

EVALUATION OF *LACTOBACILLUS PLANTARUM* GROWTH IN MILK OF INDIAN BUFFALO BREEDS BASED ON ITS PHYSICO-CHEMICAL CONTENT

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ABSTRACT

The study reports the physicochemical content of raw and pasteurization milk from Bhadawari, Mehsana and Nagpuri Indian buffalo breeds. The study demonstrated the protein, fat, lactose, total solid content, and percentage of casein of the raw milk for Nagpuri, Mehsana and Bhadawari as 4.5 ± 0.51 , 5.4 ± 0.62 , 4.8 ± 0.41 , 7.2 ± 0.32 , 6.1 ± 0.66 , 7.0 ± 0.84 , 5.4 ± 0.65 , 6.2 ± 0.65 , 5.1 ± 0.54 , 17.2 ± 1.21 , 18.8 ± 1.54 , 18.1 ± 1.22 , 10.8 ± 1.20 , 11.4 ± 1.02 and 11.5 ± 0.98 respectively. The study further extended to determine the growth profile of *Lactobacillus plantarum* (LAB) isolated from sheep milk, in the milk of different buffalo Indian breeds. The isolate was identified as a LAB by 16S rRNA sequencing technique. These LAB thrived in the different milk were characterized by colony forming unit (CFU). The study finally revealed that CFU count was found significantly higher ($P<0.05$) in Mehsana milk as comparative to other milk treated samples of Nagpuri and Bhadawari buffalos.

Keywords: *Bubalus bubalis*, buffaloes, Mehsana, Bhadawari, Nagpuri, lactobacillus, colony forming unit

INTRODUCTION

Milk is considered an essential functional food in the human diet. The major source of milk in India, especially in Maharashtra, is milking from buffalo. Along with the quantity, quality of milk, with its content and composition makes a lot of impact in dairy industries. One of the most important components of milk as a functional food includes lactic acid bacteria. These bacteria are friendly in nature boosting the natural immune system of the host and is called as probiotics (Abd-Talib *et al.*, 2013). These microbes act as a nutraceutical agent conferring health benefits. The lactobacillus (LAB) genera from the probiotics category found in the milk of different animals. These include *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* etc. used by many dairy industries as the active starter culture for many preparations including milk products and beverages (Barbosa *et al.*, 2016). Many researchers used fruit juices as a media to grow these bacteria. Milk is found to be the natural media to flourish this LAB. Till date, it was believed that the growth of LAB depends on temperature, pH, and nature of media. Many artificial media are used by the microbiologist for

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the isolation and culturing of the LAB. It proves that the composition of synthetic media not only affect but can alter the growth rate of the LAB. Considering this statistical idea the content of milk and nutrition is needed to study in case of milking buffalo. The content of milk i.e. fat, protein, lactose and total solid content vary in its concentration in a different strain of buffalo milk. LAB are nowadays flourished in different milking animal's fermented milk even in fruit-based nutraceutical beverages (Ozcan *et al.*, 2015). The demand is increased to culture the probiotics potential LAB in the milk of cow, goat, and buffalo, which significantly helped in the prevention of enteric infection, but in fact, it is not yet successfully demonstrated (Patil *et al.*, 2015). It is also reported that natural media with a different concentration of the minerals promotes the growth of LAB (Elli *et al.*, 2000).

The protein and total solid content reported for water buffalo are 4.35% and 17.40% respectively (Ahmad *et al.*, 2008) while the protein content value of about 8.7% is described by many researchers (Ariota *et al.*, 2007). Also, the quantification of lactose and casein determines the quality of milk (Aurelia *et al.*, 2009). According to 19th 2012 livestock census, 5594 buffalos were reported in Maharashtra by the National Bank of Agriculture and Rural Development Committee, India (Anonymous, 2012). In this, Bhadawari, Mehsana and Nagpuri types were found suitable for milk production in Maharashtra state. Bhadawari water buffalo are raised in Agra and Etawah region of Uttar Pradesh, India (Anonymous, 2018). The average milk yield is 800 kgs per lactation. The origin of Mehsana buffalo is from Gujarat, India. The average milk yield is 1300 kgs per lactation. The breed Nagpuri also called as Barari, is from Nagpur, India. The average milk yield of this breed is approximately 1100 kgs per lactation.

