International Journal for Research in Engineering Application & Management (IJREAM)
ISSN : 2454-9150 Vol-06, Issue-07, OCT 2020

Review on Bifidobacterium and its Applications

[1]G.Madhavi, [2]M.Shiva Prakash

[1]Research Scholar (PhD student) Clinical Epidemiology, National Institute of Nutrition (ICMR) Hyderabad, Telangana, India (madhavil75@gmail.com)

[2] Dr.M.Shiva Prakash, PhD Supervisor and Former Scientist ,Head, Department of Microbiology & Immunology, National Institute of Nutrition (ICMR) Hyderabad, Telangana, India (drmspnin@gmail.com)

Abstract - *Bifidobacteria* are Saccharolytic, hetero fermentative gram-positive anaerobes. It belongs to the phyla Actinobacteria. *Bifidobacteria* exists in the intestine of mammals and other animals. In the human intestine, Firmicutes and Bacteroides are predominant in adults and Actinobacteria in breast-fed infants. They are the first colonizing bacteria of neonates' intestines and play a prominent role in developing the neonatal gut and immune system. Bifidobacterium is considered as probiotics, based on their attributes, and it is widely used in the preparation of many dairy products and exerts more health benefits on the host and it has been used as therapeutic and as neutraceutical.This bacteria is included as a bioactive ingredient in functional foods mainly, dairy products, i.e. probiotic curd, yogurt, cheese and ice cream. This review will discuss the general characteristics of *Bifidobacteria* and the selection criteria of bacterial strains for the development of Bifidobased dairy products and their nutritional and beneficial health properties, and their use in the prevention and treatment of various diseases.

Keywords: ***Bifidobacterium, Health benefits, immune system, lactoseintolorence, Probiotics, Probioticcurd, yogurt***

I. INTRODUCTION

Probiotics are living microorganisms that are administered in enough numbers to survive in the intestine and provide a positive effect on the host. The term 'Probiotics' was first coined by Lilly and Stillwell [1] to describe the 'substances produced by one microorganism that stimulate another's growth. Probiotics include the genera Lactobacillus and *Bifidobacteria*.The *Bifidobacteria* is mostly used and studied probiotic bacteria. The *Bifidobacteria* were first isolated and identified in 1899–1900 by Tissier.[3] from the feces of breast-fed infants, he named the bacterium Bacillus Bifidus communis.The Bifidobacterium belongs to the family *Bifidobacteria*ceae. *Bifidobacteria* is characterized as Gram-positive bacteria, non-motile,nonsporing,catalase-negative anerobes.They have various shapes, including short, curved rods, club-shaped rods, and bifurcated Y-shaped rods. Presently, the branching nature and the cell shape of bifidobacteria depends on the strains and growth media [2].It was reported that depending On the composition of the culture medium, some strains of the genus Bifidobacterium may have a V or X shape and Y shape .Furthermore, the abundance of N-acetyl glucosamine, alanine, aspartic acid, glutamic acid serine and Ca2+ions in the growth medium can influence the cell shape of bifidobacteria [4]

II. THE CELL WALL STRUCTURE OF BIFIDOBACTERIA

Bifidobacterium is having gram positive cell wall and it contains various Structural components; it includes peptidoglycan (murein), teichoic acids, and polysaccharides [5] Peptidoglycan is composed of N acetyl muramicacid and N-acetyl glucosamine and it's inter peptide bridges differ among the various Bifidobacterium species. Therefore, sometimes this characteristic is used for the differentiation of the Bifidobacterium. [6] The Bifidobacterium cell wall contains polysaccharides and it is made up of glucose, galactose and rhamnose [7] and it plays an important role in their interaction with the host cells or tissues.

Growth conditions:

The optimal temperature for the growth of Bifidobacteriais37–41°C, the minimum is 25-28°C and the maximum is 43 -45° [8]. Most Bifidobacterium strains originated from humans grow optimally at 36–38°C.

Exopolysaccharide: The EPS are synthesized by Bifidobacterium extracellular by cell wall anchored enzymes, and it could have a protective role for them during Gastro intestinal transit [9]. EPS protective layer could help Bifidobacteria to survive against high acidic and high bile salt Concentration in the upper part of the gastrointestinal tract. As a result, EPS production could

 DOI : 10.35291/2454-9150.2020.0625

help to ensure the transit of strains Of Bifidobacterium from mouth to the small intestine without Destructive effect on their viability [10] EPS produced by strains of Bifidobacterium could modify the bacterial population and metabolic activity of the gastrointestinal microbiota, which is favorable for host health [11,12,13,14] Carbohydrate metabolism: Bifidobacterium is hetero fermentative organisms that produce acetic and lactic acids without generating CO_2 [15] .They can ferment fructose, Glucose, and Galactose [16] reported that some Bifidobacterium species could ferment lactose and grow in milk. *Bifidobacteria* degrade the monosaccharide by using the enzyme Xfp, it is called an F6PPK pathway. Xfp possesses dual-substrate specificity on fructose 6-phosphate or xylulose-5-phosphate. The end metabolites of the trail are ethanol, acetate, and lactate (1).F6PPK is the important biochemical test for identifying *Bifidobacteria*. More than 30species have been isolated and assigned to the genus Bifidobacterium [17] Bifidobacteria have been isolated and given from five different ecological niches the intestine the oral cavity, foods, insect gut, and sewage [18]. Species of Bifidobacterium, the most abundant species found in the human gastrointestinal tract include Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifido bacterium breve, Bifido bacterium catenulatum, Bifidobacterium pseudo catenulatum, Bifido bacterium longum subsp.infantis (B.infantis), Bifidobacterium longum subsp.longum (B.longum), Bifidobacterium angulatum, and Bifidobacterium dentium [18].Current studies indicate that *Bifidobacteria* are transmitted vertically from the mother's vagina,

