



## BACILLUS CLAUSII: A PROBIOTIC APPROACH TO COMBATING INTESTINAL INFECTIONS

Saira Rehman<sup>1</sup>, Laraib Nisar<sup>2</sup>, Gilberto Mercado Mercado<sup>3</sup>, Muhsin Ali<sup>4</sup>, Saleha Tahir<sup>5\*</sup>, Mahtab Ali shah<sup>6</sup>, Muhammad Ramzan Ali<sup>7</sup>

<sup>1</sup>Faculty of Pharmaceutical Sciences, Pharmacognosy Department, Lahore University of Biological and Applied Sciences, Pakistan, Email: [Saira.rehman@ubas.edu.pk](mailto:Saira.rehman@ubas.edu.pk)

<sup>2</sup>Institute of Microbiology, University of Agriculture Faisalabad, Pakistan  
Email: [laraibnisaruaf@gmail.com](mailto:laraibnisaruaf@gmail.com)

<sup>3</sup>Department of Chemical and Biological Sciences, Institute of Biomedical Sciences, Universidad Autónoma de Ciudad Juárez / Universidad Vizcaya de las Américas Campus Juárez, Email: [gilberto.mercado@uacj.mx](mailto:gilberto.mercado@uacj.mx)

<sup>4</sup>School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, China  
Email: [Muhsinaliswati65@gmail.com](mailto:Muhsinaliswati65@gmail.com)

<sup>5</sup>Department of Parasitology, University of Agriculture Faisalabad, Pakistan  
Email: [salehatahir999@gmail.com](mailto:salehatahir999@gmail.com)

<sup>6</sup>Quality Control Lab, National Institute of Health, Islamabad, Pakistan  
Email: [mahtabalishah69@gmail.com](mailto:mahtabalishah69@gmail.com)

<sup>7</sup>Doctor of Veterinary Medicine, Riphah College of Veterinary Sciences, Lahore, Pakistan,  
Email: [ramzan7618@gmail.com](mailto:ramzan7618@gmail.com)

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**Corresponding Author: Saleha Tahir**, Department of Parasitology, University of Agriculture Faisalabad, Pakistan  
Email: [salehatahir999@gmail.com](mailto:salehatahir999@gmail.com)

### ABSTRACT

One of the most frequent causes of doctor visits worldwide is gastroenteritis. Acute gastrointestinal disorders are a major medical and financial concern. Such conditions need to be managed more skillfully. There is still much to learn about how to lower the risk of intestinal infections through diet. Antibiotics can effectively inhibit infections, but they cannot be used prophylactically and should not be provided in the absence of sickness. Probiotics, or beneficial bacteria found in the gut, have been shown to have a variety of inhibitory effects on pathogens. Probiotics are currently gaining popularity due to the growing antibiotic resistance of pathogenic bacteria, especially in hospitals, as well as clinical data demonstrating the effectiveness of some probiotic bacteria. One probiotic that is good for human health is *Bacillus clausii*. One of its primary traits is the capacity to proliferate; the ensuing resistance to heat, acid, and salt guarantees an accessible route via the human digestive system without cellular loss. The advantageous qualities of other probiotics, like *Lactobacillus* and *Bifidobacterium* species, are more commonly known throughout the literature, despite the fact that *B. clausii* has been utilized extensively for many years.

## INTRODUCTION

The individual's intestinal microbes is made up of trillions of bacteria that reside in the human gastrointestinal (GI) tract naturally (Erkmen, 2021). This intricacy and variation in microorganisms reflect a complex microbial community (Guseva et al., 2022). The human health and disease are inextricably linked to the gut microbiota because it plays roles in neural, nutritious, defensive, fundamental, and digestive processes (Chaudhry et al., 2023). Many different intestinal and extraintestinal disorders are linked to disruptions in the natural equilibrium of the gut microbiota, commonly referred to as gut dysbiosis (Borrego-Ruiz & Borrego, 2024). Acute gastrointestinal infections rank second in the world's epidemiology of infectious illnesses after respiratory tract infections, and they are one of the main causes of illness and death in young children (Mafokwane et al., 2023). Numerous microorganisms found in food, drink, and hospital environments can lead to intestinal infections by inflaming various parts of the gastrointestinal tract (Rossi et al., 2024). Many factors, including age (children, adults), nation (developing or developed), nosocomial or community acquisition, patients' foreign travel history, and immunological state, influence the incidence of diverse bacteria that cause gastroenteritis. Gastroenteritis can have quite different clinical manifestations, ranging from minor to fatal (Acevedo-Román et al., 2024). Reactions can include nausea, vomiting, cramping in the abdomen, and/or diarrhea, depending on what's causing it (Lim & Rezaie, 2023). A diverse and dynamic microbial community that serves numerous important purposes is present in the intestine (Aggarwal et al., 2023). These include metabolic processes, interactions with the host's immune system, and trophic effects on the intestinal epithelium (Kim et al., 2021). In addition, the indigenous microbiota serves as a barrier to stop the colonization of pathogenic and opportunistic microbes (Chen et al., 2024). Consuming food that contains probiotics has been linked to a growing number of health advantages (Das et al., 2022). A committee of experts has reformulated probiotics as "live microorganisms that, when given in sufficient amounts, confer a health benefit on the host" (Sharifi-Rad et al., 2020). The expert panel also determined that gastrointestinal infections and specific bowel diseases are among the health benefits that can be treated with probiotics (Compare et al., 2022; Jakubczyk et al., 2020). The *Lactobacilli* and *bifidobacteria*, which produce lactic acid, are the microorganisms most frequently employed as probiotics (Ayivi et al., 2020). Both bacterial species belong to the typical microflora, and some of the strains generate lactose along with other antimicrobial compounds including hydrogen peroxide and bacteriocins (Ayivi et al., 2020). It is asserted that probiotics alter the host's immune response by competing with pathogens for microbial adhesion sites. However, it's still unknown exactly how they affect the immune system (Raheem et al., 2021; Zhao et al., 2021). Strains of *Bacillus*, *Saccharomyces*, *Escherichia coli*, and *Streptococcus* are additional less frequently utilized probiotic bacteria (Pereira et al., 2022). The *Bacillus* genus, which includes gram-positive, rod-shaped, aerobic or facultative anaerobic species like *B. coagulans*, *B. subtilis*, *B. licheniformis*, *B. indicus*, and *B. clausii*, is closely linked to the *Lactobacillus* genus (Payne et al., 2024). While *B. clausii* and *Bacillus licheniformis* are not typically thought of as natural inhabitants of the human GI tract, there is evidence that they has the ability to invade the epithelium of the intestinal, obfuscating the line within the transitory microflora and gut inhabitants (Chelliah et al., 2024). These bacteria have been inaccessible from adult, normal human excrement, demonstrating their abilities to endure transit via the GI tract. The spore forming bacteria are part of the genus *Bacillus* can be found in food, soil, water, air, and human digestive tracts. Spore forming bacteria go through a difficult developmental process in severe environments (Gauvry et al., 2017). During this process, a bacterial cell develops into a spore, which can withstand extremes in temperature, pH, light, water, and toxic chemicals for an indefinite period of time. The spores become vegetative cells that can proliferate and multiply when the right environmental circumstances are once

