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An Approch to Comparativemetagenomic Analysis by Study of Microbial Isolates in Fermented Milk Sample.

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ABSTRACT: Metagenomic analysis of lactobacilli from different types of fermented milk samples has become an essential tool for understanding the complex microbial communities present in these products. This approach involves the sequencing of DNA isolated from all microorganisms in a sample, enabling the identification of both known and unknown species. In this study, we analyzed the lactobacilli community present in samples of fermented milk from different regions of the world using a Metagenomic approach. Our results revealed a diverse lactobacilli community, with several known species and many previously unknown ones. We also identified several functional genes related to metabolism and stress response that may play a role in the adaptation of lactobacilli to different milk environments. This study provides a comprehensive view of the lactobacilli community in fermented milk and highlights the importance of Metagenomic analysis for understanding complex microbial communities in food products.

Keyword: Metagenome, Lactobacillus, fermented milk sample, yogurt

I. INTRODUCTION:

Lactobacillus is a type of bacteria that is commonly found in the human body, particularly in the gut and vaginal areas. There are many different strains of Lactobacillus, and each strain may have slightly different health benefits. Some of the medical benefits associated with Lactobacillus include:

1) Improved digestion: Lactobacillus helps break down food in the gut, making it easier to digest and absorb nutrients.
2) Immune system support: Lactobacillus stimulates the immune system, helping to fight off harmful bacteria and viruses.
3) Treatment of diarrhea: Certain strains of Lactobacillus have been shown to reduce the duration and severity of diarrhea.
4) Prevention of vaginal infections: Lactobacillus is important for maintaining a healthy vaginal microbiome, which can help prevent infections such as bacterial vaginosis and yeast infections.
5) Improved mental health: Emerging research suggests that gut bacteria, including Lactobacillus, may play a role in mental health by influencing the production of neurotransmitters such as serotonin.

Overall, the use of Lactobacillus as a probiotic supplement may provide numerous health benefits, but it is important to speak with a healthcare professional before starting any new supplement regimen. Lactobacillus is a genus of gram-positive bacteria that is commonly found in the human gut, mouth, and female genital tract [1].

The history of Lactobacillus goes back over a century, and its discovery has been linked to several scientists and researchers [2]. In 1885, the Russian microbiologist Ilya Ilyich Mechnikov discovered Lactobacillus bulgaricus while studying the digestive system of Bulgarian peasants who consumed large quantities of yogurt. Mechnikov observed that the bacteria present in yogurt helped to improve the health of the people who consumed it, and he coined the term "probiotic" to describe these beneficial microorganisms. Another significant contributor to the study of Lactobacillus was the German microbiologist Alfred Nissle. In the early 1900s, Nissle isolated a strain of Lactobacillus that he named after himself (now known as Lactobacillus Nissle). He found that this strain was effective in treating diarrheal diseases and was widely used in Germany during World War. In the mid-1900s, the American microbiologist Stanley L. Miller studied the role of Lactobacillus in the fermentation process of sauerkraut, which led to a better understanding of the metabolism of these bacteria. Since then,

Lactobacillus has been extensively studied for its potential health benefits, particularly in the field of probiotics. Today, Lactobacillus is commonly used as a probiotic supplement to promote digestive health and strengthen the immune system. Metagenomic analysis has a wide range of applications in microbiology, ecology, biotechnology, and

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medicine, including the development of novel therapeutics and diagnostics, the study of microbial diversity and evolution, and the optimization of industrial processes [2].

Actobacillus is a genus of bacteria that have been used for centuries in the production of fermented foods such as yogurt, cheese, sauerkraut, and kimchi. The name Lactobacillus means "milk-loving" in Latin, reflecting the fact that many of these bacteria are found in milk and other dairy products [3].

In the late 19th century, when the Russian scientist Ilya Ilyich Mechnikov first proposed the idea that certain bacteria in fermented dairy products might be responsible for the health benefits associated with consuming these foods[4]. Mechnikov's work laid the foundation for the study of probiotics, or beneficial bacteria, and he is often credited with being the "father of probiotics."

