Review Article

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Probiotic foods: Can their increasing use in India ameliorate the burden of chronic lifestyle disorders?

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Probiotics are defined as live microorganisms which, when ingested in adequate amounts, confer health benefits on the host. Chronic diseases such as diabetes, non-alcoholic fatty liver disease, coronary artery disease, a variety of chronic inflammatory disorders with an immune basis, and some forms of cancer are increasing in incidence around the world and in India, and may be attributable in part to rapid changes in our lifestyle. There is considerable public interest in India in the consumption of probiotic foods. This brief review summarizes the background of the gut microbiota, the immunological reactions induced by these, the evidence linking the microbiota to health outcomes, and the evidence linking the use of probiotics for amelioration of chronic lifestyle diseases.

Key words Bacteroidetes - *Bifidobacterium* - Firmicutes - gut microbiota - *Lactobacillus casei* - lifestyle disorders - *Prevotella* - probiotics

Introduction

Morbidity and mortality due to chronic diseases such as type 2 diabetes mellitus, metabolic syndrome, non-alcoholic fatty liver disease and coronary artery disease are common in India. These disorders are collectively called lifestyle disorders as these are associated with industrialization, dietary changes and increased life span. The implication is that lifestyle factors, including diet, smoking, alcohol and stress, may directly or indirectly be responsible for increasing burden of these diseases which also include some forms of cancer. The gut microbiota (microbes that reside in the human gut) have extensive metabolic functions that influence human health. Probiotics have been defined as "live microorganisms which, when ingested in adequate amounts, confer health benefits on the host"[1]. The oral ingestion of probiotics may be one intervention that can maintain a healthy balance of the gut microbiota and thereby help to prevent these lifestyle disorders. A symposium on "Probiotics in Prevention of Life Style Disorders" was convened by the Yakult India Microbiota and Probiotic Science Foundation in Bengaluru, India, on December 15-16, 2012. The symposium brought together an interdisciplinary group of health care professionals that included scientists, clinicians, regulators, nutritionists and students. The present opinion piece is based on the presentations at the above symposium and attempts to present an integrated

view of the human gut microbiota in context of human health and disease, reviews the current evidence for a role of probiotics in the amelioration of the burden of lifestyle disorders, and describes the prospects for the use of probiotics as food and as drugs in India.

The burden of lifestyle disorders in India and the potential for probiotics to impact health

There has been an alarming increase in the incidence of lifestyle disorders and non-communicable diseases (NCDs) in India. This is illustrated by the fact that 8 out of every 10 deaths in India are caused by non-communicable diseases[2]. This trend is also observed in other South Asian countries. With India launching a comprehensive programme to prevent and control lifestyle diseases, the potential of probiotics as a preventive intervention to be integrated in our lifestyle practices is significant. The Indian probiotic market is driven by emphasis on preventive health care, changing demographics and emergence of wellness related chains. The key challenges include lack of standardization, product stability and the need for validating product claims. There is an urgent need to examine the legal and regulatory issues that would allow probiotic use in India and to determine whether the current regulatory framework is a good fit for the range of probiotics under development or that may be developed and become available in the Indian market, in the future. Clarity in regulation of probiotic products would be imperative to improve the way probiotics are marketed in the country and to ensure that probiotic products are made available in an appropriate manner to the general public.

Probiotics - A historical overview

Dr Elie Metchnikoff, nearly a century ago, introduced the concept of probiotics as harmless friendly bacteria that could be used for improvement of health. This was based on his observations that in certain long-lived communities the consumption of certain strains of *Lactobacillus* in yogurt was associated with survival to old age[3]. Another society where lactobacilli were promoted for health uses was Japan, where probiotic drinks have been in wide use for promoting health for the last seven decades. Dr Minoru Shirota in 1930 isolated a specific strain of *Lactobacillus casei* Shirota (LcS) with probiotic properties and marketed the fermented milk drink containing this probiotic (Yakult) for over seven decades to improve intestinal health[3]. The latter has been one of the best examples of long and safe usage of a probiotic backed by preclinical and clinical studies showing objective evidence of health benefits.

