Proteomic studies on lactic acid bacteria: A review

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A B S T R A C T

Probiotics are amongst the most common microbes in the gastro-intestinal tract of humans and other animals. Prominent among probiotics are \textit{Lactobacillus} and \textit{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]. \textit{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 \textit{Lactobacillus} and \textit{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. \textit{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
Table 1
Health benefits of probiotics.

| Strain                        | Health Benefits                                                                 |
|-------------------------------|---------------------------------------------------------------------------------|
| *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 salmonellae 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 neurons 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* subsp. *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]. *Propioni* 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* subsp. *Shermani*, 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
improves the quality of protein identification. Gel-free profiling procedures, also called shotgun proteomics, use multidimensional LC-MS/MS separations of protein digests [18]. Prior to the MS analysis, an essential part of shotgun proteomics is the separation methods used, which yield a high resolving power and allow the study of complex biological samples. All of the methods are also fully automated and have a high sample throughput. In most shotgun proteomic techniques, it is not the intact protein itself that is separated and identified. Instead, proteins are cleaved into peptides using proteolytic enzymes and these peptides are subsequently separated and subjected to MS/MS analysis. Identification of these peptides by MS helps to determine the protein content of the original sample. Since peptides can be more easily separated by LC than proteins, a peptide-based proteomic analysis can be performed much more quickly and cheaply than a complete gel-based analysis and allows hydrophobic proteins and peptides to be analyzed [19]. Although 2D-MS approaches are widely used for analyzing microbial isolates, LC-MS/MS is more suitable for analyzing complex samples such as gut microbial communities [16]. The main limitation of shotgun proteomic approaches is that the data obtained only relate to a fraction of the protein, i.e., it is discovery-based in case of the traditional shotgun technique cataloguing hundreds or thousands of proteins, and where the information about post-translational modifications is lost. Therefore, most recently, the complementary and hypothesis-driven targeted proteomics is the method of choice. By contrast, 2D-MS approaches deliver a map of intact proteins where post-translational modifications result in a shift in pI (in the case of phosphorylations) or relative mass (e.g., glycosylation or truncation) and display a differential mobility on a 2D-PAGE [17,20]. It should be noted that 2-DE remains laborious and time-consuming. As for metagenome analysis, sample preparation is crucial for MS-based shotgun proteomics. The challenges arise from both the sample and from the MS analysis. In deed, on the one hand, MS analysis is highly sensitive to detergent and, on the other hand, contamination from host tissues affects quality results and interpretation. High-throughput proteomic approaches have been estimated to detect proteins from bacterial populations representing at least 1% of a community [16]. Therefore, one of the most important challenges for proteomics applications is to increase their dynamic range of detection. Using both classical gel-based and gel-free approaches with their respective advantages in a complementary manner will help to obtain a more complete picture of gut microbiota protein expression and interactions. Various proteomic studies conducted on probiotics were tabulated in Table 2.

### Table 2

Various proteomic studies conducted on probiotics, revised after [86].

| Topic | Separation and detection methods | Identification methods | Potentially probiotic strains | Reference |
|-------|---------------------------------|------------------------|-----------------------------|-----------|
| **Basic proteome research** | | | | |
| Proteome catalogue | No data | NanoLC-MS/MS, MALDI-MS/MS, CXC-LC-MS/MS | *P. freudenreichii* CIRM-BIA1, *L. rhamnous* GG and Le705 | Falentin et al., 2010 [87] |
| Proteome catalogue and comparison of strains | SDS-PAGE | MALDI-MS/MS | *L. acidophilus* NCFM | Savijoki et al., 2011 [88] |
| Proteome map, growth on lactitol | 2-D GE, Coomassie staining, 2-D DIGE | MALDI-MS/MS | | |
| **Comparison of Strains and Growth Phases** | | | | |
| Comparison of strains | 2-D GE, Coomassie staining | MALDI-MS | *B. longum* NCC2705 | Aires et al., 2010 [90] |
| Comparison of strains and growth on different media | 2-D GE, SYPRO Ruby staining | LC-MS/MS | *L. rhamnous* L-97800, *P. freudenreichii* CIRM-BIA1 | Plumed-Ferrer et al., 2008 [91] |
| **Stress** | | | | |
| Bile stress | 2-D GE, Coomassie staining | MALDI-MS | *L. delbruecki* subsp. *lactis* 200L. *plantarum* 299 V, *B. animalis* subsp. *Lactis* B107 | Burns et al., 2010 [92] |
| Bile stress | 2-D GE, Coomassie staining | LC-MS/MS | | Hamon et al., 2011 [93] |
| Effect of bile stress on cell wall proteome | 2-D GE, silver staining | MALDI-MS | | Candela et al., 2010 [94] |
| Acid stress | 2-D GE, silver staining | MALDI-MS | *L. casei* Zhang | Wu et al., 2011 [95] |
| Acid stress | 2-D GE, silver staining | MALDI-MS | *L. reuteri* ATCC 23272 | Lee and pi, 2010 [96] |
| Oxidative stress | 2-D GE, Coomassie staining | MALDI-MS | *B. longum* subsp. *lactis* BBRM68 | Xiao et al., 2011 [97] |
| Comparison of strains with different stress tolerance | LC | LC-MS/MS | *B. longum* NCC2705 | Guillaume et al., 2009 [98] |

