

# The Unexplored Role of Probiotics on the Parasitic Pathogens

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## Abstract

The beneficial bacteria coined as probiotics are used as therapeutics to the host and evidences are there to demonstrate to treat bacterial and viral respiratory infections, gastrointestinal diseases, eczema, inflammation, H. pylori infection, irritable bowel syndrome and allergic symptoms etc. In recent past, probiotics has been reported for the control of intestinal parasite infections as well as few non-gut infections spread among human and veterinary animals. Animal models and *in vitro* culture systems have been studied regarding cellular interactions between probiotics and pathogens or relevant host cells, though the underlying molecular mechanisms mediating the beneficial effects have not yet fully discovered. Hence, more evidence based studies are warranted to correlate whether probiotics through multiple mechanisms might indeed provide a strain-specific protection against parasites to use it as therapeutics. This article has described the effect of probiotics in some of the intestinal as well as non-gut parasites and suggested the scope of exploring the benefit for protozoan parasite Leishmania, as India is planning for the elimination of the disease.

## Keywords

Probiotics, Pathogen, Parasitic, Metagenomics, Leishmania

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## 1. Introduction

Probiotics are the organisms usually isolated from the gut and these live bacterial supplements are administered widely to improve human health by modulating the microflora of the gut. These are non-pathogenic symbiotic organisms which help in restoring the diversity of the gut bacterial microbiota in patients by stimulating immune system, metabolism, anti-pathogenic actions when administered in large numbers. Recent evidences gathered from *in vitro* culture systems or at best in animal models have demonstrated that probiotic bacteria can be used for therapeutic purposes on control of both intestinal parasite infections as well as few non-gut infections spread

among human and veterinary animals. However, the molecular mechanism of pathogens clearance by probiotics in the host still remains to be elucidated. Hence, it will be worth to explore the molecular interaction between probiotics with pathogens to understand the mechanisms of action of probiotics. Here, we discuss the beneficial effects of probiotics in the therapy of some gut as well as non-gut pathogens. It remains challenging to discover the anti-microbial effects of probiotics and their mechanism of action in different diseases caused by intercellular pathogens like *Leishmania*, *Trypanosoma* and *Babesia*.

## 2. The Human Gut Microbiota

The gastrointestinal (GI) tract of human is highly populated with a very complex and dynamic microbial community which remains specific for each individual and is often regulated by the genetic makeup as well as the environmental and Geographical factors [1]. This gut microbiota is comprised of hundreds of different species and varies along the GI tract, essentially plays a crucial role in human health by contributing in the digestion process, development of the gut and the immune system. Several studies have demonstrated that the specific aberrations in the composition of the gut microbiota have correlation with different infectious diseases, antibiotic treatments, ageing and also, with stress. Importantly, there is evidence that the probiotics can modulate the gut microbiota and thereby host health. There are meaningful human intervention studies which explain the beneficial effects of specific probiotic strains, however, some other reports, even promising, remain hypothetical at present. Some such commonly used microorganisms used as probiotics are *Lactobacillus*, *Saccharomyces* and *Bifidobacterium* which are instrumental in modulating the composition of intestinal microbiota and also the activity.

## 3. Metagenomics and Probiotics

Traditional culture of the human gut microbiota was conventionally used to unravel the microbiota composition and development which is currently getting replaced by several qualitative and quantitative cultures-independent latest techniques like PCR, DNA hybridization, metagenomics etc. thus remain useful means of studying the microbiota in their environment only. Importantly, the metagenomics approach has explored out a much greater diversity than the previously existing ones which ultimately revealed the community structure of many unknown gut ecosystems [2]. These new age technologies have also significantly contributed on the diverse aspects of on-going research on probiotics. Comparative analysis of microbiome of healthy individuals and diseased ones has revealed the alterations and unveiled the horizon of clear targets for the probiotic products useful to neutralize the harmful effects of several microbial toxins. Hence, extensive exploration of metagenomics is opening the underlying effect of microbiota in health; as well as other potential areas of probiotics application may be identified like the non gut body sites for which the mechanism of action yet need to be understood.

