SIX MONTHLY PROBIOTIC NEWSLETTER

# Probiotic Association of India

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## From Editor's desk

**Dear Esteemed Members,**

**Warm greetings!**

Again exhilarating time has come for Probiotic Association of India to organize its much awaited 4th Biennial Conference of PAi and International Symposium on "Probiotic Therapy: Translating to Health and Clinical Practice" on 16th& 17th February, 2018 at AIIMS, New Delhi, India for which our Readers have been anxiously waiting. I am pleased to inform you that this time, it is being organized in association with Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi and American Association for Microbiologist (ASM) under the dynamic leadership of Dr. Rama Chaudhry. Some of the eminent speakers from USA, Europe, Australia, Italy and India have already accepted to deliver talks during the conference which includes Prof. Mary Ellen Sanders, Prof. Dan Merenstein, Prof Pinaki Panigrahi, Dr John Rossen, Dr. Sanjay Patole, Prof. Claudio De Simone, Dr Dinesh Chandel, Prof S K Brahmachari, Prof. G.B.Nair, Dr. Y. S. Shouche and many more. We look forward for your active participation in the conference.

We are expecting around 400 participants from different parts of India and abroad representing various research institutes, academic organizations and representatives from the industry. We sincerely hope that you all will have a wonderful time at the two days deliberations which will enrich you with knowledge and new developments currently witnessed in probiotic research from global perspective. Hence, we look forward to your active participation. We have tried to accommodate all articles submitted by our readers. I am sure you will like the contents of this issue of the PAi newsletter which will provide you wealth of latest information on probiotic research. We always look forward to your valuable suggestions to further improve the quality of our newsletter. We also expect general articles, new scientific breakthroughs in probiotic research, launch of new probiotic formulations and any other useful information related to probiotics from all our members. Please keep sending us your inputs to make this newsletter embedded with treasure of scientific knowledge on probiotics and gut microbiota. I would like to thank Dr. Shalini Sehgal who has dedicatedly worked to bring this 13th issue of PAi Newsletter.

**Wishing you all a wonderful, pleasant and healthy time ahead!**

**(Sunita Grover)**
**Chief Editor**

**A breakthrough therapy for prevention of sepsis in neonates by Panigrahi *et al* (2017). Dr. Rama Chaudhry, the Organising Secretary of our forthcoming PAi conference at AIIMS, New Delhi is one of the co-authors of this very important study carried out in India**

# A randomized synbiotic trial to prevent sepsis among infants in rural India

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**Sepsis in early infancy results in one million annual deaths worldwide, most of them in developing countries. No efficient means of prevention is currently available. Here we report on a randomized, double-blind, placebo-controlled trial of an oral synbiotic preparation (*Lactobacillus plantarum* plus fructooligosaccharide) in rural Indian newborns. We enrolled 4,556 infants that were at least 2,000 g at birth, at least 35 weeks of gestation, and with no signs of sepsis or other morbidity, and monitored them for 60 days. We show a significant reduction in the primary outcome (combination of sepsis and death) in the treatment arm (risk ratio 0.60, 95% confidence interval 0.48–0.74), with few deaths (4 placebo, 6 synbiotic). Significant reductions were also observed for culture-positive and culture-negative sepsis and lower respiratory tract infections. These findings suggest that a large proportion of neonatal sepsis in developing countries could be effectively prevented using a synbiotic containing *L. plantarum* ATCC-202195.**

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## Anti-diabetic effect of probiotic *Lactobacillus fermentum* MTCC: 5898 in streptozotocin induced diabetic rats

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The progressive increase in the prevalence of diabetes constitutes a global public health problem as it is one of the major causes of mortality in the world. Pharmacological agents that effectively reduce blood glucose levels are available for the treatment of diabetes; however; they are expensive and are known to have side effects sooner or later. Consequently, attempts are being made to develop alternative dietary ingredients that can manage blood glucose levels. Supplementation of the diet with fermented dairy products or foods containing bifidobacteria and lactic acid bacteria have been reported to lower blood glucose levels. It is important to identify probiotic strains that exhibit significant anti-diabetic effects since the effects are strain specific. The antidiabetic effect of *Lactobacillus fermentum* (LF) MTCC 5898 was studied on streptozotocin induced diabetic rats. Six weeks of administration of probiotic fermented milk to diabetic rats caused decrease in the fasting blood glucose levels (FBG), glycylated hemoglobin ($HbA_{1C}$) levels and

and improvement in the insulin levels. LF fermented milk also found to alleviate hyperlipidemia by significantly decreasing serum total cholesterol (TC), triacylglycerol (TG), low density lipoprotein levels (LDL-C), atherogenic index (AI) and coronary artery risk index (CRI) in diabetic rats. Since high glucose levels in diabetes is responsible for oxidative stress and concomitantly imbalance in the activities of antioxidative enzymes (SOD, CAT and GPx) were also observed towards normal in liver and kidney of diabetic rats. But feeding of LF fermented milk helped in protection against oxidative imbalance by increasing the antioxidative enzymes activities. In addition to oxidative stress, the inflammatory response is the main reason for diabetes associated complications. The present study depicted that levels of inflammation markers, TNF-α and IL-6 in serum of rats decreased substantially which were fed LF fermented milk. Thus, LF fermented milk has therapeutic potential due to its anti-oxidative and anti-inflammatory properties and can be advocated to reduce the risk of diabetes and its associated complications.

