

**Review Article**

**ROLE OF PROBIOTICS IN GASTROINTESTINAL TRACT DISEASES: INFLAMMATORY BOWEL DISEASE AND NECROTIZING ENTEROCOLITIS**

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**ABSTRACT**

Bacterial microflora which is present in gastrointestinal tract cause various disorders. Probiotics provides beneficial effects in gut related disease. Previously probiotics are used as alternative medicine but now entering in mainstream medicine. Probiotics help in restoring normal bacterial microflora and regulate the gastrointestinal tract functioning through different mechanisms. There are only few evidences available which show potential benefits of probiotics. The efficiency of probiotics used as a single strain or multiple formulation is tested in various diseases such as oral health, diabetes, multiple sclerosis, inflammatory bowel disease, necrotising enterocolitis and many more. The purpose of this paper is to focus on diseases related to gastrointestinal tract including inflammatory bowel disease and necrotising enterocolitis based on the literature review available and propose future perspective to reduce the translocation of bacterial microflora in gastrointestinal tract and reducing subsequent inflammation of gut. Keywords were searched in google scholar, academic research, PubMed and Embase databases.

**Keywords:** Inflammatory Bowel Diseases, Necrotizing Enterocolitis, Gastrointestinal tract

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**INTRODUCTION**

In October 2002, probiotics was defined by World Health Organisation as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." With the advancement in technology various strains of probiotics mainly, lactic acid bacteria such as lactobacillus, bifidobacterium and other probiotics used in the treatment of various diseases are of significant importance. The intestinal microflora contains a wide variety of good bacteria such as lactobacillus, enterococcus, lactococcus, bifidobacterium and other bacteria which not only helps in improving digestion but also produces complex molecules such as vitamins and antibodies which enhances the immune response of the body. The selection criteria of probiotics obtained from human source are mostly preferred over plant sources, as chances of acceptance increases in case of human sources and also they possess the ability to bind or attach to the intestinal wall, providing resistance to acid and bile present in stomach. The main strains of lactic acid bacteria which are present in probiotics are lactobacillus acidophilus and bifidobacterium bifidum. (Saggiaro, 2004). Probiotics shows promising effects in curing inflammatory bowel disease and preventing necrotising enterocolitis.

**Inflammatory bowel disease (IBD)**

The unusual action in intestine may result from the interplay between some environmental factors and the genetic susceptibility which contributes in the Inflammatory Bowel Disease. The abnormal interaction between aberrant micro flora and intestinal mucosal immune system within the intestinal tract which majorly leads to change immunological functions and triggers the inflammatory response of Inflammatory Bowel Disease [1]

Inflammatory Bowel Disease is a chronic disease of the alimentary canal that clinically contains colitis, Crohn's disease and some more symptoms. Inflammations in intestine (mucosal) of IBD can be specified by weight loss, diarrhoea, abdominal pain, bloody stools and thus the incursion of macrophages and neutrophils which have the ability to fabricate proteolytic enzyme, cytokines and free radicals results in ulceration and inflammation [2].

Crohn's disease generally contains the cecum, terminal ileum, colon and perineal area, but they may influence any part of the gut during

their intermittent form. While colitis involves into the rectum and it can influence the surroundings of the Colon or the entire colon. Crohn's disease revealed histological thickening of sub mucosa, fissuring ulceration, transmural inflammation and granulomas while the mucosa and sub mucosa are restricted by inflammation of colitis with crypt abscesses and cryptitis [2].

**Mechanism of action of probiotics in inflammatory bowel disease**

**Tolerance of probiotics to pro-inflammatory stimulus in the gastrointestinal tract**

Chemokines, cytokines are overproduced by immune cells when a person is suffering from Inflammatory Bowel Disease (IBD) which acts on the epithelial cells of gastrointestinal tract such as TNF- $\alpha$  (Tumor Necrosis Factor- $\alpha$ ) which will cause epithelial cells to secrete IL 8 (Interleukin 8) which cause the overexpression of TLR 4 (Toll Like Receptor). TLR 4 is hyper-reactive in presence of LPS (Lipopolysaccharides). IL 8 and TNF- $\alpha$  is extensively express in response to foreign pathogen. Interaction between specific probiotic strains such as bifidobacterium cause the inactivation of inflammatory effector molecules provoked by pro-inflammatory stimulus. The role of probiotic strain is to act as immunosuppressive agent to inhibit kB/nuclear factor pathway by blocking I $\kappa$ B- $\alpha$  (Inhibitor kappaB-alpha) which will in turn inhibit translocation of active NF-kB (NF-kappaB) in the nucleus and suppress the expression of that gene [24-26].

