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## Lactobacillus Species in Breast Milk

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### Abstract

*Lactobacillus* species, present in the microbiota of breast milk, is a probiotic that deserves significant attention. It has a beneficial effect on the composition of the intestinal microflora and the intestinal immune system. In infants who were having *Lactobacillus fermentum*, a lower incidence of gastrointestinal and respiratory infections was noticed, in contrast to the control group. The significant anti-inflammatory effect of *L. fermentum* can be utilized to prevent and treat mastitis in breastfeeding women. It has also been shown to have a better clinical effect than classic antibiotics. Moreover, the higher share of *L. fermentum* in intestinal microflora of children with normal weight compared to obese ones opens other potential possibilities of the use of this probiotic.

**Keywords:** *Lactobacillus*, microbiota, probiotics, mastitis, obesity

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## 1. Introduction

Microbiota is a substantial collection of genetic and bioactive materials responsible for building and regulating our defense systems. Bacteria and their intestinal microbial proportions modulate the immune system, greatly affecting the health and illness of an individual. Gastrointestinal flora is in close and continuous contact with epithelial and immune cells. This constant stimulation is essential for the development and functioning of the immune system [1]. These types of bacteria that colonize the guts of a newborn determine how the system develops, acting as an important antigenic stimulus for developing the immune response.

In the last 20 years, probiotics, bifidobacteria, *Lactobacilli*, microorganisms, and gastrointestinal flora, all of which can modulate the aspects of both natural and acquired immune responses in the host and thus affect human health, have become of prime importance. This importance is, of course, widely emphasized commercially. However, the actual effects and actions of individual probiotic strains vary, and it is very important to know what specific

probiotics are considered in order to determine their effects. Bacteria colonize vast areas of the mucous membranes and they are also present in important body fluids like breast milk. The mother's vaginal flora and breast milk are clearly among the most important sources of bacteria for the newborn. Varying studies have reported differing quantities of live bacteria in breast milk, but most studies report median numbers of  $10^2$ – $10^3$  and a range of  $10^1$ – $10^7$  colony forming units per ml of breast milk [2]. The infant who receives 300–700 ml of milk per day receives a large amount of these bacteria at the same time. The microbiota of milk, like that of mucous membranes, is individual and changeable. The probiotic bacteria present in mucous membranes and breast milk includes *Lactobacillus fermentum*. The expected pathway by which *Lactobacilli* is received into the milk is enteromammary transport through the dendritic cells [3]. This type of transport is still a controversial subject; however, various studies suggest that dendritic cells can pick up bacteria located in the intestinal lumen and transfer them to the lamina propria. Once the bacteria get inside the dendritic cells, they can penetrate the mammary glands and other mucosal surfaces.

## 2. *Lactobacillus* species and infectious diseases of infants

Respiratory and gastrointestinal tract infections are a significant problem for young children attending daycare centers or preschool, especially in the winter season. Common infectious diseases are facilitated by a general immaturity of the immune system and of the respiratory and gastrointestinal tract function [4]. An increased number of acute diseases translate into a significant financial burden for both the family and society. The increased costs are related to medical care visits and medication as well as to time away from work and/or for payment for someone to look after a sick child [5].

The most widely used probiotic species, which belong to the genera *Lactobacillus* and *Bifidobacterium*, have shown clinically significant benefits in the treatment and prevention of childhood diarrheal and allergic diseases in at-risk populations such as allergic families, hospitalized patients, or children in daycare centers. In a study in which *Lactobacillus reuteri* was administered for 3 months in 336 otherwise healthy children attending daycare centers, it was shown that during the administration and for the next 3 months, the number of episodes of diarrhea has significantly decreased [6]. The effects of probiotics in preventing respiratory tract infections are also receiving increasing attention. In accordance with the same study mentioned earlier, the number of respiratory tract infections in the 336 children has also significantly decreased at 3 and 6 months after the administration of the probiotics [6]. There are many sources of confusion concerning probiotic intervention in children. First, the mode of probiotic administration in the general child population is challenging. Second, the selection of a specific probiotic strain or a probiotic mixture is crucial for the possible beneficial effects. The duration of breastfeeding and the use of infant formula also affect the outcome [7]. Several clinical studies have been carried out to investigate bacteria isolated from human milk. In a 6-month study [8] with 91 infants in the control group and 97 infants in the *L. fermentum* group, a reduction in the total number of infections, especially gastrointestinal tract and respiratory infections, was observed in the probiotics group (**Table 1**). *L. fermentum* was selected for the study for safety and for its anti-infective and immunomodulatory properties.

