

A REVIEW ON PROBIOTIC MICROBIOME OF HUMAN BREAST MILK THROUGH OMICS TECHNOLOGY

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ABSTRACT:

Breast milk acts as an essential natural source of good bacteria. Generally probiotics are considered as good bacteria which confer health benefits on host when administered at adequate levels. Probiotic micro flora of human breast milk renders gastro intestinal diseases, serum cholesterol. Probiotic microbiome provides resistance against pathogenic microorganisms by releasing antibacterial substances, regulates intestinal dysbiosis, anti-inflammatory, anti-carcinogenic, and anti-oxidant effects. Thus it determines several functions of the host immune system and metabolism. Probiotic microbiome keeps gut environment healthy. Native micro floras are used as potential probiotics because of its specific health benefits. Culturomics is an omics approach helps to characterize the potent native probiotic culture from human sources.

Index terms: Human Breast Milk, Omics Technology, culturomics, Probiotic bacteria, Microbiome.

HUMAN BREAST MILK:

Breast milk acts as essential, harmless and continuous sources of beneficial bacteria (Probiotics) to the infant gut (Stensballe LG *et al.*, 2013). Human milk was the gold standard for infant nutrition and it supports more life than we envision as it holds several biologically active components such as immune factors, lipids, oligosaccharides, micro RNA's and hormones among others (Hennet and Borsig, 2016). Whose concentration vary with maternal and environmental factors (Hunt *et al.*, 2011; Andreas *et al.*, 2015; Feng *et al.*, 2016; Gomez-Gallego *et al.*, 2016; Kumar *et al.*, 2016; Ruiz *et al.*, 2017). Microorganisms have emerged as significant bioactive components of human milk. Its occurrence in milk was first acknowledged at the second half of the twentieth century (Rantasalo and Kauppinen, 1959; Foster and Harris, 1960; Kenny, 1977; Eidelman and Szilagyi, 1979). The scientific interest in human milk bacteria was growing in parallel to the disclosed roles of human microbiomes in health and disease, and to the advances in the methodologies available for their study. Traditionally bacterial cells in milk were considered contaminants originating from the infant's oral cavity or the mother's skin. Later, several studies evident the presence of viable commensal, mutualistic or potentially probiotic bacteria in healthy human milk (Fernandez *et al.*, 2013) and the detection of such anaerobic species typical from gut environments has suggested that breast feeding could be an ingenious way to offer the commensal microbes to newborn gut (McGuire, 2015). However, the source of bacterial populations in milk was not completely understood yet and have been proposed to originate from maternal skin, infant's mouth, and (or) endogenously, from the maternal digestive tract through a mechanism including immune cells. In fact, bacterial interactions with the host immune system appear to differ between body sites and, understanding how the maternal immune system interacts with milk bacteria may help reveal the mechanisms allowing bacterial translocation through an entero mammary pathway (Rodríguez, 2014). Indeed, some works have proposed that selected bacteria in the maternal gut could reach the mammary gland through an endogenous route, involving complex interactions between bacteria, epithelial and immune cells (Martín *et al.*, 2004). Although the mechanisms governing this process have not been explained yet, studies with pregnant and lactating mice models have offered an acceptable scientific basis (Rodríguez, 2014; de Andrés *et al.*, 2017; Mira and Rodriguez, 2017). Certainly, bacteria from milk of

healthy women fulfill the criteria recommended for human probiotics: human origin, a history of safe prolonged intake, adaptation to mucosal (Lara-Villoslada *et al.*, 2007; Fernandez *et al.*, 2013), and to the human gut (Jeurink *et al.*, 2013), in addition to that oral administration of some *Lactobacilli* strains to lactating women led to their presence in milk supporting the existence of an endogenous gut-mammary gland associating during lactation (Abrahamsson *et al.*, 2009; Arroyo *et al.*, 2010). Nowadays, research welfares in this field were focused on (a). The roles that milk bacterial communities play on infant health and development. (b). the role on maternal health, together with breast health. (c). the maternal, infant, environmental and medical factors that shape and modify their compositions, under physiological conditions and during dysbiosis, and (d). Their origin. Healthy human milk harbors a microbial community, representing a source of commensal microorganisms for the neonate who contributes to the digestive and immune systems development in infant. Even though their limitations to access un culturable bacteria, culturing provisions preserving the strains enabling further studies and exploitation of potential biotechnological applications (Lara-Villoslada *et al.*, 2007; Arboleya *et al.*, 2011; Langa *et al.*, 2012; Cárdenas *et al.*, 2014 and 2015).

