**PROBIOTICS: RECENT ADVANCES AND FUTURE PROSPECTS**

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**Abstract:** Probiotics are the microorganism which imparts beneficial effect in the human body. The consumption of Probiotic in the form of powder, capsule and drinks restore the beneficial microflora in gut and in turn help the human being by enhancing immune system. The present review focusing on the role of Probiotics for its possible role in controlling and treating diseases such as urinary tract infection, diarrheal disease caused by bacteria, oral infection i.e. gingivitis, oral cavity and cancer. This review also focuses on regulation, side effect, safety and future prospects of Probiotics.

**Keyword:** Probiotics, UTI, Oral hygiene, Diarrhea

**INTRODUCTION**

Increasing resistant of the microbes against the multiple antibiotics has led a great threat to human health. The researchers all over the world are looking for the alternate source. One way to prevent disease is to use third generation antibiotics to control microbes while the other way is to use beneficial or antagonistic microbes that act on disease causing microbe by competition for space and nutrition. Microbe used for control of microorganisms should be harmless to the host and does not have any effect on the natural biome of human gut. This method will be better in terms of side effects as in case of chemotherapy. The term ‘Probiotics’ are combination of two word (pro-for and biotics-life). Probiotics is a mechanism that employs culture suspension of microbes for removing the pathogens from body and maintaining milieu interior of body. Probiotics plays significant role for various organs and systems of the body like it has great impact on gastrointestinal tract, cancer, vaginal infections, allergies, respiratory diseases etc.

The use of antibiotic was started after its discovery by Alexander Fleming in 1928. The discovery of antibiotic increased the life expectancy of human beings and also useful substance to control the sepsis after post-operation. Before the discovery of antibiotic and antiseptic agent the death of most of female occurs after delivery and during delivery. Now due to increase use of antibiotic and disinfectant the post operative infection has been largely controlled. The large population of the world directly or indirectly were exposed to antibiotics. The disadvantage of antibiotics is its side effect which includes liver damage, gastro intestinal disturbance, temporary or permanent deafness, nephrotoxicity, nausea, allergic reaction etc. (Table 1) and another problem associated with antibiotic is that it does not differentiate between beneficial and harmful microorganism and result in disturbing normal microflora which in turn cause deficiency of vitamins such as Vitamin B, Vitamin K etc. in human body.

