

**REVIEWS ON PROBIOTICS – IT'S USES AND APPLICATIONS**

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**ABSTRACT**

Probiotics are commonly defined as mono or mixed cultures of live microbes that when applied to an animal or human, possess a beneficial effect on the health of host possibly by improving the balance of the indigenous microflora. The field of probiosis has emerged as a new science with applications in farming and aquaculture as alternatives to antibiotics as well as prophylactics in humans. Probiotics are being developed commercially for both human uses, primarily as novel foods or dietary supplements and in animal feeds for the prevention of gastrointestinal infections, with extensive use in the poultry and aquaculture industries. This review summarizes the

commercial applications of probiotics.

**KEYWORDS:** This review summarizes the commercial applications of probiotics.

**INTRODUCTION**

Probiotics, although not a new concept, has only recently begun to receive an increasing level of scientific interest. Probiotics are generally defined as “live microbial feed supplements that can benefit the host by improving its intestinal balance” (Fuller 1989). In other words, Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller 1991, 1989). Among the large number of probiotic products in use today are bacterial spore formers, mostly the bacillus genus. The history of the probiotic effect has been well documented many times previously (Fuller 1992). The lack of fundamental knowledge about the mechanism of the probiotic effect has not deterred the development of a great many probiotic preparations destined for treatment of various conditions in man and animals. One factor that has been used in the selection of probiotic cultures has been the ability to adhere to gut epithelial cells of the animal to which

the probiotic is being fed. Adhesion is now generally accepted as an important colonization factor and since establishment in the intestine is an essential prerequisite of effective probiotic activity, it is to be applauded as the first step in a rational approach to the selection of microorganisms for inclusion in probiotic preparations (Crociani et al. 1995). However, rapid growth can achieve the same end. In some cases such as fungi (*Saccharomyces cerevisiae* and *Aspergillus oryzae*), the effect is gained without either attachment or rapid growth; mere survival is adequate. Under these conditions continuous administration is required for the maximum realization of the probiotic effect. The numerous probiotic products on the market claim to have many different effects including improved resistance to infectious disease, antitumour activity, increased growth rate and feed conversion in farm animals, improved milk production by cows and increased egg production by poultry.

### CLASSIFICATION OF PROBIOTICS

The range of microorganisms contained in the probiotic preparations is wide; comprising bacteria, moulds and yeasts. Probiotics fall under two broad classifications, those for animal use and those for human use. Probiotics used in animal feed are considered as alternatives to antibiotics and therefore used as growth promoters (Cartman and La Ragione 2004). A viable alternative to antibiotics would therefore be an important venture and for this reason the development of new probiotic products that could be licensed for animal use is receiving considerable interest. However, the transfer of antibiotic resistance traits between bacterial species is a cause for concern where large quantities of bacteria would be given to animals (McCartney 2004). Probiotics for human use, on the other hand, are subject to minimal restrictions (at least as novel foods or as dietary supplements) and come in many different forms. In supermarkets, they are often sold as dairy-type products containing “live bacteria” and in health food shops as capsules or tablets composed of lyophilized preparations of bacteria which promote “a healthy gut”, etc. Finally, on the internet some products are being sold as quasi-medicinal products which can be used for oral bacteriotherapy of gastrointestinal disorders (normally diarrhoea). Currently, there is no universal class of probiotic bacterium although the most common types available are lactic acid bacteria (e.g. *Lactobacillus* spp.). These bacteria are found normally in the gastrointestinal tract (GIT) of humans and animals: there is the vague notion that the use of indigenous or commensal microorganisms is somehow restoring the natural microflora to the gut. A second class comprises those that are not normally found in the GIT. For example, *Saccharomyces boulardii* has been shown to be effective in preventing the recurrence of *Clostridium difficile*-

induced pseudo-membranous colitis (Czerucka & Rampal 2002) as well as the antagonistic action of *E. coli* (Czerucka et al 2000). *S. boulardii* products are currently being marketed for human use. Within this group of allochthonous probiotic microbes are the spore-forming bacteria, normally members of the genus *Bacillus*. Here, the product is used in the spore form and thus can be stored indefinitely on the shelf.

