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Tackling probiotic and gut microbiota functionality through proteomics

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ABSTRACT

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Many strains exert their beneficial effects after transiently colonizing the human gut, where they interact with the rest of the intestinal microorganisms and with the host mucosa. Indeed the human gut harbours a huge number of microorganisms also known as gut microbiota. Imbalances in the relative abundances of the individual components of the gut microbiota may determine the health status of the host and alterations in specific groups have been related to different diseases and metabolic disorders.

Proteomics provide a set of high-throughput methodologies for protein identification that are extremely useful for studying probiotic functionality and helping in the assessment of specific health-promoting activities, such as their immunomodulatory activity, the intestinal colonization processes, and the crosstalk mechanisms with the host. Furthermore, proteomics have been used to identify markers of technological performance and stress adaptation, which helps to predict traits such as behaviour into food matrices and ability to survive passage through the gastrointestinal tract. The aim of this review is to compile studies in which proteomics have been used to assess probiotic functionality and to identify molecular players supporting their mechanisms of action.

Significance: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Molecular basis underlying the functional properties of probiotic bacteria responsible for the health promoting effects have been in the background for many years. Breakthrough of omics technologies in the probiotic and microbiota fields has had a very relevant impact in the elucidation of probiotic mechanisms and in the procedures to select these microorganisms, based on solid scientific evidence. It is unquestionable that, in the near future, the evolution of proteomic techniques will play a pivotal role in the generation of knowledge about the functions of probiotics and gut commensals, still a pending issue in the field of intestinal microbiomics.

Contents

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1. Gut microbiota and probiotics

Since the beginning of the 20th century we have scientific evidence that there are beneficial microbes consumed in food that exert healthy effects. Already in 1907, Elie Metchnikoff, the Nobel Prize in Physiology or Medicine in 1908, published a book with the title "The Prolongation of Life: Optimistic Studies". In this book he mentioned some observations related to the consumption of bacteria responsible for dairy fermentation, and he highlighted the association between the consumption of fermented dairy products in some Eastern European areas and an unusually large number of centenarians [1]. Later on, fermented milks including specific lactic acid bacteria strains selected for specific health purposes started to be commercialized [2] and during the 50′s the first therapies using probiotics were published in renowned medical journals [3].

Probiotics are traditionally associated with fermented foods, being lactobacilli and bifidobacteria the two main bacterial groups used by the food industry. During the last decades, probiotics have been defined in many different ways [4], but the first broad consensus definition was coined by a joint Expert Consultation Scientific Committee working on behalf of the FAO and the WHO [5]. The scientific panel defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (this definition was recently revised by the International Scientific Association of Probiotics and Prebiotics; [6]. In the FAO/WHO document, some *in vitro* tests to screen potential probiotic microorganisms were recommended, including adherence to mucus and/or human epithelial cells and cell lines, antimicrobial activity against potential pathogens, ability to reduce pathogen adhesion or displaying bile salt hydrolase activity. These screening tests became the dogma for probiotic characterization, but this phenotypic characterization does not allow going deeply into the mechanisms underlying the functionality of probiotics, a key issue to generate solid evidence-based science to support the observed beneficial effects attributed to these bacteria. Mechanistic studies have also been hampered by the lack of genetic tools to genetically modify lactobacilli and bifidobacteria; in the particular case of bifidobacteria gene silencing or protein production has been achieved only for a few model strains [7].

Maybe the key feature of probiotic microorganisms, in addition to their health promoting effects, is their ability to modulate the human microbiota. In most of the studies, this term relates to the microbial community inhabiting the human gastrointestinal tract (GIT), although a probiotic can target the microbiota from other body locations, mainly mucosae. In the case of our gut, about 10^{14} microorganisms endow us with relevant metabolic and functional attributes with their pool of genomes, also known as microbiome [8]. Currently, it is estimated that 10 million unique genes compose the human gut microbiome [9] (http://gigadb.org/dataset/100064).

