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Review Article

**PROBIOTIC- ANTIDIARRHEAL, ANTIOXIDANTS AND  
ANTIMICROBIAL****Hemant Vanjari\*, Hitesh Kachave, Gitesh Guthale, Mitali Dalvi, Mahalaxmi Mohan**  
Department of Pharmacology, MGV's Pharmacy College, Panchavati, Nasik-422003, India**Article Received:** June 2019**Accepted:** June 2019**Published:** June 2019**Abstract:**

*Probiotic is live non-pathogenic microorganisms which confers beneficial effects on host. It is available in the form of dairy products, cheese, butter, yogurt and fermented products for maintaining microbial flora. Probiotic act through different mechanisms -inhibition of proliferation of pathogenic bacteria, production of antimicrobial substances, enhance immune system by modulating different immune mechanism and by regulating intestinal permeability by strengthening pathogenic barrier. Probiotic is effective in different clinical conditions like diarrhea, intestinal inflammatory disorders, bacterial infection, allergic conditions, immunomodulation, viral infection and in oral health. Probiotic shows antioxidant activity by releasing different antioxidant enzymes which neutralize reactive oxygen species to avoid cellular damage and decrease the risk of chronic disease conditions. Probiotic shows antimicrobial property against pathogen by maintaining intestinal pathogenic barrier and production of antimicrobial peptides, H<sub>2</sub>O<sub>2</sub>, bacteriocins, organic acids by gut epithelial cells. Lactobacillus as probiotics are being used for long time due to their safety and efficacy, but other probiotics show minor side effects like thirst, constipation, nausea, vomiting, abdominal pains. Probiotics rarely show harmful effects.*

**KEYWORDS-** Probiotics, antidiarrheal, antimicrobial, antioxidants**Corresponding author:****Hemant Pramod Vanjari,**

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**INTRODUCTION:**

Golden era of antibiotics began nearly in 1928, when Alexander Fleming, a bacteriologist at London's St. Mary's Hospital, found that a mould on a discarded culture plate had an antibacterial action, and the mould was 'penicillin'. After introduction of antibiotics life expectancy has increased and antibiotics have revolutionized the field of medicines as it greatly improved quality of human life by decreasing mortality rate throughout world. But major drawback with antibiotics was that besides killing bad bacteria it also kills good bacteria and hence it disturbs ecosystem of body, causing devastating effects on body like superinfection and drug resistance. In the era of advanced technology introduction of probiotics has widened the field of medicine further. [1]

"Probiotic" means that mechanisms are employed to selectively remove only the pathogen while leaving the remainder of oral ecosystem intact. The term Probiotics is derived from a Greek word meaning 'for life' and used to define living non-pathogenic organisms and their derived beneficial effects on hosts. The term Probiotics was first introduced by Vergin, when he was studying the detrimental effects of antibiotics and other microbial substances, on the gut microbial population. He observed that probiotika was favorable to the gut microbial flora. They are usually consumed after the antibiotic therapy (for some illnesses), which destroys the microbial flora present in the digestive tract (both the useful and the targeted harmful microbes). Probiotic were redefined by Lilly and Stillwell as -A product produced by one microorganism stimulating the growth of another microorganism. Subsequently the term was further defined as Non-pathogenic microorganisms which when ingested, exert a positive influence on host health or physiology by Fuller. The latest definition of probiotic is put forward by FDA and WHO jointly is Live microorganisms which confers the beneficial effects for humans and animals.[2] Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system. Probiotics can be found in dairy and nondairy products. Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora. Probiotics in the form of yogurt, cheese, and fermented foods form part of regular diet. However, the health benefits of probiotics described firstly by Elie Metchnikoff in 1908, [3] who postulated that sour milk contains *lactic acid bacteria* whose growth in the intestine displaces disease-producing organisms. Probiotic also called as 'bio therapeutic agent' are administered to

promote health of host for treating or preventing infectious disease from some strains of pathogens.[4,5,6] Probiotic are of two types: Bacterial probiotics and Yeast probiotics, most commonly used probiotics come from the genera *Lactobacillus* and *Bifidobacterium*. Others include *Streptococcus thermophiles*, *Lactobacillus acidophilus*, nonpathogenic strains of *E. coli*, *Enterococcus*, *Bacillus*, and yeasts such as *Saccharomyces boulardii*. Although human origin of microorganisms and colonization of the gastrointestinal tract were criteria, some strains have been isolated from other animals, and probiotics are now being investigated as topical agents and vaginal suppositories as well. Another important consideration is the need for a sufficient number of microbes to survive throughout the shelf life of product.

Clinical applications of probiotic used to modify gastrointestinal flora and strengthen the pathogenic intestinal barriers are also beneficial in different disease conditions like diarrhea, inflammatory bowel diseases (Ulcerative colitis C-UC, CD-CD, Pouchitis) liver diseases, *helicobacter pylori* infection, allergic conditions, immunomodulation, diabetes, hypertension, urogenital health care, lactose intolerance, colon cancer, peptic ulcer, atopic diseases, food allergy, upper respiratory tract infection, dental caries, periodontal diseases, halitosis, oral candidiasis, viral infections, probiotic in veterinary practice.[2,1,7]

Gastrointestinal diseases disturb bowel's ecosystem. Diarrhea is associated with an increased frequency of bowel movements with production of soft or watery stool. It causes an imbalance between secretory and absorptive activities of intestine which result in increased fluidity and volume of stool leading to fluid and electrolyte loss. Diarrhea is diagnosed in millions of people per year in the world. Diarrhea is caused by pathogenic infection e.g. *E.coli*, *Vibrio cholera* or *Entamoeba histolytica*, *Giardia lamblia* or viruses. Antibiotics are also most common culprit of acute diarrhea due to loss of "colonization resistance" of the protective role of normal intestinal flora against pathogenic organism.[8] 5% to 10% of this diarrheal reason is cholecystectomy. [9] Antibiotics usage may alter the colonic microbial flora resulting in malabsorption of carbohydrate. Antibiotics kill both the good and bad microorganisms which may result into disturbance in the gastrointestinal flora. About 1.7 to 5 billion cases of diarrhea occur per year. It is most common in **developing countries**, where young children get diarrhea on average three times a year. Total deaths from diarrhea are estimated at 1.26 million in 2013 – down from 2.58 million in 1990. In 2012, it was

the second most common cause of deaths in children younger than five (0.76 million or 11%).

Reactive oxygen metabolites (ROM) are generated through normal reactions within body during several cellular processes in aerobic organisms which can cause damage in mutation in DNA, modification in low density lipoproteins, oxidation of membrane lipoproteins then excess amount of ROM can result in cellular damage which promotes chronic diseases -arthritis, diabetes, atherosclerosis, CVS and cancer.[10] To neutralize the oxidant molecules body synthesizes antioxidant enzymes and food containing antioxidants. Novel approach represents development of probiotic exerting antioxidant activity and counteracting oxidative stress in host. Properly selected probiotic strains have been developed in order to respond to specific physiological target. Probiotics can produce various metabolites with antioxidant activity, such as glutathione (GSH), butyrate, and folate. Kullisaar and colleagues found that the two antioxidant *Lactobacillus fermentum* strains, E-3 and E-18, contained remarkable levels of GSH. [11]

Probiotics possesses the antimicrobial activity. Probiotics together with other beneficial microbes are commensals of the gut and differ from pathogenic bacteria in the terms of their actions on immune cells in the gut as they do not stimulate the proliferation of mononuclear cells or trigger an inflammatory action.[12] Probiotics possess antimicrobial effect against pathogens by maintaining the homeostasis of the intestinal flora. The large amount of intestinal bacteria represents a continuing threat to intestinal barrier, gut epithelial cell produce antimicrobial peptides that act at forefront of innate immunity. Several studies confirms that resistance against AMPs (antimicrobial peptides) is less probable than observed and develop AMPs / antibacterial compounds i.e lactic acid, other organic acid, H<sub>2</sub>O<sub>2</sub>, bacteriocins. Bacteriocins are biologically active, low molecular-weight proteins or peptides that inhibit the growth of a variety of pathogenic bacteria. Specific strains of probiotics shows antagonistic effect against pathogenic microorganisms.

