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Review Paper

Methods for isolation, characterization and identification of probiotic bacteria to be used in functional foods

Ami Patel[1], J.B. Prajapati[1] and Baboo M. Nair[2]*

[1]Department of Dairy Microbiology, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat State, India
[2]Department of Applied Nutrition and Food Chemistry Lund University, Lund, Sweden

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Probiotic Science: Past, Present and Future

Some microorganisms including bacteria, yeasts, moulds and even algae are associated with fermented foods, forming a complex ecosystem. Among these organisms, lactic acid bacteria (LAB) are responsible for most of the physicochemical and aromatic transformations that are intrinsic to the fermentation process. LAB were first isolated from milk and have since been found in foods and fermented products such as meat, milk products, vegetables, beverages and bakery products (O'Sullivan *et al*, 2002).

LAB consist of a wide range of genera including a considerable number of species. Traditionally, they were divided into four genera, *Streptococcus, Lactobacillus, Leuconostoc* and *Pediococcus.* Nevertheless, a considerable change in the taxonomy of LAB was observed during the last few years (Stiles *et al.*, 1997). At present, LAB have been categorized into *Carnobacterium, Enterococcus, Lactococcus, Streptococcus, Leuconostoc, Oenococcus, Lactobacillus, Pediococcus, Tetragenococcus, Vagococcus, Weissella, Aerococcus, Alloiococcus, Globicatella* and *Dolosigranulum* genera. (Axelsson,1998). Probiotics are defined as microbial food supplements that when administered in adequate amounts exert beneficial effects on the host. Several strains of lactobacillus such as *Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus rhamnosus, etc.,* as well as *Bifidobacterium,* originally isolated from human or animal intestinal tracts have been the most comprehensively studied probiotics. Now a day they are widely added in most of the dairy and fruit based food products as dietary adjuncts (Bernet *et al*., 2004).

To gain a probiotic status, in instance for any microbial strain it is very important to accomplish several essential characteristics. The prime most property is that they

*Corresponding Author: Baboo_M.Nair@appliednutrition.lth.se

must be of human origin and must be able to survive through the gastrointestinal tract (Maruo *et al*., 2006). Resistance to acidic pH and gastric enzymes, tolerance to bile acids and phenolic compound or antibiotics and adherence to intestinal epithelial cells are the other essential properties obliged by the probiotic strains (Schillinger *et al.*, 2005). Adherence to intestinal mucosa is regarded as an imperative attribute of probiotics for colonization within the intestinal, respiratory and urogenital tracts can influence the gastrointestinal immune system and microbiota of the host (Kirjavanainen *et al*., 1998; Forestier *et al.*, 2000).

The need for precise identification of Probiotic strains

Identification and further taxonomic classification of LAB is essential not only for understanding their individual contributions to fermentation process but also to reveal their role in industrial and therapeutic applications and to study probiotic candidature. Moreover, probiotic strains are selected for potential application on the basis of particular physiologic and functional property, some of which may be determined *in vitro*. The classification and identification of a probiotic strain may give a strong indication of its typical habitat and origin. As for safety aspects, it is crucial to be able to compare clinical isolates and biotechnological strains and also to monitor the genetic stability of the strains (Donohue and Salminen, 1996; Klein *et al*., 1998).

Techniques for the isolation, characterization and identification of probiotics strains

For identification of LAB, sorting methods were initially based on physiological and chemotaxonomic features of the specific strain. Almost from the beginning of last decade, phenotypic properties are complemented or have been replaced by different molecular techniques such as DNA-DNA hybridization experiments, DNA sequence analysis, or PCR methods as they have been developed in order to get more consistent and accurate identification of LAB. The probable reason may be identification at the genus level is relatively easy to do for LAB using phenotypic methods but differentiation of species from other related genus is more problematic in most of the cases. Species level identification of LAB often relies mostly on determination of the phylogenetic position using 16S rRNA gene sequence analysis and further genotypic or phenotypic comparison with nearest neighbours. Whole-cell protein fingerprints using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is also an extensively utilized phenotypic database for the identification of LAB. Recent studies revealing genotypic characterization using 'Amplified Fragment Length Polymorphism' (AFLP) provides better differentiation in some of the phylogenetic groups and can serve as an alternative for laborious DNA-DNA hybridizations.

Phenotypic techniques use to identify and characterize Probiotic bacteria

To study any microorganism, especially a bacterium, phenotypic characterization is the indispensable step. In order to identify LAB morphological examinations,

physiological and biochemical tests are widely used phenotypic methods irrespective of source of isolation.