The human gut microbiota - Diet and cultural differences

Our understanding of the gut microbiota has expanded considerably in recent years due to the developments in molecular characterization of the gut microbiota and their metabolic capacity. Recent developments in genome sequence technology, high throughput genomics data and comparative metagenomics have all revolutionized microbiological research. New findings driven by mega projects such as the Human Microbiome Project[4] and the Meta Hit Project[5] have allowed high resolution microbial scan of the intestinal microbiota including the uncultivable species that have opened novel perspectives for clinical applications. Metagenomic studies have established that despite extensive interpersonal variability in community composition, there is a shared core of functionalities in the microbiome. The gut microbiota is typically dominated by bacteria and specifically by members of the phyla Bacteroidetes and Firmicutes. Although there is substantial range of variation in the taxa present in the gut, a recent study[6] revealed that the human populations studied so far can be divided into three distinct enterotypes based on the distribution of *Bacteroides*, *Prevotella* and *Ruminococcus*. These enterotypes are independent of the host's ethnicity, country, sex or age. There were no clear population-based or genetic explanations found for the clustering of the enterotypes, which appeared to be determined primarily by dietary effects. It was observed that a diet rich in protein and animal fat correlated with an enterotype characterized by high levels of *Bacteroides* and a carbohydrate rich diet was associated with a *Prevotella* dominated enterotype[6].

Short term dietary interventions led to rapid changes in the microbiome composition but were not sufficient to shift individuals between the enterotypes[6]. The gut microbiota has a close relationship with human health. The gut microbiota can be characterized in terms of microbiota richness (gene number) with low gene counts suggesting an atrophy of the microbiome and presence of dysbiosis[6]. Recent studies show the pronounced influence of dysbiosis of the gut microbiota in the pathophysiology of immune mediated disorders[6]. Functional metagenomic tools permit the exploration of fine interactions between food constituents, microbiota and human cells and tissues.

Cross-talk between probiotics and the intestinal microbiota

The next obvious question is whether the plasticity of the gut microbiota can be exploited by using probiotics to generate a more healthy gut microbiota and alter their metabolic activity to correct and improve lifestyle conditions. The probiotic bacteria can exert their beneficial effect on the host through various mechanisms which range from their ability to antagonize pathogenic bacteria by reducing luminal pH, inhibiting bacterial adherence and translocation or producing antibacterial substances such as bacteriocins and defensins[7]. In addition, probiotics can influence mucosal cell-cell interactions and cellular stability by enhancement of intestinal barrier function and exerting their effects on numerous cell types involved in innate and adaptive immune responses such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, and natural killer (NK) cells[7]. Metabolomic studies have shown that probiotics can modulate the gut microbiome and the metabolism of short chain fatty acids, amino acids, bile acids and plasma lipoproteins demonstrating the diversity of symbiotic co-metabolic connections between the gut microbial content and the host[8]. It is now clear that there is an ongoing cross-talk between the gut microbes, the intestinal mucosa and the host which play a significant role in deciding the state of health and wellbeing[9,10]. We now have the tools to understand this conversation and manipulate it with interventions (*e.g.* probiotics) to improve human health. However, the effects vary depending on the strain of the probiotic bacteria, dosage and route of administration and, therefore, deciphering strain specific benefits should be the next new challenge[11].