G.I. tract, or Breast milk . The number of *Bifidobacteria* decreases with an individual's increasing age and eventually becomes the third most abundant genus accounting for approx25% of the total adult gut flora.

The properties that a strain must have to be further tested for human probiotic use are (i) genera of human origin; (ii) It must be regarded as safe (GRAS) iii) it should be stable against acid, bile, oxygen, and enzyme (iii) ability to attach to intestinal mucosa (iv) colonization potentiality in the human intestine(v) production of antibiotics substances (vi) safety and demonstrable efficacy (vii) Anti carcinogenic and antimutagenic properties (viii) Good sensory and viability properties (ix) Phage resistance (x) stability of the product during storage.

Isolation of *Bifidobacteria* :Culture media used for the detection and enumeration of *Bifidobacteria* may be divided into selective complex, semisynthetic and synthetic, as well as commercial, and can be classified as non-selective media with elective carbohydrates, media with antibiotics, media with sodium propionate and lithium chloride and media with elective substance and/or low pH. Media belonging to more than one group are also used [19]. There is no standard medium for the detection of *Bifidobacteria* however, the availability of easy and inexpensive methods for detection, identification, and enumeration of Bifidobacterium spp. is important in food microbiology. The selection of an adequate culture medium for *Bifidobacteria* should be based on the following parameters: supply of nutritive substances to produce optimal growth; low redox potential; maintenance of pH value during growth by an effective buffering capacity; final pH of the prepared medium; optimal growth medium. Anaerobic conditions are also an important factor in detecting and enumerating *Bifidobacteria*. The success of *Bifidobacteria* detection in an optimal growth medium is mainly dependent upon the following factors : a) if the culture medium has no selective effect, non *Bifidobacteria* may outgrow *Bifidobacteria*; b) the ease of macroscopic identification of *Bifidobacteria* colonies which may be facilitated using indicators; c) the freshness of the ingredients of the medium; d) the composition of the culture medium which should allow the growth of different bio-types present in the material investigated [20] .

The isolation of Bifidobacterium sps from various sources has been a challenging task. Several media are used for the enumeration of Bifidobacterium sps. Reinforced Clostridia Agar and MRS Agar containing cysteine, Columbia agar medium containing lithium chloride and sodium propionate are used as selective media in dairy products and quality control laboratories.Wilkins Chalgren's agar, containing 100 mg/L Mupirocin, which is a selective medium of Bifidobacterium species from milk and cheese. MTPY medium is used for isolating *Bifidobacteria* from hen's gastrointestinal tract.Raffinose Propionate lithium Mupirocin is used for the isolation of Bifido bacteria from milk. *Bifidobacteria* is isolated from infant feces by two newly-modified Gerche's media: 1). One with stimulating *Bifidobacteria* growth maltodextrin instead of lactose. 2). Addition of lithium chloride (3g/L) and Penicillin G, Sodium salt (50 U/L). Reinforced Clostridia Medium (RCM) plus 1% lactose supplemented with 5% blood or 2% erythrocytes concentrate for total anaerobic counts separation of *Bifidobacteria* in yogurt-like products [21].. [.Mitsuoka suggested using B.L. agar for nonselective enumeration of *Bifidobacteria* from dairy products and intestinal materials. A selective medium, blood-glucoseliver agar containing oxgall(.2 mg/ml) and gentamicin (30mg/ml) was formulated for the selective enumeration of *Bifidobacteria* in fermented dairy products containing both lactobacilli and streptococci. Recovery rates of *Bifidobacteria* on this selective medium were around 90% with bloodglucose-liver agar. Strains of lactobacilli and streptococci were mostly inhibited with higher dilutions on this selective medium.[22]

Identification of *Bifidobacteria*: There are many molecular methods for the identification, characterization, and detection of Bifidobacterium, and many of these techniques are based on the 16S ribosomal gene. PCR and ARDRA are

 DOI : 10.35291/2454-9150.2020.0625

two easy and sensitive methods for the detection and identification of the Bifidobacterium genus and species. The most accurate way for differentiation at the strain level is the PFGE. The Real-Time PCR will be very popular for the detection, identification, and quantification of Bifidobacterium spp.[23,24]