## Probiotics and Female Health

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Feminine reproductive health is crucial for her well-being as well as reproduction and health of neonates. Certain microbes not only impede conception and birth of healthy baby, but can also affect maternal-fetal health, and may severely perturb the feminine reproductive health. Among the alternative strategies to prevent microbial pathogenesis, the probiotics and their metabolites have emerged as potent biotherapeutics. The probiotics have potential for preventing various genitourinary infections and consequently reduce the fetal and neonatal infections. Efforts are now underway to better understand the elemental role of human microbial communities in health and disease. While the population structure and mechanisms of human symbionts are only now being unveiled, potential health attributes are suggested by manipulating and modulating microbial functional ecology with the use of probiotics.

Over 250 species of bacteria have been detected in the vagina using genomic sequencing. *Lactobacillus iners* and *L crispatus* dominate in most women who have a clinically healthy status. Probiotics are useful for more than just gastrointestinal health. In fact, there are specific probiotic products that can prevent and treat female urogenital conditions like bacterial

vaginosis, vulvovaginal candidiasis, urinary tract infections and related complications of all three. The microbiota typically found in the vagina consists of large numbers of lactobacilli (Gram-positive rods) and small numbers of Gram-negative rods and Gram-positive coccobacilli. A milliliter of vaginal fluid contains, on average, around 100 million microorganisms from 5-10 species, 95% of which are from the genus *Lactobacillus* (Anukam,2009). It has been a long held belief that lactobacilli, in bulk, promote vaginal health by helping to maintain an acidic pH in the vagina as increased vaginal pH and decreased numbers of lactobacilli are symptomatic of various infections ergo lactic acid producing lactobacilli likely prevent infection by maintaining a low vaginal pH. This rationale has led to recommending the consumption of yogurt with the expectation that the lactobacilli particularly *L. acidophilus* and other "active cultures" should promote vaginal health. Most lactobacillus strains produce lactic acid, but only some strains produce bacteriocins and other specific regulating factors that inhibit the adhesion, colonization, growth and survival of undesirable species. Such specific factors can have prominent effects even at very low concentrations and the strains that produce them can be present in minuscule amounts, but still have a large effect on urogenital health.

Bacterial vaginosis (BV) is the most common vaginal infection and is believed to be a problem for roughly 10-29% of the female population at any given time (Allsworth, 2007). Estrogen are low at the beginning and end of the menstrual cycle or after undergoing menopause which increase the risk of infection even in healthy women. Increasing numbers of physicians are recommending that their patients follow a regimen of antibiotics with a course of probiotics for gastrointestinal health, to bolster beneficial bacteria that have been killed off. The same advice applies to urogenital probiotics. The vaginal microbiota is just as susceptible to broad-spectrum antibiotics as the intestinal microbiota. And although there is not any solid evidence to suggest probiotics alone are effective as treatment for an existing infection, probiotic supplementation can provide dividends before, during and after antibiotic treatment. Some probiotic strains can even improve the effectiveness of BV treatment with antibiotics.

Awareness about BV is particularly important because it can cause health concerns that are more serious than the immediate symptoms that women tend to notice: irritation, decreased

well-being, and diminished sexual health. BV can lead to local inflammation, increased susceptibility to sexually transmitted infections and a heightened risk of preterm labor in pregnant women. Despite medical science not having a good grasp of what causes preterm labor, it has been known for some time that there is a correlation between the incidence of BV in an expectant mother and preterm delivery. As mentioned above, antibiotics used to treat BV or other conditions can cause complications during pregnancy and disrupt the microbiota of the mother. Disruption of the microbiota is a concern because transmission of endogenous bacteria from mother to newborn occurs during birth and shortly thereafter, helping to establish the newborns own microbiota and immune system. Because of this association, the use of antibiotics to treat pregnant women has drawbacks. It has previously been hypothesized that probiotic therapy could help eliminate the conditions that cause preterm labor and hence avoid preterm labor itself (Reid, AJOG, 2003).

In case of women, the concentration of microbes inhabited in the vagina is at the concentration of approximately $10^{7-8}$ CFU per ml of fluid. The dominant species in healthy subjects is lactobacilli while in infected patients is urinary and vaginal pathogens. Other major health targets where probiotics are involved are:

- Immune enhancement
- Diarrohea
- Vaginal infections
- Colon cancers
- Allergy development
- IBD

The field of probiotics is rapidly growing with concomitant increase and developments in the research, and commercial sectors. As this field advances, new types of strains with other crucial benefits not yet explored would be identified.

**Table 1: List of Probiotic microbial strain and usage**

| Disease | Strain |
|---|---|
| Gastroenteritis | *Lactobacillus casei (*Yamada *et al.,* 2009; Kochhar, 2000; Kochar, 2014) |
| Urinary tract infection | *Lactobacillus rhamnosus GR-1, Lactobacillus reuteri RC-14* (Anukam *et al.*, 2009) |
| Lactose intolerance | *Lactobacillus acidophulus* (Hawrelak, 2003) |
| Immunity | *Bacillus circulans PB7*, *Lactobacillus plantarum DSMZ 12028 (*Bandyopadhyay and Das Mohapatra, 2009) |
| Food allergies | *Escherichia coli (*Lodinova-Zadnikova *et al.,* 2003) |
| Eczema | *Escherichia coli*, *Bifidobacterium bifidum, Bifidobacterium lactis Lactococcus lactis* (Niers *et al.,* 2009) |
| Irritable bowel syndrome | *Bifidobacterium infantis 35624*, *Escherichia coli DSM17252 (*Brenner and Chey, 2009) |
| Crohn's disease | *Escherichia coli strain Nissle 1917 (*Boudeau *et al.,*2003) |
| Traveler's diarrhea | *Lactobacillus GG*, *Lactobacillus plantarum* (Hawrelak, 2003) |

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# Eat for your Gut Microbiome and Choose MAC diet! – Your Health is guaranteed and insured too!