In the early 20th century, scientists began to isolate and identify individual strains of Lactobacillus and other probiotic bacteria. In the 1920s and 1930s, researchers discovered that certain strains of Lactobacillus could help prevent and treat diarrhea, and these bacteria became widely used in the production of probiotic supplements and functional foods [5]

Today, Lactobacillus is recognized as an important part of the human microbiome, the collection of bacteria and other microorganisms that live in and on the human body [6]. These bacteria play a key role in maintaining gut health and supporting the immune system, and they are the subject of ongoing research into their potential health benefits.

II. OVERVIEW AND BACKGROUND:

This study analyzed the microbiomes of lactobacillus sp. From isolated 5 brand of fermented dairy product samples using deep Metagenomic sequencing, as well as metagenome datasets of 20 nunu, 18 kefir, and 15 yogurt samples retrieved from public genome data- bases[2]. There were large variations in the microbiota composition between sample types, reflected by the great Differences in their profiles of lactobacillus pieces One hundred fifty-three metagenome assembled genome lactobacillus pieces One hundred fifty-three metagenome assembled genome were assembled, including four novel species belonging to the Lactobacillus, Streptococcus, Acetobacter, Rothia genera The novel genomes contained various gene elements, encoding carbohydrate-active enzymes, secondary metabolite clusters, and bacteriocins, which might influence food flavor, quality and/or safety[3]. Bacitracin encoding genes were common, which might help control undesirable microorganisms. Our Metagenomic analysis also revealed the content of bacteriophage sequences of these products, meanwhile uncovering complex Interactions between fermented milk-associated[8] bacteriophages and bacteria, including the history of bacterio phage infection and relationship between Lactobacillaceae and bacteriophages. This study highlighted the mi- crobial diversity and heterogeneity across fermented milk products. Our data demonstrated the power of deep Metagenomic sequencing in expanding our understanding of low-complexity microbial

OBJECTIVE OF THE STUDY

To study Metagenomic analysis of different type of lactobacillus sp., isolated from different brands of fermented dairy products.

RESEARCH JUSTIFICATION

Scope:

- Identification of lactobacillus sp. In different fermented milk samples.
- Studding Metagenomic in different condition.
- Sequencing cultivated anaerobic organisms from fermented milk samples.
- Sequence the genome of one organism in one time.

Importance:

Metagenomics enables the study of all microorganism, regardless of whether they can be cultured or not, through analysis of genomic data obtained directly from an environmental sample, providing knowledge of the species present, and allowing the extraction of information regarding the functionality of microbial organism.

Application

- It can be used to determine gut microbial species and their abundance, and allows to monitor human health and wellbeing (Fig. 1).
- Metagenomics sheds the light into the development of probiotic,
- Monitoring of human associated of bacterial communities allows to established ways to modulate them, so optimize human health.

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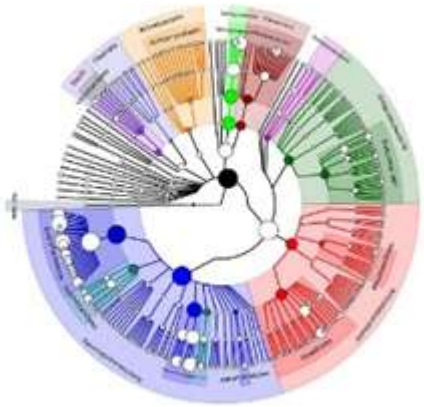

Figure 1: Phylogenetic Tree of Gut Microbiome

Future Prospects & Opportunity:

i. It can be used to determine gut microbial species and their abundance, and allow to monitor human health and wellbeing.
ii. Reveal of genomic sequence of lactobacillus sp. In one time.
iii. Sequencing cultivated anaerobic organism from fermented milk sample
iv. Metagenomic sheds the light into the development of probiotic.

