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Chapter 12

Probiotics for Prevention and Treatment of Candidiasis and Other Infectious Diseases: *Lactobacillus* spp. and Other Potential Bacterial Species

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Additional information is available at the end of the chapter

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Abstract

The resident microbiota in the human body, such as the oral cavity, gastrointestinal tract and genitourinary tract, is able to provide resistance to disease. However, imbalances in the microbial components can promote the growth of opportunistic microorganisms, such as yeasts of genus *Candida*. Fungal infections present as a major cause of infectious diseases and the microorganisms of genus *Candida* are the most frequently isolated pathogenic fungi in human fungal infections. *Bacillus* spp. and *Lactobacillus* spp. are bacteria that have probiotic effects used in commercially available products and in studies that aim for the development of probiotics able to inhibit the microbial pathogenicity and restore the balance of resident microbiota. Thus, with increasing fungus resistance to the use of antifungal agents, which are capable of causing serious side effects to the host organism unable to destroy the target microorganism, it becomes important to develop therapeutic and/or prophylactic alternatives that have a different and an effective mechanism of action with capacity to combat fungal infections without harming the patient. Probiotic bacteria provide an alternative strategy for the prevention and treatment of candidiasis and other infectious diseases.

Keywords: probiotic, *Candida* spp., *Bacillus* spp., *Lactobacillus* spp., prevention and treatment

1. Introduction

The incidence of fungal infections has increased significantly in the past 25 years [1]. Human beings are colonized by a diverse and complex collection of microorganisms, contributing all of them to host nutrition, development of the immune system, response to pathogens and mucosal cell differentiation and proliferation [2].

Probiotic bacteria are also used in human and animal nutrition to influence beneficially the balance of intestinal microbiota of the host. Probiotics have several beneficial effects related to increasing digestion, strengthening the immune system and stimulating the production of vitamin. The use of probiotics is aimed to reduce the use of antibiotics and improve animal growth, as well as feed conversion [3].

Infectious diseases along with multidrug resistance are the major public health problem in developing countries with increased mortality and morbidity [4, 5]. Apart from the threat of multidrug resistance, several studies have confirmed that the continuous use of antibiotics can damage human commensal microbiota [5, 6]. Thus, an alternative and effective research focus is necessary to combat these pathogens with no effect on normal microbiota. In this regard, the use of probiotics and their natural metabolic compounds can be a substitute in various food and pharmaceutical industries [5].

There are around 600 pathogenic fungal species for humans and this group includes the fungi that cause infection of skin (e.g., *Malassezia* species) and fungi that have the potential to cause systemic infections (e.g., *Cryptococcus neoformans* and *Candida albicans*) [7]. The yeasts of the genus *Candida* are the fourth most common cause of systemic infections acquired in hospitals in the United States with 50% mortality rates. The most pathogenic species is *C. albicans* and can cause two major types of human infections: superficial infections, such as oral candidiasis, and systemic infections [8, 9].

The genus *Candida* is commonly found in the oral cavity of healthy individuals, isolated from approximately 75% of the population with a higher prevalence of *C. albicans*, followed by *C. tropicalis* and *C. glabrata* [10]. *Candida* species are a frequent cause of recurrent infections in the mucosa when favored by risk factors such as the use of antibiotics of broad spectrum and corticosteroids for long time, human immunodeficiency virus (HIV) infection, radiotherapy in the area of head and neck, the use of orthodontic appliances, deficient oral hygiene, among other factors affecting immunocompromised patients that may result in transition of commensal phase of *C. albicans* to pathogenic [11, 12].

Under certain conditions of immunosuppression, such as individuals with acquired immunodeficiency syndrome (AIDS), oral manifestations are the most important and earliest indicators of infection. The oral candidiasis is accepted internationally as a cardinal sign of HIV infection and is present in 50% of patients with HIV infection and in 80% of patients with AIDS [13, 14].

In Brazil during the period among 1996–2006, candidiasis was the second cause of deaths in HIV-positive patients due to fungal infections, being responsible for an average of 39 annual

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deaths [15]. Moreover, oral candidiasis remains clinically relevant in these individuals, where treatment is difficult and recurrent episodes are frequent, requiring multiple antifungal treatments, which may lead to resistance selection [16, 17]. Due to this, *C. albicans* can develop resistance to antifungals used to treat oral candidiasis, such as fluconazole and miconazole [18, 19].

