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Probiotics: Selection criteria, safety and role in health and disease

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Abstract: In past few years, global neutraceutical market has witnessed an increased recognition to the probiotic supplements. Consumers are becoming more aware about food supplements which could enhance the immunity and reduce the medical incidences in their life. The probiotic approach of modulating the gut flora to re-establish the normal health has gained much creditability among modern therapeutic strategies because probiotics first colonize in our gut and perform their antibacterial, competitive and anti-inflammatory action during their inhabitation period into the host. The ongoing developments in new genomic, proteomic and metabolomic techniques have reduced the burden of researchers for identification and characterization of novel probiotic strains. Likewise, the temptation to share the remarkable benefit from current nutraceutical market has also significantly increased the misleading claims on probiotic foods. Current review comprehends and harmonizes the customary information on probiotic isolation, identification, their potential role in human health along with current guidelines and regulations.

Abbreviations: BATH, Bacterial adherence to hydrocarbons; **BSH,** Bile salt hydrolases; **EPS**, Exo-polysaccharides; **GIT,** Gastrointestinal tract; **GRAS,** Generally recognized as safe; **IBD**, Inflammatory bowel disease; **IDF**, International diabetes federation; **LAB,** Lactic acid bacteria; **MATH,** Microbial adherence to hydrocarbons; **MRS**, de-Man Rogosa Sharpe; **ROS**, Reactive oxygen species; **UC**, Ulcerative colitis; **CD**, Crohn's disease.

Keywords: Lactic acid bacteria, Probiotic, *Lactobacillus*, *Bifidobacteria*, Bio-therapeutic, Immunomodulation

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Probiotics are the living microorganisms which when ingested in adequate amounts confer some health benefits to the host (FAO/WHO, 2002). *Lactobacillus* and *Bifidobacteria* sp. are among the most frequently used and established probiotic strains. However, some *Streptococcus*, non-pathogenic *E. coli* and yeast strains have also been claimed as probiotic organisms. Foods harbouring LABs naturally, or through fortification are reported to provide several health benefits. The demand of probiotic based functional foods is growing rapidly due to increased consumer awareness about the impact of food on health. Modern lifestyle and changed dietary habits has established a necessity for the functional foods which can be used as therapeutic regime against lifestyle related health complications. Probiotics could improve human health through different modes of actions including restoration of host normal microflora, 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 (Fong et al. 2015; Pace et al. 2015; Stenman et al. 2015; Tanaka et al. 2015). 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 probiotic for health benefits has boomed the

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global food market in past few years (Di Cerbo and Palmieri 2015). Ongoing efforts in metagenomics research have generated numerous new and interesting working hypotheses for manipulating microbiota for maintaining and restoring health. Results from such 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 (Papadimitriou et al. 2015). 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. So far, appropriate guidelines for study of probiotic attribute and safety evaluation have been proposed for the safeguard of the consumers; but challenges are persisting for the identification and characterization of probiotics according to these guidelines. This review outlines the potential strategies used for the selection and evaluation of candidate probiotic strains. Also, efforts have been laid to elucidate the potential applications of probiotics in different clinical scenarios and their possible mechanism of action.

Source of Probiotics

Microbial strains serving as candidate probiotic are most commonly isolated from traditional fermented milk products. However, the isolation source varies between studies and regions and do have impact over functionality of isolates. Curd being consumed globally serves as the most preferred source. Other fermented milk products including lassi, cheese(s) etc. are explored depending on their availability (Figure 1). Table 1 enlists different regional/local fermented products that are explored for isolation of potential probiotic strains. Shelf life and functional aspects of fruits and vegetables are preserved and enhanced for long time by microbial fermentation. Fermented fruits and vegetables (Table 1) have a long history of use in human diet and are also associated with the several social aspects of different communities. Usually fermentation is carried out by the natural microflora of raw food products and progresses with succession by different microbes. Lactic acid fermentation increases shelf life, enhances nutritive value and flavours, and reduces toxicity (Swain et al. 2014). Fermented fruits and vegetables can be used as a potential source of probiotics as they include wide spectra of LABs such as *Lactobacillus acidophilus*, *Lactobacillus brevis, Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Leuconostoc fallax*, *Leuconostoc mesenteroides* etc. (Swain et al. 2014). Microbial composition varies between regions, environmental conditions and type of fermentation. Although milk, food and vegetables are explored a lot for isolation of probiotic strains, researchers believe that strains with human origin may survive better during human gastric transit compared to those of non-human origin (Del Piano et al. 2006; Ranadheera et al. 2014). Keeping this in mind, healthy human infant's faecal sample, healthy adult faecal samples and human breast milk samples are explored for selection of strains with rich probiotic potential.