III. LITERATURE REVIEW:

M F F Shuhadha, G J Panagoda, T Madhujith, N W I A Jayawardana1; et al. Curd is a potential source of probiotic Lactobacillus species. Methods this study was carried out to isolate and characterize Lactobacillus species available in curd samples sold in the market. Nine curd samples prepared using cow or buffalo milk were obtained from a local market in the Kandy district. Results Seven isolates (LB 1-7) were identified based on their colony morphology and biochemical characteristics and evaluated for probiotic attributes such as low pH tolerance, resistance to bile salts, antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa, antibiotic activity against erythromycin, chloramphenicol and norfloxacin, hemolytic activity and DNase activity. All isolates were able to grow at low pH (pH=3.0) and were able to survive at 0.3% bile salt, however, the viability decreased with time. LB7 showed very low viability with bile salt compared to others. All isolates exhibited antimicrobial activity against the two pathogenic organisms tested. Two isolates (LB1 and LB2) showed maximum zone of inhibition (18±1.13mm) against E.coli and four isolates (LB1, LB2, LB6 and LB7) against Aeruginosa. Only LB6 and LB7 exhibited resistance to all three antibiotics tested while the other isolates were sensitive. In general, a higher sensitivity was shown against erythromycin and chloramphenicol compared to norfloxacin. All isolates exhibited δ-hemolysis (non-hemolysis) while none of the isolates showed any DNase activity. Conclusions Tested isolates showed probiotic attributes such as resistance to low pH, tolerance to bile salt, antimicrobial resistance, antibiotic activity, non-hemolysis and no DNase activity.

Khubaib Ali, Muhammad Huzaifa Mehmood, Muhammad Ahmad Iqbal, Tariq Masud, Mudassir Qazalbash; et al. Curd is the most widespread traditional fermented milk product used by a large population and is a good source of vitamin B, protein, and calcium. In this study, the isolation of exopolysaccharide (EPS)-producing strains of Lactobacillus delbrueckii subsp. bulgaricus from curd samples was carried out. Identification of EPS-producing strains was done by Gram staining, catalase activity, sugar fermentation test, API 50 CHL, and PCR analysis. These EPS-producing strains were subjected for the estimation of technological properties such as titratable acidity, curdling time, acidification rate, and texture. The strains best in their technological properties were selected for the production of yogurt in combination with EPS- or non-EPS-producing strains of Streptococcus thermophiles. The EPS concentration range was from 41 to 268 mg/L in the yogurt. The highest value of EPS concentration was detected in S. thermophiles and non-EPS-producing Lb. bulgaricus after 14 days of storage.

Somnath De, Atanu Pramanik, Aditya KR Das, Suchismita Paul, Sourav Jana; et al. The isolated and identified bacteria from curd sample are of Lactobacillus sp. The bacteria of this spp. are lactic acid producers. These sp. resembles to Lactobacillus acidophilus. A probiotic is a microorganism known to be friendly and Beneficial to its host when consumed. In today's time, probiotics are a very popular subject of research among scientists and pharmaceutical companies. Due to the over consumption of antibiotics, the normal micro flora of body does not survive. The spores obtained from pharmaceuticals are not good in taste, so generally children avoid eating them, hence we have made the probiotic chocolate. It is better to eat chocolate than medicine. The chocolate containing lyophilized Lactobacillus spp. may help directly for enhancing resistance against intestinal pathogens and in the prevention of diseases.

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RENUKA GOYAL and HARISH DHINGRA; et al Lactobacillus is a genus of lactic acid bacteria. Lactic acid bacteria mainly found in fermented dairy products. This study was carried out for the identification and characterization of Lactobacillus isolated from curd. A total of 14 curd samples were collected from different places of Gurgaon (Haryana) and Lakshmangarh (Rajasthan). Their identification was carried out based on their morphological and biochemical characteristics. A total of 28 isolates were screened on the basis of their phenotypic characteristics. Lactobacillus showed cream-white colonies, rod-shaped, gram-positive, non-spore forming, non-motile, and catalase negative bacteria. Some of the Lactobacillus species are also showing the characteristics of homofermentative or heterofermentative categories.

Abhinav Pathak, Nimisha Dutta; et al Lactobacillus acidophilus comes under the genus Lactobacillus which comprises of large group of beneficial bacteria that have similar properties and all produce lactic acid as an end product of the fermentation process. Previous studies shows that L.acidophilus has its own significant importance as it improves gastrointestinal function, boosts immune system, decreases the frequency of vaginal yeast infection and helps in reducing serum cholesterol levels. The present study is directed towards isolation and identification of antagonistic L. acidophilus from different regions of Allahabad. A total of 50 curd samples were collected from different regions of Allahabad. After careful examination of morphological and biochemical characteristics, all 74 isolates of different Lactobacillus species were found to be present in which 7 strains of L. acidophilus were found. Further these 7 strains were subjected to antagonistic test against selected bacterial pathogens and results revealed that all strains of L.acidophilus were found to be good antagonistic activity against selected bacterial pathogens. This study concluded that curds contain strains of L.acidophilus which shows good antagonistic property; thus revealing that it is safe and beneficial for consumption.

