ISSN No:-2456-2165

To Investigate the Growth, Viability and Activity of Probiotic Strains in the Product Containing Prebiotics

Shradhdha M Gondaliya*
Department of Microbiology,
Kamani Science and Prataprai Arts College,
Amreli, Gujarat, India

Vimal M Ramani
Dean of Dairy Science College,
Kamdhenu University,
Amreli, Gujarat, India

Abstract:- Prebiotics are the compounds having long fibers structure which are generally stable at normal environmental condition and are used hydrolyzed easily. Now a day, they are highly used with probiotic food as a supplement to probiotic strains. Not only this, studies have shown that prebiotics also protects microorganisms against acidic and gastrointestinal conditions. Hence we have carried out a study to determine the effect of inulin and lactulose on lactic acid producing present in yogurt. For this study we have isolated two potential Lactobacilli from human breast milk. Both the strains were used to prepare yogurt and prepared yogurts were analyzed for viability of strains under storage, low pH and under gastrointestinal conditions. Results of the study have shown that prebiotic enhances the growth of probiotic microorganisms. It also maintains better viability during storage and is efficient in protecting Lactobacilli from harsh conditions of stomach. It was also observed that addition of prebiotic didn't affect the overall activity of probiotic strains.

Keywords:- *Prebiotic; Probiotic Viability; Yogurt; Gastrointestinal Condition.*

I. INTRODUCTION

Prebiotics are generally non digestible fibers which enhances the growth and activity of microorganisms including probiotic microorganism in the food. These dietary fibers can pass through the upper gastrointestinal tract and reached to large intestine where it will provide nutrition to microorganisms. [1]–[8] Marcel Roberfroid has first identified such compounds in 1995. An ideal prebiotic should have following characteristics. It should neither hydrolyze nor absorb in the upper part of the gastrointestinal tract. It should be a selective substrate for one or a few beneficial bacteria commensal to the colon, which stimulate growth and metabolic activity. It should be able to change colonic microflora to favour healthy microbial composition. It should induce luminal or systemic effects which are beneficial to health. [4], [9] Most of the prebiotic compounds are plant based oligosaccharide molecules. Fructooligosaccharides, inulin, galactooligosaccharides, pectin, beta-glucan and xylooligosaccharides are most common prebiotics obtained from various plants. [10]–[14] Lactulose is a synthetic prebiotic which is obtained by isomerization of lactose under alkaline condition. [15], [16] Probiotic strains like Lactobacilli and Bifidobacterium are capable of hydrolyzing these oligosaccharide molecules through a process known as sacchrolysis. Sacchrolysis involves various enzymes depending on the type of oligosaccharide and probiotic microorganisms. Hydrolysis of inulin can produce α-D-glucose, β-D-glucose, β-D-fructofuranose and β-D-fructopyranose. Inulin fructotransferases are the enzymes in this process and produced by probiotic microorganisms. [17] Previous studies have shown that use of prebiotic with in the food has many advantages. When prebiotics and probiotic strains both are added together then it will give synergetic effect. It is found that prebiotic maintains the acidity of food and hence protect probiotic microorganism for lower pH. It also favours the growth of microorganisms. [2], [18]–[20] Not only this, it was also noted that it contributes significantly in appearance, texture and taste of product. Viability which is one of the most vital parameter of probiotic food, prebiotics were also found to promote viability of probiotic strains under various conditions. [21], [22] In many studies it was seen that the viability was not affected for more than 5.0% at the end of storage time of 28 days at 4ºC and even after incubation in gastric juice, viability still remained high. [23]–[25] Prebiotics generally does not affect proteolytic activity of microbial strain. Knowing the advantages of prebiotics, we have also carried out a study in which we have analyzed various parameters of probiotic yogurt produced by us in presence of two prebiotic. We have taken inulin and lactulose as prebiotics. Both this prebiotics are easily available and can be used for various types of probiotic foods. We have carried out our study to determine the effect of prebiotic on three main parameters. These parameters are growth of microorganisms, viability of microorganisms and activity of microorganism. To determine the growth of microorganisms, probiotic strains were grown in presence of inulin (2%) and Lactulose (4%). The concentration of both the prebiotics were taken as per recommendation of previous studies. [26], [27] Probiotic microorganisms were allowed to grow for 96 hrs and viable cells were counted to derive the results. In Viability count, microbial strains were analyzed for viability during storage and during gastrointestinal condition. Probiotic yogurt was stored for 35 days at 4ºC and viable count was calculated each week. For viable count under gastric condition, synthetic condition was applied to the yogurt and remaining cells were counted. Probiotic activity was analyzed by determining acidifying activity of microorganisms grown with and without prebiotics. Obtained results of the study were analyzed and discussed in detail with other similar studies.

ISSN No:-2456-2165

II. METHODOLOGY

Previously isolated strains *Lactobacillus fermentum* MT308789 and *Lactobacilli oris* MY308790 isolated from human breast milk using MRS media was used for the study.

A. Preparation of Yogurt with prebiotics

Yogurt was prepared as per procedure suggested by Kailashpathy et al with slight modifications. The following steps were followed to prepare yogurt. [28], [29]. Before preparing yogurt, yogurt starter microorganisms (*S. thermophilus, L. fermentum* MT308789 and *L. oris* MT308790) were activated in respective media under favourable conditions for 4 hrs. For preparation of yogurt, 18.0 gm of skimmed milk power was dissolved in distilled water was added and heated at 85°C for 20 minutes. It was allowed to cool at 40°C. To this pre-sterile prebiotics agent inulin (final concentration 2%) or lactulose (final concentration 4%) was added. After cooled down 2% volume of active *S. thermophiles* and either of 2% volume of *L. fermentus* MT308789 or *L. oris* MT308790 were inoculated. Incubation was carried out at 42°C until the pH reaches to 4.5

B. Determination of growth

Growth of probiotic microorganisms were determined by growing them in MRS broth for 96 hours and counting the viable count of microorganism on MRS plates. For determination of growth of probiotic in presence of prebiotic, MRS broths were prepared as per standard procedure as described in earlier chapter. Broths were incubated for 24 hrs under anaerobic condition for check the presence of any contamination. After 24 hours, broths were inoculated with L. fermentum MT308789 and L. oris MT308790 in two separate flasks and were allowed to grow at 37ºC under anaerobic condition for 24 hours. After 24 hours, aliquots of freshly grown culture were transferred to another sterile MRS broth till the cell density reaches to 3 X 10^4/mL using spectrophotometer. Flasks were allowed to incubate for 96 hours and viable counts were counted at regular interval of 24 hours. For viable count, 1 ml of active culture was taken and serially diluted in sterile distilled water. From each dilution, MRS plates were spreaded with 50µL of culture suspension and allowed to incubate for 24 hrs at 37ºC. CFU was counted by keeping the dilution in mind and results were presented in form of log CFU/mL.

C. Determination of viability

Viability count was determined to check the effect of storage time on probiotics as well as to check the effect of gastrointestinal juice on probiotics. For determining the effect of storage time on viability, viability of cells during storage was counted for total 35 days. Samples were taken at regular interval of 7 days and proceed as follow. 1.0 gm of yogurt sample was taken and suspended in 100 mL of sterile distilled water. From this suspension, serial dilutions were as per standard procedure and from each tube 50 µL of sample were spreaded on prepared MRS plates. Plates were incubated at 37°C under anaerobic conditions till the visible colonies are obtained. Total microbial count per gram of yogurt was calculated by multiplying with dilution factors.