**Probiotic-**The Russian Metchnikoff was first awarded the Nobel Prize for medicine in 1908. He was the first to recognize beneficial role of select bacteria on gastrointestinal tract of humans and postulated that 'Theory of Longevity of Bulgarian peasants was due to consumption of sour milk which contains lactic acid bacteria whose growth in intestine displaces disease producing organism.[12] *Lactobacillus* and *bifidobacterium* are most common types of microbes used as probiotics

including certain yeast and bacilli. They are consumed as fermented food with added active live culture e.g. yogurt, dietary supplements.[13]

Gastrointestinal tract of human being at different site is habited by good bacteria, these bacteria have a relationship of symbiosis with host. The potentially beneficial micro-organisms are more than number of potentially harmful bacteria. This ecosystem composition is called Normobiosis. The environment where potentially harmful bacteria dominate over health beneficial bacteria is called dysbiosis. Potentially beneficial bacteria called as Probiotics. [14]

**History-**Origin of cultured dairy products dates back to the dawn of civilization, they are mentioned in Bible and sacred books of Hinduism. Climatic conditions for sure favored the development of many of the traditional soured milk or cultured dairy products such as Kefir, koumiss, and dahi. These products, many of which are still widely consumed had often been used therapeutically before the existence of bacteria was recognized. At the beginning of 20th century main functions of gut flora were completely unknown. Ilya Ilyich Metchnikoff, the Nobel prize winner in Medicine in 1908, at the Pasteur Institute linked health and longevity to ingestion of bacteria present in yoghurt. In 1907, he postulated that the bacteria involved in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, suppress putrefactive type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health. Moreover, it is the site of dangerous intestinal putrefaction processes which be opposed by introducing lactobacilli into body, displacing toxin-producing bacteria, promoting health, and prolonging life.

Tissier's discovery of *bifidobacteria* in breast-fed infants also played a key role in establishing concept that specific bacteria take part in maintaining health. In 1906, Tissier reported clinical benefits from modulating flora in infants with intestinal infections. Probiotics was first introduced by Vergin, when he was studying the detrimental effects of antibiotics and other microbial substances, on the gut microbial population. Then he observed that 'Probiotika' was beneficial to gut microbial flora.[16] The use of probiotics goes back thousands of years, with intake of probiotics in form of yogurt, cheese and fermented foods as part of a regular diet. This health benefits of probiotics were first described in 1907 by Eli Metchnikoff. In early 1920s, *L. acidophilus* milk was documented to have therapeutic effects in particular a settling effect on digestion. It was believed that colonization and growth of these microorganisms in the gut were

essential for their efficacy, and therefore, the use of intestinal isolates was advocated. In Japan in the early 1930s Shirota focused his research on selecting strains of intestinal bacteria that could survive passage through gut and on the use of such strains to develop fermented milk for distribution in his clinic. His first product containing *L. acidophilus*. He was established Yakult Honsha company. In 1953, Kollah may be credited for this definition as Probiotika as a active substance that are essential for a healthy development of life. In 1965, Probiotic as a substance produced by one microorganism and probiotic as opposite to an

antibiotics this concept is coined by Lilly and Stillwell. In 1974, Parker gives a totally different overview- Organisms and substances which contribute to intestinal microbial balance. [2] Non-pathogenic microorganisms which when ingested, exert a positive influence on host's health or physiology by Fuller. Research on probiotics, in particular *lactobacilli*, has grown exponentially during the last two decades as can be seen from the fact that compared to 180 research article were published during 1980-2000, more than 5700 research articles were published during 2000-2014 on "probiotic *lactobacillus*".[15]

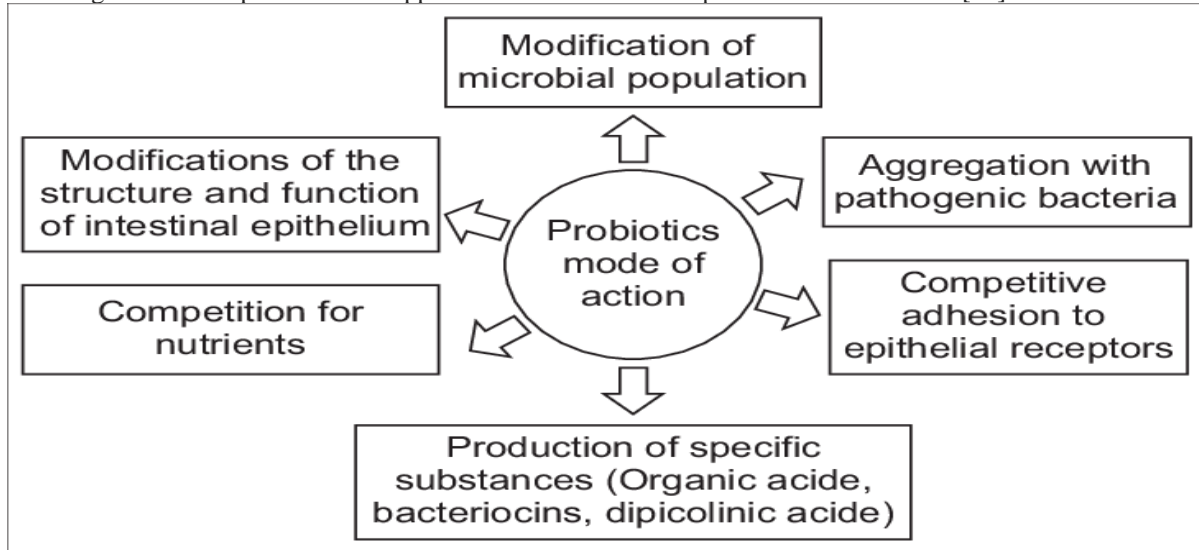


Fig 1: Mechanism of action of Probiotic

**Rationale-** Using probiotics is based on the assumption which modifies the colonic microbial flora, counteract enteric pathogens and modulate the immune systems.

**Colonization resistance-** It is property of normal colonic micro flora for protection against pathogens due to complex formation of various strains of colonic bacteria, probiotics inhibits the proliferation of pathogens during a period when normal colonic micro flora disturbed [16].

**Production of antimicrobial substances-** Probiotics produces inhibitory substances towards a broad spectrum of gram positive and gram negative pathogens, by secreting the various another antimicrobial peptides like as bacteriocins, reuterin, hydrogen peroxide, organic acids inhibiting the growth of pathogens by creating the acidic environment and act as bactericidal [17-20].

**Enhancement of the immune defense system-** Enhance the production of serum IgA as well secretory IgA, which plays a crucial role in intestinal humoral immunity [21,22]. Also enhance phagocytosis, increase activity of natural killer cells and promotes cell-mediated immunity and stimulate various other non-specific immune response against pathogens [23-25].

**Regulating intestinal permeability-** Probiotic compete with pathogens for nutrients and receptors then it enhancing the intestinal epithelial barrier by increase the production of mucin and preventing injury of epithelium from pathogens and reducing cell permeability. Probiotics regulates intestinal permeability and by modulating the epithelial tight junctions. [26-28]

According to the other theory, probiotics act by modulating the immune response at local level,

1. Induced hydrolysis of toxins and receptors.
2. Exert a trophic action on the intestinal mucosa, which leads to brush border enzyme activation, stimulation of glucose absorption and antiapoptotic effects on the enterocyte.
3. Inhibit selected intracellular mechanisms involved in viral replication (such as MEK, PKA, p38, MAPK)
4. Down regulate pro-inflammatory cytokine production, prevent apoptosis and suppress T-cell proliferation and stimulate various other non-specific immune response against pathogens.[29-31]

**Characteristic of Probiotics-** [15]

1. It should be strain specific, capable of exerting beneficial effect on host animal.
2. It should be non pathogenic and non toxic and lactic acid producer.
3. It should be present as viable cells, preferably in large numbers and it is acid and bile tolerant.
4. It should be robust and surviving processing conditions
5. It should be genetically stable under storage and field conditions.
6. It should be capable of surviving and metabolizing in gut environment e.g. resistance to low pH & acids.

