British Journal of Nutrition (2007), **98**, Suppl. 1, S96–S100
doi: 10.1017/S0007114507832910

British Journal of Nutrition

Beneficial effects of probiotic bacteria isolated from breast milk

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Breast milk is the best food for the neonate because it provides a unique combination of proteins, carbohydrates, lipids, minerals and vitamins that ensures the correct growth and development of the infant. In addition, it also contains bioactive compounds responsible for a wide range of beneficial effects such as the promotion of immune system maturation and the protection against infections. Among these bioactive agents, probiotic bacteria have been recently isolated from human milk. The present work reviews the beneficial effects of these bacteria both in animal models and in clinical trials. The promotion of immune system maturation and defence against infections as well as the anti-inflammatory properties are among the main healthy effects of these bacteria. The isolation of probiotic bacteria with beneficial effects for the host provides scientific support for the supplementation of infant formula with these bacteria, in order to advance the pursuit of the main goal of formula: to mimic breast milk and its functional effects as closely as possible.

Probiotic: Human milk: Infant nutrition: Immune system

Human milk is a complex species-specific biological fluid adapted to perfectly satisfy the nutritional and immunological needs of the neonate. It has been demonstrated that breast milk confers protection against different infectious diseases since the incidence of these disorders is lower in breast-fed than in formula-fed infants[1,2]. It has been suggested that this anti-infective effect is due to several bioactive compounds present in colostrum and/or in mature milk. These include immunoglobulins, immune cells, antimicrobial acids, polyamines, oligosaccharides, lysosyme, glycoproteins such as lactoferrin and bioactive peptides, which, acting individually or synergistically, could inhibit pathogenic microorganisms[3,4].

Recent studies have demonstrated that human milk, far from being a sterile fluid, constitutes an excellent and continuous source of commensal bacteria for the infant gut[5,6]. These bacteria could also play an important role in the reduction of incidence and severity of infectious diseases in breastfed children. This hypothesis is supported by relatively old studies reporting the loss of antimicrobial activity in pasteurised human milk[7].

Among the bacteria found in human milk, those belonging to the species *Staphilococcus*, *Lactococcus*, *Enterococcus* and *Lactobacillus* are the most frequent[5,6] (Table 1). There is increasing interest in some of these breast milk lactobacilli, such as *L. gasseri*, *L. salivarius*, *L. rhamnosus*, *L. plantarum* and *L. fermentum*, because they are considered as potentially probiotic species (Table 1).

Breastfeeding and protection against diseases

In developing countries, one of the main causes of death in the paediatric age group is the infectious disease, specially gastroenterocolitis and respiratory infections. Newborns who have not been breastfed show a 17-fold higher risk of being hospitalised due to pneumonia than those who exclusively received human milk[8]. Similarly, the risk of death due to diarrhoea increases 14.2-fold in weaned infants[9]. Breastfeeding has also been related to a lower incidence of acute otitis media[10], urinary tract infection[11] and meningitis caused by *Haemophilus influenzae*[12].

Besides its anti-infective properties, it has been demonstrated that human milk modulates the immune system of the newborn[13]. Although an anti-inflammatory activity has not yet been demonstrated *in vivo*, several epidemiologic studies suggest that breast-fed children are protected against infections without the observation of evident lesion of the intestinal or respiratory mucosa due to an inflammatory response[14]. This is probably the result of an anti-inflammatory system better regulated by bioactive components of human milk.

The immunomodulatory action of breast milk could also explain the better antibody production response in breast-fed compared to formula-fed infants after vaccination against poliomyelitis, tetanus and diphtheria[15].

Neonates who received breast milk also have a more favourable intestinal microbiota than those fed infant formula[16], which is probably due to presence of lactic acid bacteria in human milk, besides other bifidogenic compounds such as oligosaccharides[17]. It has been suggested that these differences in intestinal microbiota could be responsible for some of the beneficial effects seen in breast-fed infants. It has been known for several decades that lactobacilli and bifidobacteria inhibit the growth of pathogen microorganisms such as *Staphylococcus aureus, Salmonella typhimurium,*

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Table 1. Bacterial species generally isolated from the breast milk of healthy women

Bacterial group	Main species
Staphylococcus spp.	*S. epidermidis*
	S. hominis
	S. capitis
	S. aureus
Streptococcus spp.	*S. salivarius*
	S. mitis
	S. parasanguis
	S. peores
Lactobcillus spp.	*L. gasseri*
	L. rhamnosus
	L. acidophilus
	L. plantarum
	L. fermentum
	L. salivarius
	L. reuteri
Enterococcus sp.	*E. faecium*
	E. faecalis

Modified from Martin *et al.*[32].