**Table 1. Antibiotic and its mechanism of action and their side effects.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Antibiotic</th>
<th>Mechanism of Action</th>
<th>Side effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metronidazole</td>
<td>disrupts DNA of microbial cells by preventing nucleic acid synthesis</td>
<td>Sensory neuropathy, Gastrointestinal disturbance, neoplasia</td>
<td>Zivkovic et al. (2001); Friedman et al. (2009),</td>
</tr>
<tr>
<td>2</td>
<td>Pencillin</td>
<td>Interfere with enzymes required for the synthesis of the peptidglycan layer.</td>
<td>Skin Allergies</td>
<td>Bhattacharya (2010)</td>
</tr>
<tr>
<td>3</td>
<td>Clindamycin</td>
<td>Inhibit protein synthesis by interfering with the transpeptidation reaction.</td>
<td>Anaphylaxis, Leucopenia, Hepatotoxicity</td>
<td>Granowitz and Brown (2008)</td>
</tr>
<tr>
<td>4</td>
<td>Amanoglycoside (streptomycin, kanamycin, neomycin, gentamicin, tobramycin, and amikacin)</td>
<td>Inhibit protein synthesis by inhibiting 30S ribosomal subunit</td>
<td>Ototoxic effect Nephrotoxicity</td>
<td>Forge and Schacht (2000)</td>
</tr>
<tr>
<td>5</td>
<td>Chloramphenicol</td>
<td>Inhibit bacterial growth by</td>
<td>Aplastic anaemia</td>
<td>Cruchaud et al. (1963)</td>
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*Corresponding Author*
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<tr>
<th>Probiotics</th>
<th>Mechanism of action</th>
<th>Health Benefits</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Tetracycline</td>
<td>Inhibit protein synthesis by blocking the binding of the aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex</td>
<td>Yellowing of teeth</td>
<td>Sánchez et al. (2004)</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>Antagonist, inhibition of metabolic pathway</td>
<td>Dermonecrotic, Stevens-Johnson syndrome</td>
<td>Roujeau et al. (1995)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Inhibition of cell wall synthesis of bacteria</td>
<td>Diarrhoea, Sore in mouth, fever, swollen gland, reversible leucopenia Allergic skin reactions</td>
<td>Paavonen et al. (1989)</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Prevent replication, transcription, and repair of bacterial DNA by inhibiting DNA gyrase enzyme</td>
<td>Retinopathies, chondrotoxic effects</td>
<td>Barnhill et al. (2012)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Inhibit cell wall synthesis of Gram-positive bacteria by binding C-terminal acyl-D-alanyl-D-alanine (acyl-D-Ala-D-Ala) containing residues in peptidoglycan precursors.</td>
<td>Vancomycin can produce pseudoallergic reactions. Rapid intravenous infusion may trigger histamine release, which causes a variety of symptoms, including erythematous or urticarial reactions, flushing, tachycardia, and hypotension. The extreme flushing is called redman syndrome. The most significant untoward reactions are ototoxicity and nephotoxicity.</td>
<td>Daniel (2013)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Inhibiting the initiation process of protein synthesis</td>
<td>cause anemia by suppressing erythropoiesis and thrombocytopenia.</td>
<td>Bernstein et al.(2003)</td>
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In past few years India had witnessed the increased faith towards the traditional system of medicine. In India Ayurveda was practiced since time immemorial and people are getting good result however this system has slow acting effect on human body. The present review focuses on the beneficial effect of Probiotics in various ailments that can be controlled and treated by taking consortium of beneficial bacterial species which can be easily adapted and tolerate the gut environment of human being and impart health benefits. As per FAO/ World Health Organization (2001), Probiotics are mixture of living microorganism which imparts health benefits when administrated in adequate amount. The history of Probiotics dates back when Elie Metchnikoff gave the concept of Probiotics and discovered the process of phagocytosis in which he emphasized the role of macrophage and microphage that play important role in prevention of infection. For this contribution Elie Metchnikoff was awarded Nobel Prize in 1908. Elie Metchnikoff observed that consumption of useful microorganism (presently known as Probiotics) would modify the gut flora and harmful microorganism will be replaced by the useful bacteria. The fermentative process of bacteria produce lactic acid which inhibit the growth of proteolytic bacteria lowering down the pH of gut which in result inhibit the growth of harmful bacterial species. In Indian literature there is well documented that consumption of curd is good for health and longevity. Elie Metchnikoff also observed that Russian and Bulgarians consumed fermented milk in their diets were exceptionally long lived. Based on these observations Metchnikoff proposed that consumption of fermented milk would repopulate the intestine with harmless lactic-acid bacteria Most Probiotics are bacteria similar to those naturally found in people’s guts, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, Lactobacillus or Bifidobacterium. Within each group, there are different species (for example, Lactobacillus acidophilus and Bifidobacterium bifidus), and within each species, different strains (or varieties). A few common Probiotics, such as Saccharomyces boulardii, are yeasts, which are different from bacteria. Commercially available probiotic based products in the market are yoghurt, ice creams, milk beverages, capsules, antibiotic supplemented with Probiotics, tablet, powder etc. Lactobacillus and Bifidobacterium genera, which are found naturally in dairy products, are commonly used strains for preparation of Probiotics. **Probiotics against diarrheal disease** Indian subcontinent is very prone to diarrheal disease because of poor quality or inadequate drinking water and poor sanitation. Most of the people residing in rural areas still do not have access to clean water. The source of water for drinking is tube well, hand pump and traditional well. So, occurrence and prevalence of gastrointestinal problem is quiet common in villages and some urban areas. However, common types of diarrhea can be subside naturally, but bacterial associated disease such as Salmonella
Typhi, Shigella sp., Vibrio sp. required medical attention.