**HEALTH BENEFITS OF PROBIOTIC:**

**Diarrhea:** It is defined by WHO as three or more loose or watery stools during 24 hour period. Several studies and investigations validated the positive effect of probiotic consumption in arresting diarrhea of different type. [32]

**Acute Infantile diarrhea:** Acute infantile diarrhea caused by rotaviruses is most studied gastrointestinal condition and rapid oral rehydration is primary treatment. Probiotics have been found to be useful as rehydration therapy. [33]

**Antibiotic associated diarrhea:** Antibiotic/drug-associated diarrhea occurs after the use of various antibiotics (drugs). Its exact pathophysiology is unclear, but 5% to 10% of this diarrheal reason is cholecystectomy. Disturbance of indigenous microbial flora caused by antibiotic treatments often leads to diarrhea.[9] The main mechanism by which antibiotics cause diarrhea is through impaired resistance to pathogens as a result of disruption of gut microbial flora and subsequent alterations in metabolism of carbohydrates, fatty acids and bile acids.[34] Probiotic includes various bacterial and yeast strains that are effective in treatment of antibiotic associated diarrhea, i.e. *L. acidophilus*, *L. rhamnosus GG*, *L. fermentum* etc. and *S. boulardii*. [35]

**Traveller's diarrhea and Infectious diarrhea:** Infectious diarrhea occurs mostly due to two very important disturbances in the normal physiology of the intestine: a) increased secretion of intestinal fluid and electrolytes mainly in the small intestine; b) less absorption of fluids, electrolytes, and nutrients in the small intestine. [36-37] About 20-60% travelers suffering from diarrhea around the world. It is particularly affects people who travel from industrialized to developing countries, especially tropical and semi tropical regions. The main cause of bacteria and bacterial pathogens is *E.coli* and *Helicobacter pylori* infection. It is observed that *S. boulardii* was found to be more

effective against bacterial diarrhea and *Lactobacillus GG* showed effectiveness against viral and idiopathic diarrhea[38] and *Streptococci* have been used prophylactically to prevent traveller's diarrhea

**Inflammatory disorders:**

**Irritable bowel syndrome (IBS):** IBS is one of the most common functional gastrointestinal disorders and is a chronic condition characterized by recurrent bouts of abdominal discomfort and pain, bloating and a changeable bowel habit with an absence of any overt mucosal abnormality and flatulence. The multi-factorial pathophysiological factors for inducing IBS are: a) Psychological factors like stress and emotional status b) Social factors like upbringing and support systems and c) Biological factors like gut motility and visceral sensitivity, which interact in a complex way to exacerbate symptoms.VSL#3, a mixture of 8 probiotic strains and *Lactobacillus plantarum* decreased flatulence and relieved abdominal bloating. [39] Reduction in pain was observed with *L. rhamnosus GG*. Different studies in adults showed that *B. infantis*, *L. rhamnosus GG* and mixture of different probiotics such as *L. rhamnosus GG*, *L. rhamnosus LC705*, *B. breve Bb99* and *Propionibacterium freudenreichii JS* were found to be effective in alleviating the symptoms.[40] Probiotic *Escherichia coli* Nissle 1917 has also been proved effective in IBS treatment, especially in patients with altered enteric microbial flora. e.g. after gastro-enterocolitis or administration of antibiotics. [41]

**Inflammatory bowel disorder:** IBD is chronic, relapsing, multi-factorial disorder causing inflammation of the gastro-intestinal tract that causes severe watery and bloody diarrhea accompanied by abdominal pain. IBD affects both - the colon and small intestine and includes UC (UC), CD (CD) and pouchitis. The other reported factors involved in causing IBD are: genetic, environmental factors, dysregulation of immune system, type of intestinal microbes and oxidative stress. [42] CD and UC both are chronic inflammatory autoimmune conditions of the gastrointestinal tract and probably are due to the lack of adaptation of the innate immune system to the environment and the westernization of civilization. [43] These diseases affect 1-5 of 1,000 individuals and represent a major burden on the national health systems of many countries on different continents.

**Ulcerative Colitis (UC):** In UC only mucosa and submucosa of colon are inflamed. UC has symptoms of diarrhea and bleeding. UC like IBD mainly affects the lining of the large intestine and rectum. Long-standing UC is a risk factor for colon cancer. Use of various probiotic species like *S.*

*boulardii*, *Lactobacillus casei* and *Bifidobacterium bifidum* has shown promising results. A pilot study suggested that fermented milk containing *B. breve*, *B. bifidum* and *L. acidophilus* was beneficial to induce mild degree remission in patients.

**Crohn's disease CD:** It is also type of IBD and in this case mainly mucosa, submucosa and serosa are inflamed and the inflammation can spread to whole GIT. CD is associated with diarrhea weight loss and abdominal pain. CDs has symptoms of diarrhea and bleeding CD is usually affects the intestine, but may occur anywhere from the mouth to the end of the rectum. CD causes ulceration and inflammation that affects the body's ability to digest food, absorb nutrients and eliminate waste in a healthy way. *Salmonella*, *Campylobacter jejuni*, *Clostridium difficile*, *Adenovirus*, and *Mycoplasma* have been identified as some of the common causative agents. There are reports suggesting the effectiveness of probiotics in countering the problems of CD. [44] The exact cause of CDs is not known Probiotic are used in treatment of inflammatory bowel disease considering bacteria re involved in etiology of disease. [45] The therapeutic effects of probiotic consumption on CD are reported to be due to competitive action with commensal, pathogenic flora and an influence on the immune response system.[46] Probiotics also prevent IBD by restoring integrity of the protective intestinal mucosa. Different studies show beneficial effect of probiotic in treatment of inflammatory bowel disease in animal models. [47]

**Pouchitis:** Pouchitis is another type of IBD where ileal pouch gets inflamed especially after colectomy and ileal pouch canal anastomosis. In different studies the VSL#3 probiotic mixture was found to be highly effective for maintaining remission of chronic pouchitis. [48] The effective probiotic strains induce distinct mucosal cytokine profiles like IL-4 and IL-10. Probiotics may also influence the mucosal cell-cell interactions and cellular stability by enhancement of intestinal barrier function by modulating cytoskeletal and tight junctional protein phosphorylation, and also by producing anti-oxidant enzymes such as superoxide dismutase and catalase thus ameliorating the IBD symptoms [49].

**Lactose intolerance:** Lactose intolerance means the inability to adults to digest lactose due to lack of lactose metabolizing enzyme lactase and lack of digestion of lactose due to low level of  $\beta$ -galactosidase enzyme activity. Symptoms includes abdominal distress like diarrhea, bloating, abdominal pain and flatulence. [50] Possible pharmacological interventions for lactose intolerance are,

1] Treatment with commercially available lactase

2] Probiotics such *Lactobacillus bulgaricus* and *Streptococcus thermophiles*, reason behind for this it has been assumed that bacterial microbial flora in small intestine enhance lactose digestion.[51,52] It is mostly observed in people deficit in lactase generally tolerate lactose better from yogurt than from milk.

**Immunomodulation and Infections:** Probiotic bacteria have immunomodulatory effects, adjuvant like properties and anti-inflammatory activity and affect humoral as well as cell-mediated immunity. Probiotic bacteria are known to secrete factors responsible for modulating immune responses. For instance, secreted factors from *L.reuteri* decrease NF- $\kappa$ B dependent gene expression, resulting in diminished cell proliferation and enhanced mitogen activated protein kinase, an important event for inducing apoptosis. [53] As fermented milk drinks are popular sources of probiotics, it is important to note that *L. helveticus* is capable of producing factors during milk fermentation which are responsible for increasing calcineurin expression, causing increased formation of mast and goblet cells in the mouse gastrointestinal tract. The ingestion of the probiotic culture VSL#3, however, slowed down regulation of such response by reducing IL-8 secretion, even in the presence of a pathogen *Salmonella* Dublin mechanism for the beneficial effect of prebiotics on immune function in the gut has not been well established. However, some possible cellular events have been proposed:

1] Prebiotic fibers are able to down regulate hepatic lipogenic enzymes, through increased production of short chain fatty acids (SCFA) like propionate.

