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RESEARCH ARTICLE

QUALITATIVE ANALYSIS OF *LACTOBACILLUS* STRAINS IN MILK PROBIOTICS

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Abstract

In the present study, an attempt was taken for qualitative analysis (to detect the presence) of *Lactobacillus* strains: one of the major bacterial strains of milk probiotics by different biochemical tests, salt & pH tolerance and subsequently sugar & casein fermentation tests of cheese, curd and milk kefir. Gram staining and colony morphology of each isolated bacterial colony primarily indicated the presence of *Lactobacillus* species which was further confirmed by different biochemical and selective tests for *Lactobacillus* species. Negative catalase test as well as positive Kilger's iron agar, oxidase and Simon citrate as biochemical tests confirmed the presence of *Lactobacillus* species in the test samples. Additionally, good to moderate sugar (sucrose, fructose, glucose & lactose) and skim milk fermentation revealing deep red color and clear zone appeared around the areas in the medium where the organism had grown, proved the presence of *Lactobacillus* species in the samples. Moreover, less growth or absence of bacteria in MRS broth medium containing higher salt concentration (8% NaCl solution and above) and having higher pH value by adding 1N HCl (pH 3 and above) finally confirmed that cheese, curd and milk kefir contain *Lactobacillus* species.

Introduction:-

Probiotics termed as live micro-organisms mainly good bacteria that primarily lined in the gut upon administration in adequate amounts in our body confers a health benefit to the host [1]. Roberfroid reported that today's most of the foods contain little to no probiotics, on the contrary they contain antibiotics which kill off the good bacteria in our bodies because of refrigeration and dangerous agricultural practices. Thus adding more probiotic in foods can ensure stronger immune system, improved digestive system, reduction of cold & flu, healing from leaky gut & inflammatory bowel disease, better breath, healthier skin and vitamin production [2].

Most of the probiotics contain *Lactobacillus*, *Bifidobacterium*, *Bacillus* and *Streptoccocus* species in their formulation and milk derived probiotics are predominant with *Lactobacillus* species: these species are responsible for producing lactase, the enzyme required to break down lactose (the sugar in milk) and ferment carbohydrates in the gut producing lactic acid which helps to create an acidic environment in the digestive tract discouraging many unwanted microorganisms that thrive in an alkaline environment [3]. The genus *Lactobacillus* is gram positive, non-spore-forming, usually catalase-negative, non-motile and facultative anaerobic bacteria. It is also very heterogeneous, encompassing species with a large variety of phenotypic, biochemical and physiological properties.

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In recent decades, extensive research has been carried out on isolation and screening of microorganisms from traditional fermented foods and lactic acid bacteria (LAB) and yeasts playing an important role in numerous natural food fermentations such as curd, cheese, pickles and various other traditional foods which are closely associated with the human environment [4] and these organisms have also gained popularity as probiotics [5].

As lactic acid bacteria is regarded as a major group of probiotic bacteria, this study aimed to isolate and identify *Lactobacillus* species from milk probiotics by following different standard protocols.

Methods and Materials:-

Sample collection:-

Raw and processed cheese, sweet and sour curd, milk and coconut kefir (each 2 samples) were collected in sterile polythene bags from different markets at Dhaka city of Bangladesh (Table 1). After collection, all samples were labeled appropriately and kept in the insulated box and transported to the laboratory. All samples were processed within 24 hours form the time of collection.

Table 1:-List of collected samples

SL. No	Sample code	Sample	Sample type	Sources
1	HCR_1	Cheese	Raw	Gazipur, Dhaka
2	HCR_2			Farmgate, Dhaka
3	HCP_1	Cheese	Processed	Azimpur, Dhaka
4	HCP_2			Uttura, Dhaka
5	HDR_1	Curd	Sweet	Mirpur, Dhaka
6	HDR_2			Dhanmondi, Dhaka
7	HDP_1	Curd	Sour	Shyamoli, Dhaka
8	HDP_2			Motijheel, Dhaka
9	HKP_1	Milk Kefir	Processed	Savar, Dhaka
10	HKP_2			Kollanpur, Dhaka

Sample preparation:-

All of the collected samples (both raw & processed) were prepared for further analysis. In order to prepare ready sample, 1gm of each sample were taken into 9ml MRS (Man, Rogosa and Sharpe) broth containing test tube and mixed well using vortex machine. The entire sample containing test tubes were then incubated for 24 hours at 37° C and finally preserved the samples for further analysis.

Sample enrichment:-

RMS agar plates were prepared and one loop of broth solution containing bacteria from each test tube was taken and inoculated on plates. Then plates were incubated for 24 hours at 37° C for optimum bacterial growth.