Functional properties: To have functional effects in the intestine, probiotics have to survive in the gastrointestinal tract. Thus, it is critical for bacteria to sustain various acidic pH and gastric enzymes in the stomach,bile, pancreatin, and other intestinal enzymes in the small intestine [25]. Also, probiotics should adhere to the intestinal mucosa and significantly inhibit various entero pathogenic bacteria [26, 27]. Adhesion auto aggregation and hydrophobicity of Lactobacillus and *Bifidobacteria* have been found to be strongly related [26, 28, 29] but with the exception in Lactobacillus[30,31].Hydrophobicity and autoaggregation are based on the proteins, glycoproteins, teichoic, and lipoteichoic acids on the cell-wall surface of bacteria, and secreted factors [32]

Safety aspects: The safe use of strains of Bifidobacterium has been supported by the prolonged historical consumption of fermented dairy products containing such strains and the growing knowledge of bifidobacteria taxonomy and physiology.[33,29,34] summarised safety aspects of the selection of new probiotic strains;determination of antibiotic resistance pattern, assessment of certain metabolic activities, assessment of side effect during human studies, epidemiological surveillance of adverse incidents in consumers, toxin production (if strain belongs to a species which is known as mammalian toxin producer) and haemolytic activity(a species with known haemolytic activity).However, there is no reported case of local or systemic infections (septicemia,meningitis, and endocarditis) with the ingestion of dairy products containing Bifidobacteria [33,25].In addition, the human origin is often used as a selection criterion for Bifidobacterium to be used as probiotics in food products [35,36,37].

Technological aspect: Several technical elements have to be considered when selecting probiotic strains, such as viability and stability during development of the product and during storage, good sensory properties, and phage resistance [38].The probiotic strains should be viable at the time of consumption and It has been suggested that the minimum concentration of live probiotic bacteria at the expiry date of the product should be around 10^7 CFU/mL−1[39].Therefore, suitable strains should be able to resist throughout the shelf life. A lot of factors have been suggested to affect the viability of probiotic bifidobacteria in fermented dairy products, including the pH and acid content of the product, the levels of hydrogen peroxide which is produced by the traditional starter lacticacid cultures,and the oxygen levels within the product, which are to a large extent affected by the packaging [40,41] In general, bifidobacteria are not acid-tolerant, and poor viability and growth of most Bifidobacteriumspp. Are observed at pH below4.0 [42].The strains with a high survival rate in acidic conditions should be selected for use in fermented dairy products [16]. Furthermore oxygen and H2O2can affect the viability of Bifidobacterium in dairy products [43].Oxygen is toxic to the cells, and it enhances intercellularH2O2production, which reduces the viability of cells. Moreover, oxygen may enhance H2O2 production from other cultures in fermented milk product [41] .In general,bifidobacteria are considered as highly susceptible to oxygen, although oxygen tolerance has been shown to be species dependent. For example, B.animalis subsp. Lactis strain is olated from fermented milk was found to display good oxygen tolerance.Recently,the use of low oxygen-permeable packaging materials with glucose oxidase was shown to increase the viability of Bifidobacteria in milk products[42,43,44,45].Furthermore,N and H were shown to increase the survival of Bfidobacteria in fermented milk products during storage[46]. Bifidobacteria is a valuable dietary adjunct that can be incorporated into dairy products adding additional functional and health attributes to the milk products. Screening of suitable strains of Bifidobacteria for application in milk products is a key area in the changing functional dairy product market. Bifidobacterium, as a probiotic agent in human food, has been widely used because it is generally recognized as safe. Some strains have been used in fermented dairy products and exert a wide range of beneficial health effects, including the prevention or alleviation of infectious diarrhea, the inhibition of growth of pathogens and harmful bacteria, the modulation of the local and systemic immune responses, and so on. However, the viability of some of these probiotic strains for food use sharply decreases during the product shelf life. Moreover, many of these bacteria may be killed during their transit through the stomach's acidic conditions and be degraded or inactivated by hydrolytic enzymes and bile salts in the small intestine [47]. Therefore, the gastrointestinal survival capacity is considered as an essential criterion for the initial selection for potential probiotic strains. Moreover, the following sections discuss some of the health benefits associated with the consumption of dairy products containing *Bifidobacteria*. Bifidobacterium could increase the bioavailability of minerals, such as calcium, zinc, iron, manganese, copper, and phosphorous, by lowering the gastric pH level. Furthermore, it has been reported that the ingestion of fermented milk with *Bifidobacteria* increased the digestibility of the milk proteins [48].

Development of Bifido fermented products: The milk was boiled for one hour in the laboratory with constant stirring and then the milk was poured into steam sterilized (15 min) clay pots (500 mL per each pot) and allowed to cool to the incubation temperature, 40–45 C. 1-day old curd

 DOI : 10.35291/2454-9150.2020.0625

pot was used as the inoculum and was mixed thoroughly in a steel cup before adding to the buffalo milk.

A teaspoonful of inoculum (3 g) was added to each cup and allowed to ferment at ambient temperature at 370c. The *Bifidobacteria* inoculum was added at the same time to give a final *Bifidobacteria* population of around 10^8 CFU mL^{-1}. Curdling of milk was complete after approximately 5-6 hrs of incubation at ambient temperature. The viable counts of LAB and *Bifidobacteria* were determined by using standard microbiological methods during and upon the storage of products. After product development, the curds were tested for sensory evaluation and viability. The sensory evaluation was done by using 9-Point Hedonic scale.

