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Probiotics - A broad spectrum therapy

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ABSTRACT:

"Probiotics" are the living microorganisms which are healthful to the human population. In recent years, these probiotics are being universally used in the doctoring of broad range of human diseases, conditions and syndromes. Various researches and clinical trials have been carried out for the proper annotation of the mechanisms and their mode of action which they exert on human health. Strong evidences substantiate the efficient use of probiotics in the treatment and prevention of Candidiasis, hypercholesterolemia, diarrhoea, dermatitis and several other diseases. The probiotic agents which are most commonly used are *lactobacillus, bifidobacterium,* and *yeast*. In this review, detailed overview of the use of these probiotic agents were done for curing several ailments with manifestations of clinical trials. In future, probiotics could be used as a remedy for colon and bladder cancer, diabetes, and rheumotoid arthiritis.

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INTRODUCTION

Probiotic is a Greek word which means 'for life'. The probiotic bacteria have been of much commercial interest due the reason that they exert possible health effect. The possible health effects include immune system stimulation (Galdeano et al, 2007), anti-hypertensive effect (Yeo and Liong, 2010), anti-oxidative effect (Songisepp et al, 2004), reduction of dermatitis symptoms (Weston et al, 2005) , reduction of allergic symptoms (Ouwehand, 2007), improvement of vulvovaginal candidiassis in women (Falagas et al, 2006), treatment of acute rotavirus diarrhoea in children (Reid et al, 2003), reduction in blood cholesterol (Pereira and Gibson, 2002).

In order to consider a bacterial strain as a probiotic several criteria should be met (Lee and Salminen, 1995; McFarland and Elmer, 1997; Salminen et al, 1996). Well designed human studies with requirements similar to those for pharmaceutical studies are needed to meet these criteria.

Probiotics are generally defined as live microorganisms which when administered in adequate amounts confer a health benefit on host(Reid et al, 2003) *Lactobacillus* and *Bifidobacterium* are the two important groups of such probiotic microbes that are naturally formed in the intestine of human and have a positive influence on the intestinal microbiota. Other than these groups, species from other bacterial genera such as *Streptococcus, Bacillus*, and *Enterococcus* are also been used as probiotics but the genera of *Enterococcus* is not used as probiotics due to some specific concerns as this may contain many pathogenic species(FAO, 2002). Apart from these bacterial sources, yeast from the genera *Saccharomyces* has also been used as probiotics (Elmer et al, 1996). Commercially probiotics are marketed as powders, capsules, enriched yoghurts, yoghurt like products, and milk. Another possible health effect of probiotics was in the treatment of cancer. Probiotics should be further investigated about the negative side effects. The preferable strain of probiotics is basically based on invitro tolerance of physiologically relevant stresses: e.g. Low pH, increased osmolarity, and bile, acid tolerance.

In this review article, main focus has been given on clinical health effects on the probiotics and their side effects.

Eficiency of Probiotic in treatment of various Diseases

Probiotics have been administered for the prevention and treatment of a wide range of diseases. The use of *Saccharomyces cerevisiae* product (Amax) in the reduction of ammonia and urea excretion has been studied (Mohamad Lashkarbolouki et al, 2011). In the gut, the bacteriocins produced by various probiotics including *Lactobacillus brevis* helps in the elimination of pathogens (Karthick Raja et al, 2011). The most efficient use of probiotics is in the management of acute diarrhoea caused by rotavirus. For e. g. an analysis which is done randomly demonstrated that probiotics are effective in preventing and in the treatment of antibiotic associated diarrhoea and infective diarrhoea in both adults and children including the yeast *Sacharomyces boulardii* and the bacteria *Lactobacillus acidophilus* in combination with *Lactobacillus bulgaricus, L. rhamanosus* strain taken from American type culture collection (D'Souza et al, 2002). The controlled trials has shown that these probiotics were found to reduce the mean duration time of diarrhoea by >30 hrs. Below this, we review the current concerns of probiotics for the treatment of several ailments and also the risk using probiotics.