Morphological methods

The first discriminatory trait used to identify and characterize any bacterium is most of time based on cell morphology. Microscopic examination is the fundamental tool by which morphological studies with respect to size, shape and arrangement of the bacterium during their natural growth in suitable medium is possible. It is first criteria that provide information about genus level identification with simultaneous determination of purity of LAB. Gram's staining, endospore staining and capsule staining are the most widely employed methods in order to differentiate the LAB. On the basis of the reaction with the two stains solutions used in Gram staining, bacteria can be divided into two large groups; Gram positive organisms and Gram negative organisms. LAB belong the Gram positive group. Their shape may vary from rounded or spherical morphology are called cocci, elongated rod shaped cells are called bacilli and intermediate in shape between cocci and bacilli are called cocobacilli. They are nonsporulating rods. Several ropy strains which are considered to be polysaccharide producing LAB usually shows presence of capsule –slime layer surrounding the cell during capsule staining procedure.

Biochemical and Physiological methods

Classification of LAB was first done by Orla-Jensen in 1919 that is having a large impact on the systematic of LAB in recent years, too. With time there have been several revisions as considerable extent for classification of LAB. However, the basis of classification is noteworthy unchanged including mode of glucose fermentation, temperatures optima, pH optima, tolerance to salt, and hydrolysis of diverse hexose and pentose sugars. Moreover, some specific tests such as nitrate reduction, arginine hydrolysis, acetoin formation etc. that are based on metabolic activities also help to exemplify the strain (Stiles and Holzapfel, 1997). These characters are still very important in current classification of LAB. LAB can be divided into two groups based on their ability to ferment sugar and more preferably glucose. Some of them are known as homofermentative means in that glucose is converted almost quantitatively to lactic acid while some others are called heterofermentative in that glucose is fermented to lactic acid, ethanol, acetic acid and carbon dioxide. Miniaturized biochemical test kits API 50 CH (bioMerieux, France) were used to study the carbohydrate fermentation profiles of probiotic lactobacilli. The API 50 CH carbohydrate kit comprising 49 different carbohydrate tests is used routinely in biotyping, taxonomy and identification related research studies. Inoculation media are available for specific groups of micro-organisms such as the *Enterobacteriaceae*, lactobacilli, streptococci and bacilli. It is found to be one of the rapid versatile techniques with ease of operation and interpretation of results. In diverse studies probiotic species such as *Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus paracasei, and Bifidobacterium animalis*

have been successfully identified using API 50 CH strips (Román-Méndez *et al.*, 2009, Succi *et al*., 2005, Hamilton-Miller *et al.*, 1999).

Another characteristic used for primary identification is growth at certain temperatures. Lactococci cannot grow at 45 ºC while enterococci can grow both at 45 ºC and 10 ºC. On the other hand, steptococci do not grow at 10 ºC. Growth at different salt concentration provides differentiation especially among cocci shaped starter LAB. This character is useful in order to differentiate *Lactococci, Streptococci* and *Enterococci*. Relatedly, *Lc. lactis* subsp. *cremoris* is distinguished from *L. lactis* ssp. *lactis* by inability to grow at 40 ºC, growth in 4% salt, hydrolyse arginine, and ferment ribose (Axelson, 1998).

Apart from this, other characteristics such as production of exopolysaccharides, presence of certain enzymes, bile tolerance, type of haemolysis, growth factor requirements, growth characteristic in milk and serological typing are also plays essential role in biochemical characterization. Additional characteristics such as fatty acid composition and motility are being used as the basis of classification of the newly described genera of LAB. Francois *et al*.(2008) performed species identification of 20 LAB isolated from cow's raw using API 50 CH and API 20 STREP kits and the SDS-PAGE technique of the whole-cell proteins**.**

Drawbacks of Phenotypic methods and the transition towards genotypic techniques

In general, although phenotypic tests provide some evidence of metabolic capabilities, there are some problems such as a lack of reproducibility and a lack of discriminatory power. Designation of certain neotype strains of LAB based only on phenotypic characteristics gave confused results which were resolved only through molecular techniques.

