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The anthelmintic effects of probiotics against *Giardia intestinalis* infection: A narrative review

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Abstract

Giardia intestinalis, also known as *Giardia lamblia* or *Giardia duodenale*, is a parasite causing giardiasis, a diarrheal disease, infecting over 1 million people annually. It spreads through contaminated water or food, primarily via fecal-oral transmission. Diagnosis relies on stool examination, and treatment involves antibiotics like Metronidazole or Nitazoxanide. However, these medications may induce dangerous side effects. Probiotics, beneficial microbes with health benefits, offer an alternative approach to treating giardiasis, showing promising results in recent studies. A narrative review was employed for the study. The PRISMA method was used as an assessment tool, whereas the PICO tool was used to answer questions for this review. Investigating probiotics' potential to combat *Giardia intestinalis* infection reveals promising strategies for addressing parasitic diseases through symbiotic approaches. Understanding the mechanisms behind probiotics' anthelmintic effects is crucial for establishing their protective role in the host. This involves colonization and recovery of damaged intestinal mucosa, exclusion of pathogens, bacteriocin production, and modification of enzymatic activities, which act synergistically to regulate the normal function of tissues. The exploration of probiotics as a potential strategy for combating *Giardia intestinalis* infection has revealed promising anthelmintic effects with significant implications for preventive and therapeutic approaches to managing this prevalent gastrointestinal parasite.

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Introduction

Giardia intestinalis, traditionally known as *Giardia lamblia* or *Giardia duodenale*, commonly known as Giardia, is a microscopic parasitic organism that can cause infection in the gastrointestinal tract of humans and other mammals, causing a condition of diarrheal disease giardiasis. According to the Centers for Disease Control and Prevention (2021), Giardia is the most common intestinal parasitic disease in the United States, affecting more than 1 million people annually. It can be found on the stool of animals infected with giardia and contaminate anything it contacts. The major cause of giardiasis is ingesting contaminated water with infective cysts. Other modes of transmission include eating contaminated fruits or vegetables or via the fecal-oral route (Zeibig, 1997).

Treatment and identification of *Giardia intestinalis* pose significant challenges due to several interconnected factors. According to Mayo Clinic (2022) diagnosing Giardia infection (Giardiasis) relies on examining human feces or stool samples. Asymptomatic individuals generally do not require treatment unless they are likely to spread the parasite, but with the presence of signs and symptoms or the infection persists, doctors usually recommend such medications like Metronidazole which is the most common antibiotic for giardia infection, with a side effects of nausea and metallic taste in the mouth, strictly prohibited in drinking alcohol when taking this medicine. Nitazoxanide, another giardiasis medication, comes in a liquid form and is accessible for children and side effects may come with nausea, gas, and bright yellow urine. This medication, when taken, may impact the patient's well-being, prompting to explore probiotic treatments for giardia infection as a gentler alternative.

Recent studies have shown the significant effects of probiotics, referred to as beneficial microbes, for their prophylactic and therapeutic implications antagonistic to several diseases including Giardia. Food and Agriculture Organization of the United Nations and the World Health Organization (2006) defined probiotics as "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (p. 3). This suggests probiotics exert desirable effects when administered within the recommended dosage regimens and duration. The use of probiotics as health supplements is widely adopted. In line with this, the present review aims to summarize recent findings and elucidate the efficacy of different probiotic strains against infection with *Giardia intestinalis* and their underlying mechanisms of action. Understanding these interactions could potentially lead to new strategies for preventing and managing giardiasis.

Material and methods

A narrative review was employed for the study. The set of points that will be used was standardized by organizations of health sciences to demonstrate a quality review by assessing strengths and weaknesses (Systematic Review, 2023) and limiting bias (Page, 2021) to conclude from accurate information. Literature for review will be acquired from reliable online databases. The journals were limited by the publication date, from 2013 to 2023, and included experimental studies from reputable journals.