Table 1. List of fermented milk, fruits and vegetable products commonly explored for probiotic isolation

Fermented products	Source	Country of origin
Fermented dairy products		
Yoghurt	Cow, buffalo or mixed milk	
Tarag (Mongolian yoghurt)	Cow milk	Mongolia
Curd (Indian dahi)	Cow, buffalo or mixed milk	India
Lassi	Cow, buffalo or mixed milk	India
Kefir	Cow or Goat milk	
Kumiss	Mare or cow milk	
Rabdi	Cow or buffalo milk	India
Cheese	Cow, buffalo or mixed milk	
Fermented fruits and vegetables		
Sauerkraut	Cabbage	Europe, USA
Kimchi	Cabbage	Korea
Gundruk	Mustard, radish and cauliflower leaves	Nepal
Khalpi		
Sinki	Radish	India, Nepal, Bhutan
Other		
Sourdough	Wheat and rye flour	Egypt, USA
Dosa, Idli, Dhokla	Rice, legume	India
Miso	Rice, Soya beans	Japan

Identification and characterization of isolates

The identity of microorganism is usually ascertained primarily before evaluating them for their probiotic attributes. Microbes are subjected to battery of morphological, bio-chemical and molecular tests to assign them to one of the target group i.e. lactobacilli or bifidobacteria. Lactobacilli are identified as gram positive, catalase negative rods; bifidobacteria are anaerobic, gram positive, catalase negative rods with

Table 2. Representative primer sequences for molecular identification of common probiotic strains

Organism	Name of primers	Primer sequence	Product size (bp)	Reference
Lactobacillus	LbLMA1 R-161	CTCAAAACTAAACAAAGTTTC CTTGTACACACCGCCCGTCA	250	Dubernet et al. 2002
Bifidobacterium	Bif164 Bif662	GGGTGGTAATGCCGGATG CCACCGTTACACCGGGAA	523	Kok et al. 1996

characteristic bifid ends. Identity of isolates is ascertained at molecular level using genus and species specific PCR, serving as gold standard for identification (Figure 1). Representative sets of primers have been documented in Table 2. Closely related bacterial isolates from same species are further discriminated to sub species and strain level using newer and established techniques, including genetic fingerprinting, gene sequencing, oligonucleotide probes, pulse field gel electrophoresis etc. Additional molecular methods, such as denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis and fluorescence *in situ* hybridisation, are employed to identify and characterise probiotics. Also biochemical characterization on basis of carbohydrate metabolism is employed for such discrimination (Gomes and Malcata 1999). The ability to examine fully sequenced genomes has accelerated the application of genetic approaches to elucidate the functional roles of probiotics. Currently whole genome sequences of majority of representative probiotic strains are available for quick reference (Altermann et al. 2005; Kankainen et al. 2009; Garrigues et al. 2010).

Criteria for selection of presumptive probiotic strains

Safe transit through stomach and survival and colonization in the intestinal tract are critical and foremost parameters to qualify as a potential candidate for further screening for probiotic attributes (Kotzamanidis et al. 2010) and associated health benefits (Figure 1). Beneficial microbes to be ingested as probiotic strain has to encounter various stress factors during mobility and establishment in the gastro-intestinal tract. Probiotic bacteria must retain viability during its interaction with stomach acid, bile and high osmolarity in the small intestine (Franz and Holzapfel 2011). Gastric juice is a complex mixture of water, inorganic ions, hydrochloric acid (HCL), other inorganic ions, pepsinogen, mucus, polypeptides and intrinsic factors (Farrar and Bower 1967). HCL, secreted from stomach parietal cells contributes to the low pH range of human gastric juice, which ranges from 1.5 to 3.5 (Marieb and Hoehn 2010). However it varies during different stages i.e. empty stomach, with meal and after meal (Fordtran and Walsh 1973). Keeping this in view, a bacterial strain to serve as potential probiotic should survive the pH stress of gastric acid. Following this criteria, acid tolerance of isolates is determined at different pH (1.5 to 4.5), for different time intervals (0 to 3 hr) simulating human gastric passage. Strains capable of tolerating the pH stress for studied period are termed as acid tolerant. Bile is produced by liver hepatocytes and released in the small intestine mediated through gall bladder in response to meal stimulus. It is a complex mixture of both organic (conjugated bile acids, termed as bile salts; glycine etc.) and inorganic molecules conferring antibacterial activity, primarily