The microflora of new born is influenced by the mode of delivery in case of vaginal route the upper GI tract is colonized by vaginal and faecal microbiota of mother. The infant born with caesarean procedure are exposed to the microbiota of mother. The duration to establish the primary intestinal microflora vaginal delivery require only one month while caesarean require up to 6 month after birth. The initial bacterial count is unstable during first week after that the bacterial counts are found in the range $10^9$ – $10^{10}$ per gram of faeces (Palmer et al., 2007). The microbial flora also influence by the feeding type i.e. mother milk (Breast feeding) and formula milk feeding. In case of breast feeding microbial flora is dominated by Bifidobacteria and Lactobacilli while in formula milk feeding infant the microflora is dominated by high number of Enterobacteriaceae, Bifidobacteria, Bacteroides, Clostridia and Enterococci (Fig.1). The microflora in both cases becomes similar after infants start eating the solid foods.

In case of bacterial associated diarrhea there is administration of broad spectrum antibiotic. Several reports published which support that Probiotics are effective in treatment and prevention of diarrhea caused by Salmonella. The possible mechanism suggested for prevention of diarrhea is Probiotics that it causes the stabilization of the gut mucosal barrier, increasing the secretion of mucus, improving gut motility and it does not allow invading bacterial pathogen to colonise the gut and competing for the nutrient. It also produce bacteriocins (low molecular weight antimicrobial substance) which has property to inhibit the growth of other microorganism. (Fig.2).

Probiotics also has the ability to enhance phagocytic activity of macrophages, initiate Natural Killer cell activity, immunomodulatory activity to prevent infection (O’Hara et al., 2006; Gobbato et al., 2008). L. plantarum, L. rhamnosus, L. acidophilus, L. kefir, L. casei, St. thermophilus, L. delbrueckii ssp bulgaricus L. gasseri F71, L. gasseri L1, L. paracasei, E. faecium are few examples of bacterial species which is responsible for preventing and controlling the diarrheal disease.

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**Fig 1:** Gastrointestinal tract and microorganism associated with it (Adapted from Chandra et al. 2016)
In most of the developing countries there was reported cases of Traveller diarrhea which is result of travelling to the countries where *Entamoeba histolytica* is endemic. This protozoan is the second most common causal agent of diarrhea in developing countries. This protozoan parasite is often known to coexist as a part of the normal gut microbiota. Infection due to this parasite causes diarrhea with blood and may infect other organ if not treated in time. The treatment with metronidazole and tinidazole, used to *E. histolytica* infections. However, these drugs have adverse side effect. Due to potential side effect of antibiotics researchers are now searching for alternate treatment. Sarjapuram et al (2016) investigated the effect of Probiotics on the growth of Entamoeba. *Lactobacillus casei* and *Enterococcus faecium* showed a significant reduction of up to 71%. Higher reduction was observed when tested in combination. Decreased duration of bloody diarrhea was observed when Probiotic strain of *Saccharomyces boulardii* when administered together with metronidazole in case children infected with *E. histolytica* (Dinleyici et al., 2009).

### Probiotics for Urinary tract infection
Urinary tract infection is commonest disease in female as compared to male. It affects urinary bladder, ureter, genital organ and kidney. Frequently encountered microorganisms in case of UTI are *E.coli* followed by *Staphylococcus aureus*, *Klebsiella* sp., *Enterococcus* and *Candida albicans*. The reason of higher female susceptibility to urinary tract infections in females is a result of a variety of factors, such as the close proximity of the female urethral meatus to the anus (Lipsky, 1990) short urethra and incomplete and in coordinate voiding of urine in school girls which is often leads to infection of the urinary tract (Mond et al., 1970). UTI in most cases restricted to the infection in bladder (essentially in females) with only superficial mucosal involvement, or occasionally in organ like kidneys in both genders, and in prostate in males. The incidence of UTI was reported higher among females as compare to males and children (Hootan, 2001 and Foxman et al., 2001).

