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Prospectus of probiotics in modern age diseases

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ABSTRACT

In India food reflects the warmth, hospitality, status, symbol of wealth and aesthetics. The synergistic combination of pre and probiotics is known as synbiotics. Regular consumption of synbiotics in diets imparts health benefits like improved immune response, maintain intestinal integrity, decrease intestinal infections and down regulate the allergic response, influence digestion and gastric motility. Because of the changes in life styles due to globalization, unhealthy diets, lack of physical activity and exposure to tobacco smoke or harmful use of alcohol non communicable diseases are disproportionately affecting the 80% of low and middle income countries. This review covers the mechanism of probiotic action, use of probiotics in treatment and prevention of diseases of modern age, progress in delivery systems for the administration and finally some regulatory considerations. In conclusion, combined skills of the microbiologist, food technologist and clinician are necessary to sustain effect of probiotics. The role of probiotic organisms as alternative or complementary therapy in combating a large number of disorders can be achieved with balance and healthy life style as well as clean external environmental conditions. It is hoped that more detailed research will be conducted regarding the efficacy of probiotics so that clinically well documented and simplest formulation will be developed and can be regarded as effective for everyone. With validated results strong market will be formed and expanded in near future.

1. Introduction

People of different cultures all over the world are intricately connected with the food. In India significant consideration has been given to the consumption of type of food and spiritual virtues of mind. The Indian traditional foods are based on the customs, environmental conditions of region, life style and cultures. Indians were aware of the fact that strong body of science underpinning health benefit from foods. Milk and milk products have occupied prominent importance in Indian ceremonial aspect as well as dietary regimen since time immemorial. *Dahi, Makkhan, Lassi, Ghee, Khoa, Kheer, Chhana, Paneer, Sandesh, Shrikhand, Chhans* (Buttermilk) etc are being prepared and used regularly in Indian meals. In India food reflects the warmth, hospitality, status, symbol of wealth and aesthetics. This is evident from the ancient and most auspicious Hindu book *Shrimad Bhagavatam*[1].

Dwari dwari grihanam cha dadhi akshata phala ikshuvi
Alamkritam purnakumbhair valibhir dhupa deepakaih

Meaning: – In each and every door of the residential houses auspicious things like curd, unbroken fruits, and sugarcane and full water–pots with things for worshipping along with incense and candles all were displayed. The kindness of Indian also gave the sacred attention to plants and animals. Most sacred animal cow is believed to be treasure churned from the cosmic oceans by god. The mixture of milk, curd, butter, urine and dung–five products of cow (*Panchagavya*) have great purifying potency and have religious significance (Figure 1)[2]. In ancient India there was huge development in medicine. This is evident from the very small fraction of the total literature survived the ravages of time, but even what has survived pioneering in future research.

Russian scientist Metchnikoff (1908) observed the longevity of Bulgarian peasant was because of consumption of fermented milk and realized the role of intestinal microbes in retention of healthy life[3]. At the beginning of 20th century Metchnikoff introduced the concept of probiotic in

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western land and thus lay out scientific path for evaluating and documenting efficacy of traditional delicacies. Probiotics are defined as "live microorganisms which when administered in adequate amount confer a health benefit on the host"[4]. Instinctive desire of human for mastery over his environment has expanded the isolation of probiotics from fermented dairy product to those of human intestinal origin and hence the modified definition of probiotic is "live organisms which when ingested in adequate amounts as a single strain or combination of strains confer health benefits to the host"[5]. Prebiotics is a non-digestible food ingredient that confers benefits on the host by selectively stimulating the growth and/or the activity of one bacterium or a group of bacteria in the colon and thus improves host health and promotes the growth of certain probiotics[6]. The Synergistic combination of pre and probiotics is known as synbiotics. Synbiotics plays an important role in human health by balancing the internal microbial population (Symbionts, Commensals and Pathobionts)[7]. Regular consumption of synbiotics in diets imparts health benefits like improved immune response, maintain intestinal integrity, decrease intestinal infections and down regulate the allergic response, influence digestion and gastric motility[8].

Figure 1. Five products by cow (*Panchagavya*:-milk, curd, butter, ghee and dung) - potential therapeutic agent.

Recent report on non communicable diseases (NCD) by world health organization states 9 million of all deaths attributed to NCDs occur before the age of 60, because of the changes in life styles due to globalization, unhealthy diets, lack of physical activity, exposure to tobacco smoke or harmful use of alcohol (Figure 2), NCDs are disproportionately affecting the 80% of low and middle income countries (~ 29 million death) (WHO, 2011). Climate change has also brought changes in disease pattern globally; environmental and genetic factors have impact on *Lactobacillus rhamnosus* GG biofilm formation[9]. Increase incidence of gastrointestinal infection attributes perturbation of primed internal barrier because of imbalance between the internal environment and constant challenges by potentially pathogenic factors present in external environment. Psychological and physical stress leads to substantial changes in gastrointestinal tract physiology. These changes include inhibition of gastric acid release, reduce gastric motility, changes in bicarbonate release in duodenum, hormonal changes and decrease immune response[10]. These environmental and host factors also influence probiotics mode of actions and results in varied microbial responsiveness in colonization and/or immunomodulation leading to increase allergic predisposition of host[11].

