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Ten years of subproteome investigations in lactic acid bacteria: A key for food starter and probiotic typing

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(Article begins on next page)

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Abstract

The definition of safety and efficacy of food-employed bacteria as well as probiotic strains is a continuous, often unattended, challenge. Proteomic techniques such as 2DE, DIGE and LC/LC-MS/MS are suitable and powerful tools to reveal new aspects (positive and negative) of "known" and "unknown" strains that can be employed in food making and as nutraceutical supplements for human health. Unfortunately, these techniques are not used as extensively as it should be wise. The present report describes the most significant results obtained by our research group in 10 years of study on subproteomes in bacteria, chiefly lactic acid bacteria. Production of desired and undesired metabolites, differences between strains belonging to same species but isolated from different ecological niches, the effect of cryoprotectants on survival to lyophilization as well as the adhesive capability of strains, were elucidated by analysis of cytosolic, membrane-enriched, surface and extracellular proteomes. The present review opens a window on a yet largely underexplored field and highlights the huge potential of subproteome investigations for

more rational choice of microbial strains as food starters, probiotics and for production of nutraceuticals. These analyses will hopefully contribute to manufacturing safer and healthier food and food supplements in the near future.

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Graphical abstract

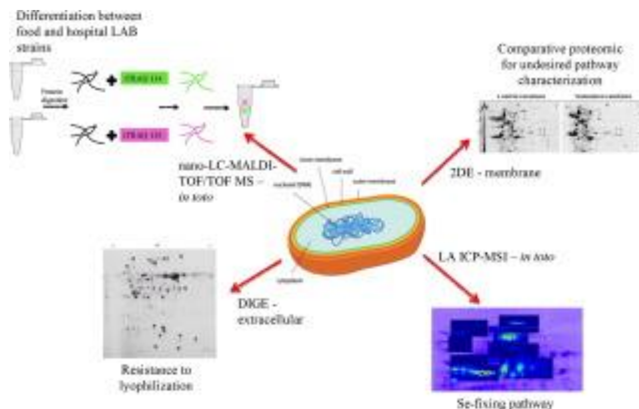

Figure options

Abbreviations

- EF, elongation factor;
- GAPDH, glyceraldehyde 3-phosphate dehydrogenase;
- PGK,phosphoglycerate kinase

Keywords

- 2DE;
- Subproteomes;
- Moonlighting proteins;
- Food bacteria;
- Adhesion;
- Biogenic amines

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1. Introduction

Safety of bacterial starters employed in food fermentations as well as efficacy of probiotic strains used as food additives or nutraceutical supplements is an urgent concern. In spite of the improved analytical controls, food-borne syndromes other than infections are still a reality in western countries [1], [2] and [3]. Probiotics have been receiving growing attention in recent years for their ability to modulate physiological functions such as

nutrition and metabolism [4] and [5], immunity [6] and [7] and gut-brain axis [8] and [9]. They play significant roles in the fight against pathological phenomena such as infections[10], cancer [11], inflammation, allergies, autoimmune diseases [7], oxidative stress[12] and [13], cardiovascular problems [5] and psychiatric disorders [14] which affect health and life quality. However, several biological activities supposed on the basis of analyses of microbial genomes still need to be proved by functional investigations. Resistance to technological treatments or phenotypic modifications occurring during lyophilization, processing and storage, are also important issues to ascertain that the viability and the beneficial properties of a particular strain (employed as starter or as bio-control agent) are conserved [15], [16] and [17].

Current genetic typing of a strain is far to be able to provide an accurate picture of bacterial physiology and phenotypes. Actually, a huge number of epigenetic modulations are known to occur in complex ecosystems such as food matrices or animal intestine where most food and probiotic bacteria live. For instance, in lactic acid bacteria (LAB), the most employed probiotics and starters, a number of metabolic pathways are controlled by pH, such as some routes activated only by acidic pH conditions and generating toxic compounds (*e.g.*, biogenic amine production or arginine deiminase pathway) [18]. The attitude to exclude *a priori* a certain starter strain for such genetic traits is incorrect if the food matrix pH is neutral or alkaline [18]. Furthermore, in the complex gut ecological niche, reciprocal interactions between bacteria and host occur: it has been demonstrated that transit through the mouse intestine changes the expression level of several bacterial genes involved in the induction of specific cross-talking signals[19] and [20], and that exposure to probiotic strains also affect colonocyte transcriptome[21]. On the other hand, it is worth reminding that gene function annotation by genomic analyses is based only on sequence homologies with genes present in databases and final validation should always be obtained by functional studies.