## 4. Beneficial Role of Probiotics

According to the definition of WHO, Probiotics are the “live organisms which when administered in adequate amounts confer a health benefit to the host” [3]. Some of such beneficial properties demonstrated by the probiotics are as follows: nonpathogenic action, protection against pathogens by increasing the total number of intestinal bacteria thereby restoring the diversity of the bacterial microbiota in patients’ immune stimulation, metabolism, resistant to low pH and acids, allowing to persist in the intestine with special capability for adherence to the gut epithelium etc. [4]. Probiotics are also efficient to control their biotic environment through regulation of intestinal motility and mucus secretion. 26 species of probiotics representing 50 strains exhibit the expected properties [5]. These are gram positive bacteria which are generally isolated from the human gut micro flora and various dairy products. The good effects of probiotics is largely dependent on the dose ingested of at least five billion colony forming units per day for at least 5 days [4] which acts as a minimum dose for the survival capacity of the ingested probiotics in the gastrointestinal system to overcome the competition with the resident bacteria.

## 5. Role of Probiotics on Intestinal Pathogens

Both prokaryotic and eukaryotic pathogens in the gut and other sites are decreased, killed or inhibited by the probiotics by strain specific mechanism through active molecular secretion (e.g. bacteriocins like lactacin,

reuterin, nisin etc.; antibiotics like 3-hydroxypropionaldehyde, hydrogen peroxide, free fatty acids, etc.) and immune induction. Importantly, lactic acid can decrease the local intestinal pH which directly disrupts the growth of the acid sensitive organisms. Recent studies have also shown the effect of probiotics on the non-gut pathogens (yeast, protozoa like *Toxoplasma gondii*, fungi etc.) through distant immune mechanism, however, the exact mechanism has still remained unexplored [6].

The beneficial effects of probiotics either singly or in combination have been observed against some of the parasites which are described in **Table 1** and **Table 2**.

The two Tables describe about some of the probiotics demonstrating effects on different eukaryotic pathogens. The gut pathogens and few non gut pathogens are listed in the first column; the probiotics strains used are in the second one; the third column shows details of the level of the studies e.g. at the cellular level like, trophozoites development and invasion capacity, cyst differentiation and survival etc., Human patients as clinical; natural or experimental animal models e.g. pig, mouse, calf, rat, chicken, gerbil as *in vivo*. The fourth column describes the time of administration of the probiotics; the efficacy has been tabulated in the fifth column followed by the corresponding references which are mentioned in the sixth column. Efficacy is reflected by the parasite load reduction as compared to the control in case of *in vivo* studies, whereas, *in vitro* assays are accounted by the reduction of infectivity or viability.

Likewise, another study with the well-defined nematode *i.e.* *Trichuris muris* infection in mice model to investigate the effects of *Lactobacillus rhamnosus* (JB-1) treatment on host defence in nematode infections demonstrated that treatment with live JB-1 accelerates parasite expulsion and up regulates goblet cell hyperplasia in resistant (to infection) mice via the IL-10 pathway [38]. Worm expulsion and goblet cell hyperplasia was noticed even in a susceptible strain (AKR) of mice, which was not observed in case of the resistant mice treated with  $\gamma$ -irradiated JB-1 or even in IL-10 knockout mice. Earlier study from this group has shown that IL-10 itself promotes goblet cell hyperplasia. Hence, the novel findings suggest the beneficial effect of the probiotic in innate defence during parasitic infections depicting new insights of its occurrence.

In a similar example in case of acute amoebiasis caused by *Entamoeba histolytica*, the probiotic yeast named *Saccharomyces boulardii* (Ultra-levure) in combination with antibiotics has demonstrated a protective effect which significantly reduced the duration of disease symptoms as well as the presence of cysts in stools in succession [39].

## 6. Role of Probiotics on Non-Intestinal Pathogen

A Mexican research group has reported the potential beneficial effect of *L. casei* ATCC 7469 on the non-intestinal eukaryotic pathogens in the case of *Plasmodium*, *Babesia* or *Trypanosoma*. Reduced parasitemia was demonstrated in *Babesia microti* (Gray strain) infected mice through the oral or intraperitoneal treatments of *L. casei* ATCC 7469, involving the stimulation of the innate immune system [34] ascribed to early appearance of IL-12 and  $\gamma$ -IFN transcripts in the spleen [40]. The lactobacilli when administered 3 days before or on the same day of parasite infection versus 7 days before, demonstrated better protective response. The underlying molecular mechanism has been attracted the investigators by testing of low and high molecular isolates from lactobacilli for their efficacy to induce early protective immune response against *Babesia microti* [35].