Bifido based products: Fermented foods are major carriers of probiotic bacteria with a positive health effect. In particular, fermented dairy products have been increasingly attracting the attention of consumers. There is a rapid increase in using probiotic bacteria in different food products due to a better understanding of these bacteria's role in maintaining the health of the host. It is reported that more than 500 probiotic products have been introduced into the global market during the past decade [43]. There are many popular delivery systems for *Bifidobacteria*, such as freshly fermented or unfermented dairy foods, including milk, yogurt, ice cream, desserts, cheese, beverages, cookies, and milk powder [44, 45, 46, 47, 48, 49,50,51,52]. Different strains of Bifidobacterium are incorporated as cell suspensions or freeze-dried form depending on the food product. The Bifidobacterium sps. are the most frequently used human probiotics because of their health benefits [53] .Improvement of the intestinal microbial balance of the host, reduction the gastrointestinal disorders, assimilation of cholesterol, and immunomodulatory effects? [54, 55, 56, 57,]

***Bifidobacteria* reduces serum cholesterol level:**

The cholesterol-lowering effect of dairy products containing Bifidobacterium strains was shown based on human and animal studies [58, 59, 60] demonstrated that Bifidobacterium milk fermented with B. longum could reduce serum lipid levels in humans and rats. In another study [61] shown in a rat model that Bifidus yogurts and yogurts fortified with whey proteins reduced the total and LDL cholesterol levels. One possible mechanism proposed for this was that the presence of organic acids, especially conjugated linolic acid (CLA) produced by these bacteria, could inhibit cholesterol production. Alternatively, it was suggested that the bacteria might have an effect on bile acids, which might hinder the absorption of cholesterol from the intestinal tract [62] However,[63] more research and extensive mechanistic studies are needed to achieve firm conclusions regarding *Bifidobacteria*'s ability to confer health benefits to the cardiovascular system.

***Bifidobacteria* and reduction of lactose intolerance:**

Probiotics are widely known to reduce the symptoms of lactose malabsorption. This condition is associated with the incomplete breakdown of lactose, the principal carbohydrate of milk, into its monosaccharide's, glucose, and galactose. It occurs due to the deficiency of the enzyme β-galactosidase, which is responsible for the breakdown of lactose [64] .When undigested lactose is passed into the large intestine; it is fermented by the indigenous micro flora into gases (CH4, CO2 and H) and short-chain fatty acids. The undigested lactose and gas production cause gastrointestinal symptoms, includes flatulence, abdominal pain, and diarrhea .*Bifidobacteria* have been reported to increase the production of β-galactosidase concentrations, which can improve the digestibility of lactose, in the alleviation of the symptoms of lactose malabsorption [65]. A later study indicated that yogurt enriched with B. animalis could modify the colonic microbiota's metabolic activities and alleviate lactose-intolerant individual [66] investigating B. longum,reported a positive effect on reducing lactose intolerance in some individuals.

***Bifidobacteria* controls diarrhea:** Many clinical studies indicate that *Bifidobacteria* containing dairy preparations can modify the course of viral and bacterial intestinal disorder [67]. A milk formula containing B. bifidum and S. thermophilus was shown to reduce rotaviral infection [68]. However [69] reported that lactose-free milk formula blended with B. lactis Bb12 and S. thermophilus TH4 could decrease the rotavirus shedding but not its duration of diarrhea in infants. Furthermore, it was shown that B. bifidum was able to neutralize some toxins. It was also reported that it could reduce the incidence of antibiotic-associated diarrhea.

***Bifidobacteria* to control inflammatory bowel diseases:** Inflammatory bowel disease (IBD) can be divided into two categories, Crohn's disease (CD) and ulcerative colitis (U.C.). However, the mechanism involved in the immunopathological and genetic basis of IBD is not yet fully understood [70,71] It was reported that mice fed with skim milk containing B. bifidum showed normal weight growth, fewer clinical symptoms, such as thickened wall and inflammatory cell infiltration, and lower levels of CD4+ T lymphocyte infiltration and inflammatory cytokine than the mice with IBD, fed with skim milk containing *Bifidobacteria* showed that the consumption of fermented milk containing B. bifidum, B. breve, and L. acidophilus could be successful in maintaining remission, and had possible preventive effects on the U.C. [71] [72].

***Bifidobacteria* and colon cancer:** Dairy products, such as yogurt, containing probiotic lactobacilli, and *Bifidobacteria* have been used to reduce the risk of colon cancer . [73] Probiotics are thought to control tumors in three ways. They can inhibit tumor cells and suppress the bacteria that produce β-glycosidase, β-glucuronidase, and azoreductase,

 DOI : 10.35291/2454-9150.2020.0625

an enzyme that catalyzes the conversion of procarcinogens to proximal carcinogens. Moreover, they can destroy carcinogens, such as nitrosamines, and suppress nitroreductase, which is involved in their synthesis [74]. However indicated that without well-defined mechanisms for the anticancer effects of probiotics, it is challenging to develop more effective, targeted probiotics, which can be evaluated for their activities in human intervention trials.[75].A few studies have investigated the potential anti carcinogenic activities of Bifidobacterium containing dairy products.