Search methodology

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is the assessment tool used to conduct this narrative review. The PRISMA method consists of a 27-item checklist addressing the specific contents of the articles and a 4-phase flow diagram describing the flow of the review process, from record identification, inclusion, and exclusion, and the reasons for exclusion (Liberati *et al.*, 2009). The PICO tool was utilized to answer the questions for this review, in which population (P) referred to the host used; intervention (I) involves the probiotic strain administered; Comparison (C) relates to the control groups or host without intervention, and outcome (O) discussing the antiparasitic effect of probiotics against *Giardia intestinalis* infection relating to the mechanisms by which they exert these effects. The study did not acquire confidential data such as patients' personal information, and no experiments were conducted on animals or any living

organisms. Previous literatures were utilized in the review. Thus, no safety risk for and from biological components will be involved.

The preliminary search on the collection of literature and studies were obtained from electronic databases, primarily Google Scholar, PubMed, Science Direct, and other academic journals. A variety of keywords are used, including "probiotics", "anthelmintic or antiparasitic effects", *"Giardia intestinalis* or *lamblia* or *duodenale* infection" and "giardiasis". The researchers collated and arranged the selected studies in Google Drive, which were then imported into Google Docs to record the research findings based on publication year, the specific probiotic strains, and notable results, mainly the mechanism of actions and antiparasitic effect of the probiotics.

Eligibility criteria

A total of 13 studies were acquired for this review. Fig. 1 illustrates the schematic diagram of the study selection process based on the PRISMA 4-phase flow diagram. The materials included in this review were chosen considering whether they satisfy the following criteria:

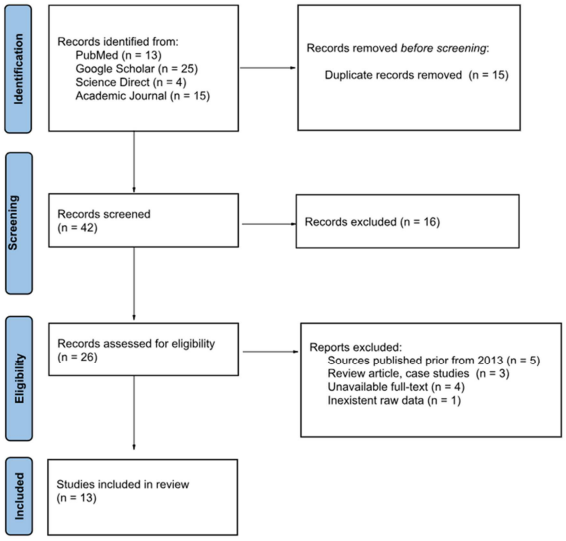

Fig. 1. Schematic diagram of the study selection process

Inclusion criteria

The studies must have been published in English within the past ten years upon submission of the review paper to ensure that the documentation materials were from recent findings and to yield significant articles of relevant impact. The chosen studies must be conducted experimentally to examine the anthelminthic effects of different strains of probiotics against *Giardia intestinalis* infection. It should note the specific probiotic strain and the mechanism by which probiotics exert their antiparasitic effects. The studies chosen must specify rats as their animal model to establish consistency and clearly understand the intended outcome. Studies must have an available full-text and be acquired from reputable, peer-reviewed journals to guarantee reliability.

Exclusion criteria

This review discusses the anthelmintic effects of probiotics against *Giardia intestinalis* infection. Therefore, journals that examined parasites of different genera or species were excluded. Articles without an available full-text and those that do not contain raw data were omitted. Also, review and editorial articles were excluded from this review.

Screening and data extraction

The screening process began with reading the title and abstract of each article. All articles that satisfied the eligibility criteria were collated in a literature matrix designed by the researchers and were subjected to full-text evaluation. Those that answered the review questions according to PICO were selected. Another full reading of the included articles was performed for data extraction. The data were typed into a spreadsheet to create an organized database.

Results and Discussion

Medication for parasitic infection often relies on anthelmintic drugs. However, challenges such as suboptimal efficacy and drug resistance persist rapidly. As an alternative, recent studies show promising effects of probiotics for their therapeutic and prophylactic implications against parasitic infections, such as giardiasis. It is important to collate findings on the anthelmintic effects of probiotics and the mechanism of actions they incurred.