OMICS TECHNOLOGY:

The ever-growing asset of omic tools was opening new avenues to understand the benefits of breast feeding from metabolomics, immunological and microbiological perspectives, to establish new microbiota-based strategies to promote maternal-infant health, including the development of procedures for the transfer of the milk microbiota to infants that were not breastfed or the development of human milk-derived probiotics (Fernández *et al.*, 2018). Application of both culture-dependent and independent techniques to assess the milk microbiomes faces some practical limitations but, together, have allowed providing novel and complementary views on its origin, composition and functioning. In the next future, the application of the ultimate advances in next-generation sequencing and omics approaches, including culturomics, will allow a detailed and comprehensive understanding of the composition and functions of these microbial communities, including their interactions with other milk components, expanding the opportunities to design novel microbiome-based modulation strategies for this ecosystem. A pooled milk sample resulting from 10 donors was analyzed through shotgun-sequencing, identifying abundant and prevalent functions in the milk microbiota, as compared to the infant gut microbiomes and identified putative immunomodulatory motifs in microbiota-derived DNA sequences, providing basis for further mechanistic studies (Ward *et al.*, 2013). Additionally, shotgun metagenomics enhance the resolution of taxonomic assessment and has demonstrated vertical transmission of strains from the mother to the infant (Asnicar *et al.*, 2017); and identified novel functions of interest from unculturable bacteria in complex communities (Berini *et al.*, 2017), though this potential has not been harnessed within the milk microbiota. NGS-based methodologies that have not yet been applied to milk microbiota studies, include RNA-based (Meta transcriptomics) or single-cell methodologies, which may offer novel information about the communities that are metabolically active at a given point and their functionality (Gosalbes *et al.*, 2012; Yao *et al.*, 2017). Finally, little attention has been paid to the associations of milk microbiota with other milk components (Roncada *et al.*, 2013; Kumar *et al.*, 2016; Bardanzellu *et al.*, 2017; Gómez-Gallego *et al.*, 2017 and 2018), although the combination of multiple “omic” approaches at DNA, RNA, protein and metabolite levels, will undoubtedly facilitate our comprehension on the inter-relationships among milk components, and ultimately, with the host. Milk micro flora and their detection through other techniques, including the specific labeling of target microorganisms - PCR or flow-cytometry based approaches or target specific RNA-based analysis, which would also confirm its functionality within the ecosystem and may provide additional proofs on the possible role of these bacteria for human health.

CULTUROMICS:

In the frame work of recent research conducted in the field of the gut microbiota, the “culturomics” approach, which is based on the utilization of a wide range of culturing and incubation conditions, was emerging as a promising strategy to aid in the discovery and description of novel microorganisms associated to the human host, including pathogenic and commensal species (Bilen *et al.*, 2018). Such approach has not been applied to the human milk ecosystem yet, although it can be anticipated that it will lead to significant advances in our knowledge of this human ecosystem and to the discovery of novel health-promoting bacterial strains. Culture-dependent approaches have been pioneering in demonstrating that healthy human milk harbors a microbial community, representing a source of commensal microorganisms for the neonate. Bacterial groups present in milk from healthy lactating mothers were more fastidious to grow under routine laboratory conditions as they require either particular atmospheric incubations or specific nutrients or ingredients in the cultivation media. They have passed unnoticed in early microbiological studies not specifically designed to recover such microbial species. For instance, *lactic acid bacteria* and *bifidobacteria*

