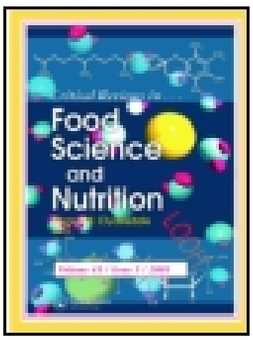

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An overview of advanced technologies for selection of probiotics and their expediency: A review

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Abstract

It has become easy to identify and select an appropriate microorganism with the advancement in various molecular biology and analytical techniques. The majority of the novel techniques is being implemented for the identification and characterization of microorganisms used for probiotic application. Standard microbial techniques such as biochemical testing and culture techniques routinely used for probiotic microbes screening, identification and selection. However, these standard techniques may not give complete information on the microbes that can be used for probiotic production. Furthermore, alternative molecular and analytical techniques such as 16S and 23S ribosomal DNA sequencing, RNA analysis by reverse transcriptase (RT-PCR), fluorescent in situ hybridization (FISH), quantitative analysis by real time PCR (RT-PCR or qPCR) and fluorescent activated cell sorting (FACS) are potentially used to confirm and select all types of lactic acid microorganism. All these approaches can be employed in the screening and selection of appropriate lactic acid bacteria which can be potentially used for the production

of human use probiotics in large scale fermentation. This review mainly focuses on various tools and techniques used for effective screening and selection of a better candidate bacterium for probiotic applications.

Keywords Probiotics. Quantitative analysis. Fluorescent in situ hybridization (FISH). Real time PCR (RT-PCR). Fluorescent activated cell sorting (FACS)

1. Introduction:

Probiotic microbial food supplements are live microflora used to facilitate advantageous effect on individual health by advancing the microbiological flora in the intestine. Most commonly used bacteria which have excellent probiotic properties are from human normal microflora and they belong to genera *Lactobacilli* and *Bifidobacteria*. These bacteria exert beneficial effects in the human gastrointestinal tract, such as, reduction of serum cholesterol, alleviation of lactose intolerance, anti-infection, antimicrobial, antioxidant, anti-allergic, antimutagenic, anticarcinogenic properties, blood lipid reduction and immune system stimulation (Mortazavian et al., 2008; Lee et al., 2014). Probiotic bacteria may produce a variety of inhibitory substances such as organic acids and bacteriocins, which can inhibit the pathogenic bacteria present in the human intestine. Organic acids lower the body pH and affect the growth of pathogens. Probiotics, prebiotics and synbiotics (combination of probiotics and prebiotics) are considered as food products, not drugs (Pundir et al., 2013). There has been a rapid rise in use of probiotic product in dairy applications. The effect of probiotics against allergies, diarrhoea, malnutrition, antibiotic side effects etc. provides the scientific basis for determining a dynamic future for dairy probiotics (Reid et al., 2015). The importance of probiotic lactic acid bacteria and Bifidobacteria in the prevention and treatment of inflammatory bowel and other associated diseases have been studied. It has been well established that probiotics act as an important prophylactic or therapeutic strategy for many mucosal and non mucosal immune-related conditions, such as celiac disease, metabolic syndrome, and diabetes (Ivanov et al., 2012). Lactic acid bacteria and bifidobacteria, have been characterized as probiotics that can modify the gut microflora and may be advantageous

for prevention and treatment of Inflammatory Bowel Disease (Lara et al., 2015). Probiotics can also induce some physiological effects even in healthy person. For example, in healthy adults four week consumption of *Lactobacillus paracasei* subsp. *paracasei* LC01 resulted in reduced faecal *Escherichia coli* and ammonia, and increases in *Lactobacillus, Bifidobacterium*, and *Roseburia intestinalis* and acetic and butyric acid (Zhang et al., 2013). The safety, functionality and quality must be fulfilled by a probiotic strain, if used as human food. The phenotypic methods have been used as an identification tool for many years to characterize a probiotic strain. These phenotypic methods include morphology examination, gram staining, biochemical methods, carbohydrate fermentation pattern, growth at different salt, pH and temperature, however temperature sometime may lead to misidentification (Margolles et al., 2009). We understand that, a successful and effective probiotic product can only be regarded as useful, if a suitable bacteria used as the product is properly identified, characterized and qualified for desired use. So, identification of probiotic strains is a chief concern for industrial, technological, and probiotic applications (Collado et al., 2006). Currently available molecular techniques such as 16S-23S rRNA sequencing, DGGE, PFGE, ARDRA contributes in a greater extent for identification, classification and characterization of these bacteria. Most commonly used identification techniques now a day's used for genotyping of probiotic bacteria are PFGE, AFLP, RAPD-PCR and Ribotyping. These identification techniques further enable identify and classify the bacteria at the strain level. Probiotic products especially for human consumption requires properly identified and well characterized microbial strains so that it does not lead to any erroneous effect to the

customers. In this review advanced as well as common techniques used recent past for identification and enumeration of probiotic bacteria are discussed.

