IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 1 Ver. II (January. 2018), PP 76-81
www.iosrjournals.org

Comparative Growth and Growing Percentage of *Lactobacillus spp.* from Different Traditional Dairy Products (Yoghurt, Cheese, Butter, Lassi and Cow milk) in Bangladesh using Stirred Tank Bioreactor

Shakti Chandra Mondal, Most. Jesmin Akhter*, Md. Nazmul Hossain, Shubhojit Saha and Md. Atikur Rahman
Department of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh
Corresponding Author: Most. Jesmin Akhter

Abstract: *One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria, which are used in making starter culture for dairy products. The growth of microorganisms may also vary in different dairy products largely depend on their components variation and the way they are manufactured. The aim of this study was to study the comparative growth of Lactobacillus in different dairy products in stirred tank bioreactor and to identify the ideal source of Lactobacillusfromdifferent dairy products namedYoghurt, Cheese, Butter, Lassi, and Cow milk.Theselected species was isolated from different samples using Lactobacillus MRS Agar media. The pH of the media was adjusted to 6.5 and maintained temperature at 37 ˚C overnight in stirred tank bioreactor..Thebacteriacounts of these samples ranged between 3.83x10^{6}cfu/ml to 2.86x10^{8}cfu/ml.The results of the present study revealed that yoghurtcontained the highest lactic acid bacteria count (2.86x10^{8}cfu/ml) than others dairy products in bioreactor and it wasthe ideal source of Lactobacillus in different dairy productsand we find that milk contains maximum of growing percentage of microorganisms.*

Keywords: *Lactobacillus spp., MRS Agar, Yoghurt, Cheese, Butter, Lassi, Cow milk, Stirred Tank Bioreactor.*

Date of Submission: 17-01-2018 Date of acceptance: 03-02-2018

I. Introduction

Different new fermented foods are introduced in the dairy field of market every year [1]. Many of these dairy products containing selected bacteria like *Lactobacillus spp*. which plays an important role in food technology and have a long history of use by people for food production and food preservation [2, 3, 4]. In the production of fermented food, Lactobacilli are highly competitive largely due to their applications [5]. In our country there are various kinds of traditional dairy products which are produced from cow milk such as yoghurt, lassi, cheese, butter, milk etc. For technological reasons or to generate a health benefit for the consumer, Lactobacilli are naturally present or added intentionally [6].The selection of microorganisms is diverse to be used in the fermentation process [7]. It is very important for us to know the physical and physiological characteristics of the type of bacterial cells which can be used in the fermentation process [8]. Bacterial cells such as Lactobacilli can survive both under aerobic and anaerobic conditions which can be cultivated in fermenters or bioreactors [9]. The function of the bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product. Stirrer tank bioreactor must be designed so that it will be able to provide the optimum environments or conditions which allow supporting the growth of the microorganisms. The design and mode of operation of a bioreactor mainly depends on the type of product, microorganism to be used, the optimal operating condition required for specific product formation, scale up of production and product value[10]. It is hardly to know exactly that is possible or not to grow the maximum range of microorganisms instirrer tank bioreactor and if grow then which types of dairy products may grow the higher range of microorganisms should have to know owing to their uses in different aspects. The growth of microorganisms may vary in different dairy products largely depend on their components variation and the way they are manufactured. Considering the information as accumulated, the research was conducted to achieve the following objectives –To study the comparative growth of *Lactobacillus spp.* in different dairy products in bioreactor and to identify the ideal source of *Lactobacillusspp*. in different dairy products with maximum growing percentage.

II. Materials And Methods

2.1 Materials

2.1.1 Collection of sample

Five samples of dairy products such as Cheese, Butter, Lassi, Yoghurt and Cow milk were collected from the local market of Dinajpur town. Immediately after collection, the samples were stored at low temperature (-4 ˚c) refrigerator except milk to protect from contamination and deterioration.