### **BENEFICIAL USES AND MECHANISM OF ACTION OF PROBIOTICS**

The potential benefits that are claimed include improved nutrition and growth and prevention of various gastrointestinal disorders. Probiotic-containing products are available for human nutrition, as animal feed supplements, and also for aquaculture (Rolfe 2000, Rowland 1999, Tournot 1989, Verschuere et al 2000). In some countries, probiotics are taken as prophylactic agents (for example, to prevent childhood diarrhea), while in Southeast Asia they are also used as therapeutic agents (Mazza 1994). Products containing endospores of members of the genus *Bacillus* (in single doses of up to  $10^9$  spores/g or  $10^9$  spores/ml) are used commercially as probiotics, and they offer some advantages over the more common *Lactobacillus* products in that they can be stored indefinitely in a desiccated form (Mazza 1994). Originally, many commercial products were sold as products that carry *Bacillus subtilis* spores, but recent studies have shown that most products are mislabeled and carry other *Bacillus* species, including *B. clausii*, *B. pumilus*, and a variety of *Bacillus cereus* strains (Green et al 1999, Hoa et. al. 2000, 2001). Germination of the spore could allow production of antimicrobial agents, such as bacteriocin-like inhibitory substances, thereby contributing to the competitive exclusion of pathogens, and it is one factor that could support the probiotic effect. A number of *Bacillus* species produce antimicrobial agents, and more than 80 different types have been reported (Mazza 1994). These antimicrobial agents are active mostly against gram-positive bacteria, but some are active against gram-negative bacteria. Recently, an antibiotic compound isolated from a strain of *B. subtilis* found in the probiotic Biosporin with activity against *H. pylori* has been reported (Pinchuk et al 2001). It was observed that at least two probiotic strains, Biosubtyl<sup>NT</sup> and Subtyl, produce antimicrobial agents (or bacteriocin-like inhibitory substances) that are active against other *Bacillus* species. Few studies have demonstrated a direct probiotic effect of *Bacillus* spores, but preliminary studies with poultry have provided evidence that there is competitive exclusion of *E. coli* 078:K80 by *B. subtilis* (La Ragione et al 2001) and a number of studies have demonstrated that *Vibrio harveyi* in shrimp is suppressed by various *Bacillus* spore formers (Rengpipat et al 1998, Vaseeharan and Ramasamy 2003). A recent study has described the characterization of an antibiotic

produced by the *B. subtilis* strain found in the commercial product Biosporin, which has been shown to inhibit growth of *Helicobacter pylori* (Pinchuk et al 2001). To generate humoral responses, spore antigens could interact with the gut-associated lymphoid tissue (GALT). As reported elsewhere, there is strong evidence that *B. subtilis* spores enter the Peyer's patches and mesenteric lymph nodes (MLN), and presumably they do this by translocation across M cells (Duc et al 2003). In the case of Biosubtyl NT, the spore-specific IgG responses were almost 10-fold higher than the responses to the other strains, showing that this strain is particularly immunogenic. In Oggioni et al (2003) study performed with human monocytes (isolated from peripheral blood mononuclear cells) stimulated with *B. subtilis* spores, significant levels of IL-1 $\beta$  and TNF- $\alpha$  were found to be produced. IFN- $\gamma$  is an activator of cellular responses, particularly the Th1 response that, in turn, is responsible for stimulating phagocytosis. IFN- $\gamma$  is also produced during inflammation (as opposed to a specific immune response), as is TNF- $\alpha$ , whose production by macrophages has been linked with chronic infections (Dornand et al 2002, Melby et al. 1994). These early responses suggest that there is an innate immune response and secretion of IFN- by peripheral blood mononuclear cells. Similarly, oral administration of various probiotic *Lactobacillus* species has been shown to enhance the innate immune system and to enhance macrophage phagocytosis (Schiffrin et al 1995), NK cell functions (De Simone et al 1993), and production of macrophage lysosomal enzymes (Isolauri et al 1995).

