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Review

Probiotics and health: An evidence-based review

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ABSTRACT

The intestinal microbiota is an ecosystem formed by a variety of ecological niches, made of several bacterial species and a very large amount of strains. The microbiota is in close contact with the intestinal mucosa or epithelial interface which is, after the respiratory area, the largest surface of the body, occupying approximately 250–400 m^2. The physiological activities of the microbiota are manifold and are just being unraveled. Based on the observations of the multiple roles played by the microbiota in health and disease, the notion of modifying it with appropriate formulations, i.e. probiotics, is being tested in several settings.

This review summarizes the current knowledge on probiotics and discusses both limitations and acquired evidence to support their use in preventive and therapeutic medicine.

Contents

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1. Introduction

The intestinal microbiota (a term that has now replaced the old denomination of "microflora" [1,2]) is an ecosystem formed by a variety of ecological niches, made of several bacterial species and a very large amount of strains [3–8]. The microbiota is in close contact with the intestinal mucosa or epithelial interface which is, after the respiratory area, the largest surface of the body, occupying approximately 250–400 m^2.

Both microbiota and mucosa, along with mucus, form the so-called mucosal barrier, an important defense system against potentially immunogenic or pathogenic factors present in the lumen. In fact, the epithelial interface separates the lumen containing the microbiota and organic residues of food and secretions (salivary, gastric, pancreatic, biliary, intestinal), from the specific gut-associated lymphoid system (GALT).

The microorganisms constituting the microbiota are unevenly distributed along the digestive tract, as summarized in Table 1. Through their metabolic activities, these organisms play an important role in the use of nutrients ingested with food; they also significantly affect the development and performance of the immune system and other functions [9–12].

The cells that constitute the immune system, responsible for the defensive responses against pathogens, are mostly concentrated in the lymphatic structures that are located in the basement membrane, or *lamina propria*, of the gastrointestinal tract. Numerous follicular structures and Peyer's patches form part of the GALT, together with T lymphocytes aggregates, antigen presenting cells (APC), and B lymphocytes, characterized by the production of IgA (secretory antibodies typical of mucosal immunity). IgA are resistant to proteolysis and do not activate the complement; therefore, they play a protective function without pro-inflammatory actions. T lymphocytes are present either as CD4+ Helper T lymphocytes in their subsets (TH1, TH2, TH17, TH9), or as CD8+ T cytotoxic cells and Regulatory T (Treg) gd cells.

The microbiota of each individual has a specific "bacteria fingerprint", a profile of its own species which is different from other individuals; nevertheless, there exists a core of at least 57 bacterial species that can be considered common to all humans. The microbial community is typically dominated by two bacterial phyla (divisions), i.e. bacteroidetes and firmicutes, which represent more than 90% of the phylogenetic groups present in the human gut, at least in its distal part.

The human gut microbiota is currently the focus of very advanced research techniques – including studies on the bacterial genome (microbiome) – whose results are published in prestigious scientific journals [3–8]. Two major projects, based on systematic DNA sequencing of the microbiota, are currently in progress: (a) the *Human Microbiome Project* (HMP), in the USA and (b) the *Metagenomics of the Human Intestine* (metaHIT), held in Europe. A report on the latter has been published and confirms the presence of a wide range of bacterial species in the microbiota, i.e. over 1000 in 124 sampled individuals and approximately 160 for each individual at study [13]. A surprising result of the meta-genomics analysis shows 15% of the sequences defined as coding known or undefined functions, suggesting that the real understanding of the role of intestinal bacterial biomass will still take many years of research and analysis.

There are two main features of the human intestinal microbiota: (1) the ability to adhere to host proteins (collagen, fibrinogen, fibronectin), which confirms a series of data collected over the years by other analytical techniques and that postulates in adherence the prerequisite for a possible persistence of bacterial strains in the gastro-intestinal system; (2) the ability to ferment carbohydrates.

The interaction between microbiota and host organism generates, for both, advantages of different kinds. The main functions of the microbiota bearing positive effects on the host organism are the following: (1) participation in the formation of the intestinal wall (see Box 1); (2) resistance to colonization: in 1916, Nissle [14] demonstrated for the first time the role of human microbiota in conferring resistance to typhoid Salmonella infection and identified in the microbiota, as was later confirmed, the first line of defense against pathogenic bacteria invasion [15]; (3) production of short chain fatty acids, metabolites that play important physiological functions in fermentation (acetic acid for muscles, heart and brain, propionic acid for gluconeogenesis; butyric acid for the enterocyte function) [16]; (4) production of vitamins: especially those of the B group and K [17]; (5) interactions with the mucosal immune system [18,19]; (6) degradation of xenobiotics, with genes capable of synthesizing enzymes having a catabolic activity towards these compounds [5].

It is important to observe that the ability of the microbiota, which depends on its composition, to "extract" calories from a diet [20,21] could explain part of the pathophysiological interpretations of overweight and obesity. Table 2 summarizes some anatomical or

Table 1
Distribution of the microbiota in the digestive system.