For determining the effect of gastric juice on viability, an experiment was carried out as per procedure suggested by Gomaa. [30] Both the strains L. fermentus MT308798 and L. oris MT308790 were activated by growing in MRS media for 24 hrs at 37ºC under anaerobic condition. After 24 hrs 5.0 mL of microbial cells were harvested by centrifugation and washed with sterile phosphate-buffered saline solution (pH 7.0). The pellet was collected and resuspended in 500 µL of the same buffer. 100 µL of this microbial suspension was added to 900 µL synthetic gastric juice solution and incubated for 1 and 3 hrs. At the end of incubation 50µL of sample was spreaded on MRS plates and incubated at 37ºC for 24 hrs. Total microbial count was calculated by multiplying with dilution factors results were presented in form of log CFU/mL.

D. Determination of pH

Before measuring the pH of yogurt pH meter was calibrated with standard buffer solutions. pH of each yogurt sample was measure as per standard procedure of pH measurement by pH meter for milk and milk products.[31]

E. Determination of titratable acidity

Titratable acidity was determined as per standard protocol suggested by food safety and standard authority of India (FSSAI). [31]. Titratable acidity as lactic acid was measured for 35 days with interval of 7 days. The following procedure was applied for TA. 10.0 gm of yogurt was taken in a clean glass dish or basin. To that 30.0 ml of warm water was added and mixed properly. To that mixture around 0.5 ml of phenolphthalein was added and mixed thoroughly. Mixture was titrated against 0.1N NaOH. Reaction was considered complete if colour stands for more than 20 seconds.

Calculation

$$\text{Titratable acidity as Lactic acid} = \frac{9 \quad AN}{W}$$

Where,

A = mL of standard NaOH required for titration

N = Normality NaOH solution

W = weight of yogurt taken for test

All experiments were carried out in triplicate and result were represented and Mean±SD.

III. RESULS

A. Determination of growth

When both the probiotic strains were grown in presence of two different probiotics, the following results were obtained. (Table 1 & 2, Figure 1 & 2)

***L. fermentum* MT308789(Log CFU/mL)**			
Incubation Time	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	4.50±0.12	4.56±0.12	4.61±0.12
24	4.40±0.13	4.92±0.16	5.23±0.23
48	4.52±0.13	6.30±0.24	7.90±0.38
72	4.19±0.11	8.10±0.41	9.70±0.51
96	3.62±0.11	6.42±0.21	8.20±0.34

Table 1:- Growth of L. fermentum MT308789 in presence of various prebiotics

***L. oris* MT308790 (Log CFU/mL)**			
Incubation Time	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	4.43±0.12	4.49±0.14	4.53±0.14
24	4.46±0.13	4.76±0.19	4.93±0.28
48	4.54±0.13	6.12±0.28	7.54±0.31
72	4.12±0.11	7.86±0.39	9.36±0.46
96	3.54±0.11	6.19±0.19	7.91±0.31

Table 2:- Growth of L. oris MT308790 in presence of various prebiotics

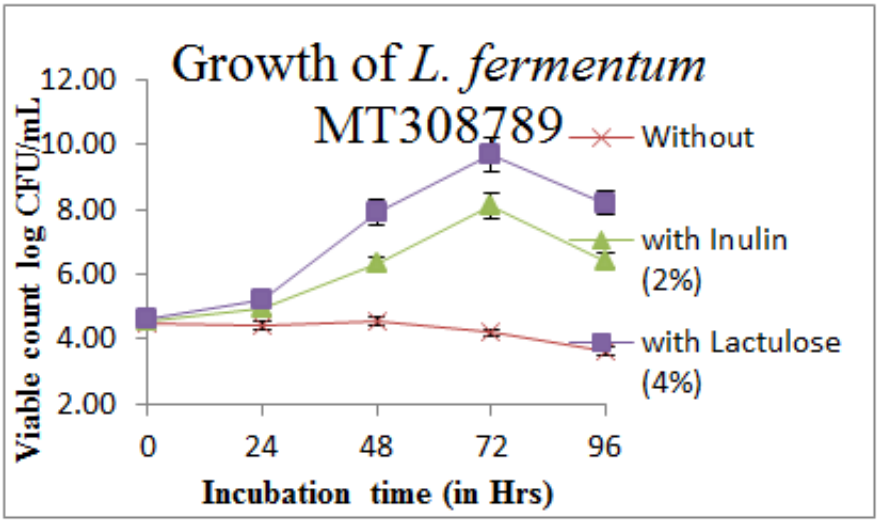

Fig 1:- Growth of L. fermentum MT308789 in presence of various prebiotics

ISSN No:-2456-2165

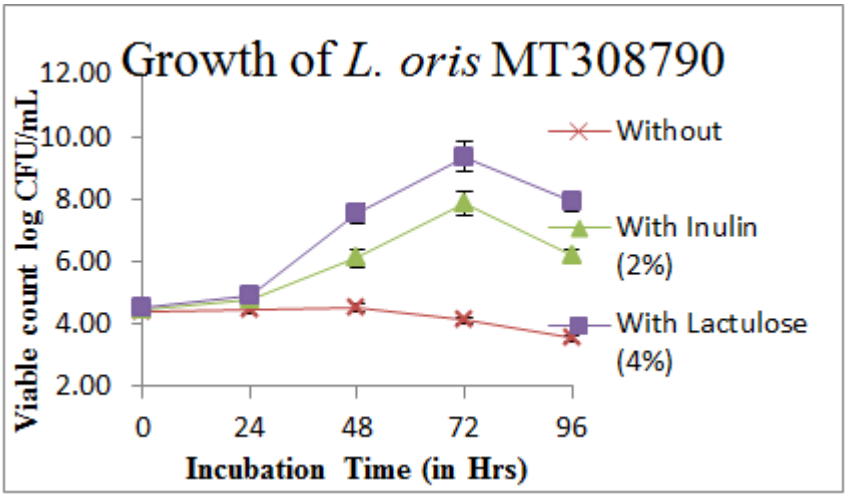

Fig 2:- Growth of L. oris MT308790 in presence of various prebiotics

Results clearly indicates that prebiotic do not have any significant effect in the initial phase of growth. This may be because of other nutrient molecules present in the growth media. After 24 hrs of growth, cultures without any prebiotic have shown very less growth. Microbes have reached to in the stationary phase. Whereas cultures with probiotics were still in their log phase and showed good growth. Growth of these culture were continues upto 72 hrs and after that they have shown decline pattern of growth. It indicated that, addition of prebiotic can provide energy to the probiotic microorganisms for longer duration and keep them alive. Probiotic microorganisms produce certain hydrolyzing enzymes which degrade these prebiotics and used them as source of energy. [15], [32], [33]

B. Determination of viability

As described earlier, viability is one of the most important criteria for any probiotic food. This viability is affected during the storage of food and interacting with gastrointestinal juice and bile juice. In this study effect during storage and after interacting with gastrointestinal juice were studied. Obtained results are noted as mentioned in the table below. (Table 3 to 6, Figure 3 & 4)

***L. fermentum* MT308789 (No of cells X107/gm)**			
Storage time in days	**Types of yogurts**		
	Without	With Inulin (2%)	With Lactulose (4%)
0	8.32±0.23	8.28±0.31	8.34±0.39
7	7.21±0.21	7.69±0.28	7.76±0.34
14	6.64±0.18	6.98±0.29	7.15±0.29
21	5.42±0.17	5.78±0.25	6.18±0.21
28	4.86±0.15	5.13±0.21	5.77±0.28
35	4.55±0.16	4.92±0.18	5.18±0.22