Yersinia enterocolitica and *Clostridium perfringens*[18]. These bacteria competitively colonise the intestine of the child, thus preventing the adhesion of pathogens. Moreover, a competition for nutrients is established and this is another mechanism that inhibits the growth of pathogenic microorganisms[19].

Intestinal colonisation by commensal bacteria also plays a key role in the maintenance of immune system homeostasis. These bacteria stimulate T_H1 responses and compensate the trend towards T_H2 responses characteristic of the neonatal immune system. It has been reported that the administration of specific probiotics to newborns reduces the incidence of atopic manifestations[20] and also of inflammatory processes where a T_H2 response is involved, such as necrotizing enterocolitis[21].

Probiotics isolated from breast milk

The description of the presence of bacteria in human milk dates back 30 years, but then it was assumed to be a contamination occurring during sample extraction[22]. At the beginning of the 21st century, two European groups independently demonstrated the presence of lactic acid bacteria in human milk and their probiotic potential[5,6]. Thus Heikkilä et al. reported that these human milk bacteria protect both mother and newborn from infections caused by *Staphilococcus aureus*[5]. In a similar way and in a series of reports, Martín et al.[6] described the isolation of lactic acid bacteria from human milk, namely, *L. gasseri* CECT5714[23], *L. salivarius* CECT5713[24] and *L. fermentum* CECT5716[23]. Besides these strains, there are other commercial strains related to human milk. One of them is *L. reuteri* ATCC55730, which is claimed to be derived from human milk but its origin has not been published yet. *L. rhamnosus* LGG, although originally isolated from intestinal sources[25], has also been found in human milk by the Finnish group[5]. Nevertheless, this work focuses on the beneficial effects of *L. gasseri* CECT5714, *L. salivarius* CECT5713 and *L. fermentum* CECT5714, which have been summarised in Table 2.

Anti-microbial effects

Protection against viral or bacterial infections is one of the most frequent claims made for probiotic consumption. Different mechanisms have been suggested to explain this anti-microbial activity (Fig. 1). *In vitro* studies demonstrate that certain probiotic strains produce anti-microbial compounds, such as organic acids, H_2O_2 and/or bacteriocins[26], that have been reported to inhibit the growth of *E. coli, Salmonella* spp. and *Listeria monocytogenes*[27]. No bacteriocin-producing *lactobacillus* has been found in human milk, although a high production of H_2O_2 has been reported[23].

Table 2. Beneficial effects of some breast milk-isolated probiotic strains

Strain	Beneficial effect	Reference
***L. salivarius* CECT5713**	Intestinal colonisation	Martin *et al.*, 2006[24]
	Production of antimicrobial compounds	Martin *et al.*, 2006[24]
	No D-lactic production	Martin *et al.*, 2006[24]
	Anti-microbial effect	Olivares *et al.*, 2006[27]
	Immunomodulatory effect	Díaz-Ropero *et al.*, 2006[30]
	Anti-inflammatory effect	Peran *et al.*, 2005[37]
***L. gasseri* CECT5714**	Intestinal colonisation	Martin *et al.*, 2005[23]
		Olivares *et al.*, 2006[38]
	Improved gastrointestinal function	Lara-Villoslada *et al.*, 2007[39]
		Olivares *et al.*, 2006[38]
	Production of antimicrobial compounds	Martin *et al.*, 2005[23]
	Anti-microbial effect	Olivares *et al.*, 2006[27]
	Immunomodulatory effect	Olivares *et al.*, 2006[35]
		Lara-Villoslada *et al.*, 2007[39]
	Anti-allergic effect	Olivares *et al.*, 2005[33]
***L. fermentum* CECT5716**	Intestinal colonisation	Martin *et al.*, 2005[23]
		Olivares *et al.*, 2006[36]
	Production of antimicrobial compounds	Martin *et al.*, 2005[23]
	Anti-microbial effect	Olivares *et al.*, 2006[27]
	Immunomodulatory effect	Diaz-Ropero *et al.*, 2006[30]
	Enhanced effects of vaccination	Olivares *et al.*, 2007[36]
	Anti-inflammatory effect	Diaz-ropero *et al.*, 2006[30]
		Peran *et al.*, 2005[37]
		Peran *et al.*, 2007[34]