Recent comparative study between biochemical profile using API CH 50 carbohydrate fermentation test and multiplex PCR technique confirmed that the use of biochemical methods does not appear to be appropriate for the identification and study of vaginal lactobacilli, since the failure rate with the former method was high compared to molecular biology techniques (Brolazo *et al*., 2011). Application of genotypic tools represented an advance in the taxonomy of lactobacilli. Even several lactobacillus species have been reclassified on the basis of fresh information from advanced molecular techniques such as *L. cellobiosus, L. pastorianus, L. arizonensis* have been reassigned to *L. fermentum* (Dellaglio, Torriani, and Felis, 2004), *L. paracollinoides* (Ehrmann and Vogel, 2005), and *L. plantarum* (Kostinek *et al.*, 2005), respectively. Several experiments based on the DNA homology of lactobacilli concluded that some strains previously classified according to their phenotype as *L. acidophilus,* in fact consists of six different groups that cannot be differentiated biochemically. These groups were then genetically classified into six different species: *L. acidophilus*, *L. crispatus*, *L. amylovorus*, *L.gallinarum*, *L. gasseri* and *L. jensenii* (Pavlova *et al*., 2002).

Genotypic methods

Although conventional microbiological methods are important for selection, enumeration and biochemical characterization it is not proficient to classify a culture taxonomically. Genotypic tools are powerful even between closely related species. There are number of alternative taxonomic organization methods including polymerase chain reaction based methods such as PCR-RFLP, REP-PCR, PCR Ribotyping, hybridization with species-specific probes and sequencing of 16s or 23s RNA using species-specific primers (Klaenhammer and Kullen 1999). Even in the natural food fermentation process when bacterial population involved has similar nutritional and environmental requirements, the applications of molecular methods resolve identification problems.

1. Hybridization techniques

Hybridization of nucleic acids either DNA-DNA or DNA-RNA using probe technology could be an alternative for faster and more reliable differentiation. Probes are synthetically prepared oligonucleotides designed to bind to the specific complimentary sequence on the target bacterial genomic DNA. Several species-specific probes have been designed. In most of the studies probes have been designed either against 16S or 23S rRNA genes (Ehrmann *et al*., 1994; Hertel, *et al*., 1993), but 16S rRNA based probes remain more popular due to the smaller size of 16S rRNA in comparison to the 23S rRNA gene. A number of *Lactobacillus* species have been identified using species-specific oligonucleotide probes (Pot *et al*., 1993; Alander *et al*., 1999; Chagnaud *et al*., 2001; Park and Itoh, 2005). However, probes based on other genes such as pyrDFE have also been successfully applied to resolve the differences among some closely related species (Briengel *et al.*, 1996). Occasionally, species-specific fragments obtained from randomly amplified polymorphic DNA - RAPD (Quere *et al*., 1997; Hayford *et al*.,1999) or DNA fragments obtained from restriction digests of plasmids or genomic DNA, have been utilized to obtain specific probes for taxonomic identification of several *Lactobacillus* species (De los Reyes-Gevilan, 1992; Giraffa and Neviani, 2000).

2. Polymerase Chain Reaction (PCR)

PCR is a method for exponential amplification of DNA or RNA sequences and is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. Amplified DNA should run on agarose gel followed by staining with ethydium bromide. Multiplex PCR, nested PCR, real Time PCR, etc. are different modifications in the basic PCR method with respect to their application**.**

3. 16S rDNA Sequencing

Genotypic characterization of microorganisms according to their 16S ribosomal

DNA sequencing was firstly proposed by Woese, in 1987. The application of 16S or 23S rRNA-targeted oligonucleotide probes is the best and most reliable approach to identify bacteria on a phylogenetic basis. The 16S rRNA gene is nearly 1540 bases long and structure is highly conserved. As the probes have the broadest specificity ranging from universal to species specificity, it is possible to use 16S rRNA gene to study phylogenetic relationships between microorganisms and identify them more accurately (Cakyr, 2003, Holzapfel, *et al*. 1998). Besides, the 16S rRNA gene is present in multiple copies in most bacterial genomes and the gene copy number is important for physiological, evolutionary and population studies of the bacteria (Acinas *et al*., 2004; Lee *et al*., 2008). The 16S rRNA gene copy number has been characterized in a few species of *Lactobacillus* and it reveals that *L. delbrueckii* has nine copies of the 16S rRNA gene that is considered to be the highest multiplicity to date (Makarova *et al*., 2006; van de Guchte *et al*., 2006).