**Role of PepT1 in promotion of colonocyte to increase defence mechanism**

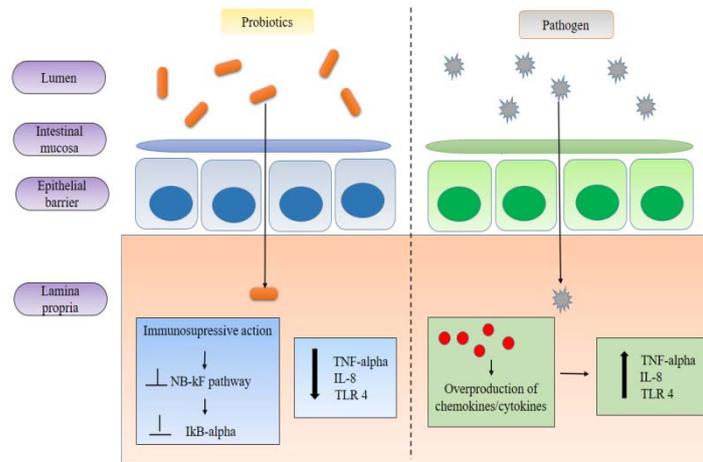
In epithelial cells of colon, MDP (Muramyl dipeptide) is drawn by hPepT1 (Intestinal apical di-/tripeptide transporter) which in turn activates the production of NF-kB and chemokines. Thus it is significant that PepT1 help in the promotion of colonocyte in defence mechanism of host by up taking MDP. Evidences had proven that Lactobacillus casei alter the function of PepT1 which leads to increase production of hPepT1 (3H) Gly-Sar uptake when incubated with Caco-2 cells which in turn increase the activity of PepT1 [27, 28].

**Table 1: Crohn disease using various probiotics**

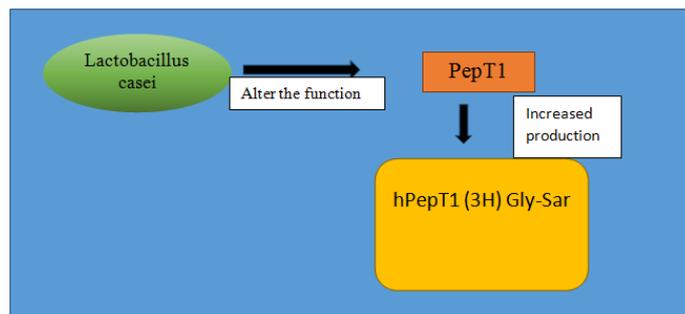
Strain	No. of patients	Outcomes	Reference
L. Rhamnosus	4 Children	Intestinal Permeability increased	Gupta et al, 2000 [3]
Saccharomyces Boulardii	32 adults	Remission maintenance was found good	Guslandi et al, 2000 [4]
L. Rhamnosus	45 adults	Endoscopic recurrence was not prevented	Prantera C et al, 2002 [5]
L. Rhamnosus	11 adults	No benefit of remission maintenance	Schultz et al, 2004
L. Rhamnosus	75 children	No time prolongation	Bousvaros et al, 2005 [6]
L. Johnsonii	98 adults	Endoscopic recurrence was not prevented	Marteau et al, 2006 [7]
L. Johnsonii	70 adults	Endoscopic recurrence was not prevented	Van Gossum et, 2007 [8]
Saccharomyces Boulardii	165 adults	No benefit of remission maintenance	Bourreille et al, 2013 [9]