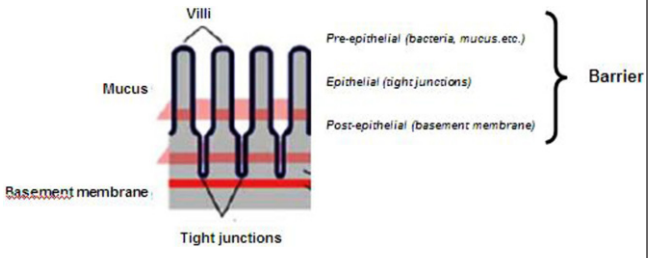
Site	Bacterial cells per gram of intestinal contents	Notes
Stomach, duodenum	$<10^3$	Lactobacilli, Streptococci HCl, peristalsis and bile inhibit the adhesion of bacteria and prevent colonization
Fasting, ileus, distal ileum	10^2–10^3	Lack of information: likely activity of fermentation of carbohydrates
	10^4–10^7	
Large intestine	10^{10}–10^{12} (prevalence of anaerobes)	Body location of most microbiota activities

Table 2
Effects of microbiota on host organism.

	GAC (germ-free animal)	MAC (ex-germ-free animal riconventionalized)
Intestinal epithelium	Thin	Thickened
Kinetics of enterocytes	Slow	Fast
Bile acid metabolism	Significant deconjugation	No deconjugation
Cholesterol metabolism	No coprostanol	Coprostanol presence
SCFA production	Reduced production	Significant production
Immunological activity	Reduced	Present

Box 1: The defense system of the intestinal mucosa.
The defense system of the intestinal mucosa can be divided into three main components:

1. *pre-epithelial*, consisting of mucus, trefoil peptides, and lipids forming a continuous gel in which a fluid rich in bicarbonates that maintain a neutral pH is secreted;
2. *epithelial*, composed of cells bound together by *tight junctions* (a complex of occluding proteins such as zonulin ZO-1, ZO-2, ZO-3, claudins, cingulines, 7H6, occludins, cadherins) that prevent the passage of material between cells: the functionality of tight junctions can be modulated by the expression of these proteins;
3. *post-epithelial*, formed by the constituents of the *lamina propria*.



functional aspects of the host organism as linked to the presence and activity of the intestinal microbiota.

2. The microbiota and the immune system

The relationships established between microbiota and host organism can be either commensal or symbiotic. The bacteria of the microbiota, as mentioned, are essential in facilitating the absorption of nutrients (for example, by allowing the hydrolysis of some otherwise non-digestible carbohydrates for the body) and in preventing the intestinal colonization by and, thus, the entering the body of pathogenic microorganisms. Therefore, it is vital that the immune system recognizes the components of the microbiota as such and establishes a state of tolerance towards them. The tolerance of the microbiota is mediated by some complementary mechanisms: (1) microbiota bacteria do not express virulence factors, e.g. secondary to chemical modifications of pathogen-associated molecular patterns, antigenic structures recognized by toll-like receptors (TLRs) on the surface of immune system cells or (2) some commensal bacteria are able to suppress inflammatory processes (for example by down-regulation of the activity of NF-κB [22]).

3. From microbiota to probiotics

According to the currently international FAO/WHO definition, established by a group of experts convened in 2001 [23], probiotics are: "*Live microorganisms which when administered in adequate amounts confer a health benefit on the host*". In Italy, the Ministry of Health [24] has defined probiotics as "*microorganisms which, once ingested in adequate amounts, have beneficial effects on the organism*" essentially resuming the definition of the two UN organizations. It should be emphasized that the FAO/WHO definition does not mention the human origin of the bacterial strain as criteria for the selection and definition of probiotics and, instead, is based on the type of effect caused [25]. This transfer of definition from scientific purpose to regulatory function has led to consider the term "probiotic", which in its original definition implies a health benefit, as a real "health claim" pursuant to Regulation (EC) n.1924/2006; this interpretation, if confirmed, on the one hand will lead to restrictions on the use of the term in foods and supplements, but will probably also allow consumers to immediately identify the strains and the products for which a beneficial effect on health has been demonstrated. From a functional point of view, experimental data suggest that probiotics may contribute to strengthening the activities of the intestinal mucosal barrier, particularly by influencing intestinal epithelial cells (IEC) and macrophages.

A recent review by Thomas et al. [26] has drawn attention to the most important mechanisms of action and to the diversity of each strain at the level of IEC and macrophages. A list of the possible mechanisms of modulation of probiotics on the signaling systems in intestinal epithelial cells (IEC) and macrophages can be found therein [26]. The most important mechanisms underlying these actions are summarized in Box 2 .

4. Critical aspects of the relationship between probiotic and host organism

The main practical aspects of the relationship between probiotics and host organisms – defined to allow a rational use of probiotics and improve the physiological functions of the host – are the following: (1) importance of live bacteria administration; functional characterization and identification of bacteria; (2) amount of bacteria to be administered (depending on the strain employed, in combination with other strains of same or different species, on the selected food matrix and industrial form of presentation) and counting techniques; (3) benefits for the host and their definition; (4) safety of use (also in the light of the two FAO/WHO documents of 2001 and 2002 and of the concept of QPS for the safety assessment of bacteria, recently introduced by EFSA).

4.1. Probiotics as live bacteria

The tradition that dates back to Metchnikoff includes both the use of a food matrix fermented by a "beneficial" bacterium and a "concentrated" bacterial supplementation of diet. Both scenarios provide consumers with live bacteria that are able to pass the gastric and ileal environments and, then, to reproduce themselves in the large intestine. This concept, ecological in nature, supports the use of live bacteria able to settle among other live bacteria, i.e. the microbiota and exercise functions involving metabolic activity. The studies supporting the efficacy of live bacteria are numerous; conversely, there are few and conflicting results on the effects of the same strain administered in either viable or non-viable forms.

