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Modes of Action of Probiotics: Recent Developments

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Abstract: Advances in probiotic research have finally confirmed the health benefits of some bacterial strains. Now, the knowledge of probiotics has entered a new and fascinating phase of research and progression in this field is likely to offer novel and useful means which put emphasis to understand the mechanisms through which the probiotics act. Understanding probiotic action may permit to utilize the right strain to protect, treat or prevent specific disorder. Several mechanisms have been proposed through which probiotics exert beneficial effects on the host. In the following review of literature, the recent knowledge regarding modes of action of probiotics is briefly discussed.

Key words: Mechanisms, probiotics, recent advances, protect, disorder, bacterial stains

INTRODUCTION

The use of antibiotics is being discouraged in many countries of the world due to their residues in milk or meat and possible resistance in bacteria of human and animal origin (Butaye *et al*., 2003). Consequently, there is growing interest in finding viable alternatives for disease prevention and enhancement of production in farm animals. The preponderance of research data in this field suggests the likelihood beneficial changes due to exposure to probiotics. The most important advantage of probiotics to antibiotics is that the former is free of any residues in meat or milk of farm animals which might have serious health implications for consumers. Probiotics has been defined in several ways. In simple term, probiotic means for life. Lilly and Stillwell (1965) were the first to define this term as those substances which are produced by one microorganisim and stimulate the growth of another. Later, Parker (1974) described the term as organisms and substances which contribute to intestinal microflora balance. Fuller (1989) modified the definition as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance.

This definition avoids the too broad term substance which could even include antibiotics. In more modern definition, Salminen *et al*. (1998) defined probiotics as food containing live bacteria which are beneficial for health whereas, Marteu *et al*. (2004) define it as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being. Despite these numerous theoretical definitions, a practical question arises what is the criteria of selection of an organism to be considered as probiotics. Several characteristics of probiotics have been described which shown as follow:

Characteristics of a good probiotic:

- Should be acid and bile resistant
- Be strain specific and possess high ability to multiply in the gut
- Should not have any side effect; should neither be pathogenic nor toxic to the host
- Culture should have a strong adhesive capability with the digestive tract of the host
- Be durable enough to withstand the duress of commercial manufacturing, processing and distribution
- Should have the ability to reduce the pathogenic microorganisms

The most commonly used probiotics are strains of lactic acid bacteria such as Lactobacillus, Bifadobacterium and Streptococcus. These bacteria are known to resist gastric acid secretion and bile salts enzymes effects, adhere well to the colonic mucosa and readily colonize intestinal tract (Jin *et al*., 1998). Moreover, these bacteria have shown strong *in vitro* inhibition against *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and *Clostridium perfringens* (Fioramonti *et al*., 2003).

Some yeast preparation such as *Aspergillus oryzae*, *Saccharomyces boulardii* and *S. cerevisiae* have also been used as probiotic agent due to its wide range characteristics like inhibit the growth of several microbial pathogens, survive throughout the intestinal

tract and it is unaffected by antibiotic therapy (Fuller, 1999; Saegusa *et al*., 2004; Zhu *et al*., 2009; Vila *et al*., 2010). Microorgnisms commonly used as probiotics for livestock animals (Ohashi and Ushida, 2009):

- *Lactobacillus* *acidophilus*
- *casei*
- *acidophilus*
- *plantarum*
- *delbruekii* ssp., *bulgaricus*
- *reuteri*
- *gasseri*
- *fermentum*
- *salivarius*
- *Bifidobacterium* *bifidum*
- *lactis*
- *Enterococcus* *faecium*
- *Bacillus* *subtilis*
- *cereus*
- *coagulans*
- *licheniformis*
- *Pediococcus* *pentosaceus*
- *Saccharomycecs* *cerevisiae*
- *boulardii*
- *Aspergillus* *oryzae*

POSTULATED MECHANISMS OF PROBIOTICS ACTION

Enhancement of epithelial barrier integrity: Probiotic bacteria can thwart the potential pathogen bacteria by enhancement of intestinal barrier function through modulation of cytoskeletal and epithelial tight junction in the intestinal mucosa (Chichlowski *et al*., 2007; Ng *et al*., 2009). Under normal physiological conditions, intestinal barrier is maintained by several factors like mucus production, water and chloride secretion and epithelial cells that form tight junction (Ng *et al*., 2009).