Table 3:- Viability of L. fermentum MT308789 during storage at 4ºC

***L. oris* MT308790 (No of cells X107/gm)**			
Storage time in days	**Types of yogurts**		
	Without	With Inulin (2%)	With Lactulose (4%)
0	7.98±0.19	8.07±0.28	8.14±0.34
7	7.14±0.17	7.48±0.32	7.61±0.31
14	6.42±0.21	6.89±0.30	7.01±0.32
21	5.21±0.18	5.68±0.26	5.93±0.29
28	4.78±0.16	5.14±0.22	5.57±0.27
35	4.46±0.14	4.81±027	5.12±0.30

Table 4:- Viability of L. oris MT308790 during storage at 4ºC

ISSN No:-2456-2165

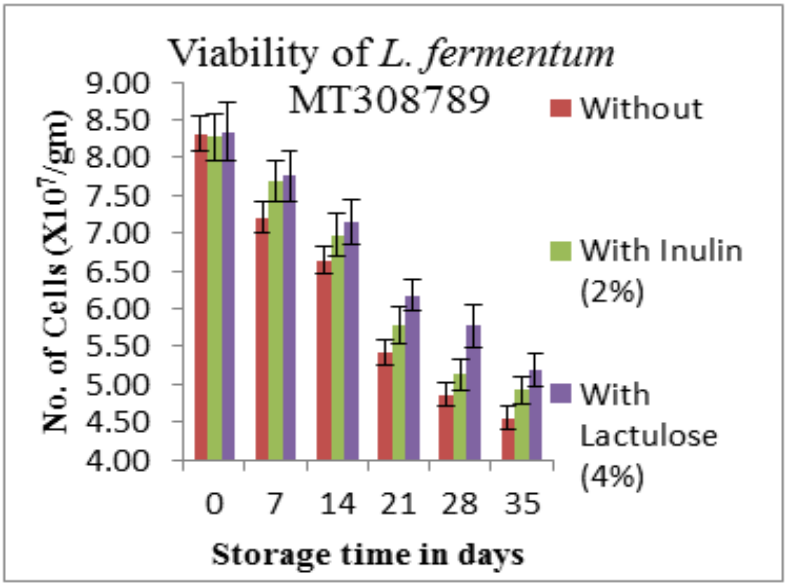

Fig 3:- Viability of L. fermentum MT308789 during storage at 4ºC

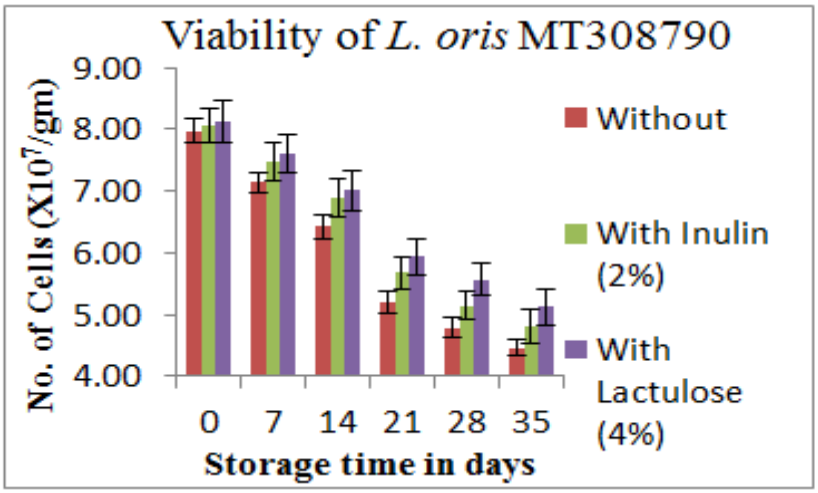

Fig 4:- Viability of L. oris MT308790 during storage at 4ºC

From the obtained results of viable count during storage at 4 ºC, it was noted that viability pattern with or without prebiotic remains the same. But the total viable count remains high in the yogurt which contains inulin and Lactulose. Data also reveal that, at the end of 35 days of storage, yogurts with prebiotics has 10.0% - 15.0% higher numbers of viable cells as compare to the yogurt without any prebiotics. It was also noted that after 14 days of incubation, viability reduces significantly. This might be because of production of certain acidic molecules during storage, resulted into lowering down the pH and destroying the probiotic microorganisms. [34]–[36]

C. Effect on viability after interacting with gastrointestinal juice

Any probiotic food consumed has to pass through the harsh condition of stomach. Stomach carried gastric juice and bile juice which are essential for digestion of food. Presence of this mixture generates highly acidic condition which destroys microorganisms quickly. Certain prebiotics are known to protect microorganisms against this condition. Here when both the probiotic strains were tested for viability by synthetic gastric juice, following results were obtained.

***L. fermentum* MT308789 (log CFU/mL)**			
Interaction time in Hrs	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	8.32±0.35	8.28±0.42	8.34±0.34
1	6.89±0.24	7.11±0.26	7.17±0.15
3	4.54±0.19	6.42±0.31	6.57±0.21

Table 5:- Viability of L. fermentum MT308789 after interaction with gastric juice

L. oris MT308790 (log CFU/mL)			
Interaction time in hrs	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	7.98±0.28	8.07±0.17	8.14±0.09
1	5.12±0.14	6.89±0.26	6.93±0.16
3	4.23±0.08	5.92±0.24	6.09±0.26

Table 6:- Viability of L. oris MT308790 after interaction with gastric juice

From the above data, it can be interpreted that microorganisms of the probiotic yogurt without any prebiotic are very susceptible to gastrointestinal juice and lost viability rapidly. At the end of 1 hour of interaction around 30.0% populations die and at the end of 3 hours of interaction, more than 50.0% populations have died. In contrast to this, when yogurts with prebiotic were studies, they showed very higher cell viability as compare to previous one. At the end of 3 hrs of interaction only 25.0% - 30.0% populations have died. This clearly indicated that prebiotic has potential to protect probiotic strains against certain harsh environmental conditions. [37]–[39]

➢ *Determination of probiotic activity*

One of the most reliable and easy method to check the activity of probiotic microorganisms is determining their post acidification ability. [40], [41] If probiotic microorganisms are alive and active than it will definitely perform some activity and produces certain acidic products which increases the acidity of the product and lower down the pH of product. Lactic acid is among the most dominant product which is produced. [13], [42] Acidification can be determined by measuring the pH and through titration. [13], [43], [44]

Here when the pH of yogurts with and without prebiotic was measured for 35 days, following results were obtained. (Table 7 & 8, Figure 5 & 6)

L. fermentum MT308789			
Storage time in days	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	4.43±0.10	4.44±0.09	4.42±0.10
7	4.38±0.08	4.36±0.07	4.37±0.09
14	4.36±0.09	4.32±0.06	4.35±0.08
21	4.34±0.06	4.29±0.06	4.31±0.07
28	4.30±0.04	4.27±0.05	4.28±0.06
35	4.24±0.05	4.20±0.06	4.22±0.06

Table 7:- Change in pH of yogurt prepared using L. fermentum MT308789 during storage

L. oris MT308790			
Storage time in days	**Types of yogurts**		
	Without	**With Inulin (2%)**	**With Lactulose (4%)**
0	4.41±0.12	4.44±0.13	4.39±0.11
7	4.38±0.10	4.35±0.10	4.35±0.10
14	4.33±0.08	4.29±0.09	4.32±0.08
21	4.30±0.07	4.25±0.08	4.27±0.09
28	4.27±0.08	4.22±0.07	4.23±0.06
35	4.23±0.06	4.18±0.06	4.19±0.04

