Probiotics and Diet for Chronic Diseases Prevention

Chapter 1

Insights on the role of probiotic strains in the prevention of chronic diseases

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1. Introduction

Probiotic as a term is a relatively new word meaning “for life” and it is currently used to describe a group of bacteria when administered in sufficient quantity, confer beneficial effects for human and animals [1]. With the growing interest of the self-care and integrative medicine coupled with our health - embracing baby bromes population recognition of the link between diet and health has never been stronger. The interest in probiotics and probiotic foods has grown enormously during the past several years across the world due to multiple health-promoting functional properties associated with probiotic cultures. Probiotic concept is now gaining momentum in India too with the entry of major global players in the probiotic production. With the growing awareness, health conscious Indian society is getting receptive to ‘Probiotic Culture’ and finds these products very attractive bioactive ingredients by serving as prophylactics or bio-therapeutics for promoting human health and well-being as well as management of specific diseases. However, still there is a long way to go before these probiotic foods change the Indian mindset and reach the common man to explore their full health benefits.

Probiotics could improve human health through different modes of action including restoration of host normal microbiota, re-establishing the intestinal barrier function, induction of homeostasis of immune system, support of normal digestive functioning and by providing several trace nutritive elements to the host [2]. These beneficial effects of the probiotics have

drawn the attention of worldwide researchers to determine the innovative approaches in the field of clinical health using probiotic formulations. The growing scientific and commercial interest in the use of probiotics for health benefits has boomed the global food market in the past few years [3]. On-going efforts in metagenomics research have generated numerous new and interesting working hypotheses for manipulating microbiota for maintaining and restoring health.

Oxidative stress is caused by an imbalance between ROS or free radical production and body antioxidant defence results into oxidative stress, which significantly alters the normal cellular functions and has been implicated in several clinical situations (inflammatory bowel disease, atherosclerosis, myocardial infarction, stroke, and vascular dysfunctionality, Alzheimer's disease, Parkinson's disease, diabetes mellitus, retinopathy etc). The consequence of free radical chain reactions can lead to severe affect on living organisms. In recent times, there is a great deal of attention for the production of natural/biological antioxidants that can substitute the chemical antioxidants [4]. As the widely used synthetic antioxidants such as butylated hydroxyltoluene (BHT) and butylated hydroxyanisole (BHA) are critically unsafe due to carcinogenicity and liver damage [5]. The bioactive compounds produced by probiotic *Lactobacillus* induce antioxidant property by the scavenging of oxidative compounds/ the prevention of their entry into the gastrointestinal system. The bioactive peptides released during the fermentation of bovine milk with mixed culture of *lactococcus and lactobacillus* showed antioxidant activities by the inhibition of lipid peroxidation process [6]. The probiotic *Lactobacillus* with antioxidant activity would be a better choice, as these are with Generally recognised as safe (GRAS) status.

Increasing use of novel probiotic species from various sources has generated a necessity for the clear regulatory framework to ensure the safe and honest use of probiotics for general population. LAB are also known to possess strong anti-oxidative activity, and are able to decrease the risk of accumulation of reactive oxygen species (ROS) [7]. Antioxidative potential of candidate probiotic can be determined by evaluating their resistance towards ROS (hydrogen peroxide, hydroxyl ions and superoxide radicals), superoxide dismutase activity and total antioxidative activity [8]. Presence of S-layer proteins, exopolysaccharides (EPS) and other cellular envelope components plays an important role in bacteria-host interaction [7]. Scientific research allowed to circumvent the tedious screening of large numbers of isolates and to identify potential probiotics from the comparison of population with different health status [9].

Elucidation of mechanisms involved and substantiation of results from animal in human studies on probiotics remain further essential research goals. Growing trends indicate that live probiotic administration would no longer be necessary; instead their active ingredients or metabolites identified as the active component will be emphasized. Although challenges remain,

the microorganisms present and involved in the treatment of diseases as well as their metabolic products may contribute considerable health benefits to consumers. Therefore, more evidence on the health impacts of beneficial microorganisms and understanding of the relationships between probiotics, and human health are essential.

2. Probiotics and Arthritis- The eternal link

Research revealed that consuming probiotics may help to treat rheumatoid arthritis and alleviate joint pain and having unbalanced gut flora may cause the disease [10]. The gut microbiome plays an essential role in the development and protection of the host immune system. Emerging knowledge about this microbial microenvironment has raised the possibility of new therapies based on this knowledge [1]. The recent evidence on the impact of the gut microbiome on arthritis and possible novel therapeutic approaches through probiotics to alter the gut microbiota is promising [10]. Studies show that probiotics can ease the biomarkers that may cause inflammation and lead to joint pain [1, 11].