2. History and definition of probiotics

The word probiotics consist of Latin word ‘pro’ means for and Greek word ‘bios’ means life (Lalitha, 2011). The idea of the beneficial effect of microorganisms on human health was proposed by Noble Prize winner Eli Metchnikoff. Metchnikoff has shown that *L. bulgaricus* is able to abolish pathogenic bacteria from intestinal microflora and result in health benefits (Metchnikoff, 1907). He suggested that the long life of Bulgarian peasants is correlated to high consumption of the fermented milk products as the lactic acid bacteria present in these products replaces the harmful intestinal bacteria (Singh et al., 2011). But the actual concept of probiotics was introduced by Lilly and Stillwell as after their work probiotics characterized as beneficial microorganisms that promotes the growth of other bacteria (Lilly et al., 1965). Parker defined probiotic microorganisms and their substances to facilitate the intestinal microflora (Parker, 1974; Goyal et al., 2013). Probiotics have been defined by Fuller as live microbial food supplements which beneficially affects the host by improving its intestinal microflora (Fuller, 1989). WHO and FAO defined probiotics as live microorganisms, its administration in adequate amount has an advantageous effect for human health. To assess the efficacy of probiotics in humans, it is necessary to have an understanding of the probiotic strains, therefore novel methods are needed for selection and characterization of these strains.

3. Selection criteria

For the selection of a microbial strain that to be used as probiotic should have certain characteristics includes (Fig. 1) Safety, production/manufacturing, viability during storage, resistance to gastric acid, bile tolerant, adherence to gut epithelial cells, colonization to the gastrointestinal tract (GIT), production of antimicrobial substances, ability to stimulate the immune response, and ability to influence metabolic activities such as vitamin production of a host (Savodago et al., 2006). The initial screening and selection of probiotics also includes testing of genotypic and phenotypic stability, plasmid stability, carbohydrate utilization patterns, antibiotic resistance patterns, ability to inhibit known pathogens, and immunogenicity. A probiotic strain should survive, proliferate and colonize in GIT, it should neither be pathogenic nor trigger allergic response to the host. Furthermore, probiotics should be easily culturable on a larger scale and must be able to resist technological manipulations such as heating and low oxygen conditions during packaging. As human stomach is highly acidic due to the presence of HCL, hence acidic environment may act as a barrier for probiotics. Therefore, the probiotic mircoflora must be able to survive gastric as well as the bile in the upper digestive tract before their entry into the small intestine (Lee et al., 2014). The probiotic species *L. casei* and *L. acidophilus* survives well in the acidic conditions of artificial gastric juice at pH 3.0 at 37^0C while *L. delbrueckii* ssp. *bulgaricus* does not (Eric et al., 2015). Microencapsulation is the most noteworthy promising and competent technology these days used for the safeguarding of probiotics against unfavorable environmental conditions like oxygen, low pH, gastric juice, bile salts, salinity, heat and cold shocks (Tripathi et al., 2014). Most common encapsulating materials are alginate, Chitosan, gums (locust bean, gellan gum, xanthan gum, etc.), gelatin, carrageenan, whey protein, starch. The

encapsulating materials significantly improved the viability of probiotic microorganisms during storage, processing, and passage in the gastrointestinal tract (Riaz et al., 2013). When exposed to simulated gastrointestinal juice, survivability of probiotic strains *Lactobacillus acidophilus* PTCC1643 and *Lactobacillus rhamnosus* PTCC1637 trapped in alginate coating, was considerably higher than their uncoated forms (Mokarram et al., 2009). A bakery product, pan bread was prepared by using sodium alginate and whey protein-encapsulated and air-dried *L. rhamnosus* GG (Soukoulis et al., 2014).

4. Health benefits

Interaction between probiotics and cells of the immune system are essential for maintaining mucosal tissue homeostasis and innate immunity. The gastrointestinal tract of human contains bacterial species which acts as a barrier against potential pathogens and help in regulating the immune response of the body. Human gut microflora comprises mainly Gram positive Firmicutes (60–80%) and Gram-negative Bacteroidetes (20–40%). *Lactobacillus* and *Bifidobacterium* are the predominant genuses in the human intestine, both have a long history of safe use and are considered as GRAS (Backhed, 2012). *Lactobacillus* is predominant in the small intestine and *Bifidobacterium* in the large intestine. However, species belonging to the genera Lactococcus, Enterococcus (*Enterococcus faecium*), Streptococcus (*Streptococcus salivarius*), yeasts (e.g. *Saccharomyces cerevisiae* and *Saccharomyces boulardii*) and filamentous fungi (e.g. *Aspergillus oryzae*) are also used as probiotics due to their health promoting effects. Some commonly used probiotic strains and their commercial applications is given in Table 1.