again present. In the manufacturing and preservation of probiotic compositions derived from food or medicine, *Bacillus* spores are more stable than vegetative bacteria because they are metabolically inert, can withstand bile salts, and can withstand the corrosive environment of the GI tract. Probiotics that are able to be spontaneously extracted from the human gut are probably capable of making it through the digestive system (Markowiak & Ślizewska, 2017). The *B. clausii* strains have been utilized in conjunction with antibiotics to lessen the intestinal adverse consequences of using antibiotics because of their innate resistance to antibiotics and the superior constitution of certain probiotic formulations.

#### **Mechanism of action of *B. clausii***

Antimicrobial substances that are secreted, inhibit the number of microorganisms or the metabolic of contaminants, the immune-modulatory effect on the lymphatic tissue affiliated with the intestines, and competitive adhesion to the gut wall, which excludes GI microbes are some of the possible the ways in which bacterial species harbor a probiotic effect (Vitetta et al., 2018).

#### **Antimicrobial activity**

The genera *Bacillus*, *Listeria*, and *Staphylococcus* are members of the order *Bacillales* of Gram-positive bacteria (Zhao & Kuipers, 2016). The *Bacillus* are expected to be a rich source of new antimicrobials, according to genome mining. These antimicrobials consist of five hundred and eighty pairs of bactericide proteins from fifty-seven species, which belong to three types of bacteriocins (Gabrielsen et al., 2014). Among the many antimicrobial compounds produced by *Bacillus* bacteria are lantibiotics, which are peptides that have undergone post-translational modification (Gabrielsen et al., 2014). One important way that probiotics stop harmful bacteria from growing in the gastrointestinal system is by producing antimicrobials like the lantibiotic clausin; this is important from a clinical standpoint when probiotics are used in conjunction with antibiotic therapy (Cesa-Luna et al., 2021). The vegetative cells of *B. clausii* are cultivated in whey and yield antibacterial peptides that suppress the growth of *Enterococcus faecalis*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Ghelardi et al., 2022).