Disruption of epithelial barrier has been reported in several clinical conditions such as enteric infections, celiac diseases and infection bowl disease (Ng *et al*., 2009). Enhancement of epithelial barrier integrity may be an important mechanism through which the probiotic bacteria benefit the host in these disease conditions. According to Chichlowski *et al*. (2007), the process of enhancement of epithelial integrity is accomplished by two mechanisms. First, the enterocytes produce a thick blanket of mucus, secreted by goblet cells which are dispersed throughout the luminal epithelium of the intestines.

The probiotic bacteria have been reported to increase the secretion of mucus by triggering inflammation in enterocytes of the intestines (Mack *et al*., 1999; Chichlowski *et al*., 2007). Caballero-Franco *et al*. (2007) reported that treatment of probiotic bacteria triggered an increase of 60% of basal luminal mucin contents by up-regulation of *MUC2* gene expression. In the same experiment, an increase in number of goblet cells were detected as an effect of probiotic treatment. Similar results were recorded by Chichlowski *et al*. (2007) who found greater number of goblet cells on chicken intestinal villi in response to probiotics treatment and suggested that metabolites produced during bacterial fermentation may play a role in the growth and maturation of goblet cells. These observations were strengthened by the study of Montalto *et al*. (2004) who reported increase production of mucin (MUC3) after treatment with several strains of Lactobacillus.

The 2nd mechanism that ensures epithelial barrier integrity is associated with a unique structure called tight junction. It is unbroken, contiguous biological barrier which prevents the entrance of macromolecules and pathogenic bacteria. The tight junction proteins are dynamic structure subject to changes that dictates their function.

The action of probiotics on cytoskeleton of tight junction came to lime light when Shen *et al*. (2006) demonstrated intact epithelial cell junction by using electron microscopy. Tight junction permeability is influenced by zonulin which is involved in the movement of molecules from intestine into the blood stream and vice versa (Shen *et al*., 2006).

The protective action of zonulin was reported by Buts *et al*. (2002) in response to administration of non-steroidal anti-inflammatory drugs when the animals were dosed with Lactobacillus. However, this mechanism is till vague and not fully understood. Some bacteria have been found to limit water and chloride secretion such as *S. thermophilus* and *L. acidophilus* reverse the *E. coli* induced chloride secretion by epithelial cells (Resta-Lenert and Barrrett, 2003). Tight junction protein called zonula occludens-1 is disturbed when exposed to pathogenic bacteria such as *S. dublin*.

The cytoskeleton arrangement was restored under the treatment of probiotic (VSL#3), suggesting the role of probiotics in preservation of barrier function and cytoskeleton architecture (Ng *et al*., 2009). Similarly, other probiotic bacteria such as *L. acidophilus*, *S. thermophilus*, individually or collectively, maintain or stabilize other cytoskeleton structures like actin, ZO-1 and occludin when disrupted by pathogenic bacteria (Resta-Lenert and Barrrett, 2003; Ng *et al*., 2009). For some bacteria, antioxidative properties have also been shown. For example, *Bifadobacterium longum* and *L. acidophilus*

have been demonstrated to scavenge α, α-diphenyl-β-picrylhydrazyl (DPPH) radical which lead to the inhibition of lipid peroxidation and reduction of DNA oxidative damage in intestinal epithelial cells (Lin and Chang, 2000).

COMPETITIVE EXCLUSION

The competition for space to adhere between indigenous bacteria and exogenous pathogens result in the competitive exclusion of pathogenic bacteria (Ohashi and Ushida, 2009). Competitive exclusion refers to physical blocking of pathogenic bacteria colonization by probiotic bacteria from their favourite site such as intestinal villus, goblet cells and colonic crypts (Chichlowski *et al*., 2007).

