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Review Article

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Survival of *Bifidobacterium* ssp. during gastrointestinal passage and their mechanism of action for pathogenic bacteria inhibition in the gut: A concise review

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ABSTRACT

Bifidobacteria genus stands out for being one of the most used probiotic bacteria for food applications. This review presents a concise and direct picture of current knowledge on bifidobacteria strains survival during the passage through the human gastrointestinal system. It also provides the necessary theoretical background and details about mechanisms of actions of bifidobacteria against pathogenic bacteria. We will also report some of the factors which make the combination of food and bifidobacteria one of the most promising research topics in the field of modern food science.

KEYWORDS: Bifidobacteria, gastrointestinal system, food pathogens inhibition, action mechanisms

INTRODUCTION

The beneficial effect of functional food and some of its components on the consumer health have been studied [1,2]. The primary objective of functional foods is to improve, maintain and enhance the health of consumers through the alimentation [3]. Among the foods with functional claims are those added with probiotic microorganisms [4]. Probiotics are live microorganisms that when continuously administered in adequate amounts, confer benefits to the health of the consumer [5]. The main action mechanisms of probiotics include competition for sites of adhesion, antimicrobial activity, neutralization of undesirable compounds [enterotoxins, ammonia, toxic biogenic amines], alteration of metabolism [aid in digestion, reduction of precarcinogenic enzymes], and increase of immunity [increased lymphocyte and macrophage activity] [6]. However, it is important to emphasize that these mechanisms of action attributed to the probiotics are species-specific, i.e., the same strain cannot be able to exert all these health benefits simultaneously [4,7,8]. In this sense, bifidobacteria stands out for being one of the majority members of the *Actinobacteria* class that inhabit the human gut [9]. Due to this characteristic, bifidobacteria strains are chosen for to be used in a wide range of food products in order to be delivered in the gut and, thus, exert their beneficial effects [4].

One of the significant challenges in developing a probiotic product with *Bifidobacterium* is to ensure a high survival rate of the bacteria during the passage through the human gastrointestinal tract [4,10,11]. The main obstacles for bifidobacteria are the extreme acid pH in the stomach and the high bile salt concentrations presence from the duodenum to the ileum [12]. However, these class of bacteria has the intrinsical advantage to accommodate their enzymatic machinery to survive along the passage through the gastrointestinal system [13]. In this way, due to this adaptive capacity, the bifidobacteria can change some metabolic/functional routs which causes an improved capacity to survive and colonize the gut [13].

When *Bifidobacterium* ssp. arrives alive until the large intestine, the probiotic bacteria present several mechanisms of action to adhere to the epithelium and also inhibit bacterial pathogens [14]. The mechanisms involved in this antibacterial activity is the ability of bifidobacteria to product inhibitory substances, inhibit the pathogen adhesion to surfaces and produce iron-siderophore [15]. *Bifidobacterium* spp. produce

some inhibitory substances such as bacteriocins, hydrogen peroxide, and lactic and acetic acids that are effective in inhibiting the growth of several pathogenic bacteria [16]. Therefore, another important factor that influence the greatly ability of bifidobacteria strains to inhibit the pathogens is their adherence to the intestinal mucosal surface and consequently the colonization of the gut [17]. The iron-siderophore production, used for the uptake of lactoferrin or transferrin by microorganisms, is also a mechanism to inhibit the survival of pathogenic bacteria [18]. Thus, the main goal of this work is to provide an overview of the current developments of bifidobacteria specific characteristics of survive passage through the gastrointestinal system and inhibit pathogenic bacteria in the intestine.

BIFIDOBACTERIA AS PROBIOTIC

Specific criteria are used in order to select a microorganism to be used as probiotic. These bacteria must be acid and bile tolerant, nonpathogenic, no transferable antibiotic resistance and present in the normal healthy gut microbiota. Also, they could produce some antimicrobial substances against pathogenic bacteria, be genetically stable (identified by appropriate molecular techniques) and adhere to intestinal mucosa (mucus and enterocytes) [19]. Moreover, one of the most important safety aspects of the use of probiotics includes a history of being non-pathogenic and a history of no association with diseases [20]. The bioavailability of nutrients, the production of antimicrobial compounds, improvement of motility, relieving of intestinal constipation as well the decrease of *Helicobacter pylori* infection in the stomach are also attributed to the consumption of probiotic bacteria [21,22].