While *L. sakei* (Dudez *et al*., 2002) and *L. salivarius* (Claesson *et al*., 2006) each possess seven copies of the 16S rRNA gene per genome, followed by *L. johnsonii* (Pridmore *et al*., 2004), *L. gaseri* (Makarova *et al*., 2006) and *L. reuteri* (GenBank accession number CP000705) that each posses six copies. *L. plantarum* (Chevallier *et al*., 1994; Kleerebezem *et al*., 2003) and *L. brevis* (Makarova *et al*., 2006) found to comprise five copies of the 16S rRNA gene and *L. acidophilus* has been reported to contain four copies (Roussel *et al*., 1993; Altermann *et al*., 2005). In general, after 16S sequencing homology searches of the rDNA sequences is usually performed using the National Centre for Biotechnology Information (NCBI) Blast Library available on the internet (www.ncbi.nml.nih.gov).

4. Random Amplified Polymorphic DNA (RAPD)

RAPD is very simple and quick genotypic method (Farber, 1996). Basic procedure for RAPD-PCR involves amplification of the genomic DNA with a single primer of short length (9 or 10 bases) arbitrary sequence, which get align with sufficient affinity to chromosomal DNA sequences at low annealing temperatures so that they can be used to initiate the amplification of bacterial genome regions. The amplification is followed by agarose gel electrophoresis, which yields a band pattern that should be characteristic of the particular bacterial strain (Caetano-Anolles *et al*., 1991; Meunier and Grimont, 1993). The technique has already been used by Nanda *et al*., (2000) to characterize rice vinegar acetic acid bacteria (AAB). They managed to discriminate among AAB strains and the patterns yielded between 7 and 8 DNA fragments. RAPD analysis is considered a reliable method to distinguish between starter and non-starter species in cheese or to monitor shifts in LAB community during cheese fermentation (Randazzo *et al*., 2009).

5. Ribotyping

In prokaryotes, the three genes coding for rRNA -16S, 23S and 5S rRNA, are separated by spacer region. Most bacterial genera contain multiple copies of the operon for rRNA, thus the spacer regions within a single strain may vary in length and/ or sequence. Ribotyping generates a highly reproducible and precise fingerprint

that can be used to categorize bacteria by the genus at the species level. In this method, after DNA extraction, basically from the actively growing bacterial culture is digested with an appropriate restriction endonuclease and resulting discrete sized fragments are separated in an agarose electophoretic gel. Followed by transferred to hybridization membrane and probed with a radiolabeled ribosomal RNA sequence. Since bacteria have multiple copies of rRNA operons in their chromosome, several fragments in the restriction digest hybridize with the probe resulting into a specific kind of band pattern.

6. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism is based on the principle that related sequences of nucleotides can be compared by exposing them to the same restriction endonucleases. Electrophoresis and staining of fragments from a given sequence yields a characteristic fingerprint, so that different sequences can be compared by comparing their fingerprints. A simple and accurate protocol, based on the direct amplification from the colony of 16S rDNA and later digestion with restriction enzymes, to identify species of LAB isolated from grape must and wine has been performed by Rodas *et al*.(2003). Deveau and Moineau (2005) used RFLP for the differentiation and rapid characterization of *Lactococcus lactis* strains producing exopolysaccharides. Yanagida *et al*.(2005) stated that 16S rDNA-RFLP tenders an efficient method to isolate and distinguish the LAB coccus from their genera into the *Enterococcus* genus, *Lactococcus* genus and *Leuconostoc* genus offering more correct results.

Terminal restriction fragment length polymorphism (TRFLP or sometimes T-RFLP) is a molecular technique initially developed for characterizing bacterial communities in mixed-species samples. TRFLP works by PCR amplification of DNA using fluorescent tagged primer pairs. The PCR products are then digested using specific RFLP enzymes and the resulting patterns could be visualized using a DNA sequencer. The results are analyzed either by simply counting and comparing bands or peaks in the TRFLP profile, or by matching bands from one or more TRFLP runs to a database of known species.

7. Amplified Fragment Length Polymorphism PCR (AFLP-PCR)

The technique was originally described by Vos and Zabeau in 1993. The amplified fragment length polymorphism has become widely used for the identification of genetic variation in strains or closely related species of LAB as this method provides simultaneous detection of various polymorphisms in different genomic regions with high sensitivity. For simple understanding, the genral procedure can be divided into three steps. In the first step, total cellular DNA is digested with one or more restriction enzymes followed by ligation of restriction half-site specific adaptors to all restriction fragments. Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences is allowed to take place in second step, and finally, electrophoretic separation of amplicons is

done on a gel matrix, followed by visualisation of the band pattern in third step. AFLP has mostly been employed in clinical studies, but its successful application for strain typing of the *L. acidophilus* group and *L. johnsonii* isolates has been reported (Ventura *et al*.,2002, Gancheva *et al*., 1999).