2] Production of SCFA from fiber fermentation especially Butyrate has been identified as a modulator of histone tail acetylation and consequently, increases the accessibility of many genes to transcriptional factors

3] Modulation of mucin production,

4] FOS and some other prebiotics have shown increased lymphocyte and/or leucocyte numbers in gut-associated lymphoid tissues (GALT) and peripheral blood,

5] Enhanced IgA secretion by the GALT is said to stimulate the phagocytic function of intraperitoneal macrophages, Probiotics improve normal immune function by increasing the concentration of Ig-A producing plasma cells, improving phagocytosis as well as increasing concentration of T-lymphocytes and Natural killer cells. [54,55]

**Cancer:** Treatment of Cancer is one of hot issue in these days. Some strains of Lactic acid bacteria (LAB) have shown antimutagenic effect because they have ability to bind with heterocyclic amines which are carcinogenic. [56,57] *L. acidophilus* is known to prolong the induction of colon tumors. It was demonstrated that feeding milk and colostrums

fermented with *L. acidophilus* resulted in 16–41 % reduction in tumor proliferation. [58] The other probiotic *L. bulgaricus* has also been reported to induce antitumor activity against sarcoma-180 and solid Ehrlich ascites tumors. [59] The proposed mechanisms by which probiotics exert anti-tumor activity include:

1] Altering the immune functions associated with immune response

2] Antiproliferative effects via regulation of apoptosis and cell differentiation.

3] Suppressing the production of enzymes like  $\beta$ -glucuronidase, urease, choloylglycine hydrolase, azedoreductase and nitro-reductase by bad bacteria especially entero-pathogens such as *E. coli* and *Clostridium perfringens*. In laboratory experimentation, some strains of LAB have shown antimutagenic effects because they have ability to bind with heterocyclic amines which are carcinogenic.[60] Animal studies proved beneficial effects of LAB against colon cancer of rodents. Human trials also suggest that some types of LAB are anticarcinogenic due to ability to decrease activity of enzyme called  $\beta$ -glucuronidase. [61] The incidence of colon cancer in people consuming dairy product has been low compared to others during population studies.

**Diabetes:** World health organization states diabetes as “Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, Diabetes has proved a silent killer for most of the patients. Diabetes management includes a large number of medications but none of them could be helpful in complete cure of disorder. Many researches are being carried out at bimolecular and pharmacological level. One of the efforts to cure this disorder is to use symbiotic. These are firmicutes (most important is *Lactobacillus* which is beneficial bacteria), *proteobacteria* and *bacteroidetes* (opportunistic pathogens which can cause gum disease and tooth decay). There was a low level bacteria from phylum firmicutes and greater level of bacteria from phylum *bacteroidetes*. Researchers also found that increased level of *bacteroidetes* and decreased level of firmicutes will lead to decreased glucose tolerance which is key problem with diabetes mellitus [62] Probiotics are supposed to treat the diabetic patients by balancing microbial gut flora. Low-fat (2.5%) dahi containing probiotics *Lactobacillus acidophilus* and *Lactobacillus casei* was tested in rats against high fructose-induced type-2 diabetes. Both these bacteria proved beneficial effect in lowering blood glucose by decreasing insulin resistance [63] It is also suggested that the use of probiotics can decrease the insulin resistance and can also lower the incident of hypertensive conditions that are closely related to diabetes. It has also been found

that *Bifidobacterium species* delivers pharmacological nutritional support in treating insulin resistance.

**Hypertension:** The incidence of increased blood cholesterol has been increased in adults, children and adolescence. Most important causes of hypertension are lipid abnormality, hypercholesterolemia and obesity [64] Mann and Sperry were among the first to illustrate that *Lactobacillus*-fermented milk has hypocholesterolemic effects [65] New researches have shown that not only the *Lactobacilli* exhibit hypocholesterolemic effects, but also *Bifidobacteria* could cause a significant reduction in serum cholesterol when cholesterol is elevated. As we know that most of the cholesterol is synthesized and absorbed in intestine, therefore intestinal micro flora has shown to effect cholesterol level in blood. Studies have shown that probiotics have been proved beneficial in lowering hypertension by decreasing blood cholesterol level and increasing resistance of LDL to oxidation [66] Angiotensin converting enzyme (ACE) which regulates the Renin angiotensin system. There are some probiotics which upon fermentation produces proteinases capable of producing ACE inhibitory peptides. And thus regulate blood pressure. [67] Several studies have demonstrated that *Lactobacillus helveticus* are involved in producing antihypertensive peptides which are ACE inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) from milk protein casein . [68]

**Urogenital healthcare:** Urogenital infections [69,70] proved to be one of the most common diseases which women are facing today. Globally, an estimated 1 billion women have bladder or vaginal infections each year[53] UTI such as bacterial vaginosis can lead to increased risk of preterm delivery [71] Mostly oral and vaginal sex play an important role in causing vaginitis and UTI, Research on the vaginal micro flora during the whole menstrual cycle has proved that only 22% women maintain a *Lactobacilli-rich* flora [72] and it is also clear that epithelial cell receptivity to *Lactobacilli* attachment enhances at peak estrogen levels midcycle [73] It is still not found what factors cause such a dramatic alteration in the flora.

**Respiratory tract infection:** Upper respiratory tract infection includes laryngitis, tracheal inflammation and common cold and these are associated with symptoms like fever, headache, and pain and cough. Most of the upper respiratory tract infections (URTs) are caused by viruses and these resolve in 3 to 7 days. To treat the symptoms like headache, pain and fever, analgesics and antipyretics are used most frequently. Fermented food containing probiotics like *Lactic acid bacteria* and *bifidobacteria* are found to reduce the episode

of URTs. Probiotics are also found to decrease the risk and incidence of respiratory tract infection (RTIs) in the children having age of 3-5 years. [54] These probiotics are also found to decrease the symptoms of pain, cough, and runny nose.

**Viral Infections:** Probiotics are useful against many viral diseases. It is also obvious that probiotics do not show antiviral effect by direct action on viruses but do so by the mechanism of

immune stimulation. [74] Those probiotics which show immune stimulation also exhibit potential antiviral effect. *Thermophilus* species have some anti-influezal effect. These species also show anti herpetic effect when administered to guinea-pigs. [75]

#### TYPES OF PROBIOTICS:

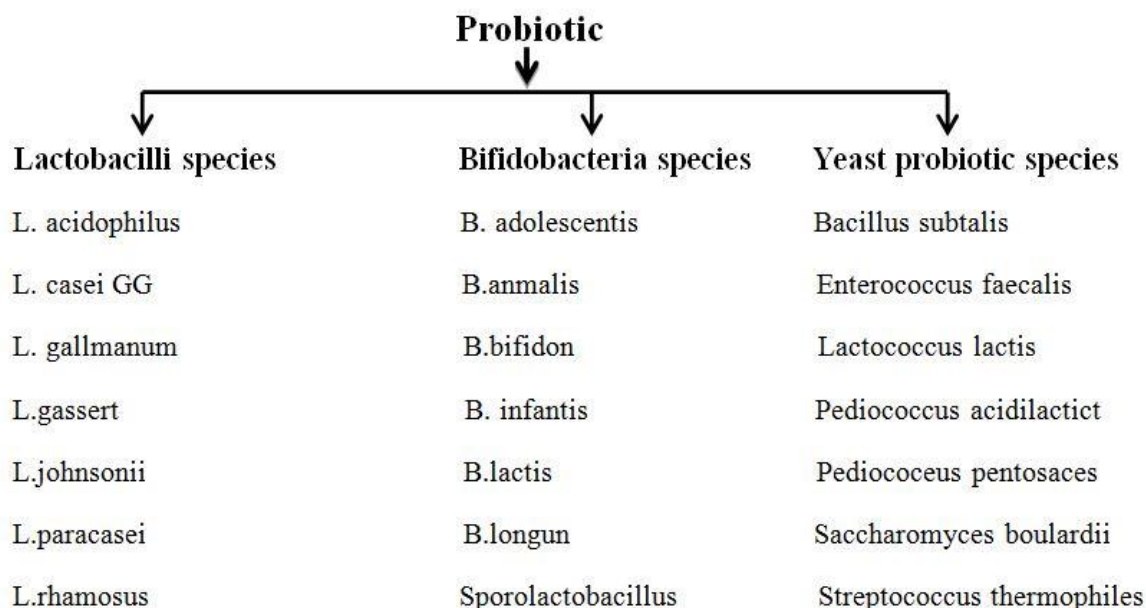


Fig. 2 Different types of bacteria which are recognized as a probiotics [76]