All these considerations underline the importance of studying bacterial protein profiles in different experimental conditions. Comparative proteomic analysis is among the most suitable tools to evaluate the phenotypic responses of a food starter or a probiotic strain to a certain environment. For instance, lactose starvation stress and the effect of the surfactant Tween-80 as a protective agent, have been evaluated in *Lactobacillus casei*GCRL163 by a comparative study based on nanoLC-MS/MS [Al Naseri 22]. A gel-free proteomic approach was used to elucidate the acidic stress response in *Lactobacillus plantarum* 423 [Heunis 23]. As far as proteomics is concerned, 2DE in-gel techniques proved to be of particular interest allowing us also to detect enzyme isoforms. For instance, it is possible to discriminate a differential biosynthesis of isoenzymes involved in either glycolytic or gluconeogenetic direction of Embden-Meyerhof-Parnas route [24]. Since the beginning of the proteomic era, several protocols have been set up to recover proteins from different cell districts allowing to separately analyze different subproteomes expressed by microorganisms (Fig. 1). The analysis of subproteomes represents a

valuable tool to obtain additional information on specific problems: membrane protein patterns can reveal induction of transporters [24] and, since the first adaptive modifications to hostile environments occur at envelope level, stress tolerance pathways [25]. On the other hand, surface and extracellular proteomes help typing probiotic bacteria by elucidating what happens in the bacterium-host interface. In this context, a very recent cutting-edge paper [Le Marechal 26] describes three methods to recover key surface proteins involved in immune-modulation in *Propionibacterium freudenreichii*.