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Diet plays an important role in shaping the structure and function of the gut microbiota. The microbes and microbial products in turn can influence various aspects of host physiology. One promising route to affect host function and restore health is by altering the gut microbiome using dietary intervention. We are what we eat and that's why everyone is prescribed for healthy food choice. Do you know the food we eat plays an essential role in maintaining the richness and function of our gut microbiota (trillions of bacteria that live within our digestive tract) and thereby dictates our health and keeps us disease-free?

**Want to be Healthy? – Choose MAC diet:** Much of the carbon and energy for members of the microbiota originate from plant- and animal-derived dietary carbohydrates and plant fibres (resistant to degradation and absorption by the host). Microbial competition within the gut is intense for metabolic access to the energy and carbon sequestered in these molecules. The complex carbohydrate portion of dietary fibre that can be metabolized by gut microbes, were proposed to be referred by the term "microbiota-accessible carbohydrate" (MAC). MACs serve as selective agents, altering the composition of the microbiota, but also dictate the functionality and metabolic output. Short-chain fatty acids (SCFAs), namely, acetate, butyrate, and propionate, are released by gut bacteria during fermentation of dietary fibers; they operate through specific receptors on the host and offer several metabolic benefits. When we adapt to MAC-deprived diet, we also lose the metabolic benefits of gut-microbiome derived metabolites.

**It's true – Modern Society consume MAC-deprived diet:** Modern diet consists of heavily processed foods, rich in fat, sugar, protein, and a variety of additives, while remaining low in micronutrients and dietary fiber. The recommended daily intake of dietary fiber is at least 30 g, although, on average, those on the Western diet only consume 15 g. People in traditional societies (for example, Hadza hunter-gatherers of Tanzania) where fiber intake can reach 50–120 g/day, are associated with a much more diverse gut microbiota when compared with people in Western countries. A diverse microbiome (microbiome richness) is associated with "good health," while low

diversity and micrbiome dysbiosis have been correlated to several non-communicable diseases including type 2 diabetes.

Low consumption of MACs over generations leads to the complete disappearance of beneficial bacterial strains in a preclinical mice model study. If we extrapolate this finding, this seems to suggest that long-term-reduced MAC consumption over generations will likely to have detrimental effects in humans. *The message is clear* – Choose MAC diet, if needed combine dietary and probiotic interventions, keep your gut microbiome happy; they in turn make you happy, healthy and keep you free from lifestyle diseases.

## Dietary metabolites derived from gut microbiota: critical modulators of epigenetic changes in mammals

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The mammalian gastrointestinal tract harbours trillions of commensal microorganisms, collectively known as the microbiota. The microbiota is a critical source of environmental stimuli and, thus, has a tremendous impact on the health of the host. The microbes within the microbiota regulate homeostasis within the gut, and any alteration in their composition can lead to disorders that include inflammatory bowel disease, allergy, autoimmune disease, diabetes, mental disorders, and cancer. Hence, restoration of the gut flora following changes or imbalance is imperative for the host. The low-molecular-weight compounds and nutrients such as short-chain fatty acids, polyamines, polyphenols, and vitamins produced by microbial metabolism of nondigestible food components in the gut actively participate in various epigenomic mechanisms that reprogram the genome by altering the transcriptional machinery of a cell in response to environmental stimuli. These epigenetic modifications are caused by a set of highly dynamic enzymes, notably histone acetylases, deacetylases, DNA methylases, and demethylases, that are influenced by microbial metabolites and other environmental cues. Recent studies have shown that host expression of histone acetylases and histone deacetylases is important for regulating communication between the intestinal microbiota and the host cells. Histone acetylases and deacetylases influence the molecular expression of genes that affect not only physiological functions but also behavioral shifts

that occur via neuroepigenetic modifications of genes. The underlying molecular mechanisms, however, have yet to be fully elucidated and thus provide a new area of research. The present review provides insights into the current understanding of the microbiota and its association with mammalian epigenomics as well as the interaction of pathogens and probiotics with host epigenetic machinery.

**Source: *Nutrition Reviews* (2017), 75 (5) 374-389.**

## Potential probiotic yeasts isolated from Indian fermented foods

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The occurrence of yeasts has been reported in a number of Indian fermented products like idli, dosa, jalebi, warries, pappad, kanji, fruit juices, bakery products, brewery products and milk products like curd and cheese etc. Out of the several yeasts isolated from fermented products, *Saccharomyces cerevisiae* according to EFSA (Europian Food Safety Authority) has a QPS (Qualified Presumption of Safety) status (Parma, Italy 2005) and is the most common yeast used in food fermentation. *Saccharomyces cerevisiae* var *boulardii* is practically the only yeast commercialized as probiotic in human medicine and has been reported to be efficacious in the prevention or recurrence of different types of diarrhoea and colitis in humans and also has been found effective in the treatment of acute diarrhoea in children and other gastrointestinal disorders.

