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Probiotics health benefits: comparison between observed data and published data with special reference to lipolytic bacteria

Madhu Rathore☼, Kanika Sharma

Department of Biotechnology, Mohanlal Sukhadia University, Udaipur (Rajasthan) 313001, India

☼Corresponding author:
Department of Biotechnology, Mohanlal Sukhadia University, Udaipur (Rajasthan) 313001, India, Email: madhurathore25@gmail.com

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ABSTRACT

Dairy and fermented foods are rich source of beneficial nutrients. Presence of probiotics or enrichment of this dairy samples with bacteria which are good for health, improved its medicinal as well as nutritional value. Present review involves the study of published data encompasses the research done on dairy and fermented foods with special focus on lipolytic probiotic *Lactobacillus spp*.

Observations as well as published data both confirms that consumption of fermented and dairy food articles help to maintain beneficial microflora of gut. This further proved by comparison made between obtained results and published data. Presence of *Lactobacillus* spp with lipolytic activity in camel milk collected from Rajasthan region, are of special importance. In present review, comparison has also been made between obtained results for dairy as well as fermented food samples and results from published data. Study also involves screening data of samples yoghurt, curd, unfermented and fermented camel milk, batter, pickle fermented fish. Present research and published data both suggested that more study of probiotics on available dairy products and fermented foods can help to solve many health related issues by natural means. However quality assurance study also matters but there are least chances of failure of such products.

Keywords: Dairy foods, fermented foods, probiotics, lipolytic, Lactobacillus, health benefits

1. INTRODUCTION

A complex association of microbes reside in lumen of the human gut. The gut microbiome collectively adds up to nutritional, physiologic and protective processes in the human host. Activity of these microbes involves the fermentation of unabsorbed dietary carbohydrate with production of short chain fatty acids, production of vitamins like biotin and vitamin K, intervention of immune responses and oral tolerance, and protection of the host against invasion by pathogenic microbes (Kau et al., 2011)

Since probiotic food products confers several health benefits like reduction of cholesterol level, improvement of immune function, resistance to infectious diseases and prevention of colon cancer, it led the development of concept of probiotic microbes hence enhanced consumption of food preparations containing LAB and Bifidobacteria (Nagpal et al., 2012).

Research for probiotic bacteria with specific enzymatic activity could be helpful to solve several health issues. As probiotics already known for their health benefits but good bacteria with significant lipolytic activity can add to their health benefits.

In this multifaceted research area on the microbiota, the premeditated use of probiotic bacteria can add significantly to gain better knowledge of constructive microbe-host interactions whereby elementary, therapeutic, dietetic, and mercantile aspects are taken into account. As concerned in the definition of a probiotic bacterium, i.e., “administration of adequate amount of a live microorganism that which confers a health benefit on the host” (FAO/WHO. 2001), this field mainly involves the health effects of specific strains after oral intake, for example, in functional food products.

While the term “probiotic” cannot be used just as a synonym for putatively valuable members of the microbiota, members of the human microbiota are often sources from which probiotics are isolated based on suggested properties such as definite health benefits, survival and persistence in the host, demonstrated protection, and constancy (Tuomola et al., 2001). While bifidobacteria and other genera are also increasingly being applied as probiotics, this review will focus on *Lactobacillus*, given their long history of traditional use in food fermentations of products derived from animals (milk and meat etc.) or plants (vegetables and olives, etc.).

The category of Lactic acid bacteria includes the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus* and others, have arriving particular interest as probiotics, since many of them are classified as generally regarded as safe (GRAS) organisms (Bernardeau *et al*. 2006; Heczko *et al*. 2006). *Lactobacillus* spp. produces antimicrobial factors and bacteriocins which make them attractive candidates for prevention and treatment of a variety of infectious diseases (Aiba *et al*. 1998). In effect, Lactobacilli have been used to reduce *Salmonella* loads and eradicate various pathogens from chickens, pigs and other animals (Pascual *et al*. 1999; Walsh *et al*. 2008; Chen *et al*. 2012), making them a sensible choice to evaluate for marine mammal health promotion.