In food products, one of the most used probiotic cultures is those of the genus *Bifidobacterium* [23–25]. Microorganisms of the genus *Bifidobacterium* are gram-positive, non-spore forming bacilli, without motility, catalase negative, and some strains develop branches morphologically appearing with bifidopartide form [26,27]. Most bifidobacteria survives only in anoxic conditions, however, some species can survive in aerobic conditions or tolerate oxygen in the carbon dioxide presence [28]. *Bifidobacterium* can grow in the temperature range of 25–45 ºC, with the optimum temperature between 36 and 38 ºC for growth of human origin *Bifidobacterium*. Bifidobacteria are demanding in relation to the pH values, which are around 6.5–7.0, with no growth below 4.5 or above 8.5 [22]. Most *Bifidobacterium* species produce vitamins such as thiamine (B1), riboflavin (B2), pyridoxine (B6), folic acid (B9), cobalamin (B12), ascorbic acid ©, nicotinic acid (PP) and biotin [29]. The principal strains of *Bifidobacterium* used for commercial purpose or studied *in vitro* and humans are summarized in Table 1.

For the beneficial effects associated with probiotic consumption to be achieved, these should be in appropriate quantities in the food and must be consumed daily [4]. Therefore, the recommended minimum daily intake is around 8-9 log Colony Forming Units (CFU g^{-1} or mL^{-1}), which can be achieved with daily consumption of at least 100g of a 6-7 log Colony Forming Units (CFU g^{-1} or mL^{-1} of the product) [24]. Compensation for possible losses during food processing and storage as well as loss during passage through the gastrointestinal tract may directly influence this count [30]. However, factors such as high levels of oxygen, pH, acidity, time and temperature of storage and processing cause sensitivity and directly affect the viability of bifidobacteria [31,32].

TOLERANCE OF BIFIDOBACTERIA TO GASTROINTESTINAL CONDITIONS

In order to exert the beneficial effects on the host, it is necessary that the probiotic microorganisms can overcome intact the human digestive system [27]. Since the effects of probiotics are related to their activity in the digestive tract, and these depend on their colonization and survival in this environment, these bacteria must be resistant to the physiological and physicochemical processes of the gastrointestinal system. For Naidu et al. [33], probiotic bacteria must survive passage through the mouth, esophagus, stomach (pH 2), and small intestine to exert their benefits in the gut. Thus, they should be able to survive through gastric juice (hydrochloric acid), pancreatic juice and bile salts, i.e. surviving in acidic (stomach) and basic (duodenum) conditions [34].

Several stress conditions influenced the survival of probiotics during the passage through the gastrointestinal system [35]. Although the loss of bacterial viability occurs throughout all the digestive process, the greater losses are reported to the stomach, due to its acidity, and the presence of bile salts in the duodenum [36]. In the stomach, many strains of *Bifidobacterium* ssp. intrinsically lack the ability to survive such acidity. In general, the acid tolerance of bifidobacteria may be considered weak, except *Bifidobacterium lactis* and *Bifidobacterium animalis* [37]. Sánchez et al. [38] reported that the survival of bacteria lacking a respiratory chain, such as *Bifidobacterium* BB-12, is associated with their F_0F_1-ATPase enzyme ability to maintain the intracellular pH under acidic conditions. Due to the damaging effects, even if probiotics are still viable in the stomach, upon reaching the colon, they may be in a sub-lethally injured state. Thus, their chances of survival may be compromised, along with their ability to colonize the intestine and have an advantageous effect on the host [35]. This behavior can be attributed to the low pH value in the stomach (~2) and the presence of pepsin [39,40]. Also, Matsumoto et al. [37] affirm that the acid tolerance of probiotic bacteria depends on factors such as growth medium, incubation conditions, enzymes profile and cytoplasmic membrane composition of each strain.