In this investigation, idli and jalebi batter were used as the source for isolation of yeasts. The medium used for isolation was malt extract-yeast extract-peptone-glucose agar (MYPG). The plates were incubated at 30ºC for 24-48 hrs. The isolates were tested for the probiotic attributes such as acid tolerance test, bile salt tolerance test, comparison of growth at 30 and 37ºC, antibiotics tolerance test, cell surface hydrophobicity, inhibitory action against enteric pathogens, enzyme based screening- β- Galactosidase, phytase, L-asparaginase, protease, amylase and lipase activities, Vitamin $B_{12}$ assay, exopolysaccharide production and cholestrol reduction assay. Safety assessment was carried out by gelatinase activity and DNase production Test. Identification of potential isolates of selected yeast isolates werecarried out by BioLog kit (IMTECH, Chandigarh) on the basis of phenotypic characterization.

Probiotics need to survive the inevitable biological barriers of gut. The primary barrier of microorganisms in the stomach is the gastric acidity (pH of 1.5-3.5). Besides the strong acid condition in the stomach, the probiotic microorganisms taken orally have to defend against the bile salts in the gastrointestinal tract. In this investigation, Id 9,11,14,15 and 18; J15 and 18 exhibited high acid and bile salt tolerance. A probiotic organism must also be able to tolerate and grow at human body temperature of 37ºC and so were these selected seven isolates. Most of the probiotic microorganisms are bacteria and many of them are not able to resist or tolerate these antibiotics, whereas, yeasts have a natural resistance against these antibiotics and can be used for patients undergoing antibiotic treatment .

Apart from tolerating the upper gastrointestinal transit, another challenge for an effective probiotic is to adhere to small intestinal cells. In this investigation, all of the yeast isolates showed high autoaggregation ability, which was observed to be upto 94 % after 3 hrs of incubation at 37 °C and after 20 hrs it was observed to be higher than 95 % for all the isolates. Strains possessing high hydrophobicity exhibit good adhesion property to intestinal cell lines. The cell surface hydrophobicity of 86.14 %, observed for J 18 in this investigation, is higher than the hydrophobicity reported till now. One of the most desirable properties of probiotic yeasts is the antibacterial activity of yeasts against pathogens that penetrate various mucosa sites. Therefore, the effect of the isolated yeast strains on the growth of selected food borne pathogens was determined and antimicrobial activity was observed against *E. coli*, *Staphylococcus aureus*, *Pseudomonas* sp., *Vibrio* sp. and *Salmonella* sp. In this investigation, all of the seven isolates were phytase producers. Present market trends clearly indicate that there is an increasing demand for phytase as a feed supplement to improve feed intake and utilization. The enzyme L-asparaginase (E.C.3.5.1.1) is used as a therapeutic agent in the treatment of acute lymphoblastic leukaemia, that depletes tumor cells of L-asparagine and the cells die because of their inability to synthesize this amino acid. Very few types of yeast have been reported to produce this enzyme and was produced by Id 9, Id 15 and J 15 strains in this study.

Yeasts are a source of vitamins, especially B-complex including B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B9 (folic acid), and H or B7 (biotin). All of the tested yeast isolates were observed to be positive for Vit $B_{12}$ production.

Extracellularpolysaccharides (EPS) producing probiotic cultures can contribute to human health by positively affecting the gut microflora. All of the strains tested positive for EPS production and can be used as immune-stimulatory adjuvants.

As a feed additive, probiotic yeast *S.cerevisiae* var. *boulardii* has been reported to reduce serum cholesterol in animals. In this study, all of the yeast strains were found to remove cholesterol, with the J18 strain showing the highest potential to assimilate cholesterol. So the results of this investigation can be considered as promising.These yeast isolates do not produce DNase or gelatinase which indicates that they are safe to be used as a probiotic in food and feed.These isolates were identified as *Saccharomyces cerevisiae*, *Candida tropicalis*, *Aureobasidium* sp. and *Pichia manschuria*.

Through this research we have isolated yeasts with potential probiotic properties. They could be prescribed to patients on prolonged antibiotic treatment, protect against food borne enteric pathogens. They have the ability to degrade anti-nutrients such as phytic acid and tannic acid, thereby, improving the nutritional status of food. They can hydrolyze lactose, thus prove beneficial to lactose intolerant patients. They also produce L-asparaginase which has anticancer property. These yeast isolates could aid in digestion because of their ability to produce lipase and protease. Enhance vitamin $B_{12}$ pool due to its production by these isolates. They can be used as immune-stimulants. Their ability to assimilate cholesterol could be of immense value to patients suffering from hypercholesterolemia. These features indicate that these yeasts could be promising probiotic agents in future and can be widely used as food and feed supplements after studying fucntionality in vivo using animal models.

## Probiotic effectiveness for inflammatory bowel disease

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Human health strives with numerous gastrointestinal disorders which cause severe damages, which leads to abnormal organ function. The inflammatory bowel disease (IBD) is one of the gastrointestinal disorders. This IBD is the collective term for a group of intestinal conditions typified by ulcerative colitis (UC) and Crohn's disease (CD). IBD is characterized by uncontrolled inflammation in the gastrointestinal tract, which has been shown to predispose to the development of colorectal cancer later stage.