Table 1. Major findings on the mechanisms and antiparasitic effect of probiotics against infection with *Giardia intestinalis*

References	Host	Probiotic strain	Mechanism	Antiparasitic effect
Al-Khaliq, 2019	Mice	*Bifidobacteria*	Conversion of naive T-cell differentiation to Th1, Th2, and T-regulatory lymphocyte and IgA production through expression of IL-10 and IL-6.	Reduced shedding of parasites in feces and normal stratified appearance of intestinal villi was observed.
Allain *et al.*, 2018	Mice	*L. curvatus, L. gasseri,* and *L. johnsonii*	Expression of bile salt hydrolase genes.	93% and 43% trophozoite reduction in treatment with *L. gasseri* and *L. johnsonii,* respectively. However, no protection was observed in treatment with *L. curvatus*.
Al-Megrin *et al.*, 2021	Mice	*Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Lactobacillus helveticus*	Enhanced IgA production through cytokine alteration in the gut mucosa and expression of TGFβ, IL-10, and IL-6.	87.5% cure rate. No alteration in the shape of the villi, with increased infiltration in the central pulp, and disappearance of trophozoites.
Amer *et al.*, 2014	Mice	*L. acidophilus* and *L. plantarum*	Bacteriocins inhibit colonization and multiplication of *G. intestinalis* trophozoite by nucleic acid degradation of microorganisms.	81.63% and 31.38% reduction in larval count, respectively.
De Freitas *et al.*, 2019	Gerbils	*B. longum* *W. paramesenteroides*	*B. longum* induced an increase in mucus production that act as a deprivation factor of *Giardia lamblia* trophozoite.	70% and 71% reduction in trophozoite adhered to the intestinal villi when treated with *B. longum* and *W. paramesenteroides,* respectively.
Goyal *et al.*, 2013	Mice	*Lactobacillus rhamnosus GG*	Release of intracellular antioxidative constituents and prevent adherence of *G. intestinalis* trophozoites to intestinal cells.	Increased intestinal disaccharides such as secrase and lactase, reduced glutathione, and decreased lipid peroxidation. Normal cellular morphology of the jejunal villous enterocytes.
Ribeiro *et al.*, 2018	Gerbils	*Saccharomyces boulardii*	Increase mucus production and intraepithelial lymphocytes.	73% parasite reduction.
Sanad *et al.*, 2020	Mice	*Acidophilus* capsule containing *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus sylvarius, Lactobacillus brevis,* and *Bifidobacterium bifidum*	Reinforcement of epithelial integrity and mucin production.	84.61% cure rate and recovery of normal villous structure.
Shaaban *et al.*, 2021	Mice	Probiotic capsule containing: *L. acidophilus, L. rhamnosus, L. crispatus, L. plantarum, L. paracasei, L. bulgaricus, L. reuteri, L. casei, L. salivarius, L. helveticus, L. gasseri, Bifidobacterium (B.) lactis, B. bifidum, B. longum, B. breve, B. adolescentis, B. infantis,*	Interference with the attachment of *Giardia* to the mucosa, competition with nutrients, and production of antimicrobial compounds.	90% and 84% reduction in *Giardia* cyst count when probiotic is given as a prophylaxis and treatment, respectively.

		Leuconostoc mesenteroides, Lactococcus lactis, and *Streptococcus thermophiles*		
Shady *et al.*, 2023	Hamster	*Lactobacillus casei* and *Lactobacillus bulgaricus*	Production of antimicrobial compounds, competition for nutrients, and interference with parasite attachment to the mucosa.	Complete eradication of *Giardia* cyst
Shukla *et al.*, 2019	Mice	*L. rhamnosus GG*	Increase nitric oxide level in serum and anti-giardial IgA antibody level in the intestinal fluid. Well-formed muscle coat, mucosal epithelial lining, normal villi, and increase in goblet cells.	Significantly reduces the life cycle of *Giardia intestinalis*.
Singh *et al.*, 2013	Mice	*Lactobacillus casei*	Increase alkaline phosphatase and intestinal disaccharides such as sucrase.	Significantly lower cyst count and eventual disappearance of the parasite. Restore intestinal mass and improve the activity of intestinal enzymes.
Yousef *et al.*, 2020	Hamster	Acidophilus capsule containing: *Lactobacillus acidophilus, L. bulgaricus, L. sylvarius, L. brevis,* and *Bifidobacterium bifidum*	Compete for limited adhesion sites and nutrients, thus preventing parasite adherence to the tissue surface.	45.9% and 49.25% reduction in trophozoite and cyst count, respectively.

