ABSTRACT: The use of a probiotics is widely accepted as natural means to promote a health which matters both for a both humans and animals as well. The mechanism of effect action of probiotics is directly associated connected with the properties of a selected probiotics strain. Selection is an important challenge that requires a platform of basic information about physiology and genetics of a candidate strain relevant to their intestinal role, functional activities, and interaction with the other micro flora. During the selection of while selecting an existing strain, it is of much importance to have a detail study about its origin, genetic makeup, and growth characteristics necessary to consider the origin of a strain, genetics and growth properties in vitro and in vivo. Probiotics must have the ability to exert a beneficial effect on a host, withstand into food stuff at a high cell count and remain viable throughout the shelf life of a product, withstand transits through a GI tract, adhere to intestinal epithelium cell lining and colonize the lumen of the tract, produce antimicrobial substances towards pathogens, technologically suitable for industrial processes and should be an associated with a pronounced health benefits. In the case of novel involvement of novel microorganisms and genetically altered modified strains as probiotics emphasizing their safety and risk while formulating new food has to be accessed. This review helps researchers to include the maximum select criteria for appropriate probiotics strain.

KEY WORDS: Functionality, Health aspects, Nomenclature, Probiotics, Safety, Survival ability

PROBIOTICS SELECTION

While selecting the probiotics strain, A safety entries must be kept in mind regarding production /manufacturing relating to the technological aspects, application, survival and colonization in the host and their health benefits. In vitro and vivo experiments can be used to justify above criteria. The use of probiotics in human will require their characterization and in vivo, human trials. Characterization of the probiotics is important in concern with gain the knowledge of the strain and mechanism of the probiotic action (Caselli et al, 2012, Kapitula et al. 2008). It is recommended to employ a combination of phenotypic and genetic techniques to accomplish the identification, classification, and typing. Vitro
studies are followed by studies in the animal models, after satisfaction turns towards human followed by clinical studies (phase 1), individual patient studies (phase 2) and finally large-scale human studies (phase 3). Ultimately appropriate target specific in vitro tests that correlate with the vivo results are recommended (Cogan 1996; Robinson, 2002).

**GENERAL ASPECTS**

**Origin**

The origin of probiotics depends upon the application of probiotics. Probiotic should be originated from a targeted animal microflora. The source can be from a human origin like human large intestine, small intestine, or a breast milk, animal origin, food source like a raw milk or fermented food. When selecting for human purpose probiotic strain should be isolated from a human microflora are more likely to adhere to human intestinal wall than others and more likely to safe. Recently, it has been indicated that the infant microflora reflects the bacterial composition of the breast milk. Therefore, the natural microbiota of human milk could be proposed as a source for the isolation of novel probiotic bacteria. The strain should be properly isolated and identified before use (Dash 2009; Elmer& MacFarland et al.2007).

**Genus, Species and Strain Identification**

According to the WHO/FAO guidelines, probiotics are the strain specific so it must be identified at genus, species and strain level. It is recommended to employ a combination of phenotypic and genetic techniques to accomplish the identification, classification and typing. For the nomenclature of the bacteria scientifically recognized names must be applied. The older or misleading nomenclatures should not be applicable on a product label. Several genera of the bacteria and yeasts have been proposed as a probiotic cultures, the most applicable on a product label. Several genera of the bacteria applied. The older or misleading nomenclatures should not be used (Nemcova 1997). The primary identification criteria used for phenotypic characterization of strains are cell morphology, determination of metabolites, enzyme activity and the ability to utilize a sugar, and molecular tool have been developed for identifying the probiotics based on the analysis of nucleic acids and other macromolecules because of high potential provided by the using polymerase chain reaction (PCR) amplification and hybridization with DNA and RNA. The DNA-DNA hybridization technique is still reference due to labor intensive and time consuming. Fatty acid methyl ester (FAME) and DNA Sequence Encoding 16SrRNA are also be used for the identification. WHO recommends all strains should be deposited in an internationally recognized culture collection (Cogan 1996; Kapitula 2008; Dash 2009).