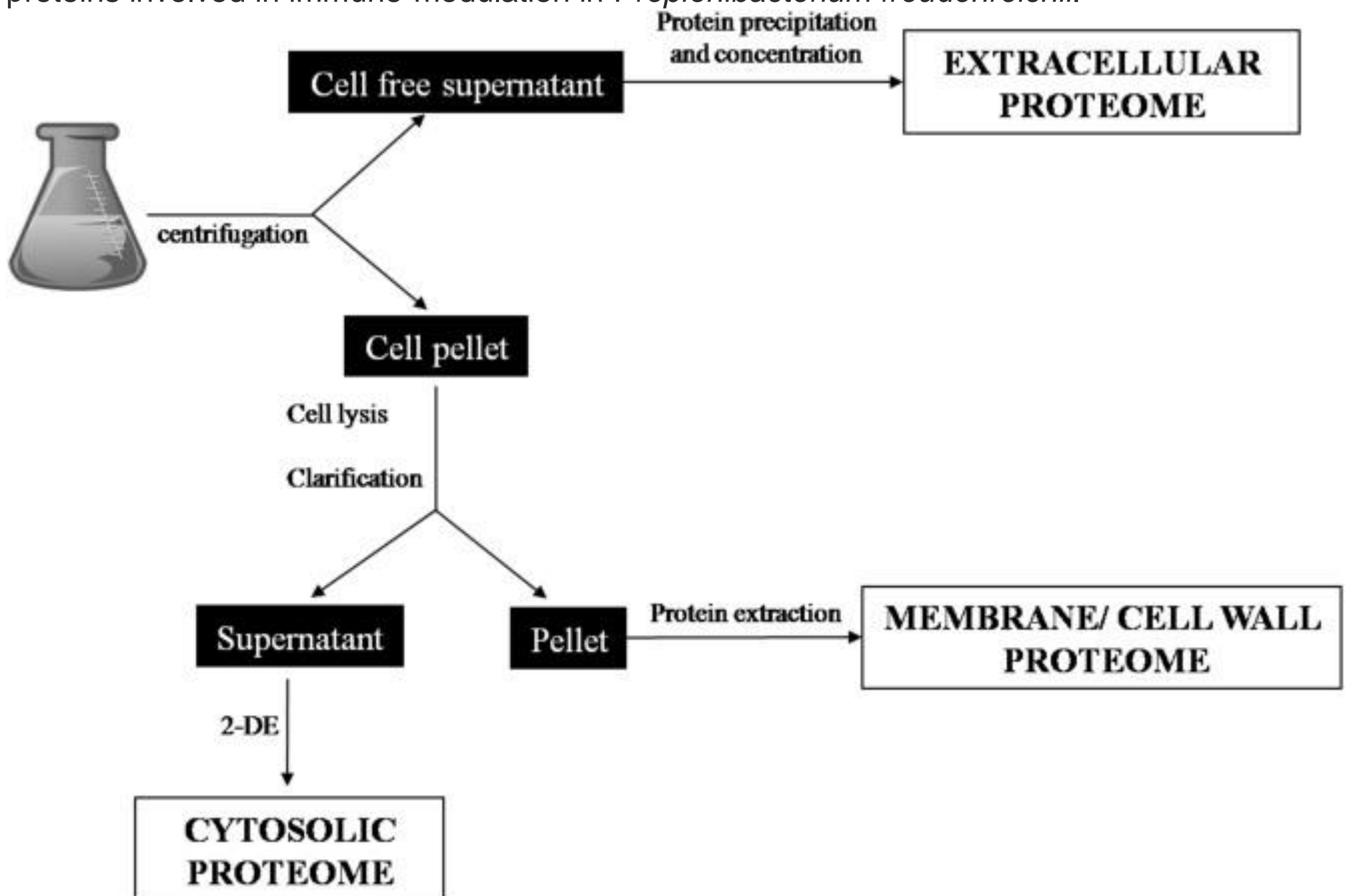

Fig. 1.

Flow chart illustrating sample preparation strategies for the analysis of the different subproteomes.

Figure options

For all these reasons we are willing to contribute with a review reporting examples in which the analysis of LAB subproteomes proved to be essential for reconstructing metabolic pathways, obtaining information about bacterial physiology and behavior and characterizing starters and probiotic bacteria in view of industrial or *in vivo* applications.

1.1. Biogenic amine risk: tracking the history of an undesired metabolic pathway by 2-DE

LAB are quite acid tolerant. Because of their inability to biosynthesize heme, they cannot get functional respiratory chains and have evolved a substrate-level phosphorylation mainly based on lactic acid fermentation. Apart from being a low-energy gain route, this pathway causes pH-lowering and LAB have to face acidification problems [18]. Some LAB strains display a natural ability to tolerate the harsh wine environment (pH = 3) as well as

the very acidic pH of stomach. This phenotype is based on several strategies to neutralizing pH lowering and/or to adapting to acidic environments and is highly appreciated in starter and probiotic LAB. Unfortunately, some of these metabolic strategies generate undesired molecules such as spoilage amines, like putrescine and cadaverine that alter the organoleptic properties of food [27]. Also bio-active amines acting on the Central Nervous System (CNS) and the vascular apparatus like histamine, an hypotensive molecule involved in allergies, and tyramine, an hypertensive metabolite sporadically involved in cerebral hemorrhage [28], [29] and [30], can be produced.

Some years ago, our research group used proteomic approach to evaluate the potential of LAB strains, either isolated from food or present in our microbial collection, to produce biogenic amines in different environmental conditions [24], [31], [32] and [33]. Once established that certain strains possess the genetic determinants for amino acid decarboxylation to amines, it still remained to elucidate if amines were actually produced and if their production was constitutive, modulated by environmental factors through regulation of gene expression or rather controlled at catalytic level.

In toto comparative proteomes of wine- and collection-isolated lactobacilli (harboring gene determinants for histamine production) grown with or without the precursor amino acid were analyzed [24] and [32]. The results obtained demonstrated that histidine decarboxylase (HDC) biosynthesis is significantly affected by the presence of histidine in the culture medium [24]. These preliminary data indicated that: i) histamine production, apart from being controlled through catalytic modulation of HDC by several effectors[34] and [35], was also regulated at biosynthetic level by histidine; ii) starter strains possessing the genetic determinants for histamine production could be safely employed in wine for malo-lactic fermentation since the content of histidine in wine is generally very low and the only amino acid present in a significant amount after alcoholic fermentation and separation of dead yeast cells is arginine [36]. However, since induction by the precursor amino acid is not the only factor triggering histamine biosynthesis, caution should be applied and other inducers should be tested, as reported in the next paragraphs.