**Table 2: Studies on irritable bowel syndrome using various probiotics**

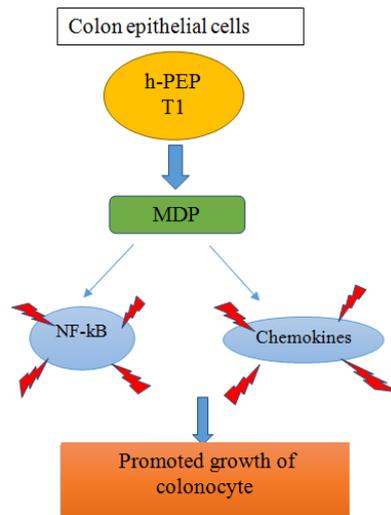
Strain	No. of patients	Outcome	Reference
L. plantarum	40 adults	Recovered	Niedzielin et al., 2001 [10]
Bifidobacterium Infantis	362 adult women	Relieve in pain	Whorwell et al, 2004 [11]
Bifidobacterium Infantis	77 adults	Relieve in pain but did not show any improvement in stool frequency.	O'manhony et al., 2005 [12]
Bifidobacterium Animalis	274 adults	Improvement in blotting	Guyonnet et al., 2007 [13]
Bacillus Coagulans	61 adults	Improvement in stool frequency	Dolin et al., 2009 [14]
L. Rhamnosus	141 children	Relieve in pain	Francavilla et al., 2010 [15]
VSL#3	59 children	Improvement in bloating but no relieve in stool frequency	Guandalini et al., 2010 [16]
Saccharomyces Boulardii	70 adults	No improvement seen	Kabir et al., 2011 [17]
Bifidobacterium Bifidium	122 adults	Overall improvement seen	Gulgliemetti et al., 2011 [18]
Saccharomyces Boulardii	67 adults	No improvement in stool frequency	Choi et al., 2011 [19]
L. Rhamnosus	80 adults	No improvement	Dapoigny et al., 2012 [20]
VSL#3 (Probiotic capsule)	50 adults	Overall improvement but benefits in particular symptoms were not shown	Ki Cha et al., 2012 [21]
L. plantarum	214 adults	Improvement in pain but no improvement in stool frequency.	Ducrotte et al., 2012 [22]
L. Salivarius	131 adults	No major improvement seen	Begtrup et al., 2013 [23]



**Fig. 1: Probiotics shows immunosuppressive effect in intestinal epithelial barrier and mucosal immune cells by downregulating the pro-inflammatory cytokines. Synopsis: TNF-alpha (Tumor Necrosis Factor-alpha), IL-8 (Interleukin-8), TLR 4 (Toll like Receptor 4), NF-kB (Nuclear Factor Kappa B), IkB-alpha (Inhibitory kappa B-alpha)**



**Fig. 2: Action of lactobacillus on PepT1 to increase production of hPepT1 (3H) Gly-Sar. Synopsis: PepT1 (Intestinal apical di-/tripeptide transporter)**



**Fig. 3: Role of hPepT1 (Intestinal apical di-/tripeptide transporter) in promoting growth of colonocyte to increase defence mechanism**

#### Modification of immune response by probiotic strains

Several studies were conducted in which mucus samples from person suffering with Crohn's disease were collected and cultured with probiotic strains such as *Lactobacillus casei*, *L. Crispatus* and *L. Bulgaricus* and *E. coli* and some samples are incubated alone. Observation shown that samples cultured with *L. casei* and *L. Bulgaricus* had reduced number of TNF- $\alpha$  and CD4 cells expression as compared to *L. Crispatus* and *E. coli*. This signifies the interaction of probiotics with immunocompetent cells to decrease the production of pro-inflammatory chemokines and cytokines [29].