Box 2: Mechanisms of interaction between probiotics and intestinal epithelial cells.

- *Induction of the synthesis of cytoprotective heat shock proteins*
  Intestinal epithelial cells (IEC), when in contact with heat, osmotic, oxidative, or other stresses, activate a system of "stress tolerance" based on the induction of heat shock proteins (hsp). Heat shock proteins in the gut include *hsp25* (that stabilizes actin) and *hsp72* (that prevents cell denaturation). These mechanisms help maintaining efficient tight junctions between IEC, thus promoting the function of the mucosal barrier [119]. Probiotics in the gut induce the production of cytoprotective heat shock proteins [120].
- *Modulation of inflammatory signaling systems in IEC*
  IEC are equipped with signaling systems to activate the immune response and face a variety of stimuli. NFκB represents the main system, which is present in the cytoplasm in its inactive form, bound to inhibitory molecules of the IκB family. In the presence of pro-inflammatory stimuli, IκB phosphorylates, detaches from NFκB and, thus, allows NFκB itself to migrate from the cytoplasm to the nucleus, activating the transcription of specific genes [121]. Some probiotics modulate the degradation of IκBα whereas others stimulate NFκB to increase the secretion of specific cytokines [122]. *Lactobacillus plantarum* inhibits the activity of NFκB and the degradation of IκB *in vitro* [123]. Another molecular target modulated by probiotics is PPARγ, a nuclear receptor that can regulate the level of intestinal inflammation and, in particular, may play a role in alleviating some intestinal inflammatory diseases by inhibiting the activity of NFκB (PPARγ is, indeed, present in small amounts in the IEC of patients with inflammatory bowel disease, or IBD) [124,125]. Treatment with specific strains of probiotics can increase the expression of PPARγ and, thereby, improve inflammation in patients with IBD [126,127].
- *Regulation of apoptosis*
  Some probiotics may regulate apoptosis of IEC. *Lactobacillus rhamnosus* GG ATCC 53103 can activate a protein with anti-apoptotic action and inhibit a protein with pro-apoptotic action in IEC stimulated with various cytokines (TNF-α, IL-1α-β or IFNγ). Some experiments show that LGG activates the production of two proteins, p75 and p40, which promote cell proliferation and activate Akt anti-apoptotic protein [128]. The ability of probiotics to regulate apoptosis may also represent a useful strategy for the control of intestinal infections [129].
- *Modulation of the signaling systems of macrophages*
  At the gut level, probiotics modulate different signaling systems of macrophages, with effects on mucosal immunity.

Therefore, due to well-established definitions and for the sake of better consumer information, the term probiotic is to be reserved for products containing living and vital cells.

The identification of the strain is necessary, both for safety reasons and to prove their beneficial action. Evidence, in fact, indicates that different strains of the same species may exert different effects on the host (Table 3). In brief, species must be identified by determining the DNA nucleotide sequence coding the 16S RNA, while the strain should be characterized by macro-restriction profile of the chromosome, as determined by pulsed field electrophoresis (PFGE). The filing in an International collection of strains is also recommended. Strains of the same species can exert different and sometimes opposite actions. The differences of actions between the various strains are well-established in the scientific literature, as cited in the FAO/WHO document: "*data obtained with one specific probiotic food cannot be extrapolated to other foods containing that particular probiotic strain or to other probiotic microorganisms*" [23], as well as in the AFSSA (*Agencie Française de Sécurité Sanitaire des Aliments*) document: "*The quantity of probiotics passing live through the gut depends on the strain, the dose ingested, factors related to the host and the vector food*" [27].

The analytical methods are well established and outlined in a series of documents: (a) 2001 FAO/WHO report [23]; (b) Italian Ministry of Health guidelines for 2005 [24]; (c) EFSA opinions issued between 2009 and 2010.

4.2. Quantities

Little is known about the optimal amount of live probiotic bacteria to be administered; this quantity is not easy to determine: it is strain-specific and it probably depends on the type of benefit sought for with the administration of probiotics (different functional effects may require different amounts of live probiotics). Of course, the overall amount cannot be low, if the aim is to markedly influence the composition of the microbiota of the host. It should be emphasized that, in case of microbial associations, each species in "competition" with a functional action must be provided in appropriate amounts.

In the absence of specific dose-response studies, however, some points reported in the AFSSA paper [27] are worth reiterating: (1) "The dose of probiotics ingested is an important factor to obtain high concentrations in the various compartments of the gastrointestinal tract"; (2) "It is often said that probiotic concentrations must be greater than or equal to 10^6 CFU/mL in the small intestine (ileum) and 10^8 CFU/g in the colon, but the scientific basis for these statements is relatively weak"; (3) "The concentrations in the colon have been proposed because they correspond to less than 1/1000 of the autochthonous flora present (which it could be reasonably expected has more chance of being active than flora present at even lower levels)". It is also necessary to remember that there is no scientific evidence proving a synergistic effect of two or more strains of bacteria in determining a functional or metabolic impact in humans. Yet, there is theoretical support to the notion that the intake of two or more bacterial species may have additional or synergistic functional effects; moreover some studies, albeit limited, seem to suggest a negative cross-effect.