Single colony isolation from enriched samples:-

After 24 hours, all incubated RMS agar [6] (Table 2) plates were analyzed by naked eye to identify the morphology of bacteria grown on those plates. Suspected characteristic single colony was isolated and colony morphology was recorded. Finally MRS slants were prepared and all of the suspected bacterial colonies were stored separately.

Table 2:-Composition of RMS broth medium (up to 1000 ml water)

Name of ingredients	Amount
Peptone	10.0 gm
Beef extract	10.0 gm
Sodium chloride	5.0 gm
pH after sterilization	7.3±0.1

Qualitative analysis:-

Gram staining of isolated bacterial colony:-

In an aseptic area, under laminar air flow a drop of saline water was placed on the slide and then a colony kept in RMS slant was taken and made a suspension on the slide to obtain thin smear. Finally smear was fixed with slight heating of the slide that was flooded with crystal violet solution and kept for 01 minute subsequently washed out

with running tap water. Smear was covered with a few drop of Gram's Iodine and kept for about 1 minute. Resultant slide was then washed out with running tap water. The slide was decolorized with absolute alcohol and then it was kept for 30 seconds and washed out with running tap water. Again alcohol was poured on the slide for a while followed by washing was continued till color ceases coming out. After de-colorization the slide was cover with a few drop of safranin red for 2 minutes and then washed out with running tap water [7]. After air drying of the slides at room temperature, a drop of emersion oil was given over the dried slide to observe color under the high power objective lenses (x100).

Biochemical tests of the samples:-

Catalase Test:-

The catalase test of the samples was performed by using 3% hydrogen peroxide (H_2O_2) solution. A drop of hydrogen peroxide (3%) was placed on a sterile glass slide. A drop of bacterial sample (kept in RMS broth) was placed on the slide and mixed well with the help of sterile loop. After few minutes slide was examined to detect the presence of bubble or froth on the slide. A positive and a negative control were used as *E.coli* and *Staphylococcus aureus* respectively [8].

Kilger's Iron agar (KIA) test:-

KIA test performs to know the mode of dextrose utilization by bacteria in oxidative/fermentative test. Kligler Iron Agar combines the principles of Russell double sugar medium and lead acetate agar into one medium (Table 2). This combination permits differentiation of *bacilli* by their ability to ferment dextrose or lactose, which produces color changes of the pH indicator (phenol) in response to acid production during fermentation of the sugars. Slants of KIA media were inoculated by stabbing the butt and streaking the slant with 24 hours culture. After incubation at 37° C for 18-24 hours, results were recorded for changing in color of the butt or slant, H_2S or other gas production [9].

Table 3:-Composition of Kilger's Iron agar (up to 1000 ml water)

Name of ingredients	Amount
Peptone	15.0gm
Lactose	10.0gm
Proteose Peptone	5.0gm
Sodium Chloride	5.0gm
Beef Extract	3.0gm
Yeast Extract	3.0gm
Dextrose	1.0gm
Sodium Thiosulfate	0.3gm
Ferrous Sulfate	0.2gm
Phenol Red	0.024gm
Agar	12.0gm
Final pH 7.4 ± 0.2 at 25ºC.	

Oxidase test:-

Oxidase test of all samples was performed on a piece of sterile what man filter paper which was soaked with 1% solution of N, N, N^1 N^1–Tetramethy1-p-phenylenediamine solution. A portion of the colony of the test organism was picked up with a sterile stick and touched on to the paper with impregnated reagent. After 5 to 10 second, the filter paper was examined to observe the color change occurred [10].

Simmon citrate test:-

Isolated bacterial colony was immersed in Simmon's citrate agar slants. Then slants were kept for incubation of 24-48 hours at 37° C and growth of bacteria and color change (to blue) was observed [11].

Table 4:-Composition of Simmon Citrateagar (up to 1000 ml water)

Name of ingredients	Amount
Sodium Chloride (NaCl)	5.0 gm
Sodium Citrate (dehydrate)	2.0 gm
Ammonium Dihydrogen Phosphate	1.0 gm
Dipotassium Phosphate	1.0 gm

Magnesium Sulfate (heptahydrate)	0.2 gm
Bromothymol Blue	0.08 gm
Agar	15.0 gm
Final pH 6.9 ± 0.2 at 25 degrees C.	

Sugar fermentation test:-
Sugar fermentation test of all samples was performed using fructose, sucrose, glucose and lactose. At first, the sugar was dissolved in distill water to make 1% W/V solution. MRS broth was prepared and added respective 1% W/V sugar solution. Then phenol red indicator was added in the broth solution which turns the color of broth solution into dark red. 10 ml media was dispensed and Darham's tube was inserted in each of these test tubes. Then the final mixture of broth and sugar solution with Darham's tube was sterilized in autoclave for 20 minutes at 121° C. After autoclaving the solution was cooled and bacterial sample was inoculated in test tubes. Then test tubes were incubated for 24 h at 37° C where only media was used as negative control. Results were observed by color changing and gas formation [12].