***Bifidobacteria* and the immune system:** The fermented products could be used to stimulate the mucosal immunity, promoting an increase in the number of Immunoglobulin A which are considered to be the first line of defence against infections [76].One of the studies reported Bifidobacterium lactis (DR10TM strain and HN019,) had been shown to enhance phagocytic cell function, and the production of interferon-gamma in laboratory animals. Several studies have been reported that the *Bifidobacteria* based dairy products shown an immune stimulation. Milk containing B. lactis was shown to be effective in enhancing some aspects of cell-mediated immunity in the elders [77]. In another study, it was demonstrated that yogurt containing probiotic strains B. lactis & L.acidophilus could modulate the non-specific cellular immune response by the increased phagocytic activity in humans [78]. In another study, supplementation of fermented milk containing B. bifidum Bb12 or L. acidophilus La1 for three weeks resulted in an increased phagocytic activity of the peripheral blood leukocytes in human [79].In addition to the probiotic bacteria, fermented milk possesses other non-bacterial components produced during the fermentation that can contribute to immunogenicity. For example, peptides and free fatty acids released during milk fermentation were shown to increase the immune response [80].

III. CONCLUSION

Bifidobacteria is considered as a probiotics and it is a valuable dietary adjunct and it can be incorporated into milk. It could be used as a therapeutic and neutraceutical agent. It is an alternative potent therapeutic in a large number of pathologies. Screening of suitable strains of *Bifidobacteria* for application in milk products is a critical area in the changing functional dairy product market. Also, there is a need for more well-designed studies to understand the actual mechanisms.

REFERENCES

[1] Lilly DM, Stillwell RH (1965) Probiotics growth promoting factors produced by micro-organisms. Science 147:747–748.

[2] Tannock, G. W. (1999). Identification of lactobacilli and bifidobacteria. Current Issues in Molecular Biology, 1(1–2), 53–64.

[3] Ballongue, J. (2004). Bifidobacteria and probiotic action. In A. V. Wright, & A. Ouwehand (Eds.), Lactic acid bacteria: Microbiological and functional aspects (pp. 67–125). New York: CRC.

[4] De Dea Lindnera, J., Canchayab, Carlos, Zhangc, Ziding, Neviania, Erasmo, Fitzgeraldb, Gerald F., Sinderenb, D.V., et al. (2007). Exploiting Bifidobacterium genomes: The mo-lecular basis of stress response. International Journal of Food Microbiology, 120(1–2), 13–24.

[5] Gomes AMP & Malcata FX (1999) Bifidobacterium spp. And Lactobacillus acidophilus: biochemical, technological andtherapeutical properties relevant for use as probiotics. Trends Food Sci Technol 10: 139–157.

[6] Kleerebezem, M., Vaughan, E.E. (2009) Probiotic and gut Lactobacilli and Bifidobacteria: molecular approaches to study diversity and activity. Annu. Rev. Microbiol., 63: 269 290.

[7] Leahy, S.C., Higgins, D.G., Fitzgerald, G., & Sinderen, D. (2005). Getting better with bifidobacteria. Journal of Applied Microbiology, 98(6), 1303–1315.

[8] Biavati, B., Vescovo, M., Torriani, S., & Bottazzi, V. (2000). Bifidobacteria: History, ecology,physiology and applications. Annals of Microbiology, 50(2), 117–132.

[9] Shah, N.P. (2007). Functional cultures and health benefits. International Dairy Journal,17(11), 1262–1277.

[10] Leivers, S., Hidalgo-Cantabrana, C., Robinson, G., Margolles, A., Ruas-Madiedo, P., & Laws, A. P. (2011). Structure of the high molecular weight exopolysaccharide produced by Bifidobacterium animalis subsp. lactis IPLA-R1 and sequence analysis of its putative EPS cluster. Carbohydrate Research, 346(17), 2710–2717.

[11] Alp, G., & Aslim, B. (2010). Relationship between the resistance to bile salts and low pH with exopolysaccharide (EPS) production of Bifidobacterium spp. isolated from infants feces and breast milk. Anaerobe, 16(2), 101–105.

[12] Salazar, N., Prieto, A., Leal, J. A., Mayo, B., Bada-Gancedo, J. C., de los Reyes-Gavilan, C. G.,et al. (2009). Production of exopolysaccharides by Lactobacillus and Bifidobacteriumstrains of human origin, and metabolic activity of the producing bacteria in milk.Journal of Dairy Science, 92(9), 4158–4168.

[13] Salazar, N., Ruas-Madiedo, P., Kolida, S., Collins, M., Rastall, R., Gibson, G., et al. (2009).Exopolysaccharides produced by Bifidobacterium longum IPLA E44 and Bifidobacterium animalis subsp. lactis IPLA R1 modify the composition and metabolic activity of human faecal microbiota in pH-controlled batch cultures. International Journal of Food Microbiology, 135(3), 260–267.

[14] Salazar, N., Ruas-Madiedo, P., Prieto, A., Calle, L. P., & de los Reyes-Gavilán, C. G. (2012).Characterization of exopolysaccharides produced by Bifidobacterium longum NB667 and its cholate-resistant derivative strain IPLA B667dCo. Journal of Agricultural and Food Chemistry, 60(4), 1028–1035.