Probiotics in augmenting innate immunity

Innate immunity is finely regulated by the gut microbiota at the interface of the intestinal mucosa. A breakdown in the balance between the putative protective and harmful intestinal bacteria leads to chronic inflammation resulting in several inflammatory disorders, including inflammatory bowel diseases[12,13]. Certain unique subsets of innate immune cells orchestrate immune responses. $CD70^+$ dendritic cells (DCs) are capable of microbiota-dependent induction of Th17 cells in the lamina propria. A unique intestinal immune cell subset, named regulatory myeloid cells, has been identified which was responsible for prevention of intestinal inflammation through inhibition of T cell responses[14]. Another regulatory T cell subset, named Tr1 cells which produce interleukin-10, has also been identified but the manner in which these cells are regulated in the intestine remains unclear[15]. Analyzing the role of two probiotic strains, *Lactobacillus casei* and *Bifidobacterium breve*, in T cell development in the intestine revealed that *B. breve*, but not *L. casei*, induced development of Tr1 cells in the large intestine[15]. Oral *B. breve* administration ameliorated colitis in immunocompromised mice given $CD4^+$ T cells from wild-type mice, but not from interleukin-10 (IL-10) knockout mice, suggesting that the probiotic prevented intestinal inflammation through the induction of intestinal IL-10-producing Tr1 cells[16].

Probiotics and amino acid uptake

With the recent WHO/FAO recommendations for the daily requirement of essential amino acids in adults being two to three fold higher than the earlier recommendations[17], the intake of essential amino acids in disadvantaged populations is likely to be marginally deficient. Although it is conventionally assumed that all the essential amino acid requirements of humans must be supplied by the diet, it is quite possible that the gut microbiota may play a significant role in the absorption of essential amino acids synthesized from endogenous urea, though this appears surprising, given that the colon is deficient in amino acid transporters[18]. There exists a significant population of bacteria in the small intestine which may exert this effect. Studies with radioactively labelled N have revealed that lysine absorption mediated by the gut microbiota almost equals the daily average requirement of a healthy adult[19,20]. With 23 per cent of the Indian sedentary male adult population being estimated to be at risk of an amino acid diet deficiency because of their dietary habits, it is possible that the microbial contribution to essential amino acid input can be between 14 to 32 per cent of the intake which may be significant in countering protein deficiencies in the population[21].

First insights into the Indian gut microbiota

Recent studies, largely from southern India, provide a snapshot of the gut microbiota in Indians in addition to revealing variations in the microbiota composition with age[22]. A detailed time series on a cohort of infants born in a hospital in southern India has demonstrated that the gut is heavily colonized even on the very first day of birth, with the transition from facultative to strict anaerobes (*Bifidobacterium* species) occurring within the first week of life[23]. The pattern is punctuated by major shifts in the composition of the microbiota with a predominance of *Bacteroides*,

Prevotella and *Clostridium leptum* seen especially after the introduction of complementary foods. Differences in gut microbiota between breast fed and bottle fed babies were difficult to evaluate since more than 90 per cent of the babies were exclusively breast fed, however marked differences were observed between children born by vaginal and Cesarean section[23]. Lactobacilli, Enterococci and *Bacteroides-Prevotella* count were higher in vaginal babies as compared to Cesarean babies. Surprisingly, these differences disappeared by day four and the reasons for the rapid change could not be identified. Significant species variations were observed in the bifidobacterial diversity in a comparative study with infant feces from New Zealand and UK who had a predominance of *Bifidobacterium longum* and *B. catenulatum* as compared to the Indian counterparts that were predominated by *B. infantis*[24]. An interesting finding of the influence of environment on the gut microbiota revealed that children living in close proximity to cattle acquired ruminal bacteria as a part of their gut microbiota[25]. Comparison between obese and non-obese children revealed that despite the fact that the dietary history was not very different, there was an increased abundance of *Faecalibacterium prauznitzii* in obese children, though that may not be the sole determining factor for obesity[26]. A plausible reason may however, be an altered ratio of Firmicutes and Bacteroidetes. Firmicutes, Proteobacteria and Bacteroidetes largely dominated the adult community although it was observed that Indians harbour far lesser numbers of Bacteroidetes than their North American counterparts (Figure)[27]. In another study, the plausible role of the gut microbiota in iron deficiency in anaemic women was elucidated and it was observed that anaemic women have much lesser numbers of *Lactobacillus* as compared to normal women[28]. The linking explanation could be that lactobacilli increase the expression of iron transporters in the caecum due to production of propionic acid, a short chain fatty acid. Feeding inulin in animals resulted in an increase in *Lactobacillus* count which had a positive effect on short chain fatty acids and thereby increased the expression of iron transporters[29].