However, the dairy industries in India not only consider the quantity of milk but overlook the quality aspects in term of fat, protein, lactose and solid content and even microbial content. Mostly dairy industries use different types of LAB genera as a starter culture for milk product preparation (Colombo *et al.*, 2014). The growth parameters of this culture vary as per the type of milk or raw material used for the flourishing. Similarly, in this context, an attempt is made to determine the growth profile of *Lactobacillus plantarum* in the milk of different breeds of buffalo. Thus, the effect of the physiological composition of Bhadawari, Mehsana and Nagpuri milk, i.e. fat, protein and total solid content is investigated to understand the growth pattern of the LAB, as it is used as natural flourishing media.

MATERIALS AND METHODS

Isolation of lactobacillus and flourishing in the milk of different buffalo breeds

The LAB was isolated from sheep milk using selective artificial De Man, Rogosa and Sharpe broth and agar media (MRS, Siffin Pharma, Germany). The isolated colonies were incubated in an anaerobic jar at 37°C for 24 h using MRS plate (Ozcan *et al.*, 2015). The colonies after incubation were transferred into MRS broth media and kept at 4°C for further experimentation. The detailed identification of isolated LAB was done by using 16S rRNA illumination Nextseq platform technique, Netherlands.

Briefly, LAB culture from MRS broth was homogenized using 50 µL of phosphate buffered saline (PBS, 8 mM phosphate, pH 7.2). Total DNA of the LAB was extracted using illustra tissue and cells genomic Prep Mini Spin kit (GE Healthcare,

Buckinghamshire, UK). Further, the pool of 16S rRNA gene from respective LAB sample was amplified and analyzed using illumination Nextseq platform.

A total of 151 milk samples were collected from three breeds of buffalo i.e. Bhadawari (n=50), Mehsana (n=50) and Nagpuri (n=51). The buffalo milk of approximately 40 ml was used for the experimentation. This was further treated by ultra-high-temperature (UHT) pasteurized for 8 seconds in an alternative cycle for a period of 60 minutes. These collected milk samples were kept in a sterile 50 ml centrifuge tube at 4°C before use. The total solid content (%), protein (%), casein (%), density (%), freezing point (%), fat (%) and lactose (%) were determined by FOSS milk scan™ 120 (calibrated with buffalo standards, Warna dairy, India) milk analyzer.

The isolated LAB from MRS Broth was transferred via nichrome loop in the milk of these different breed buffalo and incubated, evaluated for its colony forming unit (CFU) count and consistency.

Statistical analysis

Multifactorial analysis of variance was used to analyze the LAB growth in the milk of all milking animals. GraphPad Prism 5.01 (GraphPad Software, Inc.USA.) software was used for basic analysis and graphical data interpretation with the determination of a level of significance at $P < 0.05$.

RESULT AND DISCUSSION

Identification of LAB isolates and the chemical composition of milk

Isolated LAB was confirmed as *Lactobacillus plantarum* by 16S rRNA gene

sequencing technique (Balakrishna, 2013). It was confirmed by identification of avian LAB isolates with BLAST (Basic local alignment search tool) as:

CTGGGTTTGACATGTACCGGACGACTG
CAGAGATGTGGTTTCCCTTGTGGCCG
GTAGACAGGTGGTGCATGGCTGTCGT
CAGCTCGTGTCTGAGATGTTGGGTTA
AGTCCCACAACGAGCGCAACCCTTGTCCT
GTGTTGCCAGCACGTGATGGTGGGGACTC
GCAGGAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGACGACGTCAAGTCATCAT
GCCCTTATGTCCAGGGCTTCACACAT
GCTACAATGGTCGGTACAGAGGGCTGC
GATACCGTGAGGTGGAGCGAATCCCTTA
AAGCCGGTCTCAGTTCGGATCGGGGTCT
GCAACTCGACCCCGTGAAGTCGGAGTC
GCTAGTAATCGCAGATCAGCAACGCTGCG
GTGAATACGTTCCCGGGCCTTGTACACAC
CGCCCGTCACGTCATGAAAGTCGGTAA
CACCCGAAGCCGGTGGCCTAACCCCTC
GTGGGAGGGAGCCGTCGAAGGTGGGATC
GGCGATTGGGACGAAGTCGTAACAAGG
TAGCCGTACCCGAAGGTGCGGCTGGAT
CACCT. The results revealed that LAB with 98% similarity with *Lactobacillus plantarum*.

