Breast-milk lactobacilli and bifidobacteria: opportunities for the development of infant formulas

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Main Points:

- Breast milk lactobacilli and bifidobacteria constitute a promising option for the improvement of infant formulas.

- Supplementation of formula with breast-milk probiotics will allow the development of new products assisting in the establishment of a healthy microbiota and intestinal barrier.

- In the near future it is likely we will witness the routine supplementation of infant formulas with selected probiotics isolated from breast-milk.
ABSTRACT

Breast-milk is the best food for infant nutrition and development, protecting the newborn against allergies and infections. The main difference between breast and formula-fed infants regards the higher level of Bifidobacterium in the gut microbiota of the former group. This has been traditionally attributed to the presence of bifidogenic compounds, but recent studies indicate the presence of lactic acid bacteria and also bifidobacteria in breast-milk. The isolation and characterisation of these breast-milk microorganisms would allow the inclusion of specifically-selected bacteria in formulas targeted at both pre-term and full-term infants. This constitutes a very promising area for the development of new products aiming at assisting in the establishment of a healthy gut microbiota and a proper intestinal barrier resembling that of breast-fed infants.

BACKGROUND

The process of establishment and colonization of the infant gut is known to play an essential role for the maturation of the immune system and the establishment of the gut barrier [1]. Moreover, alteration in this process of colonization has been shown to predispose to disease later in life [2]. The factors driving gut colonization are diverse, among them the type of feeding is known to have a marked influence [3], with breast-milk being the best food for the infant. Breast-fed infants are protected against allergies, diarrhea, respiratory and gastrointestinal infections. Breast-feeding also affects at the establishment and development of the gut microbiota in the newborn. The main difference between breast and formula-fed infants regards the higher levels of Bifidobacterium in the microbiota of the former group. This has been traditionally attributed to the presence of bifidogenic compounds in breast-milk [4]. But recent studies indicate the presence of lactic acid bacteria and bifidobacteria in breast-milk [5-7], which may also contribute to the dominance of the latter microorganism on the breast-fed infant microbiota. Therefore, breast-milk can be considered a synbiotic food, containing beneficial microorganisms and oligosaccharides [8] which may critically contribute to the establishment of a healthy microbiota in breast-fed infants.

These facts have attracted the attention of the industry towards the supplementation of infant formula. The use of prebiotic oligosaccharides has been deeply investigated and currently it
constitutes a common practice on the manufacture of infant formulas. Attention has recently started to be paid to the addition of probiotics, and some strains have been included in formulas. Most of the currently used strains correspond to commercially available strains, not specifically selected to this end, which have not been isolated from the natural source. Only very recently bacteria isolated from breast-milk have gained attention as probiotics for driving or assisting in the development of the microbiota in the non-breast-fed infant.

NEEDS AND OPPORTUNITIES AT THE NEONATAL UNIT

Neonatal Units (NU) take care of newborns showing different problems at birth. Most of the work carried out at the NU relates to the care of preterm neonates (gestational age < 37 weeks). Among these infants those showing very low birth-weight (< 32 weeks and/or < 1500 g birth-weight) constitute the most demanding group, as they often present health complications. Currently, any baby born with a gestational age over 23-24 weeks has true possibilities of survival, although mortality and morbidity in this group of infants is extremely high.

At birth human beings are immature for most of their organs and functions. All the body systems must become mature during the first months of life to gain full functionality. Thus, these systems should adapt to extra-uterine life. In the preterm neonate this constitutes a very challenging process in which the gut microbiota establishment plays an important role. Feeding habits during the first days of life constitute a key point which will affect not only the health of the newborn, but also the future health of the individual [9,10].