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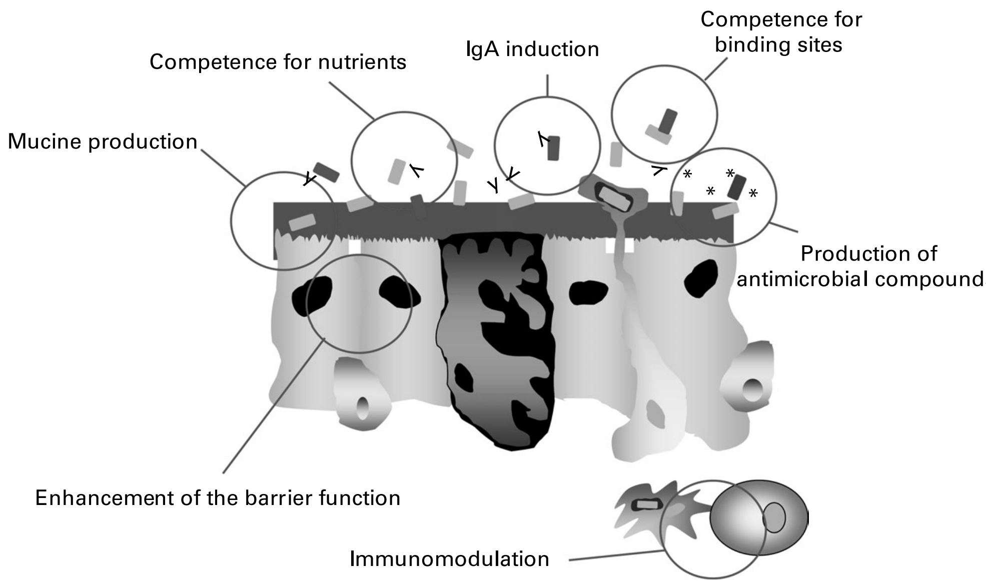

Fig. 1. Intestinal anti-infective mechanisms of probiotic bacteria.

In addition, those strains belonging to the species *L. reuteri* produce reuterin, another antimicrobial compound[28]. It has also been shown that some bacteria present in human milk improve the intestinal barrier function by increasing mucine production and reducing intestinal permeability[27]. However, competition with entero-toxigenic bacteria for nutrients and for epithelial intestinal cell receptor binding sites is probably the main anti-infective mechanism of probiotic bacteria[26,27](Fig. 1).

The human milk-isolated probiotics *L. gasseri* CECT5714, *L. salivarius* CECT5713 and *L. fermentum* CECT5714 have been reported to inhibit the adhesion of *Salmonella cholerasuis* to mucins and to increase the survival of mice infected with this pathogen[27]. It was demonstrated that the protective effect of *L. salivarius* CECT5713 is significantly higher than the effect of a reuterin producing strain[29]. This is probably due to the combination of the immunomodulatory role and the competitive activity reported for *L. salivarius* CECT5713[30].

Different clinical trials have demonstrated that, when breastfeeding is not possible, infant formula supplemented with probiotics protect children from infectious diseases. To our knowledge, most of the studies have involved supplementation with *L. rhamnosus* LGG, which have demonstrated a reduction in the incidence of rotavirus infection and in the duration of diarrhoea[31]. Currently, clinical studies are in progress to evaluate the tolerance and effectiveness of other breast milk strains, such as *L. reuteri* ATCC55730 and *L. salivarius* CECT5713.

Immunomodulatory properties

Intestinal colonisation is often the result of the first contact of the newborn with microorganisms, which is crucial for the development of the immune system of the neonate. It has been reported that differences in the composition of intestinal microbiota influence the incidence of certain pathologies with an important immunological component, such as allergic or inflammatory processes[32]. The anti-allergic effect of probiotics could be explained on the basis of the Hygiene Hypothesis and the T_H1/T_H2 balance. Probiotics induce a T_H1 response, and thus down-regulate the production of T_H2 cytokines, responsible for the allergic response.

