Proteomic studies on lactic acid bacteria: A review

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ABSTRACT

Probiotics are amongst the most common microbes in the gastro-intestinal tract of humans and other animals. Prominent among probiotics are Lactobacillus and Bifidobacterium. They offer wide-ranging health promoting benefits to the host which include reduction in pathological alterations, stimulation of mucosal immunity and interaction with mediators of inflammation among others. Proteomics plays a vital role in understanding biological functions of a cell. Proteomics is also slowly and steadily adding to the existing knowledge on role of probiotics. In this paper, the proteomics of probiotics, with special reference to lactic acid bacteria is reviewed with a view to understand i) proteome map, ii) mechanism of adaptation to harsh gut environment such as low pH and bile acid, iii) role of cell surface proteins in adhering to intestinal epithelial cells, and iv) as a tool to answer basic cell functions. We have also reviewed various analytical methods used to carry out proteome analysis, in which 2D-MS and LC-MS/MS approaches were found to be versatile methods to perform high-throughput sample analyses even for a complex gut samples. Further, we present future road map of understanding gut microbes combining meta-proteomics, meta-genomics, meta-transcriptomics and -metabolomics.

1. Introduction

Probiotics are defined as 'live microorganisms, which when consumed in adequate amounts, confer a health effect on the host'. The benefits include stimulation of the mucosal immunity, reduction of pathological alterations, and interaction with mediators of inflammation among others [1]. Lactobacillus is a common microbe in the gastrointestinal tract (GIT) of mammals and is potentially probiotic organism that contributes to the health of the host [2]. The majority of probiotic microorganisms belong to the genera Lactobacillus and Bifidobacterium. To be suitable for a probiotic use, a bacterial strain should contain certain characteristics such as it should survive the passage through gastro intestinal tract (GIT), and be resistant to GIT conditions, that involve acidic pH and bile acids [3]. The ability to adhere to the intestinal mucosa is a property of a probiotic because close contact and prolonged colonization may intensify the favorable effects of probiotics. The best proven health benefit for several probiotic strains is the reduction of risk of diarrhea. A study showed that probiotics significantly reduced antibiotic associated diarrhea by 52% and acute diarrhea of various causes by 34% [1]. Other diseases of the gut may also be alleviated with probiotics. The use of probiotics may be related to the relief of constipation and lactose intolerance. Probiotics may also be involved in increase host immune defenses and thus decrease the frequency or duration of infections like the common cold. They have also been shown to be helpful in preventing allergic disorders. Lactobacillus casei Shirota was shown to modulate immune responses of adults suffering from seasonal allergic diseases [4]. Some of the benefits offered by probiotics are listed in Table 1.

In the present article, the progress on proteomics in lactic acid bacteria including few other probiotics has been extensively reviewed in order to understand the current status of proteome research. Further, based on existing research trends the future directions of proteomics in probiotics are presented.

2. Classification

After many years of controversy regarding the classification, today, the term lactic acid bacteria is commonly used to refer to two

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Table 1
Health benefits of probiotics.

Bifidobacterium bifidum	The most dominant probiotic in infants and in the large intestine. Supports production of vitamins in gut, inhibits harmful bacteria, supports immune system response and prevents diarrhea.
Lactobacillus acidophilus	Relieves gas, improves lactose tolerance, shown 61% reduction in E. coli, lower cholesterol levels and creating of vitamin-k. Also important in GALT immune strength
Bacillus coagulans	An endospore probiotic that is heat resistant and improves nutrient absorption. Also has been shown to reduce inflammation and symptoms of arthritis
Bifidobacterium longum	Supports liver function, reduces inflammation, removes lead and heavy metals
Lactobacillus casei	Supports immunity, inhibits *H. pylori* and helps fight infections.
Bifidobacterium infantis	Reduction in diarrhea and constipation.
Lactobacillus brevis	Shown to survive the GI tract, boost cellular immunity enhanced natural T-killer cells, and kills *H.pylori* bacteria.
Bifidobacterium breve	Helps colonize healthy gut community and crowd out bad bacteria.
Bacillus subtilis	An endospore e probiotic that is heat resistant. Elicits a potent immune response and supports GALT. Suppresses growth of bad bacteria like salmonella and other pathogens.
Lactobacillus bulgaricus	A powerful probiotic strain that has been shown to fight harmful bacteria that invades your digestive system and is stable enough to withstand the acidic digestive juices stomach. It also neutralizes toxins and naturally produces its own antibiotics.
Lactobacillus rhamnosus	Supports bacterial balance and supports healthy skin. Helps fight urinary tract infections, respiratory infections and reduce anxiety by reducing stress hormones and GABA neuro transmitter receptors. Also, survives GI tract.

phylogenetically distant bacterial groups: a) Lactobacillales (Firmicutes), and b) Bifidobacteriales (Actinobacteria) [5].