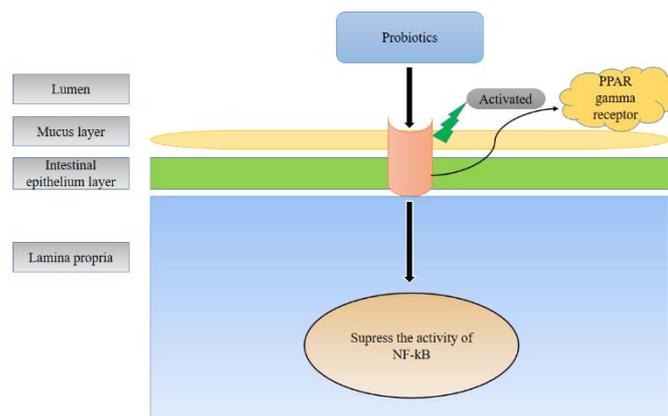
#### Role of TLR signalling in limiting intestinal inflammation

TLR (Toll-like receptors) act as pathogen recognition which is responsible to initiate the inflammatory immune response which will in turn help in destroying the foreign pathogens. TLR9 signalling helps in regulating the anti-inflammatory effect of probiotics. Evidences proved that peripheral blood T cells (PBT) cell cycling and

apoptosis is affected by *E. coli* Nissle (demonstrated by Strum *et al.* 65). When PBT consisting of anti-CD3 factors incubated with *E. coli* Nissle it causes the inhibition of cell cycling of PBT, cause in downregulation of Cyclin D2, B1 and expression of retinoblastoma which play a major role in downregulation T cell proliferation, IL2, TNF- $\alpha$ , INF- $\gamma$ , and upregulation of IL10 in PBT. *E. coli* Nissle is responsible for inhibition of proliferation of PBT which is TLR2 dependent [30].

#### Modulation of inflammatory response by PPAR (proliferator activated receptor pathway)

Colitis is inhibited by PPARc pathway by deactivating the activity of NF-kB. It's increased by *Bacteroides thetaiotaomicron*, RelA export from the nucleus through PPARc dependent anti-inflammatory mechanism is a subunit of NF-kB. PPAR forms a complex with RelA and when *B. thetaiotaomicron* come in contact with this complex it will undergo redistribution which is cause of IBD treatment [31-33].



**Fig. 4: Probiotic mechanism in order to modulate activation of peroxisome proliferator activated receptor (PPAR gamma) pathway. Synopsis: NF-kB (Nuclear Factor kappa B), PPAR gamma (Peroxisome proliferator activated receptor gamma)**

#### Necrotising enterocolitis

The premature infants which are nearly less than 32-34 w, develops a devastating gastrointestinal disease named 'Necrotising Enterocolitis' which severely effects the intestine of such infants. The term necrotising enterocolitis happens when the tissues of the intestine gets ruptured. Clinical studies suggest that this disease usually affects 1%-5% out of 2000-4000 infants [34]. It has been

also observed that this disease is very rarely found in fully matured infants. The premature infants generally are weaker as compared to the fully matured infants, so there are more chances of them to get affected by this disease [35].

The proper validated cause of this disease is still not known but several assumptions and observations have been made which determines the reasons of premature infants being majorly affected

by this severe disease. Instead of feeding premature infants with breast milk, they are provided with formula feeding which has been considered as the major cause of ill development of the infant in terms of immunity. The premature infants have weaker organs as compared to those of fully developed babies. Thus any decrement in oxygen supply to the organs especially in lungs and intestine can progressively result in damaging the linings of intestine. The damaged walls further help in the bacterial invasion in which the linings of the wall of the intestine is attacked by a number of bacteria which primarily causes local infection and later on causes irritable inflammation in the intestine of the infant. This invasion thus leads to the perforation of the intestinal walls and also the spillage of stool into the abdomen region of the infected infant [36].

#### **Study related to probiotics potential against necrotising enterocolitis**

The good bacteria i.e. the Probiotics have been considered from last few years as a good alternative source for helping in the reduction of necrotising enterocolitis. Meta-analysis of some reviews shows that there is a gradual declination in the rise of stage II NEC after supplementing the patients with probiotics. Several animal models have also been studied for knowing the proper mechanism, benefits and effects of utilizing probiotics in such cases [37].

Systematic reviews of such sample studies which were conducted on a small scale gave a brighter knowledge about the use of probiotics more precisely and strongly.

Probiotics have been widely being used for trials and studies for knowing its effect on premature infants. A number of bacterial strains like of bifidobacterium and lactobacillus were extensively used for studying. These bacterial strains were considered because of their abundance in infants. It was observed in studies that the bifidobacterium was less abundant in premature infants who were feeded without breast milk. This happens because the human milk oligosaccharide is more and more consumed by bifidobacterium which results their abundance in the fully matured infants as of compared to the premature ones [38].