As far as tyrosine decarboxylation is concerned, soluble protein analysis failed to reveal differential production of tyrosine decarboxylase (TDC) in a tyramine-producing*Enterococcus faecalis* DISAV1022 isolated from a goat cheese. For this reason, we addressed our interest to membrane protein profiles. Membrane-enriched comparative proteome, obtained by an extensive ultracentrifugation followed by extraction with thrifluoroethanol/chloroform mixture [37] and [38], enabled us to identify a spot corresponding to tyrosine decarboxylase (TDC) which was detectable only in cultures supplemented with 1 g/L tyrosine [31]. This study demonstrated that also the tyrosine decarboxylation pathway was induced at biosynthetic level by the precursor amino acid. Unfortunately, tyrosine content in cheese is very high, suggesting the exclusion of this *E. faecalis* strain as starter for cheese making.

On the other hand, our proteomic approaches revealed that the risk of undesired bio-active amine production can also be limited by alternative strategies based on induction of pathways competing with amino acid decarboxylation. It is known that similar alkalinization and energy gain can be obtained through either malo-lactic fermentation (MLF) or arginine deiminase pathway (ADI) [18]. The former route is very similar to amino acid decarboxylation and consists in the conversion of the dicarboxylic malic acid into the monocarboxylic lactic acid. This reaction contributes to both pH buffering and generation of metabolic energy (PMF). ADI pathway produces ATP and significant alkalinization as well [18]. On the basis of these considerations possible synergies/competitions among the three pathways (amino acid decarboxylation, MLF and ADI) were explored [32]. Comparative proteomic analyses of soluble extracts of *Lactobacillus hilgardii* ISE 5211 grown on high amounts of malic acid and/or arginine and/or histidine demonstrated that histidine negatively controls ADI pathway enzyme biosynthesis and that reciprocally arginine down-regulates HDC production. Although these findings offer an interesting paradigm of reciprocal regulation between these pathways, they cannot be exploited from an applicative standpoint since the ADI alkaline end-product is ammonia, a metabolite which is more toxic and dangerous than histamine for human health. Unfortunately, MLF did not show any significant competition against histidine decarboxylation or ADI in *L. hilgardii* [32]. However, recent results by Laroute and co-workers (Laroute et al., unpublished data) indicated negative modulation of amine production by malate in a *Lactococcus lactis* strain, extensively employed in the dairy industry. In such a case, malate supplementation could be considered an industrial fermentation strategy to re-direct microbial metabolism towards lactic acid instead of amine production.
A particular case of bio-active amine produced by LAB is gamma-amino butyrate (GABA) originating from glutamate decarboxylation. GABA is an appreciated molecule since it acts as a relaxing agent on the smooth muscles of the intestine [39] and also exerts positive effects on mood promoting tranquility [40], [41] and [42]. GABA-producing LAB strains show improved survival to gastrointestinal transit and have been added to several fermented foods [43] with the aim of releasing GABA directly in the gut. A combined fermentation-transcriptomic-proteomic investigation, performed on *L. lactis* NCDO2118, revealed that: i) glutamate-fortified culture medium can enhance GABA biosynthesis, and also glutamine can serve as a precursor in absence of glutamate thus allowing the recovery of a significant amount of GABA; ii) glutamate supplementation down-regulated the biosynthesis of ADI pathway enzymes [33]. As illustrated in Fig. 2 both pathways contribute to energy gain and pH lowering. These results suggest that, in strains bearing both the genes for ADI and glutamate decarboxylation, an excess of glutamate or glutamine in the food matrix allows the preventing of ammonia accumulation.

Fig. 2.

Competing routes for arginine deimination (ADI) and glutamate decarboxylation pathway which contribute to acid resistance and generation of metabolic energy in lactic acid bacteria. *ADI*, arginine deiminase; Arg, arginine; *CK*, carbamate kinase; GABA, γ-aminobutyric acid; *GAD*, glutamate decarboxylase; Glu, glutamate; Orn, ornithine; *OTC*, ornithine transcarbamylase; PFM, proton motive force.

Figure options

The overall results we obtained by comparative proteomic experiments performed on biogenic amine-producing LAB are summarized in Table 1. The presence of the precursor amino acid: i) induces a cytosolic overproduction of histidine decarboxylase (in*Lactobacillus w*53, *Lactobacillus* 30*a* and *L. hilgardii* ISE 5211); ii) triggers an overexpression of membrane-bound tyrosine decarboxylase in *E. faecalis* DISAV1022; and iii) does not induce differential biosynthesis of glutamate decarboxylase in *L. lactis*NCDO2218 as shown by both cytosol and membrane protein analysis.