4.3. Type of benefit

Probiotics can improve – within physiological limits – some functions of the digestive system, such as stool frequency or subjective characteristics. The consumption of probiotics may also be useful in reducing the risk of specific diseases, mitigating both objective and subjective symptoms, if manifest. Experimentally, it should be noted that the effects of probiotics have been observed mainly by studying groups of diseased subjects affected. This choice was and is based on purely methodological reasons: it is not easy to record significant positive effects of probiotics in the absence of functional abnormalities, as in the case of healthy populations.

This approach has allowed obtaining encouraging results, but it has also underlined the problem of data transferability to the part of the population representing habitual probiotics consumers and defined by FAO/WHO as: "*otherwise healthy people*".

We believe that, in many situations such as those of predominantly subjective symptoms (for which there is a sort of continuum between normal and clinical pathological states), the possibility to transfer to healthy "pouchitis-symptomatic" subjects data collected in patients with more complex clinical pictures dramatically improves the possibility to prove significant functional effects of probiotics. The observed effects are usually related to the presence of probiotics in the intestinal tract, ensured only by protracted administration. Long-term studies should complement the many

Table 3
Difference in probiotic action between strains of the same species. Examples refer to the action on host organism only and not to viability and persistence.

Species/strain	Action	Reference
Bifidobacterium longum W11	Low induction of IL10 and high induction of Th1	[101]
Bifidobacterium longum NCIMB 8809 and *Bifidobacterium longum* BIF53	High induction of IL10 low induction of Th1	[101]
Lactobacillus rhamnosus GG and *Lactobacillus rhamnosus* 1970-2	Difference in ability of colonization in vivo	[102]
Lactobacillus crispatus M247 e *Lactobacillus crispatus* MU5	Difference in ability of colonization in vivo	[103]

those already exist and that describe the effects of limited-duration interventions.

4.4. Safety

The safety assessment of microbial species proposed as probiotics has always been a pillar of regulatory authorities. The distribution of microbial species has, for years, been subjected to the verification of a long tradition of safe consumption or to a case-by-case evaluation that followed, in the EU, the criteria regarding novel foods, feed (zootechnical) additives, or drugs, according to their conditions of use.

In the food sector, the introduction of new rules has also led to the adoption of new instruments for the evaluation of probiotics, based on the so-called "Qualified Presumption of Safety (QPS)" [28]. Consequently, every microbial strain for which an identity has been unequivocally established and classified in a QPS group, i.e. a group that does not raise concern from the point of view of safety, is only subjected to the verification of the absence of specific "qualifications" that may cause concern for public health before the final approval of its safety standards of use. At present, the characterization of a specific strain is based on the absence of resistance to antibiotics of clinical and veterinary interest as well as of virulence factors [29].

In reality, resistance to antibiotics is not in itself a major safety issue, as different microorganisms are inherently indifferent to the activity of antibiotics, but it becomes a problem when it is accompanied by a horizontal transfer of genetic determinants [30]. For this reason, the Scientific Committee on Animal Nutrition of the EU (SCAN) and the EFSA Panel on additives, products and substances used in animal feed (FEEDAP) require the absence of transferable antibiotic resistant genes as a prerequisite for approval of a microorganism. Although there are no legally mandatory criteria for probiotics in food supplements for humans, the verification of the absence of transferable resistance is recommended for safety assessment at EU level and by, e.g. the Italian Ministry of Health.

5. Probiotics and the immune response

A series of recent observations made it possible to clarify the mechanisms of immune responses occurring in the intestine. Many of these mechanisms can be influenced by specific strains of probiotics. In the intestinal *lamina propria*, B cells are differentiated into plasma cells and secrete dimeric IgA antibodies that, on the basolateral surface of intestinal epithelial cells, bind to a specific receptor transporting them to the apical surface, where they are released into the intestinal lumen. The secretory IgA are important elements of mucosal immunity and participate in the protection of the host by binding a wide variety of dietary, bacterial, viral, and fungal antigens.

The hypothesis that probiotics might influence immunity by altering specific immune parameters, thus playing beneficial roles in human diseases is of great interest. In fact: (1) probiotics modulate and stabilize the composition of the microbiota and, therefore, may have immunomodulatory effects; (2) some probiotics are able to inhibit the inflammatory response of the intestinal immune system through inhibition of NF-κB activation or in combination with an anti-apoptotic action on intestinal epithelial cells [31,32]; (3) some probiotics are able to increase the activity of Natural Killer (NK) cells [33,34], which are first line of defense as they can perform cytotoxic activities independent from prior sensitization to antigens; (4) some probiotics increase the secretion of mucus [22]; (5) some probiotics have a direct immunomodulatory action: after being captured in the Peyer's patches, they can induce the secretion of cytokines and the expression of co-stimulatory molecules by antigen presenting cells (APC) [35]; (6) some strains of lactobacilli induce dendritic cells (DC) maturation [36]. DC can, through their particular cytostructure, pass through the layer of epithelial cells and capture antigens directly from the lumen. This characteristic of DC, combined with their ability to guide T cells response and thus stimulate the secretion of IL-10 and IL-12, underlines their role as links between microbiota, innate immunity, and adaptive immunity. With a targeted use of specific probiotic strains, it is possible to induce an immune stimulant type of response on both B (increase of humoral immunity) and T lymphocytes (increase of cell-mediated immunity) and on the phagocytic component, particularly on polymorphonuclear cells [37,38]. The ability to stimulate responses of this type is useful in very specific clinical circumstances, for example for immunoprophylaxis of upper respiratory tract infections during winter or, in addition to influenza vaccination, to increase antibody response to vaccine or, more generally, to increase non-specific immunity surveillance to pathogens of different nature [39,40].