### 5. Proteome maps

Mapping of all the proteins on a 2-D gel is the beginning for proteome studies of an organism because it facilitates further proteomic studies. Basic 2-D proteome mapping was performed for *L. casei* Zhang, a probiotic strain isolated from the Mongolian fermented dairy product koumiss [21]. From 2-D gels that covered the pI range of 4–7, 131 protein spots were identified, which represented several protein groups with carbohydrate metabolism proteins being major group. The identified proteins covered 4% of the total number of predicted open reading frames in the genome and the proteome map has since been utilized for studies on the stress responses of *L. casei* Zhang. In a more recent 2-D proteome mapping study, 275 unique proteins were identified from a probiotic *L. plantarum* strain NCFM, covering up to 15% of the theoretical proteome of this strain A [22]. 2-D proteome map of a widely used probiotic strain, *Bifidobacterium animalis* subsp. *lactis* BB-12, contained a restricted area of different proteins of basic metabolism. In *B. infantis* B107, which is found in some commercial probiotics, a protein catalogue of 136 proteins was constructed using a non-gel MudPIT approach [23]. The identified proteins were mainly enzymes involved in energy metabolism and the biosynthesis of basic building blocks or proteins required for the oligosaccharide utilization and protein synthesis. An extensive 2-D proteome mapping of a yet another probiotic strain *B. longum* NCC2705 revealed the several carbohydrate, amino acid, peptidoglycan routes, which were active in the growth of *bifidobacterial* cells. The 369 identified proteins also include various stress proteins as well as proteins without any function that is known, and in total, the identified proteins represented 21% of the predicted 1772 ORFs in the genome. This proteome map has presumably been utilized in subsequent proteomic studies of *B. longum* NCC2705 that examined the response of the bacterium to different growth conditions [24]. A proteome catalogue of *Propionibacterium freudenreichii* CIRM-BIA1 was constructed using several MS-based protein identification methods which included 490 identified proteins that covered 20% of the predicted proteins in the genome [25].

### 6. Cell surface proteins

The ability to adhere to intestinal epithelial cells is considered
important in the selection of Lactobacillus for probiotic use. Cell surface protein function as protective sheath against hostile gut environment, adapting to stress conditions. They are also involved in cell protection and surface recognition. It is demonstrated that cell surface proteins of Lactobacillus play an important role in survivability, adhesion and competitive exclusion of pathogen to epithelial cells [26].

During the last few years, a substantial body of scientific evidence has accumulated suggesting that certain surface-associated and extracellular components produced by probiotic bacteria could be responsible for some of their mechanisms of action [27]. These bacterial components would be able to directly interact with the host mucosal cells; they include exopolysaccharides, bacteriocins, lipoteichoic acids and surface-associated and extracellular proteins. Extracellular proteins include proteins that are actively transported to the bacterial surroundings through the cytoplasmic membrane, as well as those that are simply shed from the bacterial surface. Compared to the other bacterial components, the interactive ability of extracellular proteins/peptides has been less extensively studied. In a review published by Sañchez et al. [27], current findings supporting an interaction between extracellular proteins/peptides produced by probiotic bacteria (strains of the genera Bifidobacterium, Lactobacillus and Escherichia) and host mucosal cells were described in detail.