The various nutrient content i.e. protein, fat, lactose, total solid content and percentage of casein of the raw milk for Nagpuri, Mehsana and Bhadawari were 4.5 ± 0.51 , 5.4 ± 0.62 , 4.8 ± 0.41 ; 7.2 ± 0.32 , 6.1 ± 0.66 , 7.0 ± 0.84 ; 5.4 ± 0.65 , 6.2 ± 0.65 , 5.1 ± 0.54 ; 17.2 ± 1.21 , 18.8 ± 1.54 , 18.1 ± 1.22 ; 10.8 ± 1.20 , 11.4 ± 1.02 , 11.5 ± 0.98 ; respectively. No any significant ($P < 0.05$) changes were observed in the nutrient content of raw and pasteurized milk of all breeds as shown in Figure 1, Figure 2, and Figure 3 respectively. The protein and lactose content of Mehsana milk ($P < 0.05$) was found to be significantly higher as compared to milk of the

other two breeds. On contrary, the fat content of Mehsana buffalo was found to be significantly lower ($P<0.05$) as compared to the milk of Nagpuri and Bhadawari respectively. The result obtained in form of protein, fat, lactose, casein and total solid content was represented in form of Figure 1, Figure 2, and Figure 3 respectively.

The current study showed that lactose content in milk of Mehsana was higher 6.2 ± 0.65 than previously reported studies (Lingathurai *et al.*, 2009). Many other reports demonstrated that the lactose content of Nagpuri and Bhadawari is similar to the Anatolian water buffalo (Mahamood, 2010). The fat content observed in the case of Nagpuri and Bhadawari was found to significantly higher as compared to previously reported results (Lingathurai *et al.*, 2009).

In the case of Mehsana milk, the total solid content of 18.8 ± 1.54 , protein 5.4 ± 0.62 and lactose 6.2 ± 0.65 were found higher than the previously reported values (Enb *et al.*, 2009). The fat content reported in the case of Mehsana milk was found to be lower than earlier reported literature (Han *et al.*, 2007). The protein content values found in the case of Nagpuri and Bhadawari milk samples were found to be the same as that of water buffalos (Rosati, 2012). On contrary, the protein content found in the current milk samples studies of all breeds were found to be higher than reported data by Dame *et al.* (2010). The fat content values reported in the case of Mehsana milk was found to be lower as per previously reported results (Lopes, 2009). The casein content reported in the case of all Indian breeds were found to be higher than the previously reported results (Ariota *et al.*, 2007).

The growth estimation of lactobacillus in the milk of different buffalo breeds

The CFU count was determined using

serial dilution technique till 109 times by plate culture method. The analysis of CFU ml⁻¹ was carried out in the milk of Mehsana, Bhadawari, and Nagpuri. The difference in the cell count was observed with the highest CFU count 109 in the milk of Mehsana ($P<0.05$) as compared to the milk of Nagpuri and Bhadawari showing the cell count in range 108~9 as shown in figure 4. Moreover, these LAB flourished in the milk of Mehsana was found to be higher than water buffalo (Elli *et al.*, 2000). Many researchers found that LAB can be flourished in fruits juice (Anekella *et al.*, 2014). But the current study shows that LAB growth is highest in Mehsana milk as compared to other milk or other natural media used for its thriving. The reason for the growth may be the higher lactose, protein and total solid content in milk of Mehsana as compared to the other breeds.

Thus, the current study proves that the quality of milk in terms of lactose, total solid content and protein affect the growth of LAB. These nutrient values were found higher in the milk of Mehsana milk as compared to Bhadawari and Nagpuri. Thus, Mehsana milk is found suitable for the flourishing of the LAB. Additional studies are needed to be done to evaluate the other factors responsible for the growth of LAB in the milk of Mehsana buffalo.

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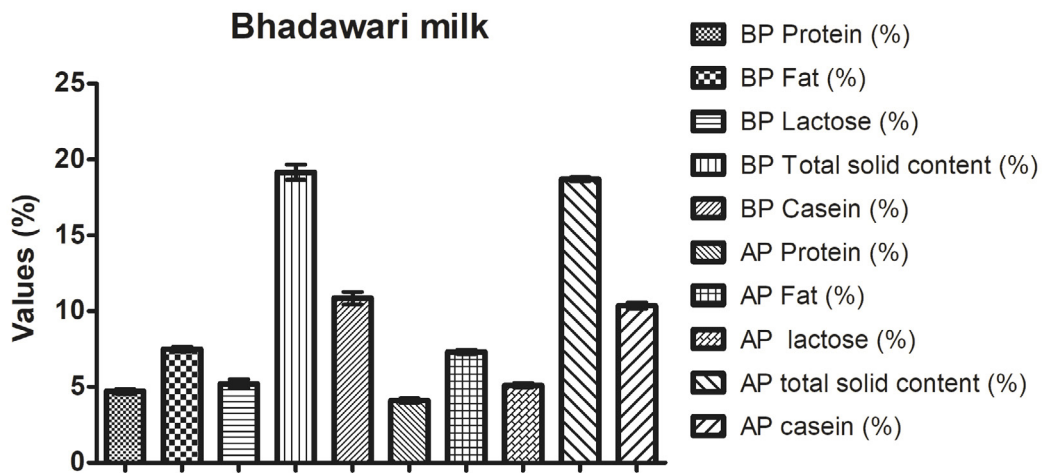


Figure 1. The physiochemical composition of Bhadawari milk.