2.1.2 Selection of Media

The growth of *Lactobacillus spp*. were isolated from Cheese, Butter, Lassi, Yoghurt and Fermented milk samples by using *Lactobacillus* MRS Agar media. The pH of the media was adjusted to 6.5.

Composition of MRS Agar media

Ingredients	gms/litre
Proteose peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Polysorbate 80	1.00
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.10
Manganese sulphate	0.05
Dipotassium phosphate	2.00
Agar	12.00

2.2 Methods

2.2.1 Preparation of media

6.715 gm. of MRS Agar media was measured with the help of electric balance and taken them in a conical flask and mixed with 100 ml distilled water. The conical flask was heated for proper mixing. In the time of heating, the mixture was rotted with the glass rod. When the mixture was properly mixed, the mouth of the conical flask was covered with aluminium foil and heated until the solution become clear. Then the conical flask with media was placed in autoclave for sterilization (Temperature: 121˚c, Pressure: 15 Ib/inch² and time: 15 minutes). After sterilization the media was cooled at 45 to 50 ˚c (approximately). Then 1 ml of sample was added into the media and properly mixed.After proper mixing, the mixing sample was poured into the stirred tankbioreactor and air as well as oxygen was supplied by compressor. Here maintained temperature at 37 ˚c overnight for 24 hours. Thus fermented sample were produced.

2.3 Determination of Bacterial Growth

2.3.1 Methods

For the total bacterial count of the samples (Cheese, Butter, Lassi, Yoghurt and Cow milk) Standard pour plate method was followed according to the method described in "Recommended method for the microbiological examination of food" (M.A. Ali, 2008).

2.3.2 Preparation of blank dilution

In order to dilute the sample consecutively 1ml of the fermented sample was diluted stepwise through a series of tubes containing 9ml of 0.85% of Sodium Chloride Solution (w/v). Sodium Chloride Solution (w/v) was prepared by 0.85 gm. of Sodium Chloride salt with 100 ml of distilled water. At first 9 ml of the Sodium Chloride Solution (w/v) was taken in a sterile test tubes and then 1 ml of the fermented sample was taken to the first test-tube with a sterile pipette. Sodium Chloride Solution (w/v) with the sample was vigorously shaken for homogenous distribution of the bacterial population in the solution. This tube was denoted as "1". From the tube "T-1" another 1 ml aliquot was transferred to the second tube and this tube was denoted as "T-2". In this way "T-3", "T-4", "T-5", "T-6" was prepared until the desired dilution is achieved. Now the tube "T-1", "T-2" "T-3", "T-4", "T-5" and "T-6" has got the dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, respectively.

2.3.3 Preparation of plating

Now from the test-tube "T-1", 1 ml of the sample solution was taken in a sterile petridish containing 9 ml of MRS Agar medium. The agar with bacterial sample was mixed by rotating the petridish. This petridish was marked as "I". In this way "II", "III", "IV", "V", "VI" marked petridishes were prepared from the tubes "T-2", "T-3", "T-4","T-5" and "T-6"respectively. Then these petridishes were placed on a level surface for few minutes for solidifying the MRS Agar medium.

2.3.4 Incubation and Isolation

The bacteria count was determined by diluting the samples serially and plating 1ml aliquot on MRS agar. MRS agar was used and incubated at 37°C for 24 hours using pour plate method after whichthe total number of bacteria per gram of sample was calculated by the following equation:

Colony count (cfu/ml) = Number of colonies (per plate) × Dilution factor.