Gut microflora differ among individuals and can vary according to genotype, age, health condition, diet, and exposure to antibiotics to host (Henao et al., 2013). The presence of bacterial species in the gastrointestinal tract is influenced by many factors such as diet, intestinal pH, bacterial adhesion, secretion of mucin and bacterial antagonism etc. It has been established by Neish, 2009 that changes in any of these factors can affect the environment that supports the gut microbiota, thus altering the number of microorganisms available to maintain intestinal homeostasis. This whole event cascade leads to excessive energy production and calorie extraction, with negative effects on metabolism and obesity which is a major cause of liver disease (Neish, 2009). Probiotics have been shown to have favorable effects when used to treat several liver diseases by reducing the production of bacterial toxins and by modulating autoimmune responses, intestinal permeability, and the inflammatory response. The mechanisms through which probiotic bacteria alter the progression of liver diseases are thought to relate to modifications in the composition of the natural intestinal microbiota and its activity. For example, supplementation with *Lactobacillus paracasei* decreases the number of *Enterobacteriaceae* and *Enterococcus spp*, and promotes the growth of species of *Lactobacillus, Bifidobacterium* and *Bacteroides* (Norberto et al., 2015). Bacteriocins produced by Probiotics, can slow down the growth of pathogenic bacteria. Organic acids produced can penetrate the cell membrane and inhibit nutrients transport and ATPase activity (Levy et al., 2006). Altered microbiota, termed dysbiosis, could lead to altered immune functions and increased risk of diseases, gastrointestinal disorders and triggers many inflammatory bowel diseases. Some evidences of probiotic bacteria are improving antibiotic-associated diarrhoea, acute infectious diarrhoea, irritable bowel

syndrome, pouchitis, ulcerative colitis, necrotizing enterocolitis and Crohn's disease (Chong, 2014). Probiotics have been validated to improve antioxidant condition, enhance immune resistance against viral infections and tumours and also boost GIT barrier functionality (Touchefeu et al., 2014; Vieira et al., 2013). Probiotics have been used as adjuvant treatment for many diseases that affect the gastrointestinal tract, such as inflammatory bowel disease, cancer, *Helicobacter pylori* infection, vaginosis, allergy, and lactose intolerance (Hill et al., 2014; McFarland, 2014; Narbona et al., 2014). It has been reported that allergic Rhinitis which is triggered by grass pollen might be mitigated using *L. paracasei* subsp Paracasei LP-33 (Costa et al., 2014). Further, a recent study suggested that IRT5 probiotics (a mixture of 5 probiotics) could suppress various inflammatory disorders, including encephalomyelitis, a T-cell mediated inflammatory autoimmune disease of the central nervous system (Kwon et al., 2013). It is illustrious that probiotics restores the gut homeostasis by involving regeneration of the intestine, which repairs infection associated damage (Vieira et al., 2013). Furthermore, the improved significance of probiotics in cancer prophylaxis and therapy has been widely studied (Patel et al., 2013). Bifidobacteria have been reported to produce a protective coat of exopolysaccharides that could help bacteria to survive against high acidic condition and high bile concentration of the gastrointestinal tract without a detrimental effect on their viability (Prasanna et al., 2014). Probiotics provide various health benefits (Fig. 2) which includes balancing intestinal microflora, improvement of the immune system, provide protection against gastrointestinal pathogens, reduction of serum cholesterol level, prevention of food allergy, protection against colon and urinary bladder cancer. Probiotics help in modulation of different signalling pathways of innate immunity through the Toll like

receptors. Different strains of probiotic *Lactobacilli* can generate different intensity of immune responses (Maldonado et al., 2015).

5. Techniques used for Strain Identification

5.1 Screening by Invitro testing

In vitro testing methods are very useful to gain knowledge on the mechanism of probiotic strain. The Invitro assay is recommedned for the testing of probiotics as it is used for human trials . The Potential probiotic microflora screened using in vitro methods have shown resistance to gastric acids (Casey et al., 2004), bile salt tolerance (Hoque et al., 2010) antimicrobial activity against pathogenic bacteria, bile salt hydrolase activity (Franz et al., 2001), adherence to human epithelial cells and resistance to 0.4% phenol (Kumar et al., 2012).

5.2 Molecular techniques for identification

5.2.1 Species specific identification techniques

Various molecular methods have been developed for identification and categorization of probiotic bacteria summarized in table 1. These techniques can be further divided into nucleic acid based and complete cell activity based identification. However, most frequently used tool is nucleic acid based because of its specificity and high throughput potential which includes ribotyping, RAPD, AFLP and PFGE. These methods have been extensively used for

identification and genotyping of bifidobacteria and LAB (lactic acid bacteria). Some of the most common and relevant methods of identifications are described below.

5.2.1.1 Amplified ribosomal DNA restriction analysis (ARDRA)

ARDRA is an excellent tool for identification and differentiation of bacteria which are isolated from composite environment and related groups of bacteria. The identification of strains at the species level is first described in 1992 (Vaneechoutte et al., 1992). It involves enzymatic amplification followed by digestion with restriction enzyme patterns, the restriction pattern obtained for new strain is compared with patterns obtained from the reference strain (Donelli et al., 2013). It is a good molecular tool for identification of human *bifidobacterium* species. A study showed that the *Bifidobacterium* species present in the intestinal tract of human can be differentiated using a six restriction pattern combination from 511bp to 525 bp 16S ribosomal fragment with *Taq*1, *Sau*3A1, *Rsa*1, *Alu*1, *Sau*961, *Nci*1 enzymes, respectively (Venema et al., 2003). ARDRA has been shown as the most appropriate method for a quick discrimination between different species of *Propionibacteria* used as probiotics (Dherbecourt et al., 2006). In addition, ARDRA has been successfully used for identification of *Lactobacilli, Bifidobacteria and Streptococci* species. (Collado et al., 2007). Recent studies on 28 faecal samples of *B. longum* suggested ARDRA as an appropriate method of distinguishing between different strains (Srutkova et al., 2011).