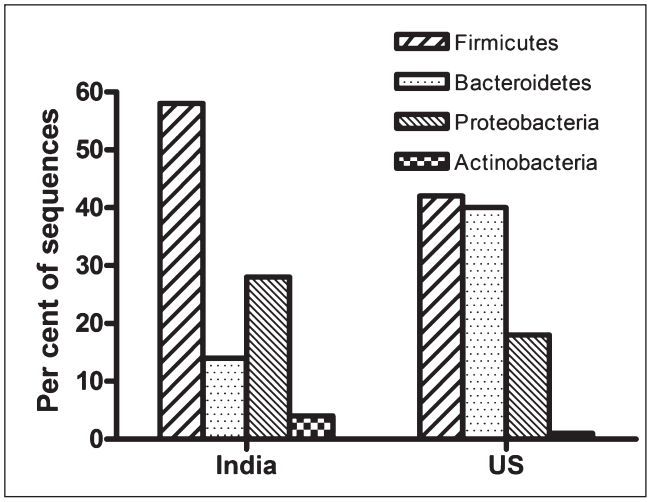

Fig. Comparison of phylum level distribution of the gut microbiota in healthy Indian and healthy US volunteers determined by cloning the fecal 16S rRNA genes followed by sequencing of the clones. Relative abundance of Firmicutes and Proteobacteria and relative scarcity of Bacteroidetes are noted in Indian compared to US volunteers. (Reproduced with permission from Elsevier).

The link between gut microbiota and gut inflammation

Studies in a mouse model of experimental colitis showed that probiotic intervention with a cocktail of four Lactobacilli and four Bifidobacteria significantly reduced severity of inflammation by down regulating tumour necrosis factor (TNF)-α secretion from distal colon explants and reducing the expression of TNF-α, and transforming growth factor (TGF)-β in distal colon, when compared to untreated mice with dextran sodium sulphate (DSS)-induced colitis[30]. Expression of barrier defense molecules was reduced in colitis mice compared to control, which was not restored by probiotic treatment. In faeces however, the relative proportions of *Bifidobacterium*, *Bacteroides*, and *Lactobacillus* were significantly reduced in the colitis mice group compared to control groups. The changes in the microbiota composition were reversed towards a normal pattern by probiotic treatment. Thus, prior administration of probiotic treatment reduced mucosal inflammation, modulated cytokine expression and restored faecal anaerobic bacterial microbiota in DSS induced colitis in mice[31]. Studies in epithelial cell lines established a basis for the suppression of chemokine and cytokine gene expression when epithelial cells were exposed to probiotic lactobacilli[31].

The link between gut microbiota, systemic inflammation and metabolic diseases

The link between an altered gut microbiota profile and obesity is linked to low grade systemic inflammation. Adipose tissue has a role as the source of obesity-associated inflammation, and is possibly directly responsible for several metabolic disorders such as insulin resistance, type-2 diabetes, hypertension,

atherosclerosis and neuro-degeneration[32,33]. Recent scientific evidence suggests that obesity is associated with increased levels of Gram-negative bacteria (GNB) that can impair intestinal permeability triggering systemic inflammation[32]. Growing evidence also suggests that systemic inflammation in adults is related to increased intramuscular fat and connective tissue will eventually predispose the offspring to diabetes and obesity, indicating that inflammation may be contributing to chronic diseases even during faetal life[34]. A low grade systemic inflammation corresponds to higher plasma endotoxin lipopolysaccharides concentrations defined as metabolic endotoxemia. In this scenario, administration of probiotics or prebiotics may be a possible dietary strategy to regulate increased levels of GNB, reduce inflammation and associated metabolic disorders[35].