Several research papers have reported on the role of extracellular proteins secreted by probiotic bacteria [28,29]. As has been reported, probiotic extracellular proteins could be linked to some of the beneficial effects ascribed to the corresponding strains, although current information is now restricted to in vitro and animal studies. To date, our knowledge of the identity of these proteins is very limited; although several studies have reported the interaction between extracellular proteinaceous compounds and human cells, few have been identified and characterized so far. Further research is needed to elucidate the precise molecular mechanism of action of each of these proteins in both epithelial and immune cells, notably in DCs. This will contribute to the understanding of how probiotics exert beneficial effects on the human host. This knowledge may lead to treatments to reverse some of the processes involved in the initiation, or perpetuation, of various gastrointestinal disorders, such as inflammatory bowel diseases, allergies and autoimmune diseases.

Lactobacilli are important commensal bacteria in the human GIT. Several Lactobacillus species are used in the food industry for the production of an array of fermented products. L. rhamnosus GG is one of the probiotic strains that has been most closely studied, and in addition has one of the most extensive safety assessment records [30]. The action of certain extracellular proteins might explain some of the beneficial effects exerted by certain probiotic lactobacilli. Enhancement of the mucosal barrier and maintenance of GIT homeostasis extracellular proteins secreted by probiotic lactobacilli have been shown to help maintain the mucosal barrier, mainly through MAPK-dependent mechanisms [31]. The signalling mechanisms of the proteins are better characterized in lactobacilli than in Bifidobacteria. Uncharacterized extracellular proteinaceous compounds secreted by Lactobacillus acidophilus PZ 1138, Lactobacillus fermentum PZ 1162 and L. paracasei subsp. paracasei LMG P-17806 have been shown to induce production of the antimicrobial peptide human β-defensin 2 (hBD2) in epithelial cells. The signal of these extracellular proteins was shown to be transduced to the nucleus through the MAPKs ERK, p38 and c-Jun terminal kinase (JNK), where hBD2 synthesis was increased through the modulation of nuclear factor kB (NF-kB) and activator protein 1 (AP-1), ending finally in an increase of IL-8 production [31]. In addition, two peptides present in L. rhamnosus GG conditioned media, NPSRQERR and PDEKN, were shown to possess antimicrobial activity against E. coli AECF 042, Salmonella enteritidisvar, Typhimurium and Staphylococcus aureus [32].

Lactobacilli modify their surface properties in response to environmental changes to maintain bacterial cell integrity. Different strains of lactobacilli are known to show great diversity in cell surface architecture with strain-specific characteristics. The cell envelope of lactobacilli is composed of the plasma membrane with embedded proteins, surrounded by the cell wall. The cell wall consists of a thick multilayered sacculus made of peptidoglycan, decorated with teichoic acids [wall teichoic acids (WTA) and/or lipoteichoic acids (LTA)], cell wall polysaccharides, pili and flagella (proteinaceous filaments), and cell surface proteins that are anchored to the cell wall through different mechanisms. Some species of lactobacilli display an additional para-crystalline layer of proteins surrounding the peptidoglycan layer, referred to as the S-layer, but it is not present in L. casei BI23 [33].

Based on the free radical theory of aging (FRTA), oxidative stress and aging is closely related with each other, and decreasing oxidative damage can extend average or maximum life span [34]. Probiotic research was first originally taken from Mechnikoff’s research on the relationship between life prolongation and eating yogurt [35]. Furthermore, more researchers paid attention to live bacteria in yogurt, and a number of lactic acid bacteria has been isolated from fermented food and screened out as probiotics. Antioxidative effect of probiotics may be an important mechanism involved in its function such as inflammatory response, because both local and systemic inflammatory responses are associated with the production of reactive oxygen species (ROS) [36].

Both, Lactobacillus and Bifidobacteria are very useful in the promotion of human health and prevention or treatment of several diseases [37]. Several lactic acid bacteria and Bifidobacteria have screened out with antioxidative effect both in vitro and vivo. L. rhamnosus GG was shown to reduce intestinal oxidative stress [38]. L. fermentum ME-3 has been not only demonstrated to possess high total antioxidative activity (TAA) and total antioxidative status (TAS) of intact cells and lysates in vitro, but it can also increase the antioxidative activity of sera and improved the composition of the low-density lipid particles (LDL) in vivo [39].