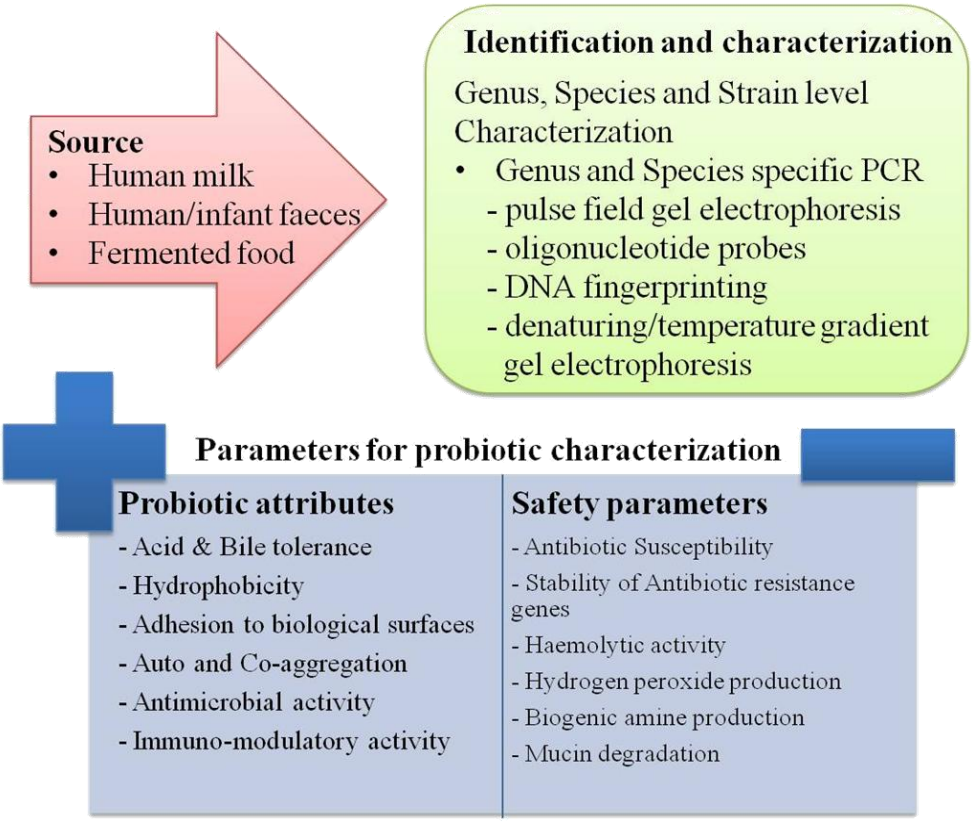

Figure 1. Possible sources, Identification strategies and parameters for probiotic characterization

through the dissolution of bacterial membranes (Sung et al. 1993; Hofmann and Eckmann 2006). Microbes are subjected to varying concentrations (0.5 to 2.0 %) of bile salts (Ox bile) with different interaction time (0 to 3 hr). Isolates showing resistance are recognized as bile tolerant. Tolerance towards bile acids is attributed to presence of bile salt hydrolases (BSH), product of *bsh* gene of bacteria (Begley et al. 2006). Presence and expression of *bsh* gene has been linked to bile tolerance and bile detoxification and is being targeted as one of the criteria for probiotic strain selection (Patel et al. 2010). BSH also plays an important role in lowering of serum cholesterol (El-Shafie et al. 2009) and is reported to be variable from strain to strain. After safe transit to the small intestine, probiotic strains need to adhere to the intestinal lining, determined by multiple factors viz. Cell wall hydrophobicity; cell adhesion potential; auto-aggregation. Adhesion to the intestinal epithelial cell lining is one of the most important characteristics of lactobacilli as well as one of the main criteria for selecting probiotic strains (Ouwehand et al. 1999). Of all, bacterial cell wall hydrophobicity plays an important role in bacterial adhesion. Hydrophobic interaction forms immediately after contact and can become stronger over time due to removal of water between two surfaces (Younes et al. 2012). Stability of this interaction relies upon composition and distribution of hydrophobic surface components of bacteria. Bacterial hydrophobicity is usually determined by BATH or MATH (bacterial/ microbial adherence to hydrocarbons) technique proposed by Rosenberg (1984) and Geertsema-Doornbusch and co-workers (1993) respectively. Strains showing higher adhesion towards hydrocarbons are termed as hydrophobic and those giving poor adhesion are recognized as hydrophilic. Higher bacterial hydrophobicity can be directly coo-related to their stronger adherence capability (Pan et al. 2006). Auto-aggregation potential of bacterial cells also plays an important role in bacterial adhesion to intestinal cells (Dunne et al. 2001). It tells about the activity of bacterial cells to interact with them in a non-specific way, which is pre-requisite for GIT colonization (del Re et al. 2000). Cell auto-aggregation can be determined by following procedure of Tomas et al. (2005). Adhesion of probiotic strain to intestinal cells can also be determined in vitro using intestinal cell line models. Usually Caco-2 and HT-29 cells are co-incubated with the test strains followed by culture based screening for bound cells (Laparra and Sanz 2009). Adherence to intestinal cells provides more interaction/ residence time and hence possibility of conferring beneficial effects is augmented; although non-colonizing bacteria have been reported to elicit health promoting effects (FAO/WHO 2006; McNulty et al. 2011).