#### **Immunomodulatory activity**

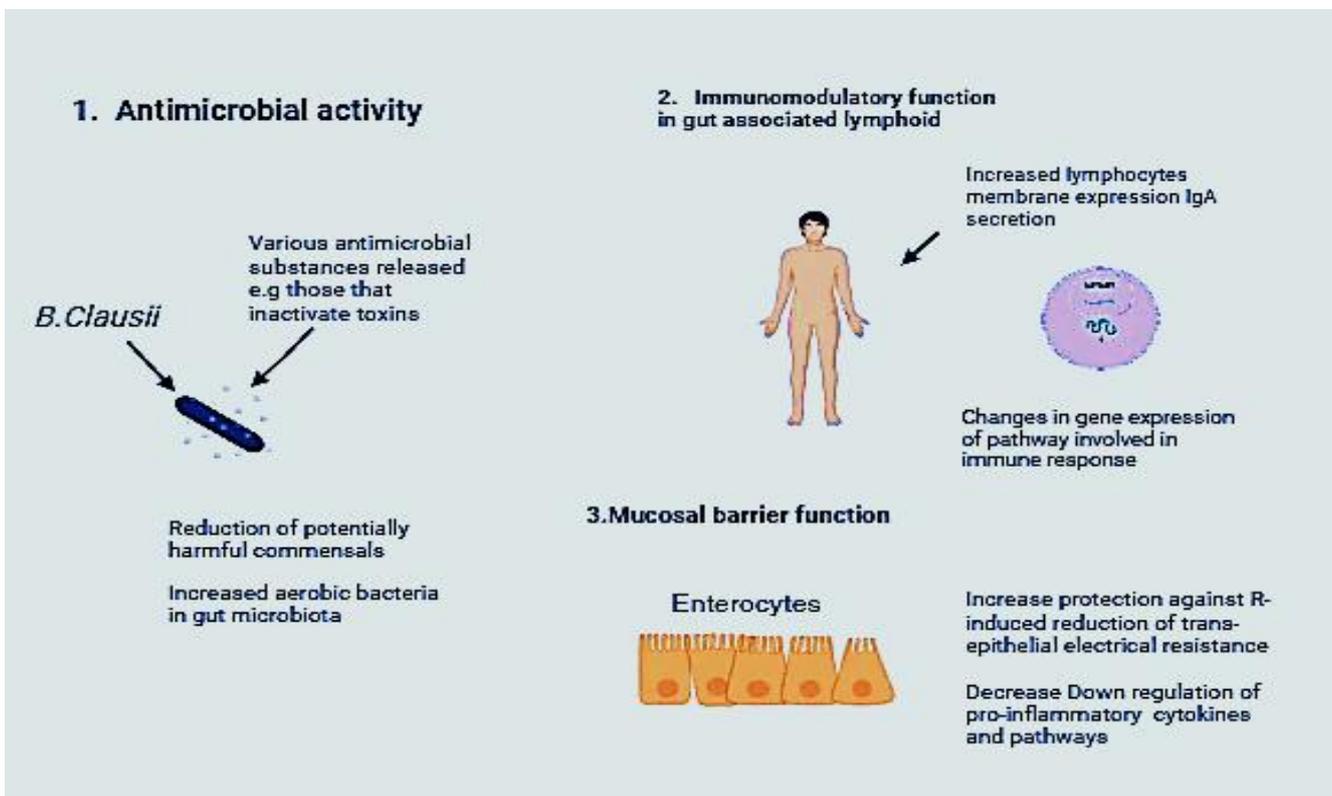
Probiotics have direct impacts on other microbes in the gut due to their antimicrobial activity, and they also have immunomodulatory properties that help the host's immune system rebalance and promote long-term health (Acosta-Rodríguez-Bueno et al., 2022). The subsequent research indicates the powerful immunomodulatory pathways through which probiotics containing *B. clausii* work. Intestinal inflammation is lessened by *B. clausii* by inducing the synthesis of anti-inflammatory cytokines (Lopetuso et al., 2016).

#### **Inhibition of Enteropathogens**

Enteropathogenic infections may be avoided by *B. clausii*. Spores of *B. clausii* strains diminish abrasions, lymphoid infiltration, as well as intestinal wastes while increasing secretion of goblet cells during pretreatment (Plomer et al., 2020). In the lymphatic vessels of the duodenum or spleen, the resulting greater mucosal integrity and elevated level of mucin

have an immune-modulatory and therapeutic impact, as shown in Figure 1 (De Castro et al., 2019).

**Figure 1:** Potential mode of action of *B. clausii*



### Gut immune function of *B. clausii*

In preclinical research employing cell lines, *B. clausii* has been demonstrated to improve the intestinal barrier in addition to *in-vitro* investigations of its functional characteristics which encourages the functioning of the intestinal barriers (Wong-Chew et al., 2024). A recent investigation showed the several ways in which *B. clausii* strains defend the stomach against rotavirus infection. The growth-promoting cells of *B. clausii* (O/C, N/R, SIN, and T) strains increase the production of the antibiotic peptides cathelicidin and humans beta defensive -2 in a human juvenile enterocyte-based rotavirus infectious model (Lippolis et al., 2013). Additionally, the strains restore cell growth that the rotavirus infection had slowed down. In addition to reducing the percentage of necrotic or apoptotic enterocytes, dealing with *B. clausii* strains as well as their residual stimulates enclosed junction protein production and mucin, strengthening the gut barrier's resilience (Kocot et al., 2022).

### Clinical applications of *B. clausii* in gastrointestinal infections

Recent years have seen a strong endorsement of the use of preparation of *B. clausii* in the management or avoidance of impairments in gut physiology, which has fueled these preparations' therapeutic applications. Practical uses and conjectures on potential relationships between *B. clausii*, the intestinal wall, and the immune system, as well as the effects of these relationships on alterations in human health (Marzorati et al., 2021).

### Acute diarrhea

Acute diarrhea continues to be a major global health concern and a common cause of mortality, particularly in developing nations. The standard treatment for this is to use oral rehydration solution, intravenous fluid when necessary, and zinc supplementation in accordance with WHO guidelines (Ugboko et al., 2020). Instead of stopping the disease's course, this treatment aims to reduce the complications that lead to diarrheal deaths. Studies

are currently being conducted in both developed and developing nations to assess the potential benefits of utilizing probiotics as an adjuvant therapy in conjunction with current anti-diarrheal medications. This concept has been around for decades. Currently, *B. clausii* is recommended for the therapy of changes in intestinal bacterial flora in both adults and children. The *B. clausii* has been demonstrated to lower stool frequency and rotavirus/adenovirus excretion in children with acute diarrhea (Kesavelu & Jog, 2023).