Lactobacilli produce lactic acid as a end product of carbohydrate metabolism hence included in the category of Lactic acid bacteria (LAB). The genus *Lactobacillus* comprises a large heterogeneous group of low-G+C gram-positive, non-sporulating, and anaerobic bacteria (Claesson et al., 2007).

Taxonomic classification of *Lactobacillus* is the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*. Nutritionally *Lactobacillus* are fastidious, grow on nutritionally rich media (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins) (Kandler and Weiss, 1986).

2. PROBIOTIC BACTERIAL TYPING

In many cases, bacterial typing is highly recommended which involve characterization of probiotic cultures at the individual strain level. It may involve screening of microbiological culture for the presence of probiotic properties, screening of selected strains during *in vitro* and *in vivo* trials, quality assurance of probiotic products, strain authentication in legal matters, and epidemiological surveys.

Molecular methods are specially liked to type probiotic microorganisms (Holzapfel et al., 2001). Typing methods are also available to identify the strains upto strain level such as fingerprinting methods that have been used for species identification such as (rep)-PCR and AFLP. Especially in the case of AFLP, the flexibility in choice of specific restriction enzymes and selective PCR primers allows to test multiple combinations, which can significantly increase the discriminatory power at strain level. On the basis of number of isolates to be processed, the necessary speed of performance, and the skill of the user, also randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) can be used for molecular typing of probiotic strains. However, RAPD is fast and consistent comparison of sets of isolates in a single PCR run, but could not provide same level of reproducibility presented by other PCR-based fingerprinting techniques, which is a chief condition for database erection. Despite of this lack of reproducibility, RAPD is still recurrently used to distinguish between probiotic strains (Manan et al., 2009; Ratna Sudha et al., 2010)

Simultaneously with AFLP, by far the PFGE is also a technique which gives highest resolution at strain level. Even though the latter scheme can be reliable, Prerequisite of this technique is, it requires dedicated electrophoresis unit and considerable technical expertise. PFGE fingerprinting has been fruitfully used to discriminate or trail probiotic strains in the genera *Bifidobacterium* (Masco et al., 2005), *Lactobacillus* (Coudeyras et al.,2008), and *Bacillus* (De Baets et al., 2009).

In addition to DNA fingerprinting, individual probiotic strains can also been discriminate and detect on the basis of sequence-based approaches. The availability of complete genome sequences of probiotic strains not only broadens our biological knowledge of their functional potential, but also offers a numerous of novel possibilities for strain identification. Based on internal nucleotide sequences of multiple (usually three to seven) housekeeping genes, several multi-locus sequence typing (MLST) schemes have been developed for discrimination between bacterial isolates at the intra-specific level. For each gene, 450–500 base pair internal fragments with a maximal number of polymorphisms are selected for amplification and subsequent gene sequence analysis. For each housekeeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the loci describe the allelic profile or sequence type. The MLST method can be automated to a larger extent and has several advantages, as well as reproducibility and data exchangeability. While MLST is mainly used to study population constitution, evolution, and phylo-geography of bacterial pathogens (Achtman, 2008), it also provides good discriminatory power to differentiate isolates for typing purposes which mainly depends on the species. Clinically relevant microorganisms such as *Enterococcus faecium* (Homan et al., 2002) and *Bacillus cereus* (Helgason et al. 2004) were the first for which MLST schemes was developed. In recent years, MLST schemes have also been developed for some other species commonly used as probiotics. MLST schemes relevant for typing of probiotic Lactobacilli include those developed for the species *Lb. casei* (Cai et al, 2007; Diancourt et al., 2007), *Lb. plantarum* (de las Rivas et al., 2006), and *Lb. Salivarius* (Raftis et al., 2011). Recently, an MLST scheme was made available for the probiotic*Bifidobacterium* species *Bf. animalis*, *Bf. bifidum*, *Bf. breve*, and *Bf. longum* (Delétoile et al. 2010). Similar to the mlst.net database for bacterial pathogens, MLST sequence type databases have been launched, for example, for *Lb. casei* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Lcasei.html) and *Bifidobacterium* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Bifidobacterium.html). These public databases allow queries and download of allele sequences and allelic STs, provide several database tools and statistics, and are also open to additional data on novel strains and species.