Recent studies have provided positive results on the effects of probiotics on the respiratory system, especially with regard to preventing and reducing the severity of respiratory infections due to an increase in IgA-secreting cells in the bronchial mucosa [41]. Positive effects were also found in regular smokers, usually affected by reduced NK cell activity [42].

Children also represent a main target of studies that investigate the effects of probiotics on human health, because of the importance of limiting the spread of diseases especially during wintertime, reducing missed days at school, and decreasing the need of antibiotics [43–45].

6. Probiotics and health

6.1. Pediatric intestinal disease

During the first weeks of life the innate defense mechanisms are more important than the acquired ones, because even healthy infants are immunologically naïve, not having being exposed – intra uterus – to nearly any antigen. In this early period of life, colostrum and breast milk can increase the resistance to enteric infections in newborns; the mechanisms for this increased resistance to infection are both passive (due to the passing through the milk of anti-microbial factors) and active, through the promotion of the development of specific immune functions, in nature. The neonatal immune system faces two major challenges: on the one hand it has to actively respond to the antigens of pathogenic bacteria, and on the other hand it must "tolerate" the antigens of both dietary and non-pathogenic bacteria. The regulation of these responses of tolerance and of active response is important for good health: the loss of these properties may lead to recurrent infections, inflamma-

tory and autoimmune diseases, and allergies. The education of the immune system in the first moments of life is critical for minimizing the onset of these immune-mediated disorders. Bacterial flora antigens, transmitted from mother to infant and which colonize the intestine already 48 h after birth (*Escherichia coli* and *Streptococci*) are fundamental for this "education" process. Diet also affects the microbiota of the newborn: breastfed babies have a predominant colonization with *E. coli* and *Streptococci bifidobacteria*, while those fed with formula milk have microbiotas with predominance of *bifidobacteria*, *bacteroidi*, *clostridia* and other enterobacteria. The gradual establishment of the flora from the early hours of life allows modulating the immune response in favor of the acquisition of oral tolerance [46,47], defined as "*specific immunological hyporesponse to a previous exposure to mucosal antigen*". As mentioned, the production of secretory IgA is one of the basic immunological mechanisms in the establishment of oral tolerance, especially during the peri-natal period, but also in the protection from pathogens. Breast milk contains secretory IgA, which may be useful for this purpose. Conversely, infants who are fed with formula milk supplemented with probiotic bacteria may experience promotion of the natural production of this immunoglobulin. Supplementation with probiotics is generally considered safe because they are identical to the microorganisms present in vaginal flora and in the human gastrointestinal tract. In recent years, clinical trials in children have multiplied and have assessed the effects of probiotics in the prevention and control of both acute and chronic gastrointestinal disorders, in addition to non-intestinal diseases such as atopy [48]. The interpretation of the results is often controversial, because studies differ in terms of microorganisms used, studied population, assessment of the doses, and frequency of administration. Hereafter, we present a critical evaluation of the available evidence regarding the use of probiotics in children, in specific para-physiological or pathological conditions.

6.2. Infectious gastroenteritis

Most of the recent studies have demonstrated the efficacy of specific probiotics in reducing the symptoms in pediatric populations affected by infectious gastroenteritis [49–52]. Probiotics reduce the duration of infectious diarrhea by 0.7 days as well as the frequency of diarrheal episodes already in the first hours [53]. According to the latest scientific evidence, their consumption is, therefore, recommended in case of acute gastroenteritis starting from the onset of symptoms. In general, probiotics should be administered for at least 5 days and, in any case, for the duration of hospitalization, in one or two daily doses. The effect is most evident in cases of early treatment of rotavirus infection with oral rehydration therapy associated with *Lactobacillus GG* [54–57]. This probiotic is able to decrease the excretion of rotavirus in the stool [58], helping to reduce the spread of the virus, in turn improving the effectiveness of preventive strategies both in communities and during hospitalization. Yet, definitive conclusions cannot be drawn based on the available results [59–61]. The effectiveness of treatment with probiotics is demonstrated in mild- to moderate-severity rotavirus gastroenteritis, while the results obtained in the treatment of rotavirus negative forms and, especially, in bacterial infections are inconclusive [54,61]. It has been hypothesized that the ineffectiveness of LGG in bacterial diarrhea is due to the ability of bacteria to produce mucinases that neutralize the effects of probiotics [62]. Conversely, a 2005 study has demonstrated the effectiveness of *Lactobacillus paracasei* ST1 in non-rotavirus gastroenteritis [63]. Data confirming the effectiveness of supplementation with probiotics in the treatment of gastroenteritis in children have also been analyzed in a recent review [64], also focusing on strain-specificity; in particular, probiotics that have shown more promising results are *Lactobacillus casei* subsp. *rhamnosus* GG, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum.*