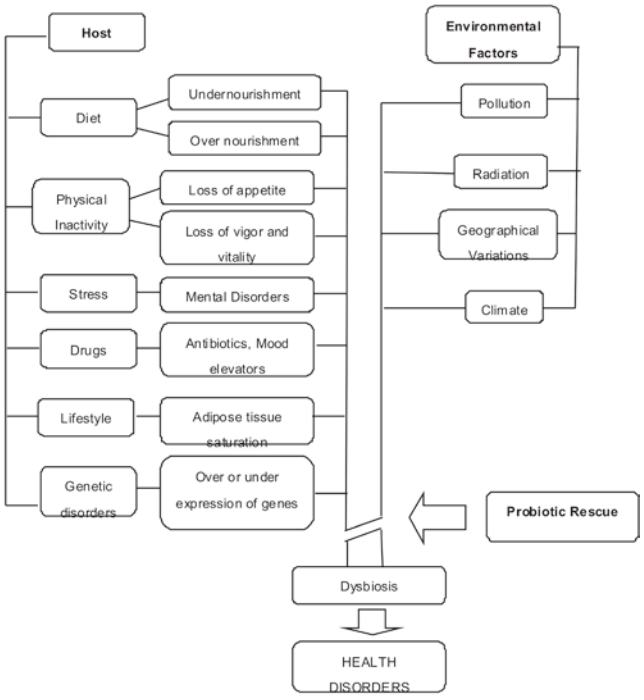

Figure 2. Demonstration of intricacy of host factors like diet, physical inactivity, stress, drugs, life style, genetic disorders, etc with environmental factors like pollution, radiations, geographical variations, regional climate to predispose to several health disorders where probiotic comes in rescue.

This review covers the mechanism of probiotic action, use of probiotics in treatment and prevention of diseases of modern age such as metabolic disorders, obesity and diabetes, allergic disorders, mental disorders and aging, progress in delivery systems available for the administration and finally some regulatory considerations.

2. Probiotic: mechanism of action

The most commonly studied probiotic include microorganisms of genera *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Pediococcus*,

Leuconostocs, *Weissella* and *Saccharomyces*. Diverse and complex community of indigenous microorganisms in gastrointestinal tract plays crucial role in both health and disease[12,13]. Emerging research and knowledge of these biotherapeutic agents revealed that administration of whole organisms is not necessary. Secretions of probiotic such as protein, Short chain fatty acid (SCFA), organic acids, cell surface active components and DNA from these microbes exert the same therapeutic effect [14]. These therapeutic agents are known as pharmabiotics or probioactive[15]. Important functions of these inhabitants are to outcompeting potentially pathogenic microbes for ecological balance and serve as important source of energy by producing metabolites through fermentation of carbohydrates to organic acids mainly butyric, propionic, acetic and lactic acid to colonocytes[16].

There are considerable evidences in support of beneficial effects of probiotic consumption in the form of dietary supplements and pharmaceuticals. These include: (1) immunomodulation by educating the naive immune system (T helper cells, Th0) and serve as non inflammatory immune stimulator (Th1, Th2 and Th3/Tr1 helper subset) in healthy individuals throughout life[3,17]. (2) Balancing colonic microbiota through colonization resistance, competing for nutrients consume by enteropathognes, modification of pH and producing strain specific antimicrobial peptides (Lantibiotics) such as nicin A (*L. lactis*), lacticin 3147 (*L. lactis*), gallidermin/epidermin (*S. gallinarum*/*S. epidermidis*), mutacin 1140 (*S. mutans*), mersacidin/actagardine (*Bacillus* subsp./*Actinoplanes* subsp.), duramycin (*Streptomyces* subsp./*Streptoverticillium* subsp.), cinnamycin (*Streptomyces cinnamoneus*), ancovenin (*Streptomyces* subsp.) pediocin (*Pediococcous* subsp.)[12,18-20]. (3) Maintaining integrity of intestinal epithelial cells by reducing production of carcinogenic metabolites like fecal azoreductase, nitroreductase and β-glucoronidase thereby reduce inflammation and improve barrier function[21]. (4) Treatment and control of diarrhea (antibiotic associated, traveler and rotavirus) by reinforcement of intestinal mucosal integrity and stimulation of secretion of antirotavirus specific immunoglobulin such as IgA[16,22]. (5) Other important therapeutic benefits of probiotics are assimilation of cholesterol, prevention of hypertension by inhibition of angiotensin- I converting enzyme and reduce blood pressure[23], relief of lactose intolerance by elevating level of lactase in intestine, prevention of urinary tract infection by production of hydrogen peroxide[24], immunomodulatory effect in patients with rheumatoid arthritis and HIV infection[25]. Probiotics have therapeutic benefits in controlling commonly occurring oral infections like Candida infection, hypo-salivation and feeling of dry mouth, dental decay, periodontal infection and halitosis [26]. Figure 3 illustrates the therapeutic benefits of probiotic on human health.

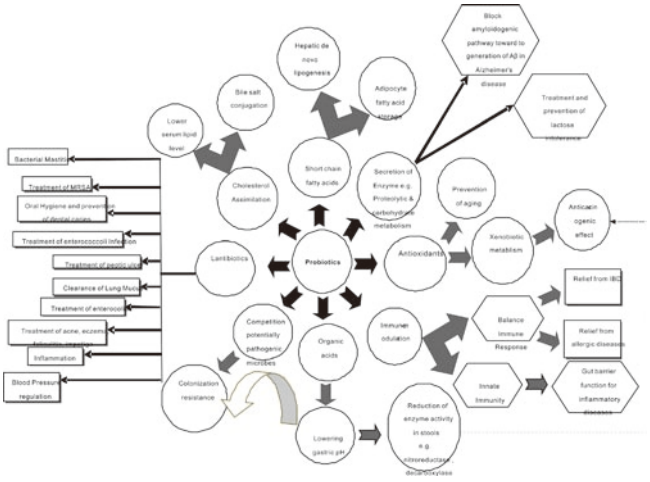

Figure 3. Probiotics for Health.