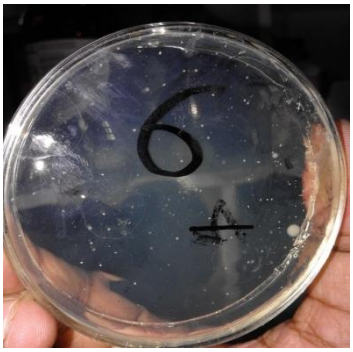
III. Results And Discussions

3.1 Bacterial Growth of various dairy product

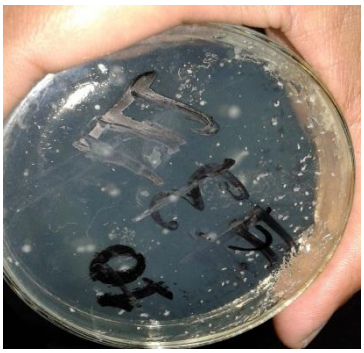
The types of sample, color, pH and after bio-reaction the total bacterial load of these samples were recorded and shown in Table: 3.1 the entire sample were found to be acidic with a pH range between 6.2 to 6.5. After 24 hours completed bio-reaction, the bacterial count per mlfor those samples ranged between $3.8398x10^6$cfu/ml to $2.8573x10^8$cfu/ml. Gauri*et al*. (2013) found thatthe optimum temperature of growth for *Lactobacillus spp*. is 37ºC. Here we showed the comparative bacterial growth at specific temperature and we used optimum temperature for *Lactobacillus spp*. was 37ºC.

A. Plate for Yoghurt

B. Plate for Milk



C. Plate for Cheese



D. Plate for Butter

E. Plate for Lassi

Figure 3.1: Bacterial growth of different product.

Table: 3.1 Bacterial growth of different collected sample

Sample name	Color of sample	pH	Mean colony count (cfu/ml)
Yoghurt	Cream	6.2	2.8573×10^8
Cow Milk	Whitish	6.3	1.2642×10^8
Cheese	Whitish	6.5	2.3710×10^7
Butter	Cream	6.3	1.0314×10^7
Lassi	Cream	6.2	3.8398×10^6

Form table: 3.1 we observed mostly Bangladeshi available dairy products in which yoghurt contained the maximum number of lactic acid bacteria and Lassi contained the minimum number of lactic acid bacteria as well as *Lactobacillus spp.*The growth rate of *Lactobacillus spp.*was higher in yoghurt than milk, cheese, butter and lassi. All of these samples were fermented dairy products except milk [12]. From table: 4.1 followed that yoghurt contained maximum number of bacterial colony. Because of processing steps of manufacturing yoghurt, needed to add the lactic acid bacteria (LAB) as a starter culture which contains *Lactobacillus spp*. and these bacteria can help to multiply the colony thus the maximum number of bacteria was observed in yoghurt sample. On the other hand showed that it was not essential to add any starter culture for manufacturing or processing steps of cheese, butter and lassi [12].

3.2 Growth comparison of *Lactobacillus spp.* between using Bioreactor vs. without Bioreactor

The production growth rate of *Lactobacillus spp*.was higher when using bioreactor than without bioreactor (Incubation only). Here we observed the growth for 24 hours production at 37ºC and from table: 3.2 we observed that the total colony count $2.8573x10^8$cfu/ml for yoghurt, $1.2642x10^8$cfu/ml for cow milk, $2.3710x10^7$cfu/ml for cheese , $1.0314x10^7$cfu/ml for butter and $3.8398x10^6$cfu/ml for lassi after Bioreactor operation. Whereas Tasneem and Jannatul (2012) or [13] showed that, the total colony count $8.8x10^7$cfu/ml for yoghurt, $5.8x10^6$cfu/ml for cow milk, $2.4x10^6$cfu/ml for cheese, $1.39x10^6$cfu/ml for butter and we got $6.28x10^5$cfu/ml for lassi after only incubation system operation. For both operations used the pour plate count methods and the pH range between 6.0 to 6.6.