**Biosafety**

Selected strains should be non-pathogenic and non-toxic. Generally the Lactic acid bacteria have good record in safety. The strains of microorganisms should be Generally Recognized as Safe (GRAS), for example *Lactobacillus species*, *Bifidobacterium species* and *Streptococcus (Enterooccus)* species, and should be following Qualified Presumptions of Safety (QPS) considering by the European Food Safety Authority (EFSA). Before selecting other probiotics, toxicological studies must be performed. Soil based organisms and spores claimed as a probiotics (Kosin and Rakshit 2006).

Probiotics strains must be characterized at a minimum with the following tests:

1) Assessment of the side effects during previous human studies.
2) Assessment of certain metabolic activities (e.g. D-lactate production, bile salt deconjugation.)
3) Determination of antibiotic resistance pattern.
4) Post market surveillance of adverse incidents on consumer (Salminen 1998 a; Salminen and Wright 1998 b).

**Functional Aspects**

**Resistance to the gastric condition**

Probiotic bacteria must be able to survive in the gastrointestinal tract. Bacterial strains in the human gastric juice are more accurate indication of the ability of the strains to survive passage through the stomach. The survival of ingested probiotics in different parts of gastrointestinal tract varies with the strain. Some strains are rapidly killed in the stomach while others, such as strains of *Bifidobacteria* or *L. acidophilus*, can pass through the whole gut at very high concentration. Lactobacilli are more acid tolerant in the food career on the other hand food matrix determines their pH tolerance. The pH of the gastric environment is 1.2 to 2.5. Numerous in vitro and vivo studies have demonstrated that probiotics organisms can survive in the gastric transit where the cells as exposed to pH values <2.0, though the time exposure (1 to 2 hr) is relatively short (Dunne, Lisa et al.2001). Survival of lactobacilli in the acidic environment has also been enhanced in presence of the metabolized sugar that allow the cell membrane proton pumps to operate and prevent the lowering of intracellular pH (Binns, 2013). The bifidobacteria however proved less acid resistance than the lactobacilli, particularly when exposed to the human gastric juice (Robinson, 2002; Salminen, Wright 1998).

**Resistance to the bile acid**

Probiotics organisms must be resistance to bile acids. Bile acids are synthesized in liver from cholesterol and secreted from the gall bladder into the duodenum in the conjugated form (500-700 ml/d). These acids undergo extensive chemical modifications (deconjugation, dehydroxylation, dehydrogenation, and degluronidation) in the colon almost solely as a result of which microbial activity ceases. (Mourad and Eddine 2006). *Lactobacillus* and *Bifidobacterium* strains isolated from the human ileum were tested against a bovine bile, porcine bile and human bile. The result observed for both the strain exhibited resistance to the bovine bile while the
porcine bile proved significantly more inhibitory to both of these bacterial groups. However, in relation to the assessment of the probiotic strain intended for the human consumption, the most relevant determination is that ability to grow in the human bile (Dunne et al. 2001; Cho 2010).

Adherence and colonization of intestinal epithelium/ tissues

Probiotic adhere and colonizes. This adhesion of probiotic to intestinal mucus and epithelial cells has long been considering one of the most important selection criteria for probiotic microorganisms. Adhesion to the intestinal mucosa may prevent the probiotic cells being washed out and therefore, enabling temporary colonization, immune modulation and competitive exclusions of pathogens. In order to produce enzymes, lactic acids, vitamins and natural antibiotics, the probiotic strain must be adhere to the intestinal wall, colonize and multiply. Probiotic strain isolated from the human gut adheres and colonizes better than probiotic strain isolated from the animal origin (Kullen, Klaenhammer 1999; Nemcova 1997).

Antimicrobial activity against potentially pathogenic bacteria

The probiotic strain should be capable of producing antimicrobial substances is most important in developing the probiotic supplement and probiotic rich foods. Several metabolic compounds produced by lactic acid bacteria (including organic acids, fatty acids, hydrogen peroxide and diacetyl) have antimicrobial activity. However, bacteriocins or proteinaceous substances with specific inhibitory activity against closely related species are most studied. At present, nisin (produced by the Lactobacillus lactis subsp. Lactis strains) is the only purified bacteriocin approved for use in the product intended for human consumption. Nisin is active against the stain mainly Staphylococcus, Micrococcus, and Listeria species. Apart of nisin, other probiotic strains are also produces a various kind of bacteriocins which adverse effect on a various pathogenic organisms. Lactococcus, Salivarcin, Acidocin 8912, Plantarcin, Lacticin A is produced by lactococcus Lactis, Streptococcus salavarius L. acidophilus L. plantarum L. delbrueckii respectively (Dash 2009; Mishra, Prasad 2000).