[15] Shah, N.P. (2006b). Probiotics and fermented milks. In R. C. Chandan (Ed.), Manufacturing yogurt and fermented milks (pp. 341–354). Oxford: Blackwell Publishing Ltd.

[16] Arunachalam, K. D. (1999). Role of bifidobacteria in nutrition, medicine and technology.Nutrition Research, 19(10), 1559–1597.

[17] Leivers, S., Hidalgo-Cantabrana, C., Robinson, G., Margolles, A., Ruas-Madiedo, P., & Laws,A. P. (2011). Structure of the high molecular weight exopolysaccharide produced byBifidobacterium animalis subsp. lactis IPLA-R1 and sequence analysis of its putativeEPS cluster. Carbohydrate Research, 346(17), 2710–2717.

[18] Ishizuka, A., Tomizuka, K. I., Aoki, R., Nishijima, T., Saito, Y., Inoue, R., et al. (2012). Effects of administration of Bifidobacterium animalis subsp. lactis GCL2505 on defecation frequency and

 DOI : 10.35291/2454-9150.2020.0625

bifidobacterial microbiota composition in humans. Journal of Bioscience and Bioengineering, 113(5), 587–591

[19] R Hartemink 1, F M Rombouts1999 Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from faecal samples Jun;36(3):181-92. J Microbiol Methods doi: 10.1016/s0167-7012(99)00031-7.

[20] Rasic, J. L., & Kurmann, J. A. (1983). Bifidobacteria and their role: Microbiological, nutritional–physiological, medical, and technological aspects and bibliography. Basel: Birkhauser Verlag.

[21] Reuter, G. (1990). Bifidobacteria cultures as components of yoghurt-like products. Bifidobacteria and Microflora, 9(2), 107-118.

[22] Roy, D. (2001). Media for the isolation and enumeration of bifidobacteria in dairy products. International Journal of Food Microbiology, 69(3), 167-182.

[23] Roy, D., Ward, P., (1992). Rapid detection of Bifidobacterium dentium by enzymatic hydrolysis of b-glucuronide substrates. J. Food Prot. 55, 291– 295.

[24] Pierre WARD, Denis ROY , Review of molecular methods for identification, characterization and detection of Bifidobacteria. Lait 85 (2005) 23–32, INRA, EDP Sciences, 2005 DOI: 10.1051/lait:2004024

[25] Sánchez B., Ruiz L., Gueimonde M., Ruas-Madiedo P., Margolles A. (2013). Adaptation of bifidobacteria to the gastrointestinal tract and functional consequences. *Pharmacol. Res.* 69 127–136 10.1016/j.phrs.2012.11.004.

[26] Del Re, B., Sgorbati, B., Miglioli, M. and Palenzona, D. (2000) Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacterium longum. Letters in Applied Microbiology 31, 438–442.

[27] .Ouwehand AC, Salminen S, Isolauri E (2002) Probiotics: an overview of beneficial effects. Ant Van Leeuw 82:279–289. doi:10.1023/A:1020620607611 [PubMed]

[28] Pan W H, Li P L, Liu Z (2006) The coorelation between surface hydrophobicity and adherence of Bifidobacterium strains from centenarians faeces. Anaerobe 12(3):148-152.

[29] Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., & Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents—Physiological effects and clinical benefits. Alimentary Pharmacology & Therapeutics, 22(6), 495–512.

[30] Salazar, N., Binetti, A., Gueimonde, M., Alonso, A., Garrido, P., González del Rey, C., et al. (2011). Safety and intestinal microbiota modulation by the exopolysaccharideproducing strains Bifidobacterium animalis IPLA R1 and Bifidobacterium longum IPLA E44 orally administered to Wistar rats. International Journal of Food Microbiology, 144(3), 342–351.

[31] García-Cayuela T., Korany A. M., Bustos I., Gómez de Cadiñanos L. P., Requena T., Peláez C., et al. (2014). Adhesion abilities of dairy Lactobacillus p *lantarum* strains showing an aggregation phenotype. *Food Res. Int.* 57 44–50. 10.1016/j.foodres.2014.01.010

[32] Goh Y. J., Klaenhammer T. R. (2010). Functional roles of aggregation-promoting-like factor in stress tolerance and adherence of Lactobacillus acidophilus NCFM. Appl. Environ. Microbiol. 76 5005–5012. 10.1128/AEM.00030-10

[33] Meile L, Le Blay G, Thierry A (2008) Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium. Int J Food Microbiol 126:316–320.

[34] Villoslada, F. L., Olivares, M., & Xaus, J. (2010). Safety of probiotic bacteria. In R. R. Watson, & V. R. Preedy (Eds.), Bioactive foods in promoting health: Probiotics and prebiotics. London: Academic Press.

[35] Arthur, C. O., Lisbeth, S. S., & Gregory, L. (2011). Probiotics: From strain to product. In K.Wolfgang, & S. Seppo (Eds.), Probiotics and health claims (pp. 37–48). Oxford:Wiley-Blackwell.

[36] Meile L, Le Blay G, Thierry A (2008) Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium. Int J Food Microbiol 126:316–320.

[37] Shah, N.P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science, 83(4), 894–907.