The incidence increases with age and sexual activity (Castellsagué et al., 2005). Rates of infection are high in postmenopausal women, because of bladder or uterine prolapse causing incomplete bladder emptying; loss of estrogen with attendant changes in vaginal flora, loss of *Lactobacilli* sp., which allows periurethral colonization with gram negative aerobes, such as *E. coli* (Pabich et al., 2003; Hu et. al., 2004 and Foxman, 2007). Catheterization is also
associated with a very high incidence of UTI (Hazelett et al., 2006). Reduction of UTI cases was observed after the administration of using vaginal route Lactobacillus rhamnosus GR1 in combination with either L. reuteri B 54 or RC 14. In the study on 52 woman who had history of reoccurrence of UTI were under single weekly dose of vaginal suppositories of Lactobacillus rhamnosus GR1/ L. reuteri B 54. The result showed the reduction of UTI reoccurrence (Reid et al., 1995).

In woman during gestation period susceptibility to UTI or bacterial vaginosis is greater as compared to normal woman. The using intravaginal applications of yogurt to treat woman with bacterial vaginosis in the first trimester of pregnancy reduced the risk of preterm birth or miscarriage was report by Neri et al. (1993). The maintenance of vaginal pH and prevalence of Lactobacillus spp. in vaginal region was essential for preventing bacterial vaginosis. The possible mechanism of Probiotics include it prevent the adhesion of uropathogens to the epithelial cells, some Probiotics produce hydrogen peroxide which kill the uropathogens, degradation of lipid, stimulation of secretory antibodies IgA and production of conjugated linoleic acid (Shalev, 2002). Lactobacillus reuteri, originally derived from mother’s milk, has been reported to survive the harsh stomach environment and maintain a healthy balance of friendly bacteria in the digestive tract.

**Probiotics for oral hygiene**

Microorganisms present in oral cavity are considered as oral microflora. Oral cavity consists of different habitats such as teeth, tongue, gingival sulcus, cheek, soft and hard palate, tonsils. These region are colonized by bacterial species which does not cause any disease, they are normal inhabitant of oral cavity. The predominant microflora of oral cavity includes Bacteroidetes, Chlamydiae, Firmicutes, Chloroflexi, Euryarchaeota, Fusobacteria, Proteobacteria, Spirochaetes, Synergistetes and Tenericutes etc (Dewhirst et al., 2010). Taking Probiotics such as L. rhamnosus GG, L. reuteri and Bifidobacterium reported to have inhibitory action on the growth of Streptococcus mutans (Näse et al., 2001; Nikawa et al., 2004; Cildir et al., 2009).

An experimental study on the effect of cheese containing L. rhamnosus strains GG and LC705 and Propionibacterium freudenreichii sps. shermanii JS on Candida spp. responsible for oral candidiasis in case of elderly persons. After 16 weeks the number of yeast count decreased but no change in mucosal lesion was observed (Hatakka et al., 2007).

The use of Probiotics enhancing the growth and number of normal microflora of oral which inturns preventing the colonization of true pathogens, and thus, preventing the microbiological shifts associated with gingival inflammation. Kragan in 1954 done the first study on the effect of lactobacillus for the treatment of inflammatory reaction of the oral mucosa.

Reduced gingivitis index and plaque index was noticed after the administration of two different L. reuteri formulations (LR-1 or LR-2) at a dose of 2 x 10⁶ CFU per day (Krasse et al., 2006). Iniesta et al. (2012) investigated the effect of oral Probiotics on the oral microbiota in which they administered tablets of L. reuteri to 40 gingivitis subjects. The effect of L. reuteri administered in tablets resulted in a reduction in the number of selected periodontal pathogens in the subgingival microbiota, without an associated clinical impact. Reduction in the number of oral microflora Streptococcus mutans and total bacteria after oral administration of fermented fermented milk containing L. rhamnosus SD11 or L. bulgaricus, once daily for 4 wk in 43 helathy young adults. (Rungsrri et al., 2017).

**Probiotics for prevention of cancer (Anticancer properties)**

Cancer is disease which can occur in any stage of life. However, this condition is very rare earlier but now it became very common and number of patient increase day by day. The increased incidence was due to lack of physical work or sedentary life style, food habit, genetic makeup and radiation exposure from the environment. The treatment of cancer in modern medicine is administration of chemotherapy which had so many side effect and painful procedure. The common side effect encountered by cancer patient are fatigue, muscle pain, headache, abdominal pain, peripheral neuropathy, suppression of bone marrow which result in low count of RBCs and WBCs, thrombocytopenia (low platelet counts), loss of hairs, weakness, loss of appetite etc. So, there is immense need of therapy which is effective and less painful to cancer patients. Some side effects are temporary and some are permanent affecting lungs, heart, kidney and other organs. In search of alternate treatment researchers have evaluated some probiotic strains for evaluation of its antitumor potential.