### Irritable Bowel Syndrome

A lower quality of life, stress, worry, sadness, and emotional issues are associated with irritable bowel syndrome, which also causes absenteeism. IBS is therefore regarded as a social and health burden. Probiotics such as *B. clausii* have been shown to be advantageous in the management of several gastrointestinal infections, and the effectiveness of current medications for irritable bowel syndrome (IBS) is limited (Vázquez-Frias et al., 2023).

### Small Intestinal Bacterial Overgrowth

An increase in the small intestine's bacterial content over normal levels is known as small intestinal bacterial overgrowth, or SIBO (Takakura & Pimentel, 2020). This causes bloating, pain in the umbilical region, and excessive gas generation in the small intestine. It can also cause malnutrition, osmotic diarrhea, and malabsorption. Even after receiving continuous antibiotic therapy, *B. clausii* can colonize the gut (Acosta-Rodríguez-Bueno et al., 2022). The probiotics of *B. clausii* have been used safely and effectively in individuals throughout a few decades, as indicated by Table 1, besides preliminary research that suggest a range of routes of action.

**Table 1.** Clinical benefits of *B. clausii* treatment

Diseases	Efficacy	References
Acute pediatric diarrhea	Reduced duration of diarrhea	(Chelliah et al., 2024)
Rotavirus infection	Reduction in weakness, swelling, abdominal pain, and fever	(Paparo et al., 2020)
Upper respiratory infection	Fewer and shorter duration of infection	(Amaral et al., 2017)
Allergic rhinitis	Reduced pro-inflammatory cytokines	(May & Dolen, 2017)
Endotoxemia	Reduced serum endotoxin and pro-inflammatory cytokines	(Jatzlauk et al., 2017)

### Compositional quality and safety assessment of *B. clausii* as a probiotic

Probiotics have become incredibly popular in recent times, but with their growing use comes controversy over how best to regulate them and whether they belong in the category of medical foods, drugs, or supplements. Probiotics are live bacteria that give the host health benefits when given in sufficient doses. The majority of bacterial spore formers in the genus *Bacillus* make up a significant probiotic product used today (El Hage et al., 2017). For them to be classified as probiotics, they must meet the necessary criteria for content, stability, and health benefits. Since bacteria are so common, they regularly enter healthy people's gastrointestinal and respiratory systems through food, water, and the air. The capacity to be

kept forever in a desiccated state without negatively affecting viability is one benefit that *Bacillus* strains have over the more popular *Lactobacillus* products. Furthermore, they are able to withstand the stomach barrier's low pH. Through immunomodulation, or the activation of cytokines, competitive exclusion of gastrointestinal pathogens through competition for adhesion sites, and secretion of antimicrobial substances, *Bacillus* species achieve their therapeutic effects. Probiotics are being produced and used more often than ever before (Berninger et al., 2018). To establish safety, toxicology tests must be conducted because probiotics are strain-specific. Despite being generally recognized as safe (GRAS), probiotics safety should not be taken for granted, and each product should be assessed individually. In order to establish safety, each possible strain must have its acute and repeated dose toxicity assessed. A Chinese study on a *Bacillus cereus* strain found that probiotics can cause food poisoning and other food-borne infectious diseases if proper safety evaluation isn't done. It is imperative to carefully adhere to the standards established by the FAO/WHO report when using a product as a probiotic or novel supplement (Gauvry et al., 2017).

#### Comparison of *B. clausii* with other probiotics

Though the specific type of probiotic to use is unknown, it has been suggested as a supplement to the usual course of treatment for acute diarrhea and gastrointestinal infections. The comparison of *B. clausii* with other probiotics are given in Table 2.

**Table 2.** comparison of *B. clausii* with other probiotics

Characteristics	<i>B. clausii</i>	<i>Lactobacillus</i>	<i>Bifidobacterium</i>	<i>Saccharomyces</i>	References
Spore forming ability	Yes	No	No	Yes	(Cai et al., 2018)
Resistance to heat	High	Moderate	Moderate	Low	(Kapse et al., 2018)
Antibiotic tolerance	High	Low-moderate	Moderate	High	(Cai et al., 2018)
Gastric acid resistance	High	Low	Low	High	(Kapse et al., 2018)
Survivability in bile salt	High	Low-moderate	Low-moderate	Low	(Cai et al., 2018)

#### Biochemical and physiological properties of *B. clausii*

Probiotics are utilized in animal feeds to avoid gastrointestinal illnesses and are being produced commercially as novel meals or dietary supplements for human usage. The product's economic viability is indicated by the market demand. The *Bacillus* spores are capable of germination and vegetative proliferation in the gastrointestinal tract of humans. Research has demonstrated that a spore mixture of *B. clausii*, which consists of four strains (O/C, SIN, N/R, and T), is resistant to a broad spectrum of antibiotics and is sold as an over-

the-counter (OTC) therapeutic supplement (Pereira et al., 2022). Specific levels of resistance to tetracycline, streptomycin, chloramphenicol, and rifampicin set these strains apart phenotypically. They were recently characterized at the bioenergetic and proteomic levels. Commercially accessible probiotics are widely utilized in clinical practice to minimize intestinal microbial imbalance during antibiotic therapy since the microorganisms in them have been proven to frequently carry some antibiotic resistance. However, the apparent advantage is at odds with the safety standards, which stipulate that bacteria intended for human consumption should not harbor any genes that facilitate the transfer of antibiotic resistance in order to reduce the worry about this resistance spreading from probiotics to pathogens in the gastrointestinal tract (Zhang et al., 2023). The goods formed from spores can be stored at room temperature without compromising their vitality because they are heat-stable and able to withstand low pH. The spore-bearing *Bacillus* species are a diverse group of microorganisms that are currently being studied to get further insight into the biochemistry, physiology, and, most importantly, molecular genetics that underlie bacterial behavior (Rajashekhar et al., 2017).

