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EVALUATION OF THE LACTIC ACID BACTERIA IN DIFFERENT TYPES OF YOGURT CONSUMED IN PAKISTAN

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ABSTRACT

Lactic acid bacteria are industrially essential components to produce milk products such as yogrut, cheese, buttermilk, and kefir. Different gram-positive species are used for this purpose, including *Streptococcus thermophilus,* and *Lactobacillus delbrueckii spp. bulgaricus* bacteria were assayed by a viable count method. Different available commercial yogurts were purchased from the local market; among these, six were selected for assay. Samples were named as Nestle Podina Raita (NPR), Nestle Smooth Yogrut (NSY), Nestle Karachi Khase (NKK), Nestle Zeera Podina (NZR), Nestle Fruit Yogrut (NFY), and National Original Yogrut (NOY). From results, it was observed that NOY has the highest mean count i.e. 12.08 ± 0.25 log10 CFU/ml and 11.02 ± 0.13 log10 CFU/ml for *S.thermophilus* and *L.bulgaricus,* respectively. Titratable acidity was highest in NOY (0.93 %) with mean viable LAB counts were found to be 11.26 ± 0.24 and 10.61 ± 0.12 for *lactobacillus bulgaricus and streptococcus thermophilus,* respectively.

Keywords: Lactic acid Bacteria (LAB) Viable counts, *Streptococcus thermophilus and Lactobacillus bulgaricus*.

INTRODUCTION

Fermented milk products have been considered to be beneficial for health. Yogurt is one of the fermented milk products and the best human diet. Presence of viable lactic acid bacteria in fermented milk like yogurt, such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus* has been correlated with vari-ous benefits for human health. It is suggested that the minimum level for probiotic bacteria should be in the range of 10^{-5} to 10^{-6} viable cells per mL (Dave and Shah, 1997). France and Spain set a mini-mum viable lactic acid bacteria number in yogurt's shelf life of 5×10^{8} CFU/ml (Birollo et al., 2000). In medical science, it is believed that yogurt starter microbes are proteolytic and produce bioactive peptides from milk protein. Fermented milk and yogurt contain peptides with angiotensin over protein inhibitory action (Guevarra and Barraquio, 2015). They have anticarcinogenic, antimutagenic effects, improvised of lactose digestion, and enhancement of the Immune system. The essential probiotic organism naturally associated with the human GIT is Lactic acid Bacteria (LAB) (Dicks and Botes, 2010). Usually, Lactic acid bacteria are classified on the bases of their morphology, mode of fermentation of glucose, and growth at various temperatures and fermentation of various carbohydrates (Axels-son, 2004; Khalid, 2011). Nowadays, the production of probiotic on an industrial scale in food and pharmaceutical has a beneficial impact on human and animal life (Mattila-Sandholm et al., 2002).

The majority of the population who use probiotics believe that the use of such products not only reduces the risk of getting many GIT related infections but also helps in many other critical diseases (Sanders, 2003). To gain a guaranteed medical advantage to humans, the minimum availability of probiotic microorganisms in the fermented milk considers that it would be around 10^{9-10} colony forming units (CFU) /g. However, the dosage is not fixed in the evaluation of probiotic (Ouwehand, 2015). A vital parameter in evaluating the quality of the product is by observing suitable for probiotic microbes selectively. To ensure that the required number of probiotic organisms in the end-product is present, fast and reliable approaches require routine count.

Moreover, such techniques are additionally essential to screen any physiological or biochemical changes in the population of probiotic bacteria amid the product's commercial storage (Vinderola et al., 2002). Besides, a few research studies have shown that some probiotic items in the market don't meet the minimal prescribed count of probiotic strain(s), particularly in those containing bifidobacteria (Fasoli et al., 2003). The presence of probiotics in yogurt is affected by many other factors such as strain of bacteria is used, the interaction between the species in it, what is the conditions of culture, production of hydrogen peroxide due to bacterial metabolism, and at the end, acrid smell, and combination of acidic acid and lactic acid. Moreover, during the fermentation of milk due to the growth of bacteria, the pH of the medium is also decreased which affects the feasible amount of probiotic in it (Shah and Jelen, 1990). The yogurt which reaches consumers may raise few doubts on the actual presence of probiotics in it because many sweeteners, preservative, flavoring ingredients are used in it (Birollo et al., 2000). Till now, no one has studied the actual presence of probiotic in commercially available yogurts in Pakistan. Now, questions can be raised here whether such products can give the said health benefits to the consumers due to the presence of probiotic in lower range. The objective of this research is to check the prescribed amount of probiotic present in commercially available products and Lactic acid Bacteria is available in such items or not. The information from this investigation will be valuable to check the various yogurt makers regarding the health benefits of live and active culture present in the products.