Table: 3.2 Bacterial load of the collected sample by using Bioreactor

Sample name	Color of sample	Mean colony count (cfu/ml) without using of Bioreactor (Incubation only)	Mean colony count (cfu/ml) by using Bioreactor
Yoghurt	Cream	8.8×10^7	2.8573×10^8
Cow Milk	Whitish	5.8×10^6	1.2642×10^8
Cheese	Whitish	2.4×10^6	2.3710×10^7
Aarong Butter	Cream	1.39×10^6	1.0314×10^7
PranLassi	Cream	6.28×10^5	3.8398×10^6

The growth comparisons of *Lactobacillus spp*. in different sample are shown in graphically in figure: 3.2. Form this graph we observe that the growth of *Lactobacillus spp.* is high for yoghurt and then cow milk, cheese, butter and lassi respectively.

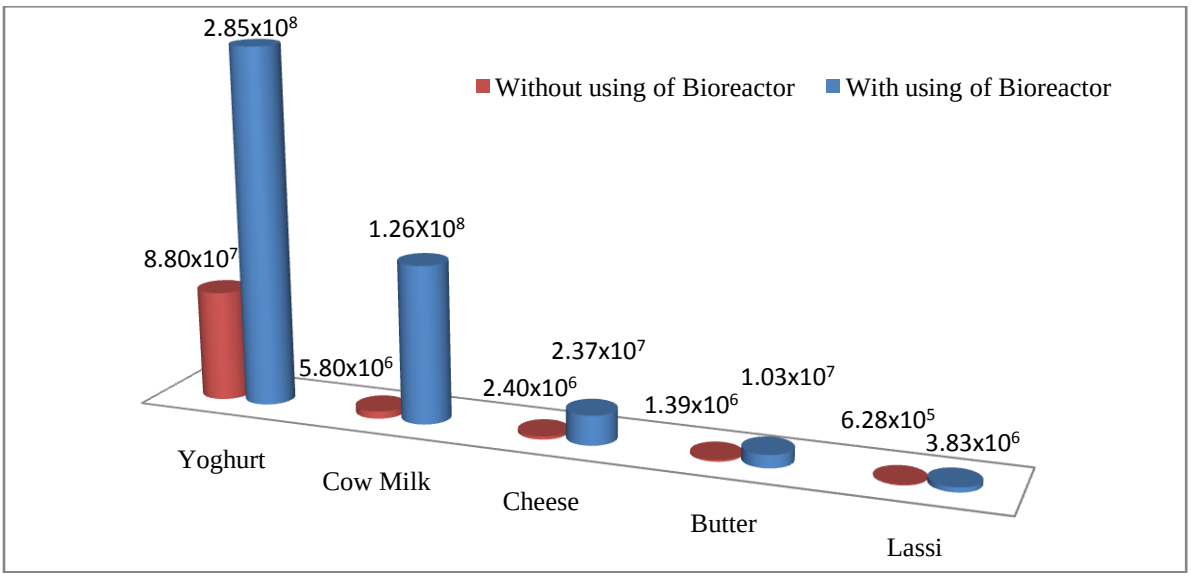


Figure: 3.2 Comparative growths for *Lactobacillus spp*. with and without using ofBioreactor

From the above result we showed that bioreactor was effective for maximum growth of *Lactobacillus spp.* Because it was operated with controls for monitoring and adjusting the many physical and chemical parameters such as temperature, pH, nutrient composition, foaming etc. and A. J. Nair, (2006) or [14] also agree with us. Here we used stirred tank bioreactor and it was the most commonly used bioreactor for microbial cultivation, in which the microbial medium was stirred with an impeller. A high density of metabolically active cells in the medium could result in sudden depletion of dissolved oxygen creating an anaerobic condition in the medium. Rapid growth also results in the depletion of essential nutrients that directly link to the growth and metabolism that caused the production of the product.