After exposure to the stomach conditions, the probiotic bacteria reaches to the small intestine and are submitted to the duodenum (i.e., bile salts, pancreatin and pH approx. 5.0) up to the ileum conditions [41]. The bile salts are known for their antimicrobial activity against probiotic bacteria, mainly due to their amphiphilic nature and ability to dissolve the bacterial cellular membrane [42]. Also, Kurdi et al. [43] observed that the bile salts cumulate in the bacterial cytoplasm, causing disturbs on membrane integrity and therefore the cell death. As observed

Table 1: Probiotic Bifidobacterium strains used in commercial products or studied *in vitro* and humans.

Strain	Benefits
B. breve NCIMB8807	Reduces symptoms of irritable bowel syndrome, reduces gastrointestinal cancer possibilities, eradication of *Campylobacter* jejuni in children
B. longum BB536	Enhance intestinal function in premature infants>1000g
B. longum BL04-3008	Decrease of azoxymethane with reduction of gastrointestinal cancer possibilities
B. lactis DR10	Reduction of *E. coli* in the gastrointestinal tract
B. animalis spp. *lactis* BB12	Prevention of traveler's diarrhea, treatment of viral diarrhea including rotavirus diarrhea, modulation of intestinal flora, improvement of constipation, modulation of immune response, atopic dermatitis symptoms alleviation in children
B. animalis DN-173 010	Increase bowel motility and reduce diarrhea caused by rotavirus

Adapted from Lerayer et al.[6] and Saarela et al. [20].

by Vinderola and Reinheimer [44], the survival of probiotic in bile environment depends on the concentration of bile, exposure time and bacterial species and strains. Moreover, the survival of probiotic strains in the gastrointestinal tract might not only depend on their number and physiological state but also on the food matrix and food consumption habits that affect bile excretion [45]. Aspects such as amount and type of protein and fat, pH, presence of specific carbohydrates or other ingredients may also influence the resistance of probiotics during passage through the gastrointestinal tract [46–49]. Begley et al. [50] also stated that some components of the food might bind to bile salts, which would protect the probiotic bacteria from their toxicity.

After passage through the stages of the stomach and duodenum, another essential aspect about probiotic bacteria is their ability to adhere to the epithelial surface and colonize the small intestine or colon [51]. The hydrophobic bacterial surface is essential for the interaction between the intestinal glycoprotein layer, the receptor on the intestinal epithelial cell and fatty acid binding sites [52]. Also, the presence of prevailing apolar groups of bifidobacteria membrane may also support the cell adherence. As observed by Wang et al. [53], the surface hydrophobicity of *B. animalis* BB-12 was 50%. However, the adhesion ability varies with the type of *Bifidobacterium*, strain and the previous damages suffered [51]. Also, it must be highlighted that Lee et al. [54] showed for an inflammatory bowel disease that the better efficacy of the treatment was strongly dependent by using milk as a carrier medium. Their results strongly indicate that dairy products might be the preferred delivery matrix for probiotic strains for benefiting human health.

In light of these observations, to evaluate the survival of probiotics during and after the ingestion process, *in vitro* gastrointestinal simulation methodologies are being used as a rapid and straightforward approach in place of *in vivo* assays, since the latter are expensive long-term studies with high variability between individuals [55]. *In vitro* studies can provide useful insights on probiotic action, safety, and efficacy of probiotics targeted for human use [45]. Several studies have conducted *in vitro* gastrointestinal trials and evaluated the survival of probiotics in foods [34,46,48,56–59], but not continuously as naturally occurs during digestion. For that reason, evaluation of gastrointestinal tolerance by these methods may not satisfactory predict the *in vivo* survivability of probiotics accompanied by the food matrix [60]. Thus, Madureira et al. [42] recommend the use of a continuous *in vitro* gastrointestinal model that includes all compartments of the gastrointestinal tract (mouth, esophagus-stomach, duodenum, and ileum). This method also includes the presence of enzymes (α-amylase, pepsin, and pancreatin) and bile salts, mechanical simulation of peristaltic movements, time retention and also a pH gradient in the stomach. By using this methodology, Verruck et al. [39] verified the protective effect of a buffalo Minas fresh cheese on *Bifidobacterium* BB-12 survival. Pinto et al. [61] using spray drying method, verified that the use of sweet whey and inulin as encapsulating agents provided better protection to the bifidobacteria when compared with the free cells after exposure to these simulated gastrointestinal conditions. Holkem et al. [32] demonstrated that *Bifidobacterium* BB-12 resists better to the passage through simulated gastrointestinal fluids and under acid conditions when the technique of emulsification/internal ionic gelation for encapsulation was used. This *in vitro* simulated gastrointestinal conditions protocol was also successfully used by Almeida et al. [62], Pinto et al. [63] and Verruck et al. [40] for probiotic mascarpone-type cheese, greek yogurt, and microcapsules with full-fat goat's milk and prebiotics, respectively. Therefore, extrapolation of data from closely related strains is not acceptable, thus, each product and strain should be tested and documented independently [45]. So, the protocol proposed by Madureira et al. [42] may be used, giving us a safe view of what happens to the probiotic bacteria viability after the passage through the gastrointestinal system.