Mystery of the probiotic mechanism is still unclear in treatment of diseases. Current knowledge of probiotic action is based on the peer reviewed data from randomized, double-blind, placebo-control clinical trials. However the data presented are inconsistent with the specific probiotic strain and variable results are observed because of differences in study design and population[14]. Another concern about this bacteriotherapy, they are not subjected to stringent review by USFDA as like as other pharmaceutical drugs and hence scientific documentation and definite health claims are not expressed by the manufacturers or scientific community accordingly[17].

3. Probiotics: modern age diseases

3.1. Lactose intolerance

Hypolactesia or lactose intolerance may be primary, secondary or congenital. Congenital lactose intolerance is extremely rare; however primary and secondary lactose intolerance may be asymptomatic and results from intestinal resection and gastrectomy respectively[27]. Colonic fermentation of lactose and increase activity of lactase was observed due to effects of *Lactobacillus acidophilus* and *Bifidobacterium longum* on in *in vitro* continuous culture system. *Streptococcous thermophilus* and *Lactobacillus delbruecki* subsp. *Bulgaricus* present in yogurt compensate lactase insufficiency and not only capable of improving lactose digestion but also slowed the gastric transit time[28]. However systematic review indicates that potential therapeutic benefits in this condition are restricted by the strain specificity[29].

3.2. Lipid metabolism

Increase in serum lipid level is one of the several

intermediate risk factors which can lead to cardiovascular disease. Probiotic mediated hypocholesteremic effects were studied in *in vitro* as well as in animal models but data presented were contradictory as the exact mechanism of the probiotic in cholesterol assimilation was not elucidated. The controversy in the results are due to difference in experimental design, amount of fermented milk administered, culture and strain specificity[30,31]. Cholesterol assimilation in resting and growing cell cultures of *Bifodobacterium longum*, *B. infantis, B. breve, B. animalis* and *B. thermophyllum* in presence of acidic (cholic acid pH 5.4) and neutral (Phosphate buffer pH 7.0) pH respectively were observed, may be due to precipitation and bacterial activity[30]. In vitro studies on *Lactobacilli, Bifodobacteria* and *Streptococcous* showed cholesterol reducing property from the media probably because of assimilation or uptake of cholesterol into cellular membrane of these bacteria[32,33]. The hypocholesteremic effect of three strains of lactic acid bacteria, *Lactobacillus acidophilus*, *L. casei* and *B. bifidum* were investigated on rats when administered with soy isoflavones[34]. Authors observed that probiotics had no significant effect on cholesterol reduction However, the lowering of cholesterol level was observed in rats fed with soy yogurt containing the lactic acid bacteria as compared to control group [35]. The role of *Bacillus subtilis* in significant reduction of lipid from muscle and liver tissue was investigated and reported when administered as dietary supplement to the freshwater species of fish Matrinxa (*Brycon amazonicus*)[36].

The effect of two stains of probiotics *L. acidophilus* GG and *Bifidobacterium* Bb-12 were investigated on differential absorption and utilization of polyunsaturated fatty acids (PUFA) in 15 infants with atopic eczema[37]. In randomized, placebo-controlled and double blind study designs this group of authors observed reduction of plasma lipids and α-linolenic acid at neutral pH. Another observation of this group was *Lactobacillus acidophilus* GG did not influence α-linolenic acid proportion whereas *Bifidobacterium* Bb-12 showed the increased proportion of α-linolenic acid in phospholipids. This effect may attribute to the physiological interaction between probiotics and PUFA. Effect of *L. rhamnosus* GG on global serum lipidomic (High dimensional lipid analysis technology) profiles were studied and investigated for changes in inflammatory variables like C-reactive protein (CRP), Tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) in 26 healthy adults[38]. In randomized and crossover trial, 14 healthy individuals with high serum cholesterol level given ordinary yogurt and probiotic yogurt composed of *L. acidophilus* and *B. lactis* for 6 weeks indicates that probiotic Yogurt lowers the serum level significantly as compared to ordinary yogurt [31].

Similar study conducted at Wien University to compare the effect of probiotic Yogurt containing two strains - *L. acidophilus* LA5 and *B. lactis* BB12 and conventional yogurt on lipid profile of 90 healthy women (Permuted block randomized trial design) (Wien University, personal communication, 2009). The study was conducted for 6 weeks and volunteers were divided in 3 parallel groups (30 each). Probiotic yogurt (300 g/d) administered to the first group, second group consumed conventional yogurt (300 g/d) and third group neither consumed probiotic yogurt nor conventional yogurt. The result of the study indicates that there were no statistically significant differences in the lipid profiles of all the three groups. Recent reports suggested that modulation of intestinal micro flora with probiotic intervention; significantly decrease hepatic triglycerides and increases PUFA as compared to plasma lipoproteins[15].