Effect of two probiotic strains i.e. Lactobacillus rhamnosus supernatant (LRS) and lactobacillus crispatus supernatant (LCS) on the human cervical and colon adenocarcinoma cell lines (HeLa and HT-29, respectively) was studied and found that Lactobacillus rhamnosus supernatant and lactobacillus crispatus supernatant are effective on HeLa cell lines while only the effect of L. rhamnosus supernatant is significant on HT-29 (Nouri et al., 2016). Antitumor activity of L. plantarum A and L. rhamnosus b was evaluated by pre-inoculating mice with lactobacilli for 14 days and then subcutaneous and orthotopic intestinal tumours were generated by pre-inoculated mice using CT26 murine adenocarcinoma cells. The oral administration with L. plantarum inhibited CT26 cell
growth in BALB/c mice and prolonged the survival time of tumour-bearing mice (Hu et al., 2015). It is commonly known that persistent infection of human papillomavirus (HPV) is responsible for the development of cervical cancer. In one study in which 54 women that administrated the daily probiotic drink for 6 months enhanced the clearance of HPV and cervical cancer precursors (Verhoeven et al., 2013). Nami et al. (2014), studied the effect of L. acidophilus 36YL strain isolated from the vagina of healthy and fertile Iranian women on four human cancer cell lines (AGS, HeLa, MCF-7, and HT-29) and one normal cell line (HUVEC) and found that the isolated strain have anticancer effects on the four tested cancer cell lines and negligible side effects on the HUVEC normal cell line.

**Regulation of Probiotics**

Probiotics are generally used for the health supplement or preventive therapy and its status is not clearly understood by the health industry. Now a day the Probiotics are gaining popularity and their demand is increasing day by day in India as well as abroad. Manufacturer are now producing Probiotics and also trying to develop a new combination of Probiotics for the health benefits of people but as far as regulation is concerned these companies have shown ignorance towards regulations laid down by the respective countries. Reported published from the different countries suggested that the manufacturing companies do not follow the quality standard as written on the products. The lower count of *Bifidobacterium bifidum* in three commercial yogurts were reported in South Africa (Lourens-Hattingh and Viljoen, 2002), Temmerman et al. (2003) found that of isolates from 55 European probiotic products, 47% of food supplements and 40% of dairy products were mislabelled. The food supplements yielded either no viable bacteria (37%) or significantly lower counts than the dairy products, contradicting the concept that health benefits derive from the presence of a minimum concentration of live probiotic bacteria.

In India, currently, food and drugs are regulated by Prevention of Food Adulteration Act (PFA) and FDA, respectively. The Food Safety and Standards Act of 2005 (FSSA) defines the foods for special dietary uses or functional foods, nutraceuticals or health supplements. Currently in India, Probiotics are characterized as functional food rather than as pharmaceutical drugs and are regulated by food laws that regulate general food items. FSSA was passed by the Indian government with the purpose to regulate different varieties of catables regulations covering nutraceuticals, foods and dietary supplements. According to FSSA, functional foods are defined legally but categorization of food categories, such as nutraceuticals, biotherapeutic agent is still unclear (Arora and Baldi, 2015). Due to increasing demand of Probiotics in these days, there may be the possibility of circulation of false, inferior and ineffective Probiotics based products. Till 2011 there is no regulatory guideline has been developed to monitor the quality of Probiotics and Probiotic based products. So, it is very necessary to have regulatory bodies to monitor or regulate the standard of probiotic products. Regulations on Probiotics were come into the existence in most of the developed countries. So, Indian Council of Medical Research had constituted a task force in which different scientist or experts were considered for making draft guideline for standardization and evaluation of Probiotics.