The inflammation of the intestines is often found in patients with arthritis, permitting for their lining to leak and allowing toxins, germs, and waste to get into the bloodstream. This can trigger inflammation to extend to other parts of the body [1]. A new level of understanding how the microbiome interacts with the immune system in recent times is evident. Environment-gene interaction is another significant element that sets the stage for initiation of autoimmune disease, that needs further investigation [12]. Probiotics could be a promising therapeutic strategy, however further interventional studies exploring the extensive interaction of microbiome and probiotics are still needed.

Recent studies prop up the growing evidence of microbiome as a causative agent for certain rheumatic diseases like rheumatoid arthritis and ankylosing spondylitis. There is interesting yet still inconclusive evidence to sustain the use of probiotics as a treatment for these diseases [11]. Probiotics as a modality of treatment will require extensive study to understand their anti-inflammatory effect, long-term efficacy, and the mechanism of action by which they exert their therapeutic role [1]. In studying a possible role of microbiota in the pathogenesis of arthritis, it has been observed that active arthritic patients have a higher serum IgA *Klebsiella pneumoniae* in comparison with healthy controls, whereas inactive arthritic patients had no such antibody elevation [13]. In these studies, it was evident that serum antibodies could be detected against pullulanase-D peptide, which possess a sequence having homology with HLA-B27 [14]. However, successive studies did not offer any confirmation of the specificity of anti-*Klebsiella* antibodies in arthritis [15].

Some of the probioitic bacteria including *Bifidobacterium longum* IPLA E44 strain and *Bifidobacterium animalis* subspecies lactis IPLA R1 have been assessed for their safety and their capability to influence the intestinal microbiota in vivo. The oral administration of *B. ani-*

malis IPLA R1 and *B. longum* E44 is considered as nontoxic, and has the ability to modulate the intestinal microbiota of rats by modulating short-chain fatty acids and the bifidobacterial population levels [16]. On the other hand, oral administration of *Lactobacillus casei* represses the type II collagen-reactive effector function of Th1-type cellular and humoral immune responses [17]. When *L. casei* was tested in rat collagen-induced arthritis (CIA), arthritis scores and proinflammatory cytokine levels were found to be lower compared with control rats and with CIA treated with indomethacin [16]. Evidence from the previous studies had showed the ability of *L. casei* to control the type II collagen-reactive effector function of Th1-type cellular and humoral immune responses in rat induced with arthritis [17].

In another study, rats with *Bacteroides vulgatus*-induced colitis have been treated with antibiotics to prevent and treat colitis, *Lactobacillus rhamnosus* GG prevented the progression/development of colitis with drastically reduced histologic scores [16]. But there are few studies on the efficacy of probiotics in human arthritis. The effect of *L. casei* was recently tested in arthritic patients and showed considerably lower serum proinflammatory cytokines (TNF-α, IL-6, and IL-12) in the probiotic-treated group, with higher level of regulatory cytokine (IL-10) as well. The disease activity score was significantly decreased with the supplementation of probiotic *L. casei* 01 [12]. Studying the possible mechanisms by which L. *casei* protects against arthritis progression exhibited the effect of oral administration of *L. casei* to repress the type II collagen-reactive effector function of Th1-type cellular and humoral immune responses in arthritic inflammation [16].

3. Psychobiotics- The modern stress alleviators

Psychobiotics are live organisms that, when ingested in adequate amounts, confer a health benefit in patients suffering from psychiatric illness [18]. But this definition, coined in 2013, is too limiting based on the latest research showing that may not be clinical depression, an anxiety disorder, or some other psychiatric disorder in order for psychobiotics to positively affect your brain [19]. Anyone suffering from chronic stress, low mood, or anxiety-like symptoms has the potential to benefit from this group of probiotics. One of the mechanisms these "mind-modulating" probiotics likely act is by their ability to produce various biologically active compounds, for example neurotransmitters. A number of molecules with neuroactive functions like gamma-aminobutyric acid (GABA), serotonin, catecholamines, and acetylcholine can be secreted by gut microbiome [20]. When these neurotransmitters are released within the gut, they may trigger cells within the gut's lining to produce molecules that signal brain function and affect the behaviour [21]. Another way that psychobiotics appear to act on the brain is by enforce effects on the body's stress response system, that involves the brain and the adrenal glands [22]. This system, known as the hypothalamic-pituitary-adrenal (HPA) axis, becomes dysfunctional in the concept of chronic stress or illness. When HPA-axis dysfunctions, the production and rhythmic timing of cortisol and other stress-related hormones be-