Table 8:- Change in pH of yogurt prepared using L. oris MT308790 during storage

ISSN No:-2456-2165

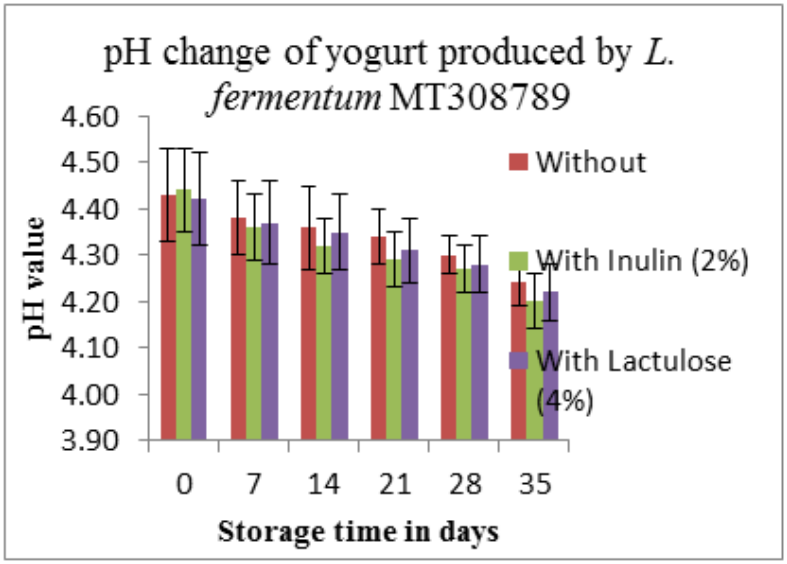

Fig 5:- Change in pH of yogurt prepared using L. fermentum MT308789 during storage

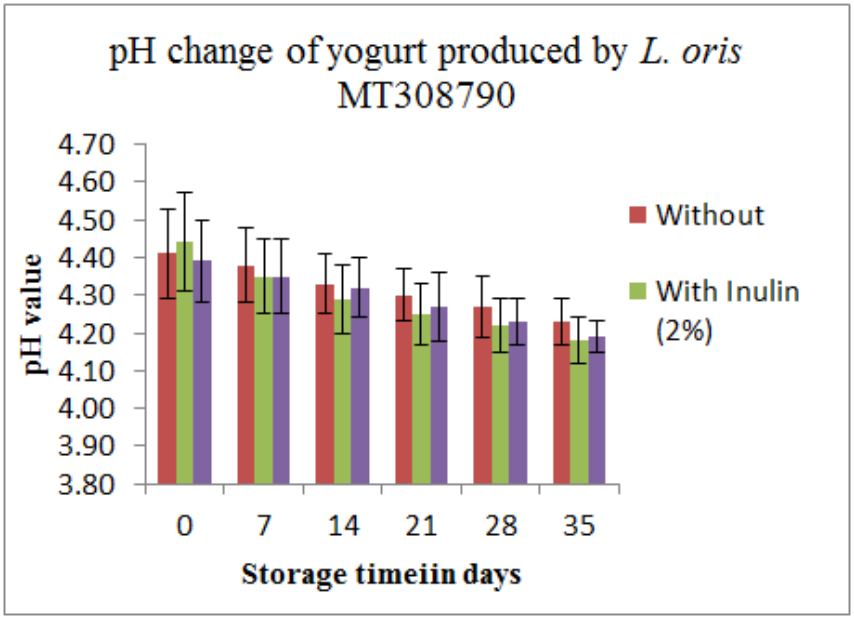

Fig 6:- Change in pH of yogurt prepared using L. oris MT308790 during storage

Based on the results obtained for pH change during the storage at 4ºC, it was observed that all yogurts posses some metabolic activity. When the metabolic activity of yogurts having prebiotic were compared to plain yogurt, it was seen that the metabolic activity is high resulting into production of acidic molecules especially lactic acid. This results into reduction in pH of yogurt. This indicates that probiotic microorganisms are still working and metabolically active. Another method which was used for determination of activity was titratable acidity. [45], [46] Results of titratable acidity are as below. (Table 9 & 10, Figure 7 & 8)

***L. fermentum* MT308789**			
Storage time in days	**Types of yogurts**		
	Without	With Inulin (2%)	With Lactulose (4%)
0	0.62±0.05	0.60±0.06	0.61±0.06
7	0.66±0.06	0.69±0.05	0.68±0.06
14	0.71±0.09	0.75±0.06	0.73±0.07
21	0.78±0.08	0.83±0.07	0.84±0.08
28	0.82±0.10	0.89±0.09	0.88±0.10
35	0.89±0.11	0.93±0.09	0.94±0.10

Table 9:- Change in titratable acidity of yogurt prepared using L. fermentum MT308789 during storage

***L. oris* MT308790**			
Storage time in days	**Types of yogurts**		
	Without	With Inulin (2%)	With Lactulose (4%)
0	0.59±0.04	0.61±0.05	0.63±0.06
7	0.63±0.06	0.65±0.06	0.69±0.08
14	0.68±0.08	0.71±0.06	0.73±0.07
21	0.72±0.07	0.75±0.06	0.76±0.08
28	0.79±0.08	0.84±0.07	0.86±0.09
35	0.86±0.11	0.91±0.10	0.92±0.08

Table 10:- Change in titratable acidity of yogurt prepared using L. oris MT308790 during storage

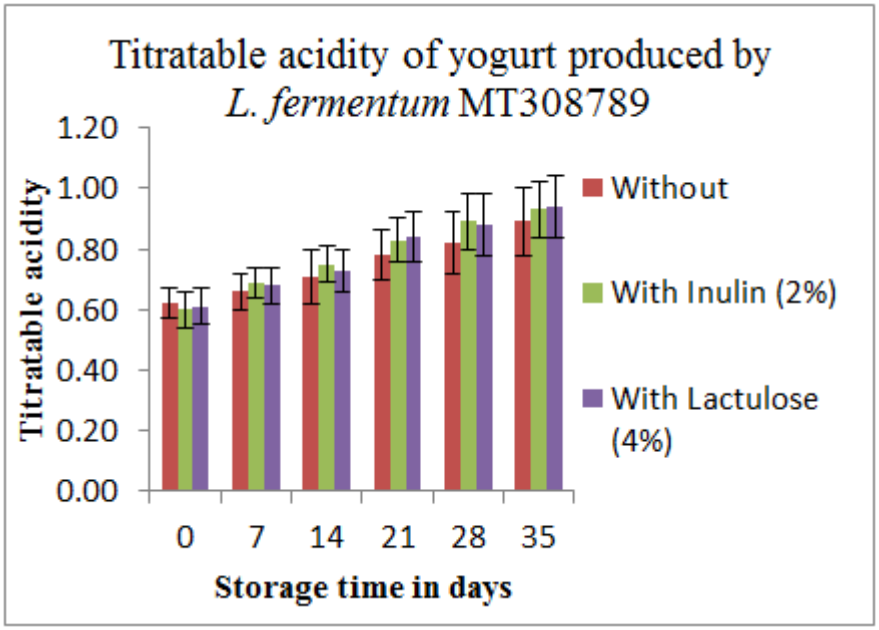

Fig 7:- Change in titratable acidity of yogurt prepared using L. fermentum MT308789 during storage

