doi:10.20944/preprints202007.0264.v1

**Review Article**

# Inhibition of *Clostridium botulinum* and its Toxins by Probiotics and Their Nisin: An Update Review

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**Running title:** bioremoval of *C. botulinum* toxins by probiotics

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 doi:10.20944/preprints202007.0264.v1

## Abstract

*Clostridium* (*C.*) *botulinum* is the causative agent of foodborne poisoning as botulism, which has a high mortality rate in animals and humans, when grows and produces toxins in food. Probiotic bacteria play a critical and functional role in food matrices, agricultural, clinical and nutritional applications. In this review, the ability of various probiotic bacteria and their metabolites to inhibit of *C. botulinum* toxicity was reviewed. For this purpose, an introduction about *C. botulinum* and its mechanism of action for pathogenicity is mentioned. After a short glance to probiotic bacteria and their beneficial health effects on human, the mechanism of their action are reviewed. Then the subject is directed to bacteriocins production by probiotic bacteria. After description of *C. botulinum* and its neurotoxins, the effects of probiotic bacteria on *C. botulinum* are reviewed with a special focus on impact of their bacteriocins on this pathogen. This study confirmed that probiotic bacteria and their bacteriocins (especially nisin) can be effective on the growth, toxin formation and toxicities of *C. botulinum* and its toxins. It could be recommended that probiotics consumption, perhaps from birth to all stages of life, would be effective in preventing or treating the toxicity of *C. botulinum*.

**Keywords:** *Clostridium Botulinum*, Probiotic bacteria, Bacteriocin, Nisin, Decontamination, Safety

## 1. Introduction

The digestive system has a significant role in the digestion and absorption of food and energy. The gastrointestinal mucosa, which covers a lot of surface, is exposed to pathogens and non-pathogens environmental agents [1]. The microorganisms present in the gastrointestinal tract especially the *Lactobacillus* Sp. and *Bifidobacterium* Sp. play an important role in health [2]. These microorganisms have the greatest impact on immune system function and lead to the development of a strong and balanced immune system [3,2,4]. Since the largest and most complex part of the human immune system is located in the tissues of the gastrointestinal tract, therefore, revival immunity system plays a serious role in protecting human against various pathogens. If individual's gut microbiota- the microorganisms that usually colonize the body - balance changes due to the use of various drugs, including antibiotics, it can increase the risk of various infectious diseases by opportunistic pathogens such as clostridium [5,6].

*Clostridium* bacteria are a group of Gram-positive bacteria, obligate anaerobes, rod-shaped and producer endospores. These bacteria are known as foodborne pathogenic and spoilage bacteria and hazardous human pathogens [7]. The most important bacteria these group are *C. botulinum* (the causative agent of botulism), *C. difficile* (causing diarrhea during antibiotic therapy), *C. perfringens* (food poisoning to cellulitis and gas gangrene) and etc. [8]. *C. botulinum* is one of the most well-known members of this group, which leads to botulinum toxin production. Botulism disease affects different individuals, especially infants (transmitted through honey), and causes many complications, including paralysis, nausea, vomiting and abdominal cramps, difficulty swallowing or speaking, weak cry, irritability, drooping eyelids, tiredness, difficulty sucking or feeding [9]. On the other hand, *C. botulinum* produces various neurotoxins, which have different effects depending on the host's final, named type A-H. Therefore, controlling these pathogens by biological, chemical and physical agents can have an effective role in providing the public health [10].

Today, the use of biologic agents in disease control has been considered by researchers due to the adverse effects of drug use on health. Among these biologic agents, can be mentioned to probiotics (health promoting) of the bacteria and their metabolites [11]. As defined, probiotics bacteria are considered as living microorganisms that, if consumed in sufficient amounts, have health effects on the host and lead to improving or restoring the gut microbiota. These microorganisms are mainly bacteria of the *Lactobacillus sp.* and *Bifidobacterium* sp. [12]. Recently, these microorganisms and their metabolite (for example bacteriocins) have been widely used in the food industries, pharmaceutical and medical due to safety (non-pathogenicity and antibiotic resistance) properties, technological and functional (survive and viability during storage, persistence in the gut-tract, anti-inflammatory, anti-mutagenic and immunomodulation) characteristics [12-14]. In addition, these microorganisms can be used to detoxify various compounds (mycotoxins, heavy metals and bacterial toxins) [15-17]. Therefore, the purpose of this review study is to investigate inhibition of *C. botulinum* bacteria by probiotics of bacteria and their metabolites.