**ANTIDIARRHEAL ACTIVITY:** Change in bowel movements with symptoms of increment in water content, volume and frequency of stool which is refer to as diuresis, and imbalance between secretory and absorptive activities in the intestine. Infection is main cause of diarrheal illness [77-79] i.e. increase frequency, fluidity, fever and abdominal pain. Diarrhea is caused by various types of bacteria, viruses and parasites, and origins from other gastrointestinal disorders and certain medication like antibiotics also causing diuresis. On the basis of duration of diuresis classified into 3 types 1) acute diarrhea- less than 2 weeks 2) persistent diarrhea- varies from 2 to 4 weeks 3) chronic diarrhea- more than 4 weeks [80] and according to pathophysiological conditions i.e infectious diarrhea becomes a major global health problem among the various types of diarrhea.[81] After pneumonia, diarrhea, especially acute infectious diarrhea, is caused for hospitalization, morbidity and mortality and second chief reason for death worldwide. [82] The threat of mortality from diarrheal disease is the greatest among children, particularly during their infancy.[83] Growth retardation, impaired cognitive

development and malnutrition are other major consequences of diarrhea in children in resource-limited countries. [84] Mostly 80% to 85% of infectious diarrhea occurs due to bacterial pathogens. Diarrheagenic *Escherichia coli* (E.coli) is the main reason for most commonly occurring pathogenic diarrhea. [85,86] Other less common reasons for diarrhea are Rotavirus or Norwalk viruses. [87]

According to the World Health Organization (WHO) and UNICEF, Diarrhea is detected in millions of people every year in both developing and developed countries and its second leading cause of death in children under 5 years of age effects of infectious diarrheal disease are higher in developing countries. About 2 billions of infectious diarrhea cases are reported every year throughout the world. Among them 1.9 millions children younger than 5 year of age die because of diarrhea each year. It means that 18% of childhood deaths are due to the diarrheal disease In estimation more than 5000 children's died every year by diarrhea and In developed countries, viruses are major cause of diarrheal illness, hospitalization, and amplified health care expenditure. [88,89] At an



adult level, the main cause of acute diarrhea are disturbance in the absorption of micronutrients, malnutrition, and growth uncertainty. [90,91] In adults 15 % were victims of antibiotics-associated diarrhea upon administration broad spectrum antibiotics. *Clostridium difficile* (*C. difficile*) is a major pathogenic agent whose growth is enhanced by the excessive use of antibiotics [92] *C. difficile* is accountable for a number of cases of infectious antibiotic-associated diarrhea and pseudo-membranous Colitis. [93,94] In 1999, a community health study in the developing countries showed that *Shigella dysenteriae-1* was responsible for 163.2 million hospital cases with 1.1 million deaths. [95] Recent studies have also supported those data. [96,97] Generally infectious diarrhea develops due to changes in intestinal microbial flora.[98]

#### **Classification of Diarrhea on the basis of pathophysiology:**

**Osmotic diarrhea:** Intestinal homeostasis depends upon balance among absorption, secretion and permeability (to pathogens and macromolecules) of the digestive epithelium. Multiple communications with endocrine, stromal, neurocrine and immune cells or with the inhabitant bacterial microbial flora controlled these functions of digestive epithelium. But upon disturbance in this homeostasis control, diarrhea and chronic inflammation may occur. A disturbance in hydro-electrolyte balance leads to water losses. The dysregulation of ion-coupled nutrient absorption or sometimes abnormal stimulation of ionic secretion may be the reason of disturbance. Water movements are mainly produced by sodium-solutes co-transport systems (Na-glucose) or chloride secretion across apical membrane of intestinal epithelial cells. Pathogenic bacterial can stick to brush-border membrane of enterocytes inducing epithelial dysfunction. This stickiness or attachment results in the discharge of enterotoxins that stimulate chloride secretion or cytotoxins unmanageable the epithelial integrity. Besides water movement mechanism specifically involved in gut, nonabsorbable compounds reaches intestinal lumen and blood due to this compounds osmotic diarrhea is induced. It is also induced by lactose malabsorption in the absence of lactase enzyme. Regulation of water movements (mast cells, phagocytosis) can also be affected by seasonal factors. Abnormal stimulation of the underlying immune system leads to release of inflammatory mediators capable of changing epithelial function. [99,100]

**Infectious diarrhea:** Pathogens including bacteria causes disturbance in absorptive and secretory mechanisms of intestinal fluid, electrolytes, nutrients in intestine.[101,102] These pathogens mainly attacking on small intestine and they are attach to mucosa and disrupt absorptive process of

enterocytes and produce active secretion without any inflammation of mucosa or destruction of mucosa. [103] The pathogen produce enterotoxins which cause major intestinal secretion. Enterotoxins-mediated diarrhea occur when the concentration of enterotoxins is increased in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). It inhibits  $\text{Na}^+$  - $\text{H}^+$  exchange and stimulates  $\text{Cl}^-$  discharge in the small intestine. [104,105] Enterotoxins of some pathogens stimulate  $\text{Cl}^-$  secretion along with  $\text{Na}^+$  absorption. Viral agents inhibits the sodium absorption by affecting function of absorptive villi. These pathogens mostly induced watery diarrhea, but some types of pathogens also induced bloody diarrhea if infection is severe or the production of enterotoxins [106] is higher and which increase intestinal permeability by changing structure of intercellular tight junction. Secretary infectious diarrhea may be induced due to other modes of secretion.

**Antibiotic/ drug associated diarrhea:** Antibiotic associated diarrhea occurs after the use of various antibiotics. Pathophysiology of antibiotic-associated diarrhea involves more than one mechanism. Antibiotics usage may alter the colonic microbial flora resulting in malabsorption of carbohydrate. Mostly drugs cause osmotic diarrhea. Some chemotherapeutic drugs also induce diarrhea by increasing the rate of proliferation of the enterocytes.[107] Chronic diarrhea can also be induced by the ileal resection. Pathophysiology of this type of diarrhea depends upon the extent of resection. Colonic secretion is stimulated because of the spilling of bile acid in the colon. Chronic diarrhea occurs because of bile acid pool depletion. [108] Probiotics are able replace and restore the microbial flora and Different studies shown that *Lactobacillus GG* significantly reduced antibiotic induced diarrhea[109] and also other *probiotics L. acidophilus* and *Lactobacillus bulgaricus* are shows beneficial effects in antibiotic induced diarrhea

**Inflammatory diarrhea:** Inflammatory diarrhea may follow more than one mechanism including exudation and inflammation of mucosa. [110,111] It may result from infectious pathogens, which affect small bowel or the colon. These pathogens elaborate cytotoxins resulting in the recruitment of inflammatory cells. [112] Pathogens induced inflammatory diarrhea by producing mucosal damage or stimulating intestinal secretion. In addition inflammatory reaction products or synthesis of inflammatory mediators such as cytokines, prostaglandins, which are both related to mucosal damage and to intestinal secretion. Intestinal bowel disease main cause of inflammatory diarrhea. [113,114,115]

**Treatment of diarrhea:**

For the prevention and control of diarrhea, rehydration therapies are thought to be the best treatments.[116] Oral rehydration solutions (ORS) are used as treatment of this disease. But nowadays their acceptance has decreased because of their efficacy to reduce the bowel movement, frequency of stool, prevention of fluid loss and to shorten the illness duration. However, it can make up the deficiency of minerals in the body. The higher dose of antibiotics, which are used for the treatment of this disease, can cause disturbance in the intestinal microbial flora and appearance of *Clostridium difficile* infection. [117] For the control of this disease, attention is given to safe, simple, and effective measures that will obviously decrease the rate of stool loss and/or the duration of illness.[118] The gut microbial flora plays an important role in the cure of gastrointestinal diseases like diarrhea.[119] This fact generates a new practice of diarrhea management by the use of probiotic foods (fermented foods) with probiotic bacteria. [120,121] Probiotic foods (*Lactobacillus* fermented foods) have been used for the establishment of intestinal microbial balance, to aid the progress of dietary digestion, or prevent colonization of pathogens [122] Probiotics are live microorganisms that beyond inherent and general nutrition provide health benefits mostly in the form of fermented milk taken in sufficient quantity. [123] Utilization of certain "*Lactic bacilli*" would augment one's health and wellbeing by aiding the in health-promoting activities of the native gastrointestinal microbial flora and reduce their potentially injurious effects. It has taken almost all these years to ascertain specific strains of "*lactic bacilli*" that are able to achieve these "probiotic" tasks.[124] Food is used as a substrate and considered as an important factor for the provision of probiotics to intestinal tract. Nutritional ingredients present in food affect growth and development of probiotics naturally present in the intestine. To deliver the probiotics to the stomach, dairy products, especially fermented milk and cheese are considered as an ideal medium.[125] Probiotic food