The extracted data from the selected studies were organized in Table 1, which presents the major findings on the mechanisms and antiparasitic effects of probiotics against infection with *Giardia intestinalis*. This is to better evaluate the administration of probiotics as an alternative prophylaxis or treatment for *Giardia intestinalis* infection to contribute to healthcare decisions.

Effectiveness of Probiotics against Giardiasis and Their Impact on rat models

Several studies have investigated potential alternative medication against *Giardia intestinalis* infection, primarily probiotics. In the study of Al-Khaliq (2019), intending to evaluate the efficacy of *Bifidobacterium* against infection with *Giardia intestinalis,* they found that oral administration of a single dose of the probiotic strain given daily has significantly reduced the shedding of parasites in the feces of the rat models. Complete disappearance of parasite shedding was observed earlier in the group administered with probiotics than those given metronidazole. The mechanism by which *Bifidobacterium* manifests its protective response towards the host includes competition of probiotics for binding sites and nutrients that prevent the adherence of the microorganism. It directly antagonizes the pathogen by stimulating the host's immune response and enhancing the synthesis and secretion of Immunoglobulin A (IgA) by expressing Interleukin 10 (IL-10) and Interleukin 6 (IL-6), which causes the elimination of *Giardia intestinalis*. A similar mechanism was expressed in a more recent study by Al-Megrin *et al.* (2021), in which the efficacy of *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Lactobacillus helveticus,* were assessed and findings showed an 87.5% cure rate. For histopathologic morphology, an unaltered villus shape, increased infiltration in the central pulp, and eventual disappearance of trophozoite was observed. Moreover, Allain *et al.* (2018) examined a different species of *Lactobacillus*, mainly *L. gasseri* and *L. johnsonii,* which induced a 93% and 43% trophozoite reduction, respectively. In their study, the bile salt hydrolase activity of the probiotics acts as a prophylactic strategy to combat giardiasis.

Furthermore, Amer *et al.* (2014) experimented on bacteriocins derived from *L. acidophilus* and *L. plantarum* which inhibited colonization and multiplication of *G. lamblia* trophozoite through the degradation of the nucleic acid of pathogens. A notable result was seen upon administration of *L. acidophilus* with an 81.63% reduction, whereas only a 31.38% reduction was observed for groups administered with *L. plantarum*. Interestingly, Goyal *et al.* (2013) discovered that medication with *Lactobacillus rhamnosus* for mice infected with *G. intestinalis* triggers an increased synthesis of intestinal disaccharides, such as sucrase and lactase, increase in antioxidant levels, primarily reduced glutathione (GSH) and superoxide dismutase (SOD), and a significant decrease levels of lipid peroxidation. Histopathologic findings of the jejunum also displayed a normal cellular morphology of the enterocytes. These factors prevent the adherence of the trophozoite form of the parasite to intestinal cells, thus protecting the host. Comparatively, Shukla *et al.* (2019) using the same probiotic strain, *L. rhamnosus,* observed a reduction in the life cycle of *G. intestinalis* following an increase in nitric oxide level in serum and anti-giardial IgA antibody level in the intestinal fluid of infected mice, along with a well-formed muscle coat, normal villi and mucosal epithelial lining, and increase in goblet cells. Additionally, Ribeiro *et al.* (2018) examined the anti-giardial effect of *Saccharomyces boulardii*, which induces a 73% parasite reduction related to increased mucus production and intraepithelial lymphocytes. Several studies also demonstrated the efficacy of the Acidophilus capsule in treating *G. intestinalis* infection. The study by Sanad *et al.* (2020) using an Acidophilus capsule containing 50 million live bacteria of 5 different probiotic strains, mainly *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus sylvarius, Lactobacillus brevis,* and *Bifidobacterium bifidum* showed an 84.61% cure rate and induced recovery of normal villous structure. Similarly, Yousef *et al.* (2020), administering the same probiotic treatment for infected mice, resulted in a 45.9% and 49.25% reduction in trophozoite and cyst count, respectively. Moreover, Shaaban *et al.* (2021) observed a 90% reduction in *G. intestinalis* cysts when the probiotic capsule was used as prophylaxis and an 84% reduction when given as treatment for infected mice. The mechanism attributed to the protective factor of the probiotic capsules includes the production of mucin, synthesis of antimicrobial compounds, and interfering with the attachment of *G. intestinalis* to adhesion sites in the host's intestinal mucosa, thereby enforcing epithelial integrity and elimination of parasites. Correspondingly, Singh *et al.* (2013) and a more recent study by Shady *et al.* (2023) using the probiotic strains, primarily *Lactobacillus casei* and *Lactobacillus Bulgaricus,* as medication resulted in complete eradication of the parasite following the augmentation of innate and adaptive response resulting to the restoration of intestinal mass and improve the activity of intestinal enzymes, such as alkaline phosphatase and intestinal disaccharides, including maltase and sucrase.