Table 1.

Summary of the results obtained from comparative proteomic analyses of different biogenic amine producing LAB. NP, not performed.

Bacterial strain	Stimulated condition	Biogenic amine	Involved enzyme	Cytosol proteome	Membrane-enriched proteome	Reference
*Lactobacillus*w53	10 g/L histidine	Histamine	Histidine decarboxylase	Enzyme up-regulated	NP	[24]
*Lactobacillus*30a	10 g/L histidine			Enzyme up-regulated	NP	[24]
*Lactobacillus hilgardii*ISE5211	4 g/L histidine			Enzyme up-regulated	NP	[32]
*Enterococcus faecalis*DISAV1022	1 g/L tyrosine	Tyramine	Tyrosine decarboxylase	Enzyme not up-regulated	Enzyme up-regulated	[31]
*Lactococcus lactis*NCDO2118	5 g/L glutamate	GABA	Glutamate decarboxylase	Enzyme not up-regulated	Enzyme not up-regulated	[33]

Table options

1.2. Surviving lyophilization without losing efficacy: what DIGE proteomics can add

The use of optimized and stable bacterial cultures both as starters for industrial processes and as probiotics is a well established practice [44] and [45]. Furthermore, the use of selected LAB (such as bacteriocin-producing strains) as bio-control agents to counteract food-borne infections and food spoilage has been expanding in the last decade [46]. Nowadays, few highly performant strains are available and used throughout the world

(*e.g.*, by food fermentation and pharmaceutical plants) for each of these applications. Hence, these bacterial strains need to be sent without damage by starter-producing companies. This can be achieved by lyophilization procedures. The same treatment is also used for probiotic strains that are not resistant to gastrointestinal transit and therefore need to be encapsulated before administration. The main problem related to the use of these standardized procedure is represented by cell death during the freeze-drying process. Several cryoprotectants have been suggested to enhance the survival rate: carbohydrates such as polyfructose, trehalose and raffinose display a protective effect on membrane and a stabilizing activity on proteins [47] and [48]. Trehalose proved to be one of the most performant cryoprotectants [49], so we decided to evaluate its effect on the cheese-isolated *L. lactis* strain 11D. The growth of this strain in the presence of trehalose as carbon source resulted in a higher growth rate than in the presence of glucose as carbon source [50]. To understand if this biomass increase corresponds to significant physiological modifications (and, notably, to potentially harmful phenotype) a 2D-fluorescence difference gel electrophoresis (DIGE) experiment was performed to highlight differences in protein profiles between cultures grown on trehalose- and glucose-containing medium. The main goal was to exclude induction of potential virulence factors by trehalose, and also to ascertain if the main probiotic characters of *L. lactis* were conserved during growth on this sugar. Inhibition of the biosynthesis of two stress proteins (GroEL and proline dipeptidase) by trehalose was observed. Such attenuation of the stress response is probably due to the protective effect of trehalose itself. Furthermore, trehalose up-regulated the production of a chitinase, suggesting that growth on this sugar could enhance the potential of this bacterial strain against fungi [50]. It has to be mentioned that in some LAB strains, chitinase gene(s) are found within an operon also encoding a chitin-binding protein involved in adhesion to chitin but also to mucins since both chitins and mucins contain N-acetylglucosamine units [51]. Hence, these data suggest that the gut adhesive potential of *L. lactis* is increased by trehalose. The biosynthesis of a cell-wall renewal enzyme was also stimulated by trehalose, confirming that this sugar can improve the growth rate of this strain.

1.3. Controversial strain typing: combining in-gel and gel-free proteomics to overcome the concept of species-pathogenicity

Some bacterial species, such as *E. faecalis*, have long been employed in food-making.*E. faecalis* is especially used in the Mediterranean area for cheese production and it is appreciated for its capability to confer specific texture and organoleptic properties to food due to its proteolytic and esterolytic activity and diacetyl production [52], [53] and [54].*Enterococcus* strains have also been proposed as probiotics, mainly for their ability to produce bacteriocins called enterocins [1], [55] and [56]. However, because of their resistance to a wide range of pHs and temperatures, enterococci are ubiquitous and able to colonize heterogeneous niches (soils, food and the

gastro-intestinal tract of humans and animals). It has recently been demonstrated that human enterococci display the same antibiotypes and toxinogenic profiles than pig-, cattle- and sheep-isolated enterococci and probably the strains hosted in the human gut are acquired from food [57]. An increasing number of multidrug resistant (MDR) enterococci has been reported in the literature: they can become the dominant flora under antibiotic pressure, predisposing immune-compromized patients to severe hospital infections including sepsis with a high mortality degree [58]. Apart from antibiotic resistance, virulence factors such as proteolytic enzymes have been also demonstrated to be present in enterococci [59] and [60]. Therefore the safe use of this bacterial species in food is questionable [61].