Various antioxidative effects of probiotic in vitro are well established, however, in vivo trials carried out were much less than trials in vitro. Linolenic acid test, TAS-method, inhibition of ascorbate autoxidation, chelating activity for Fe²⁺ and Cu²⁺ ions, superoxide anion radical, hydrogen peroxide and hydroxyl radicals scavenging activity were the useful tools to evaluate antioxidative effect in vitro [40,41]. In one study, the team investigated the antioxidative effect of L. casei Zhang on hyperlipidemic rats. Previous studies showed that L. casei Zhang, which was isolated from traditional home-made koumiss in Inner Mongolia of China, exhibited a probiotic property including higher low pH resistance, the cholesterol-removing ability and adhesion ability of human intestinal epithelial cells in vitro and enhancing immune responses in vivo [42–44]. The project of whole genome sequencing (Accession number CP001084, GenBank) and proteome has been accomplished [45].

7. Stress proteins

Bile tolerance is one of the most crucial properties probiotic bacteria should possess to survive in the small intestine. In this context, a recent study investigated the natural protein diversity within the Lactobacillus plantarum species with relation to bile tolerance, using comparative proteomics [33].

Probiotic strains encounter various stress conditions during their production, product formulation, and the passage through the GIT, which may affect the functioning of these organisms. The harsh conditions of the GIT, which involve acidic conditions and detergent-like bile acids, are a notable challenge to the survival of probiotic bacteria. To simulate GIT conditions, L. rhamnosus GG has been exposed to a sudden bile stress, and several stress response mechanisms have been revealed. Various mechanisms for recognizing various bile compounds and actively removing them from the cells were activated by bile exposure, and also several bile-induced changes in central metabolism were also detected. L. rhamnosus GG also responded in various ways to mild acid stress. Probiotic bacteria may face mild acid stress in dairy production because its pH is lowered by the production of acid by
fermenting bacteria in the fermented milk products. The acid stress response of *L. rhamnosus* GG included changes in central metabolism and in specific responses, such as the induction of proton-trans-locating ATPase, a membrane transporter used for increasing intracellular pH. These results clearly showed that *L. rhamnosus* GG possesses a large repertoire of mechanisms for responding to stress conditions, which probably explains its good survival in GIT [46]. Representative list of the stress proteins induced during proteome level studies are tabulated in Table 3.

A noteworthy phenomenon of protein phosphorylation was observed in the species, *L. rhamnosus* GG. Phosphorylation of several proteins of *L. rhamnosus* GG was detected, and there were movements showing that the degree of phosphorylation may be dependent on the growth pH. In bacteria, protein phosphorylation has been suggested to regulate the enzymatic activities or to direct proteins to different cellular locations, but here the purpose of the phosphorylation was not identified. However, in these studies, phosphorylation events were detected for the first time in a *Lactobacillus* strain [47].

A large number of studies have dealt with the response of lactobacilli towards bile exposure [48-50]. These studies have also spanned several types of species of lactobacilli and have detected considerable variable changes in the response, although it must be kept in mind that experimental conditions varied from one study to another. Furthermore, a comparative study conducted with six strains of *L. casei* has shown various significant differences between those strains [51]. Notwithstanding, some effects of bile on gene expression or protein content are usually had been observed. Induction of general stress proteins and a number of transport systems has been observed in most studies. In contrast, repression of proteins involved in the fatty acid biosynthetic pathway has been observed in *Lactobacillus delbrueckii* [48] and *L. rhamnosus* [50] whereas it was not observed in *L. acidophilus* [52], *L. plantarum* [49,53] or *Lactobacillus reuteri* [54,55]. In the above study, a transcriptomic and proteomic approach was employed to understand the response of *L. casei* BL23 to bile. The study of strain BL23 is of special interest, since it has been used as a model strain for physiological studies [57] and for its probiotic properties [56]. The authors have shown that the response of *L. casei* BL23 shares all characteristics in common with other lactobacilli and displays others specific to this strain. (Table 4)

Some strains of *L. casei* have received considerable attention for their beneficial health effects as probiotics [57]. The probiotic microorganisms are currently the focus of an intense research effort that mainly aims to determine their possible health benefits and to identify possibilities for their future use.