Chetan Sharma, Sachin Gulati, Nishchal Thakur; et al The gut microbiota plays a vital role in host well-being and lactic acid bacteria (LAB) have gained an overwhelming attention as health promoter. This perception has evolved from traditional dairy products to a money-spinning market of probiotics. The safety of probiotics is coupled to their intended use and LAB may act as pool of antimicrobial resistance genes that could be transferred to pathogens, either in food matrix or in gastrointestinal tract, which could be detrimental to host. This study evaluated the antibiotic susceptibility patterns of LAB isolated from curd (20) and human milk (11) samples. Antibiotic susceptibility was determined against 26 common antibiotics, following reference disc diffusion assay. A varied response in terms of susceptibility and resistance towards antibiotics was recorded. Among curd isolates, D7 (Lactobacillus plantarum) was the most resistant followed by D4, D8, D10 and D25. Among human milk isolates, HM-1 (L. casei) showed the highest resistance profile.

RENUKA GOYAL and HARISH DHINGRA; et al Lactobacillus is a genus of lactic acid bacteria. Lactic acid bacteria mainly found in fermented dairy products. This study was carried out for the identification and characterization of Lactobacillus isolated from curd. A total of 14 curd samples were collected from different places of Gurgaon (Haryana) and Lakshmangarh (Rajasthan). Their identification was carried out based on their morphological and biochemical characteristics. A total of 28 isolates were screened on the basis of their phenotypic characteristics. Lactobacillus showed cream-white colonies, rod-shaped, gram-positive, non-spore forming, non-motile, and catalase negative bacteria. Some of the Lactobacillus species are also showing the characteristics of homofermentative or heterofermentative categories.

T**asneem Chowdhury, Saiful Islam; et al** Curd is a widely consumed milk product in Bangladesh. Curd samples from sweetmeat stores in Chittagong city of Bangladesh were collected and analyzed for lactic acid bacteria (LAB). Bacterial load of the collected samples was determined by pour plate technique and bacterial count was found in the range of 3.27×105 to 1.05×106 cfu/mL indicating the samples as excellent nourishing environment. Based on their characteristic growth on MRS agar media six Lactobacillus isolates were isolated, similarly six isolates of Streptococcus were isolated on YGLA media. Antimicrobial properties of the isolated LABs were evaluated against four human pathogenic bacteria employing modified disc diffusion assay. All twelve isolates exhibited antagonistic activities against at least two or more of the test organisms. In our current study, Lactobacillus casei showed antagonism against all test pathogens. Among other Lactobacillus isolates L. xylosus, L. homohiochii and L. fermentum inhibited three pathogens, whereas L. salivarius and L. leichmannii showed inhibition against two pathogens. Among

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Streptococcus isolates, S. thermophiles, S. uberis, S. suis, S. faecalis and S. equnius were found to inhibit the growth three test pathogenic bacteria while S. lactis was reported to suppress the growth of E. coli and Salmonella paratyphi. The current study reports some LABs with promising antagonistic properties against test pathogenic bacteria and further investigations on the LABs will validate their appropriateness in using them for improvement of health and services.

IV. MATERIALS & METHODS

Requirements:

1. Instruments & Materials
2. Autoclave
3. Conical flask
4. Test tube
5. Beaker
6. Petri dish
7. Digital Colony counter
8. TDS meters
9. Digital PH meters
10. Digital Electro Conductivity meter
11. BOD incubator

Chemicals

1. Protease peptone
2. Beef extract
3. Dextrose
4. Sodium acetate
5. Magnesium sulfate
6. Ferric ammonium citrate
7. Agar
8. Glucose
9. Choloram phenol
10. Nutrient broth
11. Phenol red

Sample Collection

Five sample of fermented diary product including Berhumpore Local curd, Dubrajpur local curd, Theker diary curd, Red Cow curd, and Amul curd collected on different areas of Berhumpore city, Dubrajpur municipality and Birbhum corresponding area. And collect DM water from DM water plant.