[38] Deraz, S. F., Karlsson, E. N., Khalil, A. A., & Mattiasson, B. (2007). Mode of action of acidocin D20079, a bacteriocin produced by the potential probiotic strain, Lactobacillus acidophilus DSM 20079. Journal of Industrial Microbiology and Biotechnology, 34(5), 373–379.

[39] Vinderola, C. G., Prosello, W., Ghiberto, D., & Reinheimer, J. A. (2000). Viability of probiotic(Bifidobacterium, Lactobacillus acidophilus and Lactobacillus casei) and nonprobioticmicroflora in Argentinian Fresco cheese. Journal of Dairy Science, 83, 1905–1911.

[40] M. Gueimonde, S. Delgado, B. Mayo, P. Ruas-Madiedo, A. Margolles, C.G. De los Reyes-Gavilán Viability and diversity of probiotic Lactobacillus and Bifidobacterium populations included in commercial fermented milksFood Res. Int., 37 (2004), pp. 839-850Md. Rahman et al Autoaggregation and surface hydrophobicity of bifidobacteriaWorld J Microbiol Biotechnol (2008) 24:1593–1598.D OI 10.1007/s11274-007-9650-x

[41] Shah, N.P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods.Journal of Dairy Science, 83(4), 894–907Maria, S., Gunnar, M., Rangne, F., Jaana, M., & Tiina, M. S. (2000). Probiotic bacteria: Safety, functional and technological properties. Journal of Biotechnology, 84(3), 197–215.

[42] Saarela, M., Alakomi, H. L., Mättö, J., Ahonen, A.M., & Tynkkynen, S. (2011). Acid tolerantmutants of Bifidobacterium animalis subsp. lactis with improved stability in fruit juice.LWT—Food Science and Technology, 44(4), 1012–1018.

[43] Elizabeth, W. N., Yeung, M., & Tong, P.S. (2011). Effects of yogurt starter cultures on the survival of Lactobacillus acidophilus. International Journal of Food Microbiology,145(1), 169–175Lara-Villoslada, F., Sierra, S., Díaz-Ropero, M. P., Rodríguez, J. M., Xaus, J., & Olivares, M.(2009). Safety assessment of Lactobacillus fermentum CECT5716, a probiotic strainisolated from human milk. Journal of Dairy Research, 76(02), 216–221.

[44] Deraz, S. F., Karlsson, E. N., Khalil, A. A., & Mattiasson, B. (2007). Mode of action of acidocin D20079, a bacteriocin produced by the potential probiotic strain, Lactobacillus acidophilus DSM 20079. Journal of Industrial Microbiology and Biotechnology, 34(5), 373–379.

[45] Cruz, A. G., Cadena, R. S., Faria, J. A., Bolini, H. M.A., Dantas, C., Ferreira, M., et al. (2012).PARAFAC: Adjustment for modeling consumer study covering probiotic and conventional yogurt. Food Research International, 45(1), 211–215.

[46] Ebel, B., Martin, F., Le, L. D. T., Gervais, P., & Cachon, R. (2011). Use of gases to improve survival of Bifidobacterium bifidum by modifying redox potential in fermented milk. Journal of Dairy Science, 94(5), 2185–2191.

[47] Playne, M. 1994. Probiotic foods. Food Aust. 46:362–366.

[48] Cruz, A. G., Castro, W. F., Faria, J. A. F., Bogusz, S., Jr., Granato, D., Celeguini, R. M. S., et al.(2012). Glucose oxidase: A potential option to decrease the oxidative stress in stirred probiotic yogurt. LWT—Food Science and Technology, 47(2), 512–515.

[49] Cruz, A. G., Castro, W. F., Faria, J. A. F., Bolini, H. M.A., Celeghini, R. M. S., Raices, R. S. L., et al.(2013). Stability of probiotic yogurt added with glucose oxidase in plastic materialswith

 DOI : 10.35291/2454-9150.2020.0625

different permeability oxygen rates during the refrigerated storage. Food Research International, 51(2), 723–728.

[50] Cruz, A. G., Castro, W. F., Faria, J. A. F., Lollo, P. C. B., Amaya-Farfán, J., Freitas, M. Q.,et al. (2012). Probiotic yogurts manufactured with increased glucose oxidaselevels: Postacidification, proteolytic patterns, survival of probiotic microorganisms,Production of organic acid and aroma compounds. Journal of Dairy Science, 95(5),2261–2269.

[51] Cruz, A. G., Faria, J. A. F., & Van Dender, A. G. F. (2007). Packaging system and probiotics dairy foods. Food Research International, 40(8), 951–956.

[52] Rasic, J. L., & Kurmann, J. A. (1983). Bifidobacteria and their role: Microbiological, nutritional–physiological, medical, and technological aspects and bibliography. Basel: BirkhauserVerlag.

[53] Ashraf, R., & Shah, N.P. (2011). Selective and differential enumeration of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt—A review. International Journal of Food Microbiology, 149, 194–208.57.Russell, D.A., Ross, R.P., Fitzgerald, G.F. and Stanton, C., 2011.Metabolic activities and probiotic potential of bifidobacteria.International Journal of Food Microbiology 149: 88-105.

