

Although the exact ways of actions are not fully understood, the possible modes of probiotics action include the following:

### **Enhancement of epithelial barrier function**

Intestinal barrier function is maintained by several interrelated systems including mucus secretion, chloride and water secretion, and binding together of epithelial cells at their apical junctions by tight junction proteins. Integral to the gut barrier defense is mucus which is composed of mucins (MUC 2 and MUC 3) which are secreted from the goblet cells.

Mucin polymerization provides the structural foundation of the mucus, granting protection from pathogens, enzymes, toxins, dehydration and abrasion. *Lactobacillus plantarum* 299v and *Lactobacillus rhamnosus* GG have been shown to up-regulate production of intestinal mucins (MUC 2 and MUC 3) which subvert the adherence of the enteropathogenic bacterium *Escherichia coli* O157:H7 to intestinal epithelial cells, consequently preventing pathogenic bacterial translocation.

Further, some probiotic bacteria have been found to limit chloride and water secretion, as is the case with *Streptococcus thermophilus* and *Lactobacillus acidophilus* which reversed the *E. coli*- induced chloride secretion by epithelial cells. On the other hand, intestinal barrier integrity may be increased by enhancing the expression of genes involved in tight junction signaling. On this count, some of the probiotics like lactobacilli for instance, have been shown to modulate the regulation of several genes encoding adherence junction proteins such as E-cadherin and  $\beta$ -catenin in T84 epithelial cells. Moreover, incubation of intestinal cells with lactobacilli modulates tight junction protein phosphorylation.

Probiotics have also been indicated to initiate repair of the barrier function after damage. For example, *Escherichia coli* Nissle 1917 not only counteracted the disruptive effects of enteropathogenic *E. coli*, but also restored the mucosal integrity in T84 and Caco-2 cells. This effect is achieved by increasing expression and repartition of tight junction proteins of the zonula-occludens (ZO-2) and altering protein kinase C signaling.

## Increased adhesion to intestinal epithelial cells

The effective performance of a probiotic depends on their strong adhesion and colonization of the gut, which in turn improves the host immune system. *Lactobacillus plantarum* 299v has been shown to exhibit a mannose-specific adhesion by which it can adhere to human colonic cells. Once the probiotic adheres to the cell, various biological activities take place, which primarily include the release of cytokines and chemokines. These then exert their secondary activity such as stimulation of mucosal and systemic host immunity.

## Competitive exclusion of pathogenic microorganisms

Probiotic bacteria are able to exclude or reduce the growth of pathogens by any one of the following ways which includes creation of a hostile microenvironment like the lowering of the pH of the gut below than what is essential for the survival of pathogenic bacteria such as *E. coli* and *Salmonella*, by producing organic acids like acetic acid and lactic acid. The others include physical blocking of available bacterial receptor sites; compete with pathogenic bacteria for essential nutrients and energy source; secretion of antimicrobial substances and release of selective gut-protective metabolites like arginine, glutamine, short-chain fatty acids and conjugated linoleic acids.

## Production of antimicrobial peptides

Many lactic acid bacteria produce well characterized inhibitory peptides which include, but are not limited to lantibiotics (class I), peptide bacteriocins (class II), and bacteriolysins (class III). Bacteriocins are antimicrobial compounds with a molecular weight of >1,000 Dalton. Bacteriocins produced by gram-positive bacteria usually the lactic acid bacteria include lactacin B from *L. acidophilus*, plantaricin from *L. plantarum* and nisin from *Lactococcus lactis*. These have a narrow activity spectrum and act only against closely related bacteria, but some bacteriocins are also active against food-borne pathogens. The common mechanisms of bacteriocin-mediated killing include the destruction of target cells by pore formation and/or inhibition of cell wall synthesis.

The probiotic bacteria *Lactobacillus reuteri* produces an antimicrobial agent reuterin which has broad-spectrum activity against a variety of pathogens including bacteria, fungi, protozoa and viruses, and can be differentially expressed by various *L. reuteri* strains.

Defensins are a family of highly conserved small cysteine-rich antimicrobial peptides particularly abundant at mucosal sites where they contribute to the host defense by disrupting the cytoplasmic membrane of susceptible microorganisms. The probiotic *E. coli*Nissle strain has been shown to induce expression of human beta- defensin 2 in Caco-2 intestinal epithelial cells and this type of effect may contribute to an improved mucosal barrier and provide a means of limiting access of enteric pathogens.

*Adapted from "Probiotics - the versatile functional food ingredients" by Gareth Gordon Syngai, Ragupathi Gopi, Rupjyoti Bharali, Sudip Dey, G. M. Alagu Lakshmanan, and Giasuddin Ahmed. J Food Sci Technol. 2016;53(2):921-933.*

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