MATERIALS AND METHODS

All chemicals used were of reagent grade. The study was carried out from January to April 2019 in the Biotechnology lab, Government College University Hyderabad, Pakistan. The commercial yogurt starter culture containing S. *thermophilus* and *L. delbrueckii ssp. bulgaricus* was selected because its mild acidity protects bifidobacteria. Both yogurt starter cultures were obtained from local vendors. BS-MIX-28 Dancing Fairy-Vortexer Smart was used to mix the samples.

Collection of Samples: Six yogurt samples were bought as fresh as possible from the local market of Hyderabad namely: Nestle Podina Raita (NPR) Nestle Smooth Yogurt (NSY) Nestle Karachi Khas (NKK) Nestle Zeera Raita (NZR) Nestle Original Yogurt (NOY) and Nestle Fruit Yogurt (NF-Y). All the sample results were an average of 5 tests. All samples were stored in 4 to 6°C temperature to minimize the acidification. The production and expiry date of all samples were recorded in the first instance.

Isolation of Microorganisms: Pour plate count (30-300 colonies) method was used after incubation. All results are shown in log CFU/mL) (SERT et al., 2011). MRS and M17 agar were used to identify the *S. thermophilus* and *L. bulgaricus* in yogurt samples Serial dilution was performed with peptone diluents. The micropipette was used to transfer a 1mL blended yogurt sample to a test tube of 9ml sterile diluent, which stands as 10-1 dilution? Samples were mixed for 1 minute on a vortex mixer. 1ml was taken from a 10-1 dilution tube and transferred to 9 ml sterile diluent to prepare a 10-2 dilution. This activity was repeated until the expected dilution is obtained by utilizing new and sterile pipettes and diluents. To calculate the bacterial count, one ml of the prepared dilution was moved into the Petri dishes, then MRS agar medium 12 to 15 ml is added at 45°C was filled each Petri dish with the proper dilution. (Karna et al., 2007). The substance of the Petri dish was blended cautiously by rotating the Petri dish multiple times clockwise and counter-clockwise. It was left at room temperature to solidify at a smooth surface. Plates were placed in an anaerobic jar for incubation at 37^{o} C for 72 hours. Plates were then incubated anaerobically for 48 hours at 37^{o}C then 25 to 250 colonies on Petri plates counted by colony-forming unit method CFU/ml by the following formula (De Man et al., 1960)

$N = \Sigma C/[(1.0 \times n1) + (0.1 \times n2)d$

Where:

N = number of colonies per ml of sample.

∑C = sum of all of the colonies in all plates counted.

n1 = number of plates in the lower dilution counted.

n2 = number of plates in the next higher dilution counted.

d = dilution from which the first counts were obtained

Characterization on Bases of Morphology: Morphological characterization of colonies was carried out by testing their catalase reaction by observing the colony growth gram reaction, cell morphology for verifications of colony count for *S. thermophilus* and *L. bulgaricus.* Catalase activity and procedure for gram staining was performed according to reported methods (Murray et al., 1994; Smibert, 1994) Well-isolated colonies were inoculated on MRS agar for *L.bulgaricus* and M17 agar for *S. thermophiles,* incubated at their ideal temperature 37°C for 48-72 hours. The culture was Gram-stained, and a test for catalase activity was performed. Colony confirmation was accomplished for the Gram-positive, catalase-negative chains of cocci or diplococci on account of *S. thermophilus* and non-spore-forming, Gram-positive, and catalase-negative rod on account of *L. bulgaricus (Guevarra and Barraquio, 2015).*

Acidity Test: APHA method was used for the acidity test of yogurt samples. Acidity was calculated from following equation:

% Lactic Acid = (Volume of NaOH used x Normality of NaOH x 90/100)/9 ×100

Statistical analysis: Complete Randomized Design (CRD) was applied to date so that each experimental unit has the same chance of receiving any one treatment. All experiments were performed in three batches (n = 3), and the average was taken. Data were expressed as mean ± standard error using one-way ANOVA by SPSS® version 17.0. Means were compared using Duncan's multiple range tests, and statistical significance was standard by ANOVA at $p < 0.05$.