### Table 3
**List of select proteins that were induced by stress in total proteome level studies of potentially probiotic bacteria.** revised after [86].

| Classification | Name | Function |
|---------------|------|----------|
| Stress response | DnaK | Chaperon protein DnaK |
| | GroEL | 60 kDa chaperone GroEL |
| | GroES | 10 kDa chaperonin GroES |
| | GrpE | Chaperone protein GrpE |
| | Hsp | ATP dependent protease |
| | Hsp30 | A small heat shock protein |
| | Hsp70 | Heat shock induced protein Hsp70 |
| | UspA | Universal stress protein UspA |
| | – | Heat shock protein, Hsp20 family |
| | – | Repressor protein of class 1 heat shock genes |
| Clp proteins | ClpB | ATP-binding chain of ATP-dependent protease |
| | ClpC | ClpC |
| | ClpP | ATP-binding subunit of Clp protein |
| | ClpP | ATP-dependent Clp protease |
| | ClpQ | ATP-dependent protease, peptide subunit |
| DNA repair | RecN | DNA repair protein RecN |
| | RecR | Recombinase |
| | – | DNA protection during starvation protein |
| | – | Putative ATPase involved in DNA repair |
| | UvrB | UvrBC system protein B |
| | SodA | Superoxide dismutase |
| Oxidative stress | – | Thioredoxin-dependent thiol peroxidase |
| pH homeostasis | AtpA | ATP synthase alpha chain |
| | AtpF | ATP synthase beta chain |
| | AtpH | F1Fo ATP synthase subunit delta |

### Table 4
**Probiotic extracellular proteins/peptides with a known role in the interaction of potential probiotic strains with mucosal cells.**

| Protein | Microorganism | Role | References |
|---------|---------------|------|------------|
| Serpin (AAN23975) | B. longum subsp. longum NCC2705 | Inhibition of pancreatic and neutrophil elastases | Ivanov et al. (2006) [99] |
| CHWPR peptide | B. amyloliquefaciens subsp. lactis BB-12 | Upregulation of c-myc and il-6 genes | Mitsuhashi et al. (2008) [100] |
| Unidentified secreted proteins | B. longum subsp. infantis | Increase of the mucosal barrier function; attenuation of inflammation and colonic permeability in IL-10-deficient mice | Ewaschuk et al. (2008) [101] |
| Unidentified secreted proteins | B. breve C50 | Prolonged survival and maturation of DCs; increased IL-10 and IL-12 production by DCs | Hoarau et al. (2008) [102] |
| Unidentified secreted proteins | L. acidophilus PZ 1138, L. fermentum PZ 1162, L. paracasei subsp. paracasei LMG P-17806 | Induction of hBD2 production in epithelial cells | Schlee et al. (2008) [103] |
| Peptides NPSRQERR and PSEKN | L. rhamnosus GG | Antimicrobial activity | Lu et al. (2009) [104] |
| Unidentified secreted proteins | L. plantarum, L. acidophilus, L. casei and L. delbrueckii subsp. bulgaricus | Induction of mucin secretion | Caballero-Franco et al. (2007), [105] |
| Unidentified secreted proteins | L. rhamnosus GG | Increase of the production of HSP25 and HSP72 in YAMC cells | Tao et al. (2006) [106] |
| Unidentified secreted proteins | L. acidophilus and L. rhamnosus | Increase of the chloride/hydroxyl exchange activity in Caco-2 cells | Berthakur et al. (2007) [107] |
| p40 (homologous to gi|116493594) | L. rhamnosus GG | Growth promotion | Yan et al. (2007) [108] |
| p75 (homologous to gi|116493849) | L. rhamnosus GG | Reduction of the injuries caused by TNF-α; attenuation of the TLR decrease induced by hydrogen peroxide | Seth et al. (2008) [109] |
| Supernatant containing P40 and p75? | L. rhamnosus GG | Decrease of IL-8 production in epithelial cells | Choi et al. (2008) [110] |
| SlpA (YP_193101.1) | L. acidophilus NCFM | Induction of IL-10 production in DCs; DC immunomodulation | Konstantinov et al. [111] |
| Unidentified secreted proteins | E. coli Nissle 1917 | Inhibition of pathogens adhesion and colonization | Altenhöfer et al. (2004); Lasaro et al. (2009) [112,113] |
| Flagellin | E. coli Nissle 1917 | Increase of hBD2 and IL-8 production | Schlee et al. (2007) [114] |
the mechanisms through which they exert them [58]. Although there is evidence showing that dead probiotic cells also function to confer some beneficial effects upon its host [59] it is normally agreed that the probiotic micro-organisms must survive the transit through the GIT present, where they will encounter a very acidic environment in the stomach and a high concentration of bile salts in the upper small intestine [60]. Bile salts are one of the main components of bile. They are amphipathic molecules present, that play a very important role in the process of emulsification of fats and absorption of hydrophobic vitamins. In addition, bile salts have antimicrobial activity against many micro-organisms, mainly by damaging their cell envelopes [61]. Furthermore, several studies have indicated that bile salts can also damage DNA, since their exposure to bile salts induces the DNA repair systems, and strains defective in several DNA repair genes are more sensitive to bile than their parental strains [61]. Due to their amphipathic nature, bile salts may also alter the conformation of some important proteins and they may also cause oxidative stress [61,62]. Therefore, bile can act on several targets inside the bacterial cell, and the defence mechanisms that have been elicited by bacteria are likewise been diverse [61–63].