To shed light on the potential pathogenicity characters of *E. faecalis*, a proteomic investigation was carried out to compare food-derived and hospital-isolated strains [62]. A combined in-gel (exoproteome) and gel-free (*in toto* proteome by nano LC-MALDI-TOF/TOF MS) approach was used and *E. faecalis* strains of different origin proved to be significantly different. First of all, the exoproteome of the hospital-isolate contained several virulence factors, like gelatinase, extracellular serine protease and superoxide dismutase which were absent in the food-isolate protein extracts. The-gel-free approach complemented these results by revealing an increased production of additional proteins potentially involved in pathogenicity in the hospital strain. Among the latter, the endocarditis-specific antigen, a chitin-binding protein, an adhesion lipoprotein and a two-component regulatory system for alkaline phosphatase activation were identified. On the other hand, the food-isolated strain expressed proteins related to bacteria–bacteria communication suggesting a good adaptation to the complex multi-organism food matrix[62]. All these results highlight the need of detailed typing of strains to be employed in food fermentation or as probiotics and proteomics proved to be an excellent tool for this purpose.

1.4. Exploring exoproteomes for evaluating probiotic persistence, safety and efficacy

Probiotic bacteria are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host [63]. Probiotics are used either as food-additives or as (lyophilized or encapsulated) pharmaceutical preparations. They can be employed to counteract intestinal infections in the newborns, traveler's diarrhea, post-antibiotic unbalance of the gut microbiota and also as immune-modulators controlling inflammation, allergies, and autoimmune diseases [64]. Apart from these ascertained functions, they have received growing attention as cell cycle regulators since they can produce short chain fatty acids (SCFA), controlling and preventing colon cancer, and conjugated linoleic acids (CLA), acting as modulators on both lipid and sugar metabolism [65], [66] and [67]. Nevertheless, the latter functions still have to be validated and controversial results are reported in the literature [68] and [69]. The best recognized probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*. Recently, also

Propionibacteria and some yeasts, like *Saccharomyces boulardii*, have started to be considered suitable for probiotic use [70], [71] and [72].
To be recognized as probiotics, bacteria need to satisfy the following safety and efficacy requirements: 1. to be GRAS (generally recognized as safe), even in immunocompromized hosts; 2. to possess a desirable antibiotic sensitivity profile (lacking antibiotic resistance traits); 3. to be able to survive to the gastro-intestinal transit, resisting to acidic pH, gastric enzymes and bile salts; and 4. to be able to adhere to gut cells [73].
Some of the tests needed to proving these requisites are quite simple, while more sophisticated analyses are necessary to the establishment of adhesion capacity and immune stimulating properties. The ability to permanently adhere to the gut mucosa is the key for probiotics persistence in the human gut. Adhesion is based on complex and sequential events including: i) chemotactic movements towards mucus; ii) weak interactions with mucus and mucosa; and iii) production of specific adhesive molecules interacting with both gut cell surface and extracellular matrix proteins (EMP) [64]. If this process is correctly achieved, probiotics can exclude or displace other bacteria thus permanently colonizing human gut [74]. Specific adhesive molecules involved in this mechanism are present at the cell surface of probiotic strains: teichoic and lipoteichoic acids, exopolysaccharides (EPS) and, above all, proteins. In this context the study of the cell surface proteome allows to establish which adhesive proteins are involved in this phenomenon for each probiotic strain. It has to be underlined that most proteins displayed on the bacterial surface are weakly bound to the cell wall, and hence they can be recovered from the culture medium. This weak association depends on the fact that these proteins are anchored to the cell wall through non-covalent interactions [75], being “easily” released during cell-wall turnover [76], or as a result of pH changes [77] in the extracellular environment where they exert different functions. This is an interesting phenomenon allowing the use of a single protein for multiple functions. Actually, most adhesins belong to the class of moonlighting proteins, displaying several different roles in relation to the cellular compartment in which they are [78]. Roles of different moonlighting proteins are summarized in Table 2. All these considerations underline that the analysis of the exoproteome is the best method to establish how many different proteins are involved in the adhesion process. Of course, to fully elucidate the overall adhesion potential of a strain, exoproteome findings have to be complemented and validated by phenotypic tests using cell culture methods (such as adhesion tests to model gut cells) that unequivocally demonstrate adhesive capabilities.