Attributes of Probiotics

The essential characteristics of probiotics are based on assumptions more than rigorious science, like, it is basically considered that probiotics must adhere to the intestinal walls. This is supported in evidence with the in-vitro findings (Blum et al, 1999).

It is not necessary that the probiotics for human should be derived from human origin. Probiotics have

been found effective that are normally not derived from human source (for e. g. the strain of *Bifidobacterium animalis*), but the common attribute of the probiotic is assumed that they should be of human origin. The other commonly found characteristics of probiotics include strains which can be used as inoculums, should be resistant to processing conditions and should be to able to survive while its transition through the gut and thus colonize itself on mucosal surfaces. They should be capable of acting against the pathogens by multiple mechanism and show least resistance to their effect. The first and foremost beneficial effect in comparison with the time required for the vaccine to be fully protective should be rapid. The probiotics strain should act with or without antibiotics (Sanders, 2006; Reid et al, 2003 ; Lee and Salminen, 1995; McFarland and Elmer, 1997; Salminen et al, 1996).

Taxonomy

The procedure for naming is no longer based on morphological, physiological, and biochemical criteria but on the genomic characteristics and also on the molecular based phenotypic characteristics (Naidu et al, 1999; Klein et al, 1998; Klaenhammer and Kullen, 1999). The nomenclature and taxonomy of probiotics LAB (*Lactobacillus*) is under constant change.

Gene probes, DNA technology, DNA-PCR are the commonly useful tools to evaluate the mechanism of probiotics throughout the gastrointestinal tract. The researchers have used either fingerprinting or numeric analysis of biochemical reactions as phenotypic methods for the classification of strains at the species level. The genomic method used is either restriction enzyme analysis or pulsed field electrophoresis. This method constitutes the polyphasic taxonomy. This taxonomy is important for quality assurance of the probiotic products as required by the governmental law and the consumer (Klein et al, 1998).

Mechanism of action

Several mechanisms have been postulated but whether probiotic exerts protective or therapeutic effects are not fully described. Limited number of invivo studies and conclusions drawn from invitro studies that involved adults and children with intestinal disorders provide scientific evidence. Closely related probiotic strains should have different clinical effects, so the strain information should be reported with clinical and microbiological studies.

Mechanism against some types of diarrhoea:

The reason behind some type's of diarrhoea include partial lactose digestion and stimulation of the intestinal mucosal lactase activity. Hence, during the series of acute gastro entities or recurrent abdominal distress, disaccharidase activity in the small intestine may affect the transport of monosaccharide and produce osmotic diarrhoea. The use of *lactobacilli* in the fermented milk industry is to produce the lactose concentration in dairy products as they have active β-galactosidase. This may affect the severity of osmotic diarrhoea caused due to rotavirus. Rotavirus is the main cause for acute diarrhoea and vomiting. The use of the probiotic strains such as *Lactobacillus* uses the duration and severity of rotavirus diarrhoea. According to the invitro studies carried out, consumption of the *Lactobacillus* GG has shortened the diarrhoeal phase

TABLE 1: Microorganisms as probiotic agents

S. no	*Lactobacillus* species	*Bifidobacterium* species	Other species
1.	*L. acidophilus*	*B. bifidium*	*Bacillus cereus*
2.	*L. casei (Rhamnosus)*	*B. longum*	*E. coli*
3.	*L. reuteri*	*B. dreve*	*Sacharomyces cerevisiae*
4.	*L. bugaricus*	*B. infantis*	*Enterococcus faecalis*
5.	*L. plantarum*	*B. lactis*	*Streptococcus thermophillus*
6.	*L. johnsonii*	*B. animalis*	
7.		*B. adolescentis*	

intolerant individual will causes the significance reduction in the level of breath hydrogen compared with individuals that were fed with milk. The level of hydrogen in the breath is an indication of the extent of lactose metabolism in large bowl. Kolars et al observed that the ingestion of 18 g of lactose in yoghurt cause the production of 67% less hydrogen in the breath compared with that produced by a similar dose of lactose delivered in milk. Analysis of the aspirates obtained from the duodenum one hour after the consumption of yoghurt gives rise to significant levels of lactase (Kolars et al, 1984). Hence, these studies demonstrated that the delivery of lactase to the intestine via the consumption of lactase producing the probiotics is the most practical approach for the treatment of lactose mal absorption.