Exploring the potential anthelmintic effects of probiotics against *Giardia intestinalis* infection unveils promising avenues in combating parasitic diseases through symbiotic interventions. The mechanisms by which probiotics exert their anthelmintic effects are necessary to establish their protective response towards the host. This involves colonization and recovery of damaged intestinal mucosa, exclusion of pathogens, bacteriocin production, and modification of enzymatic activities, which act synergistically to regulate the normal function of tissues (Saracino *et al.*, 2021). Several studies have demonstrated defense against parasites, primarily *Giardia intestinalis,* involving intrusion with adhesion sites of the parasite and competition with nutrients (Yousef *et al.*, 2020; Shaaban *et al.*, 2021; Shady *et al.*, 2023), as well as the synthesis of antimicrobial substances, such as bacteriocin (Amer *et al.*, 2014) and nitric oxide (Shukla *et al.*, 2019). Probiotics also induce mucin production (De Freitas *et al.*, 2019; Ribeiro *et al.*, 2018; Sanad *et al.*, 2020), differentiation of T-regulatory cells, and amplify cytokines, such as IL-10 (Al-Khaliq, 2019; Al-Megrin *et al.*, 2021; Shukla *et al.*, 2013; Ribeiro *et al.*, 2018),

that enhance the host's immune response against parasitic infections (Plaza-Diaz *et al.*, 2019).

Bacteria from the genera *Lactobacillus* and *Bifidobacterium* can enhance the production of IgA through the expression of TGFβ, IL-10, and IL-6 (Hardy *et al.*, 2013). The release of IgA to the intestinal lumen potentiates immune exclusion wherein it surrounds pathogens, thereby blocking them from entering the intestinal epithelium (Breedveld and Van Egmond, 2019). As a result, IgA forms a protective barrier, along with the mucus layer, preventing penetration of parasites in the mucosal epithelium, which, in turn, facilitates the eradication of pathogens. Another mechanism involves the production of antimicrobial compounds such as bacteriocins and nitric oxide. Bacteriocins are derived from lactic acid bacteria, commonly used probiotics, that are attributed to have positive health benefits (Darbandi *et al.*, 2021). The proliferation of bacteriocins in the gut inhibits the adhesion of pathogens to intestinal mucosa as it displaces pathogens from their microbial niches without affecting the beneficial bacteria while lowering the risk of widespread microbiome damage attributed to antibiotics (Heilbronner *et al.*, 2021; Anjana and Tiwari, 2022). Furthermore, the production of nitric oxide by probiotics prevents excystation and encystation of *Giardia intestinal* trophozoite and cyst form, respectively (Shukla *et al.*, 2019). Nitric oxide inactivates enzymes necessary for mitochondrial respiration and growth of parasites, leading to decreased parasitic complications (Shen *et al.*, 2017).