supplements are also available which provide benefits to the host by stabilizing the microbial balance of the intestine and possibly accompaniment the basic nutrition.[129] Probiotics include lactic acid bacteria and among them are some *Bifidobacteria* and *Lactobacilli* that have been reported to have health benefits ranging from interfacing with harmful bacteria in the gut to the reestablishment of gut epithelial and immune homeostasis.[126] Extensive research on both species has revealed their specific benefits to the human body.[127] For the treatment of acute diarrhea, probiotics have been proposed as therapy [128] Some specific benefits provided by various species of *Lactobacilli* in the treatment of diarrheal diseases are: growth factor enhancement bioavailability of minerals,[129] mucosal barrier stabilization and reduction in the permeability of the intestine, mainly through inhibition of pathogens by lactic acid, hydrogen peroxide production, and stimulation of immune function and strengthening it.[130] The fermented camel milk is a significant remedy for diarrheal treatment [131] and also has nutritive and other therapeutic applications. Researcher performed an experiment trial on rats infected by *Rotavirus* to evaluate the effectiveness of fermented camel milk against diarrhea. After the treatment, the results indicated that probiotic camel milk contained high contents of antimicrobial agents and showed potent antiviral activity, especially against diarrhea-causing viruses.

Most of the researcher described that *Lactobacillus* strain of probiotic is effective against the diarrhea, and their utilization in various functional food. This is natural method of treatment could be an effective replacement of allopathic treatment of diarrhea without any considerable side effects.

**Lactobacillus strain of probiotic Mode of action:**

Diarrheal diseases are control through various mechanisms of lactobacillus bacteria which expressed below:

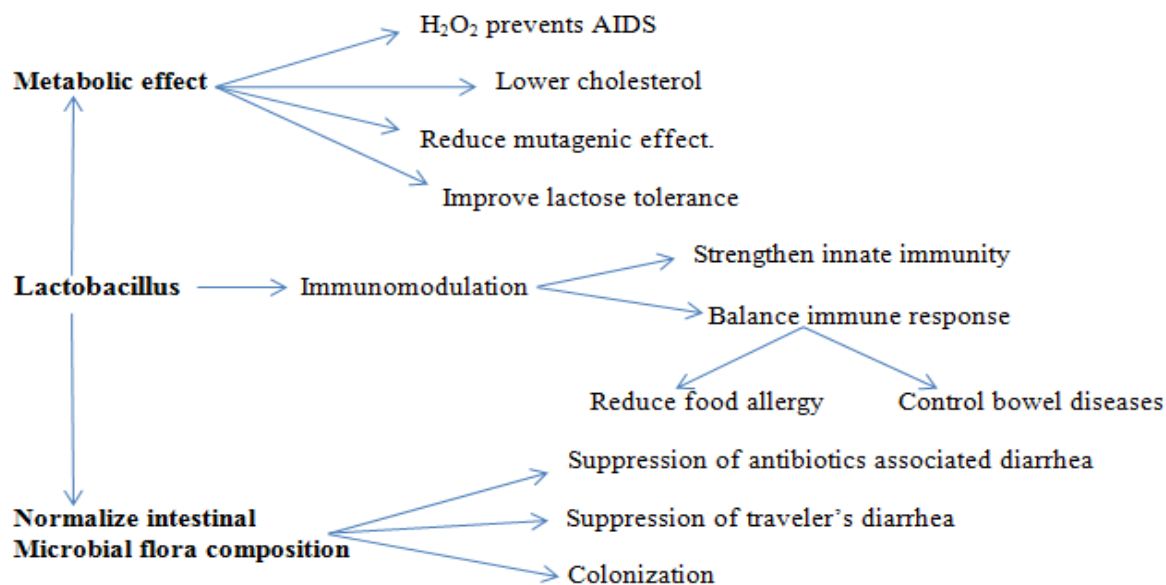


Fig. 3 Mode of action of *Lactobacillus* strains of probiotic

#### Improving epithelial barrier function:

*Lactobacillus* strains (beneficial microorganisms) play a major role in improvement of health of host.[132] Various lactobacillus strains having ability to lower blood cholesterol, improves function of intestinal epithelial barrier,[133] reduce mutagenic reactions, produces organic acids, hydrogen peroxide and bacteriocins.[134]

#### Immunomodulation:

*Lactobacillus* strains have various effects on immune system which strengthening immune system,[135] modification of immune responses of mucosa,[136] balancing of immunity which reduces the incidence of food allergy reactions [137] and addition of phagocytes,[138] and also controls irritable and inflammatory bowel disorders.[139]

#### Normalization of intestinal microbial flora:

It is recognized that gastrointestinal tract of new born baby is sterile at time of birth but as the time passes normal intestinal microbial flora established. [140] This normal microbial composition stops the adhesion of pathogens epithelial cells. i.e. it includes *Salmonella* enteric, *E.coli* and *Typhymurium* which are responsible for occurrence of infectious diarrhea.[141,142] *Lactobacillus* strains also restrain incidence of diarrhea associated with antibiotics treatment and traveler's diarrhea. In addition probiotic helpful for colonization of other beneficial intestinal bacteria. [143]

#### Production of antimicrobial agents:

*Lactobacillus* strain occupy the limited mucosal layer space and epithelial cell surface to hold back growth of pathogenic microorganisms can cause

diarrhea.[144] *Lactobacillus* strain controls pathogenic microbes by production of antimicrobial agents such as bacteriocins, organic acids and H<sub>2</sub>O<sub>2</sub>. Production of this antimicrobial agents inhibiting growth of harmful microorganisms and also suppress the transmission of diseases.[140]

#### ANTIOXIDANT ACTIVITY:

A net of pro-oxidants and the potency of an antioxidant defense system normally balanced in the body. Principal pro-oxidants are reactive species (including free radicals) divided into reactive oxygen species (ROS) and reactive nitrogen species (RNS) and they mediate the main effects of other pro-oxidative factors.[145] In the organisms the crucial ROS are superoxide radical, hydroxyl radical, lipid peroxy radical and non-radical hydrogen peroxide [146] (the latter is produced from superoxide by superoxide dismutase) and the principal RNS are nitric oxide and non-radical peroxy nitrite. The pathological efficiency of the hydroxyl radical is the most potent and it is rapidly generated via the Fenton cycle where free iron (a very potent pro-oxidant) reacts with hydrogen peroxide and also reactive oxygen metabolites (ROM), generated through normal reactions within the body during various cellular processes i.e. respiration in aerobic organisms, can cause damage in proteins, mutations in DNA, phospholipids oxidation, and modification in LDL. Most of the mentioned ROS come from endogenous sources as by-products of normal metabolic processes, while exogenous sources involve exposure to cigarette smoke, environmental pollutants, radiation, drugs, bacterial infections, excess of food iron, unbalanced intestinal microbial

flora, Several diseases are associated with the toxic effect of the transition metals (iron, copper, cadmium). Thus, abnormal formation of the ROS can occur *in vivo* and that leads to the damage of lipids, proteins, nucleic acids and carbohydrates of cells and tissues. An excessive production of ROS causes an imbalance in the pro-oxidants/antioxidants system. Any imbalance in favor of the pro-oxidants potentially leading to damage was termed 'oxidative stress'. Excessive amount of ROM can result in cellular damage which, in turn, promotes chronic diseases including atherosclerosis, arthritis, diabetes, neurodegenerative diseases, cardiovascular diseases, and cancer. To neutralize the oxidant molecules, the human body synthesizes antioxidant enzymes and molecules that, together with the antioxidants contained in food, form the biological antioxidant barrier. However, in certain circumstances, the defense system fails to protect the body against oxidative stress; consequently, the possibility of increasing antioxidant defenses is considered important in the maintenance of human health and prevention from diseases. In this direction, a novel pioneering approach is represented by the development of probiotics exerting antioxidant activity and counteracting the oxidative stress in the host.