Beneficial effects of probiotic strains may be conferred either due to their interaction with other microbes (anti-microbial activity); due to their metabolic activity (Anti-oxidative activity, EPS production etc.) or due to their role in immuno-modulation through various signalling pathways (Anti-cancerous; Anti-allergic etc.). Anti-microbial activity is considered to be a significant functional criterion for competitively inhibiting (pathogen exclusion) the pathogenic intestinal microflora through production of organic acids, hydrogen peroxide, bacteriocins etc. rendering the host safe. Antagonistic activity of different bacterial preparations is determined against common enteric pathogens (*E. coli*; *Salmonella* sp. etc.) following standard well diffusion assay. LABs are also known to possess strong anti-oxidative activity, and are able to decrease the risk of accumulation of reactive oxygen species (ROS) (Achuthan et al. 2012). Oxidative stress is caused by an imbalance between ROS or free radical production and body antioxidant defense, 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.). Anti-oxidative potential of candidate probiotic can be determined by evaluating their resistance towards ROS (hydrogen peroxide, hydroxyl ions and superoxide radicals) (Kullisaar et al. 2002); Superoxide dismutase activity (Achuthan et al. 2012) and Total anti-oxidative activity (Kullisaar et al. 2002). Presence of S-layer proteins, exo-polysaccharides (EPS) and other cellular envelope components plays an important role in bacteria-host interaction. Surface carbohydrates composing EPS and S layers have been identified as adhesion factors (Lebeer et al. 2010; Sanchez et al. 2012). Besides promoting adhesion, surface components play an important role in protection against harsh environment and also in exhibiting probiotic properties via. acting as colonization factor; pathogen displacement; modulating immune response; abolishing cytopathic effects of bacterial toxins and modification of composition and metabolic activity of intestinal microbiota (Sanchez et al. 2012). The immune-modulatory activity of probiotics relies upon interaction of their cell surface molecules and few secretory components with host cell receptors. However, there are not clearly established bacterial phenotypic markers which could be used for the prediction of the immuno-modulatory capacity of lactic acid bacteria (Voltan et al. 2007). Immune-modulating potential is usually determined by determining the effects of bacterial cell interaction over inflammatory (IL-6, TNF-a etc.) and anti-inflammatory (IL-10) markers in different cell line and *in vivo* models.

Although most of *in vitro* tests described above are laborious and also outdated, they are still applied for cost and ethical reasons. The application of new DNA, protein and metabolome based technologies is growing fast and most probably would replace traditional screening methods (Papadimitriou et al. 2015). Because *in vitro* experiments do not mimic the real environment of gastrointestinal tract, it is suggested

that health benefits exhibited by a probiotic strain in laboratory condition should also be substantiated in an animal model followed by human trials. As per FAO/WHO guidelines, health benefits of proposed probiotic candidates should be confirmed in animal models, before allotting them probiotic status. Omics technologies may also turn out to be very effective in the follow-up analysis of probiotic candidate strains resulting from *in vitro* and/or *in vivo* screening with current methodologies. The new research approaches will also facilitate the analysis and description of functional mechanisms, facilitating the construction of health claim or pharmaceutical dossiers (Papadimitriou et al. 2015).

Safety of probiotics

Lactic acid bacterial strains isolated from either of the above discussed sources are generally recognized as safe (GRAS) and are being extensively exploited for their health benefits (Cross et al. 2002; Puertollano et al. 2008). As per FAO/WHO (2001), multiple reports supports safety and efficacy of specific strains of probiotics, but these benefits cannot be extrapolated to other strains without experimentation. A full inventory of the risks is to be determined in different populations, at different doses and using different delivery modes and matrices (Papadimitriou et al. 2015). Strains to be used as probiotic should be screened for safety parameters including antibiotic susceptibility, presence of antibiotic resistance genes, haemolytic activity, hydrogen peroxide production, biogenic amine production, mucin degradation etc. (Figure 1).