Infectious diarrhoea:

Studies have been reported for the use of probiotics to either treat or prevent diarrhoea (Allen et al, 2003; Pant et al, 1996; Raza et al, 1995; Sepp et al, 1995; Szajewska et al, 2001; Mastretta et al, 2002; Oberhelman et al, 1999; Shornikova et al, 1997; Cetina et al, 1994; Ho et al, 1990). Mostly the studies were done with infants or children, etiologic agents were either rotavirus or unknown, the probiotic used was *Lactobacillus Rhamnosus* strain GG (Guandalini et al, 2000; Shornikova et al, 1997; Pant et al, 1996; Raza et al, 1995; Sepp et al, 1995; Szajewska et al, 2001; Mastretta et al, 2002; Oberhelman et al, 1999). Other strains of probiotics that has shown positive results for the treatment of acute gastro entities include *Lactobacillus reuperi* and *Saccharomyces boulardii* (Shornikova et al, 1997; Cetina et al, 1994; Ho et al, 1990). An extensive trial was done using *Lactobacillus* GG for the treatment of moderate to severe diarrhoea in children by "*The European Society for Pediatric Gastroenterology, Hapathology, and nutrition*" (Guandalini et al, 2000). This study included 287 children aged between 1-36 months from 10 countries. They were randomized to be given either placebo or *Lactobacillus* GG along with standard treatment oral rehydration solution. Those who received *Lactobacillus* GG had shown a decrease in the duration of illness (Guandalini et al, 2000). Similarly, again a study was conducted with 137 children aged between 1-36 months were admitted to the hospital with diarrhoea and were randomized to receive Placebo or *Lactobacillus* GG+ or oral rehydration solution. Children given *Lactobacillus* GG had a shorter duration of illness (Shornikova et al, 1997). In the preventive study of 81 children aged between 1 to 36 months who were hospitalized for illness other than diarrhoea, symptoms of hospitals acquire rotavirus which was prevented by the administration of *Lactobacillus* GG (Szajewska et al, 2001). Another similar finding was reported in which 130 children who were treated with *S. boulardii* found to be affective for the treatment of acute diarrhoea in children (Cetina et al, 1994). Similar findings were reported in a different study done on 92 adults.

Lowering of serum cholesterol:

Invitro studies have demonstrated that bacteria can remove cholesterol from culture medium (Gilliland, et al, 1985; Gilliland et al, 1989; Gilliland et al, 1989). Since then much attention has been given to the cholesterol lowering potential of probiotics in human. The cholesterol removal from the culture was due to the precipitation of cholesterol with free bile acids, formed in the media because of the activity of bacterial enzyme bile salt hydrolase (Klaver et al, 1993, Tahri et al, 1995). In '*in vivo*' condition there would be an increase in cholesterol excretion because of bile salt hydrolase activity.

The cholesterol lowering potential of *L. acidophillus* has been most widely studied. Two studies were performed by Lin et al (Khedkar et al, 1993). The studies were: a pilot trial without a placebo and a large placebo control trial. In the study, 23 subjects were received tablets containing 3×10^7 cfu *L. acidophillus* and *Lactobacillus bulgaricus* daily for 16 weeks whereas 15

subjects received no tablets. Fasting blood samples were taken before seven and sixteen week after the study began. It was seen that serum cholesterol in the control group remains stable at 4. 9 millimoles per litre; in the experimental group a reduction of serum cholesterol was seen from 5. 7 to 5.3 millimoles per litre after 7^{th} week and to 5.4 millimoles per litre after 16^{th} week. In India, study was performed that showed reduced serum cholesterol by 12 to 20 % after 1 month when buffalo milk fermented with specific strains of *L. acidophillus* was consumed (Lin et al, 1989). A placebo control crossover study with 30 volunteers has shown that the consumption of yoghurt enriched with a specific strain of *L. acidophillus* and fructooligosaccharides lowers serum cholesterol in faster rate when compared to placebo yoghurt (Nase et al, 2001).