The same *Lactobacillus strain* has also demonstrated a protective effect through increasing nonspecific resistance to the malarial parasite *Plasmodium chabaudi* AS infection in NIH mice [31] followed by lesser parasitemia and viability of parasites extracted from the spleen of treated mice. Also, serum of *L. casei*-treated mice has shown 1.8 (app.) times more nitric oxide concentration which provides a protective effect upon the plasmodial infection. Likewise, the oral or intraperitoneal doses of same *L. casei* ATCC 7469 given 7 days prior to the parasite infection reported a significantly reduced parasitemia of *Trypanosoma cruzi* (the causative agent of Chagas disease) over the next 50 days in NIH mice [34]. The assumption is that the stimulated host immune response by *L. casei* [41] which in turn controls *T. cruzi* infections [42] may be responsible for this protective effect.

It has been observed that the beneficial effects of probiotics are demonstrated in some of the parasites among which, majority are gut residents, very few studies have been undergone in non-gut parasites namely, *Plasmodium*, *Babesia* and *Trypanosoma* only. However the underlying cellular and molecular mechanisms have still remained unexplored. The direct effect of secretion of an active principle has been reported in case of majority of the gut parasites, where also the molecular nature of these components is still being worked upon. The stimulation of different subsets of immune system cells by the probiotics can help in production of cytokines which is followed by induction and regulation of the immune response thereby increasing the intestinal IgA immune responses as

**Table 1.** Effect of probiotics on gut parasitic pathogen.

Parasitic Pathogen	Probiotics studied	Host	Treatment	Efficacy	References
	<i>Lactobacillus (L.) acidophilus</i> NCFM/ <i>L. reuteri</i> ATCC23272	Cell culture		25% - 50% reduction	[7] [8]
	<i>L. acidophilus</i> NCFM or <i>L. reuteri</i> 4000, 4020	Mouse	7 - 15 days before infection	50% - 75% reduction	[9]
	<i>L. reuteri</i> 4000, 4020	Mouse	7 - 15 days before infection	75% - 100% reduction	[10]
	<i>L. reuteri</i> 4000, 4020	Mouse	7 - 15 days before infection	50% - 75% reduction	[11]
<i>Cryptosporidium parvum</i>	<i>L. casei shirota</i> and <i>L. rhamnosus GG</i>	Human	After infection	Clinical case of resolution	[12]
	<i>Pseudomonas (P.) alcaligenes</i> , <i>Bacillus (B) brevis</i> , <i>Enterococcus (E.) faecium</i>	calf	Concomitant administration & infection	Insignificant effect	[13]
	<i>B. brevis</i> , <i>E. Faecium</i> & <i>P. alcaligenes</i>	Cell culture		75% - 100% reduction	[14]
	<i>Bifidobacterium longum</i> ATCC 15707 or <i>B. breve</i> ATCC 15698	Cell culture		75% - 100% reduction	
	Actimel/VSL ≠ 3	Neonatal rat	0 - 3 days before infection	Insignificant effect	[15]
	<i>E. faecium</i> SF68	Mouse	3 - 7 days before infection	75% - 100% reduction	[16] [17]
	<i>L. casei</i> MTCC 1423	Mouse	3 - 7 days before infection	75% - 100% reduction	[18]
<i>Giardia lamblia</i>	<i>L. Johnsonii</i> LA1	Gerbil	3 - 7 days before infection	50% - 75% reduction	[19]
	<i>L. Johnsonii</i> LA1	Cell culture		75% - 100% reduction	[20]
<i>Giardia duodenalis</i>	<i>Lactobacilli</i> spp.	Human Dendritic Cell culture	Activated through TLR2 released by <i>Lactobacilli</i> spp.	Enhanced activation	[21]
<i>Ascaris suum</i>	<i>Bifidobacterium lactis</i> (pig isolate)	pig	More than 15 days	Undetermined	[22]
<i>Shistosoma mansoni</i>	<i>Zymomonas (Z.) mobilis</i>	mouse	3 - 7 days before infection	50% - 75% reduction	[23]
	<i>L. acidophilus</i> Lb33ac, <i>L. salivarius</i> Lb 14c7 Lb 16c6	cell culture		50% - 75% reduction	[24]
	Mitogrow	chicken	More than 15 days before infection	25% - 50% reduction	[25]
<i>Eimeria tenella/ acervulina</i>	Primalac	chicken	More than 15 days before infection	50% - 75% reduction	[26]-[28]
	Mitomax	chicken	More than 15 days before infection	25% - 50% reduction	[29]
<i>Toxocara canis</i>	<i>E. faecalis</i> CECT 7121	mouse	3 - 7 days before infection	75% - 100% reduction	[30]
<i>Trichinella spiralis</i>	<i>L. casei</i> ATCC7469	mouse	3 - 7 days before infection	25% - 50% reduction	[31] [32]

well as enhanced production of intestinal mucin. Factually, probiotics as a proposed therapeutic as an alternative to classical drug and vaccine treatment effect seems illogical; suggesting a complementary therapeutic approach for decreasing risks of infestation and thereby continuing classical treatment modality sounds more promising.