Due to the high recurrence of *Candida* lesions, and the increased resistance of conventional antifungal drugs in clinical practice, the continuous use of probiotics to prevent fungal infections may be an interesting strategy. In this chapter, we discuss how probiotics can help in the prevention and/or adjuvant treatment of candidiasis.

2. Probiotic

The history of probiotics began with the history of man; cheese and fermented milk were well known to the Greeks and Romans who recommended their consumption, especially for children and convalescents. The first association of probiotics and health benefits was made at the turn of the century when the Russian scientist, Elie Metchnikoff, systematically studied the composition of the microbiota and suggested that the ingestion of fermented milk would improve this so-called autointoxication [20].

Probiotics play an important role in human health. There is general agreement on the important role of the gastrointestinal microbiota in the health and well-being status of humans and animals [21]. Probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host. This term is defined by a United Nations and World Health Organization Expert Panel [22].

There was an increase in the number of searches, both in vivo and in vitro, related to the benefits of probiotics on health and described in the literature for the treatment of infectious diseases caused by fungi, viruses, and bacteria or diarrhea associated with the use of antibiotics, alleviation of inflammatory chronic bowel disease, decreased risk of colon cancer, reduced allergies, effect on intestinal microbiota [21], and anticancer therapies [23].

Other beneficial effects of probiotics include lowering serum cholesterol level [24–27], improving lactose intolerance, increasing the utilization of nutrients, decreasing the use of antibiotics [24, 27], and antidiabetic treatments [26, 28, 29]. In the context linking food and health, probiotics have been the subject of numerous scientific studies and publications demonstrating their therapeutic effectiveness on both systemic and gastrointestinal tract [21] (Figure 1).

Microorganisms commonly used as probiotics belong to the heterogeneous group including *Bacillus, Lactobacillus, Bifidobacterium, Saccharomyces cerevisiae*, and *Escherichia coli* [30, 31] (**Figure 1**).

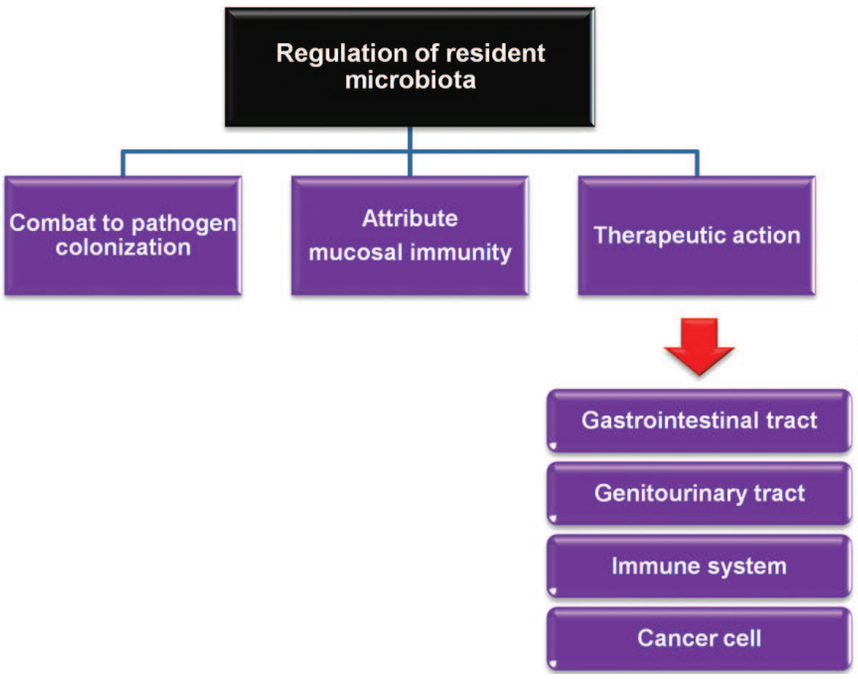

Figure 1. Some properties of probiotics.

3. *Lactobacillus* spp.

3.1. General characteristics

Lactobacillus spp. are Gram-positive bacteria, facultative anaerobic bacilli found in the normal microbiota of the gastrointestinal tract of birds and mammals, and genitourinary tract and oral cavity in the humans [31, 32]. This genre is heterogeneous and the number of species is constantly being modified due to the description of new species and reclassification of others [33]. Some members of the genus *Lactobacillus* were reclassified into *Carnobacterium* [34], *Atopobium* [35], *Weissella* [36], and *Paralactobacillus* [37]. In early 2007, 120 species composing the genus *Lactobacillus* [33] and in 2008 over 145 new species have already been identified [38, 39].

