

Analysis of Different Probiotic Strains from Emerging Concept to Application and Antimicrobial and Immunomodulatory Activity of *Bacillus Clausii* Strain in Gastrointestinal Disorders in Children

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Summary

The gut microbiota has roles in metabolic, nutritional, protective, structural, and neurological functions; disturbances in microbial homeostasis are implicated in both intestinal and extraintestinal disorders. Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host,” and many commercial preparations comprising a diverse range of species are available. While probiotics have been much researched, better understanding of the probiotic effects and applications of species such as *Bacillus clausii* is warranted.

In this narrative literature review, we review the characteristics and mechanisms of action supporting *B. clausii* as a probiotic and discuss the evidence from clinical studies evaluating *B. clausii* probiotics for the management of a variety of gastrointestinal disorders and symptoms in children, we highlight the challenges of future research and the need for more robust and diverse clinical evidence to guide physicians in the clinical application of probiotics for gastrointestinal disorders and other conditions. and its antimicrobial and immunomodulatory activity

Keywords: Probiotic; Gut immunity; Dysbiosis; Infant Formula; Immunity; Atopy; Microbiota; NEC

Introduction

Gut immune system

The composition and function of the gut microbiota are key to gut homeostasis. Gut-associated lymphoid tissue (GALT) is the largest lymphoid tissue in the body and is a major site where immune cells come into contact with antigens [1]. The gut barrier consists of the outer mucus layer, the central single layer of intestinal epithelial cells, and the inner lamina propria [2]. The mucus layer and the intestinal epithelium together constitute the physical barrier to gut microbes, whereas the immune cells of the lamina propria act as the immunological barrier [3]. The physiological barrier the mucus layer acts as the first line of defense in the gut and prevents bacteria from directly interacting with the underlying intestinal epithelium [3]. This layer contains glycosylated mucin proteins, which form a gel-like sieve structure, and the antimicrobial peptides, secretory immunoglobulins and other proteins secreted by the epithelial cells [3]. The epithelial layer is made up of enterocytes, goblet cells, and Paneth cells. The permeability of this barrier is influenced by tight junction proteins, which hold adjacent epithelial cells together [2].

biotics defines probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [4]. Probiotics exert their beneficial effects through several modes of action [5,6] and have found wide use in preventing or treating many diseases [7-18]. Probiotics belonging to *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces* spp., *Bacillus* spp., *Enterococcus* spp., and *Streptococcus* spp. are consumed around the world for their health benefits [19].

Bacillus is a genus of spore-forming bacteria found in the air, water, food, soil, and the human gut [19]. When environmental conditions are harsh, spore-forming bacteria undergo a complex developmental process in which the bacterial cell differentiates into a spore that can indefinitely survive in the absence of water, nutrients, extremes of temperature, pH, ultraviolet radiation, and noxious chemicals [20]. When favorable environmental conditions return, the spores germinate into vegetative cells that can grow and reproduce [20]. *Bacillus* spores are metabolically inactive and can tolerate bile salts, survive the acidic environment of the gastrointestinal tract, and are more stable than vegetative bacteria during processing and storage of pharmaceutical or food-based probiotic formulations [19,21,22].

The International Scientific Association for Probiotics and Pre-

Probiotics that can naturally be isolated from the human gut are likely to have the ability to survive passage through the gut [23]. *Bacillus clausii* and *Bacillus licheniformis* have been isolated from healthy human adult feces, indicating their ability to survive passage through the gastrointestinal tract [23,24]. Due to their inherent antibiotic resistance [25] and the excellent compositional quality of some probiotic formulations [26], *B. clausii* strains have been concomitantly used with antibiotics to reduce the gastrointestinal side effects of antibiotic treatment [27,28]. As an example, the probiotic strains *Bacillus clausii*, O/C (CNCM I-276), N/R (CNCM I-274), SIN (CNCM I-275), and T (CNCM I-273), are well-tolerated and have been efficaciously used in humans for several decades [24,29]. They have been available as an over-the-counter medicine since 1999 [30]. These four strains derived from a single penicillin-resistant strain, *B. subtilis* ATCC 9799 [31], were initially classified as *B. subtilis* until their reclassification as *B. clausii* in 2001 [25].