8. Amplified rDNA (Ribosomal DNA) Restriction Analysis (ARDRA)

ARDRA is the extension of the technique of RFLP to the gene encoding the small 16S ribosomal subunit of bacteria. An enzymatic amplification using primers directed at the conserved regions at the ends of the 16s gene, followed by digestion using tetracutter restriction enzymes.

The pattern obtained is said to be representative of the species analyzed. Patterns obtained from three or more restriction enzymes can be used to phylogenetically characterize cultured isolates and 16S genes obtained through cloning from community DNA.

9. Rep-PCR

Rep PCR refers to repetitive extragenic pallindromic sequence that occurs in dissimilar Gram-negative and several Gram-positive bacteria (Lupski and Weinstock, 1992). Recently, it is one of the widely used molecular tools suitable for rapid grouping and tentatively identification of LAB. Total 3 distinct families of repetitive sequences have been identified, including the 35-40 bp repetitive extragenic palindromic (REP) sequence, the 124-127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence, and the 154 bp BOX element (Versalovic *et al.,* 1994). These sequences found to be located in discrete, intergenic positions around the genome in both orientations. During PCR, the oligonucleotide primers have been designed to prime DNA synthesis outward from the inverted repeats in REP and ERIC, and from the boxA subunit of BOX, (Versalovic *et al.,* 1994). The use of these primer(s) and PCR leads to the selective amplification of distinct genomic regions located between REP, ERIC or BOX elements. The corresponding protocols are referred to as REP-PCR, ERIC-PCR and BOX-PCR genomic fingerprinting respectively, and rep-PCR genomic fingerprinting collectively (Versalovic *et al.* 1991; 1994).

In rep-PCR method, primers complementary to naturally occurring, highly conserved, repetitive DNA sequences are used. The PCR-products are separated using agarose gel electrophoresis and a species (sometimes strain)-specific pattern is obtained. Rep-PCR yields DNA molecules of various sizes once separated by gel electrophoresis that result in to a characteristic fingerprint which may be unique for a particular strain. These patterns can be analysed using e.g. BioNumerics. Isolates with similar patterns (i.e. belonging to the same species) will cluster together. Full identification can be achieved by e.g. sequencing a limited number of isolates from each group within the cluster.

A variety of rep-PCR-primers have been developed for LAB. The primer GTG

(5'GTG GTG GTG GTG GTG 3') seems to be very suitable for grouping of LAB and yeast at the species level, but other primers may prove better depending on the specific task. Rep-PCR genomic fingerprinting for LAB has become a valuable tool that permits differentiation to the species, subspecies and strain level.

10. Denaturing/Temperature gradient gel electrophoresis (DGGE/TGGE)

DGGE is based on electrophoretic separation of DNA molecules that are the same lengths but have different nucleotide sequences (Lerman *et al*., 1984). It was first used to detect single-base DNA sequence variations (Fischer and Lerman, 1983). In this technique, PCR-amplified double-stranded DNA is subjected to electrophoresis under denaturing conditions that is achieved by a solvent gradient and the migration depends on the degree of DNA denaturation. Giusto *et al*.(2007) used PCR- DGGE to identify the LAB contaminants from dry yeasts routinely used in the wine production. They amplified region V1 of 16S rDNA gene of a DNA fragment through PCR followed by a DGGE technique. *Lactobacillus* spp. and *Pediococcus* spp. were found to be the main contaminant during the study. Temperature gradient gel electrophoresis (TGGE) technique is based on electrophoretic separation of 16S rDNA fragments by using a temperature gradient.

It is generally optimized to reveal differences in the 16S rDNA V3 regions of bacteria with low G+C-content genomes. They checked the potential of TTGE to controlled dairy ecosystems with defined compositions, including liquid (starter), semisolid (home-made fermented milk), and solid (miniature cheese models) matrices and with unknown ecosystems using commercial dairy products. By using the TTGE reference database, it is possible to directly identify a species as a bacterial component of various dairy products including milk, cheese, and fermented milk. TTGE have been used to identify different bacterial species present in several dairy products, including members of the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Streptococcus*, and *Staphylococcus* (Ogier *et al*., 2002).