Yet another study investigated the acid tolerance response (ATR) in L. casei through a combined physiological and proteomic analysis. To optimize the ATR induction, cells were acid adapted for 1 h at different pH, and then the acid was challenged at pH 3.5. The result showed that the acid adaptation improved acid tolerance, and the highest survival was observed in cells adapted at pH 4.5 for 1 h. Analysis of the physiological data thus obtained, showed that the acid-adapted cells exhibited higher intracellular pH (pHi), intracellular NH4+ content, and lower inner permeability, when compared with the cells without adaptation. Proteome analysis was performed upon acid adaptation at different pHs (pH 6.5 vs. pH 4.5) using two-dimensional electrophoresis. A total of 24 proteins that exhibited at least 1.5-fold differential expression were identified. Four proteins (Pgk, LacD, Hpr, and Galm) involved in carbohydrate catabolism and five classic stress response proteins (GroEL, GrpE, Dnak, Hasl, and LCAZH_2811) increased subsequent binding of both L. casei strains, implicating the involvement of proteins in these extracts in binding. Collectively, the results in this study increase the understanding of the physiological response of L. casei when grown under the required conditions that may be encountered in fermented foods and which pose specific stress conditions, namely carbohydrate limitation and acid stress. The study presents the functional analysis of proteins which also provide new insight into the metabolic pathways engaged by L. casei in dealing with food relevant stresses.

Activation of stress proteins in response to bile has been observed in L. casei Zhang [66] and its close relative L. rhamnosus GG [50]. In contrast, a clear activation of stress proteins was not observed by [51]. A possible explanation for this difference is that [51] focused their analysis only on a subset of proteins in order to identify the biomarkers of bile tolerance. Increased modulation of these stress proteins in response to bile has also been observed in L. acidophilus [52], L. delbrueckii subsp. lactis [67], L. plantarum [49], and L. reuteri [55]. The transcriptional regulators possibly involved in the stress response were upregulated in response to bile. The transcriptional regulator, CtsR controls the expression of ClpP in L. plantarum in response to various conditions of abiotic stress [68]. An increased abundance of CtsR and ClpP in response to p-coumaric acid has been observed in L. casei [69]. Rex might also play a similar role in lactobacilli. In a recent study to evaluate the actual antioxidant potentiality of L. casei Zhang, in a hyperlipidemic rat as a model it was found that L. casei Zhang will help to alleviate the oxidative stress, by reducing lipid peroxidation and improved lipid metabolism both in blood and as well as in the liver with hyperlipidemic in vivo [50].