3.3. Oxalate metabolism

Catabolism of amino acid in liver formed decarboxylate anion oxalate. It is also present in dietary sources and food drinks such as tea, coffee, vegetables, fruits and chocolate *etc*. Oxalate forms highly insoluble salts of calcium oxalate (PH 7.0, 0.67 mg dissolve/100 mL of water) [39]. Deposition of calcium oxalate into the urinary calculi results into manifestations like hyperoxaluria, calcium oxalate urolithiosis, kidney and bladder stones and recurrent urinary tract infection in females[39-42]. Different mechanisms of increase urinary oxalate excretion were reviewed (stone formation) Figure 4[43]. Out of these four mechanisms, deficiency of oxalate degrading bacteria i.e. *Oxalobacter formigenes* due to antibiotic sensitivity and correlation with probiotics in its retention in intestine are recent areas of interest for scientists[44-47]. *O. formigenes* is Gram negative anerobe that decorboxlate oxalate to formate and carbon di oxide in intestine through enzyme Oxalyl-CoA decarboxylase and thus limits oxalate absorption in intestine[48].

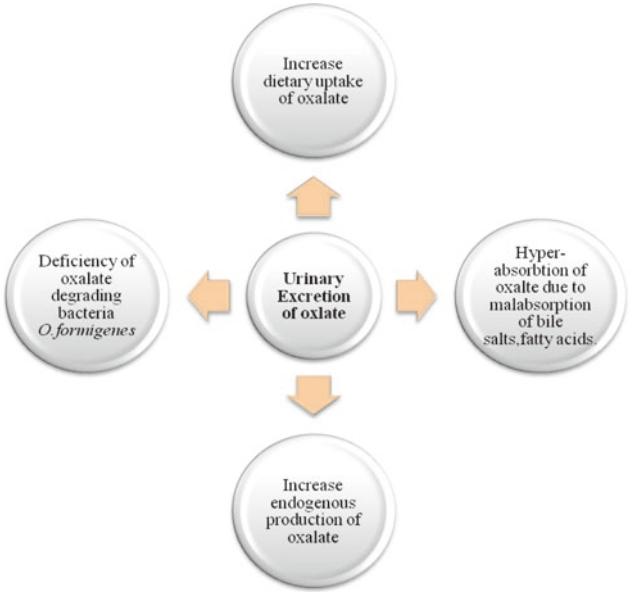

Figure 4. Mechanisms of increase urinary oxalate excretion.

O. formigenes found to be negative risk factor in severe hyperoxaluria induced by administration of ammonium oxalate in rat model for 2 weeks[49]. Authors concluded that the levels of calcium oxalate excreted in urine were significantly and rapidly reduced in rat supplemented with high doses of *O. formigenes* and returned to normal state from chronic hyperoxaluria. Two human volunteers with consistently negative oxalate degrading (OX-) ability for 2 to 4 years when administered 500 mg of *O. formigenes* strain HC1 showed significant oxalate degrading activity and confirmed positive after 9 months by PCR analysis of stool samples[40]. Same authors reported reduced excretion of oxalate immediately after 6 h in 4 human subjects when ingested 500 mg of strain HC1. In another study the oxalate degrading potential of two probiotic strains isolated from human faecal samples, *B. breve* YIT 401 and *Propionibacterium acnes* (ATCC11827) were investigated and identified by PCR and 16S rDNA sequencing[50]. The oxalate degrading activity of Oxalyl-CoA decarboxylase and formyl Co-A transferase in 60 *Lactobacilli* strains belonging to 12 different strains were evaluated [51]. This group of investigators reported that strains of *L. acidophilus* and *L. gasseri* had maximum oxalate degrading activity. Same investigators cloned and sequenced the genes coding for the enzymes Oxalyl-CoA decarboxylase and formyl Co-A transferase and activity of recombinant enzymes were determined by capillary electrophoresis. Potential effects of *Lactobacilli* and *Bifidobacteria* on reduction of urinary oxalate excretion were observed due to existence of Polygenetic relationships between the genes coding for Oxalyl-CoA decarboxylase and formyl Co-A transferase[52]. Based on the case - control study involving 247 adult patients with recurrent calcium oxalate stones and 259 control subjects inverse relationship were observed between the colonization of *O. formigenes* and recurrent calcium oxalate stones (70% reduction in overall risk)[45]. Heterologous expression of enzyme oxalate decarboxylase successfully studied and examined in *L. plantarum* NC 8[46]. The gene responsible for expression of this enzyme (Oxdc) is absent in *Lactobacillus* spp. and amplified by PCR from *Bacillus subtilis* and cloned through shuttle vector pSIP400 by using inducible promoter P_{orfx}. Thus by desirable modification in genomic sequence of lactic acid bacteria, treatment and prevention of enteric hyperoxaluria can be possible. The study conducted on 11 healthy human volunteer including 9 male and 3 female, involved ingestion of probiotic VSL#3 for 4 weeks for assessing the effect of probiotics in oxalate absorption [53]. Results of the study indicated that 4 of 11 individuals observed as oxalate absorbers at the baseline and showed marked reduction in oxalate absorption after VSL#3 ingestion. In one randomized to placebo study design, effect of controlled diet and two probiotic preparations were examined [54]. Authors concluded that there were no influence of tested probiotic on oxalate absorption and the reduction in the urinary excretion of oxalate and was solely due the oxalate restricted diet. Thus influence on oxalate metabolism is characteristic property of specific probiotic strain and not all probiotic bacteria exhibit this property. Recent review on probiotic gives an insight on role of probiotic in oxalate metabolism [55]. Studies those assessed efficacy of oral ingestion of probiotic bacteria with oxalate-degrading capacity provided an intriguing information but results are preliminary, thus this remain challenging area for future research[47].