comes interrupted. This is supposed to take part in a central role leading to mood changing problems and cognitive disorders [21]. A third way psychobiotics are supposed to act on the brain is by the action of anti-inflammatory compounds [23]. Chronically elevated levels of inflammation throughout the body and brain are recently known to be one of the major underlying mechanism of depression and other mood and cognitive problems. This inflammation can control from the gut, and some psychobiotics may have their effect in the brain by alleviating the inflammation [24].

Research is being carriedout to identify potential probiotics that have mild-modifying effects and its benefits. When tested in healthy people, different psychobiotics have been shown to boost mood and cognitive function and reduce stress and anxiety-like symptoms. Certain psychobiotics have also been proven to treat depression, anxiety, and other mental health and cognitive issues in patients with psychological disorders and/or other medical specifications. A random clinical trial on patients suffering with major depression related disorders are assigned to receive either probiotic supplements or placebo for a period of eight weeks [25]. After the treatment, patients who consumed the probiotic had significantly decreased the total scores on the Beck Depression Inventory, which is a widely used test to measure the severity of depression, when compared with placebo. On the other hand, they had significant changes in systemic inflammation as quantitated by hs-CRP, considerably lower insulin levels, reduced insulin resistance, and a major rise in glutathione, the body's master antioxidant. Other psychobiotics have some beneficial consequences on mood and anxiety-like symptoms in people without depressive or anxiety disorders. In a study to explore the possible effects on anxiety, stress, depression, and survival strategies in healthy human volunteers, a probiotic containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 alleviated psychological distress, principally depression, anger-hostility, and anxiety, and enhanced problem solving when engaged for 30 days [26]. The researchers also concluded that *L. helveticus* R0052 and *B. longum* R0175 have valuable psychological effects in healthy humans. Psychobiotics can also aid to boost mood and ease anxiety in people suffering from various chronic diseases.

Several additional psychobiotics have been shown to reduce depression and anxiety in animal studies. In a study, *Lactobacillus plantarum* strain PS128, is proven to increase dopamine and serotonin and decrease depression-like symptoms in mice [27]. In depressed mice that have been subjected to early-life stress, this same psychobiotic alliviates cortisol, normalizes the stress response system, and decreases depression [28]. Psychobiotics have also been shown to facilitate people and animals undergoing stress. A fermented milk drink (kefir) that contained *Lactobacillus casei* strain Shirota inhibited stress-related cortisol raises and increased serotonin levels in stressed medical students [29]. Furthermore, the probiotic drink decreased stress-related physical symptoms such as abdominal pain and cold symptoms.

Another probiotic bacteria, *Lactobacillus helveticus* NS8 was compared to the SSRI,

citalopram, in rats with depression, anxiety, and cognitive dysfunction due to chronic stress [30]. The probiotic worked better than citalopram in reducing stress-induced anxiety, depression, and cognitive dysfunction. It reduced cortisol and restored serotonin and other brain neurochemical levels to normal. Other *Lactobacillus helveticus*-containing probiotics have also been proven to decrease stress-related depression and anxiety by altering serotonin, cortisol, and other neuroactive compounds in animal studies [31]. In a study, *Lactobacillus helveticus* R0052 pooled with *Lactobacillus rhamnosus* R0011 normalized the anxiety-like behavior and memory impairments in immune-deficient rats related with HPA-axis dysfunction [32]. Overall, the results of these studies reveal that psychobiotics have the potential to positively impact on brain function, boostupmood, alleviate depression and anxiety, and help to handle stress. The best proven psychobiotics and the best dosages for those psychobiotics have yet to be explored, but a many of the potential probiotics used in the studies described above are commercially available as probiotic supplements.