Different *Lactobacillus* species found in the gastrointestinal tract are concerned with the balance of microbiota and it has been widely studied due to their health-promoting properties [40]. Their effects on intestinal microbiota in terms of protection include competition for adhesion sites with pathogenic microorganisms and antimicrobial substance production, such as organic acids, lactic acid, carbon dioxide, and bacteriocins [41]. In addition, the regular use of probiotic appears to prevent certain gastrointestinal disorders such as lactose intolerance [42].

In 1907, Elie Metchnikoff won the Nobel Medicine Prize because he noticed that the daily consumption of Bulgarian yogurt (known for its rich composition in lactic acid bacteria) is beneficial to health. Metchnikoff worked at the Pasteur Institute in Paris and he discovered *L.*

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bulgaricus and this strain was introduced into the commercial production of dairy products across Europe. He dedicated the last decade of his life to the study of bacteria that produce lactic acid as a means to increase human longevity. After the studies of Metchnikoff, the concept of probiotics was established and a new microbiology area started to develop [43].

3.2. *Lactobacillus* as probiotics and its mechanism of action

The main characteristics that a *Lactobacillus* strain needs to have to exercise an effective probiotic action against pathogenic microorganisms are related to three factors: the ability to inhibit the adhesion and colonization of pathogenic microorganisms in the host tissues, biosurfactant production, and hydrogen peroxide (H_2O_2). There is a collagen-binding protein called 29 kD present on the surface of some lactobacilli, which causes it to be capable of binding to collagen vaginal epithelial cells and to inhibit binding of pathogenic microorganisms to host tissues in significant numbers [44]. Some strains of lactobacilli produce biosurfactants generically known as surlactin, which are responsible for reducing the surface tension of liquid and thereby inhibiting the adherence of microorganisms. Surlactin studies are very important to help in the understanding of the urogenital tract microbiota and their maintenance for a balanced microbiota [45]. Other lactobacilli strains have the ability to produce hydrogen peroxide, which can be toxic to microorganisms that do not produce catalase [46, 47].

According to Reid and Bruce [46], not all probiotic strains have the same mechanisms of action and each has characteristics suitable for your application. For example, *L. casei* Shirota is ingested daily for about 24 million people who do not have the 29-kDa protein and do not produce H_2O_2. In the case of strain Shirota, its main action seems to be through the modulation of the host immune response.

In a recent study, Abedin-Do et al. [48] showed that some *Lactobacillus* strains exert innate and adaptive immune responses via their binding to pattern recognition receptors expressed on immune cells and many other tissues such as the intestinal epithelium. Furthermore, *Lactobacillus* can modulate the expression of genes involved in the regulation of immune system [49–53].

Members of our group evaluated the capacity of *L. rhamnosus* and its products to induce the synthesis of cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-4, IL-6, IL-10, and IL-12) by mouse macrophages. Jorjão et al. [54] used three microorganism preparations: live *L. rhamnosus* (LLR) suspension, heat-killed *L. rhamnosus* (HKLR) suspension, and the supernatant of a heat-killed *L. rhamnosus* (SHKLR) suspension. LLR and HKLR groups were able to significantly increase the production of TNF-α, IL-6, and IL-10. SHKLR also significantly increased the production of TNF-α and IL-10 but not IL-6. All the *L. rhamnosus* suspensions were not able to produce detectable levels of IL-1β or significant levels of IL-4 and IL-12. The authors concluded that live and heat-killed *L. rhamnosus* suspensions are able to induce the synthesis of different cytokines with pro-inflammatory (TNF-α and IL-6) or regulatory (IL-10) functions, suggesting the role of strain *L. rhamnosus* ATCC 7469 in the modulation or in the stimulation of immune responses.

In order for probiotic strains to have a satisfactory action, they must remain alive against stress challenges along the entire gastrointestinal tract, including the presence of bile in the small intestine. Bile is highly toxic to microorganisms not adapted to intestinal conditions. Moreover, some lactobacilli developed specific mechanisms to resist the deleterious effects caused by these compounds [55]. Among these mechanisms, we can cite the efflux pump that actively removes the acids and accumulated bile salts within the cytoplasm and the enzymatic activity of hydrolases, which are capable of neutralizing deleterious effect of bile [56–58].