	Control group	Experimental group	Incidence rate decrease (%)
Total infections	189	142	30
Gastrointestinal infections	33	19	46
Respiratory infections	134	106	26
• Upper respiratory	121	94	27
• Lower respiratory	13	12	13

**Table 1.** *Lactobacillus fermentum* administered to 6-month-old infants over a 6-month period versus *Lactobacilli*-free control group [8].

This strain is also able to colonize the mammary glands when administered to nursing mothers in capsule form. A similar effect on the health of children has been described in other probiotic strains. A multicenter, randomized, double-blind, placebo-controlled trial [4] on 126 healthy children aged 12–48 months with *Lactobacillus paracasei* (66 infants in the experimental group and 60 infants in the placebo group) showed a lower incidence of respiratory and gastrointestinal tract infections in the experimental group than in the control group (**Table 2**). An immunostimulatory effect was observed, consisting of a significant increase in the production of innate and acquired immunity peptides. Innate immunity peptides, produced by epithelial cells, Paneth cells, neutrophils, and macrophages, act as endogenous antimicrobial substances and defend the body against a broad range of pathogens (bacteria, fungi, protozoa, and viruses).

Another bacterium isolated from breast milk that has a positive effect on diseases in infants and children is *L. reuteri*. The mechanism of action of *L. reuteri* strains has been evaluated in *in vitro* and animal studies. One of the best-documented mechanisms is their antimicrobial activity. *L. reuteri* strains produce reuterin, a broad-spectrum antibacterial substance that can

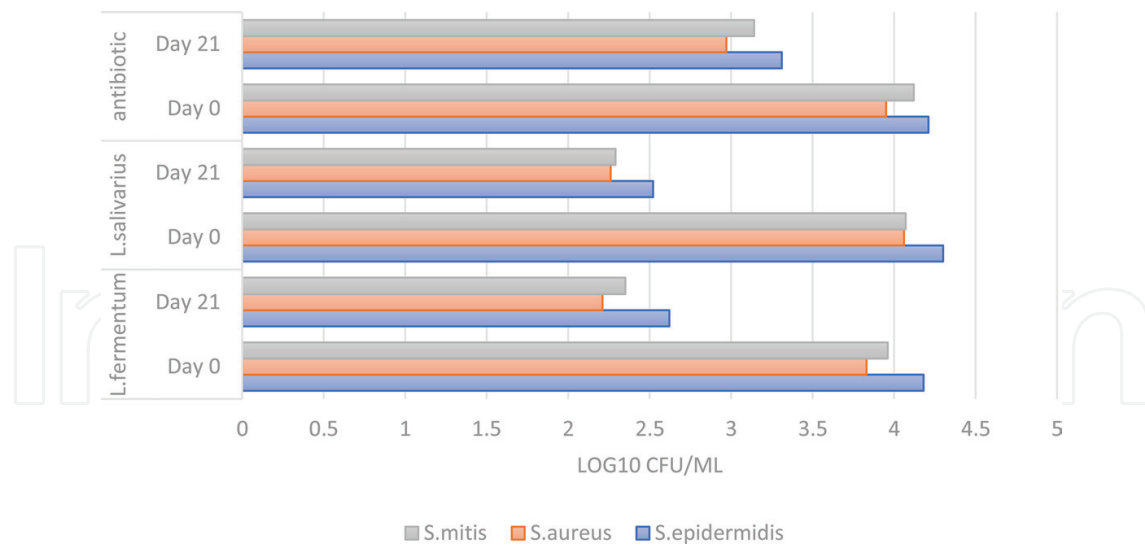
Disease	Control group	Experimental group	<i>p</i>
Acute gastroenteritis, <i>n</i> (%)	24 (40.0)	12 (18.2)	0.007
(number of episodes)	(28)	(19)	
Rhinitis, <i>n</i> (%)	24 (40.0)	22 (33.3)	0.438
(number of episodes)	(50)	(44)	
Otitis media, <i>n</i> (%)	13 (21.7)	8 (12.1)	0.151
(number of episodes)	(17)	(11)	
Pharyngitis, <i>n</i> (%)	25 (41.7)	13 (19.7)	0.007
(number of episodes)	(30)	(22)	
Laryngitis, <i>n</i> (%)	14 (23.3)	6 (9.1)	0.029
(number of episodes)	(14)	(7)	
Tracheitis, <i>n</i> (%)	19 (31.7)	11 (16.7)	0.048
(number of episodes)	(30)	(16)	