There is a wide scientific consensus supporting breast-feeding as the ideal standard for the newborn between 0 and 6 months. In the case of the preterm infant, breast-feeding is often supplemented to complete the nutritional requirements [11]. Breast-feeding provides not only adequate nutrients with a high biodisponibility, but it also facilitates an appropriate colonization of the gastrointestinal tract. In addition to nutrients, breast-milk provides prebiotic substrates and potentially probiotic bacteria, which drive the process of development of the ideal gut microbiota. This last aspect, which is often not taken into consideration, may be one of the key determinants of the relationship observed between breast-feeding and protection against different diseases.
Unfortunately, despite of the general recommendation on breast-feeding, this is not always possible. For these situations infant formula manufacturers try to develop formulas whose composition resembles, as much as possible, that of the breast-milk. Adapted formulas, for both term and preterm infants, have improved significantly over the last years, reaching a high nutritional similarity to that of breast-milk. However, there is still lot of work to be done before they are equivalent in all aspects, mainly regarding their immunomodulatory properties. In this sense, infant formulas are still far from human milk as demonstrated by the increased risk of infection observed in the formula fed infant [12].

For these reasons, current research efforts focus on obtaining improved formulas, closely resembling breast-milk also in their immunological properties, to achieve a reduction of infection risks [13]. At the NU, reducing the risk of infection is essential. An adequate, microbial colonization of the neonatal intestinal tract is critical to prevent infection and other health problems whose relationship with diet may be more important than previously considered constituting a task for future research [10].

PROBIOTICS FOR NEONATES: NEEDS AND SOURCE OF ORIGIN

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a benefit on the host [14]. Different microorganisms are used as human probiotics, being Lactobacillus and Bifidobacterium the most frequently used genera. Most of the current commercial probiotic strains have not been selected for specific applications but rather selected on the basis of their technological potential. Nevertheless, it is well known that probiotic effects are strain specific, therefore it is very likely that better performing strains may be selected when a rational, use-specific, selection process is followed [15]. The selection of strains from appropriate sources depending on the target population, such as neonates, whose microbiota differ from that of adults, constitutes a promising approach.

Infants are one of the populations that may benefit more from the use of probiotics. There is a critical “window period” during the first months of life where there is an important opportunity for immune education, when the intestinal microbiota and maturation of the immune system are not yet completed [16]. Several groups of infants: preterm, formula-fed, those who use antibiotics or require intensive care, have an increased risk of developing diseases. Such diseases, including
infections, necrotising enterocolitis or atopic eczema have been prevented by probiotics [3]. The main aim of probiotic intervention in these populations is to avoid and aberrant microbiota development which may led to impaired gut barrier function and abnormal immune responsiveness [17]. The inclusion of specifically-selected breast-milk bacteria in formulas constitutes a very promising area for the development of new products aiming at the establishment of a healthy gut microbiota and a proper intestinal barrier, resembling that of breast-fed infants [15]. Nevertheless, so far few strains have been specifically selected for human neonates, where the applications of probiotics may have a great positive impact [18].

BREAST-MILK LACTOBACILLI AS PROBIOTICS FOR NEONATES

Lactobacilli are among the most abundant microbial genera of lactic acid bacteria found in human milk [6,19,20]. The probiotic potential of some lactobacilli isolated from breast-milk has been demonstrated. Relevant studies have been carried out characterizing the antimicrobial potential, survival to the simulated gastrointestinal conditions, adhesion to intestinal cell lines, and immunomodulatory properties of strains of \textit{L. salivarius}, \textit{L. gasseri}, and \textit{L. fermentum} isolated from breast-milk [21,22]. Safety and oral tolerance assays indicated that some of these strains were adequate for human consumption [23]. This work constituted a pioneer example of the way from scientific demonstration of the probiotic potential to the commercial application of breast-milk strains in infant formulas and other products [24].

Infant formulas and fermented dairy products for children do not seem to be the only target for the application of lactobacilli originating from breast-milk, and recent studies have also proven their usefulness in adults [25].

Genetic tools, allowing sequencing and annotation of the whole genome of some of these microorganisms, as in the case of the genome of a breast-milk \textit{L. salivarius} strain [26], will undoubtedly facilitate further studies on new functional probiotic properties and applications for these strains.