6.3. Antibiotic-associated diarrhea

The incidence of antibiotic-associated diarrhea (AAD) ranges between 5% and 30% [57]. Most antibiotics may induce, during their use, diarrhea: the risk is greater in case of aminopenicillin therapies, aminopenicillin combined with clavulanic acid, cephalosporins, and clindamycin [65]. Recent meta-analyses reported a significant reduction of AAD cases when antibiotic therapy is associated with prior probiotic treatment [65–67]. *Saccharomyces boulardii* appears to be the most effective microorganism; however, LGG has also proven to be effective in children [68,69]. *S. boulardii*, for which there is a risk of hematogenous dissemination in immunocompromised patients, was effective in inhibiting the recurrence of episodes of *Clostridium difficile* infection [70]. Although not all studies have confirmed the effectiveness of probiotics treatment in the prevention of AAD, a 2009 review has shown that effectiveness is mainly related to the strain used [71]; the studies have also confirmed that there is sufficient clinical evidence to support the use of *Lactobacillus rhamnosus GG* and *S. boulardii* strains in the treatment of antibiotic-associated diarrhea [72].

6.4. Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a major cause of morbidity and mortality in premature infants; the etiology of this disease has not yet been fully clarified. Risk factors have been identified such as prematurity, enteral feeding, and bacterial colonization, which would cause the exaggerated inflammatory response responsible for ischemic necrosis of the intestine. Based on observations in animal models, some studies evaluated the effects of probiotics supplementation on the incidence of NEC in newborns. A recent review [73] examined the results of 11 studies, showing that the risk of NEC and death in the population treated with probiotics is lower and confirming significant benefits of a supplementation with probiotics in premature and very-low birth weight infants. This meta-analysis concludes that, due to the important effects achieved, the statistically significant results, and the recorded findings, further randomized trials placebo-controlled are not necessary.

6.5. Allergic and atopic diseases in children

In recent years, industrialized countries have seen a significant increase of autoimmune and allergic diseases. The factors responsible for this increase are likely to be impaired maturation of the immune function in early life – which would involve a minor TH2/TH1 switch for low or no contact with infectious agents (hygiene theory) – and altered microbial flora that enables the persistence of cytokines derived from TH2 (IL4, IL5, IL13), dominant at birth, and does not allow the shift towards a predominant TH1 response, with production of IL12 and IFNγ.

This hypothesis is supported by the observations of changes in the intestinal flora of atopic children, with a prevalence of *clostridia* [74,75]. In 2007, the first study was published postulating the role of probiotics in the recurrence of allergic respiratory symptoms in children. This study aimed at assessing whether the daily, long-term (12 months) consumption of a fermented milk containing *L. casei* DN-114 001 probiotic (with immune-modulating activity) could improve human health and modify the immunological profile of pre-school age children with allergic symptoms to inhalants [76]. This was a multicentre, prospective, randomized, double-blind study, with 187 patients (119 with asthma and 131 with rhinitis, 63 with both symptoms) of both sexes and aged between

2 and 5 years, followed by eight hospitals in Milan (Italy) and its province. The study showed that supplementation with probiotics reduces by 33% the recurrence of yearly rhinitis, with a median (IQR) of 2 episodes (1–5) *vs.* 3 (0–8); the incidence of allergic rhinitis was two times lower in children treated with probiotics in the second quarter of supplementation [OR (95% CI)] of 0.39 (from 0.19 to 0.82, $p < 0.01$). A genetic analysis of the composition of the intestinal microbial flora was performed on a subgroup of 45 patients, showing a high prevalence of probiotic flora in the gut and, in particular, the presence of numerous *L. casei* DN-114001 colonies in patients receiving the intervention as compared to the control group: gut colonization by probiotics persisted after 6 and 12 months in almost all subjects. Numerous studies have shown promising results on the effectiveness of probiotics in reducing the incidence of allergic reactions [77–80].

Unfortunately, the enormous heterogeneity of studies, strains, duration of therapy and doses used, does not allow drawing a univocal interpretation. The most recent reviews [81,82] do not conclude on the efficacy of probiotics in the treatment or prevention of major allergic diseases. The most promising data only concern the prevention of atopic eczema, although not all studies agree with these results. Indeed, two recent reviews on the use of probiotics in the treatment [83] and prevention [84] of atopic dermatitis affirm that, according to the available studies, there is insufficient evidence to support probiotic supplementation for atopic dermatitis.

6.6. Respiratory infections

As mentioned, all probiotics induce an immune response, whose characteristics are related to the strain or the combination of bacteria that have been used. Recent studies have shown positive effects of probiotics on the respiratory system, especially in preventing and reducing the severity of respiratory infections, due to an increase in IgA-secreting cells in the bronchial mucosa [41]. The role of malnutrition and deficiency of some micronutrients and vitamins has also been demonstrated in the process of viral pathogens cell entry and replication [85–87]. The preventive use of supplements containing substances active on the immune system plays an important role both before vaccination and as adjuvant in vaccines, to increase antibodies in the elderly and debilitated subjects [88,89].

Hereafter, we present the results of studies performed on different target populations aimed at investigating the effects of probiotics on infectious diseases of the respiratory system.