### **Limitations of research of *B. clausii***

Probiotics have been shown to have a number of positive effects on health, including vitamin synthesis, preventing the colonization of pathogenic bacteria, secreting bacteriocins and other antibacterial substances to sabotage other bacteria, stimulating the production of secretory IgA antibodies that sabotage other pathogens, and more. These advantages make them a viable treatment option for patients undergoing surgery, colitis linked to antibiotics, and diarrhea in both adults and children. Even while probiotics have many positive effects, the risks and disadvantages associated with them are frequently overlooked (Sánchez et al., 2017). Many probiotic bacteria, including *B. clausii*, have been linked to sepsis caused by *B. clausii* in both immunocompromised people and newborns, according to reports that have arisen in recent months. Thus, it is possible for beneficial bacteria to become pathogenic, for them to possess genes that make them resistant to drugs, or for them to resist treatment and need intensive antibiotic therapy (Swartwout & Luo, 2018). Probiotics contain bacterial strains that have the potential to develop into pathogens by becoming pathogenic. In-depth study on the subject is necessary because the mechanism behind virulence is still unclear, particularly in healthy individuals. Probiotics like *B. clausii* might be harmless, but it is highly dubious to use them in people who are immunocompromised or chronically sick. Its potential to transmigrate from the stomach into the bloodstream is the cause of this therapeutic conundrum. This case report should serve as a reminder to utilize this probiotic sparingly, especially in healthy individuals (Happel et al., 2018). We conclude by saying that while probiotics containing *B. clausii* spores might sometimes be regarded useful, their usage should be approached cautiously when dealing with severely ill patients.

### **Future perspective**

Numerous studies have been conducted as a result of the realization that the intricate communities of microorganisms that live in the human body are vital to the host's health. The metabolic tasks that this community performs are one of the main roles that have been assigned to the gut microbiota. Probiotics are commonly used to prevent and cure GI disorders, but there are still a lot of problems that need to be worked out. Immune system correlation and microbiota regulation produced by *B. clausii*. The *B. clausii* may interact directly or indirectly, maybe by influencing companion microbes, to alter the body's response to infection (Elshagabee et al., 2017). Comprehending these distinct immunological characteristics may expand its therapeutic uses, permitting its application in particular pathological circumstances based on the illness stage and the host's immune status. By employing this tactic, *B. clausii* demonstrated in a human experimental setting a definite capacity to regulate the immune system while treating acute diarrhea. Future research will be

required to better determine each strain's mechanisms of action, the best dosage, the best length of treatment, the best combination of strains that are now available, and ultimately the safety and stability of each strain. Immune system correlation and microbiota modification induced by *B. clausii* (Compare et al., 2022).

### Conclusion

The immune system, vascular and lymphatic routes, GI propria lamina, duodenal mucous membrane, gut microbiota, or collagen, neuroenteric and enteroendocrine systems, and immune system make up the complex system known as the intestinal barrier, which is in charge of maintaining the health of the host. Damage to the gut barrier is linked to numerous GI and extra-GI illnesses. Assuming these conditions are met, an ideal probiotic should be able to modulate intestinal permeability, enhance mucus production in terms of both quantity and composition, restore biodiversity within the microbiota, and facilitate equally anti-inflammatory as well as anti-fibrotic effects. Spores of *B. clausii* can withstand the gastrointestinal microenvironment as well as make it to the intestine, in which they could sprout and become vegetative. The effectiveness of probiotics in lowering the length and intensity of acute diarrhea in children, as well as the safety and effectiveness of *B. clausii* in reducing the frequency and duration of acute diarrhea, are consolidated in these recommendations. In order to understand its modes of action and explore any potential function in the extraintestinal and gastrointestinal disorders (e.g., Th-1 inducer), upcoming, organized clinical studies as well as linguistic research are required, ultimately leading to the optimization of its clinical use.

