Advances in Bioresearch
Adv. Biores., Vol 11 (4) July 2020: 84-88
©2020 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.4.8488

Advances in Bioresearch

ORIGINAL ARTICLE

Detection and Enumeration of Lactic Acid Bacteria from Human Colostrum using Traditional Microbiology Techniques

Ritesh Kumar Arya*[1] Jaspreet Singh[2] Amar Garg [3]
[1,2] School of Life Sciences, Jaipur National University, Jaipur.
[3]Vice Chancellor, Shobhit Deemed University, Meerut.
Corresponding Author : ritesharya43@dr.com, +918128137690

ABSTRACT
Human colostrum (HC) is the most basic necessity for proper growth and development of infant and it is very rich in all kind of nutrients essential for nourishment. Traditionally, HC was considered to be a sterile fluid. But, recent studies reveals that apart from all the nutritional aspects, HC also contains large number of commensals and mutualistic bacteria that has the huge potentials of probiotics. These bacteria are generally found to be Lactic Acid Bacteria (LAB). Several studies have demonstrated the presence of LAB with probiotic potentials in human milk, but very little scientific information on the number of bacteria present in HC is available till date. Therefore, LAB were isolated and enumerated from HC so that the precise number could be known. The entire study was carried out using traditional microbiology culture techniques. The study found that HC is a rich source of LAB isolation. Large number of LAB species were isolated from 60 different lactating mothers. All the species of LAB were confirmed biochemically using Bergey's Manual of Systematic Bacteriology. The study found that the average number of LAB count per ml of healthy lactating mother ranges between 10^8 to 10^9. The study also found that LAB count of mothers with C-section deliveries were observed to be very low due to intake of antibiotics during lactation period. Several other cases of low LAB count were also observed due unbalanced or poor diet. The current study deals with the detection and enumeration of LAB from HC.
Keywords *: Human Colostrum, Lactic Acid Bacteria, Probiotics, Infant gut, Gut microbiota.*

Received 22.03.2020 Revised 22.05.2020 Accepted 27.06.2020

How to cite this article:
R K Arya, Jaspreet Singh, Amar Garg. Detection and Enumeration of Lactic Acid Bacteria from Human Colostrum using Traditional Microbiology Techniques. Adv. Biores., Vol 11 (4) July 2020: 84-88

INTRODUCTION

Human Colostrum (HC) is a rich thick fluid providing complete essential nourishment for proper growth and development of the infant's organs [1]. The first milk produced by lactating mothers immediately after the delivery is called colostrum and it is biochemically and functionally different from the mature milk [2]. Colostrum, indeed, contains high concentration of lactoferrin, Immunoglobulin A (IgA), leukocytes and specific developmental factors, a low amount of lactose, potassium, calcium and a large number of Lactic Acid Bacteria (LAB). It provides immunity to the newborns[9].

Microbes are among the most important biological factors providing specific signals to guide immune system development and maturation [2]. Shifts in microbiota composition and activity appear to be related to adverse human health outcomes [7, 8]. The maternal microbial environment impacts the newborn's immune development and, consequently, the infant's health both at early and in later life.

The maternal microbiota is now recognized as a significant determinant of the maternally transferred factors that impact the child's health [1, 3, 4].Outcome can be affected by specific perinatal factors that also alter infant microbiome development, *e.g.*- excessive use of antibiotics, unbalanced diet, increasing incidence of cesarean section deliveries, unnecessarily stringent hygiene, and continuous stress influence the maternal microbiome. Alterations and disturbances in microbiota composition along with a reduction in microbial diversity or richness have been described as strong risk factors for the development of lifestyle diseases, such as allergies, diabetes, obesity, and metabolic syndrome, irritable bowel syndrome and other inflammatory-related problems [10].

The maternal microbiota and infant diet play a key role in the infant's growth, adequate microbial colonization, immune system maturation, and metabolic development. Thereafter, stepwise microbial colonization process have an impact on metabolic and immunological response, and these in turn may have an impact on programming of health later in life [6].