6.6.1. Children

A randomized, double-blind, placebo-controlled trial was performed to determine whether probiotics may reduce the risk of infections in infants. The children involved in the research were younger than 2 months of age and were daily provided with milk containing *L. rhamnosus* GG and *Bifidobacterium lactis* Bb-12, or placebo milk, administered until 12 months of age. The results suggest that probiotics may represent a mean to reduce the risk of early acute otitis media and the use of antibiotics for recurrent respiratory infections during the first year of life [90]. Similar results have emerged in a study performed on a target population of 326 children aged between 3 and 5 years, showing more than 65% decrease in the incidence of antibiotic use and 25% reduction in school missed days among children treated with probiotics [91].

6.6.2. Adults

A randomized double-blind, placebo-controlled trial assessed whether the consumption for 3 months of *Lactobacillus gasser* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5, had impacts on symptoms severity, incidence and duration of common cold. For two winter/spring seasons, 479 adults were daily treated with vitamins and minerals enriched or not with probiotics. The results indicate a reduction in the duration of episodes of common cold of at least 2 days and a decrease in the severity of symptoms among subjects receiving probiotics if compared to the randomized placebo-control group [92]. Similar conclusions were obtained in a study that assessed the effect of long-term intake of probiotics on the same pathology [93]. Another, double-blind, randomized, placebo-controlled trial, performed on 237, 234, and 250 healthy adults investigated, in three winter seasons, the efficacy of different probiotics in restoring and maintaining intestinal balance and the potential protection from respiratory tract infections [94]. The experimental protocol consisted of three phases: (1) in the first phase, an active formulation (A) was tested that contained three types of probiotics (*L. plantarum*, *L. rhamnosus* and *B. lactis*) and fructo-oligosaccharides (FOS), compared to placebo; (2) in the second phase, the same formula was compared to a similar preparation enriched with lactoferrin (B) and to placebo; (3) the third phase compared two symbiotic formulations, each containing probiotics and FOS (C) or galacto-oligosaccharides (GOS, D) with placebo. The average duration of acute respiratory infections improved with respect to ILI (influenza-like illness) and URTI (upper respiratory tract infections) in steps 1 and 2 of the study, while the incidence of cold and cough decreased in phase 3. Similar results emerge from a systematic review of clinical evidence obtained in 14 research trials (RCT) on the use of probiotics in preventing respiratory tract infections (RTI). The reduction in the severity of symptoms related to RTI was recorded in five out of six studies; in three studies out of nine the clinical course was reduced. Probiotics, therefore, have a beneficial effect on the severity and duration of RTI symptoms, although not reducing their incidence [95].

6.6.3. Elders

Two multicentre, randomized, controlled, double-blind studies were conducted in two successive vaccine seasons (pilot study and control). 86 and 222 elderly volunteers consumed, respectively, a fermented milk drink containing *L. casei* DN-114 001, a fermented yogurt or a control unfermented dairy product, twice a day for a period of 7 or 13 weeks. Vaccination took place after 4 weeks. The study showed that probiotics improve antibody responses to influenza vaccination in individuals over 70 years [96]. *L. casei* DN-114001 was also evaluated in a multicentre, double-blind, controlled study on 1.072 elderly, to assess the resistance to respiratory infections. The product containing probiotics, well tolerated, induced a reduction in the duration of respiratory infections, especially URTI and nasopharyngitis [97].

6.7. Effects on the digestive system

Many of the investigated effects of probiotics refer to the digestive system. These effects relate to both paraphysiological conditions, e.g. constipation and to situations of illness. A review has recently been published on the effect of probiotic strains on constipation [98]: five clinical studies placebo-controlled were taken into consideration on a total of 377 subjects. The results show that favorable effects on stool frequency and stool consistency were obtained in adults with *B. lactis* DN-173 010, *L. casei* and *E. coli* Nissl 1917 probiotic strains. Some strains have led to a reduction in the perception of bloating (reported by patients before and after treatment). In children, the *L. rhamnosus* Lcr35 strain showed positive effects although not statistically significant (due to the low number of subjects involved) while the *L. rhamnosus* GG strain had no impact if compared to placebo. In 2008 another placebo-controlled study was conducted in children with chronic functional constipation, treated for 8 weeks with probiotics (*L. reuteri* DSM 17938) or placebo. In this case, a significant improvement was observed,

Table 4
Effects of different strains on some gastrointestinal pathologies.