Crohn's Disease is one the inflammatory condition in gastrointestinal track which can occur in any region of the gastrointestinal tract and is characterized by transmural, granulomatous inflammation. CD is a Th1-driven immune response, characterized initially by increased interleukin-12 (IL-12) expression, followed by interferon gamma (IFN-γ). In contrast, Ulcerative Colitis is believed to be a Th2 immune response, leading to increased production of pro-inflammatory cytokines including IL-4 and IL-5. Ulcerative Colitis is restricted to the colon and generally begins in the rectum and spreads proximally, dependent upon disease severity. T helper cells are a type of T lymphocyte, which play a major role in cell mediated immunity. Two types of T helper cells are responsible of IBD. In CD, T-helper 1 (Th1) cells secrete the cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), while in UC, Th2 cells secrete interleukin-4 (IL-4), IL-5, and IL-13. In addition, Regulatory T ($T_{reg}$) cells exist that produce transforming growth factor-β (TGF-β) and IL-10, respectively. The cytokines themselves play the most critical role in T helper cells induction. Two pivotal cytokines that control Th1 and Th2 differentiation are IL-12 and IL-4, respectively. Cytokines secreted by Th1 and Th2 cells such as IL-4, IL-5, IL-6, IL-13, IL-9, IL-2 are pro-inflammatory which induces the inflammation in cells.

Schematic representation of IBD is shown in Fig.1. Figure illustrates that the intervention of antigen causes the T helper cell response which leads into secretion of pro-inflammatory cytokines. These pro-inflammatory cytokines cause tissue damage and inflammation in the cell. Descriptive mechanism is explained below.

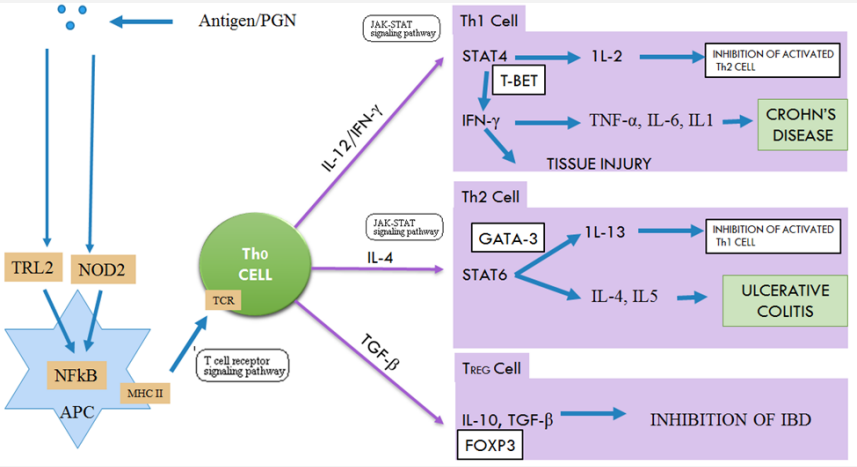


**Fig.1 Schematic representation of helper T cell response to the antigen during IBD**

**General mechanism-** When the antigen enter in to the intestine, it will be recognized by the toll like receptor (TLR). Antigen presenting cell (APC) presents the cell into the site. Depending upon the antigen, type of the TLR will activate. This TLR receptor activates NFkB (NF-kB is a protein complex that controls transcription of DNA, cytokine production and cell survival) in APC. In the presence of MHC II, the TCR (T cell receptor) activates the $Th_0$ cell (naïve cell), which is T cell Receptor signaling pathway.

**Mode of action: Crohn's Disease-** In case of Crohn's disease, from $Th_0$ cell IL-12 cytokine activates the Th1 cell (T helper cell 1). This activates STAT4 (Signal transducer and activator of transcription 4, a transcription factor belonging to the STAT protein family). This secretes IFN-γ which leads into production of cytokines such as TNF-α and IL-6 and direct tissue injury. The secreted cytokines leads into Crohn's disease. Additional cytokine IL-2 is produced and this cytokine will act as inhibitor for activated Th2 cell. Transcriptional factor in Th1 cell is T-BET.

**Mode of action: Ulcerative colitis**

In the case of ulcerative colitis, IL-4 cytokine will activate the Th2 cell (T helper cell 2) from $Th_0$ cell. This activates STAT6 protein, which also activates transcriptional factor GATA-3. This activated protein secrets cytokines IL-4 and IL-5 which leads into ulcerative colitis and additional cytokine IL-13 will act as inhibitor for the activated Th1 cell. Balance of Th1 and Th2 cells plays a major role in an organism. This balance will be maintained by Th cells by its own by inhibiting each other.

**Activation of $T_{reg}$ cell (Regulatory T cell)**

Regulatory T cell ($T_{reg}$ cell) which has anti-inflammatory reaction takes place to inhibit the Th1 and Th2 cells. TGF-β cytokine activates the $T_{reg}$ cells from naïve cell. Cytokines produced by $T_{reg}$ cells will inhibit the cytokines which are responsible for inflammation. FOXP3 is a transcriptional factor which secrets IL-10 and TGF-β. IL-10 is the major anti-inflammatory cytokine which reduces the inflammation, and as a result also leads to reduction of inflammatory bowel disease symptoms. The gut microbial environment has been shown to play a role in the development of IBD, hence targeting of the microbiota presents an option for therapeutic intervention. One potential method to manipulate the intestinal microbiota in an attempt to reduce the inflammatory response is via the administration of probiotics.

**Probiotic: Mode of action**

Probiotics in the gastrointestinal tract decrease adhesion of both pathogens and their toxins to the intestinal epithelium. Also, probiotic-derived antibacterial substances block intestinal pathogenic bacterial effect, enhance host innate immunity, suppress the pro-inflammatory cytokine production and increases anti-inflammatory cytokine production. Probiotics regulate intestinal epithelial cell functions such as barrier function, production of cyto-protective substances and prevention of cytokine-induced apoptosis. In IBD, the probiotic bacteria decrease the adhesion of pathogens and the antibiotic properties which eliminate the pathogen which causes inflammation. The useful probiotic bacteria induce the anti-inflammatory cytokines such as IL-10, which inhibits the pro-inflammatory cytokines and leads to amelioration of IBD.