ICMR-DBT guideline for Probiotics was drafted in 2011 for the evaluation and standardization of Probiotics and probiotic based products in food (Ganguly et al., 2011). The salient features of ICMR-DBT guidelines are as follows:-

- **Defination of Probiotics are same as laid down by the FAO/WHO, 2002**
- **Standardized method should be used to validate the genotypic and phenotypic identification of strain used for probiotic preparation by using modern molecular biological techniques such as 16SrRNA sequencing, DNA fingerprinting, Pulse Field Gel Electrophoresis and PCR based techniques. Nomenclature of bacterial should be as per the ICPS ( International Committee on Systematics of Prokaryotes)**
- **The strain used for probiotic preparation should have properties i.e. bile tolerant, gastric acid tolerant, show antimicrobial activity against pathogenic bacteria, reduce pathogen adhesion,**
- **Toxicity test of probiotic strain for acute, subacute and chronic toxicity if probiotic is taken in excessive amount. Before using for human being it must be tested on animal model to analyse its effect.**
- **Effect of probiotic on human body should be statistically and clinically proven.**
- **Minimal effective dose and number viable cell in cfu/ml/day should be clearly mentioned on the product.**
- **The product should be labeled with bacterial genus, species strain name as per the international nomenclature, evidence of health claim should be clearly mentioned, Serving quantity and storage condition must be written on the product.**
- **Guideline of good manufacturing process should be followed as per the HACCP ( Hazard Analysis and Critical Control Point) and Codex general principle of Food Hygiene**

**Side Effects and Risks**

Probiotics are considered safe for human uses but there is less scientific studies has been done for safety and side effect of long term use of Probiotics. The healthy individuals have a good immune system
but those people who have weakened immune system if Probiotics are given to immunocompromised patient then it may cause any health issues. Lactobacillus species have low pathogenicity and rarely cause any disease in human beings. However, in case immunocompromised person such as human immunodeficiency virus (HIV) infection or those who have undergone organ transplantation may get the disease from Probiotics strains.

Rautio et al. (1999), reported the case of a 74 year old woman having hypertension and diabetes mellitus developed a liver abscess in association with pneumonia and pleural empyema. The reason traced for the infection source was found that lady has taken probiotic milk containing L. rhamnosus GG and a strain indistinguishable from that was isolated from the abscess. Another case reported by Mackay (1999) in which a 67 year old man developed endocarditis after dental extractions. The blood cultures studies revealed the presence of a strain of L. rhamnosus indistinguishable from that in probiotic capsules he had taken.

Wolf et al.,(1998) reported no side effect or safety problem with the administration of probiotic strain Lactobacillus reuteri in a double-blind, placebo-controlled study in HIV adults. Rungnri et al. (2017) done a randomised clinical trial of Probiotics (fermented milk containing Lactobacillus rhamnosus SD11) on 43 healthy volunteer young adults and reported no side effect after the consumption of Probiotics up to 8 weeks of study period. The study stated above by different investigator suggested that more studies are required for side effect or safety problem of consuming Probiotics is required.

**Future perspectives of Probiotics research**

Increasing resistance in microorganism towards antibiotic is the big challenge to scientific community and till date, no method has been developed to revert back the antibiotic resistance. So, probiotic had given some hope to the community to prevent and treat gastrointestinal infection, antibiotic-associated diarrhea, urinary tract infection, ulcerative colitis, oral gingivitis and some types of cancer. Use of probiotic in India is not a new science but our ancestors using the Probiotics in the form of yogurts from the time since immemorial and getting benefits from this. Different types of Probiotics are now commercialized for the use of human beings and they are in great demand among the peoples of India and abroad. The manufacturing companies claiming the beneficiary effect of their products to various ailments. The regulatory bodies which are functional in India should or must analyzed the product for the claim that has been made by the manufacturer. As stated in above section about safety, whether the manufacturer had followed the safety measures so, that it could not produce any kind of adverse effect on the body of consumer. The use of probiotic is not limited only to the use of patient but it should be easily taken and used by healthy persons or young ones. The different consortium of different probiotic strains are used for the prevention of urinary tract infection, different types of diarrhea, cancer, ulcerative colitis, irritable bowel syndrome, lactose intolerance, pancreatitis, peptic ulcer caused by Helicobacter pylori, pouchitis etc.

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