**Table 2.** Common infectious diseases observed during the study period [4].

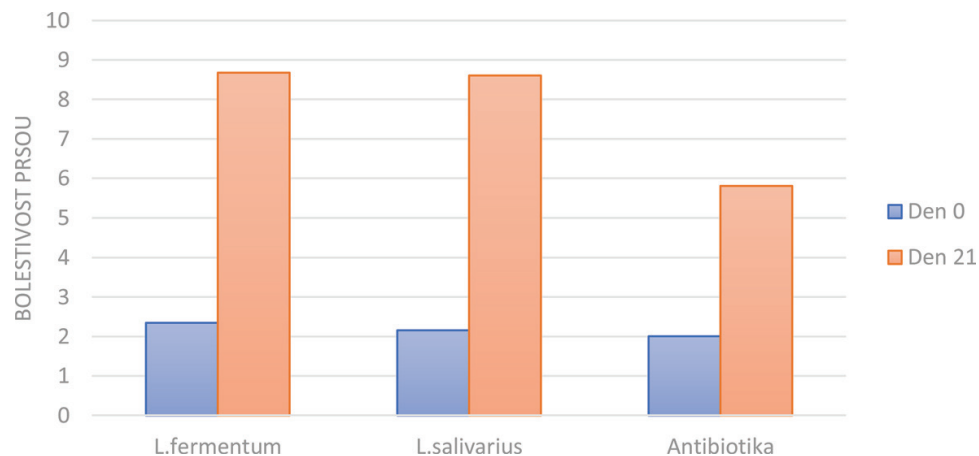
inhibit the growth of a wide spectrum of microorganisms such as Gram-positive or -negative bacteria, yeast, fungi, and parasites. *L. reuteri* strains may also regulate immune response. The results of 14 studies involving controlled trials and one systematic review indicate that the use of *L. reuteri* may be considered in the management of acute gastroenteritis as an adjunct to rehydration. There is also some evidence that *L. reuteri* is effective in reducing the incidence of diarrhea in children attending daycare centers [9]. *Lactobacillus rhamnosus*, a probiotic strain of human origin, also influences immune response both specifically by stimulating antibody production and non-specifically by enhancing the phagocytic activity of the blood leucocytes. It can promote the recovery from rotavirus diarrhea and can reduce the incidence of diarrhea associated with the use of antibiotics. In a randomized, double-blind, placebo-controlled study with 571 healthy children aged 1–6 years, there was a 17% relative reduction in the number of children with respiratory infections with complications and lower respiratory tract infections and a 19% relative reduction in antibiotic treatments for respiratory infection [10].