Probiotics dwells in GI tract and interact with the commensal flora therein. The presence of transferable antibiotic resistant genes in probiotic strain may cause serious consequences by transferring the resistance to the intestinal pathogens. Thereby, from the safety concern of consumers, it is necessary to assess the stability or transferability of antibiotic resistance of probiotic strains. Presence of genes conferring antibiotic resistance can be analyzed by following standard PCR protocols. Total genomic DNA and plasmid DNA is targeted for presence or absence of target genes. However, to generate a clear picture, it is imperative to determine the expression of target genes by RTqPCR. Genome sequencing further allows quick detection and elimination of strains that pose a potential risk, through the presence of antibiotic resistance or virulence genes. As stated earlier, production of antimicrobial compounds is considered as an important probiotic feature, conferring protection against pathogens. However, extremely potent antimicrobial activity could disrupt the normal intestinal biota with potentially detrimental consequences (Cleusix et al. 2007). Evaluating the capacity of an organism to produce these substances is an important part of characterizing its safety for human use (Sulemankhil et al. 2012). Hydrogen peroxide production is determined by streaking isolates over MRS agar plates supplemented with tetra-methyl-benzidine (0.25 mg/ml) and horseradish peroxidase (0.01 mg/ml), as previously described by Eschenbach et al. (1989). Hydrogen peroxide production is visualized by colony coloration, with white colonies indicating no production, pale blue colonies indicating poor production, and dark blue colonies indicating high production. H_2O_2 production ability of LABs varies under aerated and non-aerated conditions (Batdorj et al. 2007). Although H_2O_2 production is also taken as a predictor of long term colonization (Vallor et al. 2001), and has been documented to impart potent antimicrobial activities (Pridmore et al. 2008); but strains to be explored for functional food development should not be among high H_2O_2 producers. Another aspect considered is the production of biogenic amines e.g. Histamine (His) and tyramine (Tym), having toxicological effects. Histamine has been reported as the causative agent of histamine intoxication, while tyramine has been reported to affect the hypertensive crisis in the individuals being administrated monoamine oxidase inhibitors (Santos 1996; Suzzi and Gardini 2003; Zaman et al. 2009). Very active histamine and tyramine formation has been reported for several groups of LAB including starter cultures (Bover-Cid et al. 1999; Roig-Sague´s et al. 1997). Komprda and co-workers (2008) reported the potential of tyramine formation by some probiotic strains of *Lactobacillus*, *Bifidobacteria* and *Enterococcus* intended for processing of fermented foods. Production of biogenic amines by lactic acid bacteria is not a desirable property and is being taken as potential health risk to consumers (Ammor and Mayo 2007). Keeping this in account, only amine-negative isolates are suitable when selecting strains as probiotics, dietary adjuncts, and starter cultures (Belicova et al. 2013). A study carried out by Priyadarshani and Rakshit (2011) confirmed that biogenic amine formation is strain dependent and not related to the species. Therefore, careful screening for amino acid decarboxylase activity is recommended before selecting LABs as appropriate starter or probiotic strains in food and dairy industry. Few reports focusing on concerns regarding safety of probiotic strains indicates possibility of translocation of the probiotic strain from intestine to blood and other tissues (De Groote et al. 2005; Land et al. 2005). Bacterial translocation is mediated through invasion of bacteria through the intestinal cell wall. Mucin layer on the surface of the intestinal wall is very important to prevent bacterial translocation (Gork et al. 1999). Hence, mucin degradation activity is being used as an index of safety of probiotic strains (Zhou et al. 2001; Fernandez et al. 2005).

Probiotics in health and disease management

One of the best-demonstrated clinical benefits of probiotics is the prevention and treatment of acute and antibiotic-associated diarrhea; however, there is mounting evidence for their potential role in the treatment of allergies and intestinal, liver and metabolic disorders. These positive effects are generally attributed to the ability of probiotics to regulate intestinal permeability, normalise host intestinal

microbiota, improve gut immune barrier function and equilibrate the balance between pro-inflammatory and anti-inflammatory cytokines. The positive effects of probiotics are attributed to their ability to maintain a normal microbial community, immune homeostasis and improve digestive health of the host (Oelschlaeger 2010). Additionally, probiotics also contributes to the nutritional requirements of the host by fermenting the digestive fibers into large intestine and providing some vitamins and trace elements during their colonization period (LeBlanc et al. 2013). Nevertheless, the proposed general mechanism of action for probiotics is similar; the benefits greatly vary with different strains even in similar kind of disease conditions (Paineau et al. 2008). Several clinical trials have demonstrated the clinical benefits of probiotics in prevention of intestinal disorders, allergies, liver and hepatic malfunctioning, metabolic syndromes and even neurological disorders. Here we focus to review the current knowledge of role of probiotics in different clinical conditions.

Diarrhea

Imbalance between beneficial and harmful bacteria of intestine results into diarrhea. The factors behind, determine the type of diarrheal infection i.e. antibiotic associated diarrhea, pediatric diarrhea, travellers' diarrhea etc. In all cases, overgrowth of harmful pathogen cause severe watery bowl movements and abdominal cramps. Supplements of probiotics are very effective in restoration of the natural ratio between good and bad intestinal residents (Reid et al. 2011). Probiotics are also efficient in calming down the inflammation in intestinal epithelium through maintaining immunological homeostasis of the host (Fong et al. 2015). Probiotic strains such as LGG, *S. boulardii, L. acidophilus* NCFM and *B. bifidum* have shown most promising evidences of shortening of the duration of diarrhea and faster recovery of digestive health among different human age groups in several random placebo trials (Guarino et al. 2015). A number of cost effective probiotics supplements for the prevention and treatment of diarrhea are already popular in global nutraceutical market which shows the significance of probiotic in maintaining and improving the gut health.

Inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD) is the chronic and relapsing intestinal disorder comprising of two distinct stages: Ulcerative colitis (UC) and Crohn's disease (CD). Inflammation in UC is restricted to the rectum and colon, while CD inflammation can occur throughout the gastrointestinal tract. The degree of inflammation also differs in both conditions. In UC, microscopic lesions are limited to mucosal layer whereas more severe conditions observed in CD, wherein damages are widespread to the thickness of intestinal epithelium. The cause of IBD is associated with an unusual response for luminal antigens in a genetically susceptible host. Unfortunately, current drug strategies are not efficient against relapsing symptoms of IBD. The relation between dysregulated leuminal microflora and inflammation in IBD indicates a rationale for probiotics to control the severity and reoccurrence of the disease. Researchers have reviewed the beneficial effect of probiotics in IBD and have tried to find out the possible mechanism of action of these bacteria. The 'probiotic effect' is basically mediated by suppression of pro-inflammatory cytokines, TNF-α, IFN-γ, IL-8 and IL-12 through regulation of transcription factor, NF-κB (Petrof et al. 2004; Mikov et al. 2014). The interference of probiotics with adhesion and colonization of commensal bacteria also seems to be involved in probiotic action (Woo et al. 2013). Probiotics could control the reoccurrence of symptoms by maintaining the balance of core microbial community of the gut (Miquel et al. 2013). In this manner, we could speculate that probiotics could be used as an encouraging treatment strategy for eradication of remission of IBD. However, their application in any form of severe IBD requires a more intense research to understand multifactorial mechanism of action of probiotics.

Lactose Intolerance

A significant per cent of world adult population is unable to produce lactase enzyme which makes them intolerant to lactose containing dairy products (Swagerty et al. 2002). Reduced ability to digest dairy products also increases the risk of deficiency of calcium and several other accompanying nutrients in people with lactose intolerance. Probiotic lactobacilli in fermented dairy products are capable to reduce the intolerance symptoms by delivering the significant amount of bacterial lactase to the host stomach and intestine. Several studies have revealed that probiotics could effectively decrease the lactose mediated discomfort and improve the nutritive value of the diet in target population (Levri et al. 2005; Almeida et al. 2012).

Metabolic disorders – Diabetes, Obesity and Cardiovascular diseases

Metabolic disorders represents a cluster of commonly recognized life style disorders. Three major metabolic disorders diabetes, obesity and cardio vascular diseases are being recognized as utmost risk factors for human health and life. Genetic and sedentary life style are among the main causes of development of metabolic malfunctioning which are further supported by increased stress and bad eating habits. The visible global burden of metabolic syndrome is now significantly affecting the quality and span of human life. In 2013, a report by Murray and Lopez on global burden of disease demonstrated that metabolic syndromes are among the top 10 death causing diseases in US. According to International Diabetes Federation (IDF) the annual direct health care cost of only diabetes is approximately 286 billion dollars which will increase to 395 billion dollars by 2025.

Diabetes is characterized by inflammation mediated imbalance between overproduced reactive oxygen species (ROS) and anti-oxidative system of human body (Figure 2). It can be balanced by anti-oxidative system of probiotics along with prebiotics. In uncontrolled diabetes, the level of superoxide dismutase, the enzyme responsible for inactivating the superoxide radical is considerably reduced. Deficiency in catalase, an enzyme responsible for the removal of H_2O_2, is also noticed. Probiotics are well known for their anti-oxidative activity (Achuthan et al. 2012). Anti-oxidative system of these beneficial microbes can be utilized to provide a compensatory response to restore cellular redox balance. Potential application of probiotic strains for management of metabolic and life style disorders have been earlier reviewed in detail (Mallappa et al. 2012; Panwar et al. 2013). Besides utilizing the anti-oxidative potential of gut flora, other possible involvement has been suggested in terms of their role in alpha and beta glucosidase inhibition (Otieno and Shah 2007; Chen et al. 2014; Panwar et al. 2014); incretin hormone secretion (Simon et al. 2015); di-peptidyl peptidase 4 inhibition (Panwar et al. 2014; Zeng et al. 2015) and maintaining glucose homeostasis (Al-Salami et al. 2008).

Suggested application of probiotics as biotherapeutic for cholesterol lowering has further increased their application for humans as supplements in dairy products (Sudha et al. 2009). Cholesterol is known to be a major risk factor for cardio vascular disorders. Several research studies have shown the anti-cholesterolemic /cholesterol lowering effects of lactic acid bacteria (Kumar et al. 2011). Potential application of probiotics for management of CVD has been reviewed by Sun and Buys (2015). Besides cholesterol lowering, probiotics exert beneficial effect through reduction in total serum lipid, tri-acyl-glycerol, bile salt deconjugation and global lipid profiles in human subjects. Recently, use of *Akkermansia muciniphila* has been patented for treatment of metabolic disorders (Cani et al. 2014)

Allergy

The prevalence of allergic diseases like eczema, asthma and food allergy have significantly increased in developed world. This phenomenon is explained by hygienic hypothesis; which suggests that the reduced exposure to microbial antigens in early life establish a shift in immune system toward Th2 type of response. The shifting toward Th2 causes a reduced Th1/Th2 ratio of