3.3 Increasing growth percentage in Bioreactor for collected sample

In bioreactor the growth percentage increased about hundred to million times than in incubator. Here we showed from graph: 3.3, the growth of *Lactobacillus spp*. were increasedbioreactor than over incubator: 224 % for yoghurt, 2079% for cow milk, 887% for cheese, 642% for butter and 511% for lassi by using following formula-

$$\text{Comparison (\%)} = \frac{(\text{Colony present by using Bioreactor} - \text{Colony present by using incubator only}) \times 100}{\text{Colony present by using incubator only}}$$

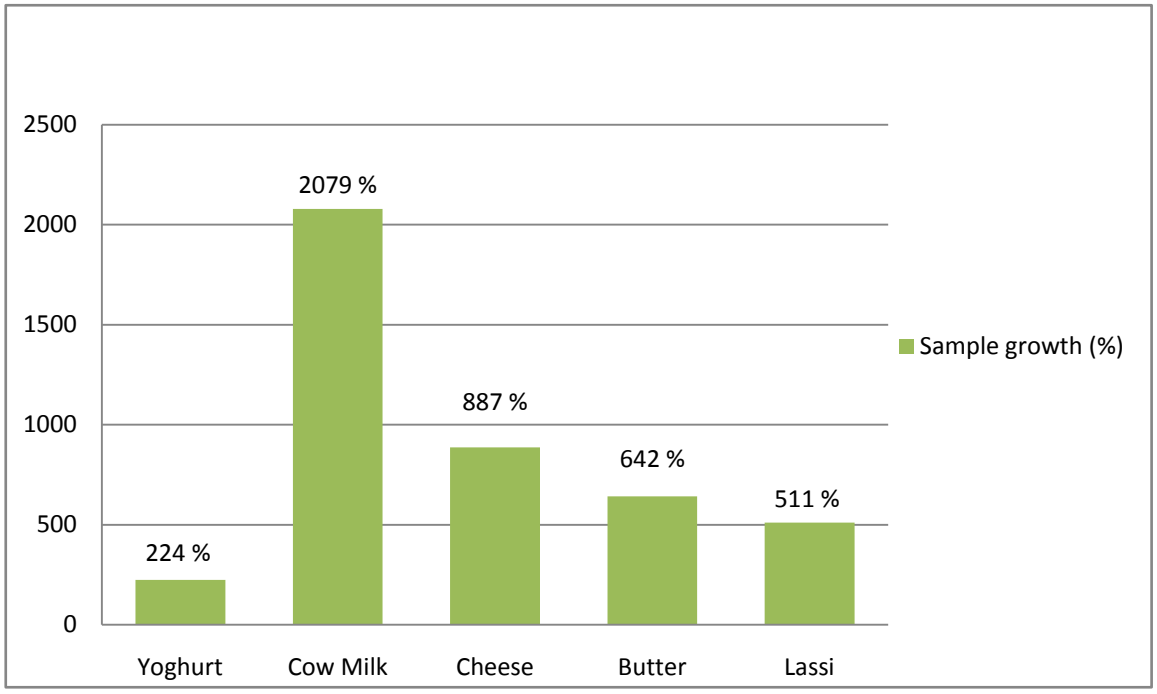


Figure: 3.3 Comparisons of growths percentage (%) of *Lactobacillus spp*. by using of stirred tankbioreactor

From figure: 3.3 we observed that maximum percentage of growth occurred in milk sample than cheese, butter, lassi and yoghurt sample respectively in bioreactor. Because milk contained less amount of *Lactobacillus spp*. than yoghurt and yoghurt was a fermented product where milk lactose could be converted into lactic acid bacteria (LAB) and as yoghurt was already a fermented product so, there were little chances to fermentation in yoghurt again. On the other hand milk was not a fermented product but it was a dairy product which could be fermented and after fermentation process it could be increased the number of total *Lactobacillus spp.* bacteria.