3.4. Diabetes and obesity

Diabetes and obesity are interdependent to each other, most of the metabolic and physiological pathways are interrupted and disordered because of the one and followed by the latter. Because of their rapid spread in developed as well as developing countries diabetes and obesity are now recognized as epidemics throughout the world. Fat enriched diet due subtle changes in food habits and life style; mainly due to increased lipoproteins like chylomicron, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and intermediate density lipoprotein (IDL) modifies the intestinal microbiota and create rise in metabolic disorders which initiate inflammation, insulin resistance and type II diabetes[8].

The aforementioned lipoproteins enable the transport of cholesterol and triglycerides within blood stream. The absorption of cholesterol from intestine has been reduced by improving the intestinal micro flora by administration of probiotics[56]. Another mechanism by which reduction of cholesterol can be done by probiotics is due to secretion exopolysacchrides which help to adhere to the cell surface and absorb cholesterol. Probiotic bacterial cell wall composed of the various peptiodoglycan and also characterized by different amino acid compositions which helps cholesterol to bind to the bacterial cell surface and subsequent utilization by the bacterial cell[56]. Ingestion of diet containing fructose in high amounts may also leads to increase in insulin resistance and hyperinsulinemia this is caused by the mobilization of fructose in liver that increases lipogenesis and increase synthesis of triglycerol[57]. As it is evident that there is strong relation between the viability and growth of probiotics with the prebiotics such fructo-oligosacchrides (FOS), gluco-oligsaccharides (GOS), xylo-oligosaccharides (XOS) etc respectively, thus by metabolizing these sugars for self growth and sustainability probiotic can minimize the occurrence of insulin resistance[55]. Bacteria are known to play an important role in activating inflammatory pathways. The underlying mechanisms which is responsible for this functioning is the involvement of Toll like receptor (TLR) and Nod like receptor (NLR); a family of membrane pattern recognizing (MPR) receptor responsible for first line of defence i.e. innate immune system[58]. *In vivo* animal modelling illustrated that TNFα continuously released in the adipose tissue during obesity to activate protein kinase C (PKC) and to increase the phosphorylation of the insulin receptor substrate on serine residue such as ser-307 leading to inactivation of insulin signalling molecule and hence to insulin resistance[8,58]. Efficacy of probiotics in reducing serum cholesterol level demonstrated *in vivo* model could subsequently improve insulin resistance.

Use of probiotics during the first six months of life demonstrates decrease in appearance of type 1-diabetes mellitus due to autoantibodies in children with genetic disorder for type 1-diabetes mellitus[59]. The recent study proposed the relevance of balance intestinal microbiota, immunomodulation, obesity and diabetic complications[60]. Thus at the concluding remarks strategies to control metabolic diseases must be developed by modification of intestinal microflora leading to maintenance of blood glucose level and reduces the contingency of obesity.

3.5. Oral diseases

Diet with inadequate nutrition has major influence on oral as well as normal health of individual. Introduction of allochthonous *Lactobacilli* are associated with the consumption of fermented and non fermented food and thus enter into the metabolic system through oral cavity [61]. The probiotic mechanism of action for maintaining the oral health is subdivided into three stages attachment, adhesion and colonization. *L. rhamnosus* GG and *L. casei* have great potential to hamper the growth of dental plaque forming Streptococci group containing *S.sanguinis*, *S. mutans*, *S. salivarius* and *S. mitior* [62]. Similarly, in two different studies it was observed that regular intake of *L. reuteri* and *Bifidobacterium* in yoghurt resulted in significant reduction of *S. mutans* population in oral cavity [63]. Antagonistic interactions (colonisation resistance) between probiotics and pathogens have significant benefits in chronic periodontitis and gingivitis[64]. The level of pro-inflammatory cytokines in gingival crevice fluid (GCF) found to be significantly reduced when individuals with periodontitis and gingivitis were administered with chewing gum containing two strains *L. reuteri* ATCC 55730 and ATCC PTA 5289[65]. The inflammation in these conditions is alkaline; LAB could change the milieu by production of lactic acid. However strain specific acidogenicity cannot be overlooked. The therapeutic effect of Weissella cibaria found to be clinically significant in prevention and treatment of Fusobacterium nucleatum induced halitosis[66]. The geriatric and immune-compromised patients found to be more susceptible to mouth infection caused by *C. albicans*. Although probiotics have appealing therapeutic benefits in maintaining the health, there are key challenges towards the wide spread of adaptation and usage of prebiotics (Figure 5)[5].

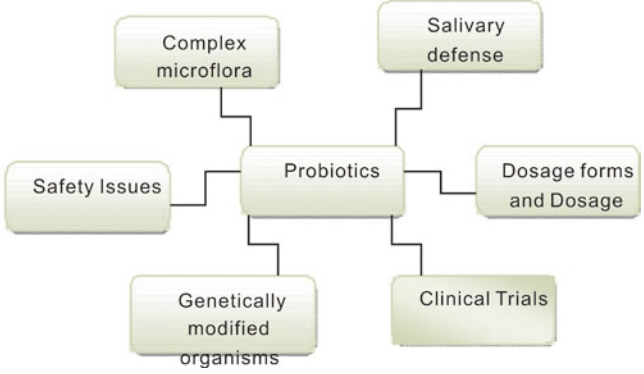

Figure 5. Challenges for therapeutic use of probiotic in oral diseases.