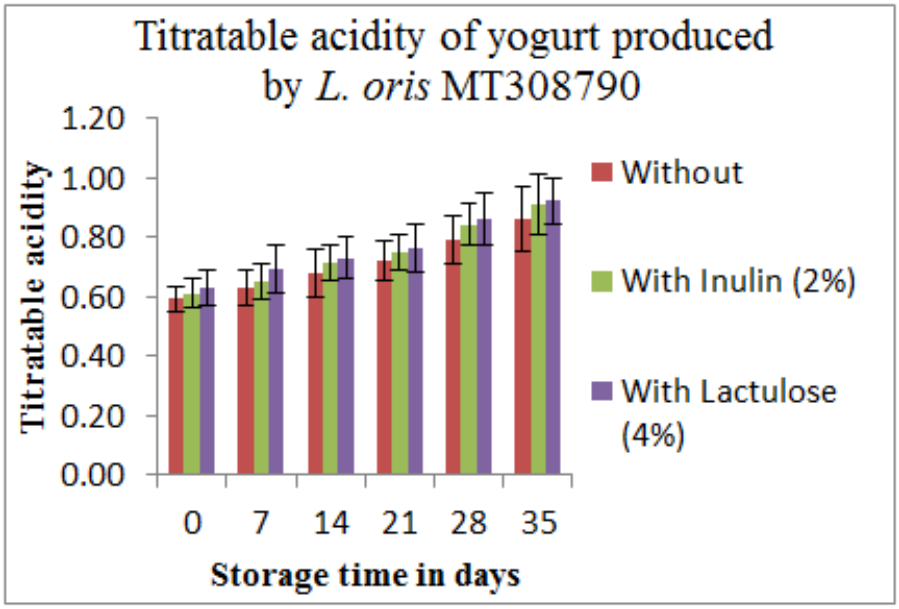

Fig 8:- Change in titratable acidity of yogurt prepared using L. oris MT308790 during storage

Obtained results have clearly indicated that during storage production of lactic acid was higher in the yogurts having prebiotics as compared to plain yogurts. This is another indication of active probiotic strain in the yogurts. [35], [46], [47]

IV. DISCUSSION

Prebiotic molecules are performing an important function of selective stimulation of intestinal bacteria, especially Lactobacilli and Bifidobacterium. Prebiotics are very stable and is not degraded by gastric enzymes in the small intestine. [7], [9] Instead of this they will be fermented by probiotic bacteria in the large intestine. Fermentation of prebiotics produced short-chain fatty acids (SCFA), mainly lactate, acetate, propionate, and butyrate and these compounds decreases pH of product. Too much production of these molecules possibly reduced the numbers of pathogenic microorganisms. [37], [48], [49] Studies have shown that fermentation of prebiotics by the probiotic bacteria enhances person's health by improving absorption of minerals such as Mg, Ca and Fe. [9], [49] Studies have also shown that prebiotic produces such compounds which can prevent colon cancer and other similar cancers. [18], [50]–[52]

Based on the atomic structure and chemical properties, current probiotics can be classified in three main categories. These categories are saccharide/sugar derivatives, proteins/peptides, and lipids. [3], [53], [54] Most preferred prebiotics used in food are saccharide derivative. Most of these saccharides are derivatives obtained from plants. Oligosaccharides (gluco-, galacto-, fructo-, isomalto-, xylo), inulin, lactulose, lactosucrose, resistant starch, pectin, guar gum, and chitosan are examples of such prebiotic molecules. [26], [32], [55] These molecules are obtained from cereals, legume, barley, wheat, chickpea, lentils, chicory, Jerusalem artichoke, onion, garlic, okra, leek, dragon fruit, jack fruit, palm fruit, nectarine and mushroom. [4], [16], [37], [56]–[58] When food is prepared using this kind of plants it may have natural contain prebiotics properties or else it can be incorporated by adding prebiotics at the time of production. Food which posses both prebiotics as well as probiotics together then this kind of food is known as symbiotic. It can be defined as combination of prebiotics and probiotics. [3], [56], [59], [60] This provides nutritional supplement with surety to added health benefits. It is known that food having both prebiotic and probiotics are able to tolerate oxygen, low pH, and unfavorable temperature. [61]–[63] Not only this, it also helps in keeping the probiotic microorganisms alive in the digestive system. [64], [65] Nowadays, prebiotics are highly used in foods, especially in dairy products. [21], [66]

Till now many studies have been carried out to analyze effect of various prebiotic compounds in the food. Most of the studies have focused on the growth of probiotics, activity of probiotics and viability of probiotic in presence of prebiotic compound. In a study of Yeo and Liong, effect of prebiotics on probiotics in soymilk was carried out. They have studied growth pattern and viability of probiotic microorganisms. At the end of the study they have concluded that addition of prebiotic enhances the growth of probiotic and also improve viability. They have used fructooligosaccharide, mannitol, maltodxtrin and pectin and got the same results with all prebiotics. [33] In our study also we have found similar kind of results. Both the probiotic strains have shown better growth and viability in presence of inulin and Lactulose. Similar results were obtained by Mishra and Mishra for inulin and fructooligosaccharide with soymilk. They have also added that prebiotic also improves the quality of product in term of texture, rheology and sensory characteristics. [46] Gustaw et al also observed the same outcome when they studied various prebiotic on bioyogurts. Even when lactulose was used as prebiotic in fermented milk similar result was obtained. The only difference is the concentration of lactulose should be used is higher than the inulin. [67] Gomma has studied the effect of various prebiotic on the fatty acid profile of *L. brevis* where he found fatty acid profile of the probiotic microorganism can be affect by various prebiotic molecules. He also noted that addition of inulin in the medium increase the level of unsaturated fatty acids which has certain health benefits. [30] When Oliveira et al tried to use more than one Lactobacilli strains as probiotic in cocktail form with fermented skim milk using inulin as prebiotic, they have found that multiple strains has less growth rate as compare to pure strains. So they have recommended to use any one probiotic microorganisms along with *S. thermophilus* for fermentation of milk. [27] Figueroa-Gonzalez et al have carried out a study to determine the actual effect of prebiotic in probiotic food using commercial saccharide. Based on their study they have recommended that actually prebiotic activity can be better understand by quantitative prebiotic parameter like prebiotic index and prebiotic activity score as compare to observing the utilization of carbohydrates. [32] Harti et al has prepared chito-oligosaccharide as used as a natural preservative for preservation of tofu. Their detailed study has revealed that 2.0% concentration of chito-oligosaccharide can preserve tofu without affecting its natural properties. [68] When Sendra et al used citrus fruit fibers as prebiotic in fermented milk; they have observed that citrus fibers enhance the survival of probiotic microorganism during storage under refrigeration. Not only this, it has also enhanced the growth of probiotics. Based on their study they suggested that citrus fruit fibers should be used as a vehicle for probiotic in variety of food products. [69] Minj and Vij have taken inulin as a prebiotic and optimized its concentration for best results. They have shown that inulin definitely have positive effects on growth, viability and activity. They have shown that addition of excess concentration inulin didn't have any additional effect. They have recommended to use maximum 2.0% - 3.0% of inulin which gives best results. [70] Study of Mumcu and Temiz have shown that addition of prebiotic didn't have any negative effect on probiotics during storage at low temperature. Rather it promotes metabolic activity by probiotic strains which results in the higher titratable acidity and power the pH value. [71] Same observation was made by Fornelli et al, they have observed

that titratable acidity was increased significantly after 7 days of storage. Even they also noted that product without probiotics and prebiotics have comparatively less acidic pH than the production have probiotics and prebiotics. [13] This may be because of higher metabolic rate of probiotic in present of prebiotic. Studies have shown that probiotics leads to production of short chain fatty acid which results into pH. This inhibits the growth of certain pathogenic microorganisms in the gut in a way protecting human from several pathogenic disease. [7], [34], [48]

