

PROBIOTICS

MICROBIOLOGY

8th Semester

Course Outlines of Probiotics

Final Exam

1. Guidelines by FAO/WHO
2. Mode of Action
3. Health Benefits/ Advantages
4. Disadvantages

Recommended Books

- 1) Probiotics and Prebiotics: Current Research and Future Trends 2015 by Koen Venema, Ana Paula do Carmo
- 2) Handbook of Probiotics and Prebiotics 2008 by Seppo Salminen, Yuan Kun Lee

Guidelines by FAO/WHO

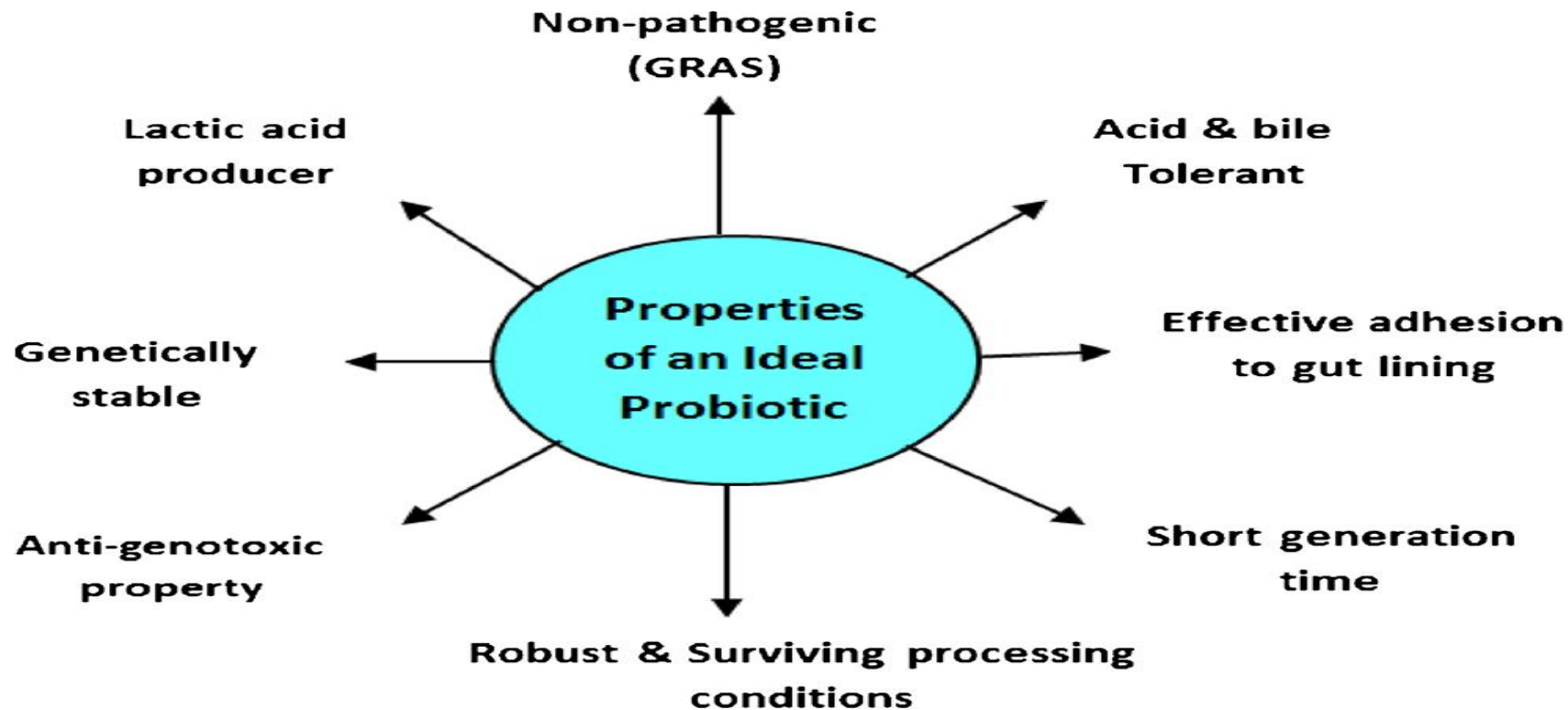
- FAO (Food and Agriculture Organization) and WHO (World Health Organization) have jointly put forward guidelines in order to set out a systematic approach for an effective evaluation of probiotics in foods to substantiate the health claims and benefits.
- The FAO/WHO guidelines on Probiotics could be used as global standard for evaluating probiotics in food that could result in the substantiation of health claims.

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- The guidelines make it necessary to perform the following activities:
 1. Strain identification.
 2. Functional characterization of the strain(s) for safety and probiotic attributes.
 3. Validation of health benefits in human studies.
 4. Honest, not misleading labelling of efficacy claims and content for the entire shelf life.