BREAST-MILK BIFIDOBACTERIA AS PROBIOTICS FOR NEONATES
As previously indicated, one of the main differences between breast and formula-fed infants is the dominance of bifidobacteria in the former group. It has been shown that allergic infants are often less colonised by bifidobacteria and present lower concentrations [27]. Interestingly, it has also been shown that allergic mothers show a lower concentration of breast-milk bifidobacteria than non-allergic ones, and their infants had lower levels of faecal bifidobacteria [28]. Thus, the early bifidobacterial composition appears to have an impact on the immune system. Interestingly, now we know that breast-milk contains bifidobacteria [5,6,29]. Thus, breast-milk provides a constant supply of *Bifidobacterium*, which may contribute to the dominance of this microorganism in breast-fed infants. This brings interest to this specific microbial group, and it suggests that these microorganisms may constitute a better option for supplementation than other microorganisms which do not play a dominant role on the breast-fed newborn gut. In fact, increasing bifidobacterial levels has often being considered a target for the formulation and development of infant formula [4].

The above mentioned facts have attracted the attention towards the isolation of bifidobacteria from breast milk. Some strains have already been isolated [5,6,7] with *B. breve* and *B. longum* being the species most frequently found, although other species such as *B. bifidum* and *B. pseudocatenulatum* have also been isolated. Therefore, these species constitute the prime choice species for the further selection of probiotic strains targeting neonates. In addition, some of the strains have been characterised and *in vitro* test have been conducted to determine their probiotic potential for neonates. The results indicate good adhesion ability for some of the breast-milk *Bifidobacterium* strains. Interestingly, higher adhesion of some strains to intestinal mucus from younger infants was observed, indicating that adhesion to intestinal mucus varies depending on the infant’s age and strain. This underlines the potential for selection of strains specifically targeting young infants [18].

**OTHER BREAST-MILK MICROORGANISMS AS PROBIOTICS FOR NEONATES**

To date, little is known about the potential health benefits (if they exist) that other breast-milk bacteria, different from bifidobacteria and lactobacilli, may have on the newborn. The search for strains able to exert beneficial effects has been mainly focused on the natural inhabitants of our gastrointestinal tract belonging to *Bifidobacterium* and *Lactobacillus*. However, a substantial and
recent body of scientific evidence suggests that other bacterial genera could rationally be screened for their use as probiotics [30]. Interestingly, members of the genera Staphylococcus and Streptococcus comprise up to 90% of the total microbial strains isolated from human milk [6,31]. This pool of enterococci, staphylococci and streptococci provides a continuous source of microbial inoculation for the breast-fed infant gut. Therefore, it is plausible and reasonable to think that due to the levels at which they are ingested, these microorganisms might play important roles in the infant development.

Obviously, a major safety concern appears immediately: the presence of pathogen representatives within these taxa. Typically, streptococci and staphylococci strains isolated from human breast-milk lack the genetic determinants coding for virulence factors, and are susceptible to a high number of antibiotics [31,32]. This lack of virulence factors and the higher sensitivity to antibiotics create a clearly differentiated barrier between the commensal microbiota of the human breast-milk and their pathogen relatives. Care should be taken about the presence antibiotic resistances and virulence factors encoded in mobile genetic elements, since they may be transferred from pathogenic to commensal strains through horizontal gene transfer. This mechanism must be clearly evaluated prior to the final strain selection. In addition, the establishment of differential phenotypic and genotypic traits that may group this commensal microbiota apart from their pathogen relatives is mandatory.

It is thus conceivable that the more abundant microorganisms of human milk might have important functions in the development of the infant gut. Even in certain cases these bacteria, with the appropriated methodology of selection, could be prospected for potential and specific health benefits [30].

CONCLUSIONS

Human breast-milk constitutes an interesting source for obtaining new and specific probiotic strains for neonates, aiming at assisting a proper development of the gut microbiota and the immune development in infants who, for different reasons, cannot be breast-fed.

Although clinical data would be needed before drawing any conclusion on their probiotic properties, some of the strains isolated from breast-milk may have good probiotic potential for their inclusion in infant formula. To this regard, the further improvement of the formula
composition constitutes a scientific challenge as well as a future opportunity to improve the health status of the neonate. It is also important to underline that infants constitute a highly susceptible population. Therefore, although the currently available data on probiotics indicate a good tolerability and safe consumption, a detailed safety assessment would be needed before any new strain is included in infant formula.

In the near future it is likely that we will witness routine supplementation of infant formulas with specifically selected probiotic strains isolated from breast-milk. To closely resemble the natural scenario, these supplements should not include exclusively lactobacilli and bifidobacteria, but perhaps also other breast-milk microorganisms.

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REFERENCES AND NOTES