Prebiotics are known to have various functions. These includes calcium and other mineral absorption, enhance immunity, reduced cholesterol and triacylglycerol, destroying pathogenic microorganisms, maintain balanced microbiota, reduction of colon cancer, lowering down bowel inflammation and reduction in allergic symptoms. [43], [49] World gastroenterological organization has issued global guidelines for probiotics and prebiotics. They have mentioned about clinical application of prebiotics in colorectal cancer, diarrhea, hepatic encephalopathy, ulceratice colitis, Crohn's disease, lactose malabsorption, nectrotizing enterocolitis and fatty liver. [72] George et al has studied the effect of prebiotic and probiotic on chronic kidney disease. They have observed that use of appropriate prebiotic and probiotic can reduce production of certain uremic toxins like creatinine, nitrogen, uric acid in case of chronic kidney disease. [73] Ferrarese et al have carried out an experiment where they have studied the effect of prebiotics on weight loss and metabolic syndrome. From entire study, they have concluded that for each prebiotic dose dependent effect and length of probiotic should be standardized to reduce excess weight and enhance metabolism. [74] Orel and Rebersak have studied the effect of prebiotics to pediatric population for constipation, poor weight gain and eczema. They have observed benefit effect of prebiotics in the children having complains of constipation, poor weight gain or eczema. They have suggested incorporation of prebiotics in the ORS for rapid and easy supplementation. [75] Indrio et al have studied the effect of prebiotic on gastrointestinal motility of newborn. In the study glucooligosaccharide and fructooligosaccharide in ratio of 9:1 was given to newborns for 30 days and various clinical parameters were studied. Results of the study revealed that supplementation of prebiotic may stimulate gastric emptying and improve maturation of the EGG activity. [76] Recent research in prebiotics has focused on use of biotechnology to enrich the plants with prebiotic molecules naturally. An attempt was made by Dwivedi et al to breed prebiotic rich nutritious food crops. They have suggested to use genetic based approach for production of plants which better quality of prebiotics. [57]

V. CONCLUSION

Based on the entire study for prebiotic carried out here and from the obtained results, it can be concluded that addition of prebiotic molecules surely enhance probiotic microorganisms growth and viability. They will also keep them active during storage at low temperature. Not only this, they will also protect them against harsh conditions of gastrointestinal juice. Even it also keeps the microorganisms potentially active during storage.

REFERENCES

[1]. E. M. Dewulf, P. D. Cani, S. P. Claus, S. Fuentes, P. G. B. Puylaert, A. M. Neyrinck, L. B. Bindels, W. M. De Vos, G. R. Gibson, J. P. Thissen, and N. M. Delzenne, "Insight into the prebiotic concept: Lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women," *Gut*, vol. 62, no. 8, pp. 1112–1121, 2013.

[2]. V. Singla and S. Chakkaravarthi, "Applications of prebiotics in food industry: A review," *Food Sci. Technol. Int.*, vol. 23, no. 8, pp. 649–667, 2017.

[3]. D. M. Gatlin III and A. M. Peredo, "Prebiotics and Probiotics : Definitions and Applications," *South. Reg. Aquauclture Cent.*, no. 4711, pp. 1–8, 2012.

[4]. P. L. Conway, "Prebiotics and human health: The state-of-the-art and future perspectives," *Scand. J. Nutr.*, vol. 45, no. 1, pp. 13–21, 2001.

[5]. A. Choudhari, S. Shinde, and B. N. Ramteke, "Prebiotics and probiotics as health promoter," *Vet. World*, vol. 1, no. 2, pp. 59–61, 2008.

[6]. P. Markowiak and K. Ślizewska, "Effects of probiotics, prebiotics, and synbiotics on human health," *Nutrients*, vol. 9, no. 9, 2017.

[7]. L. B. Bindels, N. M. Delzenne, P. D. Cani, and J. Walter, "Towards a more comprehensive concept for prebiotics," *Nat. Publ. Gr.*, pp. 1–8, 2015.

[8]. S. H. Al-Sheraji, A. Ismail, M. Y. Manap, S. Mustafa, R. M. Yusof, and F. A. Hassan, "Prebiotics as functional foods: A review," *J. Funct. Foods*, vol. 5, no. 4, pp. 1542–1553, 2013.

[9]. T. Manigandan, S. P. Mangaiyarkarasi, R. Hemalatha, V. T. Hemalatha, and N. P. Murali, "Probiotics, Prebiotics and Synbiotics - A Review," *Biomed. Pharmacol.*, vol. 5, no. 2, pp. 295–304, 2012.

[10]. G. Fan, J. Chang, Q. Yin, X. Wang, and X. Dang, "Effects of probiotics, oligosaccharides, and berberine combinations on growth performance of pigs," *Turkish J. Vet. Anim. Sci.*, vol. 39, no. 6, pp. 637–642, 2015.

[11]. M. a. Underwood, "Probiotics and human milk oligosaccharides in premature infants," *Neoreviews*, vol. 20, no. 1, pp. e1–e11, 2019.