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- Some of the pre-requisites of an ideal probiotic organism are depicted in diagram.



IDENTIFICATION OF STRAINS OF PROBIOTICS

- Effects of probiotics are strain specific.
- Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies.
- Both phenotypic and genotypic tests should be done using validated standard methodology.
- Nomenclature of the bacteria must conform to the current, scientifically recognized names as per the International Committee on Systematics of Prokaryotes (ICPS).

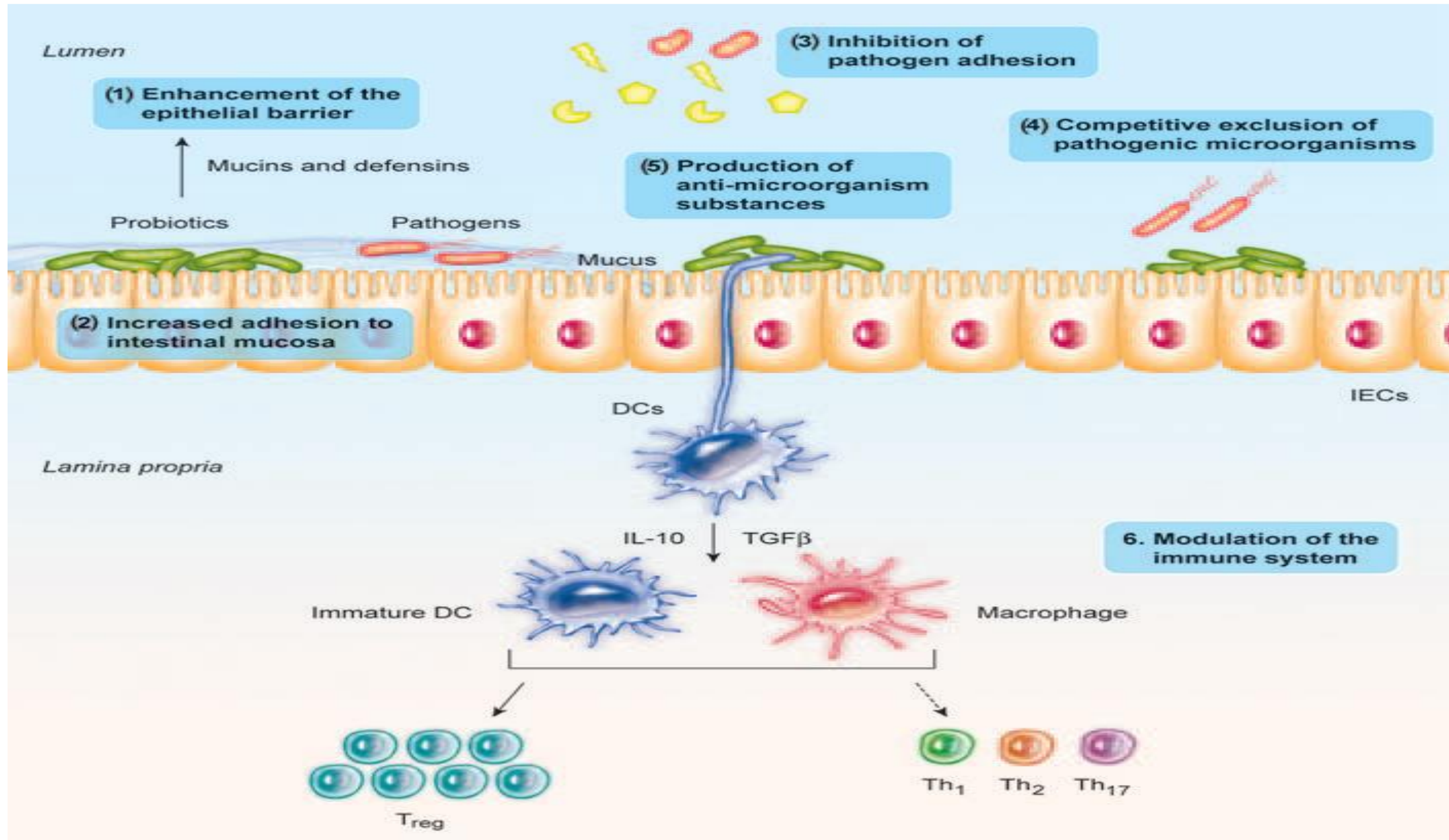
Joint FAO/WHO Guidelines for the Evaluation of Probiotics in Food London, Ontario, Canada, April 30 and May 1, 2002

- In recognition of the importance of assuring safety, even among a group of bacteria that is Generally Recognized as Safe (GRAS), the Working Group recommends that probiotic strains be characterized at a minimum with the following tests:
 1. Determination of antibiotic resistance patterns
 2. Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation).
 3. Assessment of side-effects during human studies.