According to FAO WHO [22], the ideal characteristics of a probiotic strain of *Lactobacillus* considered are as follows:

- Not pathogenic;
- Stable in acid and in the presence of bile;
- Adhesion ability in human mucosa;
- Colonize the intestine;
- Remain viable during storage and use;
- Have beneficial physiological effects and safe.

3.3. *Lactobacillus* in prevention and treatment of *Candida* infection

In vitro assays are important to evaluate the antifungal activity of each strain and characterization of the mechanisms of action, performing as a screening to in vivo tests with experimental models.

Sookkhee et al. [59] isolated and identified different species of lactic acid bacteria from the oral cavity of 130 volunteers in Thailand and they studied probiotic action against *C. albicans* in vitro. The authors found 3790 different samples of lactic acid bacteria including the genera *Lactococcus, Lactobacillus, Streptococcus, Leuconostoc*, and *Pediococcus*, and it was concluded that *L. paracasei* and *L. rhamnosus* strains were two species that had the greatest number of clinical isolates able to inhibit *C. albicans*.

Noverr and Huffnagle [60] examined the effect of living cultures, heat-killed cultures, and supernatants of probiotic bacteria (*L. casei, L. paracasei*, and *L. rhamnosus*) on the morphogenesis of *C. albicans* and observed an inhibition in the formation of germ tube when *C. albicans* interacted with living cells or supernatant of *Lactobacillus*. It was also found that supernatants obtained from cultures of 2 h inhibited germ tube formation of *C. albicans*. However, the addition of 24-h growth cultures took complete inhibition, suggesting that the accumulation of a soluble compound of the supernatant is responsible for this inhibition.

Coman et al. [61] evaluated the antifungal activities of two probiotic strains, *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®, and their 1:1 combination, named SYNBIO®, using agar well-diffusion method and liquid coculture assay. They tested probiotic strains in eight strains of *Candida*, including *C. albicans, C. krusei, C. glabrata, C. parapsilosis*, and *C. tropicalis*. All the *Candida* strains are strongly inhibited, except *C. glabrata* and *C. tropicalis*, and during the

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coculture assay, the inhibitory activity of probiotic bacteria against *Candida* strains was approximately 40% in some cases and absent in other cases, in particular against some strains of *C. albicans* and *C. tropicalis*. The authors concluded that in vitro screening of *Lactobacillus* strains according to their activity in various environmental conditions might be a valuable method that could precede clinical efficacy studies for adjunct treatment with probiotics in cure of different infections.

Parolin et al. [62] identified 17 clinical strains of *Lactobacillus* from the vaginal cavity of healthy premenopausal women, including the following species: *L. crispatus, L. gasseri*, and *L. vaginalis*, and evaluated their in vitro activity against *Candida* spp. (nine strains) and characterized their antifungal mechanisms of action. In general, the strains tested were more active toward *C. albicans*. No *Lactobacillus* strains showed activity against *C. krusei* and *C. parapsilosis*. All strains produced hydrogen peroxide and lactate, and in particular, *L. crispatus* BC2, *L. gasseri* BC10, and *L. gasseri* BC11 appeared to be the most active strains in reducing pathogen adhesion. It was concluded that these in vitro assays are prerequisites for the development of new therapeutic agents based on probiotics for prophylaxis and adjuvant therapy of *Candida* infection.

Some in vivo studies also show the effectiveness of probiotics in *Candida* infection. Wagner et al. [63] demonstrated that the inoculation of probiotics (*L. acidophilus, L. reuteri, L. casei* GG, and *B. animalis*) in immunodeficient mice reduced the density of *C. albicans* in gastrointestinal tract, incidence of systemic candidiasis, and prolonged the survival of adult and neonatal mice. Probiotic bacteria also modulated antibody and cell-mediated immune responses to *C. albicans*. The authors demonstrated that probiotic bacteria can protect immunodeficient mice from candidiasis; however, none of the probiotic bacteria we studied completely eliminated *C. albicans* from the alimentary tract.

Matsubara et al. [64] evaluated the oral colonization by *C. albicans* in experimental murine immunosuppressed and treatment with *L. acidophilus* and *L. rhamnosus*. The colonization by *C. albicans* on the oral mucosa, started on day 1 after inoculation, remained highest from day 3 until day 7 and then decreased significantly. Probiotic bacteria reduced *Candida* colonization on the oral mucosa significantly compared to the untreated group of animals (negative-control group). The reduction of yeast colonization in the group treated with *L. rhamnosus* was significantly higher compared to the group receiving nystatin (positive-control group). The authors concluded that the treatment with probiotics in this model may be an effective alternative to prevent it.