Currently, more systematic studies of probiotic effects on parasites are needed to explore major factors involved in the physiological and molecular level with special emphasis on standardized dosage, mode of administration, time duration etc. of probiotics; strain level characterization of the probiotics used remain preferable; wide spectrum of probiotics either individually or in combination may be studied on greater number of pathogens in their corresponding animal models etc. It has also been emphasized that animal models with inbred strain and controlled

**Table 2.** Effect of probiotics on non-gut parasitic pathogen.

Parasitic Pathogen	Probiotics studied	Host	Treatment	Efficacy	Reference
<i>Plasmodium chabaudi</i>	<i>L. casei</i> ATCC7469	mouse	7 - 15 days before infection	25% - 50% reduction	[33]
<i>Babesia microti</i>	<i>L. casei</i> ATCC7469	mouse	0 - 3 days before infection	75% - 100% reduction	[34] [35]
			3 - 7 days before infection	25% - 50% reduction	
<i>Trypanosoma cruzi</i>	<i>L. casei</i> ATCC7469	mouse	3 - 7 days before infection	75% - 100% reduction	[36]
<i>Trypanosoma brucei brucei</i>	<i>Saccharomyces cerevisiae</i>	rat	28 days before infection	Significantly lower	[37]

micro flora may provide uniform result, more so, gnotobiotic mice remains more preferable in term of better efficacy analysis of probiotics use for gut microorganisms.

From all of the above mentioned reports, it is proven that probiotics can eliminate the gut pathogens through direct secretion, which help in: dissolving the toxins, restricting the adhesion or invasion of pathogen, and even compete for nutrients. Whereas, effect of probiotics on *in vivo* studies on non-intestinal pathogens like *Trypanosoma*, *Plasmodium*, *Babesia* suggest a remote effect through a nonspecific immune stimulation which needs more serious exploration. One such study has described the effect of probiotics to ameliorate the immunosuppressive effect of *T. brucei brucei* infection.

## 7. Probiotics and Its Effect on Animal Parasites

Apart from the efficacy studies of probiotics on human parasites, few other animal parasites have also been targeted for elimination or reduction of numbers as evidenced by the studies in fish parasites [43], in case of inland bearded dragons [44], other animals like cats and dogs in animal shelter [45] and dairy goat kids [46].

## 8. Suggested Future Study

Leishmania, being a parasite pathogen in human and other few mammals, remains responsible to cause cutaneous and Visceral Leishmaniasis which is categorized under one of the neglected tropical diseases. Leishmaniasis is prevalent in 88 countries, and there is an estimated 2,000,000 new cases per year, of which 500,000 are cases of Visceral Leishmaniasis (VL). In the Indian subcontinent alone, around 300,000 cases occur yearly, which represents an estimated 70% of the total global burden of VL. India and Bangladesh are among the most severely affected countries in the world [47]. The disease is affecting poorest of the poor and India has targeted for its elimination by the year 2015 [48]. Visceral Leishmaniasis infections are caused by the group of *Leishmania donovani* complex (*L. donovani*, *L. infantum syn.* and *L. chagasi*) and develop fever, swelling of the liver and spleen, and anaemia and it is commonly named as Kala azar.

An important fact for control and elimination of the disease is that the existing antileishmanial drugs are extremely costly and remain very toxic. Vaccines against VL are still not in the horizon, though several efforts are in progress. Hence, at this juncture, based upon the evidence of beneficial effects of probiotics on some of the parasitic pathogens, it is suggested that the probiotics may be tested for VL *in vivo* and their effect may be wise to analyse keeping in mind the study designs for other non-gut parasitic pathogens like *Trypanosoma*, *Plasmodium* and *Babesia*. The outcome analysis may be of useful information as an emerging therapeutic strategy as an adjunct to the conventional chemotherapy for this non gut parasitic disease which in turn may benefit the mankind in the developing countries plagued by the disease.

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