Recent an adapted new conception of oxidative stress was advanced as a disruption of redox signaling and control emphasizing [148] an impact of the redox ratio as good tools for the quantification of oxidative stress. It is remarkable that the glutathione redox ratio has a crucial impact concerning this conception. A large body of evidence confirms that high-grade oxidative stress is one of the crucial players in the pathogenesis of diseases. To maintain the physiological grade of oxidative stress needed for a number of biofunctions like intracellular messaging, growth, cellular differentiation, phagocytosis, immune response. The human body has an integrated antioxidant defense system (IADS, Table. 1). Several antioxidant components for this human IADS are derived from foodstuffs and provided by GI microbial flora. Interestingly, it became more apparent to the IADS of the host and GI microbial

flora are tightly linked and some specific strains with physiologically effective antioxidant activity may have a great impact on the management of the oxidative stress level in the gut lumen, inside mucosa cells and even in the host blood, to support the functionality of the IADS of the human body. Thus, experiments to find out strains with physiologically relevant antioxidant properties/effects as well as trials (including special clinical trials) using capsules of such strains or foodstuffs enriched with antioxidant strains are needed. Unfortunately, scientific data on probiotic LAB with physiologically relevant antioxidant properties is very limited and the data of experiments/trials about both intestinal antioxidant protection/influence and systemic antioxidant protection/influence (effects of Oxidative stress-related indices) are scarce. Probiotics are believed to stimulate growth and healthy as well as to modify the ecology of the intestine in a beneficial manner for the host. [149] An increasing number of scientific reports have appeared on the effects of probiotic combinations on the health of the host than using single probiotic strain [150] Probiotic bacteria are widely used in human and animal nutrition and beneficially influence the balance of the intestinal flora of the host. Probiotic bacteria provide an array of health benefits which include competition, antagonistic effects, enhancement of digestion, strengthening of the immune system and stimulation of vitamin production.[151] Also have demonstrated antioxidant potential studied from different assay. Probiotic exhibit antioxidant activity in all major way, they may reinforce the inherent cellular antioxidant defense by secreting enzymes like superoxide dismutase (SOD). They also release and promote the production of the major non-enzymatic antioxidant and free -radical scavenger glutathione (GSH). Moreover, they promote the production of certain antioxidant biomolecules, such as the exopolysaccharides (EPSs).And finally, the exhibit metal chelating activity. All these data suggest that probiotics may have a potential therapeutic role in reactive oxygen species (ROS), characterized gastrointestinal disorders.

Oxidative stressors (pro-oxidant)	Integrated antioxidant defense system
Ischemia/reperfusion	Vitamin E, C, Q, A
Smoking, Inflammation, xenobiotics	Enzymes as antioxidants
PUFA mega doses	(SOD, GPx, CAT, HO1)
Iron or copper excess	Other antioxidants
Radiation, Exhaustive exercises	(GSH, plasma albumin, uric acid,
Prolonged severe emotional stress	Bilirubin, carotenoids, etc)

Table 1. Effect of oxidative stressors and the potency of the integrated antioxidant defense system (IADS) of the body are normally balanced. An imbalance leads to potentially harmful oxidative stress. PUFA, polyunsaturated fatty acids; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; HO1, haem oxygenase1; GSH, reduced glutathione.[148]

Probiotics strains have developed, in order to respond to specific physiological targets probiotics beneficial effects, some authors have reported the protection against oxidative stress and the capability to decrease the risk of accumulation of ROS. The antioxidant mechanisms of probiotics could be assigned to ROS scavenging, metal ion chelation, enzyme inhibition, and to the reduction activity and inhibition of ascorbate autoxidation. Probiotic metabolic activities may have an antioxidant effect via the scavenging of oxidant compounds.[152]

#### **Role of probiotics in intestinal anti-oxidative protection (Mechanism of action):**

The most documented effects of LAB in humans are the stimulation of the immune system, the prevention and the reduction of the intensity and duration of diarrhea, and reduction of lactose intolerance.[153] LAB also have some other beneficial effects such as vitamin synthesis, improvement of mineral and nutrient absorption, modulation of GI physiology and reduction of pain perception. Special probiotic strains may induce the expression of receptors on epithelial cells that locally control the transmission of nociceptive information to the GI nervous system.[154] LAB may thus be of benefit to health and help protect against diseases, like CVD, diabetes, metabolic syndrome, etc. As far as oxidative stress is at least one of the components of initiation and/or the development of the mentioned diseases thus any kind of agent which can prevent the development of harmful oxidative stress has a principal impact. They can during the consumption period adhere to the epithelial cells of GI modulating the human physiological status via the gut associated immune system and/or directly due to the expression of receptors of GI. LAB beneficial effects are strain-specific. *In vitro* and cellular models, the probiotic properties of lactobacilli have been limited to few parameters such as the ability to survive low (pH 2-3) and bile salts, to produce pathogen inhibitory compounds (including hydrogen peroxide), to compete with energy availability or adhesion sites, and to enhance immune response.[155] Along with the probiotics themselves, there are metabiotics i.e., the metabolic by products of probiotics. Metabiotics are beneficial in promoting a healthy GI by creating an environment most favorable to probiotics, by nourishing the enterocytes, reinforcing mucosal barrier function, by maintaining or supporting epithelial integrity or signaling the immune system to limit inflammatory responses both in the gut and through influencing T-cells throughout the body. Glutathione (L-gamma-Glu-L-Cys-Gly or GSH) is a major cellular non-enzymatic antioxidant. It eliminates lipid- and hydro peroxides, hydroxyl radical and peroxynitrite

mainly via cooperation with glutathione peroxidase. The GI surface is an important host organism-environment boundary and the interactions of gut microbes inside the intestinal lumen and mucosal cells are important for the host. An impaired environment such as the imbalance of GI microbial flora, but also the increase of LP and decrease of the reduced GSH both at the GI surface and in the GI cells, are the mighty modulators causing different unhealthy outcomes in the host. In this process the involvement of the glutathione system is crucial as GSH, besides its role as a crucial antioxidant, is the principal redox controller for a number of processes in cells. GSH has essential role in maintaining mucosal integrity. Evidently some probiotics are able to promote an elevation of the level of beneficial bacteria in the GI.

#### **ANTIMICROBIAL ACTIVITY:**

Throughout the history of microbiology, most human studies have been focused on the disease causing organisms found in people; while fewer studies have studied benefits of the resident bacteria. However, humans are surrounded by beneficial microorganisms that live in human body. The intestinal microbial flora is very well adapted, exceptionally stable and very specific for each individual. In normal conditions of stable functioning of the digestive system, neutral and beneficial microorganisms dominate. It is estimated that there are 100 trillion microorganisms in the intestine of a human adult and this is 10 times larger than the number of cells in the human body.[156,157] However, the balance of the intestinal microbial flora is negatively influenced by advance lifestyle, which leads to increased numbers of pathogenic microorganisms that disrupt microbial balance and cause a reverse from beneficial to harmful functioning. In such cases, the external support with probiotics is very welcome and supported by several scientific studies.[158] The most common probiotic bacteria are certain strains from the genera *Lactobacillus* and *Bifidobacterium*. Other probiotic bacteria include, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Enterococcus faecium*, *Streptococcus thermophilus*, *Escherichia coli* Nissle 1917, etc. Certain yeasts such as *Saccharomyces boulardii* are also probiotics. [159,160]

Probiotics together with other beneficial microbes are commensals of the gut and differ from pathogenic bacteria in the terms of their actions on immune cells in the gut as they do not stimulate the proliferation of mononuclear cells or trigger an inflammatory action[161] probiotic should have the ability to adhere to mucus and/or epithelial cells, and/or other surfaces; a probiotic should be

susceptible to antibiotics; a probiotic should exhibit antimicrobial activity against pathogens the important attributes of probiotics is their antimicrobial effect against pathogens by maintaining the homeostasis of the intestinal flora.

