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Full Length Research Paper

List of Lactiplantibacillus Plantarum Strains' Surface Proteins for the Dairy and Vegetable Niches

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With its pertinent probiotic and technological characteristics, Lactiplantibacillus plantarum (previously Lactobacillus plantarum) is employed extensively in the food industry to enhance the flavor, texture, and organoleptic qualities of fermented goods. As the primary players in bacterial-host interactions, cell-surface proteins play a crucial part in the molecular processes that produce beneficial effects. In order to obtain a complete picture of variations in protein profiles that might be connected to the strains' particular characteristics and habitat of origin, the proteins found on the surface of four L. plantarum strains—two of which were isolated from vegetable matrices and two from dairy products—were identified using proteomics. The findings showed that strains from vegetable matrices had a more varied pattern of surface proteins than bacteria from dairy matrices (>500 proteins vs. roughly 200 proteins, respectively). A core of 143 proteins was shared by the four strains, although 445 of those proteins were unique to strains derived from vegetable matrices, while 26 were unique to strains originating from dairy. Only strains from vegetable matrices exhibited choloylglycine hydrolase (bile salt hydrolase) and sortase A, which are involved in adhesion. These strains, especially L. plantarum S61, may offer substantial probiotic and biotechnological promise based on the unusual molecular activities of the discovered proteins.

Key words: Lactiplantibacillus plantarum, Surface proteins, Proteomics, Probiotics, Adhesion.

INTRODUCTION

The Lactobacillus genus includes a large number of bacterial species known to possess probiotic properties. Of them, Lactiplantibacillus plantarum (previously Lactobacillus plantarum) is one of the most intriguing and extensively researched species for its potential in biotechnology. It is "generally recognized as safe" (GRAS) by the US Food and Drug Administration (US FDA) and has a qualified presumption of safety (QPS) designation from the European Food Safety Authorities (EFSA) (EFSA Panel on Biological Hazards (BIOHAZ), 2024). Additionally, strains of L. plantarum have probiotic properties, which means they may help manage or avoid inflammatory illnesses (Fidanza et al., 2021).

As a starting culture, L. plantarum enhances the flavor, texture, and organoleptic qualities of fermented products and is also frequently utilized in food processing and preservation. With one of the largest genomes of any lactic acid bacteria (LAB, 3.3 Mb), L. plantarum has been isolated from a variety of habitats, including plants, food matrices, and the gastrointestinal tracts of humans and animals. This justifies a high level of genetic diversity within the species (Carpi et al., 2022; Martino et al., 2016; Siezen et al., 2010).

The ability of probiotics to adhere to epithelial cells is a critical characteristic because it promotes colonization and persistence in the gut, which are necessary prerequisites for probiotics to have positive effects like immune system stimulation and the exclusion of pathogenic microorganisms from the gut mucosa (Marco et al., 2006). The complex repertoire of proteins on the bacterial surface, known as the proteosurfaceome by Desvaux et al. (2018), is directly

involved in many bacterial functional and technological traits as well as the dynamic communication between bacteria and host. Numerous genomic studies have been conducted to predict the exo-proteome of this species due to the significance of surface proteins. This has revealed the presence of multiple protein classes that are differently linked to the cell surface (Albarracin et al., 2022; Boe-khorst et al., 2006; Huang et al., 2020; Kleerebezem et al., 2010).

However, to the best of our knowledge, only a small number of studies have used proteomic approaches to examine the repertoire of surface proteins of L. plantarum, despite the fact that proteomics has emerged as the preferred method for examining the surface architecture of bacterial cells, helping to define protein location and topology (Siciliano et al., 2019). Izquierdo et al. (2009) discovered 27 cytoplasmic proteins that may be connected to bacterial adhesion characteristics after comparing the cell-wall proteome that was isolated from three strains of L. plantarum with varying adhesion rates. Similar to this, Beck et al. (2009) discovered 29 outer-surface proteins of L. plantarum 299v, including ribosomal proteins, stress-related proteins, and glycolytic enzymes. More recently, Du et al. (2022) presented a catalog of the surface proteins of the probiotic L. plantarum HC-2 strain, which resulted in the identification of 201 surface proteins, including cytoplasmic proteins, membrane-integral proteins, transmembrane proteins, and proteins that carry a signal peptide.