The probiotic bacteria alter the physical environment of the intestines in such a way that pathogenic bacteria cannot survive. Probiotic bacteria exclude the opportunistic bacteria in two ways. First, the probiotic bacteria compete with pathogenic bacteria for nutrients and energy source thus, preventing them from acquiring energy r equired for growth and proliferation of pathogenic bacteria in the gut environment (Cummings and Macfarlane, 1997).

Second, probiotics produce several organic acid and Volatile Fatty Acids (VFA) as a result of their metabolism and fermentation. Consequently, the pH of the gut is lowered below that essential for survival of pathogenic bacteria such as *E. coli* and Salmonella (Marteu *et al*., 2004; Chichlowski *et al*., 2007). Probiotic bacteria also eject the colonization of pathogenic bacteria by attaching themselves to the surface of the gut thus preventing the adhesion of the pathogenic bacteria to gastrointestinal epithelium.

Probiotic bacteria such as Lactobacillus plantarum induces the transcription and excretion of the mucins, MUC2 and MUC3 from goblet cells, thereby inhibits the adherence of enteropathogenic such as *E. coli* to the intestinal wall (Fooks and Gibson, 2002). Other such examples include the detachment of *Salmonella typhimurium*, *Shigella flexneri*, *Clostridium difficile* and other pathogens (Mead, 1989; Isolauri *et al*., 2004).

SECRETION OF BACTERIOCINS

Lactobacilli and *B. cereus* have been reported to produce various metabolites which have inhibitory effect on pathogenic bacteria (Oscariz *et al*., 1999; Vila *et al*., 2010). *Lactobacillus acidophilus* has been reported to produce acidophilin, lactocidin and acidolin and *L. plantarum* produces lactolin (Vila *et al*., 2010). Nicin and diplococcin are among the anti-metabolites produce by Streptococci.

Bacillus cereus produces bacteriocin like substances which presents high activity in the pH range 2.0-9.0. (Risoen *et al*., 2004). These bacteriocins have been demonstrated *in vitro* experiments for their inhibitory action against range of bacteria like *Bacillus*, *Klebecella*, *Pseudomonas*, *Proteus*, *Salmonella*, *Shigella*, *Staphylococcus*, *Vibrio* species and *E. coli* (Vila *et al*., 2010).

INTERFERENCE WITH QUORUM SENSING SIGNALLING AGENTS

From the recent research, it has been concluded that quorum sensing regulates the virulence expression in probiotics which may interfere with the signalling system avoiding the onset of virulence in pathogenic bacteria (Vila *et al*., 2010). Bacteria communicate with each other as well as with their surrounding environment through chemical signalling molecules called auto-inducers (Schauder and Bassler, 2001; Vila *et al*., 2010).

This phenomenon is called quorum sensing. The probiotic bacteria such as Lactobacillus, Bifadobacterium and *B. cereus* strains degrade the auto-inducers of pathogenic bacteria by enzymatic secretion or production of auto-inducer antagonists which render the quorum sensing bacteria mute and deaf.

Medellin-Pena *et al*. (2007) demonstrated that *Lactobacillus acidophilus* secretes a molecule that inhibits the quorum sensing signalling or directly interact with bacterial transcription of *E. coli O157* gene, involved in colonization and thus, bacterial toxicity is thwarted. Medina-Martinez *et al*. (2007) and Cerda-Cuellar *et al*. (2009) recorded similar conclusions using *B. cereus* and *B. toyoi* probiotic bacteria.

EFFECTS OF PROBIOTICS ON EPITHELIAL CELLS

One of the major question before the bacteriologist was how the intestinal epithelial cells distinguish probiotic and pathogenic bacteria. This concept was cleared by Lammers *et al*. (2002) and Otte and Podolsky (2004) who concluded that distinction is based upon the production of cytokines.