Modulation of immune system

Strains of probiotics should be able to stimulate as well as regulate a several aspects of the natural and the acquired immune response. There is a significant difference between the ability of the Bifidobacterium and a Lactobacillus strain to influence the functioning of the immune system appears. The intake of specific strains of the probiotics has also been shown to enhance the immune response to natural infections and systematic or oral immunization in the human subjects. Probiotics are thus suggested to confer the protection against enteropathogenes by stimulating the cytokine production; Enhancing the phagocytic capacity of polymorphonuclear cells and microphages; Enhancing the specific antibody response to the pathogens (Dunne et al. 2001; Kosin, Rakshit 2006).

Health Aspects

The selection of the probiotic organisms depends upon a health claims. Probiotic must be able to exert their benefits on the host through the growth and/or activity in the human body. Most proven probiotics strains are human origin, a strong case can be made that they are normal commensals and, therefore, safe to use. To achieve the health benefits, probiotic bacteria must be viable and available at high concentration, typically 10^8 to 10^10 CFU/g of product (Mishra, Prasad 2000). There is a need for refinement in vitro tests to predict the ability of probiotics to give health benefit to human being. They should provide benefit against gastroenteritis, irritable bowel syndrome, and Inflammatory Bowel Disease (IBD; Crohn’s disease and ulcerative colitis), diarrhea, cancer, depressed immune function, inadequate lactase digestion, infant allergies, failure-to-thrive, hyperlipidaemia, hepatic diseases, helicobacter pylori infections and AIDS (see the all issue of International J of Probiotics and Prebiotics edited by Masci et al, 2013).

Production Aspects

Acid production

The rate of acid development is a critical criterion for the selection of the probiotics in milk-fermented products. A rapid acid production in the raw material not only helps to prevent the growth of unwanted microorganisms but is also essential for the aroma, texture, and flavor of the end product, which has positive influence on its overall acceptability in the form of pH, texture, flavor, and aroma of the product. (Hussain, Khan 2008; Dunne et al. 2001).

Proteolysis

Proteolysis (Casein hydrolysis) contributes to the texture, flavor and body development in the end product like different varieties of cheeses and yogurts. Hence this criteria is an important consideration in lieu of the above products. The ability to produce cell wall bound Extra Cellular Proteinases (CEP) is very important feature of T-LAB in the hydrolysis of milk proteins (casein) which supply amino acids to the cells that are essential for growth of LAB. Protein degradation is mainly associated with Lactobacillus bulgaricus and Streptococcus thermophilus (Kosin, Rakshit 2006).

Probiotic stability and viability

Probiotic must have the capabilities for its survival in the food, feed and dietary supplements. Manufacturer has given a great attention to probiotic stability. More importantly the probiotics strain should be stable enough to withstand a conventional industrial production process. Stability is also a strain specific.

Probiotic stability is affected by the high temperature, oxygen humidity and high water activity in the culture. The criteria for probiotic selection to study its smooth stability depend on the following factors:

1. Ability of probiotics to survive and maintaining in
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the storage without loss of viability.
2. Ability to grow quickly to maximum concentration in a simple and cheap fermentation medium.
3. Ability to grow and survive in microaerophilic or aerobic condition.
4. Ability to withstand physical handling without significant loss of viability.
5. Ability to survive in the food matrices and during the processing (Salminen and Wright, 1998).

Microencapsulation, appropriate selection of acid and bile resistance strains, use of oxygen impermeable containers, two step fermentation, stress adaptation and incorporation of the micronutrients such as peptides and amino acids are the methods used to increase the probiotic stability. Good manufacturing practices (GMP) must be applied in the manufacture of the probiotic food and supplements with quality assurance and shelf life conditions established. The codex general principles of food hygiene and guidelines for application of Hazard Analysis and Critical Control Point (HACCP) should be followed (Gueimonde and Salminen 2006; Dash 2001).