### 3. *L. fermentum* in the treatment of mastitis

Mastitis is a common disease during lactation, affecting 3–33% of lactating mothers. Inflammation of the mammary glands usually has an infectious origin involving staphylococci, streptococci, and/or *Corynebacterium*. Traditionally, *Staphylococcus aureus* has been considered the main etiological agent of acute mastitis, although *Staphylococcus epidermidis* is emerging as the leading cause of chronic mastitis. Multidrug resistance and/or the formation of biofilms are very common among clinical isolates of these two staphylococcal species. This explains why mastitis is difficult to treat with antibiotics and why it constitutes one of the main reasons to cease breastfeeding. In this context, the development of new strategies based on probiotics, as alternatives or complements to antibiotic therapy for the management of mastitis, is particularly appealing. The anti-inflammatory effect of *L. fermentum* can be successfully used to prevent and treat mastitis in a breastfeeding woman. Given as a nutritional supplement to a woman with breast inflammation, it demonstrated a better clinical effect than conventional antibiotics. Moreover, a higher proportion of *L. fermentum* in breast milk is beneficial to the child by favorably modulating the child's intestinal microflora, with beneficial consequences for the immune system and health. A study [11] of 352 women with symptoms of mastitis demonstrated a beneficial effect of treatment with lactobacilli. The women were divided into three different groups: one group using *L. fermentum* and one group using *Lactobacillus salivarius*, both strains isolated from human milk and a third group receiving antibiotics. After 21 days, a reduction in the number of the main etiological agents causing mastitis (*S. epidermidis*, *S. aureus*, and *Streptococcus mitis*) was observed. This reduction was greater in the probiotic groups (**Figure 1**). The groups in which *Lactobacilli* were used also experienced greater pain reduction (**Figure 2**). A similar study of 225 women with severe mastitis caused by staphylococci demonstrated a beneficial effect of *L. fermentum* treatment. There was a faster retreat than in the control group treated with antibiotics. Mastitis relapse was more common among the women treated with antibiotics (31% versus 10%). The principle of the antibacterial action of *L. fermentum* could be explained by its high ability to adhere



**Figure 1.** Bacterial counts from breast milk at the beginning (day 0) and at the end (day 21) of the trial [11].



**Figure 2.** Breast pain score at the beginning (day 0) and at the end (day 21) of the trial. Pain is expressed as extremely painful (0) to no pain (10) [11].

to epithelial cells and inhibit the adhesion of pathogenic bacteria by producing antimicrobial compounds (lactic acid,  $H_2O_2$ ) and by its effect on increased mucin production. The action of *L. fermentum* is immunostimulatory.

#### 4. Individually different microflora of normal-weight and obese individuals and the role of probiotics

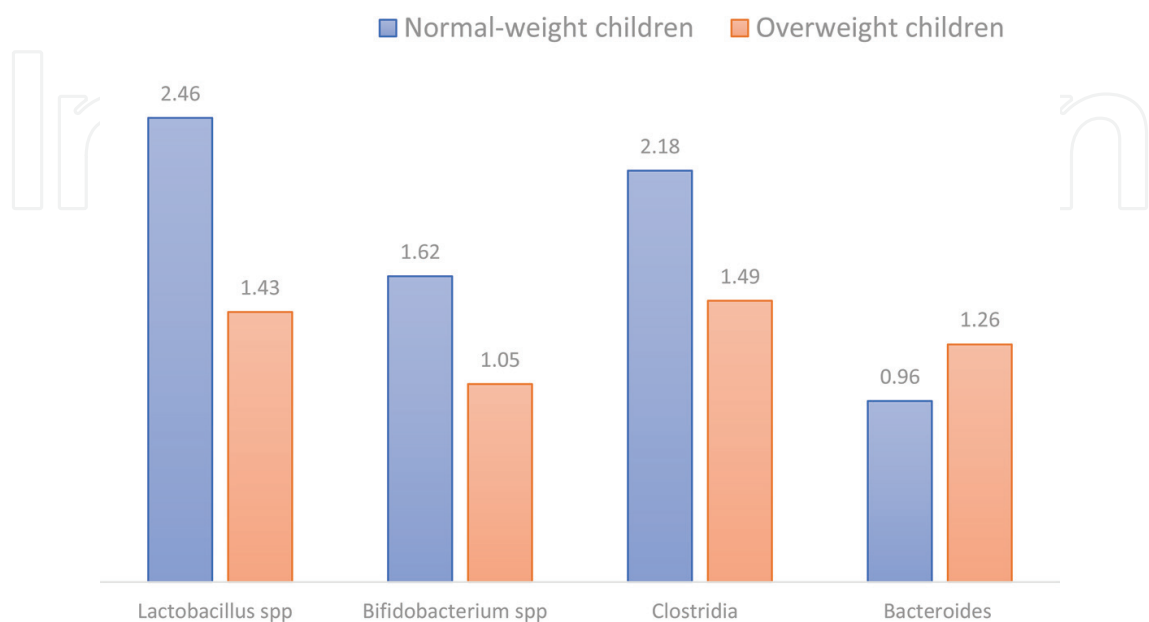
Obesity is viewed as one of the more important public health problems of our time, and the velocity of propagation is highest in children. This can lead to a vicious circle: obese children often become obese adults, and maternal obesity overnourishes the fetus, thereby programming adult size and health with a heightened risk of obesity later in life. Recent scientific