Many studies have been performed using probiotic on Inflammation. Published literature shows that *L. plantarum* 299v reduced IL-12 and IFN-γ production. Studies demonstrates a role for *Lactobacillus reuteri* in prevention of colitis in IL-10 mice. *L. salivarius* was also shown to modify the intestinal microflora where *C. perfringens*, coliforms, and enterococcus levels were significantly reduced. The efficacy of *L. salivarius* UCC118 and *Bifidobacterium infantis* 35624 in attenuation of colitis in the IL-10 mouse model has been demonstrated. Reduced inflammatory scores and reduced production of pro-inflammatory cytokines have been observed in IL-10 mice that had been injected subcutaneously with *L. salivarius* UCC118. *L. acidophilus* La-5*, L. delbruckii* subsp. *bulgaricus, Bifidobacterium* Bb-12 and *Streptococcus thermophilus* significantly reduced inflammation in HLA-B27 model. As a preventive strategy, challenging probiotic *Bacillus* spp alone or in combination with the bifidobacteria has not been attempted. Employing these probiotics could help in better gastro-intestinal homeostasis including therapeutic means for IBD treatment.

**Our initiative**

In our lab, we have attempted to characterize the anti-inflammatory properties of native probiotic *lactobacillus* spp*. in vitro* in cell line and animal models and found *that Lactobacillus fermentum* as well as *L. plantarum* has potential ability to induce anti-inflammatory cytokines. Besides, we have evaluated the potentiality of spore-forming *Bacillus* spp. for probiotic properties. Our native isolate of probiotic *Bacillus* sp. and

Bifidobacteria is being evaluated to study the mechanism of IBD reduction. *Bacillus* spp is a spore forming bacteria which has the better efficiency to survive in harsh gut system with low pH, acidic condition. In addition, our native isolates of *Bacillus* spp possess antimicrobial properties which can eliminate the pathogens causing IBD. Bifidobacteria is a dominant gut microflora, which has a major impact on development of immune system. Bifidobacteria though reported in reduction of IBD, molecular mechanism need to be investigated. We aimed to develop the native isolates of probiotic cultures to suppress IBD related disorders.

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## Harmful Bacteria beware, here comes thy enemy – the Probiotics

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Probiotics are defined as dietary supplements that contain live non-pathogenic microorganisms, which when administered in adequate amounts can be beneficial in the treatment and prevention of pathological conditions. Microorganisms constituting probiotics are mainly bacteria, similar to the beneficial bacteria that occur naturally in the human gut. Many genera of bacteria, such as *Lactobacillus*, *Enterococcus*, *Bacillus*, *Escherichia*, and *Bifidobacterium* have been used as probiotics due to their beneficial effects. The use of probiotic microorganisms for the prevention or therapy of gastrointestinal disorders is an obvious measure and perhaps the most usual application of probiotics, because most health effects attributed to them are related directly or indirectly (i.e., mediated by the immune system) to the gastrointestinal tract. The mechanisms and the efficacy of a probiotic effect often depend on their interactions with the specific microflora of the host or immunocompetent cells of the intestinal mucosa. The gut (or its associated lymphoid system, GALT) is the largest immunologically competent organ in the body, and maturation and optimal development of the immune system after birth depend on the development and composition of the indigenous microflora and vice versa. In this era of antibiotic abuse, developing countries are facing a massive setback to control different types of gastrointestinal infections. Use of probiotics, in appropriate procedure may come as a boon to mankind.

Enteroaggregative Escherichia coli (EAEC) is a pathotype of *E. Coli* associated with acute and chronic diarrhoea in both the developing and developed countries and are now recognised as an emerging enteric pathogen. EAEC has been associated with chronic infections in malnourished and immunocompromised children leading to death of the infected. The symptoms associated with EAEC infections include watery diarrhoea, nausea, anorexia, low-grade fever. Patients show increase in IL-8, IL-1 B, leukocytes indicating a substantial gastrointestinal inflammatory response. In brief, EAEC infection is one of the leading causes of childhood morbidity in developing countries. The lack of proper model system to study the *in vivo* effects of EAEC infection and the host response is a bottleneck in the development of proper therapy.

In our laboratory, we are working to establish a mouse model for the study of EAEC infection and to develop a sustainable way to treat the infection by using probiotics instead of antibiotics. We have already established a persistent diarrhoea (> 2 wks) model due to EAEC infection by challenging malnourished BALB/C mice with EAEC-042 strain ( fig:1).

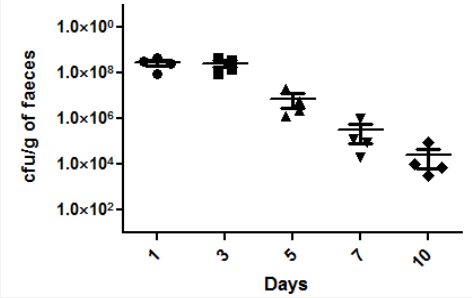


**Fig 1: Establishment of model system to study EAEC-induced persistent diarrhoea.**

Next, we tried to use indigenous Lactobacillus strain (LBS2) as a therapeutic agent to treat EAEC-induced diarrhoea. Seven day post-infection, we treated the mice with two doses of LBS2 on alternative days, which caused a massive reduction of EAEC numbers in the stool.