### References

- Acevedo-Román, A., Pagán-Zayas, N., Velázquez-Rivera, L. I., Torres-Ventura, A. C., & Godoy-Vitorino, F. (2024). Insights into Gut Dysbiosis: Inflammatory Diseases, Obesity, and Restoration Approaches. *International Journal of Molecular Sciences*, 25(17). <https://doi.org/10.3390/ijms25179715>
- Acosta-Rodríguez-Bueno, C. P., Abreu y Abreu, A. T., Guarner, F., Guno, M. J. V., Pehlivanoglu, E., & Perez, M. (2022). *Bacillus clausii* for Gastrointestinal Disorders: A Narrative Literature Review. *Advances in Therapy*, 39(11), 4854–4874. <https://doi.org/10.1007/s12325-022-02285-0>
- Aggarwal, N., Kitano, S., Pua, G. R. Y., Kittelmann, S., Hwang, I. Y., & Chang, M. W. (2023). Microbiome and Human Health: Current Understanding, Engineering, and Enabling Technologies. *Chemical Reviews*, 123(1), 31–72. <https://doi.org/10.1021/acs.chemrev.2c00431>
- Amaral, M. A., Guedes, G. H. B. F., Epifanio, M., Wagner, M. B., Jones, M. H., & Mattiello, R. (2017). Network meta-analysis of probiotics to prevent respiratory infections in children and adolescents. *Pediatric Pulmonology*, 52(6), 833–843. <https://doi.org/10.1002/ppul.23643>
- Ayivi, R. D., Gyawali, R., Krastanov, A., Aljaloud, S. O., Worku, M., Tahergorabi, R., Silva, R. C. da, & Ibrahim, S. A. (2020). Lactic Acid Bacteria: Food Safety and Human Health Applications. *Dairy*, 1(3), 202–232. <https://doi.org/10.3390/dairy1030015>
- Berninger, T., González López, Ó., Bejarano, A., Preininger, C., & Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. *Microbial Biotechnology*, 11(2), 277–301. <https://doi.org/10.1111/1751-7915.12880>
- Borrego-Ruiz, A., & Borrego, J. J. (2024). An updated overview on the relationship between human gut microbiome dysbiosis and psychiatric and psychological disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 128(July 2023), 110861. <https://doi.org/10.1016/j.pnpbp.2023.110861>
- Cai, J., Zhao, C., Du, Y., Zhang, Y., Zhao, M., & Zhao, Q. (2018). Comparative efficacy and tolerability of probiotics for antibiotic-associated diarrhea: Systematic review with network

meta-analysis. *United European Gastroenterology Journal*, 6(2), 169–180. <https://doi.org/10.1177/2050640617736987>

Cesa-Luna, C., Alatorre-Cruz, J. M., Carreño-López, R., Quintero-Hernández, V., & Baez, A. (2021). Emerging applications of bacteriocins as antimicrobials, anticancer drugs, and modulators of the gastrointestinal microbiota. *Polish Journal of Microbiology*, 70(2), 143–159. <https://doi.org/10.33073/PJM-2021-020>

Chaudhry, T. S., Senapati, S. G., Gadam, S., Mannam, H. P. S. S., Voruganti, H. V., Abbasi, Z., Abhinav, T., Challa, A. B., Pallipamu, N., Bheemisetty, N., & Arunachalam, S. P. (2023). The Impact of Microbiota on the Gut–Brain Axis: Examining the Complex Interplay and Implications. *Journal of Clinical Medicine*, 12(16), 1–20. <https://doi.org/10.3390/jcm12165231>

Chelliah, R., Hyeon, N., Rubab, M., & Yeon, S. (2024). Robust and safe : Unveiling *Bacillus clausii* OHRC1 ' s potential as a versatile probiotic for enhanced food quality and safety. *LWT*, 203(May), 116291. <https://doi.org/10.1016/j.lwt.2024.116291>

Chen, Y., Xiao, L., Zhou, M., & Zhang, H. (2024). The microbiota: a crucial mediator in gut homeostasis and colonization resistance. *Frontiers in Microbiology*, 15(August), 1–15. <https://doi.org/10.3389/fmicb.2024.1417864>

Compare, D., Sgamato, C., Nardone, O. M., Rocco, A., Coccoli, P., Laurenza, C., & Nardone, G. (2022). Probiotics in Gastrointestinal Diseases: All that Glitters Is Not Gold. *Digestive Diseases*, 40(1), 123–132. <https://doi.org/10.1159/000516023>

Das, T. K., Pradhan, S., Chakrabarti, S., Chandra, K., & Ghosh, K. (2022). Current status of probiotic and related health benefits. *Applied Food Research*, 2(2), 100185. <https://doi.org/10.1016/j.afres.2022.100185>

De Castro, J. A. A., Guno, M. J. V. R., & Perez, M. O. (2019). *Bacillus clausii* as adjunctive treatment for acute community-acquired diarrhea among Filipino children: A large-scale, multicenter, open-label study (CODDLE). *Tropical Diseases, Travel Medicine and Vaccines*, 5(1), 1–9. <https://doi.org/10.1186/s40794-019-0089-5>

El Hage, R., Hernandez-Sanabria, E., & Van de Wiele, T. (2017). Emerging trends in “smart probiotics”: Functional consideration for the development of novel health and industrial applications. *Frontiers in Microbiology*, 8(SEP), 1–11. <https://doi.org/10.3389/fmicb.2017.01889>

Elshagabee, F. M. F., Rokana, N., Gulhane, R. D., Sharma, C., & Panwar, H. (2017). *Bacillus* as potential probiotics: Status, concerns, and future perspectives. *Frontiers in Microbiology*, 8(AUG), 1–15. <https://doi.org/10.3389/fmicb.2017.01490>

Erkmen, O. (2021). *24U ) Beneficial microbiota of gastrointestinal tract. April 2018.*

Gabrielsen, C., Brede, D. A., Nes, I. F., & Diep, D. B. (2014). Circular bacteriocins: Biosynthesis and mode of action. *Applied and Environmental Microbiology*, 80(22), 6854–6862. <https://doi.org/10.1128/AEM.02284-14>

Gauvry, E., Mathot, A. G., Leguérinel, I., Couvert, O., Postollec, F., Broussolle, V., & Coroller, L. (2017). Knowledge of the physiology of spore-forming bacteria can explain the origin of spores in the food environment. *Research in Microbiology*, 168(4), 369–378. <https://doi.org/10.1016/j.resmic.2016.10.006>