MATERIAL AND METHODS

Sample Collection:

All the samples examined in our study were collected from JNU Hospital, Jaipur (Rajasthan, India) during the period of June 2018 to September 2019. Total 60 HC samples were collected from different lactating mothers who voluntarily offered to become a part of the study. A written consent was taken from all the mothers who participated in the study. Sample collection was started only after getting permission from Institution Ethics Committee. All the samples were collected very carefully under aseptic conditions. All the lactating mothers were declared to be in good and healthy conditions. A detailed medical history was recorded using a proforma designed consulting a gynecologist as well as pediatrician. The breast nipples of lactating mothers were first cleaned with cotton dipped in alcohol. The tubes used for sample collection were autoclaved to avoid contamination. First, 1000µl of HC sample was discarded to avoid contamination from body flora. The mid flow of HC was carefully collected in sterile tubes and were immediately transferred to laboratory for further processing.

Isolation of LAB:

The fresh samples of HC were serially diluted by mixing 1ml of HC with 9ml of sterile Peptone water. Aliquots of dilution were prepared upto 10^{-6} by transferring 1ml of sample from each tube. The dilution of 10^{-4}, 10^{-5} and 10^{-6} were selected for inoculation. The Man, Rogosa and Sharpe(MRS) [HiMedia] agar plates were used for inoculation as it is selective media for growing of LAB. The spread plate technique was used for inoculation of inoculums on the MRS plates. 0.1ml of inoculum was transferred from each selected dilution. The plates were properly sealed with parafilm and were incubated at 37 □ for 48h under anaerobic conditions using anaerobic gas jar. Plates were observed for bacterial colonies after incubation period.

Enumeration of LAB:

After the period of incubation, plates were removed from the anaerobic gas jar and were observed for bacterial colonies. All the bacterial colonies were counted using digital colony counter. The colony forming unit per ml of sample was counted using the formula.

$$\text{CFU/ml} = \frac{\text{Number of colonies X Dilution Factor}}{\text{Volume Plated}}$$

The average number of CFU/ml were calculated by calculating the mean of three different plates of a single sample.

Identification of LAB:

All the distinct colonies grown on the MRS agar plates were further sub-cultured and were confirmed to be LAB using Gram's staining, Catalase test and Sugar Fermentation test.

Gram's Staining:

The pure culture of isolates were used for Gram's staining and other biochemical characterization. Slides were prepared for each isolate and were tested for its Gram's reaction using standard protocols [3]. All the slides were observed under microscope at 100x for confirming the cell morphology.

Catalase test:

Catalase is a type of enzyme produced by several microorganisms that breaks down hydrogen peroxide into water and oxygen and forms bubbles of gas. The formation of gas bubbles in the reaction indicates the presence of catalase enzyme.

$$2H_2O_2 \rightarrow 2\ H_2O + O_2$$

Catalase test was performed for all the isolates to check their catalase reactions. The overnight cultures of isolates grown of MRS agar plates were used for this test. 3% of hydrogen peroxide solution was dropped on pure culture and was observed for gas bubble formation.

Sugar Fermentation Test:

Sugar Fermentation test were performed for each isolate to confirm their Genus and species. Different sugars such as Maltose, Glucose, Lactose, Galactose, Mannitol, Xylose, Fructose were used. The isolates were inoculated in different sugars using standard protocol and were incubated and observed for acid and gas production. The results were interpreted using Bergey's Manual of Systematic Bacteriology [5].