In order to obtain a more comprehensive understanding of the variations in protein profiles that may be connected to strain-specific characteristics, we conducted a proteomic study employing shotgun and quantitative label-free techniques to identify the proteins found on the surface of four distinct L. plantarum strains (two isolated from vegetable foods and two from dairy products).

According to the unusual molecular roles of the proteins that were found, strains derived from vegetable matrices may have substantial probiotic and biotechnological potential.

2. Materials and methods

2.1. Bacterial strains and growth conditions

In this investigation, four strains of Lactiplantibacillus plantarum were employed.

L. plantarum ITEM 17218 was isolated from the raw milk cheese Valtellina Casera, which was produced in farms in the Sondrio province of Italy; L. plantarum ITEM 18335 was isolated from the raw milk cheese Formagela Val Seriana, which was produced in the Val Seriana region of Bergamo, Italy, and was listed as a typical product of the Lombardy Region (decree n. 4079 of 2010). With Regulation (EC) n. 1263/1996, this cheese's Protected Designation of Origin (PDO) was recognized on a European level. These two strains are designated as D strains and are part of the Agro-Food Microbial Culture Collection, CNR, Bari, Italy.

A company in Oujda, Morocco, sold L. plantarum S61, which was isolated from fermenting green olive brine; DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) supplied L. plantarum DSM 20174, which was isolated from pickled cabbage. The designation V strains is used to these two strains.

Subcultures of L. plantarum strains were kept as frozen stock at —80 ◦C in Man, Rogosa and Sharpe broth (MRS) (Thermo Fisher Scientific, Waltham, MA, USA) with 20% (v/v) glycerol (Carlo Erba, Milan, IT) for 24 hours at 28 ◦C. 100 mL of MRS test tubes (1 % v/v, pH 6.8) were inoculated with these cultures and then incubated for 14 hours at 28 °C (the incubation period corresponding to the early stationary growth phase).

2.2. Extraction of surface proteins

Centrifugation (7500g for 10 min at 4 ◦C) was used to harvest the cells, and PBS buffer (phosphate buffer) was used twice for washing.

With modest changes, the LiCl procedure of Lortal et al. (1992) was used to extract surface proteins (Mazzeo et al., 2022). In short, 10 mL of 5 mol/L LiCl was used to suspend 1 g of bacterial pellets, which were then incubated for 1 hour at 4 °C. Following a 15-minute centrifugation at 7000 g at 4 °C, the samples were filtered through 0.22 µm nitrocellulose membranes (Merck, Darmstadt, Germany) to remove the supernatants. After two hours of incubation at —20 ◦C, surface protein extracts were precipitated using pure ethanol that had been pre-chilled (1:9 v/v). Centrifugation (15,000g for 15 min at 4 ◦C) was used to extract the protein pellets, which were then repeated with 1 mL of 70% ethanol and dissolved in 0.1 mol/L Tris-HCl, 8 mol/L urea, and pH 8.5. The Bradford assay (Bio-Rad, Hercules, CA, USA) was used to determine the protein concentration.

Sample preparation for proteomic analyses and LC-MS/MS analysis Protein samples (20 µg) were reduced in 0.1 mol/L Tris-HCL, 8 mol/L urea, pH 8.5 with 1.5 µL of 0.2 mol/L dithiotreitol for 45 minutes at 37 ◦C. The protein samples were then alkylated in the same buffer with 6 µL of 0.2 mol/L iodoacetamide for 30 minutes at room temperature in the dark. Six microliters of 0.2 mol/L dithiotreitol were used to quench the alkylation process.