#### Antimicrobial properties of probiotics:

The online Merriam Webster dictionary defines “antimicrobial” as “destroying or inhibiting the growth of microorganisms and especially pathogenic microorganisms” and “antagonistic” as “showing dislike or opposition: showing antagonism”. This antimicrobial/antagonistic ability is especially important for probiotics as one of the functional beneficial requirements of probiotics is a broad antimicrobial spectrum as well as antagonism against pathogenic bacteria with strong antimicrobial activity. The antagonistic activity of one microorganism against another can be caused by competitive exclusion, immune modulation, stimulation of host defense systems, production of organic acids or hydrogen peroxide that lower pH, production of antimicrobials such as bacteriocins, organic acids, hydrogen peroxide, antioxidants, production of signaling molecules that trigger changes in gene expression.[162,163] Antimicrobial substances produced by beneficial microorganisms are known to include lactic acid, acetic acid, formic acid, phenyllactic acid, benzoic acid as well as other organic acids, short chain fatty acids, hydrogen peroxide, carbon dioxide, acetaldehyde, acetoin, diacetyl, bacteriocins and Bacteriocins like inhibitory substances and others.[164-166] The most common bacteriocins include lacticin, lactocin, pediocin, pisciolin, enterocin, reuterin, plantaricin, enterolysin and nisin[167-168]

Use of probiotics for the betterment of human and animals is an established fact now. Recently a plenty of studies have been emerged in support of their antimicrobial effect from the good quality clinical trial with randomized placebo controlled design and results from the properly performed invitro studies [169-173]. Probiotics with a variety of application are reported to enhance the intestinal health and immune system, as well as anticarcinogenic, antidiarrheal and hypocholesterolaemic effects, improve lactose utilization[174,175] *Lactobacilli* are known to produce many types of bacteriocins like acidophilin acidolin, lactocidin, lactobrevin [176,177] These organic acids not only lower the pH thereby affecting the growth of pathogens but also are toxic to microbes. Besides producing antimicrobial toxins,

Probiotics have ability to adhere to cells, reducing pathogenic bacteria adherence hence causing pathogen exclusion. Probiotics interact with

epithelial cells and dendritic cells and immunomodulatory effect. Many *Lactobacilli*, *streptococcus* and *saccharomyces* species have been reported to found safe for the prevention and treatment of various infectious diseases.[178,79] Indiscriminate use of costly antibiotics leading to the emergence of multi drug resistance in pathogenic bacteria is a major clinical concern throughout the world.[180] The severe side effects of antibiotic therapy has raised the demand for an alternative safer therapeutic agent.[181] Probiotic could be a good candidate in this regard.[182] The antimicrobial activities either in-vivo or in-vitro against the wide range of pathogens including *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been reported by the various species of *lactobacilli*, *saccharomyces* and *streptococcus*. [183,184] Researches related to the use of probiotic as a complete alternative of antibiotic are in early phases. So, it is envisaged that a judicious combination of antibiotics & probiotics must be used to treat acute phase of the infection as the enhancement in the antimicrobial activity of the antibiotic by probiotic strain will not only cut the duration but also the cost of the antibiotic treatment booning the poor people in developing countries on one hand and reducing the increasing drug resistance in pathogenic micro-organism on the other hand.

**SIDE EFFECTS OF PROBIOTICS:** Normally probiotic are considered as non-harmful bacteria that exert health benefit to the host. This is true in most cases. *Lactobacillus* are used for a long times due to their safety. Probiotics which are mostly considered as safe are *Lactobacillus* and *Lactococcus*. It has been well established that the intestinal microbial flora plays an important role in the metabolic activity and immune system of the host, and probiotics help to promote microbial flora. However, it can also be argued that manipulation of the normal microbial flora by probiotic use may theoretically increase the risk of adverse metabolic and immunomodulatory effects. Some minor adverse effects, including thirst, constipation, bacteraemia and meningitis with *S. boulardii* use [185,186] bloating and flatulence with *L. rhamnosus GG* use [187] nausea, vomiting, abdominal pain, rash, diarrhea and constipation, have been reported [188] Although serious complications from probiotic use are exceedingly rare, given that probiotics are live microorganisms, it is conceivable that they may rarely result in invasive infections. Mackay et al reported a case of *Lactobacillus* endocarditis with probiotic use in a patient with underlying mitral valve disease [189]. There was a case of liver abscess due to *Lactobacillus rhamnosus* reported in a diabetic patient who was consuming dairy products containing *L. rhamnosus GG* [190]. A case of

recurrent *Bacillus subtilis* septicemia has also been reported in an immunocompromised patient [19] after the use of probiotics containing *B. subtilis*. There have also been several reported cases of *S. boulardii* fungemia associated with probiotic use. Most cases of invasive infections associated with probiotic use have occurred in patients with intravenous catheters [194-196], the elderly [197] and immunocompromised population [198,199]. Safety of the probiotics containing strains of *Lactobacillus* and *Bifidobacterium* was evaluated in infants and showed no adverse events. Pharmaceutical companies should keep in mind the potential of health risk of probiotics before adding these into particular products. Before launching any probiotics product, safety profile of that probiotic species should be assessed to avoid incidence of any unexpected harmful effect.

**SAFETY OF PROBIOTICS:** The safety of viable microorganisms is difficult to establish using current assessment methods, theoretically almost any microorganism may under, certain circumstances, either cause an infection or alter its virulence due to internal or external factors. The balance between intestinal microbes, the intestinal barrier and whole body forms a cohabitate in which the human system accepts the microbial interference and ecology in its current form. No evidence of opportunistic infection or other ill effects been observed in controlled clinical studies with *lactobacillus* and *bifidobacteria*. [200]

Probiotic are generally regarded as being safe and side effects in ambulatory care have rarely been reported. Safety issues are related to bacterial translocation and sepsis, and to the risk of carrying antibiotic resistance transposons that may spread resistance to antibiotics. The latter has been reported for some probiotics, among which are *L. reuteri* ATCC 55730 and *enterococcus faecium*. [201] Also the non-pathogenic *S. boulardii* yeast has been shown to be safe in animal models and although the yeast has been available for over 40 years and used throughout the world side effects, are uncommon. Nonetheless a few cases of fungemia have been reported although only 13 well documented cases of *S. bularadii* infection have been described in the literature In one study constipation and increased thirst were reported to occur. The biotherapeutic agents are extremely rare, cases reported in literature are limited to sporadic cases of transient bacteremia or fungemia.

#### PRECLINICAL TECHNIQUES FOR SCREENING OF ANTIDIARRHEAL DRUGS:[202]

##### In vivo models:

1. Gastrointestinal transit time using charcoal meal
2. Castor oil induced diarrhea

3. Castor oil induced enteropooling
4. Magnesium Sulphate induced diarrhea
5. Serotonin induced diarrhea
6. Prostaglandins induced diarrhea

##### Intestinal enteroids models:

1. Enterotoxigenic E.coli induced diarrhea
2. Pathogenic E.coli induced diarrhea
3. Cholera toxin induced diarrhea

##### Ex. Vivo models:

1. Effect of test sample on isolated tissue
2. Effect K induced contraction

##### Innovative techniques:

1. Enkephalinase inhibition as a potential drug target for secretory diarrhea
2. Enkephalinase inhibition assay
3. Intestinal ion channels as anti-diarrheal screening target
4. Screening of drugs against inflammatory bowel disease (IBD) associated diarrhea
5. Farnesoid X receptor (FXR) as a potential target for anti-diarrheal screening

##### CONCLUSION:

Live microorganisms confers beneficial effects majorly on gastrointestinal tract. It is used in treatment of diarrhea, inflammatory bowel disease, lactose intolerance, cancer, hypertension, diabetes. Probiotic act through different mechanisms like as colonization resistance, release of antimicrobials agents, enhance immune response, and strengthen intestinal pathogenic barriers. Probiotic classified into bacterial probiotic and yeast probiotic. Its metabolites possess antioxidant activity by protecting against oxidative stress and reduce accumulation of reactive oxygen species. Probiotic also shows antimicrobial activity against various pathogenic agents and inhibits adhere to intestinal membrane, destroy them by releasing various peptides like as bacteriocin, H<sub>2</sub>O<sub>2</sub>, Probiotics might be best replacer of allopathic treatment with benefits of no side effects, cost effectiveness, easily availability and natural way of treatment. The concept of probiotics as cure of gastrointestinal diseases is no doubt an emerging technology but still it needs more research.

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