In contrast, the anti-inflammatory effect of probiotics is more difficult to explain. *In vitro* studies have demonstrated that the immunomodulatory effects of probiotics depend on the cell environment. Thus, in the absence of additional stimulus, the breast milk probiotics *L. salivarius* CECT5713 and *L. fermentum* CECT5716 enhance the production of T_H1 cytokines such as IL-2 and IL-12 and the inflammatory mediator TNF-α[30]. However, when cells are incubated in the presence of lipopolysaccharide, together with the probiotics, a reduction of T_H1 cytokines is observed[30]. This regulatory mechanism is probably based on the production of IL-10, an immunosuppressive cytokine, which has been reported to be increased by these probiotic strains[30].

The immunomodulatory effects of probiotics have also been reported in animal models of pathologies where the immune system is involved. Different probiotic strains isolated from human milk have been reported to enhance the immune defence of mice, increasing both natural and acquired immune responses[30]. This immune-stimulating activity could be also involved in the anti-infective role previously mentioned for these bacteria in an animal model of *Salmonella* infection[27]. In addition, the breast milk probiotic *L. gasseri* CECT5714 in combination with *L. coryniformis* CECT5711 reduces the incidence and severity of the allergic response in an animal model of cow's milk protein allergy[33]. In a recent report, *L. fermentum* CECT5716 showed a beneficial effect in an animal model of intestinal inflammation, reducing the inflammatory response and the intestinal damage[34].

Probiotics have also been reported to modulate the immune response of healthy humans, as shown by a recent study which reports an increase in phagocytic activity, in the number of natural killer cells and in the plasma concentration of IgA in healthy humans consuming human milk-isolated probiotics daily for 3 months[35]. A more recent report demonstrates that the consumption of *L. fermentum* CECT5716 enhances the response to influenza vaccination in healthy volunteers aged 26-40 and reduces the incidence of influenza-like illness[36].

In addition, the beneficial effect of probiotics in allergic processes has been widely reported. In this sense, the consumption of probiotics present in human milk, especially *L. rhamnosus* LGG, has been shown to reduce the incidence and severity of atopic dermatitis in children[20]. Although less is known about other allergic disorders, there is data to support a positive effect of *L. gasseri* CECT5714 in adults with respiratory allergy[33].

Gastrointestinal benefits

There is increasing interest in the manipulation of intestinal microbiota with the aim of improving gastrointestinal function and nutrient absorption. Different reports demonstrate that human milk probiotics colonise the intestine and increase faecal lactobacilli counts thus modifying intestinal microbiota both in rodents[37] and humans[38]. In addition, molecular analysis show that these bacteria are metabolically active in the human gut, increasing the production of functional metabolites such as butyrate[38], which is the main energy source for colonocytes and plays a key role in the modulation of intestinal function. In the previously mentioned clinical trial[38], an increase in faecal moisture, and in stool frequency and volume was observed which could be related to the increase in the faecal concentration of butyric acid.

Similarly, the administration of *L. gasseri* CECT5714 also caused an increase in faecal lactobacilli counts in a clinical trial in children aged 3-12[39]. In the same study the cytotoxicity of the faecal water of children who received the probiotic has been shown to be lower than that of the control children[39]. Finally, in another clinical trial the supplementation of infant formulas with *L. rhamnosus* LGG has been demonstrated to improve neonate growth pattern, which could suggest an increased bioavailability of nutrients in these infants[40].

Conclusions

Breastfeeding is the main determinant of the intestinal colonisation of the neonate, which, apart from other components, is due to the recently discovered presence of probiotic bacteria in human milk. In addition to gastrointestinal benefits, modulation of microbiota by probiotic bacteria has been shown to regulate the immune function and to enhance defence against intestinal pathogens. Thus, the addition of breast milk probiotics to infant formulas could be a new alternative to mimic some of the functional effects of human milk in children who are not breastfed.

Conflict of interest statement

All the authors except JMR are employees at Puleva Biotech SA. All the studies presented have been funded by Puleva Biotech's own founds. This review has mainly been written by JX with collaboration of all the other authors.

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