5.2.1.2 The 16S and 23S rRNA sequencing

Phenotypic identification of bacteria has been improved with the introduction of 16S/23S-5S rRNA sequencing methods (Collins et al., 1991). Some regions of 16S and 23S rRNA are

conserved throughout all bacteria, hence these sequences can be used as a source of species specific bacterial identification (Klijn et al., 1991). The primers encoding consensus sequences of 16S and 23S rRNA are used for PCR amplification of the genomic DNA of identifying probiotic bacteria and further the PCR products obtained can be analysed on agarose gel electrophoresis (Bested et al., 2013). The intergenic spacer region of 16S& 23S rRNA, generally used for the identification of *Lactobacillus* genus (Han et al., 2005). Rapid identification of *Lactobacilli* can be done by using colony PCR method, crude cell lysate and species specific primers targeting 16SrRNA (Luo et al., 2012).

5.3 Strain specific identification techniques

5.3.1 Ribotyping

Ribotyping is used for identification and classification of bacteria based on differences in the rRNA make-up which provides high discrimination of bacteria at the species and subspecies level. DNA extraction from bacterial colony, enzymatic digestion with appropriate restriction endonuclease followed by agarose gel electrophoresis analysis, transferred to hybridization membrane followed by incubation with a specific enzyme linked DNA probe. After washing, the enzyme substrate produces chromogenic signals, DNA fragments are visualized by adding on membrane made up of nylon, the pattern observed is then used for detection of strain. This technique has been used to differentiate *lactobacilli* (Rodtong et al., 1993). It has been also applied to characterize *Lactobacillus* and *Bifidobacterium* strains (Zhong et al., 1998; Giraffa et al., 2000). A current taxonomic study has been paying attention on typing of *Lactobacillus* spp. occurring in dental caries (Svec et al., 2010).

5.3.2 Random amplified polymorphic DNA (RAPD)

RAPD have been broadly reported as simple, quick, economical technique for genetic typing of diverse strains of bifidobacteria and lactic acid bacteria. A large number of samples can be analyzed within a short period of time (Satokari et al., 2003). It is a PCR based method that makes use of arbitrary primers or short primers which anneals to multiple random target sequences and directed to the formation of fingerprints of DNA bands differentiated between bacterial species (Fujimoto et al., 2008). This method has been successfully applied to distinguish between various species of lactobacilli at interspecies as well as intraspecific level. It allows to distinguish between bifidobacteria (Vincent et al., 1998) and *Lactobacillus acidophilous* group and related strains (Gancheva et al., 1999). Many reports on RAPD assay are available used to differentiate at strain and genus among different probiotic lactobacilli species (Du plesses et al., 1995; Roy et al., 2000) and bifidobacteria (Schillinger, 1999; Pal et al., 2012). It is also studied that RAPD method has a very low degree of discrimination among *L. fermentum* and *L. gasseri* in comparison with PFGE methods, (Gosiewski et al., 2012). However, RAPD has been used as a suitable technique to validate lactobacilli results received by Pulse field gel electrophoresis method (Herbal et al., 2013).

5.3.3 Pulse field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP)

PFGE allow the separation of large sized DNA fragments (larger than 10 kb). DNA fragments are subjected to periodic change in polarity of the electric field. The fingerprints of DNA obtained depend on the restriction enzyme specificity as well as the genome sequence

of the bacteria. It is reported that this method has been used for discrimination among significant probiotic bacterial strains such as *B. animalis* and *B. longum*, (Roy et al., 1996), *L. casei and L. rhamnosus* (Tynkkynen et al., 1999). PFGE has been used to categorize different lactobacilli strains in various probiotic products for human use (Coeuret et al., 2004). It has been also used in diversity and dynamics study of lactobacilli isolates (Henri et al., 2008). In a recent study PFGE has been used as an identification tool to evaluate vaginal epithelial cell colonization by a mixture of orally administrated different *Lactobacilli* (Strus et al., 2012). Furthermore, few studies on AFLP suggest that this technique is a hybrid of RFLP and RAPD techniques. It has better reproducibility, greater resolution and sensitivity at genome level as compared to RAPD-PCR. AFLP has become one of the most amplified techniques for bacterial taxonomic studies (Janssen et al., 1996). AFLP have been used as strain specific identification technique for probiotic strain Lactobacilli (Sisto et al., 2009) and *Bifidobacteria longum subsp*. (Makino et al., 2011).

5.3.4 PCR-Denaturing gradient gel electrophoresis (DGGE)

This method provides an easy evaluation of microorganisms used in probiotic products as it does not require prior culturing of cells or individual strains separation. The separation of similar length PCR products in denaturing gradient gels is based on sequence differences (Herbel et al., 2013). DGGE a molecular fingerprinting method allows separation of up to 500 bp DNA fragment. The melting point is an important factor in this method as the days DNA migrating through gel stops at a specific melting temperature which results in separation (Logan et al., 2003). Various studies reveal that the bacterial population

identification in the fermentation of Italian sausages (Cocolin et al., 2001), traditional sourdough (Randazzo et al., 2005) and wine making (Renouf et al., 2006) is done using this technique. It has been used also used for the identification of *Lactobacillus* species in the gastrointestinal microflora of mice (Walter et al., 2000). Study of intestinal persistence of orally administrated probiotic *Lactobacillus* sp. strain in healthy adults has been also done using PCR-Denaturing gradient gel electrophoresis (Hutt et al., 2011). To assure results this technique should be used in combination with 16S rRNA sequencing of the V3 region (Liu et al., 2012).