Deng et al. [65] evaluated the probiotic action in vitro and the anticolonization capacity of *L. paracasei* FJ861111.1 in vivo in mice infected with other selected pathogenic microorganisms. In vitro results showed that *Shigella dysenteriae, Staphylococcus aureus, Cronobacter sakazakii, E. coli*, and *C. albicans* were inhibited by *L. paracasei* FJ861111.1 that presented elevated survival at pH 2.5 and bile salt concentration at 0.3%. In vivo results demonstrated that the fermented milk with *L. paracasei* improved significantly the total population of bacteria, and the presence of *Lactobacillus* in the feces of mice. The colonization by *C. albicans* was significantly inhibited in the intestine of mice after infection and demonstrated the potential of this strain used as a probiotic organism for the production of functional fermented milk.

Although mice and rats are the gold standard for *Candida* studies, economic and ethical issues limit the use of mammals in these experiments, especially when a large number of strains need to be analyzed [66]. Invertebrate models have been used to study the microbial pathogenicity and pathogen-host interactions, which provided considerable insight into different aspects of microbial infection [67]. In this respect, *Galleria mellonella* has been found to be an interesting invertebrate model for the study of the pathogenicity of *C. albicans* [68–71]. Recently, our laboratory developed pioneering in vivo study to evaluate the probiotic action of *L. acidophilus* in the experimental candidiasis in *G. mellonella*. Vilela et al. [31] demonstrated that the inoculation of *L. acidophilus* into *G. mellonella* infected with *C. albicans* reduced the number of yeast cells in the larval hemolymph and increased the survival of these animals. However, *L. acidophilus* exerted no inhibitory effect on *C. albicans* filamentation in *G. mellonella* tissues. In this study, we verified that *G. mellonella* is an adequate model for the study of the probiotics.

4. *Bacillus* spp.

Bacillus spp. were classified a long time as only soil microorganisms, but they are also commensal microorganisms of the gut of humans and animals due to the great adaptability to the intestinal environment, representing part of your natural life cycle [72–74]. Some *Bacillus* species have been used as probiotics for at least 50 years, but scientific interest for these microorganisms has occurred mainly in the last 15 years [30, 75].

Among the large number of probiotic products in use today are bacterial spore formers, mostly of the genus *Bacillus. Bacillus* bacteria have been used widely as putative probiotics because they secrete many exoenzymes [76–78]. The species that have been most extensively examined include *B. subtilis, B. clausii, B. coagulans, B. licheniformis*, and *B. polyfermenticus* [26, 30, 79]. Although it requires an evaluation in each case, many species of *Bacillus* are considered as nonpathogenic and safe for animal and human consumption [79–81].

Used primarily in their spore form, these products have been shown to prevent gastrointestinal disorders and the diversity of species used and their applications are astonishing [30], then, demonstrating that exert immune stimulation, antimicrobial activity, and competitive exclusion. Studies have shown that these bacteria are able to grow inside the intestinal tract and could be considered temporary residents. This is important because it indicates that they are not exogenous microorganisms but may have unique symbiotic relationship with the host [74].

4.1. General characteristics

The members of genus *Bacillus* are Gram-positive, aerobic or facultative anaerobic, catalase-positive, and spore-forming bacteria [82, 83]. These microorganisms are saprophytic common in soil, water, dust, and air [84] and also involved in food spoilage [85]. These bacteria are considered allochthonous and enter the gut by association with food [30] or in an endosymbiotic relationship with their host, being able to survive temporarily and proliferate within the gastrointestinal tract [30, 86].

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B. subtilis is a model microorganism for studies involving the genus *Bacillus* [87]. This species is a widely used oral vaccine delivery system since it has been classified as a novel food probiotic for both human and animal consumption [88, 89]. The beneficial effects of *B. subtilis* on the balance of the gastrointestinal microbiota justify its use as probiotic in pharmaceutical preparations, for the prevention and treatment of intestinal disorders and the reduction of inflammation [90–92].

4.2. Spores as probiotics

Sporulation of *Bacillus* spp. represents a protection process, which is usually induced by low levels of nutrients and conditions unfavorable to the survival of the bacteria in vegetative form [93]. The spores are extremely resistant cell structures that when exposed to appropriate abiotic factors, through the germination, they can return to vegetative form [94].