have been isolated from milk following the utilization of specific growth media and longer anaerobic incubations. (Martin *et al.*, 2003 and 2009; Abrahamsson *et al.*, 2009; Solís *et al.*, 2010; Arboleya *et al.*, 2011; Murphy *et al.*, 2017). In view of the high inter-individual, inter populations and inter-study variations reported up till now, milk microbiomes research needs an urgent standardization of samples collection and processing as previously proposed for other human microbiomes (Costea *et al.*, 2017). Factors like maternal diet, known to affect the gut microbiota composition (Dingess *et al.*, 2017; Bzikowska-Jura *et al.*, 2018). Most studies on milk microbiomes up till now have exclusively identified bacterial population shifts, but have not provided any functional insights into these communities. Results from gut microbiomes studies have demonstrated that microbial ecosystems are more conserved at functional than at taxonomic levels, reflecting the existence of redundant functions among community members and suggesting that some species might be interchangeable without affecting the functional attributes of the community (Moya and Ferrer, 2016). Thus, functions may be better biomarkers for health-disease states than taxonomical composition though they have been scarcely studied on milk. Bacterial isolation coupled to phenotypic and genotypic characterization, including whole genome sequencing analyses, were essential steps to characterize and evaluate the safety and the probiotic potential of bacterial isolates from human origin, including those isolated from milk. Generally, the invitro assessment of probiotic properties in human milk isolates has been included their antimicrobial activity against pathogenic microorganisms and the compounds (organic acids, bacteriocins, hydrogen peroxide) that may be responsible for such activity, the ability to survive when exposed to conditions similar to those found in the human gastrointestinal tract, their adherence to human intestinal cells and mucin, their ability to degrade mucin or to produce biogenic amines or their susceptibility to antibiotics (Martín *et al.*, 2005, 2006 and 2010). The safety and health-promoting properties of some microbial strains have also been confirmed in vivo, including the use of animal models (Olivares *et al.*, 2006) and human clinical trials (Jiménez *et al.*, 2008b; Arroyo *et al.*, 2010; Maldonado *et al.*, 2010, 2012; Gil-Campos *et al.*, 2012; Espinosa Martos *et al.*, 2016; Fernández *et al.*, 2016). Finally, a few strains isolated from breast milk have been analyzed to date through Whole Genome Sequencing, including representatives from the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (Jiménez *et al.*, 2010a, b, 2012; Martín *et al.*, 2012, 2013). Approaches offer novel opportunities to address studies in this field contributes to identify the mechanisms governing the milk microbiota assembly and its impact on maternal-infant health, paving the way to design novel microbiota-based strategies to promote maternal-infant health. Despite of the large inter-individual and inter population variations, most studies agree to identify the genera *Staphylococcus*, *Streptococcus*, and *Propionibacterium* as core members of the milk microbiota (Hunt *et al.*, 2011; Jiménez *et al.*, 2015), as they appear to be universally present in healthy milk irrespectively of maternal and infant characteristics studied to date. The presence of potentially beneficial bacteria including *Lactobacilli* and *Bifidobacteria* species, was also commonly reported in human milk, though these were not consistently detected across all samples and populations studied and usually represent a minority of the bacterial populations detected (Chen *et al.*, 2018). Intriguingly, Nucleotide Genome Sequence (NGS) -based studies have also identified in milk DNA from strictly anaerobic gut-associated microbes (*Bacteroides*, *Blautia*, *Clostridium*, *Collinsella*, *Coprococcus*, *Eubacterium*, *Faecalibacterium*, *Roseburia*, *Ruminococcus* etc.) which were either non-culturable or hard to culture in the laboratory, and that have not been recovered through milk culturing to till now. (Cabrera-Rubio *et al.*, 2012; Jost *et al.*, 2013, 2014; Jiménez *et al.*, 2015; Gomez-Gallego *et al.*, 2016). Confirming their presence within healthy human milk would require its isolation through culturomic approaches.

HISTORY AND DISCOVERY OF PROBIOTICS:

The term probiotic, meaning 'for life', is derived from Greek word 'pro' and 'biota'. The concept of probiotics dates back to pre-biblical times and humans were known to consume live bacteria more than 2000 years ago in the form of fermented milk and other foods. According to FAO/WHO in 2002 a universal definition for probiotics as 'live microorganisms that, when administered in adequate amounts, confer health benefit on the host (Hill *et al.*, 2014). Elie Metchnikoff in 1908 proposed the theory of good bacteria, he observed that putrefactive microbes in the gut had adverse effects, normalised by consumption of sour milk (noticed in Bulgarian peasants), and increases the longevity of life. The first clinical trials were conducted in the 1930's to test the effects of probiotics on constipation. In 1965 Lilly and Stillwell described as contrasting to antibiotic i.e., "substances secreted by one microorganism which stimulates the growth of another microorganism". Parkers in 1974 first use the term 'probiotic'. Mann and Spoerig in 1974 discovered that people who take yogurt fermented with wild strains of *Lactobacillus* sp. results very low