The gut microbiota exerts a fundamental role in human health by promoting intestinal homeostasis, stimulating development of the immune system, providing protection against pathogens, and contributing to the production of micronutrients and energy [10]. Therefore, it can be easily deduced that microbiota plays a pivotal role in human health, notably at the level of the relative compositions of their single microbial species [11]. Indeed modifications in its composition have been related to a number of metabolic disorders and diseases, notably with an autoimmune or chronic inflammatory component, including inflammatory bowel diseases, systemic lupus erythematosus (SLE), metabolic syndrome, rheumatoid arthritis, type-1 diabetes, and obesity [12–17]. Currently, the interaction between intestinal microbiota and different organs, such as gut-liver axis and gut-brain axis, is becoming evident; thus dysbiosis in this microbial community has been associated with liver disease, mood, autism or brain development disturbances [18].

Microbiota increases in number, density and complexity from the oral cavity to the colon [19], and it contains microorganisms belonging to the three domains of life: Eucarya, Bacteria and Archea. Bacteroidetes and Firmicutes are the dominant bacterial phyla in adults, whereas the main archaea identified to date is the methanogenic *Methanobrevibacter smithii* [9,20]. Almost all bacteria members can be ascribed to nine phyla, with *Bacteroides* and *Firmicutes* accounting from almost 90% of these populations; other phyla such as *Actinobacteria* – in which bifidobacteria are is included – constitute subdominant groups [9,21].

In this populated scenario, orally ingested probiotics must deal with stressful conditions characterizing the human GIT (acidic pH, bile and digestive enzymes), starving conditions and microbial antagonism interrelationships. The advent of omics techniques during the last decade has allowed overtaking many of the inconveniences associated with the molecular characterization of probiotic functionality, and proteomics plays a pivotal role in this process. Using different proteomic methods, mainly, but not exclusively, gel-based approaches, scientists have been able to identify the molecular players involved in different stress responses critical for survival during industrial processing [22] and/or along the gastrointestinal tract transit [23,24], and to know the proteins involved in important metabolic functions, such as mucin utilization [25], as well as in adhesion, immune stimulation and other host-microbial interactions [26,27].

2. Proteomic approaches

In microbiology, the classical definition of proteome can be adapted to "the complete protein complement of a cell or subcellular fraction of a microorganism in a defined growth phase under concrete and precise physiological conditions" [28]. During the last decades a huge amount of genetic information has been obtained thanks to the development of genomics (mainly DNA sequencing technologies and platforms) and Bioinformatics (algorithms, massive data storage and query and data integration). However genomics is not enough to explain the complex biological events that are mediated by proteins, as the presence of a simple gene says very few about its expression and the production of a bioactive protein. Therefore, in the *omics* era, proteomics has become more interesting since they allow detecting proteins involved in the main cellular functions such as catalysis and stress responses. The proteomic approaches involve all the techniques used to identify and quantify the complete set of proteins present in a sample, cell or tissue under defined experimental conditions. A detailed review of common techniques was written by Monteoliva and Albar [29] and further reviewed by Abdallah and co-workers [30]. Further reviews for the application of proteomics to the study of probiota/microbiota functionality are also available in the scientific literature [31,32]. Setting a proteomics experiment involves all parameters affecting sample preparation (basically protein extraction and purification), followed by gel-based/gel-free protein separation coupled to a mass spectrometer step in which the polypeptides/proteins are finally detected through their mass-to-charge ratio (Fig. 1). The most common approach is the so-called "Bottom-up proteomics", in which proteins are digested (usually through the action of trypsin) and the resulting mix of peptides detected in the mass spectrometer. This contrasts with the "Top-down" proteomics, in which proteins are not digested prior separation, and which is very useful for the detection of protein degradation products, isoforms, posttranslational modifications or truncated proteins [33]. In the identification step,