Bacterial spore formers are being used as probiotic supplements for use in animal feeds, for human dietary supplements, as well as in registered medicines [74]. The use of spore-based products raises a number of questions. Since the bacterial species being used are not considered resident members of the gastrointestinal microbiota, how do they exert a beneficial effect? According to Cutting [74], while often considered soil organisms this conception is misplaced and Bacilli should be considered as gut commensals. Therefore, in fact, the question to be answered is what produces the probiotic effect: the vegetative cells (spores germinated) or the spores themselves? The natural life cycle of spore-forming microorganisms involves spore germination, sporulation, and re-proliferation when nutrients are scarce [30]. According to these authors, although it is unlikely that they are true commensals, a unique dual life cycle of spore formers in the environment and within the gut of animals could represent a mechanism that may be responsible for probiotic action.

Bacillus spp. forms thermostable spores and shows advantages over other microorganisms non-spore-forming, but also have probiotic activity. Thus, the product can be stored at room temperature in the dried form without any deleterious effect on the viability. Furthermore, since spores are extremely stable and resistant, they are able to survive low pH of gastric barrier [95, 96]. Therefore, a particular dose of ingested spores can be stored indefinitely without refrigeration and the desired dose of vegetative bacteria will reach the small intestine intact [74].

The research efforts and the search for new perspectives for clinical and nutritional applications with probiotic preparations that last comparatively more than other pharmaceutical drugs are justified because the spores are more resistant than the vegetative cells. This allows for greater reliability in the treatment method with probiotics and reduces the cost of production [79].

4.3. Mechanism of action of *Bacillus* probiotic

Before a bacterial strain can be considered probiotic, some criteria must be assessed as inhibition capacity in the growth of harmful microorganisms, not toxic, not pathogenic, and be tolerant to acid, bile salt conditions, and pancreatic secretions in order to reach the small

and large intestines, its ability to adhere to intestinal epithelial cells [82, 97–99], remain viable during transport and storage, exert beneficial effects on the host, stabilize the intestinal microbiota, adhere to the intestinal epithelial cell lining, and produce antimicrobial substances toward pathogen [82, 98].

Many authors have proposed that the properties of adhesion are a decisive factor for the selection of new probiotic strains. The mechanisms of action of probiotics against gastrointestinal pathogens consist principally on the following:

- Competition for nutrients and sites of accession;
- Production of antimicrobial metabolites [21, 100];
- Changes in environmental conditions;
- Modulation of the immune response of the host [21, 101].

The principal mechanism by probiotics is the production of antimicrobials that inhibit pathogenic microorganisms. *Bacillus* species produce a large number of antimicrobials and include bacteriocins and bacteriocin-like inhibitory substances, subtilin and coagulin, as well as antibiotics, surfactin, iturins A, C, D, E, and bacilysin [30, 102]. In 1979, Ozawa et al. [103] demonstrated that *B. subtilis* var. *natto* inhibited the growth of *C. albicans* in the intestinal tract and [104] showed that a surfactin had activity against yeast.