values of blood serum cholesterol (Parvez *et al.*, 2006). In 1994, the World Health Organization judged probiotics to be the next-most important immune defense system when commonly prescribed antibiotics were rendered unworkable by antibiotic resistance (Kailasapathy and Chin, 2000). Harrison in 1975 reported that cells of *Lactobacillus acidophilus* added to infant formula decreases levels of serum cholesterol. Later, Tissier in 1984 acclaimed the administration of *bifidobacteria* were predominant in the gut flora of breastfed infants for infantile diarrhoea. According to Fuller in 1989, a probiotic can be defined as “a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 2012). Schrezenmeir and De Vrese (2001) defines probiotic as “A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the micro flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host.” Later, FAO/WHO in 2002 developed a universal definition for probiotics. *Lactic acid bacteria* in acidic fermented foods have an ancient history of promoting health and life, especially in the treatment of gastroenteritis (Schrezenmeir and de Vrese, 2001). The use of probiotics in antibiotic resistance was called microbial interference therapy (Parvez *et al.*, 2006).

COMPOSITION OF PROBIOTIC STRAINS AT DIFFERENT CONDITIONS:

Several microbes have been studied as potent probiotics. Majority of the probiotic organisms considered were of human origin. Numerous species and strains belong to the genera of *Lactobacillus* and *Bifidobacteria* are considered as potential probiotics (Kleerebezem and Vaughan, 2009). In addition to these major genera, species of *Streptococcus*, *Enterococcus*, *Propionibacterium*, *Bacillus*, and Gram- negative *E. coli* Nissle -1917 and *Saccharomyces* exists and are also being used as probiotic microorganisms (Gareau *et al.*, 2010 : Iannitti and Palmieri, 2010). The microorganisms belonging to *lactic acid bacteria* (LAB) were studied as probiotics.

Few microorganisms studied as probiotics includes *Bifidobacterium animalis*, *B. animalis subsp. lactis*, *B. adolescentis*, *B. bifidum*, *B. longum subsp. infantis*, *B. longum*, *B. breve*, *Lactobacillus acidophilu*, *L. delbrueckii*, *L. casei*, *L. fermentum*, *L. helveticus*, *L. paracasei*, *L. johnsonii*, *L. plantarum*, *L. sakei*, *L. gasseri*, *L. salivarius*, *L. reuteri*, *L. rhamnosus*, *Bacteroides* such as *Bac. Coagulans*, *Bac. Subtilis*, *Bac. cereus*, *Bac. Clausii*, *Bac. licheniformis*, *Bac. Mesentericus*, *Enterococcus faecalis*, *E. faecium*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *P. pentosaceus*, *Streptococcus salivarius*, *Str.thermophiles*, *Escherichia coli*, *Saccharomyces cerevisiae subsp. Sub sp. boulardii* (Foligne *et al.*, 2013). Understanding the composition, functions and assembly of the human milk microbiota has important implications not only for the infant gut microbiota establishment, but also for the mammary health since dysbiosis in the milk bacteria may lead to mastitis. Besides, host, microbial, medical and environmental factors may affect the composition of the human milk micro biome, with implications for the mother infant health. Globally, over 200 different bacterial species representing approximately 50 different genera have been isolated from milk, including new bacterial species, such as *Streptococcus lactarius* (Martín *et al.*, 2011). Bacteria will start to colonize in baby’s digestive system after birth and type of bacteria that reside in baby’s intestines are dependent on his or her diet, environment, and mothers own normal flora. (Rautava S *et al.*, 2012). Indeed, among the microorganisms occurring in milk, some strains belonging to the species *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, and *Bifidobacterium longum subsp. infantis* have demonstrated potential to promote mother and infant health, including the prevention or treatment of lactational mastitis, the promotion of a normal gut bacterial colonization in preterm neonates ,in IBS patients (Arroyoetal.,2010; Chiuetal.,2014; MaldonadoLobónetal.,2015; Molesetal.,2015; Fernández *et al.*, 2016; Shin *et al.*, 2018). Some *Lactobacillus* and *Bifidobacterium* species (*L. gasseri*, *L. salivarius*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. reuteri*, *B. breve*, *B. longum* etc.) from milk have awakened strong interest as potential probiotic bacteria, enjoying the GRAS (Generally Recognized As Safe) and the QPS (Qualified Presumption of Safety) status conceded by the Food and Drug Administration and the European Food Safety Authority, respectively.