5.3.5 FACS (Fluorescent activated cell sorting) method

FACS is a high throughput tool allows sorting of heterogenous biological cell mixtures based on the fluorescent characteristics and light scattering. It allows large number cellular examination at a given time (i.e. 200- 2000 cells/second). There are numerous diverse parameters that can be linked to various cellular characteristics (Tracy et al., 2010). Different fluorescent probes can be used for the examination of physiological characterization of a live cell, these include membrane integrity and membrane potential, pH of cytoplasm, intracellular enzyme activity etc. These characteristics provide viability measure of bacteria (Chen et al., 2012). Hence FACS can be potentially used for high throughput cell sorting which could minimize the time required for determination of size of cell, abundance and cellular metabolic activity of probiotic strains (Davis, 2014).

5.3.6 Nucleic acid-based enumeration methods (PCR methods)

PCR is a method of obtaining genomic DNA fingerprints of bifidobacteria (Roy et al., 1996). It uses primers complementary to intersperse repetitive consensus sequences that can amplify sequences between the repetitive elements (Olive et al., 1999). Numerous studies have used three different primers which are BOX, ERIC and REP (Gomez et al., 2000). Primers ERIC and BOX have been used for classification and differentiation of Bifidobacteria (Ventura et al., 2002). RT-PCR uses reverse transcriptase enzyme to transcribe RNA into its complementary DNA. mRNA is short lived, extremely labile molecule in bacteria, hence transcription of bacterial mRNA should provide a useful marker of viability (Davis, 2014). Hellyer and Nadeau (2004) used RT-SDA (Reverse transcriptase-strand displacement amplification) as a marker for bacterial viability of bacteria. qPCR is culture independent, rapid amplification method that uses fluorescent dyes for the amplification and detection of DNA and RNA. Intensity of fluorescent signal produced by fluorescent dyes is proportional to the amount of DNA/RNA produced per cycle of PCR. This technique allows discrimination of different species and quantification of microbial populations by measuring the abundance of the target sequence in the DNA samples which are extracted from various food products (Postellac et al., 2011). It is evident from the literature that commonly used qPCR techniques use TaqMan labelled primers, molecular beacons and SYBR Green. TaqMan and molecular beacons are probe based techniques on reporter-quencher system (Bustin et al., 2012) whereas fluorescent dyes e.g. SYBR Green has a binding affinity to dsDNA and it is a quantitative approach for enumeration of bacterial communities (Miller et al., 2012; Castoldi et al., 2013). Interestingly, qPCR is also used to detect minor bacterial populations in the dominant population of bacteria and using this method even the non

cultivable bacterial species can be detected and quantified (Postellac et al., 2011). Recently a more understanding on these aspects was provided and a map has been published for obtaining more reliable results using MIQE guidelines (Taylor, 2013).

5.3.7 Fluorescent in situ hybridization (FISH)

Culture-independent detection methods have been used to enumerate probiotic strains based on viability. These techniques quantify viable cells either by using dyes to differentiate live and dead cells, by direct observation, measure the membrane integrity or characterize metabolic activity of cell such as nucleic acids synthesis. Probiotic bacteria can be directly visualized by microscopic techniques, but to enumerate viable bacteria requires differentiation of live and dead bacteria. FISH involves direct imaging and visual enumeration of bacteria. The technique enables rapid and accurate way to identify and quantify bacterial species by using epifluorescence, confocal microscopy, flow cytometry techniques (Zwirglmaier, 2005). This technique combines the versatility of microscopic and DNA/rRNA hybridization methods. The technique is based on hybridization of a rRNA-targeted fluorescent probe (a small stretch of oligonucleotide) to a specific region of 16S/23S rRNA ribosomal sequences of bacteria. Cells marked with fluorescent probe can be differentiated by using specific light filters (Lahtinen et al., 2006). It can be accomplished in a few hours, which allows fast in situ analysis (Juste et al., 2008). Now a days, an alternative to DNA probes is peptide nucleic acid (PNA) probes which have emerged as a rapid microbial detection method (Stender et al., 2002). PNA probes recognize and bind to specific region of 16S and 23S rRNA with higher thermal stability. The Lac663 probe bound

exclusively to *Lactobacillus* strains and capable of directly quantifying Lactobacillus spp. at concentrations which considered as effective on human health. The advantage of this technique is possible to localize and observe target cells within their native environment by the help of this technique. The technique also used in aquaculture systems for monitoring of pathogen and probiotics. Bacterial identification in intestinal tract of tilapia species have beed studied using this technique. Also, it can also help to control the release of pathogenic bacteria to the environment results from pond water exchange, which could be a serious health problem for wildlife. The results of this study evidently verified that the FISH is a potent tool used in aquaculture (Alessandro et al., 2015). A new Histo-FISH method which is based on specific fluorochrome labeled probes, able to detect *Lactobacillus* spp. and *Bifidobacterium* spp. within biofilms on the mucosal surface of the GIT embedded in paraffin in histological slices. This is also appropriate method for visualization of bacterial populations in gastrointestinal tract (John et al., 2016). The Histo-FISH method permits us to analyse bacterial colonization as well as biofilm formation in stomach and caecum of BALB/c and germ-free mice (Madar et al, 2015). It has been also used in food industry for specific lactic acid bacteria detection for the production of hard cooked cheeses (Bottari et al., 2010). In addition it is also noteworthy to mention that FISH methods in combination with qPCR can also be used for enumeration of probiotic strains into a varied range of cheese based products.