4. Probiotics and Autism

Autism is categorized by a collection of neurological, gastrointestinal and immunological dysfunctions that include deficiency of eye contact, as well as deficiencies in socialization and communication, abnormal theory of mind function, language dysfunction, restrictive, repetitive, and stereotypical behaviours, food allergies, constipation [33]. The autism spectrum disorders will fit in to an “umbrella” sort of five childhood-onset conditions identified as pervasive developmental disorders (PDD). Interchangeably the terms pervasive developmental disorder and autism spectrum disorder are widely used. Three most common PDDs associated with autism spectrum disorders are autism, Asperger’s syndrome, pervasive developmental disorder- Not or else specified as (PDD-NOS) (ASM). Autism is called a “spectrum disorder” since it affects individuals differently and to varying degrees. The connection between gut microbiome, intestinal disease and autism is a potential area of research. Recently, Hsiao et al. [34] investigated on the link between gut microbiota and autism spectrum disorders (ASD) in a animal model and found that the ASD symptoms are elicited by microbes and its associated metabolite compositional and structural shifts. However, the conditions are relieved by a potential probiotic *Bacteroides fragilis*. Consequently probiotics may also provide therapeutic strategies for neurodevelopment disorders.

Another research team, Gilbert et al. [35] in their profound study provided the evidence of probiotic effect and therefore it set an excellent concept of how a combination of microbial profiling, mouse models, germ-free mice, and bacterial metabolomics could be used to mechanistically understand the effects of the gut microbiome on health and disease states and to build up therapeutic strategies to treat ASD conditions. Reports also indicate that probiotics can be used to treat anxiety and post-traumatic stress disorder (PTSD) in mouse models, including one specific model that involve an intact vagus nerve for gut-brain signalling [36]. Children

suffering with autistic symptoms often have more digestive health complications compared to normal population. One among 4 children with autism are thought to have at least one chronic gastrointestinal symptom [37] and several studies have also discovered abnormal digestive health problems in people with autism [35, 36, 38]. Unrecognized gastrointestinal disorders especially disaccharide malabsorption and reflux esophagitis, may contribute to the behavioural problems of autistic patients [39]. The intense association of gastrointestinal symptoms with autism severity reveal that children with more severe autism are often to have more severe gastrointestinal symptoms and vice versa. The use of probiotic alters the metabolic functions of gastrointestinal tract that include increased absorption of fatty acids and gut permeability [38]. These probiotics may thus provide therapeutic strategies for neurodevelopmental disorders. Therapies that target the gut microbiome may clutch the key for making progress against a wide range of notoriously difficult psychiatric illnesses. Thus, supplementation of probiotics in the early life may reduce the risk of neuropsychiatric disorder development later in childhood possible by mechanisms not limited to gut microbiota composition.

5. Cholesterol lowering probiotics

Cholesterol binding in the small intestine may possibly reduce the amount of dietary cholesterol absorbed by Lactic acid bacteria (LAB) [39]. Some of the mechanisms for lowering the dietary cholesterol absorption have been hypothesized [40], including enzymatic deconjugation of bile acids by the enzyme bile-salt hydrolase (BSH), co-precipitation with deconjugated bile, cholesterol assimilation, binding of cholesterol to the cell walls of probiotic bacteria, cholesterol incorporation into the cell membranes of probiotic bacteria, translation of cholesterol to coprostanol, and production of short-chain fatty acids by the potential probiotic bacteria in the presence of prebiotic substrates.

The enzyme BSH hydrolyzes conjugated glycodeoxycholic acid and taurodeoxycholic acid, resulting in the deconjugation of glyco- and tauro-bile acids. Once deconjugated, bile acids are lesser soluble and much absorbed by the intestines, leading to their exclusion in the faeces. Cholesterol is also used to synthesize new bile acids in a homeostatic response, leading to the lowering of serum cholesterol. However, the ability of probiotics for cholesterol-binding appeared to be growth and strain specific. According to a study, there is difference in faecal cholesterol-associated short chain fatty acids model in normal rats fed with the oat-based diets with probiotics in comparison to the group fed with cereal-based diet [41]. Probiotic bacteria are also able to reduce cholesterol in blood that can be obtained from carnivores as they normally eat meat containing high fat yet rarely develop cardiovascular conditions. Isolation and characterization of such potential strains of probiotic bacteria has a possible application in controlling cholesterol levels in humans [42].Cholesterol lowering peptides are originated by beta-lactoglobulin, casein and soy proteins proteolysis [41]. They are able to lower the total cholesterol of rats under *in vivo*. Their mechanism of action possibly consists of the decrease