In addition to MLST approaches, unique gene sequences can also identified through comparative genomics that allow to discriminate a given probiotic culture from other members of the same species. Such strain-specific target sequences can then be used to notice a given probiotic in complex environments, such as food matrices or fecal samples, without the need for culturing. As an alternative, culture-independent methods such as fluorescent in situ hybridization and real-time PCR are employed for direct detection and enumeration of the target strain in the sample.

3. PUBLISHED DATA ON PROBIOTIC *LACTOBACILLUS* STRAINS SPECIALLY REPORTED FROM DAIRY AND FERMENTED FOOD SOURCES

Bassyouni et al., (2012) obtained the collection of fifty four isolates. Among these fifty isolates, eight isolates from different dairy products were observed as potential probiotic safe for human use. He also checked these isolates for probiotic properties. The isolated bacteria were studied for antagonistic effects on clinically isolated *E.coli*, *Salmonella spp*. *Micrococcus spp*., *Staphylococcus spp*.; where they found to be tolerant to low pH and bile salt and effective against isolated *E.coli*, *Salmonella spp*. *Micrococcus spp*. API ZYM Kits and antibiotic sensitivity kits were used to check enzymatic activity of isolates. Biochemical and physiological results indicated that they were found to be related to the genus Lactobacillus and suggested to belong to *L. casei* (4 isolates), *L. Acidophillus* (3 isolates) and *L. Lactis* (1 isolates) and showed antagonistic activity against *E.coli* ,*Salmonella spp*. *Staphylococus spp*. These isolates produce β- galactosidase enzyme, which is beneficial for lactose intolerance. *Lactobacillus spp*. produced enzymes including leucinearylamidase, crystinearylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosamidase so authors concluded that human milk, yogurt and raw milk are considered a good source of potential probiotic strains also the isolated bacteria had no haemolytic activity so it consider as a great potential probiotic and safe for human use.

Haghshenas et al. (2015) isolated *Lactobacillus* isolates from sheep dairy products (yogurt and ewe colostrum) with probiotic and anticancer activity. 125 lactic acid bacteria were isolated from total 100 collected samples of colostrums and yogurt samples. Out of these, 17 *Lactobacillus* strains belonging to five species (*L. delbrueckii*, *L. plantarum*, *L. rhamnosus*, *L. paracasei*, and *L. casei*) were identified. Colostrums samples were found to possess *L. plantarum* 17C and 13C, showed remarkable results such as resistant to low pH and high concentrations of bile salts, susceptible to some antibiotics and good antimicrobial activity hence show good candidature of potential probiotics. Seven strains (1C, 5C, 12C, 13C, 17C, 7M, and 40M), the most resistant to simulated digestion, were further investigated to evaluate their capability to adhere to human intestinal Caco-2 cells. *L. plantarum* 17C was the most adherent strain. The bioactivity assessment of *L. plantarum* 17C showed anticancer effects via the induction of apoptosis on HT-29 human cancer cells and negligible side effects on one human epithelial normal cell line (FHs 74). The metabolites produced by this strain can be used as alternative pharmaceutical compounds with promising therapeutic indices because they are not cytotoxic to normal mammalian cells.

Research study conducted on isolation of Lipolytic *Lactobacillus* isolates from yogurt. Pin pointed colony with catalase negative activity was isolated and Biochemical profiling suggested them as *Lactobacillus* sp. This isolates also showed good resistance towards pH in the presence of pepsin and amylase. Bile acid tolerance was also observed in the same. *Lactobacillus* isolate also showed good antioxidant activity yet to be checked for cell line adhesion property. This isolate showed good antimicrobial activity against *E. coli*. Dairy products are potential source of probiotic bacteria and consumption of the same can exert several health benefits to the human beings.