RESULT AND DISCUSSION

Bacterial count of Yogurt samples: Table 1 shows the mean viable lactic acid bacterial counts in log10 CFU/ml. It was observed the there is no significant difference in all six commercial yogurt samples for both bacterial strains. The highest mean value was overserved in NOY sample for S*. thermophilus* and *L. delbrueckii ssp. bulgaricus* i.e. 12.08 ± 0.25 and 11.02 ± 0.13, respectively. NKK shows least all yogurt samples with mean viable counts of 10.01 ± 0.58 and 9.51 ± 0.35 *Thermophilus* and *L. delbrueckii ssp. Bulgaricus,* respectively.

Table 1: *S. thermophilus* and *L. bulgaricus* Mean viable count

Yogurt samples	Mean viable LAB counts (log10 CFU/ml) n = 5	
	S. thermophilus	***L. bulgaricus***
Nestle Podina Raita (NPR)	11.36 ± 0.41	10.39 ± 0.44
Nestle Smooth Yogrut (NSY)	10.25 ± 0.36	9.98 ± 0.22
Nestle Karachi Khas (NKK)	10.01 ± 0.58	9.51 ± 0.35
Nestle Zeera Raita (NZR)	10.68 ± 0.49	10.22 ± 0.62
Nestle Original Yogrut (NOY)	12.08 ± 0.25	11.02 ± 0.13
Nestle Fruit Yogrut (NFY)	10.57 ± 0.67	10.14 ± 0.38

n = number of samples examined per yogurt brand

Properties of lactic acid bacterial colonies: Properties of *S.thermophilus* and *L. bulgaricus* colonies were observed by a well-isolated colony that appeared on the Petri plate. A single colony was aseptically transferred on stab/slat to observe the growth pattern on tangible media. The colony of *S.thermophilus* on M17 agar plate seemed to be smooth white, around 0.5 to 3.0 mm in circular, diameter, low convex demonstrated unequal development on a slant. While Lactobacillus generally has little size settlements having 2–5 mm size with the whole margin, convex, shiny, and cloudy, without pigment (Vos et al., 2011).

Table 2: *Streptococcus thermophilus* and *Lactobacillus* viable lab counts, and Titratable acidity in a yogurt samples.

Yogurt sample	% Lactic acid	Mean viable LAB counts[a] (log10 CFU/ml)	
		Streptococcus thermophilus	***Lactobacillus bulgaricus***
NPR	0.71	9.67 ± 0.25	9.55 ± 0.36
NKK	0.85	10.25 ± 0.68	9.63 ± 0.40
NZR	0.83	10.67 ± 0.46	10.21 ± 0.6
NOY	0.93	11.26 ± 0.24	10.61 ± 0.12
NFY	0.69	9.54 ± 0.19	8.84 ± 0.14
NSY	0.79	10.9 ± 0.49	10.32± 0.37

[a]Mean viable counts with the same superscript are not significantly different ($P \leq 0.05$)

Morphology of Lactic acid bacteria: Gram-positive staining of 48-hour old culture was used to characterize the morphology of LAB samples. *A bacterium* for *S. thermophiles* and gram-positive staining of 72-hour old culture was used for the determination of morphology of *L. bulgaricus*. Microscopic visualization showed that S. thermophilus cells were bowl and circular, and they usually occur in aggregated form. They were non-motile, Gram-positive, catalase-negative, and non-spore forming. *Lactobacillus delbrueckii ssp. Bulgaricus* is a rod-shaped cell; sometimes, they appear almost coccoid and in a short chain. *L. bulgaricus* facultatively anaerobic, Gram-positive, and non-sporing. Their ideal temperature is 30–40°C and their digestion is fermentative and saccharolytic, in any event, half of the finished result of carbon is lactate (Holt, 1977).

Comparison of acidity and viable counts: Table 2 shows lactic acid acidity in different yogurt samples. Acidity was observed highest in NOY sample, which correlated with viable counts of the same sample. No official standards have been set on minimum titratable acidity for frozen yogurts yet. So it is suggested that food industries should ensure the minimum base titratable acidity value of 0.25 % lactic acid (Holt, 1977).

Conclusion

In this study, we have assayed commercially available yogurts in Pakistan to conform to the presence of *S. thermo- philus* and *L. bulgaricus*. Six samples were taken and each sample was tested for mean viable count LAB of (*S. Thermophilus* and *L. bulgaricus*). Among all samples, the mean viable count was significantly lower ($P \leq 0.05$) in NKK. The lower mean of LAB count in NKK is due to the freezing of the sample. There was no significant difference between the two bacterial strains for acidity and mean viable counts, tested in this study. The Viable count of *S. thermophilus* and *L. bulgaricus* yogurts assayed and found under prescribed value for the proposed therapeutic impacts and wellbeing benefits for the consumers

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