

# Postbiotics: A new member in the biotics family

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## ABSTRACT

Postbiotics were defined in 2021 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as a “preparation of inanimate microorganisms and/or their cellular components that confers a health benefit to the host.” The field of postbiotics is a new area within the biotics family; numerous products have already been developed for clinical applications, such as immune stimulation, the management of diarrhea in children and adults, the management of irritable bowel syndrome, and 3 infant formulas.

In particular, infant formulas with postbiotics obtained from milk fermented with *Bifidobacterium breve* C50 and *Streptococcus thermophilus* O65 —and their metabolites—, including the oligosaccharide 3'-GL, have demonstrated to be safe and to contribute to the development of the gut microbiota and the gut-associated immune system. These modifications help to prevent and manage functional gastrointestinal disorders in infants.

**Keywords:** *microbiota; human milk; infant formulas; fermentation; postbiotics.*

doi: <http://dx.doi.org/10.5546/aap.2023-10168.eng>

**To cite:** Toca MC, Burgos F, Tabacco O, Vinderola G. Postbiotics: A new member in the biotics family. *Arch Argent Pediatr.* 2024;122(5):e202310168.

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**Funding and conflict of interest:** Meetings to organize the work were held through digital platforms provided by Nutricia-Bagó. The manuscript was written with the help of all authors in an autonomous manner; the company did not interfere with the editorial management or the final article. The authors state that their only relationship with Nutricia-Bagó was their participation as lecturers in conferences and symposiums organized by the company. GV is a member of the board of directors of the International Scientific Association of Probiotics and Prebiotics (ISAPP).

**Received:** 7-26-2023

**Accepted:** 8-17-2023



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## INTRODUCTION

Breast milk (BM) contains a wide variety of live and non-viable microorganisms, whose transmission to the infant contributes to determine their present and future health, to shape the neonatal gut microbiome, and to modulate the immune system. The development of massive sequencing techniques allowed to characterize the composition, diversity, and variability of BM microbiota,<sup>1</sup> which revealed a complex and dynamic ecosystem.<sup>2</sup> Numerous external factors, such as medication use, diet, and emotional factors, among others, may induce BM dysbiosis.<sup>3</sup> The administration of antibiotics in the perinatal period and during breastfeeding has been associated with reduced bacterial diversity in BM.<sup>4</sup> Dietary patterns rich in fiber and the intake of vitamins C and B complex have also shown to improve BM composition.<sup>5</sup> Maternal post-natal psychosocial distress has been linked to lower BM bacterial diversity at 3 months post-delivery.<sup>4</sup>

As for donated BM, it is subjected to heat treatment to achieve stability and ensure microbiological safety. The heat treatment used by most milk banks is the Holder pasteurization method, which is characterized by heating BM to a temperature of 62.5 °C for 30 minutes, then cooling it rapidly to 4 °C in less than 15 minutes. Although pasteurization causes the loss of some of the biological, structural, and functional properties of BM, pasteurized BM is the preferred alternative when breastfeeding is not available. However, new heat treatments are being developed to minimize the impact on the functional components of BM, such as the high temperature short time (HTST) or the high-pressure processing (HPP) methods.<sup>6</sup>

Heat treatments do not modify the prebiotic effect of BM; they eliminate the viability of its microorganisms, but not its functionality entirely because non-viable microorganisms also have an immunomodulatory ability.<sup>7</sup> If we then consider the concept of postbiotics (a composite term from the Greek prefix meaning “after” and “biotic” meaning “life”), which is explained in the section below, pasteurized BM would exert its postbiotic effect through non-viable, but intact, microbial cells, microbial cell fragments, and their metabolites.<sup>7</sup>

The objective of this study was to conduct a non-systematic review of the functional characteristics of infant formulas that contain postbiotics.

## POSTBIOTICS: DEFINITION, SCOPE, AND DIFFERENCE FROM PROBIOTICS

The knowledge about the beneficial effects of non-viable microorganisms and fermentation products is not recent. However, scientists have used a series of divergent terms to refer to this phenomenon, such as heat-inactivated probiotics, inactivated probiotics, non-viable probiotics, tyndallized probiotics, cell fragments, cell lysates, paraprobiotics, or postbiotics.<sup>8</sup> In an effort to establish a common term and propose a consensus definition, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined postbiotics as a “preparation of inanimate (non-viable) microorganisms and/or their cellular components that confers a health benefit to the host.”<sup>7</sup>

Both probiotics and postbiotics must be identified at the genus, species, and strain level, in addition to sharing the requirement that their beneficial effects be demonstrated by safety and efficacy studies. In the case of postbiotics, inactivation or elimination of viability does not imply a loss of activity or functionality, since non-viable cells and fermentation metabolites may interact with the gut-associated immune system. In terms of safety, postbiotics do not entail a risk of translocation, especially in vulnerable populations or in case of a weakened gut barrier. A postbiotic is not a probiotic that lost viability throughout its shelf-life. A microorganism is considered a postbiotic if the efficacy study was conducted with the microorganism deliberately inactivated. Unlike probiotics, the definition of postbiotics also contemplates the possibility of fermentation metabolites being present, due to the health impact that microbial metabolites from the fermentation of a substrate can have.<sup>7</sup>