Figure 2. The physiochemical composition of Mehsana milk.

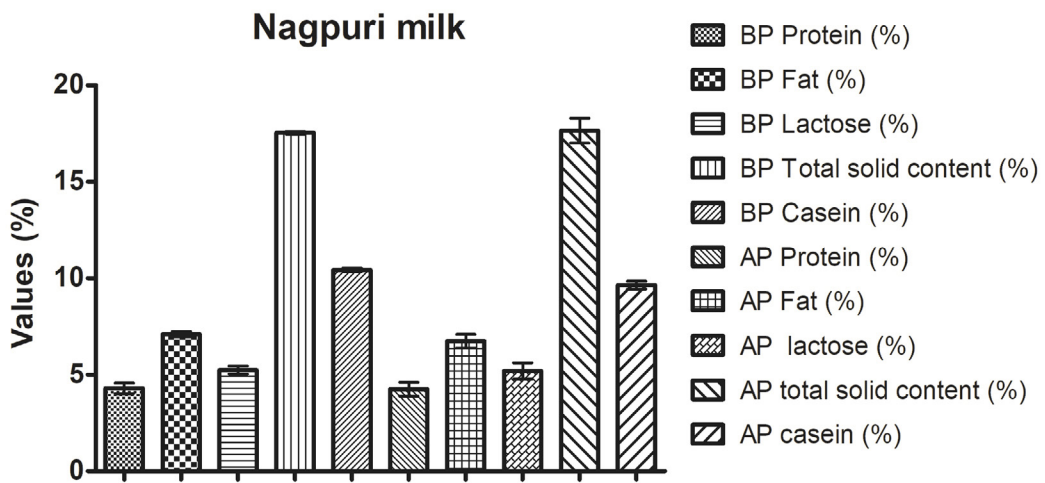


Figure 3. The physiochemical composition of Nagpuri milk.

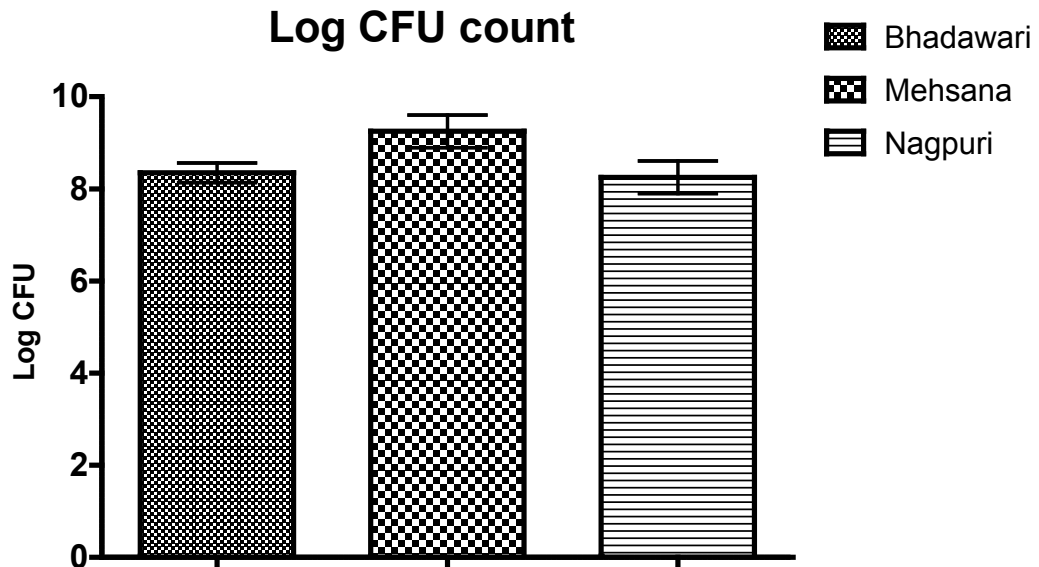


Figure 4. The CFU count of the LAB in the milk of different breeds of Indian buffalos.

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