The 'gut connection' to type 2 diabetes is being investigated. Experimental models connect an altered microbiota composition to the development of obesity, insulin resistance, and diabetes in the host through several mechanisms such as increased energy harvest from the diet, altered fatty acid metabolism and composition in adipose tissue and liver, modulation of gut peptide PYY and glucagon-like peptide (GLP)-1 secretion, activation of the lipopolysaccharide toll-like receptor-4 axis, and increased inflammation[36]. An association of pro-inflammation and oxidative stress has been demonstrated not only in patients with type 2 diabetes but also in subjects with impaired glucose tolerance (prediabetes)[36]. Recent data have characterized the intestinal microbiota in type 2 diabetic patients. The proportions of phylum Firmicutes and class Clostridia were significantly reduced in the diabetic group compared to the control group[37]. A recent study has identified twenty-two bacterial species and four operational taxonomic units that were either positively or inversely correlated with metabolic syndrome traits, suggesting that certain members of the gut microbiota may play a role in these metabolic derangements[38]. More importantly, the recent metagenome-wide association study showed that patients with type 2 diabetes were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens[39]. While these studies suggest plausible bacterial sequence signatures as risk factors for type 2 diabetes, these also warrant an imperative need for such metagenomic studies in relation to type 2 diabetes in India. Considering the link between alterations in intestinal mircobiota and metabolic diseases, probiotics are claimed as potential modulators of gut-microbiota that change gut-microbiota composition in a beneficial manner and exert various health beneficial effects *i.e.* anti-hyperglycaemic, anti-hyperlipidaemic, anti-oxidant and anti-inflammatory[40].

Several studies have examined the effect of probiotics on other metabolic parameters that correlate with metabolic syndrome. In one such study, a blend of *Aloe vera* and probiotic was useful in reducing the total serum cholesterol, triglycerides, VLDL and LDL and increased HDL[41,42]. The faecal *Lactobacillus* counts also increased significantly in the probiotic plus *Aloe vera* group[43]. A recent study with probiotic *dahi* (curd) also indicated a hepatoprotective effect of LcS and *L rhamnosus* GG against aflatoxin B1 induced hepatic damage in male Wistar rats[44].

Role of gut microbiota in neonatal sepsis

Neonatal sepsis is another condition with a systemic inflammatory response, where the gut microbiota may play a crucial role in pathogenesis. In a study exploring the association of Gram-negative bacteria in the gut of neonates with subsequent sepsis, a diverse array of GNB was isolated from the gut of the neonates. *Klebsiella pneumoniae* was the most common followed by *Escherichia coli*. *K. pneumoniae* was also the most frequently isolated GNB from the blood[45]. Neonates with GNB in the gut had higher incidence of clinical sepsis than those without. In 50 per cent cases the genotypes of the organisms found in the blood were indistinguishable from their gut counterpart[46]. This provided strong evidence in support of an association between gut colonization and neonatal sepsis.

The link between gut microbiota, probiotics, and cancer

The role of probiotics in the prevention and treatment of a variety of disorders that go beyond gut health (cancer, metabolic diseases, *etc.*) is increasingly being recognized. Cancer is one of the leading causes of death in most parts of the world. The lethal cancers arise mostly in the mucosal lining of various organ systems and are diagnosed at the advanced stages calling for multi-modal treatments which have a huge financial burden for governments and care giving families. Cancer prevention is a favoured and cost-effective option. There is emerging evidence that the microbiota residing in the mucosa of the body cavities (*e.g.* gastrointestinal, respiratory, genitourinary) may influence the development of cancers by various