Ultimately, protein samples were digested using 0.2 µg of Sequencing Grade Modified Trypsin (1:100 w/w) (Promega, Madison, WI, USA) after being diluted to 1 mol/L urea by adding 0.1 mol/L Tris-HCl, pH 8.5. For eighteen hours, the digestion was conducted at 37 °C. Following acetonitrile (ACN) conditioning and a 0.1% FA rinse, the resulting peptide mixtures were desalted by solid phase extraction using C18 ZipTip columns (Merck). The peptides were then loaded in 0.1% FA, eluted with 70% ACN in 0.1% FA, dried in a Speed-Vac centrifuge (Savant), and solubilized in 0.1% FA. As previously described (Mazzeo et al., 2022), tryptic peptide mixtures were examined using a Q-ExactiveTM mass spectrometer (Thermo Fisher Scientific) interfaced

with an UltiMate 3000 RSLCnano LC system (Thermo Fisher Scientific). Two distinct bacterial cultures (biological replicates) had their proteins extracted, and each sample (technical replicates) underwent three LC-MS/MS analyses.

2.3. Protein identification and label-free quantification analysis

Protein identification and quantification were obtained by processing the MS raw files using the Sequest search engine and the Proteome Discoverer (PD) search engine platform (version 2.4.1.15, Thermo Fisher Scientific). The following criteria were applied to the database searches: A contaminant protein database (provided by the manufacturer) and the Lactiplantibacillus plantarum database (downloaded from UniProtKB on July 2024, https://www.uniprot.org/) include trypsin as a proteolytic enzyme, up to two missed cleavages, carbamidomethyl as a fixed modification for cysteine residues, oxidation of methionine residues as a dynamic modification, 20 ppm mass tolerance for precursor ions, and 0.02 Da mass tolerance for MS/MS fragments. Both the protein and peptide levels had a false discovery rate (FDR) of 1%. The integrated peak area, or "abundance," for every protein that was quantified was obtained using the PD method.

The PD data was processed using Perseus software (version 1.6.0.7) (Tyanova et al., 2016). Reverse hits and contaminants were eliminated from the dataset. The dataset utilized for quantitative analysis contained proteins that were deemed reliably identified based on a number of peptides ≥2 and valid abundance values in three replicates of at least one strain. Only proteins that met these requirements in a single strain were thought to be specifically found on its surface.

The bio-informatic tool MAFFT Multiple Sequence Alignment (MSA, https://www.ebi.ac.uk/jdispatcher/msa/mafft?stype=protein&format=clustalw) was used to compare the sequences of discovered proteins in order to prevent protein redundancy (Madeira et al., 2024). Proteins that shared more than 80% of their sequences were not included in further bioinformatics analysis.

To find statistically significant differences (p value < 0.05) in protein abundance values between strains of different origin (V strains vs. D strains) and L. plantarum S61 vs. L. plantarum DSM 20174, quantitative analysis was performed using the two sample test (Student's t-test). The surface proteins of V strains and D strains were quantitatively compared, taking into account those proteins that were consistently found in at least one of the two strains isolated from the same habitat. Protein Fold Change Values (FCV) were determined by calculating the log2 difference between the mean protein abundance values of the couples under consideration. A substantial difference was defined as an FCV > ±2.

Perseus software was used to perform cluster analysis, and the results were visualized by creating a heatmap that showed the abundance values for each protein in each of the six replicates of each strain.

2.4. Bioinformatics and functional analyses

SignalP 5.0 (https://services.healthtech.dtu.dk/services/SignalP-5.0), SignalP 6.0 (https://services.healthtech.dtu.dk/services/SignalP-6.0), and InterPro (https://www.ebi.ac.uk/interpro/) were used to analyze the identified proteins. These programs can predict the presence of specific non-cytoplasmic or trans-membrane domains as well as the presence and location of signal peptide cleavage sites in amino acid sequences for translocation across cell membranes (Almagro Armenteros et al., 2019; Paysan-Lafosse et al., 2023). Gene Ontology annotations were used to derive the functional classification of surface proteins from UniProtKB. Putative moonlighting characteristics of the discovered proteins were obtained using the MoonProt database (http://www.moonlightingproteins.org/) (Chen et al., 2021). The online program Inter-actiVenn (http://www.interactivenn.net/) was used to create the Venn diagram (Heberle et al., 2015).

