all the samples used for the study

Fig 4, 5 Pure cultures of the bacterial strains on MRS media



Inference: The above isolates are obtained from all the 3 curd samples. Several other pure cultures were also made in addition to the plates shown above.

Bacterial Identification Results

Table 1 showing the results of Gram's staining and microscopy

	1	2	3	4	5
SAMPLE 1 Natural curd	Gram positive, bacilli (P1)	Gram Positive, strepto bacilli (P2)	Gram positive staphylococci (P3)	Gram positive bacilli in chains and single (P4)	Gram positive diplobacilli (P5)
SAMPLE 2	Gram positive Streptobacilli (Q1)	Gram positive Bacilli (Q2)	Gram poitive small bacilli (Q3)	---	----
SAMPLE 3	Gram positive bacilli (R1)	Gram postive coco bacilli (R2)	Gram positive long bacilli (R3)	Gram positive very small bacilli (R4)	------

Inference: According to the results a total of 5 different bacteria were identified in home made curd P, 3 different bacteria in store brought curd Q, 4 different cultures in store bought curd R. The results of their microscopy are also furnished in the above table.

The above cultures were further subjected for Biochemical tests which include Catalase, Starch hydrolysis, Glucose fermentation, Voges Proskauer etc and the bacteria were identified to be the following

Table 2: List of bacteria isolated from the samples

S.NO	Sample	Name of the bacteria
1	P1	Lactobacillus casei
2	P2	Lactobacillus lactis
3	P3	Lctobacillus delbrueckii
4	P4	Bacillus subtilis

5	P51	Lctobacillus delbrueckii
6	Q1	Lactobacillus lactis
7	Q2	Lactobacillus casei
8	Q3	Bacillus coagulans
9	R1	Lctobacillus casei
10	R2	Lctobacillus delbrueckii
11	R3	Lctobacillus fermentum
12	R4	Bacillus coagulans

Inference: The above table shows all the common bacteria especially lactobacillus found in the selected curd samples. Further these results show that the home made curd and commercial curd have same type of bacterial species without any additional bacteria. *Bacillus subtilis* was the only species which was additionally found in the natural curd.

PCR Primer and conditions used for amplification

- The primers used for the amplification of 16S rRNA gene:
- F CGAAACCAAGAGGGGTTAAA -44F
- R CGAGCTGTCTTGGATGTGAA -244R

- PCR Conditions

1 cycle: 94°C for 5 min (Initial denaturing)
35 cycles: 94°C for 60 sec (denaturing)
53°C for 45 sec (annealing)
68°C for 90 sec (extension)
1 Cycle: 68°C for 10 min (final extension)

Molecular identification

Sequencing results of the above cultures:

Table 3: Sequencing results for the bacterial samples

S.NO	Sample	Sequence of the bacteria
1	P1	AGGTAACGGCTCACCAAGGCGATGA TACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC AGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTC GGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAA CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTT ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGC GCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT GGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTT TCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT TACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
2	P2	TTGAGAGACTGATCGGCCACATTGGGACTGAGAC ACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAAC GCCGCGTGAGTGAAGAAGGTCTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGCAGTAAC TGGTCTTTATTTGACRGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAATGATAAGTCTGATGTGAA AGCCCACGGCTCAACCGTGGAACTGCATCGGAAACTGTCATTCTTGAGTGCAGAAGAGGAGAGTGGAACT CCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCA ACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACG ATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCTCAGTGCCGCAGCAAACGCATTAAGCGCTCCGCCTGGG GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCNGCACAAGCGGTGGAGCATGTGGTTT AATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTGCGCTACACCTAGAGATAGGCCGTTCC CTTCGGGGACGCAGAGACAGGTGGTGCATGGCTGTCGT
3	P3	GCGGCGTATTAGCTAGTTGGTGAGGT AACGGCTCACCAAGGCAATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACG GCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCC GCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGT

		TGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG TGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGC CCTCGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACT GACGCTGAGGCTCGAAAGCATGGGGTAGCGGAAACAAGGATTAGATACCCTGGTAGTCCATGCCGTAAAC GAATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTG GGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTC CCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTGTCGT
4	P4	CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAA TTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAG GGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCG TAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC CTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGCAGAGTGACAGGTG GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATC TTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGA AACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTG AAGCTGGAATCGCTAGTAATCGCGGATCAGCAT
5	P51	AAACGGCCCGGCGCCCAGCAGCGAAAGAATGC TGGAGCCCCAGCCGCAGGCCAAGCCCGAGAAAGCCCGGCCGCAAGGCCGCGAATTCACCAAGGACCTGCG CTTAAACGAGAAGTACACCTTCGAGAACTTCATTCAAGGCGAGGGCAACAAACTGGCTGCCGGGGCCGCT TTGGCGGTTGCCGACAACCCGGGGACCTTCTACAACCCCTTGTTCATCTTCGGGGGCGTGGGCCTTGGTA AAACCCACTTGATGCAGGCGATCGGCCACCAGATGCTGGCGGAAAGACCAGATGCCAAGGTTGTCTACAT TCAAAGTGAGACTTTTGTCAACGACTTCATCAATTCCATCAAGAACAAGACCCAGGACAAGTTCCGGGAG AAATACCGGACAGCTGACCTGCTCTTGGTCGACGACATCCAGTTCTTCGCCAAGAAGGAAGGGATTCAGG AAGAATTCTTCCATACTTTTGAGACTCTCTATAATGATCAAAAGCAGATCGTCATGACCAGTGACCGGCT GCCAACGGAAATTCCCGACCTGTCCGAGCGGCTGGTCTCGCGTTTTGCCTGGGGACTGCAAGTTGAAATC ACCCCGCCAGACCTGGAAACCCGGATCGCCATCCTGCGCAAGAAGGCTGAATCCGAAGGCCTGGAGATCG ATGAAAGCACCCTGGACTATGTGGCCAGCCAGGTCGATACTAATATCCGGGAGCTGGAAGGGGCCCTGGT CAAAGTCCAGGCCCAGGCCACCATTCAAAAGCAGGACAT
6	Q1	CAGGTTTAACATTTAATGCTTCAAGAAGACGACGTTCCT CACCAGGTCCAACTGAACTTGAATGTGATTTTACAAGTAAGCCAATTTCTTGGGCTTTCTCAACCAAGAC ACGGTTTTGAACTCCTGTTTCTTTCGAAATTTGACTTATTCTTTTTTTATTTGCTGCCAATTCGATCCTC CTCTTTGGGTAAAACTACTGACAAAACTGTCATTAAATAGACTAGCCAATTGTCAGTAAAAAAATACTAT AATTTTTTCTTTTGATTAATAATCTGAAGGGTTAACTCTAACATACGTTCTTTATTTCCTGCTTTTTCTG CAGTTGCTAATTGTTGCTGAAGTTCTTCAAATTTTATTTGTTCCATTTCATTTGAAAAAATGGAAACCAA GTCATTAATTTCCTGACTTGATGCAGTCTCTGGTAAATCTAAACTTATAATCTGATAAAAGAGACTACGC TGATCCTCTGACAACTCACTTGCCAAATGGGATTCATCAATCTGCTCATAAACCATTGCCTCAAGTAATA TCTTTTCAAATATATCCTGATAACGTTTATGGACAAATCTAAAATTATCATCCTGAGAAAATTTTTTCAA AACAGATGAATGATAAATCATTCGATGAAGGAGCATCTCCTCAGCTCTTTCTGAACGAGACAATTTAGGA ACCTGAATCGTTGGTTGAAAAACTTGAGCTGGTTCATTTCTTCTATCAGCTACTTGAGGTTGAATATTAT TTTCATAAAAGATTTCTTCAAAATCAATAGGTATGGCCGAATCAAGATTAGAATAATCTCTCTCGTCAGT AAAATTTTCAACTAGATTTGAAGCCCCATAATCACTAACAGAATGGTCAGTAATATTCATATTTTCACGG CGCAAATTTACAGCCTGCTCCACTTGATTATACT
7	Q2	GGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC AGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTC GGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAA CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTT ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGC GCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT GGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTT TCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT TACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG TGCATGGTTGTCGTCAGCTCGTGTCGTGA
8	Q3	AAGGCCTTCGGGTCGTAAAACTCTGTTGCCGGG GAAGAACAAGTGCCGTTCGAACAGGGCGGCGCCTTGACGGTACCCGGCCAGAAAGCCACGGCTAACTACG TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGG CGGCTTCTTAAGTCTGAGGTGAAATCTTGCGGCTCAACCGCAAGCCGGTCATTGGAAACTGGGAGGCTTG AGTGCAGAAGAAGAGAATGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAAGAACACCAGTG GCGAAGGCGGCTCTCTGGTCTGGAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAAAAGGGTTTCCGCCCTTTAATGCTGCAGC TAACGCATTAAGCACTCCGCCTGGGGAATACGGCCGGCAAGGCTGAAACTTCCAAGGAATTGACGGGGGC CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCT CTGACCTCCCTGGAGACAGGGCCTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAG CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGACCTTAGTTGCCAGCATTCAG TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC

		CTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAG
9	R1	AGGTAACGGCTCACCAAGGCGATGA TACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC AGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTC GGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCCAA CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTT ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGC GCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCAT GGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTT TCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCT TACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
10	R2	AACGGCTCACCAAGGCAATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACG GCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCC GCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGT TGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG TGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGC CCTCGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCA TGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACT GACGCTGAGGCTCGAAAGCATGGGGTAGCGGAAACAAGGATTAGATACCCTGGTAGTCCATGCCGTAAAC GAATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTG GGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTC
11	R3	TACCAAGGCGATGAT GCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCA GCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCG GCTCGTAAAGCTCTGTTGTTAAAGAAGAACACGTATGAGAGTAACTGTTCATACGTTGACGGTATTTAAC CAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTA TTGGGCGTAAAGAGAGTGCAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTG CATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAG ATATATGGAAGAACACCAGTGGCGAAGGCGGCTACCTGGTCTGCAACTGACGCTGAGACTCGAAAGCATG GGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTT CCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTT ACCAGGTCTTGACATCTTGCGCCAACCCTAGAGATAGGGCGTTTCCTTCGGGAACGCAATGACAGGTGGT GCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACT AGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCG
12	R4	GTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGG CGGCTTCTTAAGTCTGAGGTGAAATCTTGCGGCTCAACCGCAAGCCGGTCATTGGAAACTGGGAGGCTTG AGTGCAGAAGAAGAGAATGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAAGAACACCAGTG GCGAAGGCGGCTCTCTGGTCTGGAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAAAAGGGTTTCCGCCCTTTAATGCTGCAGC TAACGCATTAAGCACTCCGCCTGGGGAATACGGCCGGCAAGGCTGAAACTTCCAAGGAATTGACGGGGGC CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCT CTGACCTCCCTGGAGACAGGGCCTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAG CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGACCTTAGTTGCCAGCATTCAG TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC CTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAG

Inference: The above results indicate the output of sequencing for all the bacterial samples. The sequences can be subjected for BLASTN analysis to identify and confirm the bacterial species.

Fig 6: BLASTN analysis for the above sequence P1 :

Inference: The above BLAST result confirms the identity of bacteria to be *Lactobacillus casei* strain JXHH4.
Similar analysis was performed for all the other bacteria and their identity was confirmed.

Conclusion

The current study aimed at revealing the microbial quality of home made natural curd and compare it to the commercial curd samples. In achieving this the samples were subjected for microbial isolation followed by identification. Identification was performed both by biochemical and molecular techniques. The results revealed the presence of 5 to 6 varieties of lactobacillus species. Based on the above study it was confirmed that the bacteria present in curd samples were nearly the same without much variation. Some of the common bacteria identified were *Lactobacillus casei, Lactobacillus lactis, Lctobacillus delbrueckii and Bacillus coagulans, Lactobacillus fermentum* were the additional bacteria found in store bought curd.

Reference

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