MECHANISMS OF ACTION OF PROBIOTICS FOR PATHOGENIC BACTERIA INHIBITION

The gastrointestinal tract is one of the access ways of pathogens, and if the defense mechanisms fail, these pathogens can colonize and/or penetrate the cells and tissues of the host [64]. As summarized in Figure 1, probiotic can inhibit the growth of pathogenic bacteria in the gut by several mechanisms, such as the capacity to synthesize antibacterial substances, inhibit the adhesion of pathogenic bacteria on the intestinal surface and also produce iron-siderophores [15]. Siró [45] reported that probiotics, such as *Lactobacillus* spp. and *Bifidobacterium* spp., have developed different mechanisms to survive in competition with pathogenic bacteria. In the case of *Bifidobacterium* spp., one of their important proprieties is the ability to produce organic acids (e.g., lactic and acetic acids), hydrogen peroxide and bacteriocins to suppress the colonization of pathogenic bacteria in the gut [22]. Makras and De Vuyst [65] tested 37 *Bifidobacterium* strains, including *Bifidobacterium animalis* subsp. *lactis* BB12, and reported a great organic acids production

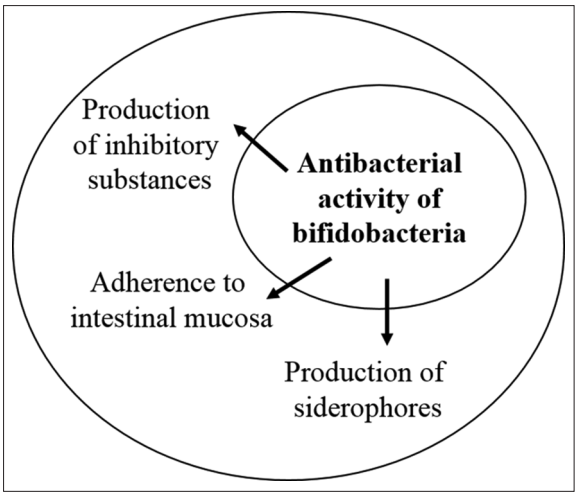

Figure 1: Summary of antibacterial activities of bifidobacteria. Adapted and modified from Fung et al.[15]

with a decrease in the medium pH. This behavior led to the inhibition of *Salmonella enterica* ser. Typhimurium SL1344 and *Escherichia coli* C1845.

Although the antimicrobial effect of the *Bifidobacterium* is often ascribed to the inhibitory action of organic acids and the related pH decrease in the bowel, the bifidobacteria has a high potential as bacteriocins producers [66]. Bacteriocins are ribosomally-synthesized peptides that have antimicrobial activity against other bacteria by creating pores in the cellular membrane causing the dissipation of proton motive force, ATP depletion, and leakage of nutrients that subsequently lead to cell damage or cell death [67]. Fung et al. [15] stated that numerous genera of pathogenic and nonpathogenic bacteria might be affected by bacteriocins. However, as cited by Lima et al. [68], the efficacy of probiotic bacteria against pathogens is based on a combination of bacteriocin action and production of antimicrobial substances such as hydrogen peroxide and organic acids. The antagonistic activity of *Bifidobacterium lactis* BB12 and *Bifidobacterium longum* 46 against six target pathogens was evaluated using different assays (liquid and solid media, microaerobic and anaerobic cultivation) and high activity against *Shigella sonnei* and *E. coli* was reported [69]. Gibson and Wang [70] reported that bactericidal or bacteriostatic substances were excreted by eight strains of bifidobacteria and could inhibit the growth of *Salmonella* spp., *E. coli*, *Listeria monocytogenes*, *Campylobacter* spp., *S. sonnei* and *Vibrio cholerae*. Martins et al. [71] reported production of antagonistic substances by *Bifidobacterium* BB12 against *Bacillus cereus*, *Clostridium difficile*, *Clostridium perfringens* Type A, *E. coli* ATCC 4328, *Enterococcus faecalis*, *L. monocytogenes*, *Pseudomonas aeruginosa*, *S.* Typhimurium, *Salmonella* Typhi, *Shigella flexneri*, *S. sonnei*, and *Candida albicans*. The better ability to colonize the gastrointestinal tract of mice is linked to higher hydrophobic property of the cell wall of *Bifidobacterium* BB12. Also, Saleh and El-Sayed [72] reported two bacteriocins: bifilact Bb-12 and bifilong Bb-46, the first produced by *Bifidobacterium lactis* Bb-12 and the second by *B. longum* Bb-46. These bacteriocins shown strong activity against *Staphylococcus aureus*, *S.* Typhimurium, *Bacillus cereus* and *E. coli*.