These beneficial microorganisms may act through a variety of ways, such as producing organic acids [32] and antimicrobials [33,34], resisting colonization by pathogens [35–38], improving barrier function [39–42], and modulating the pro- and anti-inflammatory responses of the immune system [43–53]. Other modes of action include producing enzymes that aid in digestion, manufacturing small chemicals that have systemic effects (such as cortisol, tryptophan, and others), and mediating interactions with host cells by means of cell surface structures [reviewed in 7,8]. Specific species and strains of probiotics that manipulate the gut microbiota have shown promise in the treatment or prevention of specific diseases. Immunomodulation by probiotics is strain-specific and their influence on immunity depends on the physiological environment in which they act [54]. Therefore, their use needs to be personalized to the individual's intestinal milieu [55].

Gut Immune Function

Clausii Enhancing Gut Barrier Function

As well as in vitro studies of its physiological properties that support gut barrier function, *B. clausii* have been shown to enhance the gut barrier in preclinical studies using cell lines. A recent study has shown how *B. clausii* strains protect the gut from a rotavirus infection by multiple modes of action. In a human pediatric enterocyte model of rotavirus infection, the vegetative cells of *B. clausii* (O/C, N/R, SIN, and T) strains induce synthesis of human beta defensin 2 and cathelicidin, which are antimicrobial peptides. The strains also rescue cell proliferation that has been slowed by rotavirus infection. Treatment with *B. clausii* strains or their supernatant also reduces the proportion of necrotic or apoptotic enterocytes as well as increases mucin production and synthesis of tight junction proteins, increasing the gut barrier integrity. In addition, they inhibit ROS production by rotavirus and the release of pro-inflammatory cytokines, such as IL-8, IFN- β , and TLR-3 pathway genes [48]. Thus, this study shows the mechanistic basis for the clinical efficacy of *B. clausii* in pediatric viral acute gastroenteritis [48].

Apart from the context of clinical disorders, vegetative cells of *B. clausii* affect the global reprogramming of gene expression in the gastrointestinal tract of relatively healthy individuals. In duodenal cells derived from patients with mild esophagitis, *B. clausii* affect the expression of genes involved in immunity and

inflammation, apoptosis, cell growth and differentiation, cell-cell signaling, cell adhesion, signal transcription, and transduction [49].

Contributing to Gut Homoeostasis

Patients undergoing chemotherapy often suffer from a dysbiotic gut microbiome, which leads to several general side effects of chemotherapy, such as nausea, vomiting, abdominal pain, and diarrhea [50]. Patients with pancreatic adenocarcinoma who survive longer than five years harbor tumors with a microbiome signature that includes *B. clausii*, *Pseudoxanthomonas* spp., *Streptomyces* spp., and *Saccharopolyspora* spp. [50]. Specifically, the presence of *B. clausii* is associated with longer survival times [50]. In mice with pancreatic cancer, transfer of long-term survivors' gut microbiomes can alter tumor microbiome composition, tumor growth, and tumor immune infiltration [50]. Thus, use of fecal microbiota transfer may represent an attractive clinical option for increasing the life expectancy of patients with pancreatic adenocarcinoma.

In an in vitro simulation of the human gastrointestinal tract, a synbiotic formulation consisting of *B. clausii* SC-109 spores along with other probiotic bacteria and prebiotic ingredients increased butyrate production by the microbiome and the diversity of gut microbiota, especially the levels of *Faecalibacterium prausnitzii*, *Bifidobacterium* spp., and *Lactobacillus* spp. [51], which exert anti-inflammatory effects in the gut, contributing to gut homeostasis.

Uremia is a major syndrome of chronic kidney disease and presents with high levels of urea in the blood of patients. In a rat model of uremia, administration of *B. clausii* UBBC07 spores reduced serum urea, creatinine, and malondialdehyde levels that were induced by acetaminophen treatment [52]. The authors of this study attributed this observation to an antioxidant effect exerted by *B. clausii*. Other studies have also shown a decrease in serum urea levels in patients with chronic renal failure administered probiotics [65]. Therefore, this may represent a novel clinical use of probiotics in chronic kidney disease.

Antimicrobial and Immunomodulatory Activity

Antimicrobial Activity

The Bacillales are an order of Gram-positive bacteria, which include the genera *Bacillus*, *Listeria*, and *Staphylococcus*. Based on genome mining, Bacillales are predicted to be a rich source of novel antimicrobials. These antimicrobials comprise three classes of bacteriocins, amounting to 583 bacteriocin gene clusters from 57 species [66]. Bacteria belonging to the genus *Bacillus* produce a wide range of antimicrobial substances, including lantibiotics, which are post-translationally modified peptides [67]. The production of antimicrobials such as the lantibiotic clausin is a key route by which probiotics prevent the growth of pathogenic bacteria in the gastrointestinal tract; this is clinically relevant when administering probiotics alongside antibiotic therapy.