## 2. Probiotic Bacteria

The term probiotic, which means 'for life' comes from the Greek 'pro bios'. The history of probiotics began with the history of man; So that it was well known by the Greeks and Romans with cheese and fermented dairy products. They recommended consumption these substances, especially for children, seniors and convalescents for their health effects [3]. Moreover, prebiotics usually known as non-digestible food compounds (Fibers, Oligosaccharides, Chicory root, Garlic, Leek, Onion, Banana and etc.) that are selectively used by gut microbiota for fermentation. These

 doi:10.20944/preprints202007.0264.v1

compounds stimulate the growth or activity of beneficial microorganisms. Furthermore, these bacteria are related with lucrative health aftereffects can be specifically targeted. Considering the available evidence that prebiotics can alter the gastrointestinal microbiota. But it's not yet clear quietly how changes in the microbiota composition and performance by prebiotics, how stable these alters and how the effects of these changes on human health. Therefore, these characteristics will need to be investigated further [5]

Probiotics can be found in dairy and non-dairy products [3]. Currently, preparation of probiotics is chiefly based on acid-lactic bacteria (Lactobacilli, Streptococci, and Bifidobacteria). These bacterial genera are usually harmless to health and have proven to be important components of the gut-tract microbiota [18]. Lactic acid bacteria (LAB) are Gram-positive bacteria, non-sporulating, anaerobic or facultative aerobic cocci or rods, which, by fermentation and metabolism of carbohydrates, produce lactic acid as one of the main metabolite [19]. Acceptable level of bacteria in probiotic food products at the end of their shelf life is to have up to $10^7$ CFU/g [20]; but, since the probiotics must withstand some conditions and stresses in the target location to elicit their effect. Therefore, the human gut tract must be contains up to $10^{13}$ - $10^{14}$ cells to ensure they reach the sufficient numbers [21].

The term probiotic is mostly related to lactic acid bacteria (*Lactobacillus* and *Bifidobacterium* Sp.), but, it can also be applied to other microorganisms. For example, some *Bacillus* (*B.*) species including. *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans* and *B. licheniformis* have been used as probiotics for at least 50 years in an Italian product commercialized as Enterogermina ($2{\cdot}10^9$ spores) [22].

The main sections of the human digestive tract (the stomach, the small intestine, and the large intestine) have their own distinct microbiota [21,23-25]. The aerobic Gram-positive bacteria are

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 July 2020 doi:10.20944/preprints202007.0264.v1

mostly inhabited in the stomach ($<10^3$ CFU/g). The genera *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp., and *Streptococcus* spp. are inhabited in the small intestine ($10^3$ - $10^4$ CFU/g), and the genera *Bacteroides* spp., *Fusobacterium* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and *Eubacterium* spp. are inhabited in the large intestine in large numbers ($10^{11}$ - $10^{12}$ CFU/g). There are many studies of the probiotics effects of LAB [26]. The most popular probiotic microorganisms with claimed health benefits for humans and animals are represented in Figure 1 [27,3,28-30]. They have been isolated from various sources like dairy products, plants, fermented meat products, pickled fruits and vegetables, beverages, soy sauce, marinated fish products and fermented cereal products.