3.6. Allergic disorders

The world wide rise in atopic diseases (eczema, food allergy, hay fever and asthma) is generally related to the two environmental factors- hygiene and nutrition. The diets of ancient people were enriched with the plethora of useful microbiota as the methods of food preservations were of natural fermentation or by sun drying[10]. The imbalance between internal gut microflora, the primed barrier for food derived antigens and microorganisms from external environment leads to fundamental failure of underlying immune regulation. The lack of allergic diseases in early infancy leads to increase in atopic diseases in later stages of life whereas infants with higher number of siblings, living in rural areas or in contact with live stock are at lower risk for developing atopy[67]. The proposed mechanisms of probiotics in prevention of allergic disorders may attribute to maturation of immune system, enhance production of anti-inflammatory cytokines e.g. interleukin-10 (IL10) and transforming growth factor β (TGF β) by blood leucocytes, enhanced interleukin-2 (IL2) response and increase Th1/Th2 ratio, increase immunoglobulin A (IgA) production and up regulation of mucin-encoding genes in host intestinal epithelium and finally enhancement of intestinal epithelial repair factor through TLR activation[68]. Data on clinical studies were reviewed systematically and meta-analysis were done on subject of probiotics and allergic disorders by[69,70]. The results of the clinical trials were inconsistent. Some trials showed reduction in eczema but no significant effect had been demonstrated on other allergic disorders after administration of probiotics. In summary, understanding of linked with lifestyle, geographic factors, socio-economic status and heterogeneity in allergic disorders prevalence throughout the world needs immediate focus to resolve the complex and unanswered questions[71].

3.7. Mental disorders

Functional foods have potential therapeutic benefits on mental health. Recently, European commission report (2010) quoted optimal mental state, mental performance and behavioral changes can be significantly influence by intake of functional food in regular diet. Vitamins of B-complex group have significant effect on the cognitive performance and maintain mental health in older people. Ethanol precipitate from fermented milk with *L. helveticus* IDCC3801 when administered to the scopalamine (1 mg/kg dissolved in normal saline) induced memory impaired rats for 14 consecutive days suggests that there were significant improvement of amyloid protein precursor (APP) metabolism and memory deficit[72]. Same group of authors reported strong decrease in wild type APP and β - secretaes enzyme level in cell based assay when treated with the ethanol precipitate from fermented milk cultured with *L. helveticus* IDCC3801. In a pilot study, 39 patients suffering from chronic fatigue syndrome randomly assigned to receive daily supplements of either *L. casei* strain shirota (24 billion

CFU) or placebo for 2 months shown significant reduction of anxiety symptoms compared to placebo group[73]. Recent study highlighted the role of gut microbiota in bidirectional communication of the gut – brain axis and suggested that certain probiotic strains proved to be therapeutically beneficial in the treatment of the anxiety and depression[74]. Authors reported, after chronic treatment with *L. rhamnosus* JB-1 there were region dependent reduction in GABAB1b mRNA expression and increase expression of GABAA α 2 in brain of mice (n=36). Thus study indicates *L. rhamnosus* (JB-1) reduced stress induced corticosterone, anxiety and depression related behaviour in mice. Administration of formulation containing two probiotic strains of *L. helveticus* R0052 and *B. longum* R0175 demonstrated the decrease of the sub-scores of somatization, depression and anger-hostility spheres in 25 healthy human volunteer[75]. Although these study results showed promising role of certain probiotic strain on single neuronal signaling pathway, role of other neurotransmitter and neuropeptide systems those are related to the anxiety and psychiatric disorders cannot be rule out.

3.8. Ageing

Development of science and medicine led to increase in life expectancy and substantial increase in elderly population[76]. Strain specific implication of in therapeutic regimen for modulation of composition and specific health benefit are crucial to maximize the potential benefit of probiotics in treatment of particular disease group or those at dynamic life stages – young and older adults[77]. The marked shift in age demographics is due to rapidly growing elderly subpopulations in many countries accompanied by increase in infectious and age- related diseases[78]. Intestinal mucosal response is compromised in elderly animal and human. Because of this deficient immune response efficacy of mucosal vaccines reduces and increase the incidences of infectious diseases in elderly[78]. With the increase in age the levels of lactase and β -galactosidase decreases, thus there are more incidences of lactose indigestion in elderly. Probiotic strains such as *Lactobacillus GG, L. reuteri, L. acidophilus, L. johnsonii, B. bifidum, S. faecium, S. boulardii etc* reported to have beneficial therapeutic effect in prevention and treatment of microbial diseases in humans[79]. While the role of probiotic in antiaging is intriguing extensive research needs to go forward for more detail and clearly evidence-based approach.

4. Probiotics

4.1. Formulation prospective

Delivery of probiotics for mitigation or treatment of diseases in viable form without altering the sensory characteristics of the formulation is challenging. Currently intake of probiotics in functional foods such as dairy and non dairy products is most popular. The dairy products include fermented milk, curd; yoghurts, flavoured milk, butter milk etc and non dairy products such as chocolates, juice, and biscuits are more demanding among the consumers of all age groups. Because of proven health benefits and cost effective sources pharmaceutical companies are attracted towards the probiotics. Probiotic are now available as neutraceuticals in both prescribed and over-the-counter (OTC) drug ranges. The available marketed dosages forms include capsules, tablets, powders (Sachets), suppositories and pessaries.