[12]. T. Revathy, S. Mythili, and A. Sathiavelu, "Assessing The Growth Of Probiotic Bacteria In Selected Prebiotic Foods Rich In Oligosaccharides," *Int. J. Appl. Biol. Pharm. Technol.*, vol. 2, no. 1, pp. 483–487, 2011.
[13]. A. R. Fornelli, N. S. Bandiera, M. D. R. Costa, C. Hoch, and B. De Souza, "Effect of inulin and oligofructose on the physicochemical, microbiological and sensory characteristics of symbiotic dairy beverages," *Semin. Agrar.*, vol. 35, no. 6, pp. 3099–3112, 2014.
[14]. S. Lockyer and S. Stanner, "Prebiotics – an added benefit of some fibre types," *Nutr. Bull.*, vol. 44, no. 1, pp. 74–91, 2019.
[15]. R. Pinheiro, D. S. Oliveira, A. Carolina, R. Florence, and P. Perego, "Use of lactulose as prebiotic and its influence on the growth , acidification profile and viable counts of different probiotics in fermented skim milk," *Int. J. Food Microbiol.*, vol. 145, pp. 22–27, 2011.
[16]. S. Wichienchot, P. Thammarutwasik, A. Jongjareonrak, W. Chansuwan, P. Hmadhlu, T. Hongpattarakere, A. Itharat, and B. Ooraikul, "Extraction and analysis of prebiotics from selected plants from southern Thailand," *Songklanakarin J. Sci. Technol.*, vol. 33, no. 5, pp. 517–522, 2011.
[17]. T. Barclay, M. Ginic-markovic, M. R. Johnston, P. D. Cooper, and N. Petrovsky, "Analysis of the hydrolysis of inulin using real time 1 H NMR spectroscopy," *Carbohydr. Res.*, vol. 352, pp. 117–125, 2012.
[18]. N. Khorshidian, M. Y. Asli, H. Hosseini, M. Shadnoush, and A. M. Mortazavian, "Potential anticarcinogenic effects of lactic acid bacteria and probiotics in detoxification of process-induced food toxicants," *Int. J. Cancer Manag.*, vol. 9, no. 5, 2016.
[19]. H. Majamaa and E. Isolauri, "Probiotics: A novel approach in the management of food allergy," *J. Allergy Clin. Immunol.*, vol. 99, no. 2, pp. 179–185, 1997.
[20]. D. Song, S. Ibrahik, and S. Hayek, "Recent application of probiotics in food and agricultural science," in *Probiotics*, 2012, pp. 1–34.
[21]. N. Jose, C. Bunt, and M. Hussain, "Comparison of Microbiological and Probiotic Characteristics of Lactobacilli Isolates from Dairy Food Products and Animal Rumen Contents," *Microorganisms*, vol. 3, no. 2, pp. 198–212, 2015.
[22]. W. H. Holzapfel, P. Haberer, R. Geisen, J. Björkroth, and U. Schillinger, "Taxonomy and important features of probiotic microorganisms in food and nutrition," *Am. J. Clin. Nutr.*, vol. 73, no. 2 SUPPL., 2001.
[23]. A. M. Mahasneh, S. Hamdan, and S. A. Mahasneh, "Probiotic Properties of Lactobacillus Species Isolated from Local Traditional Fermented Products," *Jordan J. Biol. Sci.*, vol. 8, no. 2, pp. 81–87, 2015.
[24]. M. Pamela, "Probiotics: Are they worth taking?," *Pharm. J.*, vol. 278, no. 7445, pp. 373–376, 2007.
[25]. E. Y. Ann, Y. Kim, S. Oh, J. Y. Imm, D. J. Park, K. S. Han, and S. H. Kim, "Microencapsulation of Lactobacillus acidophilus ATCC 43121 with prebiotic substrates using a hybridisation system," *Int. J. Food Sci. Technol.*, vol. 42, no. 4, pp. 411–419, 2007.
[26]. D. Banerjee, R. Chowdhury, and P. Bhattacharya, "the prebiotic influence of inulin on growth rate and antibiotic sensitivity," *Int. J. Pharm. Pharm. Sci.*, vol. 8, no. 4, pp. 181–184, 2016.
[27]. R. P. D. S. Oliveira, P. Perego, M. Nogueira, D. Oliveira, and A. Converti, "Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk," *LWT - Food Sci. Technol.*, vol. 44, no. 2, pp. 520–523, 2011.
[28]. A. Talwalkar and K. Kailasapathy, "A Review of Oxygen Toxicity in Probiotic Yogurts: Influence on the Survival of Probiotic Bacteria and Protective Techniques," *Food Sci. Food Saf.*, vol. 3, no. 3, pp. 117–124, 2004.
[29]. K. Kailasapathy, I. Harmstorf, and M. Phillips, "Survival of Lactobacillus acidophilus and Bifidobacterium animalis ssp. lactis in stirred fruit yogurts," *LWT - Food Sci. Technol.*, vol. 41, no. 7, pp. 1317–1322, 2008.
[30]. E. Z. Gomaa, "Effect of Prebiotic Substances on Growth , Fatty Acid Profile and Probiotic Characteristics of Lactobacillus brevis NM101-1 1," *Microbiology*, vol. 86, no. 5, pp. 618–628, 2017.
[31]. Food Safety and Standards Authority of India, *Lab Manual - Manual of methods of analysis of foods*. 2015.
[32]. I. Figueroa-gonzález, G. Rodríguez-serrano, L. Gómez-ruiz, M. García-garibay, and A. Cruz-guerrero, "Prebiotic effect of commercial saccharides on probiotic bacteria isolated from commercial products," *Food Sci. Technol.*, vol. 2061, no. 3, pp. 747–753, 2019.
[33]. S. Yeo and M. Liong, "Effect of prebiotics on viability and growth characteristics of probiotics in soymilk," *J. Sci. Food Agric.*, vol. 90, pp. 267–275, 2010.
[34]. R. Karimi, A. M. Mortazavian, and A. G. Da Cruz, "Viability of probiotic microorganisms in cheese during production and storage: A review," *Dairy Sci. Technol.*, vol. 91, no. 3, pp. 283–308, 2011.
[35]. M. K. Tripathi and S. K. Giri, "Probiotic functional foods: Survival of probiotics during processing and storage," *J. Funct. Foods*, vol. 9, no. 1, pp. 225–241, 2014.
[36]. D. E. P. Sumalapao, J. A. R. T. Mesina, E. C. Cabrera, and N. G. Gloriani, "Viability kinetics of Lactobacillus casei Shirota strain in a commercial fermented milk drink during refrigerated storage," *Natl. J. Physiol. Pharm. Pharmacol.*, vol. 7, no. 11, pp. 1242–1246, 2017.
[37]. O. Ozcan, T. Ozcan, L. Yilmaz-ersan, A. Akpinar-bayizit, and B. Delikanli, "The Use of Prebiotics of Plant Origin in Functional Milk Products," *Food Sci. Technol.*, vol. 4, no. 2, pp. 15–22, 2016.
[38]. A. Mohammad, R. Mohammadi, and S. Sohrabvandi, "Delivery of Probiotic Microorganisms into Gastrointestinal Tract by Food Products," *New Adv. Basic Clin. Gastroenterol.*, 2012.