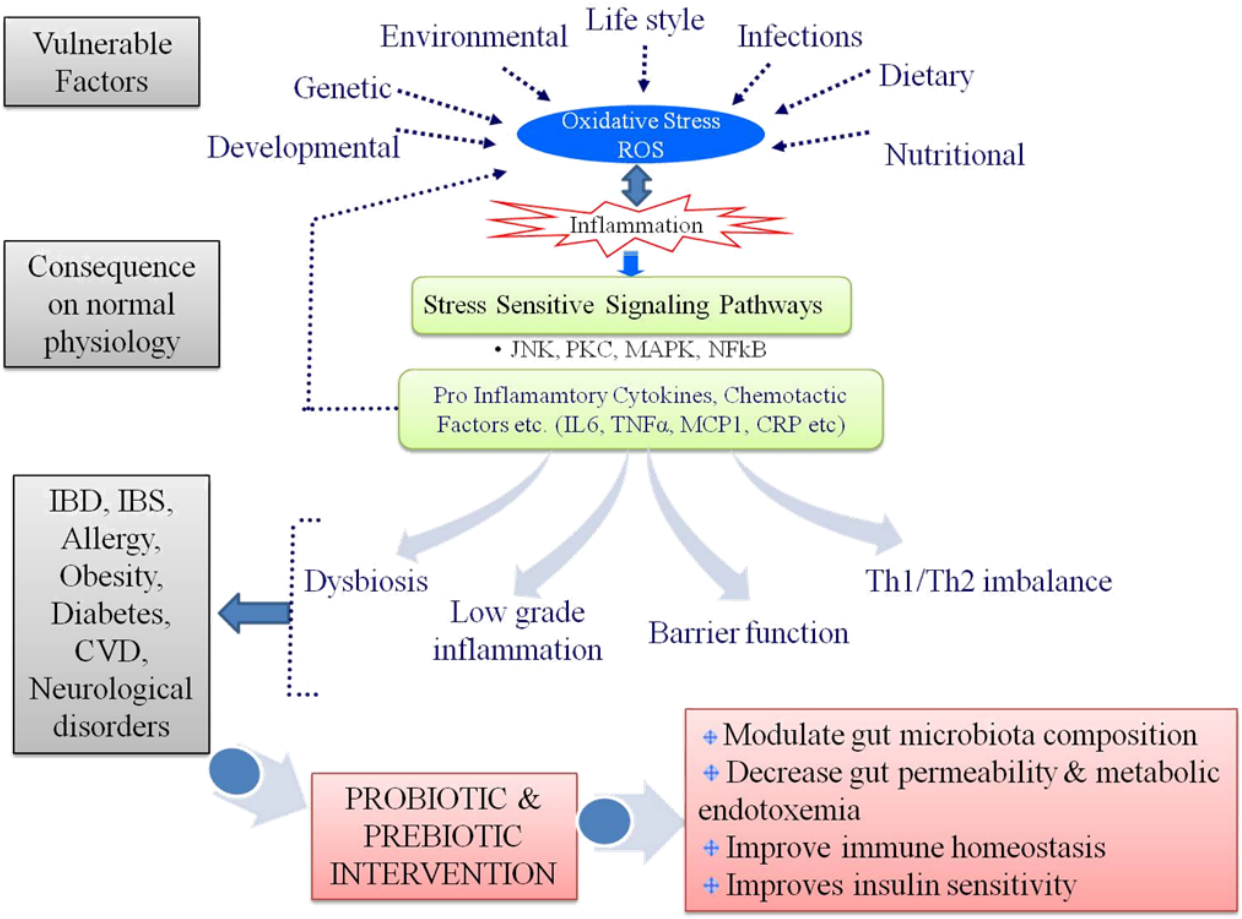

Figure 2. Factors involved in pathogenesis and possible probiotic and prebiotic intervention for different clinical scenarios

immunological machinery and develops an allergic type mucosal immunity. It is well known that the composition of intestinal microbial community has an important role in development and shifting of mucosal immune response by inter-mediating various signalling pathways (Macpherson et al. 2005). Thus, over the past few years probiotics has attracted the attention of researcher in context to find out an alternative treatment strategy for the prevention of pathology of allergic response (Cuello-Garcia et al. 2015). Several studies have reported that probiotic poses a strong effect on immune-physiology of intestine by regulating luminal dysbiosis and restoring the intestinal barrier function (Rautava and Isolauri 2002; Osborn and Sinn 2007). However, research in this front is still in its primary stage and further clinical evidences in large target population are required before their future applications.

Additional health benefits

The mechanism of action of probiotic is conferred by its interaction with host as well as the resident microflora dwelling within us or on our mucosal and skin surface. Thereby, the beneficial effect of probiotics completely depends upon the genetic and microbiological structure of the human body. The discrete beneficial functional effects of probiotics have been described in humans with specific immunological conditions such as pregnant women (Lindsay et al. 2013), Elderly (Eloe-Fadrosh et al. 2015), Infants (Bin-Nun et al. 2005), and athletes (Nichols 2007; Pyne et al. 2015). Besides, the role of probiotics in controlling human behaviour using gut brain axis is also recognized in recent years (Hemarajata and Versalovic 2012). Moreover, probiotic research has also shown promising role in modulating brain activity and central nervous system disorders (Tillisch et al. 2013; Wang and Kasper 2014). Thus it is now well established that the proven strains of probiotics can promote human health in several clinical scenarios. However, elucidation of mechanisms involved and substantiation of results from animal studies in humans remain further essential research goals. Growing trends indicates that live probiotic administration would no longer be necessary; instead their active ingredients or metabolites identified as the active component will be emphasized.