Similarly, Balamurugan et al.(2014) reported in their published paper that Fifteen LAB (12 lactobacilli, 1 lactococcus, 2 Leuconostoc) and one yeast purified from home-made curd were evaluated for resistance to acid, pepsin, pancreatin and bile salts; antimicrobial resistance; intrinsic antimicrobial activity; adherence to Caco-2 epithelial cells; ability to block pathogen adherence to Caco-2 cells; ability to inhibit interleukin (IL)-8 secretion from HT-29 epithelial cells in response to *Vibrio cholerae*; and ability to induce anti-inflammatory cytokine expression in THP-1 monocyte cells. Results showed that *Lactobacillus* abundance in fermenting curd peaked sharply at 12 h. Nine of the strains survived exposure to acid (pH 3.0) for at least one hour, and all strains survived in the presence of pancreatin or bile salts for 3 h. None showed haemolytic activity. All were resistant to most antimicrobials tested, but were sensitive to imipenem. Most strains inhibited the growth of *Salmonella typhimurium* while five inhibited growth of *V. cholerae* O139. Seven strains showed adherence to Caco-2 cells ranging from 20-104 per cent of adherence of an adherent strain of *Escherichia coli*, but all inhibited *V. cholerae* adherence to Caco-2 cells by 20-100 per cent. They inhibited interleukin-8 secretion from HT-29 cells, in response to *V. cholerae*, by 50-80 per cent. Two strains induced IL-10 and IL-12 messenger ribonucleic acid (mRNA) expression in THP-1 cells. LAB in curd had properties consistent with probiotic potential, but these were not consistent across species. LAB abundance in curd increased rapidly at 12 h of fermentation at room temperature and declined thereafter.

Curd is also a source from which potential probiotic lipolytic *Lactobacillus* sp purified with 14mm Zone of clearance on Tributyrin agar plate. Isolate showed good probiotic potential as: resistance to low pH in the presence of pepsin and amylase enzyme, bile acid tolerance, antimicrobial activity against *Bacillus subtilis* and good antioxidant activity also.

According to the Davati et al. (2015) camel milk is amongst valuable food sources in Iran. Researchers described the presence of probiotic bacteria and bacteriocin producers in camel milk. Isolation and molecular identification of lactic acid bacteria were done from camel milk and also evaluate them for the presence of probiotic properties. A total of ten samples of camel milk were collected from the Golestan province of Iran under aseptic conditions. A total of 64 isolates were analyzed based on biochemical tests and morphological characteristics. The most frequently isolated LAB was *Enterococci*. *Weissella*, *Leuconostoc*, *Lactobacilli* and *Pediococci* were less frequently found. Based on restriction analysis of the ITS, the isolates were grouped into nine different ARDRA patterns that were identified by ribosomal DNA sequencing as *P. pentosaceus*, *Enterococcus faecium* strain Y-2, *E. faecium* strain JZ1-1, *E. faecium* strain E6, *E. durans*, *E. lactis*, *Leuconostoc mesenteroides*, *Lactobacillus casei* and *Weissella cibaria*. The results showed that antimicrobial activity of the tested isolates was remarkable and *P. pentosaceus* showed the most antibacterial activity. In addition, *E. durans*, *E. lactis*, *L. casei* and *P. pentosaceus* were selected as probiotic bacteria.

In present research fermented camel milk and unfermented camel milk screened for the presence of *Lactobacillus* using selective media i.e. MRS agar. Isolated bacteria were found to possess significant lipolytic activity. These bacteria were also checked for probiotic properties and showed good response against low pH in the presence of pepsin and amylase enzyme, show bile salt tolerance activity and show antimicrobial activity against *Pseudomonas aeruginosa* and *Proteus vulgaris*.

Fermented fish, dosa batter, pickle also screened for the presence of lipolytic probiotic lactic acid bacteria. Purified isolates also showed significant lipolytic activity. These isolates were checked for probiotic potential, and found to possess good probiotic potential as showed good growth on low pH in the presence of amylase and pepsin enzyme, show bile acid tolerance, exhibit antimicrobial potential etc.