RESULTS

All the 60 HC samples examined in our study showed the positive growth of LAB on MRS agar plates as shown in [Figure 1]. The characteristics of colonies formed on plates varied from sample to sample. The LAB count in each ml of HC of lactating mothers ranged between 10^2 to 10^8 as shown in [Table 1]. All the isolates tested in our study were gram positive and catalase negative which strongly signified the confirmation of LAB at initial stage. The Gram's morphology of bacteria observed under microscope varied from isolate to isolate as shown in [Figure 2]. The results of sugar fermentation test helped us in identifying species of LAB using Bergey's Manual of Systematic bacteriology. The most common LAB found in majority of the HC samples were *Bifidobacterium longum, Lactococcus lactis, Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium bifidum, Lactobacillus fermentum.*

Table 1: Enumeration of LAB from HC using Standard Plate Count.

HC1	1.2×10^7	HC21	6.1×10^7	HC41	4.9×10^3
HC2	5.8×10^6	HC22	6.7×10^3	HC42	5.7×10^6
HC3	3.5×10^7	HC23	6.8×10^6	HC43	3.8×10^4
HC4	1.9×10^2	HC24	5.4×10^5	HC44	6.3×10^7
HC5	2.2×10^6	HC25	6.2×10^7	HC45	6.7×10^8
HC6	8.0×10^4	HC26	4.2×10^3	HC46	4.8×10^6
HC7	6.2×10^7	HC27	9.2×10^5	HC47	5.2×10^6
HC8	2.6×10^5	HC28	8.8×10^4	HC48	6.6×10^5
HC9	5.4×10^7	HC29	3.5×10^8	HC49	5.2×10^3
HC10	7.4×10^7	HC30	4.6×10^6	HC50	7.2×10^7
HC11	6.3×10^7	HC31	7.3×10^7	HC51	4.6×10^5
HC12	2.8×10^5	HC32	5.8×10^5	HC52	8.5×10^6
HC13	7.4×10^7	HC33	6.4×10^7	HC53	5.4×10^2
HC14	3.8×10^5	HC34	7.6×10^5	HC54	7.4×10^6
HC15	4.5×10^7	HC35	3.4×10^3	HC55	3.1×10^3
HC16	6.3×10^6	HC36	5.8×10^8	HC56	5.8×10^8
HC17	3.8×10^7	HC37	4.6×10^3	HC57	7.7×10^6
HC18	4.6×10^4	HC38	7.7×10^7	HC58	4.8×10^7
HC19	5.7×10^7	HC39	4.8×10^5	HC59	4.6×10^5
HC20	3.4×10^6	HC40	6.4×10^6	HC60	5.1×10^3