Recent findings suggest that Bile-Salt-Hydrolase (BSH)-like activities from the probiotic strain of *Lactobacillus johnsonii* La1 may contribute to the anti-giardial activity displayed by this strain (Allain *et al.*, 2018). Conjugated bile salts are, thus, directly consumed by Giardia without being detoxified (Farthing *et al.*, 1985). When Giardia comes into contact with conjugated bile salts, it can integrate them into its metabolic activities without requiring prior neutralization or alteration. This suggests a mechanism through which Giardia interacts with its surroundings and obtains vital nutrients or substances necessary for its survival and growth. In contrast, bacterial deconjugation aims at reducing the detergent properties of conjugated bile salts, which are more toxic for bacterial cells than secondary bile salts, i.e., cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) (De Boever and Verstraete, 1999). Following numerous research endeavors, it has been firmly established that DCA induces disturbances in the composition of eukaryotic membranes through its influence on the arrangement of membrane lipid microdomains. Based on this understanding, we hypothesized that secondary bile salts, with a specific focus on deoxycholic acid (DCA), might possess the capability to effectively eliminate Giardia trophozoites by causing significant damage to their cellular structure. This hypothesis is rooted in the observed effects of DCA on membrane integrity and its potential implications for disrupting the vital functions of Giardia trophozoites.

Numerous studies have extensively documented that an individual's susceptibility to infections relies on the composition of intestinal microbiota that interfere with establishing pathogens. In this study, the well-acknowledged fact is that *G. intestinalis* induces structural and functional derangement of the small intestine, leading to damaged brush border microvilli and impaired activities of brush border membrane enzymes (Khanna *et al.*, 1988). The enzymes, including alkaline phosphatase, lactase, sucrase, and maltase, are crucial for the digestion and absorption of nutrients in the small intestine. The damage caused by Giardia species leads to a decrease in the activity of important brush border enzymes, particularly those located in the upper villus region, which are biomarkers for intestinal damage. Interestingly, it was observed that probiotic supplementation to Giardia-infected mice further prevented the brush border injury in the small intestine by significantly enhancing the specific activities of brush border enzymes (alkaline phosphatase, sucrase, and lactase) and subscribes with the previous studies (Humen *et al.*, 2005). With this, we can conclude that probiotics may protect against the damage caused by Giardia

infection. Previous research, including that by Southcott *et al.* (2008), has provided evidence to support this statement. They observed heightened activity of brush border enzymes, such as sucrase and lactase, in rats experiencing methotrexate-induced mucositis after receiving supplementation with sheep yogurt containing distinct probiotic strains. Likewise, Humen *et al.* (2005) noted an increase in sucrase activity in *Lactobacillus jonsonii La1*-treated rats

Lipid peroxidation measured in terms of Malondialdehyde (MDA) levels was found to be significantly higher in Giardia-infected mice during infection compared with control mice. However, probiotic-fed Giardia-infected mice had significantly decreased lipid peroxidation levels compared with Giardia-infected mice (Goyal *et al.*, 2013). The data indicates a reduction in MDA levels in the small intestine of probiotic-fed mice infected with Giardia, suggesting a decrease in lipid peroxidation. This reduction in lipid peroxidation potentially prevents intestinal tissue damage in mice infected with Giardia and supplemented with probiotics. Therefore, probiotic supplementation protects against lipid peroxidation and subsequent tissue damage caused by Giardia infection. It is believed that during transit through the gastrointestinal tract, lactobacilli may release their intracellular antioxidative constituents, reducing tissue injury and thereby protecting the host (Goyal et a., 2013). Thus, probiotics can be administered as a potential alternative medication against *G. intestinalis* infection as exhibited by the mechanisms by which they exert their anthelmintic effects

Conclusion

The exploration of probiotics as a potential strategy for combating *Giardia intestinalis* infection has revealed promising anthelmintic effects with significant implications for preventive and therapeutic approaches in managing this prevalent gastrointestinal parasite. The current review combines papers discussing the antiparasitic effects of probiotics and highlighting the mechanism of actions associated with their effectiveness in fighting against *G. intestinalis*. Findings from the included studies revealed that probiotic strains in the genera *Bifidobacterium* and, largely, *Lactobacillus* could be a potential prophylactic and therapeutic medication against the specified parasitic infection.

Recommendation(s)

Most research on the impact of probiotics on parasites has been conducted through animal experiments and in vitro cultures. Human trials in this area are marginally reported. Other factors, including the molecular dynamic demonstrated by these beneficial bacteria, remain undecipherable. Hence, advanced investigation on the interactions of probiotics and pathogens with humans as hosts could elucidate knowledge on the greater potential of probiotics.

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