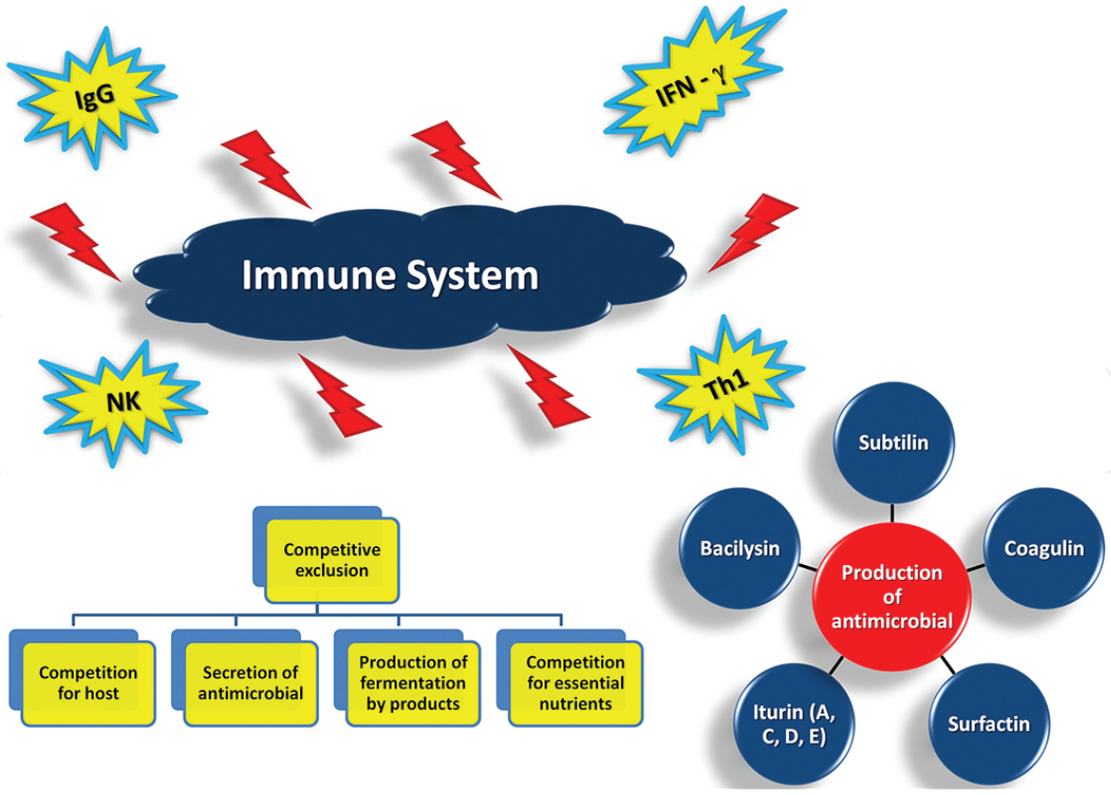

Figure 2. Mechanism of action of *Bacillus* probiotic.

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Stimulation of the immune system or immunomodulation is considered an important mechanism to probiotics. Studies in humans and animal models have provided that the oral administration of spores stimulates the immune system, and this confirms that spores are neither innocuous gut passengers nor treated as a food. Helper lymphocyte (Th1) responses are important for IgG synthesis but more importantly for cytotoxic T-lymphocyte recruitment, and for the destruction of intracellular microorganisms, and involve presentation of antigens on the surface of the host cell by a class I major histocompatibility complex (MHC)-processing pathway [30].

Studies have shown that small amount of inoculum of *B. subtilis* spores can germinate in the small intestine, grow, proliferate, and then again sporulate [105, 106]. Thus, the spores of *Bacillus* spp. can germinate in significant numbers in the jejunum and ileum [107], and stimulate and regulate the synthesis of immunoglobulin A, the pro-inflammatory cytokines such as tumor necrosis factor and interferon γ, and the helper T lymphocytes [108]. Therefore, through colonization, immune stimulation, and antimicrobial activity developed by these bacteria it is possible to prove that they have the potential probiotic effect [109].

Different mechanisms have been proposed for competitive exclusion agents including competition for host-mucosal receptor sites, secretion of antimicrobials, production of fermentation by-products, such as volatile fatty acids, competition for essential nutrients, and stimulation of host immune functions [30] (**Figure 2**).

4.4. Studies with *Bacillus* spp. as probiotics

In literature, there are in vivo and in vitro studies of *Bacillus* spp. about the benefits of their probiotic action in humans and animals. However, despite its recognized probiotic action and its benefits to human and animal health, to date, there are no studies on the effect of *Bacillus* spp. in the genus *Candida*. Subsequent text describes some studies with the genus *Bacillus* as probiotic.

Lee et al. [26] studied the potential probiotic characteristics of *B. polyfermenticus* KU3 isolated from *kimchi*, a Korean dish made from fermented vegetables. The spore cell of *B. polyfermenticus* KU3 was highly resistant to artificial gastric juice and survived for 24 h in artificial bile acid. *B. polyfermenticus* KU3 did not generate the carcinogenic enzymes, β-glucosidase, N-acetyl-β-glucosaminidase, and β-glucuronidase, and adhered strongly to HT-29 human intestinal epithelial cell lines. The authors found that *B. polyfermenticus* KU3 strongly inhibited the proliferation of cancer cells such as HeLa, LoVo, HT-29, AGS, and MCF-7 cells. The supernatant of *B. polyfermenticus* KU3 had an anticancer effect against HeLa and LoVo cells. Conversely, the proliferation of normal MRC-5 cells was not inhibited. They also demonstrated the anti-inflammatory activity of *B. polyfermenticus* KU3 under inflammatory conditions, as shown by the reduction in nitric oxide and pro-inflammatory cytokines (TNF-α, IL-10, TGF-β2, and COX-2). This study demonstrated the probiotic characteristics of *B. polyfermenticus* KU3 and provided evidence for the effect of this bacterium against various cancer cells.