4.2. Capsules

The viability of probiotic cells is important in technological point of view to maintain formulation effective during the shelf life. Studies showed survival of probiotic cells improved when encapsulated with polymers (microencapsulation) or filled in empty hard gelatin capsules[80]. Out of these two methods of microencapsulation (ME) is most widely studied for delivering the bioactive compounds. ME is the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged periods of time under the influence of specific conditions[81]. At least eight ME methods have been applied to probiotics. These methods along with their characteristics, benefits and problems are discussed elsewhere[82].

In broad sense the ME is advantageous over the other formulation methods with aid of naturally occurring polymers such as such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin and pectin so that one can stabilize the core material, control the oxidation, provide the time controlled release medication, mask the odours, flavours or colours and protect the cells when pass through gastrointestinal tract[83]. Although future prospective of ME in relation to probiotics seems to be bright two basic problems in ME are size of probiotics (1-5 μ in diameter) and most important they must kept alive after processing[84]. Non uniformity in ME and evaluation of the process parameters for optimizing the encapsulation were discussed elsewhere[85]. More research is still needed to overcome the challenges in ME technology (Figure 6).

Figure 6. Challenges in microencapsulation.

Table 1

Country specific regulatory requirements for probiotics.

Sr. No.	Country	Regulatory act	Comments
1.	China	The health food control act, general standard for health food and china food safety law	Require assessment for safety of food and effectiveness of the functions for health. No registration of probiotics.
2.	Korea	The Korean health/ functional food act of 2004	Foods are classified in two categories: 1.Generic food 2.Health functional food: – food manufactured or processed in the form of tablets, capsule, powder, granule, liquid, pill, drinks or foods with ingredients or components that possess the functionality useful for the human body.
3.	Hong Kong	Food and drug regulation, requirement for nutrition labelling and nutrition claim, effective in 2008	–
4.	Taiwan	The health food control act, effective in 1999	Foods capable to solicit health claims are called health food. Health food denotes the foods with health care effect, decrease harm and risk of diseases.
5.	India	Prevention of food– adulteration act and rules (PFA) and drug and cosmetics law	PFA does not regulate any functional or probiotic dairy products. ICMR established guidelines for probiotics." guidelines for probiotic functional foods".
6.	Thailand	Guidelines for probiotics (2008). food law (1979) and health ministerial announcements (2000)	Probiotic foods are under the regulation of food law (1979) and health ministerial announcements (2000)
7.	Vietnam	Ordinance on food hygiene and safety 2003 and guideline for the management of functional food products 2004	Functional foods are categories under: 1.Nutritious food 2.Supplementary food 3.Health defence food 4.Medicated nutritious food
8.	Singapore	–	No regulation system for functional food and probiotics
9.	Malaysia	The food act of 1983 and regulations	Ministry of health currently working on developing new regulation on probiotics
10.	Philippines	Philippines national standard on fermented milk, the guidelines for use of nutrition and health claims, 2007 and guidelines on probiotics, 2004	Guidelines are based on codex guideline for use of nutrition and health claims.
11.	Indonesia	The regulation for functional food , 2005	Government allowed probiotic fermented food according to number of available cells under this regulation.
12.	USA	1.The 1990 nutrition labelling and education act 2.Dietary supplement health and education act 1994 3.The 1997 food and drug administration modernization act 4.The 2003 FDA consumer health information for better nutrition initiative. 5.Guidelines for industry on complementary and alternative medicine products and their regulation by the FDA, 2006 6.Guidance for industrial evidence– based review system for the scientific evaluation of health claim:2009	The 1990 nutrition labelling and education act
13.	European Community Legislation	Regulation (EC) no. 1424/2006 of the European parliament and of the council of 20 December 2006	Food containing probiotics fall under general group of functional foods.
14.	Canada	1.Food and drug act 2003 2.Food and drug regulation 2009 3.Guidance document for preparing a submission for food health claims 2009 4.Subsection 5 (1) of the food and drug act 2003 5.Guidance document–the use of probiotic microorganisms in food 2009.	Food and drug act 2003
15.	Australia and New Zealand	1.Australia and New Zealand food standard code 2.Nutrition, health and related claims	Australia and New Zealand food standard code
16.	Mexico	Regulation for sanitation control act of product and service (1999)	The definition of health claim mentioned in article 174 of this law.
17.	Argentina	Codigo Alimentino Argentino (2006)	No formal definition of health claims
18.	Brazil	Resolution no. 18 and 19 of 30 April 1999	The resolution states functional claim and usefulness for health

Filling freeze dried powder in hard gelatin capsule is other way of delivering the probiotics to its site of action in viable form. Appropriate combination of cryoprotective agents, bioactive components, antioxidative agents, vitamins and amino acids may maintain probiotic concentration in health benefit conferring amount (106-109)[86].

Enterococcus faecium M74 pellets coated with different concentration of shellac (naturally occurring polymer) were formulated and studied for release characteristics[87]. Examination of the survival and stability of probiotic properties of three vaginal lactobacilli after freeze drying with addition of individual and combined excipients showed that hard gelatin capsule provide the protective covering around the probiotic bacteria and prevent direct exposure to gastric acid by forming gelatinous mass[86]. Suitable selection of excipients enhances the viability of probiotic cells during storage.