Table 2.

Established role of different moonlighing proteins.

Moonlighting protein	Role	References
α-enolase	Adhesion, plasminogen binding	[77] and [79]
Glyceraldheyde 3-phosphate dehydrogenase	Adhesion	[77]
Phosphoglycerate mutase	Plasminogen binding	[79]
EF-Tu	Adhesion	[80] and [81]

Moonlighting protein	Role	References
GroEL	Adhesion	[50]
DnaK	Plasminogen binding	[79]
Ornitine transcarbamylase	Immunogenic surface protein	[82]

Table options

In our recent researches, the exoproteome and the adhesion attitude of three*Lactobacillus* strains (*Lactobacillus reuteri* Lb2 BM DSM 16143, *L. plantarum* S11T3E and *Lactobacillus pentosus* S3T60C) were investigated to correlate adhesion potential to specific proteins [83] and [84]. The adhesive ability of all these strains had been previously proved, by using Caco-2 cells as a model of gut mucosa. All the exoproteomes were analyzed during the middle exponential phase to avoid cytosolic contaminations due to cell lysis during the stationary growth phase. *L. reuteri* exoproteome revealed the presence of several moonlighting proteins (PGK, GAPDH, EF-Ts, EF-Tu and trigger factor). Some of them (EF-Tu, GAPDH and trigger factor) have been previously referred to as adhesins. The fact that most of these proteins are enzymes involved in central metabolism or protein biosynthesis, strongly underlines the importance of subproteome evaluation. Currently available bioinformatic tools can hardly predict if the product of a gene will be secreted or not, since some proteins are known to be translocated across the cell membrane even if they do not possess a "traditional" signal peptide for secretion[85]. As a consequence, gene-function predictive software can hardly help us to ensure if a gene product with a proved intracellular function (involvement in central energy metabolism) also possesses a more sophisticated and interactive role in the cell wall or extracellular environment. It is interesting to underline that some moonlighting proteins found in *L. reuteri* exoproteome (like EF-Tu) can have immunomodulating functions [86]and this is a valuable information about a probiotic strain. Actually, probiotic and gut bacteria can act on dendritic cells by means of interactions with Toll-like receptors and direct them towards specific differentiation [87]. In this way, different immune functions (*e.g.*, production of pro-inflammatory or anti-inflammatory cytokines, T helper/T regulatory cell ratio) can be controlled.

L. plantarum S11T3E exoproteome revealed the presence of several proteins with adhesive functions (adherence protein with chitin-binding domain, GAPDH, M23 family peptidase) confirming the hypothesis that its adhesive ability mainly depends on this family of macromolecules. On the contrary, only one potential adhesive protein (M23 family peptidase) was present in *L. pentosus* S3T60C exoproteome, indicating that its adhesive capability could be mediated chiefly by macromolecules different from proteins. Notably, exopolysaccharides could be responsible for this property as confirmed by its ability to produce high amounts of these compounds [84].

1.5. Subproteomes for reconstructing metabolic pathways: *L. reuteri* story of selenium fixing and releasing

L. reuteri is a probiotic species possessing both acidic pH and bile salt resistance[88] and [89] and appreciated for its ability to produce microbial interference