Studies performed by Thirabunyanon and Thongwittaya [99] investigated the activity of isolates of *Bacillus* spp. for possible use as potential probiotics, and their protective inhibition activity against *Salmonella enteritidis* infection. The gastrointestinal tracts of native chickens were evaluated for use as a potential probiotic. *Bacillus* demonstrated higher growth inhibition of seven food-borne pathogens, including *S. enteritidis, S. typhimurium, E. coli, B. cereus, S. aureus, Listeria monocytogenes*, and *Vibrio cholerae*. The authors concluded that *B. subtilis* NC11 has a protective activity against *S. enteritidis* infection, and is able to competitively exclude it from its original site in the gastrointestinal tract, which is the beginning of the route of food-pathogenic contamination.

Rhee et al. [110] studied the effect of bacteria administered orally on the development of the gut-associated lymphoid tissue (GALT) in infant rabbits and *B. subtilis* showed greater importance in GALT development. Besides, *B. subtilis* secretes antimicrobial agents, as coagulin, amicoumacin, and subtilisin, which may have probiotic effect by suppressing the growth of competing microorganisms, such as enteric pathogens.

Pinchuk and colleagues [90] demonstrated that a probiotic strain *B. subtilis* 3, originally isolated from animal feed, has inhibitory effect against *Helicobacter pylori* due to the production of antibiotics, including amicoumacin A. The group of isocoumarin antibiotics (which the amicoumacin A belongs) can exert, among other properties, anti-inflammatory and anti-tumor actions, and present potential for use in the treatment of *H. pylori* infection.

In the human and animal consumption, the spores of *B. subtilis* were used as probiotics and competitive exclusion agents [107, 111], and, in some countries, *B. subtilis* was applied in oral bacteriotherapy of gastrointestinal disorders [107].

Bacillus probiotics were developed for topical and oral treatment of uremia [30]. *B. coagulans* had the ability to secrete a bacteriocin, coagulin, that has activity against a broad spectrum of enteric microbes [112] and since 1983 [113] showed the beneficial effects of *Bacillus* probiotics on urinary tract infections.

Ghelardi and colleagues [114] aimed to investigate the survival and persistence of *B. clausii* in the human gastrointestinal tract following oral administration as spore-based probiotic formulation. The authors concluded that *B. clausii* strains can have different ability to survive in the intestinal environment. *B. clausii* spores administered as a liquid suspension or a lyophilized form behave similarly in vivo and *B. clausii* spores survive transit through the human gastrointestinal tract, and they can germinate, outgrowth, and multiply as vegetative forms.

The use of *Bacillus* species as probiotic is expanding rapidly with increasing number of studies demonstrating immune stimulation, antimicrobial activities, and competitive exclusion by these microorganisms. Most research with *Bacillus* has been performed in animals and some clinical studies also in humans. Thus, the question is: Are the findings relevant to probiotic research in humans?

Therefore, if the results are promising and not only the bacteria are becoming superbacteria, but also other microorganisms such as fungi, why not apply the probiotic properties of *Bacillus* spp. in the genus *Candida*?

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5. Conclusion and future perspective

This chapter sought to provide the reader knowledge about the probiotic action of bacteria *Bacillus* spp. and *Lactobacillus* spp., describing the characteristics of microorganisms, the probiotic mechanism of action, and the studies described in the literature.

The high prevalence of *Candida* spp. associated with the increased resistance of microorganisms to conventional antifungal treatments boosts the development of research for new treatments to infections caused by *Candida*, such as probiotics. The treatment with probiotics promotes the reestablishment of the natural condition of microbiota with advantages over conventional antifungal because they do not induce microbial resistance, are nontoxic when administered in adequate amount, and therefore do not produce undesirable side effects, and also stimulate the immune system.

Infectious diseases along with the resistance of microorganisms to drugs represent serious problem in health. The knowledge of microorganisms that have characteristics capable of influencing the pathogenicity of *Candida*, and that characterize possible methods of prevention and treatment for candidiasis, is important, mainly, to provide alternative for microbial resistance without causing harmful side effects to the human organism and do not cause resistance to the fungus.

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