Guideline for selection of probiotics

The probiotic therapy has been adapted by humans long ago. Earlier, a general approval of a therapeutic strain was considered by few physiological characteristics i.e. non-pathogenic and capable to colonize in intestine. The criteria of selection of probiotic varied largely in different global organizations. Thereby, a general guideline for the evaluation of probiotics was proposed by FAO/WHO (2002). The guideline recommended strain level identification of probiotic followed by *in vitro* and *in vivo* evaluation for functional and safety characteristics which should also be confirmed in humans by clinical trials. For the awareness of consumers, FAO/WHO guideline also recommended the labelling of probiotic food with complete information about strain, composition, minimal number, shelf life along with manufacturer details. In Indian context, till 2010, probiotics and their products were evaluated without any systemic approach. Thereby, to control the false claims of probiotic products, Council of Medical Research (ICMR) along with the Department of Biotechnology (DBT), framed a specific guideline for the characterization of probiotics for Indian market (Ganguly et al. 2011). Like FAO/WHO guideline, ICMR/DBT guideline also deals with safety, health claim and labeling issues of probiotic products. Briefly, the parameters to define a probiotic include:

- Strain level nomenclature as per international standard
- *in vitro* screening for
  - Acid and bile resistance
  - Antimicrobial activity
  - Pathogen exclusion
  - Bile salt hydrolase activity
- *in vivo* safety studies
- To substantiate in vitro functionality in vivo efficacy study
- Efficacy study in humans by a clinical trial
- Labeling of probiotic product with strain designation, minimum viable number for the claimed health benefit, storage stability and manufacturer information.

Further, the guidelines also suggest that before introduction of an overseas probiotic in Indian market, its efficacy should be evaluated in Indian population. Thereby, ICMR/DBT guideline has implemented a strong support to the consumers against misleading approaches of probiotic manufacturers in Indian market.

Regulation of probiotic foods

It is anticipated that the probiotic market of India will grow at a CAGR of around 19% till 2019 (www.techsciresearch.com/3044). The market share of probiotic products is divided between pharmaceutical supplements and functional foods of probiotics. The evaluation of safety and reliability of these products is currently regulated by Food and Drug administration (FDA) along with Food Safety and Standards Authority of India (FSSAI). FSSAI, with the help of Food Safety and Standards Act 2005 (FSSA), has recommended minimum scientific standards for the regulation of manufacturing, storage and sale of such products into the market. Moreover, Prevention of Food Adulteration Act (PFA) also regulates the execution of maximum manufacturing, compositional and packaging information on the food products. Nevertheless, after constitution of ICMR-DBT guidelines, efforts are being made to control the false claim of probiotic products by a more specific legislative framework which will

favour the safety and well being of Indian consumers.

Status of probiotic industry

A recent survey by Ali et al. (2015) has reported that the global nutraceutical market has been estimated at 749.6 billion USD. However, the demand of probiotic food alone in global market was 27.9 billion USD in 2011 and is expected to increase at a 6.8% CAGR in 2016 (Raghuwanshi et al. 2015). In India, the value of probiotic market was 12 million USD which will grow at a CAGR of 11% by 2016 (Sharma et al. 2013). The data clearly indicates that the graph of probiotic business will flourish very rapidly in upcoming years. Thereby, there is an urgent need for a strong regulatory framework which could control the misleading product range from global and Indian market. Currently probiotic industry is captured by few key players globally with application of few established probiotic strains (Table 3).

Table 3. Representative list of probiotic strains being commercially explored by different company/institutes

Probiotic Strain	Company/ Institute	Country of origin
Lactobacillus acidophilus NCFM *Bifidobacterium lactis* HN019	Danisco	Wisconsin
Lactobacillus rhamnosus GG	Valio Dairy	Finland
Lactobacillus casei Shirota *Bifidobacterium breve* Yakult	Yakult	Japan
Lactobacillus johnsonii La1	Nestle	Switzerland
Bifidobacterium lactis BB12 *Lactobacillus acidophilus* LA-1/LA5	Chr. Hansen, Inc.	Wisconsin
*Lactobacillus plantarum*299v	Probi AB	Sweden
Lactobacillus salivarius UCC118	University College Cork	Ireland
Lactobacillus rhamnosus R0011 *Lactobacillus acidophilus* R0052	Institute Rosell	Canada

Future perspectives

Although there are many probiotic products currently available to consumers, both as functional food and nutraceuticals; there is still uncertainty surrounding the structure of regulation of these products. As the area is growing and establishing, changes to existing regulatory process may be required to cater the needs and safety of patients and users globally. Further oriented research is required to answer the key questions involving the actual mode of action; optimal dosage and duration of probiotic therapy; origin and source of probiotic strain; strain specificity and stability of health claims.

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