Conclusion

This review describes the updated information towards use of various tools and techniques for identification and selection of probiotic microorganisms. The advanced tools and techniques used in this prominent area of research are also recapitulated in this review. Both conventional as well as advanced alternative techniques are reviewed which offers various options for selecting an appropriate microbial strain towards testing its potential for use of probiotic isolate in a variety of applications. These approaches are summarized in figure 3 which gives an overall idea of using such techniques in this field. Although the cost of advanced tools are high compared to traditional techniques, but nevertheless, these tools offer various advantages over traditional techniques. The high throughput screening tools such as FACS and Q-PCR offers fast and reliable identification of probiotics which in turns reduces the cost of experiments with high success rate. The cell based bioassay methods could predominantly use to check the activity as well as side effects of identified bacterial strains. The advanced techniques such as 16S and 23S RNA analysis, FACS and q-PCR can be potentially used for the identification as well as a selection of such microorganisms for industrial as well as academic applications.

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Table 1 Commercially available probiotic products, their strain designations and commercial applications

Probiotic strain	Product (company)	Applications and proven Health benefits	References
L. acidophilus NCFMs	HOWARU® Premium Probiotics (Danisco A/S)	Beverages, confectionery, dairy, dietary supplements and frozen desserts, anti allergic	West et al. 2014; Patel et al. 2015
L. acidophilus La-14	Flora FIT® (Danisco A/S)	Food and beverages, skin health	Caglar et al. 2015; Sadana et al. 2015
L. acidophilus LA5	Biorich® (Chr. Hansen A/S)	Starters for yoghurt manufacture, anti-diarrhoea, immune boosting, anti-infection	Reid 2015; Sadana et al. 2015
L. casei Shirota	Yakult® (Yakult Honsha Co. Ltd)	Probiotic beverage, intestinal flora reposition, improve digestion	Raghuwanshi et al. 2015; Hajela et al. 2015
L. casei DN114001	Actimel® (Danone)	Protection against pathogens	Martin et al. 2014; Galdeano et al. 2015

Lactobacillus casei rhamnosus Lcr 35	Ginophilus® (Probionov)	Lowers vaginal pH, prevents harmful pathogenic bacteria from colonizing and proliferating	Stefan et al. 2014; Paturi et al. 2015
Lactobacillus johnsonii La1	SVELTY® Gastro Protect (Nestle)	Controls *H. pylori* infection and stomach discomfort, immune boosting	Gotteland et al. 2008; Rebolledo et al. 2014; Schulz et al. 2015
Lactobacillus plantarum 299v	LP299V® (Probi AB)	Anti-diarrhoea, immune boosting	Jain et al. 2014; Patel et al. 2015
Lactobacillus rhamnosus GG	Actifit®, Gefilus®, LGG®, Vifit® (Valio)	Antiallergy, anti-diarrhoea, immune boosting, oral health	Caglar et al. 2005; Rebolledo et al. 2014; Schulz et al. 2015
L. paracasei CRL431	L. casei 431® (Chr. Hansen A/S)	Immune boosting, anti-infection	Galdeano et al. 2015; Paturi et al. 2015
L. paracasei GMNL-33	GenMont biotech	Anti allergic	Patel et al. 2015 Schulz et al. 2015
L. reuteri ATCC 55730 (Protectis)	BioGaia (Biogaia)	Antiallergy, anti-diarrhoea, oral health, immune	Paturi et al. 2015

		boosting, anti-infection	
B. animalis ssp. lactis Bb12	Chr. Hansen	Reduced salivary counts of *S. mutans*	Caglar et al. 2005; Jothika et al. 2015
B. animalis DN173010	DanActive fermented milk (Danone)	Digestive health improvement, Inhibits growth of *S. mutans*	Caglar et al. 2005; Gungor et al. 2015
B. lactis HN019 (DR10)	Foneterra	Immune boosting, anti-infection	Gungor et al. 2015; Paturi et al. 2015
B. longum BB536	Morinaga Milk Industry Co. Ltd.	Anti allergy	Caglar et al. 2015; Schulz et al. 2015
B. infantis 35624	Align (Procter y Gamble)	Maintain the digestive balance fortifying the digestive system with healthy bacteria	Raghuwanshi et al. 2015; Tian et al. 2015
Enterococcus faecium SF68	Bioflorin (Cerbios – Pharma)	Prevention and treatment of intestinal disorders, anti-diarrhoea	Hajela et al. 2015; Siepert et al. 2014
Streptococcus salivarius K12	BLIS technologies	immune boosting, anti-infection, oral health	Gungor et al. 2015; Patel et al. 2015; Paturi et al.