Ghelardi, E., Abreu Y Abreu, A. T., Marzet, C. B., Calatayud, G. Á., Perez, M. I. I. I., & Castro, A. P. M. (2022). Current Progress and Future Perspectives on the Use of *Bacillus clausii*. *Microorganisms*, 10(6), 1–16. <https://doi.org/10.3390/microorganisms10061246>

Guseva, K., Darcy, S., Simon, E., Alteio, L. V., Montesinos-Navarro, A., & Kaiser, C. (2022). From diversity to complexity: Microbial networks in soils. *Soil Biology and Biochemistry*, 169(March), 108604. <https://doi.org/10.1016/j.soilbio.2022.108604>

Happel, A. U., Barnabas, S. L., Froissart, R., & Passmore, J. S. (2018). Weighing in on the risks and benefits of probiotic use in HIV-infected and immunocompromised populations.

- Beneficial Microbes*, 9(2), 239–246. <https://doi.org/10.3920/BM2017.0106>
- Jakubczyk, D., Leszczyńska, K., & Górská, S. (2020). The effectiveness of probiotics in the treatment of inflammatory bowel disease (Ibd)— a critical review. *Nutrients*, 12(7). <https://doi.org/10.3390/nu12071973>
- Jatzlauk, G., Bartel, S., Heine, H., Schloter, M., & Krauss-Etschmann, S. (2017). Influences of environmental bacteria and their metabolites on allergies, asthma, and host microbiota. *Allergy: European Journal of Allergy and Clinical Immunology*, 72(12), 1859–1867. <https://doi.org/10.1111/all.13220>
- Kapse, N. G., Engineer, A. S., Gowdaman, V., Wagh, S., & Dhakephalkar, P. K. (2018). Genome profiling for health promoting and disease preventing traits unraveled probiotic potential of bacillus clausii B106. *Microbiology and Biotechnology Letters*, 46(4), 334–345. <https://doi.org/10.4014/mbl.1804.04001>
- Kesavelu, D., & Jog, P. (2023). Current understanding of antibiotic-associated dysbiosis and approaches for its management. *Therapeutic Advances in Infectious Disease*, 10, 1–18. <https://doi.org/10.1177/20499361231154443>
- Kim, E. T., Lee, S. J., Kim, T. Y., Lee, H. G., Atikur, R. M., Gu, B. H., Kim, D. H., Park, B. Y., Son, J. K., & Kim, M. (2021). Dynamic changes in fecal microbial communities of neonatal dairy calves by aging and diarrhea. *Animals*, 11(4). <https://doi.org/10.3390/ani11041113>
- Kocot, A. M., Jarocka-Cyrta, E., & Drabińska, N. (2022). Overview of the Importance of Biotics in Gut Barrier Integrity. *International Journal of Molecular Sciences*, 23(5), 1–32. <https://doi.org/10.3390/ijms23052896>
- Lim, J., & Rezaie, A. (2023). Irritable Bowel Syndrome - Like Symptoms in Quiescent Inflammatory Bowel Disease : A Practical Approach to Diagnosis and Treatment of Organic Causes. *Digestive Diseases and Sciences*, 68(11), 4081–4097. <https://doi.org/10.1007/s10620-023-08095-w>
- Lippolis, R., Siciliano, R. A., Mazzeo, M. F., Abbrescia, A., Gnoni, A., Sardanelli, A. M., & Papa, S. (2013). Comparative secretome analysis of four isogenic Bacillus clausii probiotic strains. *Proteome Science*, 11(1). <https://doi.org/10.1186/1477-5956-11-28>
- Lopetuso, L. R., Scaldaferri, F., Franceschi, F., & Gasbarrini, A. (2016). Bacillus clausii and gut homeostasis: state of the art and future perspectives. *Expert Review of Gastroenterology and Hepatology*, 10(8), 943–948. <https://doi.org/10.1080/17474124.2016.1200465>
- Mafokwane, T., Djikeng, A., Nesengani, L. T., Dewar, J., & Mapholi, O. (2023). Gastrointestinal Infection in South African Children under the Age of 5 years: A Mini Review. *Gastroenterology Research and Practice*, 2023. <https://doi.org/10.1155/2023/1906782>
- Markowiak, P., & Ślizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9(9). <https://doi.org/10.3390/nu9091021>
- Marzorati, M., Van den Abbeele, P., Bubeck, S., Bayne, T., Krishnan, K., & Young, A. (2021). Treatment with a spore-based probiotic containing five strains of Bacillus induced changes in the metabolic activity and community composition of the gut microbiota in a SHIME® model of the human gastrointestinal system. *Food Research International*, 149, 110676. <https://doi.org/10.1016/j.foodres.2021.110676>
- May, J. R., & Dolen, W. K. (2017). Management of Allergic Rhinitis: A Review for the Community Pharmacist. *Clinical Therapeutics*, 39(12), 2410–2419. <https://doi.org/10.1016/j.clinthera.2017.10.006>
- Paparo, L., Tripodi, L., Bruno, C., Pisapia, L., Damiano, C., Pastore, L., & Berni Canani, R. (2020). Protective action of Bacillus clausii probiotic strains in an in vitro model of Rotavirus infection. *Scientific Reports*, 10(1), 1–10. <https://doi.org/10.1038/s41598-020-69533-7>
- Payne, J., Bellmer, D., Jadeja, R., & Muriana, P. (2024). The Potential of Bacillus Species as