Fermented fish products are important dietary components in the protein deficient far East especially in southeast Asia. It is an old technology to preserve fish by adding salt in it. This method of preservation still enjoys popularity in many developing countries owning to its simplicity and low cost of processing. When fatty fishes are salted there is usually a certain degree of fermentation involved. Fermentation of fish is brought about by autocatalytic enzymes from fish and microorganisms in the presence of high salt concentration (Majumdar & Basu, 2010). *Lactococcus lactis subsp. cremoris*, *Lactococcus plantarum*, *Enterococcus faecium*, *Lactobacillus fructosus*, *L. amylophilus*, *L. corneformis subsp. torquens* are predominant LAB species reported in fermented fish. *Lactobacillus sake*, *L. curvatus*, *L. divergens*, *L. carnis*, *L. sanfrancisco*, *Leuconostoc mesenteroides*, *E. faecium*, *L. plantarum*, *L. brevis*, *Pediococcus pentosaceous* are reported in fermented meat products of Eastern Himalayas. These lactic acid bacteria showed inhibitory activity towards *Klebsiella pnemoniae* which is a contaminant of stored meat. Also, they demonstrated probiotic characters such as enzymes production and hydrophobicity (Rai et al., 2010).

Cereals and legumes are fermented by several groups of bacteria in the large intestine, yielding a variety of fermentation products, particularly short-chain fatty acids (SCFA). The resulting SCFA are known to provide an acidic environment in the large intestine, which stimulates the proliferation of probiotic cultures (Roopashri and Vardaraj, 2009; Macfarlane et al., 2006). Mostly batter is prepared from these basic ingredients and batter is left overnight at room temperature for fermentation, occasionally sodium bicarbonate is added to provide anaerobic conditions for the growth of yeast and lactic acid bacteria. During the preparation of Kallappam fermented toddy is added as additional source of LAB. Fermented batter is either prepared as steamed cakes (idli) or pan cakes (dosa, appam) before it gets too soured. Predominant microflora isolated from batter of these foods include: *Weissella paramesenteroides*, *Lactobacillus fermentum*, *L. plantarum*, *Streptococcus faecalis*, *Pediococcus acidilactici*, *P. cerevisiae*, (*Leuconostoc mesenteroides*. *L. plantarum* AS1 isolated from south Indian fermented food Kallappam successfully prevented colonization of entero-virulent bacterium *Vibrio parahaemolyticus* in HT-29 cell line (Satish *et al*., 2011).

Presence of probiotic lactic acid bacteria in dairy products or enrichment of these dairy sources with the same can leads improvement of nutritional level of a person as well as can help in dilation of effect of several diseases in human being. These studies also support the fact that regular consumption can be helpful to build up immunity against several diseases.

Research has been conducted in Rajasthan region, where camel milk is more easily available. Camel milk is also well known for their therapeutic properties hence enrichment of the same with purified LAB after quality assurance can further improve its value for therapeutic purpose as well as nutrient supplement.

Present review encompasses the value of research done on dairy samples. As dairy samples, are regularly consumed by the person of all the age group hence enrichment of the same with probiotics will add more to its nutritive value. Especially camel milk is known for their therapeutic properties like in treatment of malaria, jaundice, gastro intestinal disorder and strong cough (pneumonia). Research by Indian scientists supports the therapeutic value of camel milk in the treatment of several diseases including tuberculosis (Ilse, 2004).

In addition to this, camel milk is different from other ruminants milk, having low cholesterol, low sugar, high minerals, (sodium, potassium, iron, zinc, and magnesium), high vitamin C, low protein and large concentration of insulin. The values of trace minerals were significantly higher in camel milk as compare to bovine milk (Agarwal et al., 2002; Agarwal, 2005).

Furthermore presence of probiotics with lipolytic activity will increasingly add to its nutritive value. Consumption of such products will reduce the complications associated with heart diseases. Main reasons of which is alteration or changes in lipid profile of an individual.

Probiotic lactic acid bacteria with lipolytic activity assume to be lower the lipid level by metabolise it. Hence could be beneficial for peoples suffering from complications associated with higher lipid level. In addition to this consumption of these nutrient rich supplement could be helpful to improve health of suffering individuals.

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