			2015
Streptococcus oralis KJ3 *Streptococcus uberis* KJ2 *Streptococcus rattus* JH145	ProBiora3 (Oragenics Inc.)	Low-acid–producing oral inhabitants quickly colonize the oral cavity to inhibit the growth of the pathogenic streptococci	Caglar et al. 2005; Gungor et al. 2015; Jothika et al. 2015
Escherichia coli Nissle 1917	Mutaflor (Ardeypharm)	Prevention and treatment of intestinal disorders	Siepert et al. 2014; Singh et al. 2015
Escherichia coli	URO VAXOM® (Apsen)	Immunotherapy, prevention recurrent infections of the lower urinary tract	Stefan et al. 2014; Paturi et al. 2015; Singh et al. 2015
Saccaromyces boulardi	Florastor (Biocodex)	Immune boosting	Patel et al. 2015; Paturi et al. 2015

Table 2 Genotypic methods and their applications

Method	Strain/species specific typing	Applications	References
ARDRA	Species specific	Identification and discrimination between closely related bacterial species	Srutkova et al. 2011; Shehata et al. 2012; Gianfranco et al. 2013
16S/23SrRNA sequencing	Species specific	Identification based upon 16S/23S conserved region	Luo et al. 2012; Bested et al. 2013
Ribotyping	Strain specific	Identification and classification based upon differences in rRNA	Svec et al. 2010
RAPD	Strain specific	Identification and distinguish between different bacterial species based upon arbitrary primers	Pal et al. 2012;Gosiewski et al. 2012
PFGE	Strain specific	Identification and	Coeuret et al.

		differentiation between different bacterial strains	2004; Henri et al. 2008; Strus et al. 2012
PCR-DGGE	Strain specific	Strain level identification	Hutt et al. 2011; Liu et al. 2012; Herbel et al. 2013
AFLP	Strain specific	Taxonomic studies of bacteria	Sisto et al. 2009; Makino et al. 2011