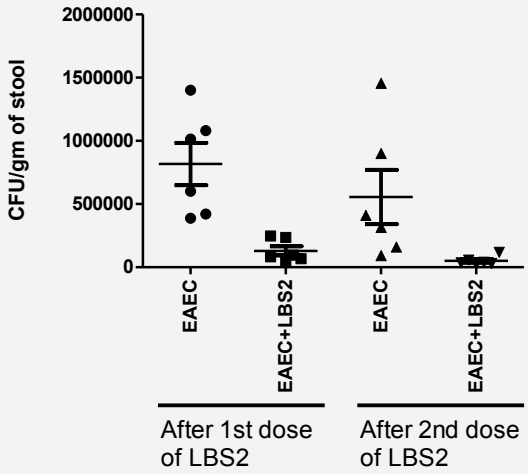


**Fig 2: Use of probiotics reduce harmful EAEC in stool**

With the above findings, we hope that indigenous probiotics may be used for effective treatment of EAEC-induced persistent diarrhoea. However, an elaborate study is needed to establish it further. We also argue that this study will increase the awareness in the society for the use of probiotics instead of antibiotics in the early stages of gastrointestinal infections that would reduce antibiotic

## PSYCHOBIOTICS : GUT MICROFLORA AS A TOOL TO CURE DEPRESSION

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### INTRODUCTION

Use of probiotics to benefit mood is a recent advancement and their use as an adjuvant treat-ment (add-on treatment) to standard care for major depressive disorder. In 2013, scientists de-fined Psychobiotic as, "*a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness,"* recommending probiotics as a novel class of psychotropic (mind-altering) treatment. Probiotics have been found to act as delivery vehicles for neuroactive compounds (compounds that stimulate the nervous system), and cer-tain probiotic strains actually secrete neuroactive compounds. Studies indicate that the probiotic organism *Bifidobacterium infantis* increases the secretion of serotonin precursor tryptophan In animal models Serotonin is the feel-good hormone, and many antidepressant medications work by increasing the availability of serotonin. Human studies have also found benefit of probiotics on mood, reduction in psychological distress and decrease in cortisol levels (cortisol is released under stress). Thus, Probiotics can be used as antidepressants in near future.

### MECHANISM OF ACTION

1. Psychobiotics alter the levels of inflammatory mediators circulating in the body, which may cause depressive symptoms.

2. Ability of gut bacteria to metabolise certain fibres into specific fatty acids. These acids are then absorbed in the large intestine, with a small amount reaching the brain which improves the mood.

### HOW DO THESE BACTERIA AFFECT MENTAL HEALTH?

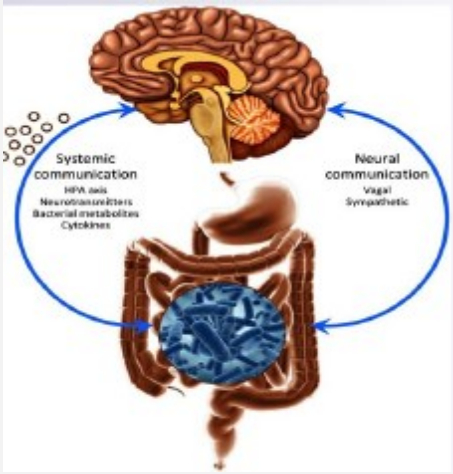


**Figure 1: Bidirectional communication channels between the gut microbiota and the brain (Mayer et al, 2014).**

## GUT-MICROBIOME SIGNALING

✦ Recent compelling research into the microbiome-gut-brain axis and the evidence for the anxiolytic and antidepressant action of *Bifidobacteria* or *Lactobacilli* in rodents and humans after their ingestion as live cultures has been documented.

✦ Several preclinical studies showed a link between specific probiotics and beneficial behav-ioral effects. These included one in which rats with depressive behaviors resulting from maternal separation displayed normalized behavior and an improved immune response after ingesting the *Bifidobacterium infantis* probiotic.

✦ Healthy volunteers who received *Lactobacillus helveticus R0052* plus *B longum* for 30 days reported significantly lower stress levels than those who received placebo, as well as signifi-cantly reduced urinary free cortisol levels.

✦ Another study of 124 volunteers (mean age, 61.8 years) showed that those who consumed probiotic-containing yogurt for 3 weeks had significantly improved mood compared with those who received placebo.

Depression is also associated with an alteration in the microbiota. Psychobiotics are good bacte-ria that have the potential to increase microbial diversity and treat the symptoms of depression.

## FUTURE PROSPECTS

Overall, the rationale for using psychobiotics is sound, and their development and application might well be easier than drug treatments, given that they are unlikely to require the same regu-latory procedures as those used for psychotropic drugs.

This field needs large-scale, placebo-controlled trials to provide definitive evidence of benefit and to detect which probiotics have psychobiotic potential.

Nevertheless, "there is no doubt that many patients would value the emergence of nonconven-tional antidepressants in the form of psychobiotics.