Salt tolerance test:-
In order to perform salt tolerance test of the samples, MRS broth containing 2%, 4% and 8% NaCl solution were prepared. Then 5 ml of the media was dispensed in test tube and sterilized in autoclave at 121° C for 20 minutes. After autoclaving, the solution was cooled down and each bacterial sample was inoculated in three test tubes containing 2%, 4% and 8% NaCl broth solution and kept for incubation at 37° C for 24 hours. After 24 and 48 hours microbial growth was determined by observing turbidity of the MRS broth medium [13].

pH tolerance test:-
In order to perform pH tolerance test, MRS broth was prepared and pH was adjusted to 2, 3, 4, 6, 8 and 10 and the adjusted broth was dispensed in 6 test tubes (each 5ml). These test tubes were sterilized in autoclave in 121°C for 20 minutes. Samples were inoculated in each test tube and incubated for 24 hours at 37° C where media was used as negative control [14].

Casein digestion test:-
In order to perform casein digestion test, 1% skim milk agar plate was prepared and sterilized by autoclaving for 20 minutes at 121°C. Then bacterial samples were inoculated with streaking and incubated for 24 hours at 37°C. After 24 hours, clear zone on skim milk agar plate was observed indicating the positive result [15].

Result and Discussion:-

In this study processed, raw, sweet and sour type of milk probiotics (cheese, curd & kefir) were subjected for different biochemical tests along with sugar fermentation, salt, pH tolerance and casein digestion tests for qualitative analysis of *Lactobacillus* species.

Identification of isolated bacterial colony:-
Colony morphology:-
Total ten morphologically distinct single colonies were isolated from four cheeses samples (raw & processed), four curds samples (raw & processed) and two milk kefir samples (processed) on MRS plates (Table 5 and Figure 1). All the isolates were gram positive bacteria with rod shaped structure (Figure 2).

Table 5:-Observation of colony morphology

SL. No	Sample code	Sample	Sample type	Morphology of colony			
				Shape	Size	Color	Number
1	HCR_1	Cheese	Raw	Round, Centered	Small	Pale Yellow	Plenty
2	HCR_2						
3	HCP_1	Cheese	Processed	Round, Centered	Small	Pale Yellow	Plenty
4	HCP_2						
5	HDR_1	Curd	Sweet	Round, Centered	Small	Pale Yellow	Plenty
6	HDR_2						
7	HDP_1	Curd	Sour	Round, Centered	Small	Pale Yellow	Plenty
8	HDP_2						

9	HKP_1	Milk Kefir	Processed	Round, Centered	Small	Pale Yellow	Plenty
10	HKP_2						

Gram staining:-
All isolated single colony bacteria were subjected for gram staining test and all sample revealed positive result. All isolates were morphologically in rod shape (Table 6 & Figure 2) which represents *Lactobacillus* species present in these samples.

Table 6:-Observation of gram staining

SL. No	Sample code	Sample	Sample type	Gram staining	
				Observation	Morphology
1	HCR_1	Cheese	Raw	+	Rod
2	HCR_2				
3	HCP_1	Cheese	Processed	+	Rod
4	HCP_2				
5	HDR_1	Curd	Sweet	+	Rod
6	HDR_2				
7	HDP_1	Curd	Sour	+	Rod
8	HDP_2				
9	HKP_1	Milk Kefir	Processed	+	Rod
10	HKP_2				

Figure 1:-Colony morphology on MRS plate

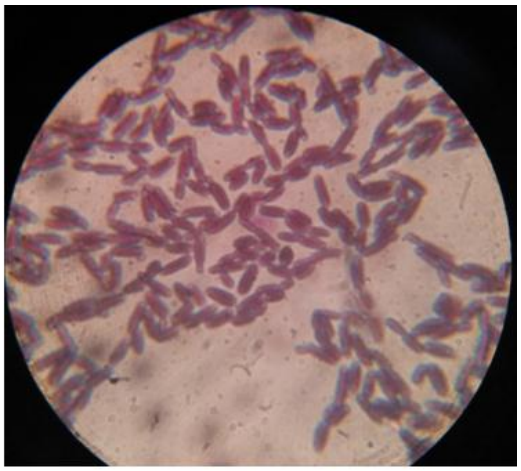


Figure 2:-Gram staining

Biochemical tests of the samples:-
All the samples were subjected for different biochemical tests like catalase, oxidase, Simon citrate and KIA test for confirmation of *Lactobacillus* species present in samples.

All of the test samples were catalase negative because no bubble or gas produced due to lower percentage of H_2O_2 conversion into water and oxygen (Table 7). Microorganisms can produce catalase enzyme to neutralize toxic forms of oxygen metabolites (H_2O_2) living in oxygenated environments and protects them. Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. This catalase negative test results indicated the present of *Lactobacillus* species in milk probiotics tested in this study.