[54] Pereira, D. I. A., & Gibson, G. R. (2002a). Cholesterol assimilation by lactic acid bacteria andbifidobacteria isolated from the human gut. Applied and Environmental Microbiology,68(9), 4689–4693.

[55] Pereira, D. I. A., & Gibson, G. R. (2002b). Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Critical Reviews in Biochemistry and MolecularBiology, 37(4), 259–281.

[56] Beena, A., & Prasad, V. (1997). Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and lactose-hydrolysed condensed whey on serum cholesterol and triacylglycerol levels in rats. Journal of Dairy Research, 64(03), 453–457.

[57] Shortt, C., & O'Brien, J. (2004). Handbook of functional dairy products. Florida: CRC.

[58] Shah, N.P. (2007). Functional cultures and health benefits. International Dairy Journal,17(11), 1262–63.

[59] Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Jose, A. F. F. (2010). Functional foods and nondairy probiotic food development: Trends, concepts, and products. Comprehensive Reviews in Food Science and Food Safety, 9(3), 292–302.

[60] Shortt, C., & O'Brien, J. (2004). Handbook of functional dairy products. Florida: CRC.

[61] Parracho, H., McCartney, A. L., & Gibson, G. R. (2007). Probiotics and prebiotics in infant nutrition. Proceedings of the Nutrition Society, Vol. 66. (pp. 405–411). : Cambridge Journals Online.

[62] Shah, N.P. (2007). Functional cultures and health benefits. International Dairy Journal,17(11), 1262–63.

[63] Jiang, T., Mustapha, A., & Savaiano, D. A. (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing Bifidobacterium longum. Journal of Dairy Science, 79(5), 750–757.

[64] Shortt, C., & O'Brien, J. (2004). Handbook of functional dairy products. Florida: CRC.

[65] Bouma, G., & Strober, W. (2003). The immunological and genetic basis of inflammatory bowel disease. Nature Reviews Immunology, 3(7), 521–533.

[66] Mao, M., Yu, T., Xiong, Y., Wang, Z., Liu, H., Gotteland, M., et al. (2008). Effect of a lactose-free milk formula supplemented with bifidobacteria and streptococci on the recovery from acute diarrhoea. Asia Pacific Journal of Clinical Nutrition, 17(1),30–34.

[67] Saavedra, J. M., Bauman, N. A., Perman, J. A., Yolken, R. H., & Oung, I. (1994). Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. The Lancet, 344(8929), 1046–1049

[68] Mao, M., Yu, T., Xiong, Y., Wang, Z., Liu, H., Gotteland, M., et al. (2008). Effect of a lactose-free milk formula supplemented with bifidobacteria and streptococci on the recovery from acute diarrhoea. Asia Pacific Journal of Clinical Nutrition, 17(1),30–34.

[69] Kim, N., Kunisawa, J., Kweon, M. -N., Eog Ji, G., & Kiyono, H. (2007). Oral feeding of Bifidobacterium bifidum (BGN4) prevents CD4+ CD45RB T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clinical Immunology,123(1), 30–39.

[70] Ishikawa, H., Akedo, I., Umesaki, Y., Tanaka, R., Imaoka, A. I., & Otani, T. (2003). Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. Journal of the American College of Nutrition, 22(1), 56–63

[71] De Moreno, D. L. A., & Perdigon, P. (2005). Antitumour activity of yoghurt. In J.D. Martinez (Ed.), Focus on colorectal cancer research (pp. 97–123). New York: Nova Science Publishers 74.Rowland, I. (2004). Probiotics and colorectal cancer risk. British Journal of Nutrition, 91(06), 805–807.

[72] Bomba, A., Nemcov, R., Gancarc˘ı´kova´, S., Herich, R., Guba, P., & Mudronov, D. (2002). Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. British Journal of Nutrition, 88(SupplementS1), S95–S99.

[73] Galdeano, C. M., De LeBlanc, A.M., Dogi, C., & Perdigón, G. (2010). Lactic acid bacteria as immunomodulators of the gut-associated immune system. In Fernanda Mozzi, Rául R. Raya, & G. M. Vignolo (Eds.), Biotechnology of lactic acid bacteria: Novel applications (pp. 125–141). Iowa: Wiley-Blackwell.

[74] Gill, H. S., Rutherfurd, K. J., Cross, M. L., & Gopal, P. K. (2001). Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. The American Journal of Clinical Nutrition, 74(6), 833–839.

[75] Lollo, P. C. B., de Moura, C. S., Morato, P. N., Cruz, A. G., de Freitas Castro, W., Betim, C. B.,et al. (2013). Probiotic yogurt offers higher immune-protection than probiotic wheybeverage. Food Research International, 54(1), 118–124

[76] Klein, A., Friedrich, U., Vogelsang, H., & Jahreis, G. (2007). Lactobacillus acidophilus 74-2 and Bifidobacterium animalis subsp. lactis DGCC 420 modulate unspecific cellular immune response in healthy adults. European Journal of Clinical Nutrition, 62(5),584–593.

[77] Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am J Clin Nu.August 1997DOI: 10.1093/ajcn/66.2.515S.

 DOI : 10.35291/2454-9150.2020.0625