Disorder	Strain	Dose	Ref.
Treatment of acute infectious diarrhea in children	*L. rhamnosus GG*	10^{10}–10^{11} ufc	[104]
	L. reuteri ATCC 55730	10^{10}–10^{11} ufc × 2/d	[104]
	S. cerevisiae (boulardii)	10^{9} ufc × 3/d	[104]
Treatment of acute infectious diarrhea in adults	*Enterococcus faecium LAB SF68*	10^{8} ufc × 3/d	[104]
Prevention of antibiotic-associated diarrhea	*S. cerevisiae (boulardii)*	10^{9} ufc × 2/d	[105]
	L. rhamnosus GG	10^{10} ufc × 1–2/d	[105]
	B. lactis Bb12 + S. thermophilus	10^{7} + 106 ufc/g formula	[105]
	Enterococcus faecium LAB SF68	10^{8} ufc × 2/d	[106]
	S. cerevisiae (boulardii)	1 g or 3 × 10^{10} ufc × 1/d	[107]
	L. rhamnosus GG	10^{10}–10^{11} ufc × 2/d	[108]
	L. casei DN-114 001 in fermented milk with L. bulgaricus + S. thermophilus	10^{10} ufc × 2/d	
	B. clausii	2 × 10^{9} spores × 3/d	
	L. acidophilus CL1285 + L. casei	5 × 10^{10} ufc × 1/d	
Prevention of rotavirus nosocomial infection in children	*L. rhamnosus GG*	10^{10}–10^{11} ufc × 2/d	[105]
	B. lactis Bb12 + S. thermophilus	10^{8} + 10^{7} ufc/g formula	[105]
	B. lactis Bb12	10^{9} ufc × 2/d	
	L. reuteri ATCC 55730	10^{9} ufc × 2/d	
Prevention of C. difficile infection in adults	*L. casei DN-114 001 in fermented milk with L. bulgaricus + S. thermophilus*	10^{10} ufc × 2/d	[106]
	S. cerevisiae (boulardii)	2 × 10^{10} ufc × 1/d 2 × 10^{10} ufc × 1/d	[105]
Adjuvant in therapies for Helicobacter pylori eradication	*L. rhamnosus GG*	6 × 10^{9} ufc × 2/d	[109]
	B. clausii	2 × 10^{9} spores × 3/d	[109]
	S. cerevisiae (boulardii)	1 g or 5 × 10^{9} ufc × d	[109]
	L. casei DN-114 001 in fermented milk with L. bulgaricus + S. thermophiles	10^{10} CFU × 2/d	[110]
Reduction irritable bowel syndrome symptoms	*B. infantis 35624*	10^{8} ufc × 1/d	[111]
	L. rhamnosus GG	6 × 10^{9} ufc × 2/d	[112]
	B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. delbrueckii subsp. bulgaricus, L. plantarum, S. salivarius subsp. thermophilus	4.5 × 10^{11} ufc × 2/d	[113]
	L. rhamnosus GG, L. rhamnosus LC705, B. breve Bb99 and P. freudenreichii subsp. shermanii JS	10^{10} ufc × 1/d	[114]
	B. animalis DN-173 010 in fermented milk with L. bulgaricus + S. thermophilus	10^{10} CFU × 2/d	[115]
Remission of ulcerative colitis	*E. coli* Nissle 1917	5 × 10^{10} × 2/d	[116]
Remission of pouchitis	*B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. delbrueckii subsp. bulgaricus, L. plantarum, S. salivarius subsp thermophilus*	4.5 × 10^{11} ufc × 2/d	[117]
Prevention of necrotizing enterocolitis	*B. infantis, S. salivarius subsp. thermophilus, B. bifidum*	3.5 × 10^{8} ufc per strain × 1/d	[118]
	L. acidophilus + B. infantis	10^{9} ufc per strain × 2/d	[73]

after the second week of treatment, in stool frequency while no effect was registered in stool consistency [99].

The microbiota, however, participates in the function of the mucosal barrier against the adhesion of pathogenic bacteria, which is at the base of infectious processes. When this barrier function is altered by chemical agents, antigens, or other stress factors, different types of intestinal disorders can occur, sometimes due to pathogenic bacteria proliferation. Several experimental data suggest that probiotics may contribute to the reinforcement of the activities of the intestinal mucosal barrier, particularly affecting the functionality aspects of intestinal epithelial cells or macrophages (Table 2). Table 4 [100] reports published data of controlled studies on the use of probiotics in prevention and treatment of certain disorders or digestive diseases.

7. Conclusions

Some effects of probiotics on normal or pathological functions of the organism are well documented and their use, either alone or in combination with other therapies, may be considered as "evidence-based". However, for other clinical conditions further studies are needed, as the available evidence is not sufficient to demonstrate the efficacy of probiotics.

In general, as discussed in this paper, the effects are "strain-specific" and may not be extended to other probiotics of the same genus or species. More specifically: (1) the influence on the microbiota composition, through probiotic bacteria consumption, may contribute significantly to human health and well-being; (2) the evaluation of the possible beneficial effects must be specific to each combination of strains and not limited to the impacts of different mixed strains; (3) the amount of probiotic bacteria used to induce beneficial effects, as well as the possible effects of the (food) matrix, must be extrapolated from experimental data; the intake must be prolonged over time; (4) in healthy subjects, some probiotics significantly contribute to lessening hive and to the reduction of intestinal discomfort; (5) some probiotics may contrast intestinal pathogens by direct antagonism, e.g. through the production of cytokines, defensins, etc., or by competitive exclusion; (6) some probiotics contribute to the prevention of infectious diarrhea in children; (7) some probiotics are associated with an overall improvement in functional intestinal disorders (bloating, abdominal discomfort, etc.) typical of the irritable bowel syndrome; (8) some probiotics reduce the frequency and severity of necrotizing enterocolitis in premature infants; (9) there is the possibility of using probiotics to accelerate clinical remission in inflammatory bowel disease (IBD) and pouchitis; experimental evidence, however, is not solid; (10) some probiotics, probably due to the stimulation of nonspecific immune pathways, appear to reduce the duration and/or the severity of seasonal viral infections; (11) preliminary, though not univocal, reports suggest that specific strains

of probiotics can reduce the incidence or some dermatological aspects of allergic diseases in children; (12) foods containing probiotics have proven to be safe both in the healthy population and in patients with some diseases; (13) the complexity of research on probiotics suggests the adoption of tools to assess their effects different from those classically used to evaluate the actions of drugs.

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