**Figure 1**

**Fig. 1** Probiotic microorganisms with claimed health benefits for humans and animals

### 2.1 Mechanisms action of probiotic bacteria

Probiotics may be applied their beneficial health effects in the three modes [11]: a) Adjusting the host defense system including the inherent and acquired immune systems; b) Direct or indirect effects on other microorganisms, pathogens, and commensal.; c) Effect on the metabolites of microorganisms like toxins and host products, e.g. bile salts and food ingredients. Deactivating toxins and detoxifying host and other food compounds in the digestive tract may be done by various activities. For this purpose, probiotics may use a dual effect, prevent or reduce the colonization of pathogen microorganisms in the intestines [31], or interacting with the gut-associated lymphoid tissue (GALT) to prevent inflammatory responses and reinforce their own tolerance and possibly to food [32]. The useful effects of probiotics are usually varied and specific [33]. Some species participate in the treatment of acute diarrhea associated with rotavirus [34],

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ulcerative colitis [35,36], *Clostridium difficile*-associated diarrhea [37], and *Helicobacter pylori* infection [38,39].

**2.2 Bacteriocins produced by probiotic bacteria**

Bacteriocins, defined as probiotics metabolites, are classified into four groups on the basis of to their molecular mass, thermo-stability, enzymatic sensitivity, presence of modified amino acids, and mechanism of action [40]. Class I: are small peptide inhibitors and include nisin and other lantibiotics; Class II: This group comprises heat-stable peptides with molecular weight smaller than 10 kDa and with no modified amino acid; Class III: This group consists of peptidic antibiotics that are heat-labile proteins with a molecular weight larger than 30 kDa; Class IV: This group contain either glycoproteins or lipoproteins that require non-protein moieties for their activity [41]. As presented in Table 1, there are several of bacteriocins which can be produced by types of probiotic bacteria. Nisin, one of the most important bacteriocin, is the prototype lantibiotic (amphipathic antibiotic peptide) from *Lactococcus lactis* and *Streptococcus lactis* which is active against certain Gram-positive and Gram-negative bacteria at the nanomolar range. The biosynthesis of nisin involves synthesis of ribosomal peptide, dehydration of serine and threonine amino acids residues, cyclization through sulfhydryl increment of cysteine to a dehydrated residue, transmission of the precursor peptide and proteolytic activation (Figure 2a). The Hinge region of nisin, formed of 3 amino acids (residues NMK), is implicated to play an important task during insertion of nisin's C-terminus into the cell membrane [42]. As shown in figure 2b, there are two inhibition or killing mechanisms of nisin in a bacterial cell. It can be bind to lipid II (is located in the cell membrane and plays a fundamental role in wall synthesis) and causes pore formation. The second mechanism is interfering and prevention of cell wall biosynthesis [43-45].

 doi:10.20944/preprints202007.0264.v1

**Figure 2**

**Fig. 2 a)** Structure of nisin A. Dhb, dehydrobutyrine; Dha, dehydroalanine; Abu-S-Ala, β-methyllanthionine; Ala-S-Ala, lanthionine; Hinge region (Asn-Met-Lys) [43]. **b)** Mechanism of action of Nisin in the cell of bacteria [46].

**Table 1 near here**

## 3. *C. botulinum* and its Neurotoxins

Clostridium bacteria, an anaerobic spore former, are widely found in nature, environment and the intestines of humans and animals and foodstuffs, especially fresh meat, drinks, milk and canned food [9]. These bacteria produce spores and are very resistant to heat. Among the various species, *C. botulinum*, causes serious food poisoning associated with meats, fish, and vegetables [50]. *C. botulinum* strains are categorized into four biotype groups based on their toxin type and proteolytic capabilities (Table 2). Group I consists of all type A and some (proteolytic) type B strains, and is characterized as highly proteolytic, group II strains are only weakly or non-proteolytic and consists of non-proteolytic type B and all type E strains. Strains of both groups different in their relative heat resistance, maximum and minimum growth temperatures, and tolerance to acid and salt. Strains in groups III and IV are not commonly considered with foodborne human botulism [51,10,52]. Optimum and minimum temperature of growth for proteolytic strains are 37°C and 10°C, respectively. Non proteolytic strains grow and produce toxins at temperatures as low as 3.3 to 4.0°C [10]. Therefore, preserve of minimally processed refrigerated foods at temperatures higher than 10°C will result in botulism hazard if spores survived the pasteurization process [53]. The sodium chloride and nitrite, at refrigeration temperatures, have synergistic impact on *C. botulinum* spores, especially in cured meats [53]. The role of nitrite in nitrosamine formation in cured meats caused to the search for an anti-botulinum agent [54]. ]. Botulinum neurotoxins are a