8. Cell functions

Oxidation processes are indispensable to life for energy and metabolism. Oxidative stress may cause a little damage and also produce toxic substances, especially for those patients who are facing the problem of obesity [70]. Biomarkers of oxidative stress or antioxidant enzymes that were associated with diseases of the blood circulation system and tissues have been developed [71]. Turgut investigated many changes in MDA and GSH levels of mice serum, spleen and liver for evaluating the oxidative injury of aluminum. Other studies showed that T-AOC, liver GPT and GOT levels and enzymatic antioxidants activities of SOD, CAT and GSH-Px could also be used as the indexes of oxidative damage [72,73].

As one of main reactive species, ROS can lead to the damage of lipids, proteins, nucleic acids and carbohydrates of cells in vivo [74]. In the process of peroxidation of lipids, polyunsaturated fatty acids in cell membrane are primarily oxidized by ROS, and this proceeds by a free radical chain reaction mechanism. Furthermore, the oxidative degradation of lipids on cell membrane causes damage in the cell structure and function [75]. As a secondary product of lipid peroxidation, MDA is a mutagenic and carcinogenic reactive substance present in human cells by the deterioration of biological molecules [76].

MDA is accompanied by the free radical mediated lipid peroxidation, and its level is considered as a good marker of oxidative stress [77]. Gil et al. (2006) [77] found significantly decreased levels of serum and liver MDA in the L. casei Zhang groups with different doses.
compared to the hyperlipidemic group. Particularly, liver MDA levels of both medium and high dose recovered to a normal level. In vitro experiment also indicated that L. acidophilus and B. longum could scavenge MDA on cultured mammalian cells [78]. Other in vivo consistent researches showed that both B. infantis DSM 15159 and Ecologiec® 641 (a kind of multispecies probiotics) resulted in a significant tissue MDA level compared to control [79,80]. Thus, all the results above suggested that the probiotic treatments could protect these rats from oxidative stress to some degree, especially higher dose had a complete protective effect on the liver of rat with aspect of lipid peroxidation. These findings indicated that the free radicals being released were being more effectively scavenged in the liver, that has also been observed in pre-treatment with B. Catenulatum ZYB0401 or the mixture of B. Catenulatum ZYB0401 and L. fermentum ZYL0401 [81]. Moreover, this MDA level reducing effect to liver was dependent on the dosage of administered probiotics. On the other hand, administration of L. casei Zhang in healthy rats kept a normal MDA level when compared to control rats.

As one of key scavengers of ROS, superoxide dismutase (SOD) can also protect host cells from oxidative damages [82]. High-fat diet could also induce a decrease of SOD activity, both in blood and liver tissue. Lactic acid bacteria usually possess SOD, and its specific activity could not be influenced by the aerobic environment [83]. In an experiment it was pointed out that SOD production of L. casei Zhang could keep scavenging free radicals to a normal level in healthy rats. As another key antioxidant enzyme, GSH-Px is a glutathione-utilizing peroxidase and plays a very important role in protecting the organelles from oxidative injury [84]. High-fat diet can induce a decreased GSH-Px activity. Similar to SOD activity above, L. casei Zhang had showed an ideal preventive effect in Group C with both sera and liver.

In order to understand the effect of probiotics on hepatic function, serum GOT and GPT activities have been determined. GOT and GPT (also known as AST and ALT) will release into blood if the liver cell necrosis. So it is the most commonly used as an indicator of liver function [85]. It was found that GPT values of administered probiotic dropped to normal level, and GOT values also significantly decreased when compared to the model level. In a previous study, SD rats supplemented with L. fermentum MG590 showed a decreased activity of GOT; the rats were fed a medium containing alcohol drink [86]. Moreover, Xing et al. [87] reported that the process of supplementation with Bifidobacterium and Lactobacillus dropped the alanine aminotransferase values (ALT). It was also noted in a combined study that ALT and AST decreased significantly in mice with treatment of Se-enriched Lactobacillus in comparison with liver injury model group [88]. Therefore, it was indicated that L. casei Zhang might reduce the liver injury induced by high-fat diet in rats. Besides, there was no difference among three doses, suggesting that different doses exerted a similar extent of protective effect on liver.