In oxidase test, all samples produced dark purple color within 5-10 second was considered positive for *Lactobcillus* test where *V. cholera* was used as positive control (Table7 & Figure 4). It is known that Simmons citrate agar tests the ability of organisms to utilize citrate as a carbon source. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, nutrients and the pH indicator-bromothymol blue. Organisms degrading citrate must also use the ammonium salts, producing ammonia [16], thus increasing the pH of the medium. The increase in pH then causes color change in the bromothymol blue indicator,

turning it blue. In case of Simmon Citrate test, all the bacterial isolates (T-1 to T-5 in figure 5) turned the medium color into blue indicated that the organism (Table 7) was citrate positive which confirmed the presence of *Lactobacillus* species in the test samples. Cheese, curd and milk kefir were subjected to perform KIA test and all initially produced yellow slants and finally converted into red slants with slightly gas production into butt revealed positive result for *Lactobacillus* species (Table 7 & figure 3). Test samples initially produced a yellow slant and butt as a result of dextrose fermentation indicated the acid formation from dextrose and the concentration of dextrose was only one percent and, therefore became rapidly exhausted. Once the dextrose was depleted, the reaction reverts to alkaline (red slant) due to the oxidation of acids. Additionally gas production was demonstrated by the presence of bubbles in the butt or ring formation on the top of the butt indicated the positive result [17].

Table 7:-Results of biochemical tests

SL. No	Sample code	Sample	Sample type	Biochemical tests						
				Catalase test	Oxidase test	Simmon Citrate test			KIA test	
						24hr	48hr	color	Initial	Final
1	HCR_1	Cheese	Raw	-	+	+	+	Blue	Yellow/ acidic	Red/ alkaline
2	HCR_2									
3	HCP_1	Cheese	Processed	-	+	+	+	Blue	Yellow/ acidic	Red/ alkaline
4	HCP_2									
5	HDR_1	Curd	Sweet	-	+	+	+	Blue	Yellow/ acidic	Red/ alkaline
6	HDR_2									
7	HDP_1	Curd	Sour	-	+	+	+	Blue	Yellow/ acidic	Red/ alkaline
8	HDP_2									
9	HKP_1	Milk Kefir	Processed	-	+	+	+	Blue	Yellow/ acidic	Red/ alkaline
10	HKP_2									