molecules such as reuterin, reutericin and reutericycline useful to counteract infections [90],[91] and [92]. It is also able to compete with pathogenic bacteria for adhesion [93] and to attenuate toxin production in both bacteria [94] and fungi [95]. *L. reuteri* Lb2 BM DSM 16143 is able to grow on sodium selenite, recovering it from the culture medium. Part of this selenite is reduced to elemental selenium and accumulated on the cell surface as nanoparticles [12], but a significant part follows another way. In order to clarify the metabolic destiny of selenium (Se) into the cell, a comparative proteomic investigation of the *in toto* fraction by 2DE was set up by growing *L. reuteri* Lb2 BM DSM 16143 with and without sodium selenite [12]. An enhanced biosynthesis of selenocysteine lyase was detected in stimulated condition, suggesting, for the first time, a possible ability of this strain to fix selenium into selenocysteines. Selenium is usually inserted into two amino acids: selenomethionine (SeMet) and selenocysteine (SeCys). While SeMet is formed by a simple replacement of sulfur with selenium in methionine, SeCys is defined as the 21st amino acid and it is genetically encoded by the UGA codon[96]. Further investigations on this metabolic ability were performed by laser ablation inductively coupled plasma mass spectrometry imaging (LA-ICP MSI) of Se-containing proteins in 2D gel electrophoresis, followed by their identification by capillary HPLC-electro-spray Orbitrap MS/MS assisted by the quantitative control of selenium elution by ICP MS [97]. This approach provided a highly reliable demonstration that this *L. reuteri*strain fixes Se exclusively into SeCys, whereas Met/SeMet substitution did not occur. This study also allowed the identification of the proteins in which Se is stored as SeCys (phosphoketolase, pyruvate kinase, arginine deiminase, 6-phosphogluconate dehydrogenase, GAPDH, ornithine carbamoyltransferase, ribonucleoside hydrolase RihC), as well as the exact position in which SeCys is inserted within the protein primary structure (Fig. 3). This work was a further demonstration of the power of different and combined proteomic approaches in the study and characterization of properties and abilities of probiotic bacteria.

Fig. 3.

Incorporation of selenium as selenocysteine in specific proteins of *L. reuteri* LB2 BM DSM16143 as revealed by a 2D LA-ICP MSI approach.

Figure options

The study of the exoproteome of *L. reuteri* Lb2 BM DSM 16143 grown in the presence of sodium selenite and the manual interpretation of the raw data of the LC-MS/MS, revealed the presence of two peptides containing a SeCys and belonging to GAPDH and phosphoketolase, respectively [13]. This experimental evidence has strengthened the probiotic potential of this strain since it demonstrated its ability to secrete organic bio-available forms of Se (Se-containing proteins). Furthermore, this is also a very appreciated feature in the perspective of using the strain as nutraceutical supplement to treat Se deficiencies, or as an antioxidant to prevent viral infections and aging-related diseases [98].

2. Conclusions

Ten years of investigations on probiotic and food bacteria proteomes by 2DE, DIGE and gel-free approaches have demonstrated that proteomic techniques can offer highly valuable and efficient help in typing strains to be employed as food starters, probiotics and nutraceutical supplements. The main results discussed in this review are summarized in Table 3. However, such a challenge is still at its beginnings. Nowadays, the probiotic/starter potential is still largely underexplored and underexploited, and further investigations of the protein profiles can add consistence to claims and open new relevant perspectives in the fields of food industry and human health.

Table 3.

Contributions concerning proteomic analyses revised in this manuscript.

Physiological Function Studied	Method	Reference
Lactose starvation	LC-MS/MS	[22]
Acid stress response	LC-MS/MS	[23]
Transporter induction	Total in-gel proteome	[24]
Envelope stress response	High isoeletric point subproteome	[25]
Acclimation before MLF	Total in-gel proteome	[17]
Se-fixing pathway	Total in-gel proteome	[12]
Moonlighting proteins	Envelope-enriched and exoproteome by in-gel proteome	[13]
Extracellular protein modulation by human gut factors	SDS-PAGE and MALDI MS/MS	[20]
Tyrosine and phenylalanine decarboxylase enzymes	Membrane in-gel proteome	[31]
ADI and histidine decarboxylation reciprocal competition	Total in-gel proteome	[32]
Membrane proteins solubilization	Membrane-enriched in-gel proteome	[37]
GABA production	In-gel proteome and transcripts	[33]
Separation of membrane proteins	Membrane in-gel proteome	[38]
Lyophilization resistance	DIGE in-gel proteomic	[50]
Virulance factors in food and hospital Enterococci	In-gel exoproteome and gel-free total proteome	[62]
Exported proteins	In-gel exoproteome	[76]
Adhesion	In-gel exoproteome	[83]
Bile salts response	Total in-gel proteome	[88]
Adhesion	In-gel exoproteome	[84]
Selenocysteine-containing proteins	LA-ICP MSI	[97]

Table options

Conflict of interests

The authors declare no conflict of interests.

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