Some examples of postbiotic products already available on the international market are those based on a combination of heat-inactivated *Limosilactobacillus fermentum* CNCM MA65/4E-1b and *Lactobacillus delbrueckii* subsp. *delbrueckii* CNCM MA65/4E-2z —and their fermentation metabolites— for the management of diarrhea in children and adults;<sup>9</sup> on a spray-dried inactivated culture of *Aspergillus oryzae* —and its fermentation metabolites— for animal nutrition;<sup>10</sup> on a heat-inactivated strain of *Sacharomyces cerevisiae* as an immune booster;<sup>11</sup> or on heat-inactivated *Bifidobacterium bifidum* MIMBb75 for the management of irritable bowel syndrome.<sup>12</sup>

## MECHANISMS OF ACTION AND SAFETY OF POSTBIOTICS

The mechanisms of action of prebiotics, probiotics, and synbiotics are targeted at the development of a healthy gut microbiota, represented in the first months of life by the *Bifidobacterium* and *Lactobacillus* species.<sup>13</sup> Molecules (especially short-chain fatty acids) responsible for the development of the gut mucosa,<sup>14</sup> immune maturation,<sup>15</sup> metabolic programming<sup>16</sup> and neurodevelopment<sup>17</sup> are generated from the metabolic activity of these bacteria. In relation to postbiotics, due to the technology used to obtain them (bacterial fermentation of a milk matrix followed by sterilization by spray drying), the oligosaccharides responsible for the prebiotic effect and the metabolites derived from bacterial fermentation are preserved, in addition to inactivated microorganisms and traces of DNA, cytoplasmic components, and cell membranes.<sup>7</sup>

Based on these elements present in postbiotics, the mechanisms of action responsible for their effects on health are the modulation of the gut microbiota, the local and systemic immune response, and the general metabolism; the enhancement of the barrier function of the gut mucosa; and the molecular connections of the gut-brain axis.<sup>7</sup> Most likely, given their combined action, such physiological effects are greater than those expected with each of the components individually.<sup>18</sup>

Given that they are inactivated microorganisms, the microbiological safety of postbiotics is warranted to prevent the potential translocation of live microorganisms to the internal milieu. In addition, such inactivation allows a wider range of storage conditions compared to products with viable microorganisms. However, the safety assessment also contemplates that the bacterial fermentation products do not have potentially toxic molecules, which is achieved with the proper selection of strains and the control of fermentation conditions.

## POSTBIOTICS IN INFANT FORMULAS

The primary objective of clinical studies with infant formulas (IFs) is to establish the safety, tolerance, and normal development of infants as per the standards established by the World Health Organization (WHO). Their secondary objective is to prove the benefits of IF on the gut microbiota, attempting to bring it closer in composition and activity to that received by infants fed with breast

milk, in addition to achieving a positive impact on the maturation of the gut immune system.

The aspects frequently studied include anthropometric data to ensure normal growth; the development of adverse effects; stool consistency, frequency, and pH (stool acidity is an indirect indicator of increased concentration of lactobacilli and bifidobacteria); thymus growth as a marker of immune system development and maturation; and improvement of functional symptoms, such as crying, cramps, and regurgitation. In recent years, investigations have extended to include the study of immunoglobulin A (IgA) content in stools, gut microbiota composition, and metabolic activity, with the measurement of short-chain fatty acid (SCFA) and lactate levels.

An IF fermented with *Lactobacillus paracasei* CBA L74 was developed; the microorganism was heat-inactivated after fermentation. The formula was given for 3 months to children aged 12 to 48 months; a decrease in infections (acute gastroenteritis, pharyngitis, laryngitis, and tracheitis) was observed, with an increase in the level of defensins, secretory IgA (S-IgA), and cathelicidin in stools.<sup>19,20</sup> In another study, it was shown that the gut microbiota of children who received this IF was similar to that of children fed with BM, with reduced diversity, intermediate IgA levels, and a metabolome similar to that of the group who received BM.<sup>21</sup>

In the efficacy study of the fermented formula containing a postbiotic derived from *Bifidobacterium animalis* subsp. *lactis*, BPL1, it was observed that infants fed with this formula, in their follow-up up to 1 year of age, showed adequate growth curves, good tolerance, and fewer gastrointestinal symptoms than infants fed with standard infant formula (SIF), with significantly less events of atopic dermatitis, bronchitis, and bronchiolitis.<sup>22</sup>

Clinical studies with partially fermented IF with *Bifidobacterium breve* C50 and *Streptococcus thermophilus* O65 showed an adequate development in infants according to the WHO growth curves, in terms of both weight and height as well as head circumference.<sup>23–27</sup> This was even demonstrated in preterm newborn infants born at 30–35 weeks of gestation.<sup>28</sup>

The gastrointestinal tolerance was adequate.<sup>24–27</sup> In the study of preterm newborn infants,<sup>28</sup> subjects had less abdominal distension after 2 weeks of receiving fermented infant formula (FIF) for preterms compared to those who received standard preterm formula ( $p < 0.016$ ),

with no differences in gastric residuals and stool characteristics between both groups. No significant differences were observed in the development and severity of adverse events.