These researchers found that probiotic bacteria did not induce IL-8 secretion by epithelial cells compared with intestinal pathogens such as *E. coli*, *Salmonella dublin*, *Shigella dysenteriae* and *Listeria monocytogenes*. In addition, combined culture of pathogenic bacteria, *S. dublin* and probiotic, VSL≠3, decreased the production of IL-8 indicating that probiotic bacteria can override the effect of pathogenic bacteria.

Another effect of probiotic bacteria on epithelial cells is recognition of these bacteria through production of

Toll-like receptors such as TLR-2 and TLR-4. Such interaction result in production of protective cytokines that enhances epithelial cell regeneration and inhibition of epithelial apoptosis (Rakoff-Nahoum *et al*., 2004; Ng *et al*., 2009).

ANTI-INFLAMMATORY EFFECT OF PROBIOTIC BACTERIA

Ng *et al*. (2009) described that pathogenic bacteria induce proinflammatory response in intestinal cells by activating the transcription factor NF-kB. In contrast, non-pathogenic bacteria can attenuate the proinflammatory response by secreting the counter-regulatory factor IkB. This phenomenon was demonstrated in non pathogenic bacteria which attenuated IL-8 secretion elicited by pathogenic *S. typhimurium* (Neish *et al*., 2000). Kelly *et al*. (2004) demonstrated in a case of *Bacteroides thetaiotaomicron* that the anti-inflammatory response of the bacteria is achieved by blocking the transcription factor NF-kB in the nucleus through nuclear hormone receptor, resulting in attenuation of NF-kB-mediated inflammatory gene expression in pathogenic bacteria.

Other studies show that the ani-inflammatory response of the probiotic bacteria has been achieved through variety of mechanisms like inhibitition of soluble chymotrypsin-like activity of proteosome of the intestinal epithelial cells, production of cytoprotective heat shock protein, delayed activation of NF-kB and stabilizing level of IkB which result in attenuation of proinflammatory affect of the pathogenic bacteria (Petrof *et al*., 2004; Ng *et al*., 2009).

IMMUNOMODULATION

The gut is often referred as the largest immune organ of the body as more lymphocytes reside in the gut than any other organ of the body (Chichlowski *et al*., 2007). The enterocytes of intestines provide such a barrier which prevents the passive loss of nutrients on one hand and on the other prevent the access of pathogens into the body. The lamina properia of the intestines is enriched with lymphocytes, macrophages, heterophils and dendritic cells all of them fighting against the pathogens. Probiotics have the ability to enhance the capacity of the host immune system against the pathogens and ultimately improve their health.

Probiotic bacteria are able to influence the inflammatory response elicited by pathogens through specific signalling pathway (Yurong *et al*., 2005; Chichlowski *et al*., 2007). Probiotic bacteria may exert its beneficial effects and modulate the immune system of the host against potentially harmful antigens via activation of lymphocytes and antibody production (Ng *et al*., 2009). For example, *L. rhamnosus* admistration resulted in enhanced non-specific humoral response reflected by an increase production of IgG, IgA and IgM from circulating lymphocytes.

Similar results were recorded by feeding yogurts containing *L. acidophilus*, *L. bulgaricus*, *S. thermophilus*, *B. bifidum* and *B. infantis* probiotic bacteria (Tejada-Simon *et al*., 1999). Accumulated body of evidence have shown that the protective effect of probiotics is associated with elevated humoral and cellular immune response which is achieved through increased production of *T lymphocytes*, CD+cells and antibody secreting cells, expression of pro and anti-inflammatory cytokines, interleukins, IFN-γ, natural killer cells, antibody production, respiratory burst of macrophages and delayed type hypersensitivity reaction (Panda *et al*., 2003; Oyetayo and Oyetayo, 2005; Chichlowski *et al*., 2007; Zhu *et al*., 2009; Ng *et al*., 2009; Ohashi and Ushida, 2009).

CONCLUSION

Probiotics are microbial cell culture that produces beneficial effect on the health and well-being of the host. The beneficial effects of probiotics are the consequence of their proposed mechanisms of action. Understanding the modes of probiotics may permit to improve the production of livestock and minimize the side-effects associated with the use of antibiotics.

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