mechanisms. Gastric cancers may develop due to biochemical changes created by the growth of pathogenic bacteria that synthesize nitrosamines. Characteristic alterations in the gut microbiota, probably resulting in production of toxic microbial metabolites, have been found in colon cancer[47]. The role of probiotics as an alternative approach to normalize the microbial microbiota and thus prevent cancer is undergoing intense investigation. Interleukin-6 (IL-6) *trans*-signaling plays a role in the development of inflammation-based colon tumourigenesis. The soluble form of IL6R (sIL6R), which is cleaved by TNF-α converting enzyme (TACE) from extracellular domain of membrane bound IL6R, as well as IL6 played a central role in this non-canonical IL6 signaling[48-50]. LcS supplementation suppressed disease activity in patients with active ulcerative colitis and prevented the occurrence of colon tumours in high risk groups. Treatment with LcS induced improvement of both chronic inflammatory bowel disease (IBD) and colitis associated cancer (CAC) in mice accompanied by the downregulation of IL-6 production in lamina propria mononuclear cells[51]. It was further confirmed that specific-polysaccharide chain (PSPG-I) in the cell wall of LcS was critical for the anti-IL6 action of LcS because mutant strains of LcS, which lacked PSPG-I, did not induce an improvement of CAC in mice or did not demonstrate an anti-IL6 action[52,53]. These results indicated that several species of intestinal microbiota are involved in the development of colorectal cancer via accelerating IL-6 *trans*-signaling in mucosal dendritic cells and LcS may play an important role in the prevention of intestinal inflammatory disorders through the suppression of IL-6 action.

Market overview of probiotics in India

Although presently India represents less than 1 per cent of the global probiotic share, it is the most potential market for probiotic products because of a growing interest in health care and need for preventive medicine. The domestic market that is comparatively nascent at this stage and is estimated at ₹ 20.6 million with a projected annual growth rate of 22.6 per cent unitil 2015[54]. Probiotics are available both as foods (to improve health) and as drugs (to treat a specific medical condition). Drugs that are not included in Pharmacopoeia are referred to as Proprietary medicines and require to comply with the standards as per Schedule V of the Drugs and Cosmetics Rules 1945. Probiotics drugs account for over 90 per cent of the market in India, followed by milk and milk products. Probiotic foods, on the other hand, are regulated by a set of food laws that aim to regulate general food items as defined by the recently established Food Safety and Standards Authority of India[55]. The Indian Council of Medical Research and Department of Biotechnology have formulated guidelines for probiotics being marketed as foods[56]. These guidelines specify the criteria that need to be borne when launching a probiotic food in the country. These guidelines also specify the labelling requirements for probiotic products in India. These guidelines may be used as a reference to establish the much needed probiotic standards in the country by the Food Safety and Standard authority of India.

Gaps in understanding and the way forward

There is a growing body of evidence to support the potential use of probiotics in the prevention and treatment of various human diseases, the caveat being that only specific organisms may be effective for certain disease manifestations. The use of probiotics to manipulate the gut microbiota and to advance health in India remains an exciting proposition. Several issues need to be addressed in order to take this forward. These include an improved characterization of the variability in the gut microbiota, a better understanding of how such a variability can result in similar or different functional profiles, and more integrative studies that take into account the interaction between the microbiota, the host and the environment to produce a phenotype. Substantiation of probiotics claims for gut and immune health benefits is hampered by a relative lack of relevant and validated biomarkers[57]. A combination of cultural and molecular studies for delivering new insights on the impact of different diets on the individual variation in microbiota composition and subsequently upon health will become important. In addition, establishing rules and regulations for the proper identification of organisms for specific uses and clearly demonstrating underlying mechanisms of action will shape the future of probiotics research with respect to various disease interventions. Further research, in the form of controlled human studies, is needed to determine which probiotics and in what dosages are associated with the greatest efficacy. Carefully selected and fully tested probiotic strains will probably provide alternative options for individuals in whom conventional medical therapies have failed to promote health and perhaps, in the future, serve as a first-line choice of therapy for some patients. This targeted translation of science for consumer benefit coupled with effective regulations will play an important role for paving the path ahead for probiotic use in the country.

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