LACTIC ACID BACTERIA: (LAB):

LAB are one of the native flora colonizing the gut of humans belongs to Gram-positive, low G+C content, non- sporulation, anaerobic bacteria. They are fastidious organisms requiring media rich in nutritional components (de Vrese and Schrezenmeir, 2008). *Lactobacillus* strains have been shown to modulate the composition and metabolism of intestinal flora (Kuisma *et al.*, 2003). The group LAB comprises including *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, *Lactosphaera*, *Macrobacterium*, *Bifidobacterium*, *Carnobacterium*, *Propionibacterium* and *Weissella* (Holzapfel *et al.*, 2002). Among different genera of *lactobacilli*, *bifidobacteria* and *Lactococcus* are known to be health beneficial (Tannock, 1998). The

phenotypic properties in LAB characterization and classification includes morphology, glucose and various other carbohydrates utilization pattern, growth at different temperatures, pH and salt concentrations, methyl esters of fatty acids, protein pattern in the cell wall or in the whole cell (Decallone *et al.*, 1991; Gatti *et al.*, 1997; Mohania *et al.*, 2008). Nevertheless, these methods are not accurate and tedious to perform (Mohania *et al.*, 2008) and affect accurate identification of cultures at the genus and species level. Molecular methods have been used as an alternate or complementary tool to phenotypic characterization for the identification of bacteria. Molecular characterization of probiotic LAB is an advancement of molecular biology led to the development of rapid, sensitive and automated molecular detection methods for several probiotic bacteria. Primers specific to target sequences like 16S ribosomal RNA (rRNA) encoding gene, 16S-23S rRNA intergenic spacer sequence, RecA and IdhD genes have been used for identification. The techniques used for analysis includes RAPD, RFLP, AFLP, PAGE in probiotic taxonomy and characterization (Mohania *et al.*, 2008). Genome sequencing techniques are used for the analysis of the bacterial genome is the best tool to identify and characterize potent and novel strains however it is a laborious and expensive. 16S rRNA gene sequence analysis are widely used for the phylogenetic classification of organisms and the sequences are compared based on available databases like GenBank, DDBJ, EMBL etc. (Amor *et al.*, 2007). LAB was initially isolated from milk and subsequently found in different fermented foods namely meat, dairy products, vegetables, bakery items and beverages (Klaenhammer *et al.*, 2002; Metchnikoff, 1908). LAB gained importance as fermentation of food and feed, probiotics, pharmaceuticals and as bio control agents (Harzallah and Belhadj, 2013), extensively used as starter cultures in the food industry (Leroy and De Vuyst, 2004), found to inhibit spoilage (Klaenhammer *et al.*, 2002) as it produces bacteriocins and antioxidant, extensively applied in bio preservation of foods (Devi and Halami, 2013). *Bifidobacteria* and *Lactobacilli* are known to be important for functionality of small intestine and colon (O'Sullivan and Halami, 2013). Due to their long history of usage as fermented food, genera of *Lactobacillus*, *Bifidobacterium* and *Lactococcus* 'generally recognized as safe' (Salminen *et al.*, 1998), acclaimed as probiotics even in immune compromised hosts (Daliri and Lee, 2015; de Vrese and Schrezenmeir, 2008).

IDENTIFICATION OF PROBIOTIC BACTERIA:

Taxonomy of the microorganisms of interest is the primary step while selecting a potential probiotic strain. It is a polyphasic approach comprising phenotypic and genotypic methods (Vandamme *et al.*, 1996) Such as sugar fermentation profile, physiological and biochemical characteristics. Phenotypic methods are used for the initial characterization while genotypic tools help in the characterization at the molecular level. Criteria/properties for selection of probiotics and their health benefits are Resistance to pancreatic enzymes, acid and bile resistance, Adhesion to the intestinal mucosa, immune modulation, pathogen exclusion, repair of damaged mucosa, prolonged transient colonization and maintained viability, Production of antimicrobial substances Antagonism against pathogenic microorganisms, Health safety, Good technology properties such as strain stability, production at large scale, oxygen tolerance.