- Probiotics in the Food Industry: A Review. *Foods*, 13(15), 1–23. <https://doi.org/10.3390/foods13152444>
- Pereira, W. A., Mendonça, C. M. N., Urquiza, A. V., Marteinsson, V. Þ., LeBlanc, J. G., Cotter, P. D., Villalobos, E. F., Romero, J., & Oliveira, R. P. S. (2022). Use of Probiotic Bacteria and Bacteriocins as an Alternative to Antibiotics in Aquaculture. *Microorganisms*, 10(9). <https://doi.org/10.3390/microorganisms10091705>
- Plomer, M., Perez, M., & Greifengberg, D. M. (2020). Effect of *Bacillus clausii* Capsules in Reducing Adverse Effects Associated with *Helicobacter pylori* Eradication Therapy: A Randomized, Double-Blind, Controlled Trial. *Infectious Diseases and Therapy*, 9(4), 867–878. <https://doi.org/10.1007/s40121-020-00333-2>
- Raheem, A., Liang, L., Zhang, G., & Cui, S. (2021). Modulatory Effects of Probiotics During Pathogenic Infections With Emphasis on Immune Regulation. *Frontiers in Immunology*, 12(April), 1–32. <https://doi.org/10.3389/fimmu.2021.616713>
- Rajashekhar, M., Shahanaz, & Kalia, V. K. (2017). Biochemical and molecular characterization of *Bacillus* spp. isolated from insects. *Journal of Entomology and Zoology Studies*, 5(5), 581–588. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
- Rossi, F., Santonicola, S., Amadoro, C., Marino, L., & Colavita, G. (2024). Recent Records on Bacterial Opportunistic Infections via the Dietary Route. *Microorganisms*, 12(1), 1–27. <https://doi.org/10.3390/microorganisms12010069>
- Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., & Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition and Food Research*, 61(1), 1–15. <https://doi.org/10.1002/mnfr.201600240>
- Sharifi-Rad, J., Rodrigues, C. F., Stojanović-Radić, Z., Dimitrijević, M., Aleksić, A., Neffe-Skocińska, K., Zielińska, D., Kołożyn-Krajewska, D., Salehi, B., Prabu, S. M., Schutz, F., Docea, A. O., Martins, N., & Calina, D. (2020). Probiotics: Versatile bioactive components in promoting human health. *Medicina (Lithuania)*, 56(9), 1–30. <https://doi.org/10.3390/medicina56090433>
- Swartwout, B., & Luo, X. M. (2018). Implications of probiotics on the maternal-neonatal interface: gut microbiota, immunomodulation, and autoimmunity. *Frontiers in Immunology*, 9(December), 1–14. <https://doi.org/10.3389/fimmu.2018.02840>
- Takakura, W., & Pimentel, M. (2020). Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome – An Update. *Frontiers in Psychiatry*, 11(July), 1–9. <https://doi.org/10.3389/fpsy.2020.00664>
- Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., & Oyewale, J. O. (2020). Childhood diarrhoeal diseases in developing countries. *Heliyon*, 6(4), e03690. <https://doi.org/10.1016/j.heliyon.2020.e03690>
- Vázquez-Frias, R., Consuelo-Sánchez, A., Acosta-Rodríguez-Bueno, C. P., Blanco-Montero, A., Robles, D. C., Cohen, V., Márquez, D., & Perez, M. (2023). Efficacy and Safety of the Adjuvant Use of Probiotic *Bacillus clausii* Strains in Pediatric Irritable Bowel Syndrome: A Randomized, Double-Blind, Placebo-Controlled Study. *Pediatric Drugs*, 25(1), 115–126. <https://doi.org/10.1007/s40272-022-00536-9>
- Vitetta, L., Vitetta, G., & Hall, S. (2018). Immunological tolerance and function: Associations between intestinal bacteria, probiotics, prebiotics, and phages. *Frontiers in Immunology*, 9(OCT), 1–15. <https://doi.org/10.3389/fimmu.2018.02240>
- Wong-Chew, R. M., Nguyen, T. V. H., Rogacion, J. M., Herve, M., & Pouteau, E. (2024). Potential Complementary Effect of Zinc and *Alkalihalobacillus clausii* on Gut Health and Immunity: A Narrative Review. *Nutrients*, 16(6). <https://doi.org/10.3390/nu16060887>
- Zhang, L., Liu, Y., Wang, X., & Zhang, X. (2023). Physical Exercise and Diet: Regulation of Gut Microbiota to Prevent and Treat Metabolic Disorders to Maintain Health. *Nutrients*, 15(6), 1–16. <https://doi.org/10.3390/nu15061539>

Zhao, X., & Kuipers, O. P. (2016). Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics*, *17*(1), 1–18. <https://doi.org/10.1186/s12864-016-3224-y>

Zhao, Y., Zeng, Y., Zeng, D., Wang, H., Zhou, M., Sun, N., Xin, J., Khalique, A., Rajput, D. S., Pan, K., Shu, G., Jing, B., & Ni, X. (2021). Probiotics and MicroRNA: Their Roles in the Host–Microbe Interactions. *Frontiers in Microbiology*, *11*(January), 1–12. <https://doi.org/10.3389/fmicb.2020.604462>