Storage

Storage of probiotic supplement at 4 to 5°C is recommended to maintain the viability of the micro-organisms and they should be used prior to the expiration date of the product. Probiotic supplements must be kept in refrigerated conditions otherwise they have not stay viable. There are some probiotic products, which may be shelf-stable according to their manufacturers, and their storage and shipping requirements must be met (Dash 2009).

Quality Control Aspects

The quality control criteria are important in concern with the approval of the probiotic over the specific health claims. Thus functional food regulations should take into account strain properties and their stability during the industrial processing and use.

Consideration for the probiotic manufacturing includes quality control procedure such as:
1. The criteria and procedures for quality control must be determined and implemented.
2. Verification of genetic identity of selected species.
3. Assuring the probiotic potency.
4. Ensuring the purity of probiotics.
5. Ratifying the finished product through independent testing (Tuomola, Crittenden et al. 1998)

Criteria Used For Multistrain Selection

Functionality of a multistrain probiotic could be more effective and more consistent than that of the monostrain probiotics. Interaction among the lactic acid starters and probiotic bacteria has been investigated to establish adequate combination of strains to manufacture probiotic dairy products using strains of *Streptococcus thermopiles*, *Lactobacillus delbrueckii* sp. *bulgaricus*, *Lactococcus lactis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium spp.* The strains used in multistrain and multispecies probiotics should be compatible and preferably synergistic. The design and use of multistrain and multispecies probiotics need to be studied well before use. However the special attention should be given to avoid a combination of probiotic strain showing inhibitory properties i.e. production of hydrogen peroxide, or bacteriocins. Probiotics are strain specific, condition specific and dose specific. The probiotic formulation must not contain a multi strain/ species and a low moisture and low moisture activity (Kosin, Rakshit 2006; Dash 2009).

PROBIOTIC LABELING

India, for an example, is in a nascent stage, as far as development and marketing of probiotic products is concerned. Therefore, stringent labeling should be laid down to prevent misleading to the consumers. The term ‘probiotic’ should only be used on the labels of the products if physiological (health) benefit in humans is well established. Regulatory framework established to better address issues related to probiotics including efficacy, safety, labeling, fraud and claims. The International Probiotic Association (IPA) and World Health Organization (WHO) provide the guidelines for the labeling of probiotics. Under these guidelines the probiotic product should be contain following information:

- Genus, species and strain designation following standard international nomenclatures.
- The minimum viable numbers of each probiotic strain should be specified at the level which efficacy is claimed and at end of shelf life.
- Evidence based health claims should be clearly stated.
- The suggestion serving size deliver the minimum effective quantity of the probiotic related to the health claims.
- Nutrition supplement facts
- Manufacture lot number and expiration date
- Address of manufacturers
- Proper storage condition and shelf life should be mentioned (Elmer et al.2007; Salminen 1998).

HOW TO IMPROVE A PROBIOTICS

The use of biotechnology for the improvement of microorganisms probably offers great impact for future development of probiotic strains. Only by using biotechnology, it is possible to isolate the genes that are responsible for specific traits such as acid production, flavor production, adherence, stress response and bacteriophage resistance and transfer these genes to a probiotic strain without altering other beneficial properties and adverse effects (Cogan 1998). Microencapsulation may also provide a novel frontier in their active delivery through the gut (Soodbakhsh et al, 2012).
CONCLUSION

In the case of new products, the objective is to produce products that are at least as safe as conventional counterparts regardless of potential health benefits. So the proper selection and design of probiotic is important. Generally the origin, strain, and safety characteristics of the targeted strain must be studied. For generality and functionality of the probiotics in vitro and in vivo test should be there for knowing the functional and health aspects of probiotics. Both the test is correlated to each other for output. The production aspects, like stability of probiotics under different manufacturing conditions and food environment is also taken into consideration for their selection and GMP should be follow at the manufacturing level (Levin, 2011). Quality control aspects have to be considered for the desirable characteristic and output of the probiotics. Multistrain probiotics can be selected for improvement of functionality (Saxelin et al, 2010).

REFERENCES


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