Evaluation of parameter of DM water & Curd

DM (Deionized or Demineralized) water is a type of purified water that has had all of its minerals ion removed, making it highly pure and suitable for a variety of laboratory applications physical parameter is very important for curd because it helps to identify healthy curd. When testing the physical parameters of DM water & curd, some important parameters to consider include:

pH: pH is a measure of the acidity of alkalinity of a solution. The pH of DM water should be neutral (pH7) because it is free of any acidic or basic ion. (Fig: 2)

Figure2: pH check of DM water by digital pH meter

EC: Conductivity is measure of the ability of water to conduct an electric current. DM water should have a very low conductivity due the absence of mineral ions, typically, the conductivity of DM water is less than 1µS/cm (fig 3.)

Figure 3: Digital EC meter

TDS: it is the measurement of the total amount of the dissolved solids in the water, including both mineral and non-minerals ion. DM water should have a very low TDS because it has been demineralized. Typically, the TDS of DM water is less than 1mg/L. (fig 4)

Figure 4: Digital TDS meter

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Temperature: DM water temperature maintain of an room temperature that is should be 25 ° C

Figure 5: Thermometer

Media Preparation:
Nutrient Agar media:
Nutrient Agar media is general purpose, Nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms.
Composition of Nutrient Agar
0.5 % Peptone, 0.3% beef extract/yeast extract, 1.5% Agar, 0.5% NaCl, Distilled water with pH adjustment to neutral (7.4) at 25 ° C

Nutrient Agar Medium	Quality for 250 ml media	Quality for 500 ml media in g
Agar-agar	3.75	7.5
Beef Extract	0.75	1.5
Peptone	1.25	2.5
Glucose	0.625	1.25
NaCl	1.25	2.5
DM water	qs	qs

Table 1: Composition of Nutrient Agar

Preparation of Nutrient Agar:
- Weigh all above chemical ingredients properly and dissolved in 250 ml DM water.
- Heat this mixture stirring to fully dissolve all components in water both at 45°C.
- Autoclave the dissolved mixture (Fig: 6) at 121 degrees Celsius for 15 minutes.
- Once the nutrient agar has been autoclaved, allow it to cool but not solidify. (Fig: 8)

Pour nutrient agar into each plate a leave plates on the sterile surface until the agar has solidified. Replaced the lid of each Petri dish and store the plates in refrigerator [9].

Figure 6: Nutrient Agar Media

Prepare 10 % solution different curd samples:
To prepare a 10% solution, mix curd and water in a specific ratio. Here I had prepare 10 ml curd solution. (Fig: 7)

Hare the steps to follow:
Measure the 1 gm amount of curd
Calculate 10 % of 10 mlMeasure 1 gm of curdAdd curd into the previously measure10 ml of Distilled water Prepare 10 % solution of curd (w/v)

Figure 7: 10% Solution of Different Curd

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Figure 8: Autoclave Media

INOCULATION OF CURD SOLUTION

1. 5 sterile plates are taken and numbered as (1), (2), (3), (4) and (5). (Fig: 9)
2. The Nutrient agar media which is cooled to 45° C after autoclaving, is poured on the sterile petri plate to form a thick layer (6 mm) and allowed to rest undistributed for 2 hours.
3. After 2 hours when the media solidifies, from the Sample I curd solution are innoculated in petri plate (A) under the laminar air flow. (Fig: 10)
4. In the same way inoculation in done in Petri plate (B) from the sample no 2.
5. In the same way inoculation in done in Petri plate (c) from the sample no 3.
6. In the same way inoculation in done in Petri plate (D) from the sample no 4.
7. In the same way inoculation in done in Petri plate (D) from the sample no 5.
8. Then petri plate placed into the BOD incubator to incubate for 48 hours and maintain 37° C. (Fig: 11)

Figure 9: Media Plate

Colony Count:

Colony counting is a method used to determine the number of viable bacteria in a sample [10]. It involves counting the number of colonies that grow on a culture plate after incubation. The colony count is often used in medical and food microbiology to assess the microbial load of a sample, and to monitor the effectiveness of antimicrobial treatments. To perform a colony count, a small amount of the sample is spread over the surface of a solid agar medium, and the plate is then incubated under appropriate conditions for bacterial growth. After incubation, the colonies that have formed on the plate are counted manually or using automated colony counters. The number of colonies counted is then used to Estimate the number of viable bacteria in the original sample, taking into account dilution factors and the size of the sample analyzed. The results of colony counting can be reported as colony forming units per milliliter (CFU/mL) or per gram (CFU/g) of the original sample [11]. The accuracy and precision of the colony count depend on several factors, such as the quality of the agar medium, the incubation conditions, and the skill of the person performing the count. (Fig: 10)

Bacterial Identification:

Bacterial identification is the process of determining the specific type or species of a bacterial organism. There are various methods for identifying bacteria, including phenotypic, genotypic, and biochemical methods. Final identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and oxidase tests, indole producing, growth at 15° C, and carbohydrates fermentation (lactose, mannitol, salicin, sucrose, a trehalose) test.