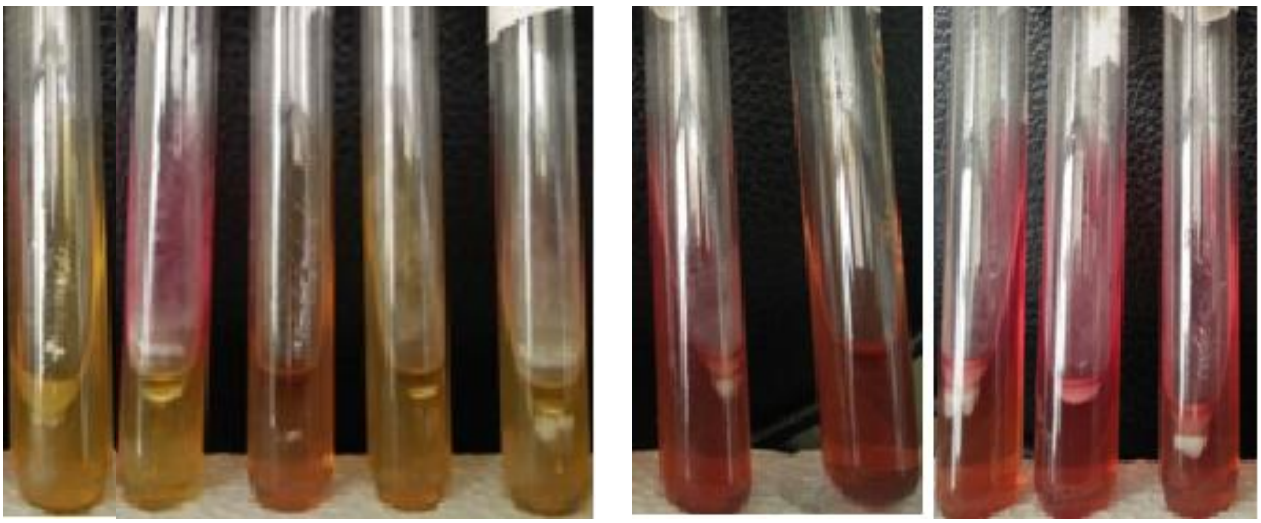


Figure 3: KIA test

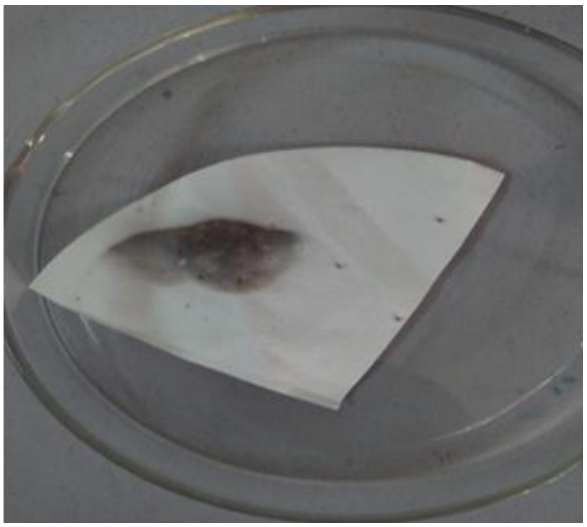
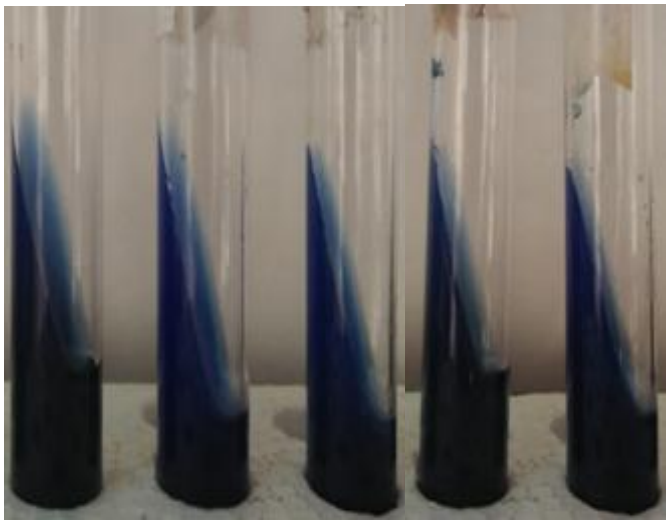


Figure 4: Oxidase test



T-1 T-2 T-3 T-4 T-5

Figure 5: Simon citrate test

Sugar fermentation test:-

All of the bacterial samples were performed sugar fermentation test in sucrose, fructose, glucose and lactose media. All of the samples performed good fermentation except sour curd and milk kefir revealed moderate fermentation in sucrose; sweet curd and milk kafir performed moderate fermentation in fructose and sour curd and milk kefir showed moderate fermentation in glucose and lactose medium respectively (Table 8 & Figure 6). Due to the lowering of the pH of the medium because of acid production through fermentation of sugar by the sample was observed by the color change of the pH indicator-phenol red into deep red in the medium. Additionally small inverted tubes called Durham tubes were also immersed in the medium to test for the production of the gas (hydrogen or carbondioxide). It was observed that all of the test samples produced gas because of different degree of sugar fermentation was an indication for *Lactobacillus* species [18].

Table 8:-Results of sugar fermentation

SL. No	Sample code	Sample	Sample type	Sugar fermentation			
				Sucrose	Fructose	Glucose	Lactose
1	HCR_1	Cheese	Raw	+, r, g	+, r, g	+, r, g	+, r, g
2	HCR_2						
3	HCP_1	Cheese	Processed	+, y, g	+, r, g	+, r, g	+, r, g
4	HCP_2						
5	HDR_1	Curd	Sweet	+, r, g	±, y, g	+, r, g	+, r, g
6	HDR_2						
7	HDP_1	Curd	Sour	±, y, g	+, r, g	±, r, g	±, r, g
8	HDP_2						
9	HKP_1	Milk Kefir	Processed	±, y, g	±, y, g	±, y, g	±, y, g
10	HKP_2						