As the colonization of the gastrointestinal tract is based on the ability of the bacteria to adhere to the intestinal epithelium, another vital factor in the competitive exclusion of pathogenic bacteria is the ability of the probiotics to adhere to the mucosal surface [20]. Collado et al. [73] reported in an *in vitro* study that *Bifidobacterium* BB-12 was able to adhere to the intestinal mucosa and inhibit several pathogens, such as *Bacteroides vulgatus*, *Clostridium histolyticum*, *C. difficile*, *Enterobacter aerogenes*, *L. monocytogenes*, *S.* Typhimurium, and *S. aureus*. Bernet et al. [74] reported that bifidobacteria isolated from human gastrointestinal tract were able to adhere to Caco-2 cells due to the presence of a proteinaceous adhesion-promoting factor which affected the adherence of pathogenic E. coli and *S.* Typhimurium. In another study, Collado et al. [75] investigated the protective effect of *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* LGG alone and in combination on the adhesion of *S.* Typhimurium, *C. perfringens*, *C. difficile*, and *E. coli* K2. They demonstrated that in combination, the probiotic strains enhanced each other's adhesion, mainly in pig large intestinal mucus and reduced the adhesion of all the tested pathogens. Additionally, Jungersen et al. [7] affirmed that *Bifidobacterium* BB-12 is able to compete for mucosal adhesion and, thus, inhibit serious gastrointestinal pathogens by antimicrobial substances production.

Beyond the capacity of producing antimicrobial substances and inhibit the adhesion of pathogenic bacteria, the production of iron-siderophores was also reported as an antimicrobial ability of probiotics [15]. In general, the iron plays a vital role in the metabolism of bacteria that requires iron for growth acting as a global regulator of gene expression [76]. To obtain the necessary iron to survive, bacteria developed several metabolic routes to excel over other lineages. One of their approach is to synthesize and export siderophores, which are chelators with high-affinity to iron [77]. Certain bacteria or fungi produce and release siderophores to scavenge extracellular iron and redeliver the metal to the cell [78]. Vazquez-Gutierrez et al. [79] evaluated the siderophore production of 86 bifidobacteria strains (30 from culture collections and 56 isolates from infants) and reported that 35 strains exhibited high siderophore activity, 31 showing intermediate and 20 low activity. They conclude that the mechanisms used by bifidobacteria to sequestrate and use iron confers an great advantage to their survival and competition agaist pathogenic bacteria.

CONCLUSIONS

Bifidobacteria strains are used in a wide range of food products in order to be delivered in the gut for exert their beneficial effects.however, these strains have some obstacles to overcome, as survive through the acid pH in the stomach the presence of high bile salt concentrations in the intestine. However, when these bacteria arrives alive until the large intestine, several mechanisms of action to inhibit pathogenic bacteria are present, i.e., production of inhibitory substances, inhibition of the pathogen adhesion to surfaces and production of iron-siderophores. In the light of these observations, this concise review showed the necessary theoretical background and

some details about survival during the passage through the human gastrointestinal system and mechanisms of actions of bifidobacteria against pathogenic bacteria.

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