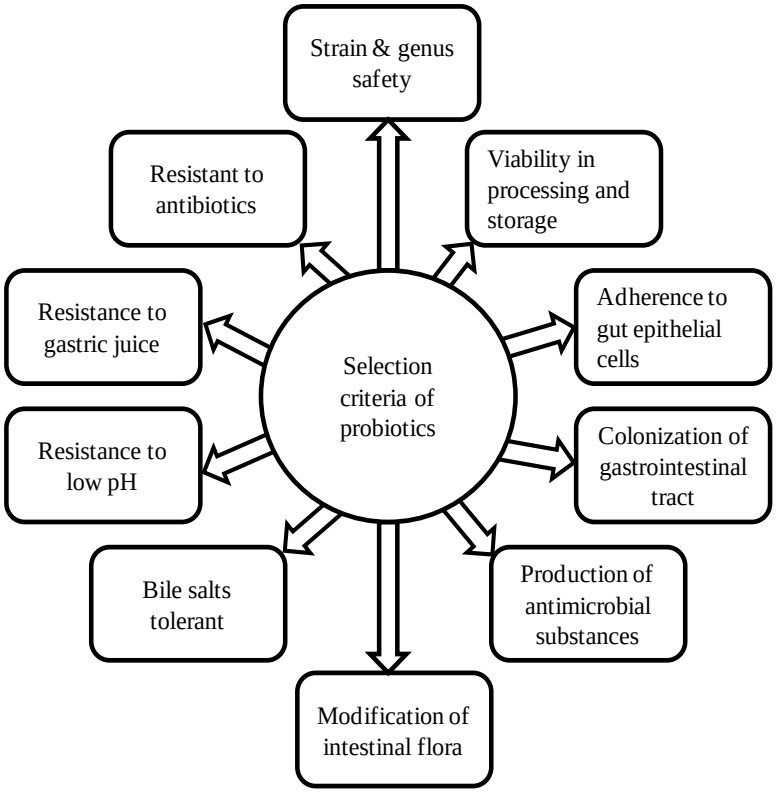

Fig. 1 Criteria for selection of probiotics

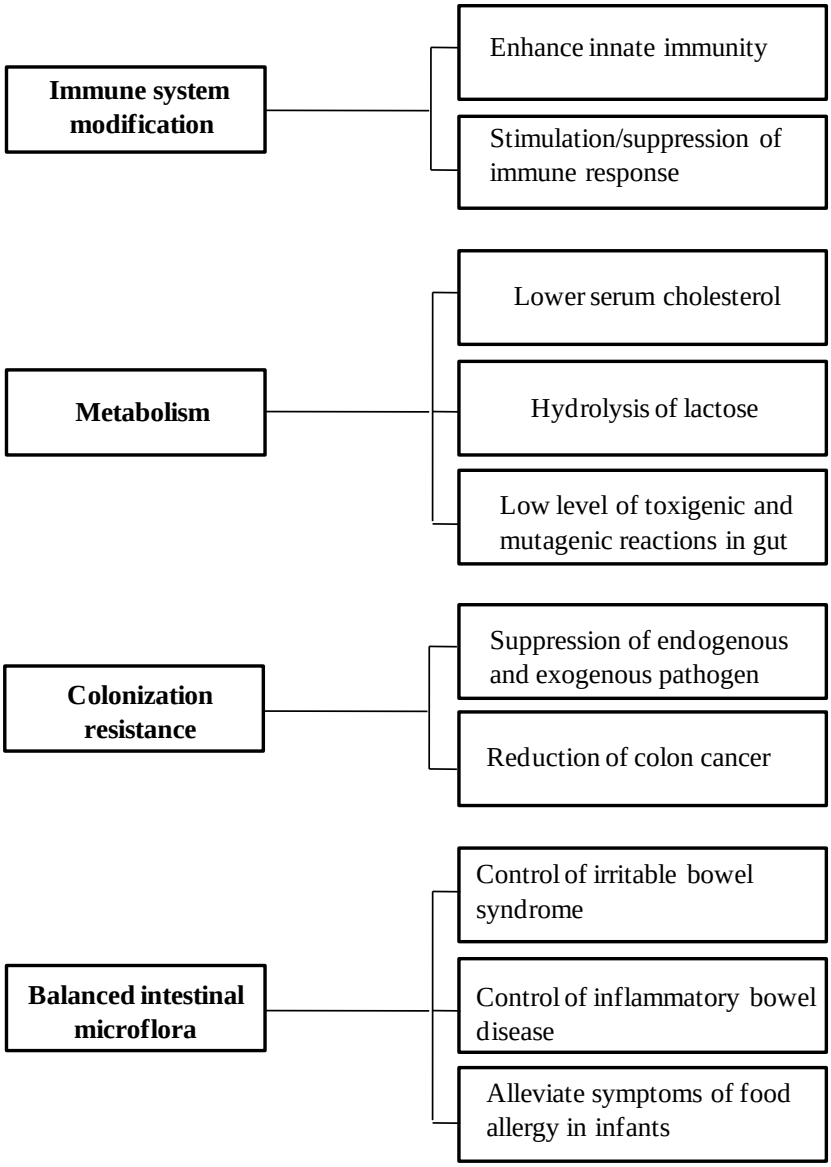

Fig. 2 Health benefits of probiotic bacteria

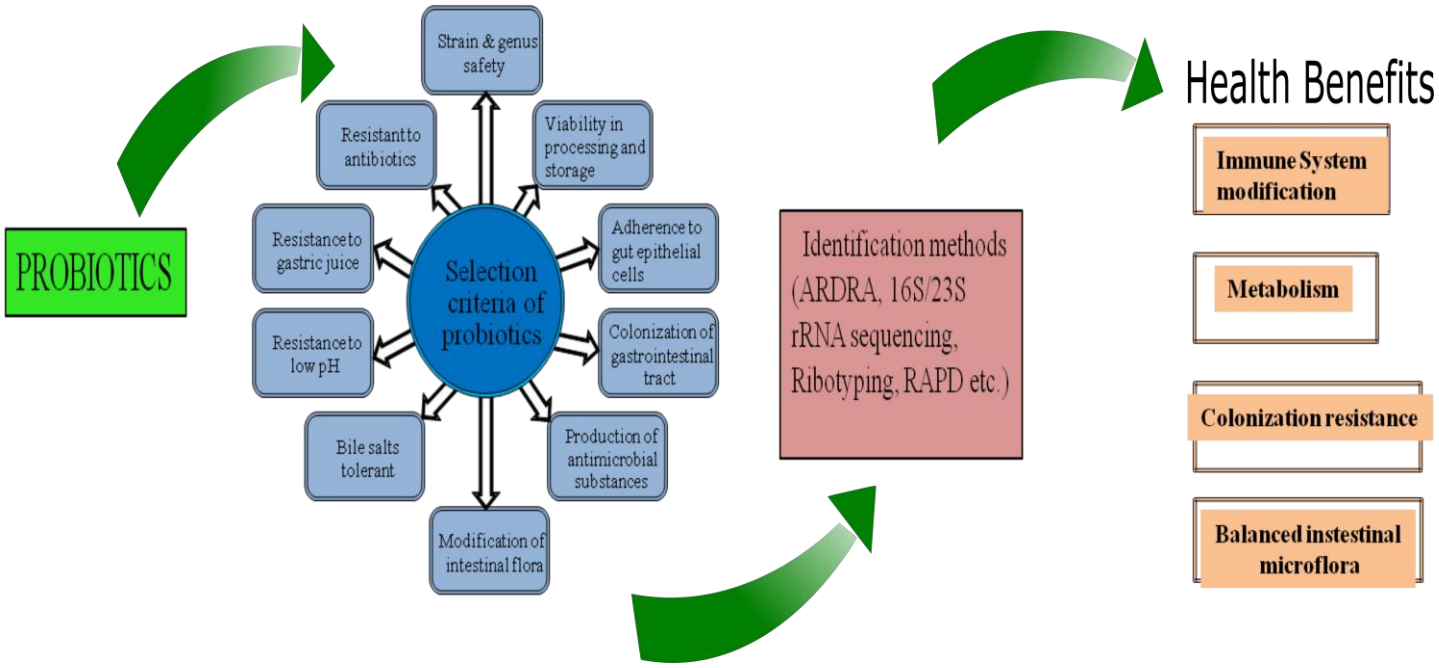

Fig. 3 A combinatory approach for selection of probiotics