4. Epidemiological surveillance of adverse incidents in consumers (post-market)
5. If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition.
6. If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required.

Mode of Action of Probiotics

- Major probiotic mechanisms of action include the following.
 1. Enhancement of the epithelial barrier.
 2. Increased adhesion to intestinal mucosa.
 3. Inhibition of pathogen adhesion.
 4. Competitive exclusion of pathogenic microorganisms.
 5. Production of antimicrobial substances.
 6. Modulation of the immune system.



Enhancement of the Epithelial Barrier

- The intestinal epithelium is in permanent contact with luminal contents and the variable, dynamic enteric flora.
- The intestinal barrier is a major defence mechanism used to maintain epithelial integrity and to protect the organism from the environment.
- Defences of the intestinal barrier consist of the mucous layer, antimicrobial peptides, secretory IgA and the epithelial junction adhesion complex.

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- Once this barrier function is disrupted, bacterial and food antigens can reach the submucosa and can induce inflammatory responses, which may result in intestinal disorders, such as inflammatory bowel disease.
- Consumption of non-pathogenic bacteria can contribute to intestinal barrier function, and probiotic bacteria have been extensively studied for their involvement in the maintenance of this barrier. However, the mechanisms by which probiotics enhance intestinal barrier function are not fully understood.

Increased adhesion to intestinal mucosa

- Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotic strains and the host.
- Adhesion of probiotics to the intestinal mucosa is also important for modulation of the immune system and antagonism against pathogens.
- Thus, adhesion has been one of the main selection criteria for new probiotic strains and has been related to certain beneficial effects of probiotics.

Inhibition of pathogen adhesion

- Lactic acid bacteria (LABs) display various surface determinants that are involved in their interaction with intestinal epithelial cells (IECs) and mucus. IECs secrete mucin, which is a complex glycoprotein mixture that is the principal component of mucus, thereby preventing the adhesion of pathogenic bacteria.

Competitive exclusion of pathogenic microorganisms.

- The mechanisms used by one species of bacteria to exclude or reduce the growth of another species are varied, including the following mechanisms:
 - a) Creation of a hostile microecology
 - b) Elimination of available bacterial receptor sites
 - c) Production and secretion of antimicrobial substances and selective metabolites.
 - d) Competitive depletion of essential nutrients.

Production of antimicrobial substances

- One of the proposed mechanisms involved in the health benefits afforded by probiotics includes the formation of LMW compounds (1000 Da), such as organic acids, and the production of antibacterial substances termed bacteriocins (11000 Da).
- Organic acids, in particular acetic acid and lactic acid, have a strong inhibitory effect against Gram-negative bacteria, and they have been considered the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens.
- The undissociated form of the organic acid enters the bacterial cell and dissociates inside its cytoplasm.

- The eventual lowering of the intracellular pH or the intracellular accumulation of the ionized form of the organic acid can lead to the death of the pathogen.
- Many Lactic acid bacteria (LABs) produce antibacterial peptides, including bacteriocins and small antimicrobial proteins (AMPs).
- Bacteriocins produced by Gram-positive bacteria (usually LAB, including lactacin B from *L. acidophilus* , plantaricin from *L. plantarum* and nisin from *Lactococcus lactis*) have a narrow activity spectrum and act only against closely related bacteria, but some bacteriocins are also active against food-borne pathogens.

Modulation of the immune system

- It is well known that probiotic bacteria can exert an immunomodulatory effect.
- These bacteria have the ability to interact with epithelial and dendritic cells (DCs) and with monocytes/macrophages and lymphocytes.
- The immune system can be divided between the innate and adaptive systems.
- The adaptive immune response depends on B and T lymphocytes, which are specific for particular antigens.

- In contrast, the innate immune system responds to common structures called pathogen-associated molecular patterns (PAMPs) shared by the vast majority of pathogens.
- The primary response to pathogens is triggered by pattern recognition receptors (PPRs), which bind PAMPs.
- In addition, extracellular C-type lectin receptors (CLRs) and intracellular nucleotide binding oligomerization domain-containing protein (NOD)-like receptors (NLRs) are known to transmit signals upon interaction with bacteria.

Health Benefits/Advantages of Probiotics

- The most important and documented beneficial effects of probiotics include the prevention of diarrhea, constipation, enhancement of antibacterial activity and anti-inflammatory.
- Furthermore, they also contribute to the synthesis of nutrients and improve their bioavailability; some probiotics are known to exert anti-oxidative activity in the form of intact cells or lysates.
- Probiotics have also demonstrated their inherent effects in alleviating symptoms of allergy, cancer, AIDS, respiratory and urinary tract infections.