Prevention of dental caries:

In a day care centre, children who were given *Lactobacillus* GG for 7 months were examined for dental caries. It was found that the children in the age group of 3 to 4 years had significantly lower rate of dental caries and reduced oral count of *streptococcus mutans* compared before the treatment. (Kalliomaki et al, 2001)

Prevention and treatment of allergic reactions:

Extensive studies have been carried out for the modification of allergic reactions that have been reported for atopic eczema with *Lactobacillus* GG as probiotics (Kalliomaki et al, 2001; Rautava et al, 2002; Kalliomaki et al, 2003; Isolauri et al, 2000; Majamaa et al, 1997). Similarly a study was reported which showed that *Bifidobacterium animalis* Bb12 reduced the severity of atopic dermatitis (Kalliomaki et al, 2003). In a study conducted 159 pregnant women with a family history of atopic disease were either given *Lactobacillus* GG capsules or a placebo for two to four weeks before their expected delivery (Kalliomaki et al, 2001). Mothers who chose to breast fed their new born continued to receive *Lactobacillus* GG or placebo for 6 months, and women who did not breast fed gave the *Lactobacillus* GG or placebo to their infants. In the first two years of the children's life for the group given *Lactobacillus* GG, a 50% reduction in the frequency of atopic eczema was seen. The mother who breast fed their new born in the *Lactobacillus* GG group had higher levels of transforming growth factors β_2. A follow up study showed that the group that received *Lactobacillus* GG still had significant lower percentage of atopic eczema for four years after birth compared with placebo group. In another trail study with 27 infants of atopic eczema history were randomly divided into three groups, given *Lactobacillus* GG, *Bifidobacterium* lactase Bb12 or placebo. SCORAD score was obtained after two months which reflected the extent and severity of atopic eczema, showed a positive result in the improvement of skin conditions of patients after consuming probiotics with supplemented formula (Isolauri et al, 2000).

Prevention of recurrent vulvovaginal Candidiasis:

Vulvovaginal candidiasis (VVC) is a very common infection among women. Foxman et al (2000) conducted a survey in U. S and it was found that 6. 5 % and 8% of women older than 18 years reported ≥ 1 and ≥ 4 episodes of VVC during two months and one year prior to survey. The total annual cost for treating VVC (1995) was estimated to be $ 1.8 billion. Therefore the need for the development of effective agents for the prevention of VVC was very high. Probiotics was then potentially used for the treatment and prevention of VVC. *Lactobacilli*, specially *Lactobacillus crispatus*, *Lactobacillus jensaii* and *Lactobacillus iners* are the commonly found microorganisms in the vagina of a healthy premenopausal women. *Lactobacilli* produce lactic acid and other substances so that a low pH is maintained in the vagina. Hence, the overgrowth of those pathogens causes Bacterial Vaginosis (BV) and Gonorrhoea. Studies conducted by Hilton et al (1992) showed a significant deduction in vaginal colonization with *candida* species after the oral administration of *L. acidophillus*, and clinical improvement within a seven