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## Workshop on "Probiotics in Clinical Practice"

The workshop on "Probiotics in Clinical Practice" was organized at ICAR-National Dairy Research Institute, Karnal during April 20 to April 21, 2017 under the aegis of "Probiotic Association of India (PAi)" and "Indian Society of Veterinary Pharmacology and Toxicology (ISVPT). The workshop was inaugurated at 9.00 AM on April 20, 2017. The brief inaugural session was chaired by Dr. R.R.B.Singh, Director, NDRI, Karnal and Co-chaired by Dr. V.K. Batish, Secretary, PAi. Dr. Sunita Grover, Head, Dairy Microbiology Division and Organising Secretary PAi welcomed the guests and participants. Dr. V.K. Batish, Secretary, PAi presented the overview of workshop. The workshop was attended by ten participants from all corners of the country who are directly associated with probiotics research and development.

Three guest speakers from AIIMS, New Delhi and PGI, Chandigarh delivered lectures and interacted with the participants. Prof Rama Chaudhry (AIIMS), discussed her experience of "Probiotic Clinical Trial at AIIMS with the participants. Dr. Alka Mohan Chutani (AIIMS) delivered a lecture on the topic entitled "Medical Nutritonal therapy and Non communicable diseases". Dr. Bhavaneet Bharti (PGIMS, Chandigarh) talked about "Malnutrition and Probiotics". The participants showed their keen interest in the topics and discussed their ideas and experiences thoroughly.

The detailed practical oriented deliberations were made by NDRI faculty during the two days programme of workshop. Practicals involving Isolation, Identification, and characterization of probiotic strains with molecular techniques, their adhesive properties and safety assessment parameters were discussed in detail. Practical of bio-availability, gut barrier integrity along with techniques used in evaluation of immunomodulatory potential of probiotic bacteria were also demonstrated. The participants discussed the protocols in detail and enquired about their doubts and difficulties they face in their own laboratories. The interactions between faculty and participants will definitely improve the quality of probiotic research.

Workshop was concluded at 5.30PM on April 21, 2017 with a lecture by Dr. A.K. Srivastava (Member ASRB and Ex Director NDRI) President, PAi and ISVPT and distribution of participation certificates.

Dr. Sunita Grover, Organising Secretary gave the concluding remarks and Dr. Rajeev Kapila, Co-organising secretary thanked all the guests and participants.

## Guest faculty:

| | | |
|---|---|---|
|  |  |  |
| Dr. Rama Chaudhry | Dr. Alka Mohan Chutani | Dr. Bhavneet Bharti |

## Participants attending the workshop :


# Upcoming Events

1. **4th Biennial conference of PAi and International Symposium on "Probiotic Therapy: Translating to Health and Clinical Practice" will be held on February 16-17, 2018 at JLN Auditorium, All India Institute of Medical Sciences, New Delhi in association with AIIMS.**

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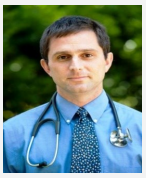
|  |  |  |
| Dr. Mary Ellen Sanders | Prof. Daniel Merenstein M | Dr. Pinaki Panigrahi |

**KEY NOTE SPEAKER: Dr. Mary Ellen Sanders, Dairy & Food Culture Technologies, USA**

**EMINENT SPEAKERS:**

- **Prof. Daniel Merenstein M, Georgetown University School of Medicine (Sponsored by ISAPP)**
- **Dr. Pinaki Panigrahi, University of Nebraska Medical Center, Nebraska, USADr.**
- **Sanjay Patole, KEM hospital, Perth, Western Australia**
- **Prof. Samir K. Brahmachari, Former DG-CSIR**
- **Dr. Yogesh Shouche, NCCS, Pune**

**Organizing Secretary : Dr. Rama Chaudhry Co-organizing Secretary : Dr. Sunita Grover**

2. **International Scientific Association for Probiotics and Prebiotics (ISAPP) - 2018 Meet at Singapore ( 5 - 7th**

## " List of New Members of PAi (March 2017-October 2017)

| S. No. | Name | E mail ID | Membership ID |
|---|---|---|---|
| **Life Members** | | | |
| 1. | Dr. Bhavneet Bharti | bhavneetsahul@gmail.com | 452 |
| 2 | Dr. Alka Mohan Chutani | alkamohan@yahoo.com | 453 |
| 3 | Dr. Sarang Dilip Pophaly | sarang01@gmail.com | 454 |
| 4 | Dr. Tasneem Hussain Ravat | tnavagharwala@gmail.com | 459 |
| 5 | Dr. Aysha, C.H. | chaysha@gmail.com | 462 |
| 6 | Dr. V.D.Bamola | vdbamola@gmail.com | 465 |
| 7 | Dr. Jasvir Singh | dr.jasvir.singh@gmail.com | 466 |
| **Corporate Members** | | | |
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| 2 | M/S Yakult Danone India Pvt. | neerja.hajela@yakult.co.in | 455 |
| 3 | M/S Sarvotham Care Ltd | mohan_krishna@sarvothamcare.com | 460 |
| 4 | M/S Mother Dairy Fruit and Vegetables Pvt. Ltd. | tsr.murali@mohterdairy.com | 461 |
| 5 | M/S Tablets (India) Pvt. Ltd. | ts@tabletsindia.com | 463 |
| 6 | M/S Synergia Life Sciences Pvt. Ltd. | siddharth.daftary@viridisbiopharma.com | 464 |
| **Ordinary Members** | | | |
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| 2 | Dr. Saumya Chaturvedi | | 456 |
| 3 | Dr. Vandana Kumari | vandu01arya@yahoo.co.in | 457 |
| 4 | Dr. Chaynika Verma | Chaynika1388@gmail.com | 458 |
| **Student Members** | | | |
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