Health Benefits/Advantages of Probiotics

- Probiotics have also beneficial effects on aging, fatigue, autism, osteoporosis, obesity and type 2 diabetes.
- As shown below a number of mechanisms are thought to be associated with probiotic beneficial effects:
 1. Production of inhibitory substances like H₂O₂, bacteriocins, organic acids, etc.,
 2. Blocking of adhesion sites for pathogenic bacteria.
 3. Competition with the pathogenic bacteria for nutrients,
 4. Degradation of toxins as well as the blocking of toxin receptors.
 5. Modulation of immune responses.

Health benefits in summarized form

1. Antibiotic associated diarrhea
2. Probiotics and Cancer
3. Irritable bowel syndrome (IBS)
4. Lactose intolerance
5. Immunomodulation
6. Treat high cholesterol

Antibiotic associated diarrhea

- Disturbance/destruction of the indigenous microflora caused by antibiotic treatments often leads to diarrhea.
- The main mechanism by which antibiotics cause diarrhea is through impaired resistance to pathogens as a result of disruption of the gut microbial flora and subsequent alterations in the metabolism of carbohydrates, short-chain fatty acids, and bile acids.
- Probiotics including various bacterial species like *L. acidophilus*, *L. rhamnosus* GG, *L. delbrueckii*, *L. fermentum* etc. and the yeast *S. boulardii* are effective in reducing the incidence of antibiotic- induced diarrhea.

Probiotics and Cancer

- *L. acidophilus* is known to prolong the induction of colon tumors.
- It was demonstrated that feeding milk and colostrum fermented with *L. acidophilus* resulted in 16–41 % reduction in tumor proliferation.
- The other probiotic *L. bulgaricus* has also been reported to induce antitumor activity against sarcoma-180 .

Probiotics and Cancer

- 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 β - glucuronidase, urease, choloylglycine hydrolase, azedoreductase and nitro-reductase by bad bacteria especially entero-pathogens such as *E. coli* and *Clostridium perfringens*.

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.
- 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.
 - c) Biological factors like gut motility and visceral sensitivity, which interact in a complex way to exacerbate the symptoms.

Irritable bowel syndrome (IBS)

- A mixture of 8 probiotic strains and *Lactobacillus plantarum* decreased flatulence and relieved abdominal bloating.
- 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.
- Consumption of *Bifidobacterium bifidum* and Probiotic *Escherichia coli* for 4 weeks have effectively alleviated global IBS, as well as its related symptoms.

Lactose intolerance

- Lactose intolerance is most common type of carbohydrate intolerance and attributed to lack of digestion of lactose due to low levels of β galactosidase enzyme activity.
- Symptoms include abdominal distress like diarrhea, bloating, abdominal pain and flatulence.
- Two possible pharmacological interventions for lactose intolerance are:
 - 1) Treatment with commercially available lactase (tablets).
 - 2) 2) With probiotics such as *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

Lactose intolerance

- It is also observed that consumption of milk containing *Bifidobacterium longum* and *L. acidophilus* cause significantly less hydrogen production and flatulence.
- The combination of *Lactobacillus caseishirota* and *Bifidobacterium breve* Yakult has shown better effect and improved the symptoms of lactose intolerance significantly.

Immunomodulation

- 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.
- 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 immunity.

Treat high cholesterol

- Mann and Spoerry were the first to suggest the possible effects of probiotic consumption on lipid metabolism.
- The reduction in serum cholesterol levels has been observed in the people on consumption of fermented milk and lactic acid bacteria.
- *L. bulgaricus*, *L. reuteri*, *B. coagulans* are some of the probiotic strains with reported hypocholesterolemic effects.
- Consumption of low-fat yogurt containing *B. longum*BL1 in a trial involving 32 hypercholesterolemic patients, showed a significant decline in triglycerides, total serum and cholesterol level.

Disadvantages of Probiotics

1. Taking probiotics in larger amounts might produce digestive issues, such as gas and bloating, but they are usually temporary.
2. Discomfort in the gastrointestinal tract.
3. It causes allergy.
4. People having on underlying disease or compromised immune system cause potential health problems like skin rash, fever, bloody stools.

CONCLUSION

- Due to the many benefits of probiotics in the absence of any risk, they have become a useful tool for the medical community. They not only boost the functioning of the immune system, they naturally aid in fighting bad bacteria by supplying the body with enough good to keep the body in symbiosis.
- Unlike most traditional therapies for disease and illness, there are no side effects or risks associated with them, nor is there a risk of overdose that leads to the need for a prescription, or the monitoring of a physician.

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