+ = Good fermentation
± = Moderate fermentation
- = no fermentation

y = yellow in color
r = red in color
g = gas production

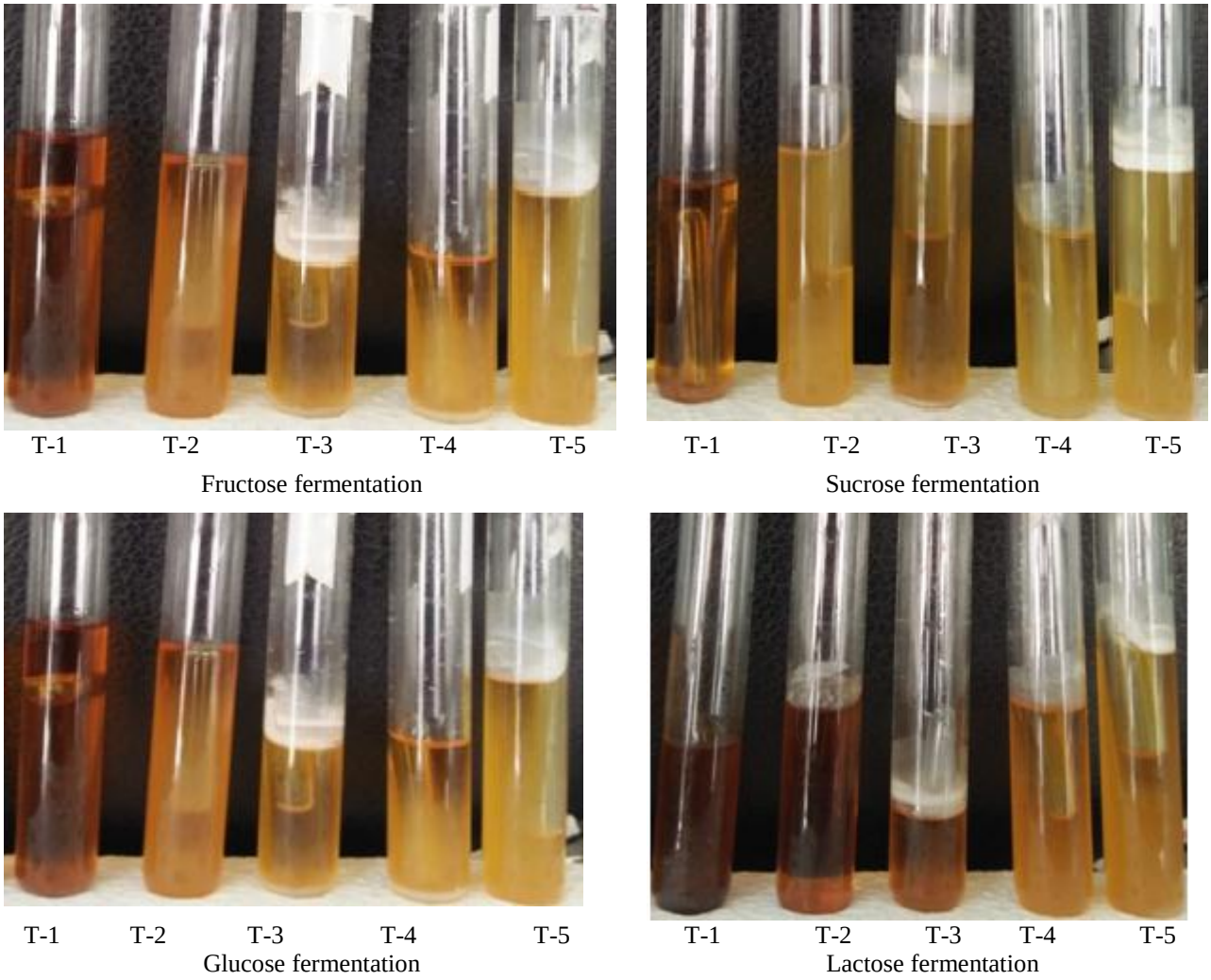


Figure 6: Sugar fermentation

Salt tolerance test:-

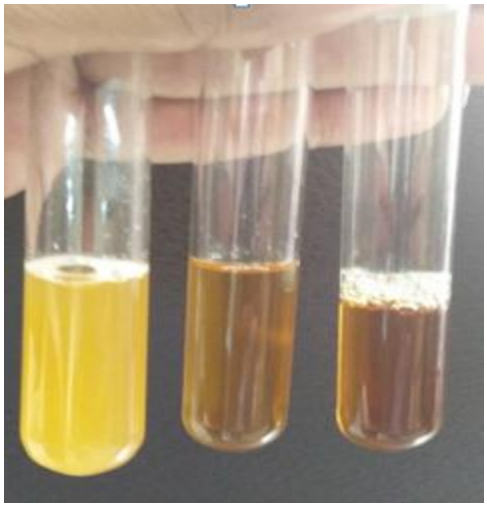
Cheese, curd and milk kefir were performed salt tolerance test at 2%, 4% and 8% salt solution containing MRS broth. As it is a selective medium, this tests the ability of an organism to survive in a salt-rich environment. A positive salt tolerance test was indicated by growth and/or turbidity in media without indicator after 24 and 48 hours in 2% and 4% salt medium (Table 9 & Figure 7). But in case of 8% salt solution, there was very less turbidity (less growth of bacteria) and no turbidity (no growth of bacteria) after 48 hours in MRS broth medium [19].