4.3. Tablets

Study results on viability of *L. fermentum* 2311 showed significant improvement when entrapped in hydroxyl propyl methyl cellulose (HPMC) phthalate as tablet forming matrix [88]. Tablets are comparatively more advantageous than other dosage forms; they are unit dosage forms hence high accuracy in dosages can be achieved, easy to scale up, have low moisture content hence less water activity shown by probiotic bacteria and improved stability during the shelf life, protect probiotics from degradation by gastric juices and deliver them to intestine and more importantly tablets have good patient acceptance. Use of compression coating in viability of *L. acidophilus* ATCC 4356 in developing the colon specific delivery system reported elsewhere[89]. However selection of suitable coating material is important for achieving the effective delivery and protection of the probiotic cells. Recently gel forming polymers such as gelatin, pectin, guar gum, HPMC etc received more importance than calcium alginate because later could not improve the acid tolerance and allows diffusion of hydrogen ions into the cells because of porous nature of calcium alginate[85,89].

4.4. Powders

Probiotic powders comprise the balance blend of multiple strains of probiotic species and frequently used as supplements. To increase the bulk of the formulation prebiotics are added into the powder for restoring the viability of probiotic cells. Recent review discussed the problems related to viability of probiotic strains composed in commercially available products in powdered form and suggested the method for enumeration of viable cells and analyzed the reasons for variations in plate count results[84]. Commercially powders are available in three forms; dried cultures, free flowing powders and powders for unit dosage forms like capsules and tablets[84,86,89]. Probiotic cells in powder undergo different processing stresses. Thawing, freeze drying, homogenization, pH changes, oxygen contents, compositional compatibilities, moisture content (water activity aW > 0.3) and temperature variations compromise product stability and bring viability of cells in question[90]. Advances in analytical tools such as Flow Cytometry, PCR etc explored the multiple dimensions of cell survivability and distinguished cells into sub lethally damage but culturable and sustainable/or viable but not culturable[91]. These data may suggest the clinical use of probiotic powder as additive in oral rehydration solution (ORS) for treatment or prevention of diarrhea in children, specific recommendation for the use of probiotic awaited till the further human trials.

4.5. Suppository and pessaries

Increasing use of antibiotics leads resistance to pathogenic microorganisms of high potency drugs and repeated use decrease the efficacy in treatment or prophylaxis of urogenital disease[92]. Emergence of probiotic as Comparative alternate medicines (CAM) to traditional medication owe to their unique properties of producing bioactive compounds (probioactive) such as hydrogen peroxide, bacteriocin, lactic acid, biosurfactants, short chain fatty acids, which imparts inhibitory action on uropathogens[93]. Topical application of freeze dried *L. acidophilus* suppository lowers the recurrence of bacterial vaginosis and vulvogenital candidiasis in patients as compared to other dosage forms such as powders, tablets or capsules[94]. Pilot study conducted on 9 female patients who had experienced 2 episodes of UTI and recurrence for 2 years demonstrated that administration of vaginal suppository of *L. crispatus* GAI 98332 reduced the recurrence of UTI because of its adhesion to vaginal epithelial cells and hydrogen peroxide producing ability[92]. Data describing the efficiency of probiotic in treatment of UTI are available; still the role of vaginal suppository and pessaries on recurrent UTI is uncertain.

5. Regulation

Probiotic effects are strain specific and not a single strain possesses all the beneficial characteristics required for the effective treatment[4]. Hence consortia of different strains are recommended to get the synergistic effects. For selection of non human origin species, the *in vitro* assays followed by *in vivo* assays are carried out and called as generally regarded as safe (GRAS)[95]. According to Indian Council of Medical Research (ICMR, 2011) guidelines for newly identified or less reported strain with no history of safe human use and strains not included in the GRAS status preclinical evaluation should be done for demonstration of safety. Toxicity (acute, sub acute and chronic) studies are also recommended for all new strains when ingested in large amounts. The current market for probiotics is difficult to assess because proprietary information available for probiotic products is subtle[96]. The usual approach for safety of probiotics is presumption of long history of use in fermented dairy

products. Food and Drug Administration (FDA) authority in United States compiled a list of microorganisms considered as probiotic on its "Partial List of Microorganisms and Microbial-Derived Ingredients that are Used in Foods" (http://vm.cfsan.fda.gov/~dms/). Each country has different regulatory system. Table 1 represents the country wise available regulatory act and promulgation for probiotic products in stated guidelines.

6. Conclusion

In conclusion, combined skills of the microbiologist, food technologist and clinician are necessary to sustain effect of probiotics. The role of probiotic organisms as alternative or complementary therapy in combating a large number of disorders can be achieved with balance and healthy life style as well as clean external environmental conditions. Assessment of postulated or partially claimed therapeutic benefits with consistent results and appropriately designed and adequate sample sized human study with strict alignment to guidelines are obligatory. For now, on an everyday basis millions of people are dying or suffering in region of the world where no properly documented probiotics are available. It is hoped that more detailed research will be conducted regarding the efficacy of probiotics so that clinically well documented and simplest formulation will be developed and can be regarded as effective by everyone. With validated results strong market will be formed and expanded in near future.

Conflict of interest statement

We declare that we have no conflict of interest.

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