3. Results and discussion

Since surface proteins are essential for microbial probiotics and technological characteristics, it would be crucial to ascertain the makeup of surface protein patterns in order to gain understanding of the molecular mechanisms behind the functional characteristics of bacteria.

3.1. Surface proteins of the four L. plantarum strains

Surface proteins extracted from four L. plantarum strains were identified using shotgun proteomic techniques. Specifically, two strains isolated from dairy products (ITEM 17218 and ITEM 18335) were identified as D strains, and two strains isolated from vegetable matrices (S61 and DSM 20174) were identified as V strains.

Seven hundred surface proteins were found to be present in at least one of the four strains of L. plantarum (Table S1, sheet raw data). The bioinformatic tool MAFFT was used to verify protein redundancy (Madeira et al., 2024).

73 proteins were predicted to have a signal peptide for translocation across cell membranes based on bioinformatics processing of the protein sequences in this dataset using the SignalP tools. Furthermore, 39 proteins had trans-membrane domains (three of which also had a signal peptide), nine proteins had cell membrane subcellular locations, and one protein had cell envelope subcellular locations, according UniProtKB annotations. Furthermore, based on the category of cellular components, two proteins were designated as extracellular and two as membrane.

Based on the Phobius program, the bioinformatics resource InterPro predicted the presence of a signal peptide for two extracellular proteins, two cell surface proteins, and the proteins pepti- dase M10 (A0AAE2LP93), PTS system, cellobiose-specific EIIB component (F9URP9), OppA (A0A837P6C3), and Oligopeptide ABC transporter periplasmic oligopeptide-binding protein. Peptidoglycan glycosyltransferase (A0A1E3KSC2) was expected to have a transmembrane domain (Table S1, sheet functional classification).

Some of the proteins, including enolase, glyceraldehyde 3-P-dehydrogenase, chaperonin GroEL, elongation factor Tu, glucose-6-phosphate isomerase, and glutamine synthetase, have been found on the cell surface of LAB (Beck et al., 2009; Izquierdo et al., 2009). Twenty-five proteins were identified in the MoonProt database as moonlighting proteins of Gram-positive bacteria (Chen et al., 2021). These proteins may be involved in adhesion processes by acting as mucin, plasminogen, fibronectin, and laminin binding proteins. They were also found in the secretoma of these L. plantarum strains that our group had previously examined (Mazzeo et al., 2024) (http://www.moonlightingproteins.org/ accessed on July 2024) (Chen et al., 2021). Furthermore, the Gram-negative organism Escherichia coli was shown to have eight ribosomal proteins that moonlight (Table S1, sheet functional classification).

Numerous cytoplasmic proteins were found in our investigation, despite the fact that we purposefully decided to examine bacterial cells obtained during the early stationary growth phase in order to reduce the possibility of cell lysis and intracellular protein contamination. Other proteosurfaceome studies that found a large proportion of cytosolic proteins were in agreement with these data (Du et al., 2022; Espino et al., 2015; Klotz et al., 2017). The possibility that some of these proteins might also moonlight by taking on secondary roles on cell surfaces, however, cannot be completely ruled out (Jeffery, 2018, 2019).

Remarkably, the majority of the cytoplasmic proteins and moonlighting proteins (chaperones, glycolytic enzymes, elongation factors, and ribosomal proteins) were among the 84 proteins found in this study that were also recently found in the surface protein set of the probiotic L. plantarum HC-2 strain (Du et al., 2022).