from an average age of 3. 5 to 2. 5 days in children hospitalized are treated at home for rotavirus infection (Isolauri et al, 1991; Isolauri et al, 1994; Kaila et al, 1995; Kaila et al, 1992; Majamaa et al, 1995; Shornikova et al, 1997; Pant et al, 1996). Kaila et al found that the concentration of IgA antibodies against rotavirus increased drastically in serum of children treated with probiotics than in untreated children which affects the recovery. The immune response to vaccination with the live oral rotavirus vaccine was better in children receiving *Lactobacillus* GG than in control children. This was postulated by Isolauri et al. Antimicrobial substances such as organic acids, fatty free acids, ammonia, hydrogen peroxide and bacteriocins are produced by *Lactobacillus* invitro. This metabolites produced are used to increase the shelf life of food and to suppress spoilage and food borne pathogens in dairy products (Isolauri et al, 1991; Isolauri et al, 1994; Kaila et al, 1995; Kaila et al, 1992; Bin, 1995). *L. casei* strain GG was isolated by Gorbach et al is an example of a probiotics with the above said properties. This strain was recently classified as "LGG" (Bengmark, 1998; Klein et al, 1998). LGG was found by natural selection and it is easy to identify in a mixed culture of *Lactobacilli* and *streptococci* by its unique colony forming morphology (Gorbach, 2000). This strain has the ability to produce antibacterial substance of low molecular weight that inhibits both gram positive and gram negative enteric bacteria in the intestinal flora of mice (Silva et al, 1987; Naidu et al, 1999). Enzymatic mechanisms for the modification of the toxin receptors and blocking of toxin mediated pathology can be used by probiotics. For e.g. *Clostridium difficile* toxin receptors in the rabbit ileum are degraded by *S. boulardii* (Pothoulakis et al, 1993; Wilson and Perini, 1988) and blocks the cholera induced secretion in rat jejunum by the production of polyamine.

Colonization of pathogenic microorganism by competitive inhibition is prevented by probiotic agents. For e.g. : *L. plantarum* and LGG have been shown to competitively inhibit the attachment of E. coli 0157H7 to HT-29 human colonic cancer cells (Michail et al, 1994). Antamoeba histolytica's invitro attachment to erythrocytes was decreased by *S. boulardii* (Rigothier et al, 1997). Macrophage's phagocytic activity was increased against several intracellular bacteria in mice after oral or parenteral administration of *L. casei* (Nanno et al, 1986; Perdigon et al, 1986). Compliment and reticulo endothelial activation was seen after the oral activation of *S. boulardii* (Caetano et al, 1986). Recently it was found that mucosa associated *Lactobacilli* could be the potent stimulators of IL-12. This enhances the cell mediated immunity if they are able to translocate over the gut barrier and interact with the gut mucosal system (Hessle et al, 1999). Probiotics also play an important role in allergy prevention as they help in decreasing IL-4 and IgE production.

Potential indications in infectious Diseases

Probiotics can be used to control certain infections and to re-establish the human microbial microbiota has gained acceptance in recent years. With renewed interest due to the alarming rise of antimicrobial resistance and the application of molecular techniques to select the probiotic agents and to evaluate their clinical impact. Well conducted clinical trials are very few which can clearly demonstrate the benefits of probiotics in human beings. Recently carried out studies on probiotics have helped us in improving or understanding of their potential use in infectious diseases, which are discussed below:

Lactose mal absorption:

The level of lactase β-galactosidase decreases with the people's age. This deduction causes lactose to be incompletely absorbed which results in the abdominal cramp, bloating, flatus and severe to moderate diarrhoea. This causes limited consumption of dairy products among the elderly people. Kim and Gilliland (Kim and Gilliland, 1983) found that feeding yoghurt to the lactose

day course of vaginal suppositories of LGG were shown in women with current vaginitis. H_2O_2 producing *Lactobacillus* (LB+) are isolated from vaginal mucosa of most healthy women but are present in less than 23% of women with BV (Eschenbach et al, 1989). LB+ organisms are toxic to *Gardenella vaginalis* at high concentration, but their absence may allow a overgrowth of catalyst negative organisms present in women with BV. Also, LB+ may inhibit growth of *Naiffaria gonorrhea* by several mechanisms, including acidification of their environment, H_2O_2 secretion and production of protein inhibitors (Klebanoff et al, 1991).