The following three basic mechanisms have been proposed for how orally ingested nonindigenous bacteria can have a probiotic effect in a host: (i) immunomodulation (that is, stimulation of the GALT) (e.g., induction of cytokines), (ii) competitive exclusion of gastrointestinal pathogens (e.g. competition for adhesion sites), and (iii) secretion of antimicrobial compounds which suppress the growth of harmful bacteria (Fuller 1991). Immune stimulation as a mechanism for a probiotic effect is difficult to define, but this must result from induction of proinflammatory cytokines that increase phagocytosis (by macrophages or dendritic cells) and perhaps also stimulation of cytotoxic cells. Various ways exist in which probiotics could be beneficial. They can act either singly or in combination. Immunostimulants vary according to their mode of action and the way they are used. Certain derivatives, such as polysaccharides, lipoproteins, nucleotides and  $\beta$ -glucans, have the capability to increase phagocytic abilities by activating macrophages. Rengpipat et al (2000) indicated that the use of *Bacillus* sp. provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*Penaeus monodon*). The mechanisms by

which probiotics exert biological effects are still poorly understood, but the nonspecific terms such as colonization resistance or competitive exclusion are often used to explain their mode of action. Colonization resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract (Biourge et al. 1998). Youngman et al. (1984) reported that the effects of probiotics may be classified in three modes of action: (i) able to modulate the host's defences including the innate as well as the acquired immune system. This mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of (chronic) inflammation of the digestive tract or parts thereof. In addition, this probiotic action could be important for the eradication of neoplastic host cells; (ii) a direct effect on other microorganisms, commensal and/or pathogenic ones. This principle is in many cases of importance for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut; (iii) Finally, affecting microbial products like toxins and host products, e.g. bile salts and food ingredients. Such actions may result in inactivation of toxins and detoxification of host and food components in the gut. It was also stated that the kind of effect(s) a certain probiotic executes depends on its metabolic properties, the molecules presented at its surface or on the components secreted. Even integral parts of the bacterial cell such as DNA or peptidoglycan might be of importance for its probiotic effectiveness. The individual combination of such properties in a certain probiotic strain determines a specific probiotic action and as a consequence its effective application for the prevention and/or treatment of a certain disease.

### 1. Immune stimulation

Stimulation of the immune system, or immunomodulation, is considered an important mechanism to support probiosis. A number of studies in humans and animal models have provided strong evidence that oral administration of spores stimulates the immune system. *Bacillus firmus* vegetative cells have been shown to stimulate the proliferation of human peripheral blood lymphocytes in vitro (Prokesova et al 1994). An interesting study has shown that *B. subtilis* in combination with *Bacteroides fragilis* promoted development of the GALT in rabbits and led to the development of the pre-immune antibody repertoire (Rhee et al. 2004). Proinflammatory responses cannot necessarily be considered a beneficial feature of a probiotic since they have been linked to a number of autoimmune diseases such as inflammatory bowel diseases including ulcerative colitis and Crohns disease (Sartor 1996).

## 2. Synthesis of antimicrobials

The production of antimicrobials by probiotics is considered one of the principal mechanisms (microbial interference therapy) that inhibit pathogenic microorganisms in the GIT. *Bacillus* species produce a large number of antimicrobials. These include bacteriocins and bacteriocin-like inhibitory substances (BLIS) (e.g., Subtilin and Coagulin) as well as antibiotics (e.g., Surfactin, Iturins A, C, D, E, and Bacilysin). Some *Bacillus* species contained in commercial products are known to produce antimicrobials. *B. subtilis* strains carried in the commercial products Promarine and Bio Plus 2B have also been shown to produce antimicrobials (Urdaci & Pincluk 2004). Probiotic strains of *B. coagulans* are found in a number of commercial products often mislabeled as *Lactobacillus sporogenes*. *B. coagulans* produces coagulin, a heat-stable, protease-sensitive BLIS with activity against Gram-positive bacteria (Hyronimus et al 1998). *B. subtilis* var. natto has also been shown to inhibit the growth of *Candida albicans* (Ozawa, et al 1979) in the intestinal tract and a surfactin has been identified with activity against yeast (Nagal et al 1996).