Lactobacilli are a group of Gram-positive, non-spore-forming, rod-shaped bacteria that are commonly found in the gastrointestinal tract,

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mouth, and vagina of humans and other animals. These bacteria are often used in the production of fermented foods such as yogurt, kefir, and sauer-kraut [13].

There are several methods for identifying lacto-bacilli, including:

1. Gram staining: Lactobacilli are Gram-positive, which means that they will stain purple with a Gram stain.
2. Microscopic examination: Lactobacilli are rod-shaped bacteria, so they will appear as long, thin cells under a microscope.
3. Biochemical tests: Various biochemical tests can be used to identify lactobacilli, including the catalase test (lactobacilli are catalase-negative) and the fermentation test (lactobacilli are capable of fermenting various sugars).
4. DNA sequencing: DNA sequencing can be used to identify lactobacilli based on their genetic makeup.

Overall, a combination of these methods can be used to accurately identify lactobacilli.

Figure-10: Digital Colony Counter

Figure-11: Laminar Air Flow (Left) And B.O.D. Incubator (Right)

V. RESULT:

Table 2: Physical Parameter of Demineralized Water.

Physical parameter of Demineralized Water			
pH	**EC**	**TDS**	**TEMP**
7	14.5	5	25

Table 3: Physical Parameter of Different Curd.

Physical parameter of Different Marketed Curd Samples		
Curd sample	**pH**	**EC**

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Berhumpore Local	4.5	4.5 mS/cm
Dubrajpur local	4.7	3.9 mS/cm
Red Cow	4.2	4.7 mS/cm
Amul Masti	4.1	5 mS/cm
Thacker Diary	4.3	4.2 mS/cm

Table 4: Identification Test of Lactobacillus.

Lactobacillus	catalase	Indole	Oxidase	Growth at 15 ^{o} c	Sucrose	Lactose
Lactobacillus colli-noides	-	-	-	+	-	-
Lactobacillus Sake	-	-	-	+	+	+
Lactobacillus alimen-terius	-	-	-	+	+	-

Table 5: Lactobacillus in Different Marketed Curd Sample.

Sample	*Lactobacillus stains*
S1: Berhumpore Local	*Lactobacillussake*
S2: Dubrajpur local	*Lactobacilluscollnoides*
S3: Red Cow	1. *Lactobacillussake* 2. *Lactobacilluscollnoides*
S4 : Amul masti	1. *Lactobacilluscollnoides* 2. *Lactobacillussake* 3. *Lactobacillusalimenterius*
S5: Thacker Diary	*Lactobacillusalimenterius*

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Table-6: Colony Count of Different Curd Sample.

Sample	Total Count	CFU/ml
S1 (Berhumpore Local)	58	0.58
S2 (Dubrajpur local)	31	0.31
S3 (Red Cow)	13	0.13
S4(Amul masti)	27	0.27
S5(Thacker Diary)	59	0.59

Table 7: Similarity analysis between Resulting strain and other lactobacillus. strain

Organism Name	Organism Groups	Strain	Assembly	Size(Mb)	GC%
Lactobacillus sp. CBA3605	Bacteria;Terrabacteria group;Bacillota	CBA3605	GCA_002970915.1	2.55258	42.8191
Lactobacillus sp. CBA3606	Bacteria;Terrabacteria group;Bacillota	CBA3606	GCA_002970935.1	2.55974	43.1529
Lactobacillus sp. 3B(2020)	Bacteria;Terrabacteria group;Bacillota	3B(2020)	GCA_013487845.1	2.18761	42.5039
Lactobacillus sp. ESL0677	Bacteria;Terrabacteria group;Bacillota	ESL0677	GCA_029392875.1	2.0288	38.7
Lactobacillus sp. JM1	Bacteria;Terrabacteria group;Bacillota	JM1	GCA_009834385.1	2.08541	35.0463

Table 8: Comparative Metagenomic analysis of isolates of lactobacillus species from samples.