advances point to systemic low-grade inflammation and local gut microbiota as contributing factors for overnutrition. The gut microbiota enables the hydrolysis of indigestible polysaccharides into easily absorbable monosaccharides and the activation of lipoprotein lipase by direct action on the villous epithelium. Consequently, glucose is rapidly absorbed and fatty acids are excessively stored, with both processes boosting weight gain.

Bacterial milk composition in obese mothers differs from the bacterial milk composition of mothers with standard body weight [11]. Since breast milk is one of the most important means of colonizing infants with bacteria, there is an idea that there is a relationship between obesity and the transmission of microbial flora from mother to infant. It is known that obese infants and obese children generally have very different microbial flora from infants who are lean and healthy (**Figure 3**). The results reported by Kalliomäki et al. suggested that gut microbiota deviations predispose individuals toward energy storage and obesity. The genus *Bifidobacterium*, affecting both the quantity and quality of the microbiota during the first year of life, was shown to be higher in children who remained normal weight than in children developing overweight. The microbiota aberrancy during infancy in children becoming overweight was also associated with a greater number of *S. aureus* than in children remaining normal weight as assessed by real-time qRT-PCR. These findings imply that high numbers of probiotics and low numbers of *S. aureus* in infancy may provide protection against overweight and obesity development.

Perhaps it would be advisable to think about intervention in cases of obese mothers. When is the right time for such an intervention? We know that some bacteria are transmitted from mother to infant. For an obese mother, it would be most helpful to choose an appropriate intervention before or during pregnancy, in any case before giving birth. If the microbial flora has already been transferred to the infant, it could be optimized during breastfeeding through specific probiotics. *L. fermentum*, a strain isolated directly from breast milk in the form of a food supplement, is available as a possible solution. Whether the expected effect of normalization of the intestinal microflora can be produced by such a solution should be confirmed by further studies.



**Figure 3.** Bacterial counts in fecal samples analyzed by fluorescent *in situ* hybridization during infancy (6–12 months) [12].

## 5. The effect of probiotics deserves further clinical trials

The mucosal microbiota is formed by millions of bacteria. The *Lactobacillus* species are undoubtedly important bacteria for the development of humoral and cellular immunity. However, in the human gut, they are only a part of a huge mosaic where each particle has its place and function. After decades of research, probiotics are still an open chapter of great and unimagined opportunities to influence the immune system and to treat some of civilization's diseases. Most of these diseases are multifactorial. Influencing the mucosal microflora seems to be a promising step. Available data suggest that some probiotics such as *L. fermentum*, *L. reuteri*, *L. paracasei*, and *L. rhamnosus* may have some effect on community-acquired infections; however, confirmation studies are still needed.

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