[39]. R. Ghasemnezhad, V. Razavilar, H. Pourjafar, K. Khosravi-Darani, and K. Ala, "The viability of free and encapsulated lactobacillus casei and bifidobacterium animalis in chocolate milk, and evaluation of its pH changes and sensory properties during storage," *Annu. Res. Rev. Biol.*, vol. 21, no. 3, pp. 1–8, 2017.
[40]. F. Sarvari, a. M. Mortazavian, and M. R. Fazeli, "Biochemical characteristics and viability of probiotic and yogurt bacteria in yogurt during the fermentation and refrigerated storage," *Appl. Food Biotechnol.*, vol. 1, no. 1, pp. 55–61, 2014.
[41]. A. Terpou, A. Papadaki, I. K. Lappa, V. Kachrimanidou, L. a. Bosnea, and N. Kopsahelis, "Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value," *Nutrients*, vol. 11, no. 7, 2019.
[42]. A. G. Cruz, J. A. F. Faria, E. H. M. Walter, R. R. Andrade, R. N. Cavalcanti, C. a. F. Oliveira, and D. Granato, "Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology," *J. Dairy Sci.*, vol. 93, no. 11, pp. 5059–5068, 2010.
[43]. T. Turgut and S. Cakmakci, "Probiotic Strawberry Yogurts: Microbiological, Chemical and Sensory Properties," *Probiotics Antimicrob. Proteins*, vol. 10, no. 1, pp. 64–70, 2018.
[44]. F. Tamjidi, a. Nasirpour, and M. Shahedi, "Physicochemical and sensory properties of yogurt enriched with microencapsulated fish oil," *Food Sci. Technol. Int.*, vol. 18, no. 4, pp. 381–390, 2012.
[45]. R. Ferdousi, M. Rouhi, R. Mohammadi, A. M. Mortazavian, K. Khosravi-Darani, and A. H. Rad, "Evaluation of probiotic survivability in yogurt exposed to cold chain interruption," *Iran. J. Pharm. Res.*, vol. 12, no. SUPPL., pp. 137–142, 2013.
[46]. S. Mishra and H. N. Mishra, "Comparative study of the synbiotic effect of inulin and fructooligosaccharide with probiotics with regard to the various properties of fermented soy milk," *Food Sci. Technol. Int.*, vol. 24, no. 7, pp. 564–575, 2013.
[47]. E. Mani-López, E. Palou, and a. López-Malo, "Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria," *J. Dairy Sci.*, vol. 97, no. 5, pp. 2578–2590, 2014.
[48]. L. J. Fooks and G. R. Gibson, "In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens," *FEMS Microbiol. Ecol.*, vol. 39, pp. 67–75, 2002.
[49]. P. M. Rolim, "Development of prebiotic food products and health benefits," *Food Sci. Technol.*, vol. 35, no. 1, pp. 3–10, 2015.
[50]. D. Gayathri and B. S. Rashmi, "Anti-Cancer Properties of Probiotics: A Natural Strategy for Cancer Prevention," *EC Nutr.*, vol. 5, no. 4, pp. 1191–1202, 2016.
[51]. M. Raman, P. Ambalam, K. K. Kondepudi, S. Pithva, C. Kothari, A. T. Patel, R. K. Purama, J. M. Dave, and B. R. M. Vyas, "Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer," *Gut Microbes*, vol. 4, no. 3, pp. 181–192, 2013.
[52]. D. Gayathri, Asha, and T. N. Devaraja, "Lactobacillus sp. as probiotics for human health with special emphasis on colorectal cancer," *Indian J. Sci. Technol.*, vol. 4, no. 8, pp. 1008–1014, 2011.
[53]. W. Sybesma, R. Kort, and Y. K. Lee, "Locally sourced probiotics, the next opportunity for developing countries?," *Trends Biotechnol.*, vol. 33, no. 4, pp. 197–200, 2015.
[54]. B. Petschow, J. Doré, P. Hibberd, T. Dinan, G. Reid, M. Blaser, P. D. Cani, F. H. Degnan, J. Foster, G. Gibson, J. Hutton, T. R. Klaenhammer, R. Ley, M. Nieuwdorp, B. Pot, D. Relman, A. Serazin, and M. E. Sanders, "Probiotics, prebiotics, and the host microbiome: The science of translation," *Ann. N. Y. Acad. Sci.*, vol. 1306, no. 1, pp. 1–17, 2013.
[55]. R. Pinheiro, D. S. Oliveira, P. Perego, M. Nogueira, D. Oliveira, and A. Converti, "Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk," *LWT - Food Sci. Technol.*, vol. 44, no. 2, pp. 520–523, 2011.
[56]. B. Bandyopadhyay and N. C. Mandal, "Probiotics, Prebiotics and Synbiotics - In Health Improvement by Modulating Gut Microbiota: The Concept Revisited," *Int. J. Curr. Microbiol. adn Appl. Sci.*, vol. 3, no. 3, pp. 410–420, 2014.
[57]. S. Dwivedi, K. Sahrawat, N. Puppala, and R. Ortiz, "Plant prebiotics and human health: Biotechnology to breed prebiotic-rich nutritious food crops," *Electron. J. Biotechnol.*, vol. 17, no. 5, pp. 238–245, 2014.
[58]. Y. Vandenplas, G. Veereman-Wauters, E. De Greef, S. Peeters, A. Casteels, T. Mahler, T. Devreker, and B. Hauser, "Probiotics and prebiotics in prevention and treatment of diseases in infants and children," *J. Pediatr. (Rio. J).*, vol. 87, no. 4, pp. 292–300, 2011.
[59]. R. Frei, M. Akdis, and L. O'mahony, "Prebiotics, probiotics, synbiotics, and the immune system: Experimental data and clinical evidence," *Curr. Opin. Gastroenterol.*, vol. 31, no. 2, pp. 153–158, 2015.
[60]. R. Patel and H. L. Dupont, "New approaches for bacteriotherapy: Prebiotics, new-generation probiotics, and synbiotics," *Clin. Infect. Dis.*, vol. 60, no. Suppl 2, pp. S108–S121, 2015.
[61]. R. George Kerry, J. K. Patra, S. Gouda, Y. Park, H. S. Shin, and G. Das, "Benefaction of probiotics for human health: A review," *J. Food Drug Anal.*, vol. 26, no. 3, pp. 927–939, 2018.
[62]. I. Stamatova and J. H. Meurman, "Probiotics: Health benefits in the mouth," *Am. J. Dent.*, vol. 22, no. 6, pp. 329–338, 2009.
[63]. E. M. M. Quigley, "Prebiotics and Probiotics in Digestive Health," *Clin. Gastroenterol. Hepatol.*, vol. 17, no. 2, pp. 333–344, 2019.
[64]. M. Derrien, M. W. J. Van Passel, J. H. B. Van De Bovenkamp, R. Schipper, W. De Vos, J. Dekker, M. Derrien, M. W. J. Van Passel, J. H. B. Van De Bovenkamp, M. Derrien, M. W. J. Van Passel, J. H. B. Van De Bovenkamp, R. G. Schipper, and W. M. De Vos, "Mucin-bacterial interactions in the human

oral cavity and digestive tract," *Gut Microbes*, vol. 1, no. 4, pp. 254–268, 2010.
[65]. N. Suharti, A. Kumala, E. Chundrayetti, and A. E. Putra, "The Effect of Dadiah Feeding of Breastfeeding Women with Normal Flora of Digestive Tract," *ICOMHER*, pp. 1–7, 2018.
[66]. A. Coeuret, S. Dubernet, M. Bernardeau, M. Gueguen, and J. Vernoux, "Isolation, characterization and identification of Lactobacilli focusing mainly on cheeses and other dairy produces," *Lait*, pp. 269–306, 2003.
[67]. W. Gustaw and M. Kordowska-wiater, "The influence of selected prebiotics on the growth of lactic acid bacteria for bioyoghurt production," *Acta Sci. Pol. Technol. Aliment.*, vol. 10, no. 4, pp. 45–466, 2011.
[68]. A. S. Harti, D. S. Haryato, Sunarto, W. Setyaningsih, and S. Yatmihatun, "The Potential Chito-Oligosaccharide (COS) as Natural Prebiotic and Preservatives on Synbiotic Tofu in Indonesia," *Int. J. Pharma Med. Biol. Sci.*, vol. 4, no. 3, pp. 204–208, 2015.
[69]. E. S. Ã, P. Fayos, Y. Lario, J. Ferna, A. Pe, E. Sayas-barbera, and J. A. Perez-Alvarez, "Incorporation of citrus fibers in fermented milk containing probiotic bacteria," *Food Microbiol.*, vol. 25, pp. 13–21, 2008.
[70]. J. Minj and S. Vij, "Effect of Prebiotic Inulin on the Fermentation and Growth Kinetics Pattern of Probiotic Yoghurt Bacteria," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 6, no. 12, pp. 1755–1768, 2017.
[71]. A. Ş. Mumcu and A. Temiz, "Effects Of Prebiotics On Growth And Acidifying Activity Of Probiotic Bacteria" *GIDA*, vol. 39, no. 2, pp. 71–77, 2014.
[72]. F. Guarner, M. E. Sanders, Rami Eliakim, R. Fedorak, A. Gangl, J. Garisch, P. Kaufmann, T. Karakan, A. G. Khan, N. Kim, H. S. (Poland) Juan Andrés De Paula Balakrishnan Ramakrishna Fergus Shanahan (Ireland), and A. L. M. (The N. Alan Thomson (Canada), *World Gastroenterology Organisation Global Guidelines - Probiotics and prebiotics*. 2017.
[73]. M. George, L. Joseph, D. George, and D. George, "Role of prebiotic and probiotic in the management of chronic kidney disease patients," *Int. J. Med. Res. Heal. Sci.*, vol. 5, no. 2, pp. 50–53, 2016.
[74]. R. Ferrarese, E. R. Ceresola, a. Preti, and F. Canducci, "Probiotics, prebiotics and synbiotics for weight loss and metabolic syndrome in the microbiome era," *Eur. Rev. Med. Pharmacol. Sci.*, vol. 22, no. 21, pp. 7588–7605, 2018.
[75]. R. Orel and L. V. Reberšak, "Clinical Effects of Prebiotics in Pediatric Population," *Indian Pediatr.*, vol. 53, no. 12, pp. 1083–1089, 2016.
[76]. F. Indrio, G. Riezzo, F. Raimondi, M. Bisceglia, L. Cavallo, and R. Francavilla, "Effects Of Probiotic And Prebiotic On Gastrointestinal," *J. Phyisology Pharmacol.*, vol. 60, no. 6, pp. 27–31, 2009.