Figure 1: Isolated LAB on MRS agar plates

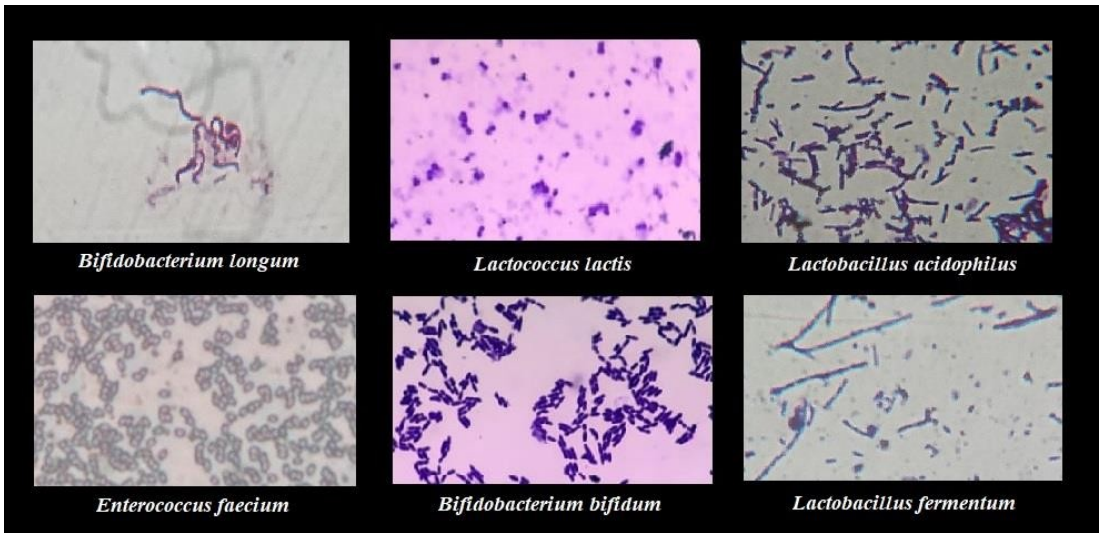

Figure 2: Gram's staining observation under compound microscope.

DISCUSSION

HC was traditionally considered to be a sterile thick fluid. But, this dogma has been revised over years. Large number of studies in the past have showed the presence of LAB in human milk, but several data regarding origin of LAB in Human Colostrum and its positive effects on infants is still a mystery. Traditional techniques of microbiology were used in the study because it is believed to be more reliable than modern microbiology techniques. Modern techniques have some limitations such as the viability of milk microbes cannot be analyzed, total bacteria counts may be over- or underestimated because of cell-wall composition, DNA extraction methods and the number of microbial 16S gene copies which may lead to the over- or underestimation of bacteria counts. Contamination in DNA extraction kit and reagents was also reported in the past studies [7]. Large number of variations were observed in the enumeration of LAB from HC, this could be due to several factors such as maternal diet, hygiene, intake of antibiotics during pregnancy, etc.

CONCLUSION

HC is not just a thick fluid but a rich source of LAB isolation with best probiotic potentials. Therefore, it should be promoted maximum. LAB with probiotic potentials from HC is relatively a new field which can revolutionize the market of probiotics by commercialization of intestinal probiotic bacteria. In several cases, such as, lactation failure and viral diseases which is very common is developing countries, many mothers fail to feed the first milk to infant. In such cases, some artificially genetically modified product should be brought to the market which can fulfill all the required criteria's of probiotics needed for proper growth of infant. Human Colostrum is a vast area of research and still needs more genuine studies to be carried out to justify the direct effects of LAB on infant's health.

ACKNOWLEDGEMENT

The entire work was supported by Jaipur National University, Jaipur (Rajasthan, India). We are greatly thankful to all the faculty members of School of Life Sciences who directly and indirectly contributed to our study. A special thanks to Mrs. Mamta (Nursing Assistant) for helping us in collecting HC samples from the maternity ward of JNU Hospital.

REFERENCES

1. Ballard O and Morrow A. L. (2013). Human milk composition: nutrients and bioactive factors. Pediatr. Clin. North Am , **60**: 49–74.
2. Bendiks M, Kopp MV. (2013). The relationship between advances in understanding the microbiome and the maturing hygiene hypothesis. Cure Allergy Asthma Rep,**13**(5): 487-494.
3. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. (2015). Maternal microbiome and pregnancy outcomes that impact infant health: a review. Adv Neonatal Care 2015, 15(6):377-385.
4. Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al.(2016). The maternal microbiota drives early postnatal innate immune development. Science, **351**(6279):1296-1302.
5. Hammes P H and Hertel C, In: Vos P.D., Garrity, G.M., Jones, D, et al. (2009).Bergey's Manual of Systematic Bacteriology, Springer, New York, Edi 3rd , 456-479.

6. Hooper LV and Macpherson AJ. (2010). Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol, **10**:159-169.
7. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. (2016). The gut microbiota and host health: a new clinical frontier. Gut **65**(2):330-339.
8. McGuire MK and McGuire MA. (2015). Human milk: mother nature's prototypical probiotic food?. Adv Nutr, 6(1):112-123.
9. Pang, W. and Hartmann P. E. (2007). Initiation of human lactation: secretory differentiation and secretory activation. J. Mammary Gland Biol. Neoplasia,**12**: 211–221.
10. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. Microbe Ecol Health Dis , **26**:26050.