## 3. Other mechanisms

The competitive exclusion (CE) concept is a term mostly used in the poultry industry and refers to the ability of orally administered bacteria to stimulate the host's resistance against infectious disease (Nagal et al 1996). Different mechanisms have been proposed for CE agents including competition for host mucosal receptor sites, secretion of antimicrobials, production of fermentation by-products, such as volatile fatty acids, competition for essential nutrients and stimulation of host immune functions. *Bacillus* species (*B. subtilis*, *B. firmus*, *B. megaterium* and *B. pumilus*) have recently been shown to convert genotoxic compounds to unreactive products in vitro and this has been proposed as a probiotic mechanism, if this could occur in the intestine (Caldini et al 2002).

## COMMERCIAL HUMAN PRODUCTS

Members of *Lactobacillus* are well-recognized as safe probiotics, available in commercially probiotic products. Recently several studies have been carried out by the researchers around all over the world on the use of spore formers as probiotics (Khalil et al 2007, Patel et al 2010). Products fall into two major groups, those for prophylactic use and those sold as health food supplements or novel foods. Bona fide *Bacillus* species being used include *B. subtilis*, *B. cereus*, *B. licheniformis*, *B. pumilus*, *B. clausii* and *B. coagulans*. Other spore-



formers being used are *Paenibacillus polymyxa* and *Brevibacillus laterosporus*, both being former *Bacillus* species and now belonging to the *Bacillus sensu lato* group.

### 1. Prophylactics

These are marketed for prophylaxis of gastrointestinal disorders particularly child-hood diarrhoea (mainly rotavirus infections) or as an adjunct to antibiotic use. Another well-known product is Bactisubtil which carries one strain of *B. cereus* termed IP5832. The *B. subtilis* component of Biosporin (*B. subtilis* strain 3 or 2335) is known to produce an isocoumarin antibiotic, aminocoumarin A, active against *Helicobacter pylori* (Pinchuk et al 2001).

### 2. Health foods and dietary supplements

Probiotics have been widely used in aquaculture as a means of controlling disease, enhancing immune responses, providing nutrients and enzymatic contributions, and improving water quality (Qi et al 2009). A large number of *Bacillus* products are used as novel foods or as dietary supplements with various claims of enhancing the well-being of the user, restoring the natural microflora to the gut, etc. Natto is a food made by fermenting cooked soybeans with *Bacillus subtilis* (natto) or *B. subtilis* var. natto shown to have probiotic properties and the *B. subtilis* var. natto component is thought to stimulate the immune system, produce vitamin K<sub>2</sub> and have anti-cancer properties.

**COMMERCIAL ANIMAL PRODUCTS** In the case of Toyocerin, this contains a strain of *B. cereus* var. toyoi that has been deemed safe for animal use because of its failure to produce enterotoxins and its failure to transfer antibiotic resistance. The extensive application of probiotics in aquaculture is relatively recent and widely becoming recognized as important for disease control. However, the aquatic probiotics differ from the use of terrestrial based probiotics and it is a field that is expanding rapidly in countries with intensive farming of fish, and particularly shellfish (e.g., SE Asia) (Verschuere et al 2000). Shrimps have a non-specific immune response and vaccination (even if feasible) can only provide short-term protection against pathogens. Probiotic treatments on the other hand provide broad-spectrum protection. *Bacillus* spp. are being used as probiotics and as biocontrol agents since bacteria used for bioremediation are usually nitrifying bacteria and are used to degrade the detritus generated from fish and shellfish in rearing ponds. Biocontrol refers to the use of bacterial supplements that have an antagonistic effect on pathogens (Gatesoupe 1999). Some possible benefits for fish and shrimp linked to the administering of probiotics have already been suggested: *B. subtilis* and *B. licheniformis* fed fish displayed a significant improvement of feed conversion

ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). It has also been shown that survival rates of European eels (*Anguilla Anguilla*) fed with *Enterococcus faecium* were significantly higher than in the control groups after challenged with *Edwardsiella tarda*. Furthermore, *Enterococcus faecium* was also found along the shrimp digestive system when fed diets containing this probiotic strain.

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