Future perspective

Some of the following are the future perspective of probiotics in the field of infectious diseases:

Mucosal vaccine and immunomodulation

Medaglini et al (1998) have recently developed the genetic system for the expression of heterologous antigens from human papilloma virus and HIV type 1 on the surface of human commensal *Streptococcus gordoonii* and *L. caesi*. Local and systemic immune responses were detected in Cynomolgus monley after vaginal colonization with above mentioned recombinant strains. Both macrophage activation and IL-12/γ-IFN pathway stimulation are the promising areas of research with regard to the resistance of pathogens by enhanceing of mucosal and systemic immunity (Miettinen et al, 1998; Hessle et al, 1999). LAB can be used as live vectors for oral immunization. More experimental and clinical studies are needed to give a detailed explanation about the role of probiotics as immune modulative.

Prevention of transmission of AIDS and STD'S.

The role of vaginal microbiota in determining the efficiency of HIV and other STD transmission is not fully understood. *Lactobacillus* plays a very important role in the regulation of vaginal microflora. The production of H_2O_2 rather than the particular species of *Lactobacillus* is essential for the regulation of vaginal micro biota. This is the toxic molecule which is the most potent local microbicide present in the human vagina. Experimental findings have suggested that LB+ given at high concentration is viricidal for HIV-1 (Van't et al, 1989; Gill et al, 2001).

In the treatment of cancer:

Supplementation with certain specific strains of lactic acid bacteria (probiotics) could prevent the establishment, growth and the metastasis of transplantable and chemically induced tumor cells which was studied in several animals. In humans too, the probiotic therapy is assumed to reduce the rates of colon cancer by inhibiting the transformation of procarcinogens to active carcinogens, binding/ inactivating mutagenic compounds, suppressing the growth of procarcinogenic bacteria, reducing the absorption of mutagens from the intestine and enhancing the immune function (Medaglini et al, 1998; Miettinen et al, 1998). However, an inverse relationship between the intake of fermented dairy product, containing *Lactobacilli* or *Bifidobacterium* and the incidence of colon and breast cancer has been reported in epidemiological and population based case control studies (Miettinen et al, 1998; Hillier et al, 1998). There is a lack or little experimental evidence regarding the effectiveness of probiotics against cancer in humans. Howsoever, Aso et al (1992) demonstrated the protective effect of *L. casei* strain on the recurrence of superficial bladder cancers in randomized, controlled, multicentre studies. Within two weeks of the study subjects were enrolled after removal of bladder tumours. Tumor recurrence after 1 year was drastically lower in subjects receiving *L. casei* (57%) as compared with control group (83%). Thus, it gave a conclusion that *L. casei* delayed the onset of tumor recurrence. Similar findings were seen in a placebo controlled study which involved 125 patients by Aso et al. This is due to increase in the percentage of T-helper cells and NK cells in adult colorectal cancer patients suggested that stimulation of immune system by *L. casei* have an important role in

suppressing the development of tumour. Long term research and studies are needed to establish a basis for probiotic therapy in cancer prevention.

CONCLUSION:

The probiotic agents are the live microorganism belonging to normal flora, with low or no pathogenecity that exerts a positive effect on the health of the host. The probiotics used cover a wide range of diseases and ailments, therefore probiotics can be referred as a broad spectrum therapy. The mechanism of the action of probiotics therapy uses bacterial interference in several infectious diseases. Probiotics are safe for use in healthy persons but it should be used with cautions in some persons because of the risk of sepsis being involved. The probiotic strains which are newly developed first and foremost should be thoroughly evaluated for safety and then marketed. The mode of action of probiotic will depend on the strains of the probiotics used, as each different strain have their own specific effects. Various studies and researches have resulted in the use of probiotics for the treatment and evaluation of a variety of infections that involves mucosal surfaces, including pediatric gastroentities and vaginatis. The future perspective of probiotics will depend on further detailed explanation of basic mechanism, which can only be done by carrying out more researches and clinical trials.

In view of the increasing use of probiotics, as health supplements and therapeutic agents, clinicians need to be aware of the risks and benefits of this treatment.

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