When FIF was used, a significant decrease in cramps was observed,<sup>24,25</sup> stool consistency<sup>24–26</sup> as well as stool pH,<sup>27,29,30</sup> were similar to those of infants fed with BM.

It was shown that infants fed with FIF had less severe diarrhea<sup>23</sup> and greater thymus development.<sup>29</sup> Feeding with FIF was not shown to decrease the incidence of cow's milk protein allergy, but it does decrease sensitization to food allergens, such as cow's milk protein.<sup>31</sup>

At 4 months of age, the mean IgA level in the group receiving FIF with 3-galactosyl-lactose (GL) and galacto-oligosaccharides and fructo-oligosaccharides (GOS/FOS) was significantly higher than in the group receiving SIF ( $p < 0.03$ ) and was the most similar to the group receiving BM. In addition, in previous studies, it was observed that IgA levels increased with formulas containing GOS/FOS. In this study, the effect on IgA levels was demonstrated to be greater when FIF supplemented with GOS/FOS was used. In addition, stools showed significant increases in acetate and lactate, with no changes in butyrate.<sup>27,30</sup>

In relation to the gut microbiota, infants fed with BM showed the highest percentage of bifidobacteria, but the composition of the gut microbiota in infants fed with FIF showed favorable changes as of the fourth month, with an increase in bifidobacteria compared to SIF.<sup>27,30</sup> The development of several bacterial genera (7 at 8 weeks of age, 16 at 17 weeks of age) with significant difference in abundance in infants fed with FIF versus SIF was also demonstrated.<sup>30</sup> At 17 weeks, the samples showed bacterial clusters that were more closely aligned with the levels detected in the group that received BM. As for the fecal metabolome, this study observed more than 400 different metabolites in BM-fed infants, with a wide difference to those found in infants fed with IF. However, the data demonstrated that the administration of FIF and prebiotics may induce responses in gut microbiota composition that are closer to the fecal metabolite profile of those fed with BM than to that of those fed with SIF.

A 2022 systematic review that included 11 randomized studies concluded that the IFs with postbiotics assessed so far are safe and well-tolerated by infants who cannot be breastfed. However, and depending on the IFs considered

in that systematic review, the authors could not reach a conclusion on the clinical effects and benefits of these formulas over others.

According to the authors of that systematic review,<sup>32</sup> it is reasonable to discuss with the healthcare providers involved the characteristics of these IFs in terms of decision-making.<sup>32</sup> The referenced systematic review<sup>32</sup> did not include evidence from a previous study because it focused on preterm infants<sup>24</sup> nor the most recent study that included metabolome analysis<sup>30</sup> because it was published after the review. Therefore, future reviews including this evidence may result in different recommendations due to the evidence that is being accumulated about the combination of prebiotics and postbiotics.

## CONCLUSION

The concept of postbiotics refers to the benefits of inactivated microorganisms and their metabolites on health. Heat treatment of BM for its preservation in milk banks does not modify its prebiotic content. Although the microorganisms are inactivated, they partially maintain their functionality, acting as putative postbiotics.

The field of postbiotics is a new area within the biotics family; products with different clinical applications have already been developed. For the time being, available evidence for postbiotics in IF analyzed in a systematic review does not allow to determine their superiority over other IFs, so their use remains at the discretion of healthcare providers. A recent study, not included in the systematic review, showed that prebiotic- and probiotic-containing IFs maintain the metabolome closer to that of infants receiving breast milk than IFs without these biotics.

IFs with postbiotics obtained from milk fermented with *Bifidobacterium breve* C50 and *Streptococcus thermophilus* O65, and their metabolites, have demonstrated to be safe and to contribute to the development of the gut microbiota and the associated immune system. These modifications help, for instance, to prevent and manage functional gastrointestinal disorders in infants.

Short-term studies may assess non-clinical parameters which may be indicative of a potentially positive impact on the child, such as thymus size, stool type and frequency, IgA levels, and the composition and function (metabolome) of the gut microbiota. The studies described here demonstrate indirect or non-clinical, potentially advantageous benefits. Future long-term studies

with larger populations may demonstrate clinically significant benefits. ■

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