Both DGGE and TTGE are now methods of choice for environmental microbiologists and have been used to determine the genetic diversities of natural microbial communities such as the communities in biofilms, soil, ocean depths, hot springs, lakes, a biodegraded wall painting, and fermented foods. Heilig *et al*.(2002) stated that the combination of specific PCR and DGGE analysis of 16S rDNA amplicons allows the diversity of important groups of bacteria that are present in low numbers in specific ecosystems to be characterized, such as the lactobacilli in the human GI tract.

11. Pulsed Field Gel Electrophoresis

In this method, the genomic DNA is treated with a restriction enzyme followed by separation of fragments on an agarose gel. It provides resolution at subspecies and strain level. Initially, live cells are embedded in agarose and then lysis is achieved.

Hence, genomic DNA is digested with infrequent cutting restriction enzymes. This method provide alternative electrical field with predetermined intervals. Direction of electrical field is changed at these intervals so called pulse times. Thus, based on this property higher molecular weights DNA fragments could be separated with this method.

Reuter et al.(2000) used these classical phenotypic methods for the identification of species, biotypes and even special strains within a species for probiotic cultures. They also applied molecular methods for confirmation of species and for the distinction of special strains, comprised plasmid content of strains, fingerprinting of total soluble proteins of whole cells with SDS-PAGE (Klein, Hack, Zimmermann, and Reuter, 1994; Pack, 1997), RAPD-PCR and the PFGE-technique methods fostrain-specific differentiation (Piehl, 1995; Klein *et al*., 1998), and finally dot blot hybridisation-technique with gene probes for easier identification on the species level (Goldberg *et al*., 2000). In one of the study conducted by Tynkkynen *et al*., (1999)., PFGE was found to be the most discriminatory method, revealing 17 genotypes for the 24 strains studied that were biochemically identified as members of the *Lactobacillus casei* group while ribotyping and RAPD analysis yielded 15 and 12 genotypes, respectively. The high discriminatory power of PFGE has been reported for the differentiation between strains of probiotic bacteria, such as *B. longum* and *B. animalis*, *L. casei* and *L. rhamnosus* (Tynkkynen *et al*., 1999), *L. acidophilus* complex (Roy *et al*., 2000), *L. helveticus* (Lortal *et al*.,1997), and *L. johnsonii* (Ventura *et al*., 2002).

12. Fluorescence in situ hybridisation (FISH)

FISH is a one of the versatile technique used for the rapid identification of LAB. It permits the direct identification and quantification of bacterial species at microscopic level without previous cultivation. It consists of fluorescent labelled DNA probes that would specifically hybridise each of the species or genera. FISH in situ hybridization enables the direct enumeration of whole bacterial cells in samples using either fluorescence microscopy or flow cytometry (Brunser *et al*., 2006). Flow cytometry is a rapid and sensitive technique that can determine cell numbers and measure various physiological characteristics of each individual cell, using appropriate fluorescent dyes. In 1999, Franke and co-workers used this method to detect *Ga. sacchari* and other wine related microorganisms including some species of LAB. Blasco *et al*. (2005) identified species of LAB commonly found in wines with the aid of same technique using fluorescent oligonucleotide probes, homologous to 16S rDNA.

13. DNA micro-arrays

Miniaturization of DNA hybridization techniques has led to the development of DNA chips or DNA micro-arrays with respect to taxonomy and 'omics' studies, (Bae *et al*., 2005). In this technique, an array of oligonucleotides, cDNA, or genomic DNA can be immobilized on a small glass slide in such a way that one sample can be tested against a large number of probes simultaneously.

Conclusion

Current molecular tools along with conventional methods have significantly enhanced our knowledge of the complexity of LAB from various different sources like food matrixes. It is observed that some molecular methods such as Real Time PCR, Fluorescence in situ hybridisation (FISH), PCR-RFLP and DGGE help to characterize the bacterium up to species level whereas in most of the circumstances with respect to RAPD-PCR, AFLP, Rep-PCR it is possible to identify the LAB up to strain level, too. To date, the most widely used techniques for bacterial identification and quantification are based on the 16S rRNA gene. All these phenotypic and molecular approaches play a key role for the screening and selection of probiotic bacteria and assessing their role in diverse fermented products during fermentation. Furthermore, they also help to access the viability and vitality of probiotic LAB during processing and analysis of their presence, persistence, and performance in the gastrointestinal tract.

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