Table 9: Results of salt tolerance test

SL. No	Sample code	Sample	Sample type	NaCl %					
				2%		4%		8%	
				24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
1	HCR_1	Cheese	Raw	+++	+++	+++	+++	±	-
2	HCR_2								
3	HCP_1	Cheese	Processed	+++	+++	+++	+++	±	-
4	HCP_2								
5	HDR_1	Curd	Sweet	+++	+++	+++	+	±	-
6	HDR_2								
7	HDP_1	Curd	Sour	+++	+++	+++	++	±	-
8	HDP_2								

9	HKP_1	Milk Kefir	Processed	+++	+++	+++	++	±	-
10	HKP_2								

+++ = more turbid, ++ = moderately turbid, + = less turbid, ± = very less turbid, - = no turbidity



2% 4% 8%

Figure 7: Salt tolerance test

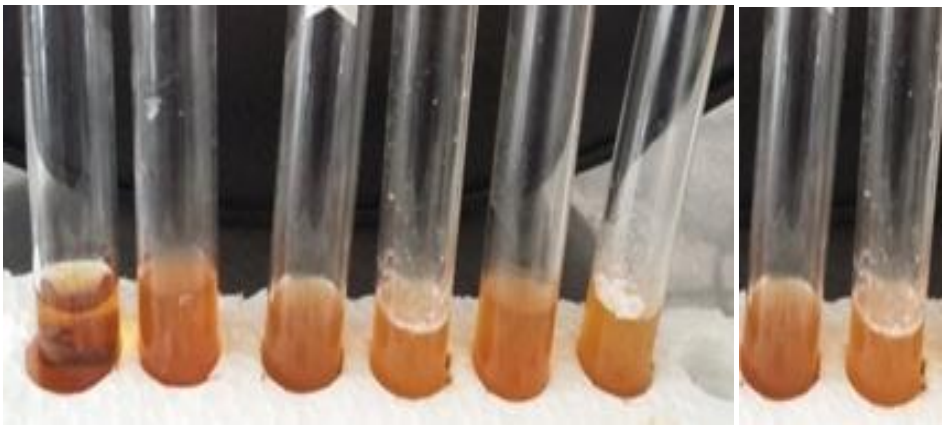
pH tolerance test:-

pH tolerance test measures the capability of bacterial survival in acidic media and the results of pH tolerance study are shown in table 10 and figure 8. The entire test samples i.e. *Lactobacillus* species survived up to pH 3 indicated-by more turbidity of the medium by bacterial growth after 24 and 48 hours respectively. Additionally there was less to moderate turbidity at pH 4 and no turbidity at pH 6, 8 and 10 respectively proved absence of bacterial growth at high pH of the medium. As extreme pH affects the structure of macromolecules, it broke the hydrogen bonds holding together the strands of DNA and hydrolyzed the lipids at basic pH. If H^+ ions are neutralized by hydroxide ions, the concentration gradient of H^+ across the plasma membrane collapses and impairs energy production promoting denaturation and destroying activity thus decreasing of the rate of bacterial growth [20].

Table 10:- Observation of pH tolerance test

SL. No	**Sample code**	**Sample**	**pH**											
			2		**3**		**4**		**6**		**8**		**10**	
			24 hrs	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
1	HCR_1	Cheese/ Raw	+++	+++	+++	++	+	±	-	-	-	-	-	-
2	HCR_2													
3	HCP_1	Cheese/ Processed	+++	+++	+++	++	+	±	-	-	-	-	-	-
4	HCP_2													
5	HDR_1	Curd/ Sweet	+++	+++	+++	++	+	±	-	-	-	-	-	-
6	HDR_2													
7	HDP_1	Curd/ Sour	+++	+++	+++	++	+	±	-	-	-	-	-	-
8	HDP_2													
9	HKP_1	Milk Kefir/ Processed	+++	+++	+++	++	+	±	-	-	-	-	-	-
10	HKP_2													

+++ = more turbid, ++ = moderately turbid, + = less turbid, ± = very less turbid, - = no turbidity



T-1 T-2 T-3 T-4 T-5 T-6 T-7 T-8

Figure 8: pH tolerance test

Casein digestion test:-

Skim milk agar is a differential medium that tests the ability of an organism to produce an exoenzyme, called casease that hydrolyzes the casein into smaller polypeptides, peptides and amino acids that can cross the cell membrane and be utilized by the organisms [21]. The entire test samples in casein digestion test except milk kefir, a clear zone or halo appeared around the areas where the organism had grown proved the presence of *Lactobacillus* species in the samples (Table 11).