2.1. Lactobacillus

Taxonomically, the *Lactobacillus* genus is diverse and it contains at least 12 separate phylogenetic groups. More than 150 species have been named with the *Lactobacillus* genus, which were isolated mainly from human and animal GITs and mucous membranes and from surface of plants. Several *Lactobacillus* strains are used in the preparation of fermented dairy products and in the production of sauerkraut, pickles, and silage. One of the most important probiotic *Lactobacillus* strain is L. *rhamnosus* GG, which is the most intensively studied probiotic bacterium. L. *rhamnosus* belongs to an L. *casei* phylogenetic group together with L. *casei*, L. *paracasei*, and L. *zeae*. The health effects of L. *rhamnosus* GG are based on several mechanisms which was reviewed separately [6]. Further, L. *rhamnosus* GG strain has numerous effects on the host immune system. The best proven health benefit of L. *rhamnosus* GG has been lowered risk and reduced treatment days for acute diarrhea in (Guandalini et al., 2000) [7]. L. *rhamnosus* GG can also reduce the risk for antibiotic-associated diarrhea and other intestinal side effects associated with the use of antibiotics.

2.2. Bifidobacteria and propionibacteria

The other two important genera that consist of probiotic strains are *Bifidobacterium* and *Propionibacterium*. *Bifidobacteria*, important inhabitants of the GIT, are considered positive indicators of health. The most widely studied probiotic *Bifidobacterium* strain is probably *B. animalis* subspp. *lactis* Bb-12, the use of which is to reduce the risk for respiratory infections in infants, to have some protective effect against diseases like diarrhea in children, and also to reduce the severity of atopic eczema in infants. They are also typically stress-tolerant when compared to other *Bifidobacterium* species, which is important for their use in probiotic preparations [8]. *Propionii* bacteria are used as starter cultures in the dairy industry, especially in Swiss-type cheeses, and have less probiotic properties than what are available for probiotic *Lactobacillus* and *Bifidobacterium* strains. Potentially probiotic *Propionibacterium* strain, *Propionibacterium freudenreichii* subspp. Shermanii JS, has been shown to have non-inflammatory effects during *Helicobacter pylori* infection in vitro. Furthermore this strain has been shown to reduce serum C-reactive protein level in healthy adults.

3. Proteomic studies on lactic acid bacteria

Some of the sought-after benefits of probiotic bacteria mentioned earlier are widely researched. In the research of the molecular biology of probiotics, one important technique is proteomics. Proteomic research of lactic acid bacteria is relatively recent. Proteomics of lactic acid bacteria has been used i) to map proteome of a bacteria which is an overview of bacterial protein content, ii) to study and understand adaptation of gut conditions such as low pH and bile acids and various stress conditions, iii) to study proteins localized on the cell surface, and iv) as a tool to answer special questions about the molecular biology of bacteria.

4. Methods used to carry out proteomics analyses

Aires & Butel [9] have reviewed various methods employed to carry out proteomics studies. Proteomic investigations of microbial communities initially depended on 1D electrophoresis (sodium dodecyl sulfate-PAGE) to generate protein fingerprints of communities [10]. However, a major drawback to this technique is that it cannot identify individual proteins. With the advent of technologies, the two-dimensional (2D) gel-based proteomics technique was made available to research community wherein proteins are separated according to their isoelectric point. Next level of proteomics had mass or gel-free profiling procedures based on liquid chromatography (LC) separation. Both strategies relied on mass spectrometry (MS) for protein identification. In gel-based approaches, intact proteins are separated before an in-gel enzymatic digestion to generate proteolytic peptides, which are subsequently identified by MS. Gel-independent LC approaches can be performed on intact proteins or proteolytic peptides derived from a digested complex sample.

Most of the proteomic studies on probiotics have been performed using 2D-MS [11,12], which relied on two major strategies for the separation of proteins. In 2005, a shotgun proteomics approach was used to study a natural acid-mine drainage biofilm community at the microbial and strain-resolution level [13,14]. Only two studies have focused on the human GI tract microbiota using a classical 2D-gel electrophoresis, trypsin in-gel digestion and MS identification [15], and a gel-free profiling procedure based on LC-MS/MS [16]. The proteome analysis was widely performed using 2D-MS and this methodology currently provides the highest protein species resolution capacity with relatively low instrumentation costs. However, this methodology has few limitations. It is difficult to automate and hence was found to be time-consuming, expensive and labor intensive. The method can only be used to separate highly hydrophobic and alkaline proteins, or proteins with an extreme isoelectric point or molecular weight. 2D-MS also has a low dynamic range, and gel-to-gel variability depends largely on staining and visualization techniques [17]. Owing to these limitations, 2D-MS approaches are usually used to analyze low-complexity proteomes, such as those from model organisms. The advantage of using model organisms is that their genome can be sequenced, and this