A cumulative study represented a report which was considered as the largest trials on probiotics in term of knowing its effect on necrotising enterocolitis. A total of 1315 infants were kept under surveillance in a PiPS trial. In this study it was noted that no harm was seen by the use of probiotics, so there were less chances of risk for the treatment [39].

Several interventions were kept under observation to know the effect of probiotic. A total of 35 trials were conducted on 10,520 patients where it was seen the difference in the condition of necrotising enterocolitis by dividing some of them with treatment of probiotics while some of them were deprived of it. The result showed mixed results [40]. So later it was described that for having a positive impact of probiotics in the prevention of necrotising enterocolitis there must be proper choices of product with keeping in mind of certain specific parameters which includes low risk factors. To give a detailed view of this a survey report in an article was conducted on for 16 different products of probiotics which was being used for this purpose in US neonatal intensive unit cares (NICU). The reports of this survey compelled the authors to declare that there was a huge dominance of the products which were having common bacterial strain preparation of Lactobacillus rhamnosus or lactobacillus reuteri of about 50% of its positive results [41].

Also, a cohort study of France revealed that there was tremendous decrease in the cases of necrotising enterocolitis after treating the infected infants with these lactobacillus strain labelled products [42].

#### **Mechanism of action of probiotics NEC in premature infant**

There are number of *in vitro* studies including mechanism occurring in the gut, specific to commensal bacterial strain in order to prevent NEC disease in preterm infant [43].

#### **Up-regulating the cytoprotective gene expression**

Commensal bacterial strain named as Bacteroides thetaiotaomicron regulate the mRNA expression of gene related to gastrointestinal function which included improvement in nutrient absorption capacity of the host and processing. Establishment of colonies of Bacteroides thetaiotaomicron showed the increase ileal Na/glucose co-transporter (SGLT-1) mRNA expression, after the administration of B. thetaiotaomicron in the host led to the induction of immunoglobulin A (T-cell independent) producing B-cells by increasing the expression of polymeric immunoglobulin receptor (pIgR) that helps in transportation of IgA covering epithelium lining and provide coherency, increases host capacity towards metabolization of xenobiotic and endotoxin, Colonization of this bacteria up regulate the mRNA of angiogenin-3, specifically in intestinal crypt epithelium [44].

#### **Reducing the mRNA expression of pro-inflammatory molecules**

Commensal bacteria such as Lactobacillus rhamnosus GG that help in blocking of inflammatory responses produces in intestinal epithelia by generating reactive oxygen species (ROS) that responsible for inhibition of NF-kappaB through oxidative deactivation of modulate Ubc12 enzyme and TNF-alpha that was also included in switching on of NF-kappaB, ROS generation by the LGG *in vitro* and *in vivo* in intestinal epithelium layer. From the study it was suggested that Salmonella Strain (non-virulent) provide tolerance towards pro-inflammatory stimuli by blocking (Inhibitor kappaB-alpha/NF-kappaB) through the phosphorylation and polyubiquitination of IkappaB-alpha and led to its degradation [45].