When cultured in whey, vegetative cells of *B. clausii* produce antimicrobial peptides that inhibit the growth of *Salmonella typhimurium*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis* [53]. These bacterial species are also inhibited by spent coffee grounds fermented with *B. clausii* Sinubrase® [68], indicat-

ing that this *B. clausii* strain secretes the antimicrobial peptides into the growth or fermentation medium.

The vegetative cells from two strains of *B. clausii*—UBBC07 and O/C—have been shown to produce clausin [47,54,69]. The clausin from *B. clausii* UBBC07 exhibits antimicrobial activity against some Gram-positive bacteria [47]. The O/C strain of *B. clausii* produces clausin that exhibits antimicrobial activity against some Gram-positive bacteria and inhibits the cytotoxic effects of *Clostridioides difficile* [58,59]. The clausin from O/C has also been shown to target lipid intermediates of bacterial peptidoglycan synthesis [54].

Immunomodulatory Activity

Whereas the antimicrobial activity of probiotics has direct effects on other microorganisms in the gut, the immunomodulatory activity of probiotics rebalances the host immune system, enabling long-term health effects for the host. The following studies point to the potent immunomodulatory mechanisms by which *B. clausii* probiotics exert their effects.

Chronic inflammation, due to an aberrant immune response involving Th2 cells, can lead to asthma [70], which is characterized by airway inflammation involving eosinophils, and structural changes to the airways, termed airway remodeling [71]. When administered to mice with ovalbumin-induced asthma, *B. clausii* isolated from tidal mudflats have been shown to reduce the numbers of eosinophils, neutrophils, and lymphocytes and reduce the thickening of the airway epithelium [55]. *B. clausii* also reduce IL-4 and IL-5 levels and the expression of hypoxia-related genes in these mice [55], pointing to their potential use in reducing airway inflammation in clinical settings. Vegetative cells of *B. clausii* MTCC-8326 induce a controlled inflammatory response in RAW264.7 murine macrophage cells by increasing pro-inflammatory cytokines at earlier time points and anti-inflammatory cytokines at later time points [56]. This strain also protects murine macrophages from *S. typhimurium*-induced cytotoxicity [56]. It colonizes the mouse gut and protects BALB/c mice, but not C57BL/6 mice from *S. typhimurium* infections [57].

Macrophages in the intestine play a key role in either increased inflammation following an infection, or in decreased inflammation to enable wound repair [72].

Infection with *C. difficile* causes diarrhea, pseudomembranous colitis, and septicemia, and it may also be fatal. It is often transmitted as a nosocomial infection and following antibiotic therapy. Equally, other pathogens, such as *Bacillus cereus*, secrete enterotoxins, such as hemolysin BL and cytotoxin K, which damage the intestinal epithelium, causing diarrhea, emesis, or hemorrhage. In vitro, the vegetative cells of the O/C strain of *B. clausii* secrete a serine protease, which protects intestinal cells from the cytotoxic effects of *C. difficile* and *Bacillus cereus* [58]. Two hours of co-incubation with *B. clausii* O/C can rescue the low viability, low proportion of cell attachment, and decreased mitochondrial activity induced by *C. difficile* or *B. cereus* infection [58]. These studies highlight the clinical relevance of *B. clausii* probiotics in protecting patients at risk of *C. difficile*-associated diarrhea.

Following an infection, macrophages stimulate nitrite production, which leads to destruction of the pathogen. Pro-inflammatory cytokines and CD4⁺ T cells also play a role in mounting a

coordinated response. Vegetative cells of *B. clausii* (O/C, N/R, SIN, and T) have been shown to stimulate nitrite production in Swiss murine peritoneal cells and induce the pro-inflammatory cytokine, IFN- γ , and increase the proliferation of CD4⁺ T cells in murine BL/6j spleen cells [59]. In addition, lipoteichoic acid from the O/C strain of *B. clausii* induces nitric oxide production in RAW 264.7 macrophages and may underlie the immunomodulatory ability of *B. clausii* [60].

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