FACTORS INFLUENCING GROWTH OF PROBIOTICS:

The description of commensal bacteria in milk permits a deeper resolution of microbial communities (Jeurink *et al.*, 2013; McGuire, 2017). Most NGS-based studies conducted on milk have focused on metataxonomics to define bacterial population's presence and their variation with selected factors. Briefly, such studies revealed the existence of large interindividual variations (Hunt *et al.*, 2011; Cabrera-Rubio *et al.*, 2012), some of which have been associated with geographical and lifestyles differences, with a general higher microbial diversity in samples from developing and (or) rural locations (Kumar *et al.*, 2016; Sakwinska *et al.*, 2016; Drago *et al.*, 2017; Li S.-W *et al.*, 2017; Lackey *et al.*, 2019), in agreement with observations with in the gut microbiota ecosystem. Other specific factors frequently associated with overall variation in the milk micro biome structure include delivery mode (Cabrera-Rubio *et al.*, 2012 and 2016; Khodayar-Pardo *et al.*, 2014; Toscano *et al.*, 2017) and time from birth (Cabrera-Rubio *et al.*, 2012 and 2016; Khodayar-Pardo *et al.*, 2014; Toscano *et al.*, 2017, Ma *et al.*, 2015; Yu *et al.*, 2016; Patel *et al.*, 2017), though some inconsistencies have been reported among studies, differences in the milk micro biome structure of women who gave birth vaginally and those delivering by C-section have been frequently reported, some authors have even reported a higher *Lactobacilli* representation following vaginal delivery in a Taiwanese population (Chen *et al.*, 2018), and other studies found no differences in milk micro biomes depending on delivery type (Sakwinska *et al.*, 2016; Urbaniak *et al.*, 2016). Remarkably, particular cohort-dependent factors that might be driving variation in the human milk micro biomes have not been thoroughly explored, but might contribute to explain these apparent discordances. Similarly, differences between colostrum, transition and mature milk microbiota have been reported by some authors, who reported an increased abundance of typical oral inhabitants in transition and mature milk, though such differences have not been consistently observed in different studies (Cabrera-Rubio *et al.*, 2012; Chen *et al.*, 2018). Other

factors less explored but described to affect the milk micro biome includes environmental exposure to disinfection agents (Bever *et al.*, 2018), chemotherapy (Urbaniak *et al.*, 2014b), maternal nutrient intakes (Boix-Amorós *et al.*, 2016; Williams *et al.*, 2017a), and the structure of human social networks, including cooperative breeding (Meehan *et al.*, 2018). Correlation and network analyses have also related variation in the milk micro biome with variation in other milk components (Gómez-Gallego *et al.*, 2017; Williams *et al.*, 2017b).

MECHANISM OF ACTION OF PROBIOTIC BACTERIA:

The mechanism of action of probiotics can be categorized into (i) Inhibition of pathogens and restoration of gut homeostasis, (ii) Enhancement of intestinal barrier function, and (iii) Modulation of the host immune response.

The process of digestion in the human body occurs by the action of salivary enzymes, gastric juices and bile acids followed by absorption in the small and large intestine. However, our digestive system itself is not equipped to breakdown the microbiota present in the gut symbiotically rendering them absorbable by the host cells. Probiotics when ingested have to outstrip the harsh conditions of the gastro intestinal tract. The cells that survive the gut transit then colonize and adhere to the intestinal mucosa interacting with the host system. One of the first mechanisms of probiotic action is the inhibition of pathogenic bacteria by secretion of several metabolites and additionally competing for sites of adhesion thus preventing pathogens from binding to the host epithelium. By enhancing mucus secretion, promoting the function of tight junction proteins and communicating with the underlying immune system helps in the maintenance of barrier function and positive influence on the immune response. The immune response evoked is strain dependent and strain specific. Some strains evoke adaptive response in favour of T-helper cells, while some may evoke Th2 response which further secrete cytokines and induce the immune production of immune cells. Probiotics are also known to stimulate T regulatory cells such as IL-10 and TGF β known to regulate the extent of immune response in the host. Induction of T reg cells is said to elicit an anti-inflammatory response. (Tsai *et al.*, 2012).

IMPORTANCE OF PROBIOTICS:

Certain microorganisms yield vitamins especially B-group vitamins (LeBlanc *et al.*, 2011). Probiotics may inhibit pathogenic bacteria by production of bacteriocins, metabolites which creates unfavorable pH for gut bacteria (or) bio surfactants with antimicrobial effect (Barrier effect) which influences the colonization of pathogenic bacteria by competing with binding sites thus preventing adhesion of pathogens. This inhibition activity is shown by different *Lactobacillus*, *Bifidobacterium* and *Propionibacterium* strains in in vitro models (Collado *et al.*, 2006; Collado *et al.*, 2007a). In addition, *lactobacilli* and *bifidobacteria* are able to produce antimicrobial agents such as organic acids, hydrogen peroxide, diacetyl, short chain fatty acids and bacteriocins (Servin, 2004). Probiotics enhance barrier function by improving mucus secretion, influencing the synthesis of antimicrobial peptides, mucins, preventing apoptosis of epithelial cells and up regulating cryoprotective heat shock proteins. (Johnson-Henry *et al.*, 2008; Mack *et al.*, 2003; Petrof *et al.*, 2004; Yan *et al.*, 2007). Probiotic strains of *S. thermophilus* and *L. acidophilus* have shown to alter the expression of tight junction proteins in in vitro and in vivo studies (Resta-Lenert and Barrett, 2003). Anderson *et al.*, (2010) reported altered expression of genes encoding occludin, tubulin, proteasome and cytoskeleton anchoring proteins by *L. plantarum* MB452. Probiotics such as VSL-3 probiotic mixture, *E. coli* Nissle and *L. rhamnosus* GG, can also strengthen the mucosal barrier function in mice and in vitro models (Johnson-Henry *et al.*, 2008; Madsen *et al.*, 2001; Ukena *et al.*, 2007). Strengthening of the mucosal barrier function may be due to normalization of gut permeability, regeneration of epithelial cells and strengthening of tight junctions, as shown for *L. rhamnosus* GG in rat model and for *E. coli* Nissle in in vitro model (Isolauri *et al.*, 2001). Probiotics were natively present in our intestines as normal flora. Many types of bacteria are harmful to health and result infections but the normal flora is beneficial bacteria which helps us. The ratio between good and bad bacteria effects on health. Imbalances in the ratio of good bacteria may causes damage to the intestine and leads to some health disorders (Sohn K, 2017). By Supplementing with probiotics we can restore, correct the imbalance and reestablish the healthy balance of normal flora of intestine (Anabrees J *et al.*, 2013). This influences the richness of breast milk bacterial composition and thus makes progress the health of babies. The World Allergy Organization (WAO) released guidelines in 2015 about the use of probiotics in pregnancy and breastfeeding to avoid allergic conditions in babies Exposure to antibiotics is common in early life especially in caesarian section deliveries. It impacts on infant gut resistome and leads to development of antibiotic resistant pathogenic strains. During the period of antibiotic medication due to elimination of normal flora such imbalances occur in the mother's intestine and intake of antibiotics during pregnancy results lower amounts of beneficial bacteria in breast milk and ultimately may take away the baby's normal flora necessary for proper immune

system development. Having such imbalance during pregnancy can upturn the risk that baby will have allergic conditions after birth and childhood asthma (Stensballe LG *et al.*, 2013). The potential benefit of probiotics includes preventing allergic conditions, colic in newborns (Dani C *et al.*, 2016, Elias J *et al.*, 2011), improve the frequency and volume of your baby's stools and reduce the possibility of babies' infections (Anabrees J *et al.*, 2013). Probiotics are widely known to influence the immune system and maintain immune function balance. They are thought to bring about these effects by virtue of their cell structures such as DNA, LPS, peptidoglycan, flagellin and/or metabolites like SCFAs. They may act directly or indirectly by modulating the gut microbiota (Butel *et al.*, 2014).