 doi:10.20944/preprints202007.0264.v1

metalloprotease (150-kDa) and including a heavy chain (100-kDa) and a light chain (50-kDa) that linked by a disulfide bond [10,51]. The neurotoxin blocks releasing acetylcholine from the motor nerve-endings and leading to paralysis in human and animal [55]. *C. Botulinum* causes three major diseases in humans including foodborne botulism, infant botulism and wound botulism. The toxin usually is destroyed by heating (80˚C/20 min or 85˚C/5 min). Since the neurotoxin is completely tasteless and odorless, so foods that contain neurotoxin may not show any warning signs to the consumer [55,56].

**Table 2 near here**

## 4. The effects of probiotic bacteria on *C. botulinum*

Botulinum neurotoxins are generated by the gram-positive, anaerobic spore-forming Clostridium species and are the causative agent of botulism [58]. There are at least 7-8, different serotypes of Botulinum neurotoxins that four serotypes A, B, E, and F causes of botulism in humans [59]. Botulinum neurotoxins are highly poisonous to humans with the have lethal and oral dosage of 0.1–1 ng/kg and 1 µg/kg, respectively. Botulinum neurotoxins lead to a public health and safety threat in the form of foodborne, wound, and infant botulism. Because of its mortality and morbidity, there is a considerable economic burden associated with the long-term handling of intoxication [60,61]. The first function in botulinum neurotoxins defection and foodborne illnesses is, surviving in gastrointestinal tract, then should be bind and translocate through the intestinal epithelium to reach the bloodstream. Based on previous research a complex of botulinum toxin serotype A- which is combination of holotoxin with neurotoxin-associated proteins- binds and transits through the intestinal epithelia to disseminate in the blood faster than botulinum toxin serotype A holotoxin alone [55,62]. Hence, perception the mechanism(s) in which botulinum neurotoxins bind to and breach this epithelial barrier is of excellent scientific benefit because of the potential expansion of novel therapeutics to prevent this required first step of oral intoxication [62].

Nowadays; researcher have provided incentive document that probiotic microorganisms are precious in the prohibition and treatment a number of diseases and disorder. Today, the use of probiotics has increased dramatically due to our growing awareness of the beneficial effects of these microorganisms and how each of the strains acts in specific conditions [63]. It should be noted that these definitions are consistent with the definitions provided by WHO organization [63,64]. Recent study about probiotic such as lactobacillus, concentrate on the interaction of them with immune system [65], and their effect as an anticancer and bio-therapeutic agent. Probiotics microorganism have a great potential in treatment some diseases such as *Helicobacter pylori* infection, irritable bowel syndrome, and inflammatory bowel disease as well as boosting the immune system of healthy individuals [66-68]. The most commonly probiotic strains used are *Lactobacillus spp*., *Bifidobacteria* spp., and the yeast strain *Saccharomyces cerevisiae* var. *boulardii*. Lactic acid bacteria and Bifidobacteria have good ability in removing heavy metals [69] cyanotoxins [11], and mycotoxin from in vitro aqueous solutions[70]. The probiotic impacts seen are both strain and species dependent showing that combinations of vary strains and species may require to be suitable to the special subject at hand rather than having one "universal" probiotic therapy. Although many advantage characteristics of probiotics have been displayed in both in vivo and in vitro research, the accurate mechanism(s) that is responsible for these beneficial effects remains to be fully elucidated. These mechanisms which have related to probiotics are: (a) conservation of the gut epithelial barrier, (b) competitive elimination of pathogenic microorganisms, (c) secretion of antimicrobial products, and (d) regulation of the mucosal immune system in favor of the hosts [11,71].