9. Omics approaches

Due to high diversity of gut bacterial communities there was confusion between gut microbiota and their health status. New DNA techniques based on 16S rRNA gene sequencing, have greatly improved our knowledge of the gut microbiota. Comparative genomic sequence analyses have led to metagenomic approaches that have offered new insights into the genetic diversity of this ecosystem. However, genetic data on their own do not help to elucidate the functions of the microbial communities in this ecosystem. Microbial functionality can be characterized either by the analysis of mRNA transcripts (i.e., transcriptomics) or analyses of proteins (i.e., proteomics). During the last decade, progress in protein analysis has stimulated interest in proteomic analyses. As proteins are involved in biotransformation processes, proteome analyses constitute a suitable way of characterizing the dynamics of microbial functions. Traditionally used for the study of pure cultures, proteome analyses are now being applied to detect expression profiles and to provide functional insight directly from mixed microbial environmental samples (metaproteomics). Despite the limited number of investigations concerning the gut microbiota, these approaches have already demonstrated their potential to provide functional insights. Metaproteomics approaches may therefore become a useful tool to monitor the functional products of the gut microbiota in relation to dietary interventions, length of life, health and diseases. Probiotics have been used to prevent several diseases and there is now increasing interest in their use. However, their actual efficacy in terms of health benefits is still debated. Progress in basic knowledge of probiotic strains, in strain selection and in understanding their mechanisms of action is needed to give credibility to the health claims of probiotics. In this regard, proteomic analyses of potential probiotic strains, as well as metaproteomic analyses of fecal or intestinal samples throughout clinical studies, can provide useful information on the potential benefits of probiotic supplementation.

10. Conclusion

There is a need to understand the composition of the microbial communities of gut microbiota, as well as their functions in their respective environments. Large metagenomic sequencing projects that analyze genomic DNA directly from samples are providing a great deal of data on the genetic diversity and potential within specific environments. However, we are only just beginning to understand the interactions between the microbiota and the host. Among novel techniques that are being developed, (meta)proteomics is a useful means of identifying functional genes and relating genetic and taxonomic diversity to the functionality of the microbial communities in their complex environment. However, any single ‘omics’ approach, such as (meta)genomics or (meta)transcriptomics or (meta)proteomics or (meta)metabolomics, may not be sufficient on its own to characterize the complexity of biological systems. Indeed, integrative ‘omics’ approaches are likely to help further decipher complex biological systems. Improvements still need to be made in ‘omics’ technologies and experimental protocols, as well as in computational methodologies and statistical tools. This will help integrative analyses of multiple large-scale ‘omics’ datasets to generate new knowledge that cannot be derived from the analysis of a single data type alone. Integration of knowledge at different levels, from genes to proteins and metabolites, will be a powerful tool to help us understand gut microbiota-host interactions. This will lead to the development of relevant hypotheses on the relationships between microbiota and health, and will also yield better disease markers for diagnosis and therapy monitoring.

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Significance

Due to immense benefits of probiotics in maintaining gut health of humans and animals, the need for compiling a progress of research work published in the area with focus on proteomics was required. Health promoting benefits of the probiotics (Lactobacillus and Bifidobacterium) to the host further extend to reduction in pathological alterations, stimulation of mucosal immunity and interaction with mediators of inflammation among others. Thus this paper reviews proteomics of probiotics, with special reference to lactic acid bacteria.
along with the utilized 2D- MS and LC-MS/MS approaches even for a complex gut samples are reviewed.

Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2018.04.009.

References

[1] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[2] K. Koskenniemi, K. Pi, K.-J. Jouhkimainen, J. Vehmas, E. Vanio, L. Soininen, T. Pihlajamäki, H. Kauppinen, et al., Proteomic and transcriptomic characterization of bile stress response in probiotic Lactobacillus Acidophilus DSM 1338, Mol. Cell. Proteom. 13 (2014) 687–697.
[3] B. Forti, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[4] K. Lee, H.G. Lee, K. Pi, Y.J. Choi, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[5] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[6] K. Lee, H.G. Lee, K. Pi, Y.J. Choi, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[7] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[8] K. Lee, H.G. Lee, K. Pi, Y.J. Choi, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[9] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[10] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[11] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[12] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[13] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[14] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[15] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[16] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[17] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[18] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[19] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[20] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[21] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[22] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[23] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[24] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[25] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[26] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[27] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[28] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[29] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[30] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[31] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
[32] S.J. Salminen, M. Gueimonde, E. Isolauri, Probiotics that modify disease risk, J. Nutr. 135 (2005) 1294–1298.