of the micellar cholesterol solubility or also of an improved ability to bind taurocholate [43]. Their overall action prevents cholesterol absorption by CacO2 cells under *in vitro* and enhances faecal steroid excretion under in vivo. Several peptide molecules are essentially play a key role involved in these activities. Some of the bioactive peptide sequences involved in the cholesterol lowering attributes are Ala-Leu-Pro-Met-His, Ile-Ile-Ala-Glu-Lys, and Gly-Leu-Asp-Ile-Gln-Lys [43]. Studies also conducted to discover the role of probiotic bacteria in the reduction of serum cholesterol illustrated promising results during in vitro studies where cholesterol precipitated out with a possibility of the same being eliminated under *in vivo* conditions [42]. However, much research needs to be conducted in this regard as these reports were inconclusive.

In conclusion, the WHO has pointed out that substitute disease control approaches such as the use of probiotics may be needed in the future in the prevention and treatment of certain chronic diseases. Probiotics are live microbial preparations with established health benefits that maintain or improve intestinal microbiota. They have become an imperative part of the nutrition as the microbial population in our bodies are transformed by the use of antibiotics and other substances that are designed to inhibit pathogens and disease (Figure 1). While antibiotics are effective at killing pathogens, on the other hand, they are also effective in killing beneficial bacteria. By introducing friendly microbiota to the gut flora, this can support the resident microbiota that leads to the healthy gut system and there by reduce the risk of chronic diseases.

6. Figure

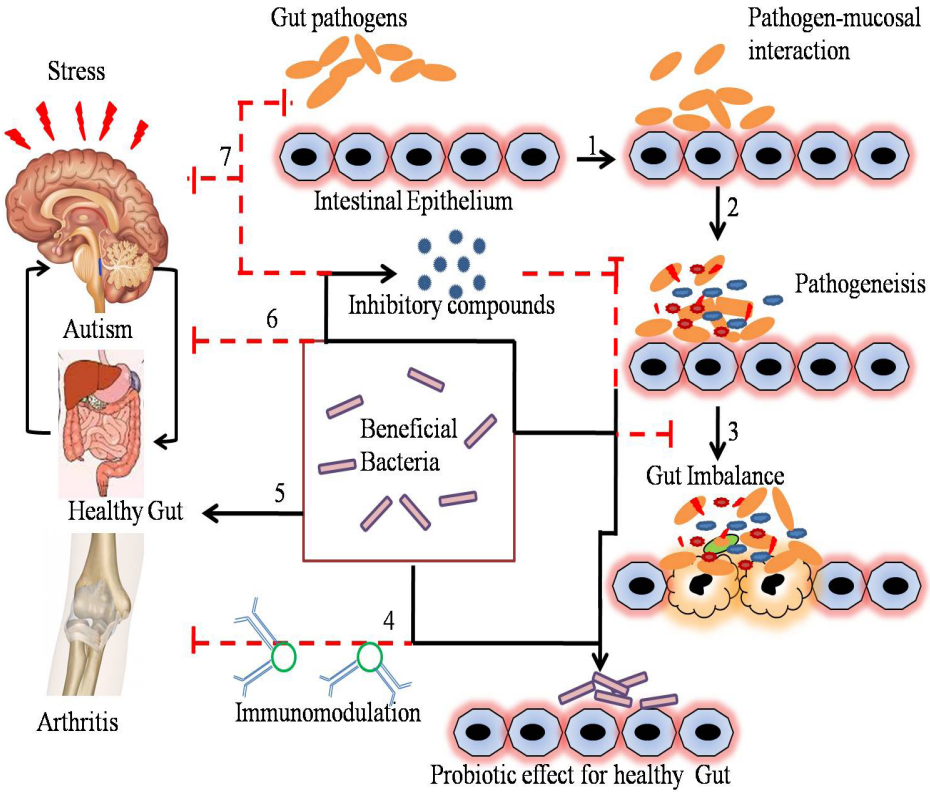

Figure 1: Model mechanism of effect of probiotics in controlling some of the chronic diseases by defending human gut intestinal epithelium at different check points. Probiotics and their inhibitory compounds serve as the blocking points for pathogen interaction and pathogenesis steps (1-3). This probiotic effect leads to direct intestinal modulation through indirect immune response that can control the arthritis (4). On the other hand, the probiotic bacteria maintains and protects the gut environment (5) directly prevent the adhesion of pathogens and favour the probiotic therapy for a healthy gut- brain barrier that can prevent the stress related diseases (6-7).

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