IV. Conclusion

From the above study we can conclude that, Lactobacilli are naturally present in various Dairy products. They are important organisms recognized for their fermentative ability as well as health and nutritional benefits. We also ensure that the rate of growing condition of microorganisms at different dairy products can be maximized by using bioreactor.The results obtained from the study that Yoghurt is the highest lactic acid bacteria counts than others dairy products in bioreactor because of its components variation but in terms of comparing between maximum growing percentage of microorganisms between incubation system and bio-reaction system we find that milk contains maximum of growing percentage of microorganisms. As

contained the maximum number of *Lactobacillus spp.* so we can considered,Yoghurt is the ideal source of *Lactobacillus spp.* in different dairy products in various aspects.

References

[1]. Ventling, B.L. and Mistry, V.V. (1993). Growth characteristics of bifidobacteria in ultra-filtrated milk. J Dairy Sci 76, 962–971

[2]. Colombel, J.F., Cortot, A., Neut, C. and Romond, C. (1987). Yoghurt with Bifidobacteriumlongum reduces erythromycin induced gastrointestinal effects. Lancet North Am Ed 4, 43–45.

[3]. Ishida, Y., Nakamura, F., Kanzato, H., Sawada, D., Hirata, H., Nishimura, A., Kajimoto, O. and Fujiwara, S. (2005). Clinical effects of *Lactobacillus* acidophilus strain L-92 on perennial allergic rhinitis: a double-blind, placebo-controlled study. J Dairy Sci 88, 527–533.

[4]. GuessasBettache, AdjoudjFatma, HadadjiMiloud and KihalMebrouk (2012). Isolation and Identification of Lactic Acid Bacteria from Dhan, a Traditional Butter and Their Major Technological Traits. World Applied Sciences Journal 17 (4): 480-488.

[5]. Gauri Dixit, Deepti Samarth, Vidya Tale, Rama Bhadekar (2013). Comparative studies on potential probiotic characteristics of *Lactobacillus acidophilus* strains. EurAsian Journal of BioSciences Eurasia J Biosci 7, 1-9.

[6]. M.Z. Hoque, F. Akter, K.M. Hossain, M.S.M. Rahman, M.M. Billah and K.M.D. Islam (2010).Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. World Journal of Dairy & Food Sciences 5 (1): 39-46.

[7]. Hitesh Jagani, KarteekHebbar, Sagar S. Gang, P. Vasanth Raj, Raghu Chandrashekhar H. and J.Venkata Rao (2010). An Overview of Fermenter and the Design Considerations to Enhance Its Productivity. Newsletter, *Pharmacologyonline*1: 261-301.

[8]. Jana Smetanková, ZuzanaHladíková, FrantišekValach, Michaela Zimanová, ZlaticaKohajdová, Gabriel Greif, MáriaGreifová (2012). Influence of aerobic and anaerobic conditions on the growth and metabolism of selected strains of *Lactobacillus*plantarum 5(2): 204-210.

[9]. Hitesh Jagani, KarteekHebbar, Sagar S. Gang, P. Vasanth Raj, Raghu Chandrashekhar H. and J.Venkata Rao (2010). An Overview of Fermenter and the Design Considerations to Enhance Its Productivity. Newsletter, *Pharmacologyonline*1: 261-301.

[10]. Sukumar De (1991). Outlines of Dairy Technology.Published in India by Oxford University Press, First published in 1991, ISSN 0-19-561194-2.

[11]. Tasneem Chowdhury and Jannatul Ferdouse (2012). Isolation, Characterization and Antimicrobial Activity of Lactic AcidBacteria from Local Milk and Milk Products. Bangladesh J Microbiol, Volume 29, Number 2, December 2012, pp 76-82.

[12]. A. J. Nair (2006).Introduction to biotechnology and genetic engineering. Infinity Science Press LLC, Hingham, Massachusetts/ New Delhi, India, ISBN no: 978-1-934015-16-2.

Shakti Chandra Mondal "Comparative Growth and Growing Percentage of Lactobacillus spp. from Different Traditional Dairy Products (Yoghurt, Cheese, Butter, Lassi and Cow milk) in Bangladesh using Stirred Tank Bioreactor." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.1 (2018): 76-81.