## 5. The effects of bacteriocins on *C. botulinum*

Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are various structurally, functionally, and ecologically. Applications of bacteriocins are being tested to assess their application as narrow-spectrum antibiotics. Activity of bacteriocin is depended to the properties of the given food system [72]. Generally, nisin is most effective at a pH of $<6.0$ in low-fat and -protein foods [73]. The use of bacteriocins to prevent pathogens is particularly attractive in some components such as minimally processed refrigerated meats. *Listeria* (*L.*) *monocytogenes* and *C. botulinum* are of particular concern in minimally processed refrigerated meats because of their susceptibility and heat resistant spores of these product which caused to grow and produce toxin in temperature-abused food [74,75]. Many lactic acid bacteria have excellent ability in producing bacteriocins to inhibit of *L. monocytogenes*, *C. botulinum* and a broad range of Gram-positive and Gram-negative foodborne pathogens [76,77]. *C. botulinum* is one of the more nisin-resistant clostridial species [78]. Twenty-three strains of Lactic acid bacteria were assessing for bacteriocins-like activity against types A and B spores from proteolytic and non-proteolytic *C. botulinum* strains [79]. *Pediococcus pentosaceus* ATCC 43200, *Pediococcus pentosaceus* ATCC 43201, *Lactococcus lactic subsp. lactic* ATCC 11454, *Lactobacillus acidophilus* N2, *Lactobacillus plantarum* Lb75, *Lactobacillus plantarum* Lb592, and *Lactobacillus plantarum* BN demonstrated bacteriocins-like inhibition of all *C. botulinum* strains tested. Based on the minimum inhibitory concentrations, *Pediococcus pentosaceus* 43200 was most inhibitory to *C. botulinum* [80].

Nisin is one of common bacteriocin produced by *Lactococcus lactic* and show wide activity against Gram-positive bacteria including Listeria, Bacillus, Clostridium, Staphylococcus, Streptococcus, Lactobacillus, and Micrococcus [81-83]. In the United States, nisin has a generally-recognized-as-safe (GRAS) application to inhibit *C. botulinum* spore. Application of nisin was approved in the United States in April 1988, although its function is limited to certain pasteurized process cheese [84]. As regards *C. botulinum* can grow in minimally processed refrigerated foods [85] which might be destroyed by usage of nisin [86-88]. The synergistic impacts of nisin and heat treatment on *C. botulinum* have been study by Scott and Taylor [89]. Some study investigated the sensitivity of six *C. botulinum* strains to nisin and exhibit that type A are more resistance followed by B and E. While this study evaluated that proteolytic strains are more resistant to nisin than saccharolytic strains [89], but Rayman et al. have found proteolytic and non-proteolytic type B spores were equally resistant to nisin [90]. Growth conditions and food components also affect nisin's effectiveness. Factors decreasing nisin's ability to inhibit *C. botulinum* growth include low acid environment, short heat-shocking periods, high spore load, high protein and phospholipid concentrations, and increased incubation temperature [91,89].

## 6. Conclusion

Considering the importance of pathogenicity and the mortality rate of by *C. botulinum*, and its role in food poisoning, Therefore, control of this pathogen and neurotoxins produced by it, is required by various factors. Probiotics and their metabolites (bacteriocins) as biological control agents play an important role in detoxification and reduce the risk by these pathogens. On the other hand, the relation of antagonism between *C. botulinum* and bacterial members of the ecosystem are well known. Accordingly, researchers concluded that the use of probiotics and metabolites could help in the prevention of *C. botulinum* colonization and reduce botulinum neurotoxin production.

**Acknowledgments**

This study is related to the project NO. 1397/68940 from Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Research & Technology Chancellor" in Shahid Beheshti University of Medical Sciences for their financial support of this study.

**Funding** The work was supported by the financial support of Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran, project NO. 1397/68940.

**Compliance with Ethical Standards**

**Conflict of Interest** No potential conflict of interest was reported by the authors.

**Ethical Approval** We state that this article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** For this type of study, formal consent is not required.

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