Table 11:-Results of casein digestion test

SL. No	Sample code	Sample	Sample type	Reaction with Casein
1	HCR_1	Cheese	Raw	+
2	HCR_2			
3	HCP_1	Cheese	Processed	+
4	HCP_2			
5	HDR_1	Curd	Sweet	+
6	HDR_2			
7	HDP_1	Curd	Sour	+
8	HDP_2			
9	HKP_1	Milk Kefir	Processed	-
10	HKP_2			

+ = clear zone indicating positive result
- = no clear zone indicating negative result

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References:-

1. Degnan, F.H. (2008): The US Food and Drug Administration and probiotics: regulatory categorization. Clin. Infect. Diseases, 46(2): 133–136
2. Roberfroid, M. B. (2000): Prebiotics and probiotics: are they functional foods? American journal of clin. nutrition, 71: 1682-1687.
3. Arora, M.and Baldi, A. (2015): Regulatory categories of probiotics across the globe: A review representing existing and recommended categorization. Indian J. of Med. Microbiol., 33(5): 2-10.
4. Stiles, M. E. and Holzapfel, W. H. (1997): Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol., 36(1):1-29.
5. Rivera-Espinoza, Y. and Gallardo-Navarro, Y. (2010): Non-dairy probiotic products. Food Microbiol., 27(1): 1-11.
6. Greenberg A. E., Trussell R. R. and Clesceri L. S. (1985): Standard methods for the examination of water and wastewater, 16th edition., APHA, Washington, D.C
7. Cunningham, G., Seghrouchni, K., Ruffieux, E., Vaudaux, P., Gayet-Ageron, A., Cherkaoui, A., Godinho, E., Lew, D., Hoffmeyer, P. and Uçkay, I. (2014): Gram and acridine orange staining for diagnosis of septic arthritis in different patient populations. Int. Orthop., 38(6): 1283-90.
8. Wahyuni, A. E. T. H., Tabbu, C. R., Artanto, S., Setiawan, D. C. B. And Rajaguguk, S. I. (2018): Isolation, identification, and serotyping of *Avibacterium paragallinarum* from quails in Indonesia with typical infectious coryza disease symptoms. Vet. World., 11(4):519-524.
9. Ryck, R. D.,Struelens, M. J. and Serruys, E. (1994): Rapid biochemical screening for *Salmonella*, *Shigella*, *Yersinia*, and *Aeromonas* isolates from stool specimens. J. Clin. Microbiol., 32(6): 1583-5.
10. Sheikh, A. F., Yadyad, M. J., Goodarzi, H., Hashemi, S. J., Aslani, S., Assarzadegan, M. A. and Ranjbar, R. (2018): CagA and vacA allelic combination of Helicobacter pylori in gastroduodenal disorders.Microb. Pathog., 20(122): 144-150.
11. Brown, A. and Smith, K. (2015): Benson's microbiological applications, laboratory manual in general microbiology, 13th edition, pp 277.
12. Promon, S. K., Kamal, W., Rahman, S. S., Hossain, M. M. and Choudhury, N. (2018): Bioethanol production using vegetable peels medium and the effective role of cellulolytic bacterial *(Bacillus subtilis)* pre-treatment. Version, 7:271.
13. Aarti, C., Khusro, A., Varghese, R., Arasu, M. V., Agastian, P., Al-Dhabi, N.A., Ilavenil, S. and Choi, K. C. (2018): In vitro investigation on probiotic, anti-candida, and antibiofilm properties of *Lactobacillus pentosus* strain LAP1. Arch. oral. biol., 89: 99-106.
14. Delekta, P. C., Shook, J. C., Lydic, T. A., Mulks, M. H. and Hammer, N. D. (2018): *Staphylococcus aureus* utilizes host-derived lipoprotein particles as sources of exogenous fatty acids. J. Bacteriol., 26:717-28.
15. Fisk, R. T. and Mordvin E. O. (1943): Digestion of casein by *staphylococci* on milk agar containing serum. J. Bacteriol., 46(4): 392–393.
16. Brown, A. and Smith, K. (2015): Benson's Microbiological Applications, Laboratory Manual in General Microbiology. 13th edition, pp 277.
17. Pyar, H. and Peh, K. K. (2014): Characterization and identification of *Lactobacillus acidophilus* using biology rapid identification system. Int. J. Pharm. and Pharm Sci., 6(1): 189-193.
18. Hossain, M. S., Al-Bari, A. and Wahed, M. I. I. (2016): Biochemical characterization of probiotics available in Bangladesh. J. of Sci. Res., 8(1): 101.
19. Hajna, A. A. and C. A. Perry. (1943): Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal *Streptococci*. A. J. of Pub. Health, 33:550-58.
20. Sahadeva, R. P. K., Leong, S. F., Chua, K. H., Tan, C. H., Chan, H.Y., Tong, E.V., Wong, S.Y.W. and Chan, H.K. (2011): Survival of commercial probiotic strains to pH and bile. I. F. Resh. J., 18(4): 1515-1522.
21. Thakur, M., Deshpande, H. M. and Bhate, M. A. (2017): Isolation and identification of lactic acid bacteria and their exploration in non-dairy probiotic drink. I. J. of C. Micro. and Apl. Sci., 6(4):1023 -1030.