Sl.No	Organism	Stain	Distance	TSL	TNCP	GC%
1	Lactobacillus sp	LAC5	0.498	14,87,044	0	45.8
4	Uncultured Lactobacillus sp.	Chicken_10_mag_88	0.614	17,52,336	0	48.9
5	Uncultured Lactobacillus sp.	UMGS258	0.053	24,70,970	0	53.4
7	Uncultured Lactobacillus sp.	ERR276892_bin.4_meta_WRAP v1	0.692	28,73,148	0	46.8
10	Lactobacillus sp	UBA11204	0.991	23,48,886	0	44
11	Lactobacillus sp	LAC4	0.822	33,35,227	0	40.2
16	Lactobacillus sp. wkB10	wkB10	0.175	19,22,347	0	35.4
19	Lactobacillus sp. JM1	JM1	0.389	20,85,415	3	35.04

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VI. DISCUSSION:

It is the long time that scientist are trying to substitute synthetic drugs with natural drugs with natural products.[14] Nowadays, various natural materials and method are used to prevent or treat diseases.[15] The use of Probiotics is one of these methods. Lactobacilli as normal intestinal flora which by preventing intestinal infection, lowering cholesterol, stimulating the immune system, and reducing the risk of colon cancer play and important role in human health. Probiotic bacteria produces Lactic acid and organic acid, reduce the pH environment and dry to prevent the growth of many bacteria. This bacteria produce anti-microbial compound such as bacteriocin which can be uses as natural preservatives.

In this study it was found that the sample 4 (Amul Masti) has acidic pH of 4.1 with electro conductivity of 5 mS/cm (Table 3). Lactobacillus species has been identified by various biochemical tests (Table 4).

In table 5 presence of different lactobacillus species has been observed. But Marketed sample 4 (Amul Masti) has different variety of lactobacillus species (identically Lactobacillus collnoides, Lactobacillus sake, Lactobacillus alimenterius).

Marketed sample 3 (Red Cow) has two different variety of Lactobacillus species (identically Lactobacillus sake, Lactobacillus collnoides). And rest of the samples are having only one variety of lactobacillus species (identically either Lactobacillus sake / Lactobacillus collnoides / Lactobacillus alimenterius).

Bacterial Quantification (Table 6) shows highest CFU/ml of 0.59 in sample 5(Thacker diary) & least CFU/ml of 0.13 in sample 3 (Red Cow). Sample 4 (Amul Masti) has a nominal CFU/ml of 0.27.

The Genomic similarity analysis (Table 7) shows for lactobacillus organism strain CBA3606 with 43.15% Genomic of compatibility.

Organism Name	Organism Groups	strain	Assembly	Size(Mb)	GC%
Lactobacillus sp. ESL0731	Bacteria;Terrabacteria group;Bacillota	ESL0700	GCA_029395555.1	2.05231	38.9237
Lactobacillus sp. ESL0700	Bacteria;Terrabacteria group;Bacillota	IBH004	GCA_029392095.1	2.03643	38.9636
Lactobacillus sp. IBH004	Bacteria;Terrabacteria group;Bacillota	IBH004	GCA_026185255.1	1.98132	36.4742

Table 8 shows various lactobacillus species with comparative Metagenomic analysis by BLAST.

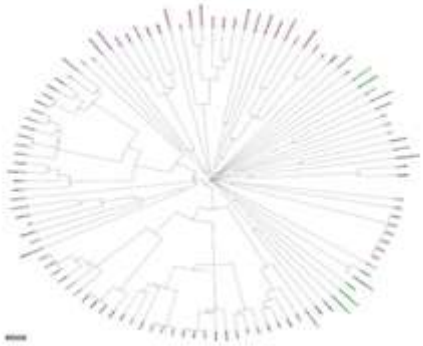

Figure 12 shows specificity & origin of lactobacillus.

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VII. CONCLUSION:

The result of this study shows variety of lactobacillus species present in sample 4(Amul Masti) curd sample, Having important role in food preservation and human health. These bacteria can be raised for the production of various kinds of food and pharmaceutical product, they can also be used for the production of new functional food. Therefore, increasing use of diary product containing probiotics and most effective lactobacilli are recommendedin daily diet. Metagenomics Analysis shows a correlation of Genomic similarity any lactobacillus species.

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