#### **Synthesis of butanoate and short chain fatty acids metabolites**

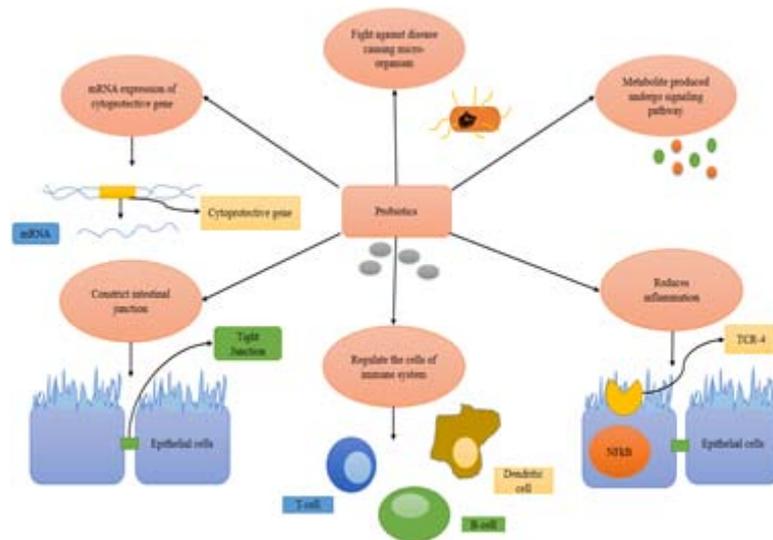
Synthesised metabolite helps in nourishment of epithelial cells of colon and decreases the pH and oxygen stress in intestinal lumen which led to the retardation of growth of bacteria e. g. clostridia and enterobacteria, it also decreases the intestinal inflammatory calprotectin and increases IgA. Number of studies support probiotic bacterial strain that improved the integrity of intestinal epithelial barrier by restricted the diffusion of small solutes, macro-molecules and endogenous toxins and regulate the expression of intestinal mucosa related factor which are involved in modulation and maintenance, which include TLR4 (Toll-like Receptors 4), TLR9, mucin and trefoil factor (TFF) and antimicrobial polypeptide molecules [46].

#### **Maturation of intestinal barrier and its proper functioning**

Previous report show that commensal bacteria mainly LGG colonization in intestinal region promote intestinal barrier coherency and its maturation by inducing the mRNA level of claudin 3 by introduction of LGG either live or non-live formulation as probiotics. Study based on MyD88-/-mice show that TLR2 perform essential role in tight junction protein (TJ protein) expression and promote maturation of intestinal barrier through TLR signalling pathway. As seen in premature infant affected with NEC, study related to this suggested that claudin 3 ubiquitous TJ protein transcriptionally up-regulated during the phase of postnatal growth [47].

#### **Regulate immune response through stimulation of dendritic cells**

Probiotics helping in activation of T-cell activation and their balancing of Th1:Th2. Two commensal bacteria in order to treat clinical NEC named as L. lactis and B. infantum able to alter mRNA level of gene responsible for innate immunity and at same instance decreases the IL-8 expression. Commensal bacteria perform a crucial function in balancing of T-helper cells. In intestinal region, undeveloped dendritic cell (DCs) localised in the lamina propria extend the appendages in between enterocytes goes into intestinal lumen. These appendages expose TLR-2,4 through these receptors commensal bacteria interact, immature DCs cell converted into mature DCs that provide the surrounding favourable for maturation and balance of Th1 and Th2 cells [48].



**Fig. 2:** This fig. shows number of mechanism that probiotic undergo to prevent NEC in premature new born and improve the gastrointestinal immaturity. Synopsis: TLR4 (Toll-like receptor 4), NFkB (Nuclear Factor Kappa B)

## CONCLUSION

Probiotics serves a potential source for treatment of various diseases. Studies which were conducted on Mice, rats or other animal models given us positive results which states that probiotics has prominent effect on treatment of various diseases related to gastrointestinal tract. Along with this one major advantage of probiotics is that, they do not possess any significant side effects. They play an important role in decreasing the time duration of infection in the body and its susceptibility.

The major evidence is that probiotics are strain specific that means specific strain is responsible for treating specific disease. In case of inflammatory bowel disease, lactobacillus plantarum, lactobacillus bifidobacterium and lactobacillus rhamnosus shown prominent effect for its treatment. Moreover, the working mechanism of every probiotics and health conditions caused by them are different in each case.

In case of necrotizing enterocolitis, studies of Meta analyses have shown decrease in NEC Stage 2, when administered with probiotics. Many studies across the world have proven evidence of success when administered with prophylactic probiotics. In this review, different mechanism of working of different probiotics in case of NEC and their therapeutic efficiency has proven. Cluster randomized clinical trials comparing commercially available probiotic strains sufficiently determine the difference on NEC. Researches have proven potential side effects possess by nutritional supplements and hence banned them. There is a wide scope of probiotics to be one of the finest therapy to treat various gastrointestinal disease. Moreover, the focus of study is shifted to properties, functions and composition of probiotics.

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Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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