Bifido bacterium probiotics strain *breve* Yakult (Tokyo, Japan) alleviation of diarrhea and *Campylobacter enteritis*, promotion of preterm infant growth and treatment of ulcerative colitis *B. lactis* Bb-12 Chr. Hansen (Horsholm, Denmark) supports bowel function, enhances immune response, reduces respiratory tract infections *B. lactis* HN019 (DR10) New Zealand Dairy Board immune enhancement, alleviation of gastrointestinal maladies *B. longum* BB536 Morinaga Milk Industry co., Ltd (Zama-City, Japan) promotes gastrointestinal health *B. longum* SBT-2928 Snow Brand Milk Products co., Ltd (Tokyo, Japan) antimicrobial and immune stimulation activity *Lactobacillus* probiotics *L. acidophilus* DDS-1 Nebraska Cultures, Inc. (Lincoln, NE) supports healthy immune system and microbiota, promotes digestion and nutrient absorption, inhibits pathogens and improves metabolism *L. acidophilus* LA-1 Chr. Hansen (Horsholm, Denmark) anti-oxidant and anti-tumour activity *L. acidophilus* LB Lacteol Laboratory (Houdan, France) prevention of diarrhea, improves immune function, alleviates lactose intolerance, vaginal disorders etc. *L. acidophilus* NCFM Rhodia, Inc. (Madison, WI) reduces risk of colon cancer, alleviates lactose intolerance and antimicrobial activity *L. acidophilus* R0052 Institut Rosell (Montreal, Canada) anti-inflammatory, anti-oxidative property *L. acidophilus* SBT-2062 Snow Brand Milk Products co., Ltd (Tokyo, Japan) anti-cancer effect *L. casei* DN014001 (Immunitas) Danone Le Plessis-Robinson (Paris, France) reduction of diarrhea and rhinitis, enhancement of immune function *L. casei* Shirota Yakult (Tokyo, Japan) maintenance of gut microbiota, modulation of immune system, regulation of bowel movement and reduction of gastrointestinal infections *L. crispatus* CTV05 Gynelogix, Boulder, CO reduction of vaginal and urinary tract infections. *L. delbrueckii* subsp. *bulgaricus* 2038 Meiji Milk Products (Tokyo, Japan) thermophilic starter culture for yoghurt and cheese *L. fermentum* RC-14 Urex Biotech Inc. (London, Ontario, Canada) prevention and treatment of urogenital infections *L. johnsonii* La1 Nestle (Lausanne, Switzerland) pathogen inhibition and immunomodulation *L. paracasei* CRL 431 Chr. Hansen (Horsholm, Denmark) enhancement of immune response, reduction of common cold and influenza like-symptoms *L. paracasei* F19 Arla dairy (Stockholm, Sweden) enhancement of gastrointestinal function and immune response *L. plantarum* 299V Probi AB (Lund, Sweden) reduction of diarrhea, enhancement of immune response, reduction of IBD and IBS symptoms *L. reuteri* SD2112 BioGaia (Raleigh, NC) reduction of cholesterol levels, inhibition of *Helicobacter pylori* promotes vaginal health and infant gut health *L. rhamnosus* 271 Probi AB (Lund, Sweden) antimicrobial and immune stimulation activity, promotion of gut health *L. rhamnosus* GG Valio Dairy (Helsinki, Finland) prevention of diarrhea, gastrointestinal infections, reduction of respiratory infections, atopic dermatitis and eczema, prevention of urogenital infections *L. rhamnosus* GR-1 Urex Biotech Inc. (London, Ontario, Canada) prevention and treatment of vaginal and urinary tract infections *L. rhamnosus* LB21 Essum AB (Umea, Sweden) maintenance of gut health *L. rhamnosus* R0011 Institut Rosell (Montreal, Canada) prevention of diarrhea, maintenance of gut health *L. salivarius* UCC118 University College (Cork, Ireland) antimicrobial and anti-inflammatory activity Other probiotic strains *Lactococcus lactis* L1A Essum AB (Umea, Sweden) promotion of gut health *S. boulardii* Biocodex Inc. (Seattle, WA) treatment and prevention of gastrointestinal disorders, stimulation of immune function (Nagpal *et al.*, 2012).

PROBIOTIC BACTERIAL PROTEINS AND THEIR IMPORTANCE:

Probiotic bacteria produces anti-microbial compounds, biogenic amine adheres intestinal cells which offers antibiotic resistance. As it consists of different antimicrobial compounds, bacteriocins, immunoglobulin's and other immune component cells it provokes the growth of helpful bacteria in neonate gut. Specifically, the activities most commonly studied on milk isolates include virulence and antibiotic resistance (Jiménez *et al.*, 2008a; Kozak *et al.*, 2015), but also bacteriocins production (Heikkilä and Saris, 2003; Olivares *et al.*, 2006; Lara - Villoslada *et al.*, 2007; Kozak *et al.*, 2015; Sharma *et al.*, 2017) and probiotic traits (Fernández *et al.*, 2013; Reis *et al.*, 2016). The variations in milk components such as immune cells, polyamines and fatty acids are observed in some studies, though such observations reported in limited studies their potential implications have not been thoroughly explored to monitor or improve maternal-infant health.

DISADVANTAGES:

Probiotics rarely cause dangerous infections in people with weakened immune systems, serious illnesses, certain medical conditions, or those who have recently had surgery. They may also be dangerous to babies who have health problems. However, a few reports have indicated that giving probiotic supplements to babies caused blood infections. Probiotic supplements are not regulated by (Food and Drug Administration) (FDA).

SIGNIFICANCE:

This review summarizes the main advances on our comprehension of the human milk microbiota gained through the refinement of tools available for microbial ecology studies, identifying opportunities to address existing knowledge gaps.

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