A cluster analysis was performed using quantitative proteomic data to identify differences and similarities among the surface protein patterns of the four strains. A correlation between the proteosurfaceome of these strains and the biological niches from which they were isolated is evident from the heatmap that was produced, which displayed a first cluster that included L. plantarum ITEM 18335 and ITEM 17218 (isolated from raw milk cheese) and a second cluster that included L. plantarum S61 (isolated from fermented green olives) and L. plantarum DSM 20174 (isolated from pickled cabbage) (Fig. 1). Differences in the quantity of proteins found in isolates originating from dairy and those isolated from vegetables also clearly demonstrate this relationship. Actually, with 533 and 584 proteins, respectively, L. plantarum DSM 20174 and L. plantarum S61 (V strains) have the most surface proteins. According to Table S1, sheet raw data, the two L. plantarum strains ITEM 17218 and ITEM 18335 (D strains) have 205 and 208 surface proteins, respectively. Only 26 proteins were unique to strains from dairy products (Table S1, sheet D strains, Fig. 2), whereas 445 proteins were specifically found on the surface of strains isolated from vegetable matrices (Table S1, sheet V strains, Fig. 2) and the four strains shared a common core made up of 143 proteins, including several moonlighting proteins (21 out of the 33 identified ones) (Table S1, sheet core proteins).

Functions like serine-type endopeptidase activity, serine-type D-Ala-D-Ala carboxypeptidase activity, peptidyl-prolyl cis-trans isomerase activity, and kinase activity were similarly represented in the proteosurfaceomes of the D and V strains, according to the classification of surface proteins using the GO "molecular function" category.

However, the surface protein repertoire of strains from plant matrices primarily contained proteins with predicted molecular functions, including oxidoreductase, metal ion binding, magnesium ion binding, cysteine-type peptidase, unfolded protein binding, transferase, glycosyltransferase, and transmembrane transporter (Fig. 3).

Notably, the proteosurfaceomes of V strains showed more proteins with glycosyltransferase activity than those of D strains (Table S1, sheet functional classification, Fig. 3), which is consistent with findings from the Cen et al. (2020) investigation. Through a comprehensive phylogenomic analysis of 140 strains of Lactose plantarum from various niches, these authors showed that strains from dairy niches had significantly fewer genes annotated as glycoside hydrolases (GH) and glycosyltransferases (GT) than strains from vegetable isolates. This was likely because the carbohydrates in dairy products (primarily lactose and galactose) are less complex.

Interestingly, Sortase A (StrA), a transpeptidase that connects surface proteins to the peptidoglycan of Gram-positive bacteria, was also found among the proteins that were exclusively found in V strains. According to reports, StrA plays a crucial role in bacterial contact with host cells and biofilm formation in L. plantarum WCFS1 by facilitating the covalent binding of 27 predicted surface proteins to the cell wall (Fernandez Ramírez et al., 2018; Remus et al., 2013). The absence of the strA gene in a mutant of the L. plantarum CMPG5300 strain resulted in a distinct loss of the auto-aggregative phenotype as well as a decreased ability to form biofilms and adhere to vaginal epithelial cells (Malik et al., 2013).

Furthermore, strains from vegetable habitats had ten moonlighting proteins exclusively on their surface. Among these, it is noteworthy that DSM 20174's surface contains choloylglycine hydrolase, also known as bile salt hydrolase or bsh, a protein that can bind plasminogen (Candela et al., 2007). Furthermore, one of the factors for choosing probiotic strains is the capacity to hydrolyze bile salts (Begley et al., 2006). Numerous clinical trials have indicated that bile salt hydrolase active probiotic bacteria are effective at decreasing cholesterol (Jones et al., 2013). Additionally,

Carpi et al. (2022) found probiotic flag genes in their pan-genomic investigation of L. plantarum, including genes encoding sortase A and bile salt hydrolase.

A quantitative proteomic analysis was performed on Fig. 2 to further evaluate the proteosurfaceome of the V strains to the D ones. Venn diagram illustrating the proteomic findings. To create the diagram, lists containing the entries of proteins found in each strain have been entered into the computer. Each strain's surface protein count is indicated in parenthesis. Red and blue circles, respectively, indicate the quantity of proteins that are exclusively found on the surface of strains that are of dairy and vegetable origin. (The reader is directed to the online version of this article for an interpretation of the color references in this figure legend.) dataset that contains 143 proteins that are shared by all strains. In the proteosurfaceome of the V strains, this analysis revealed that 11 proteins were less abundant and 23 proteins—including six moon-lighting proteins (RpsJ, RpsG, Pgk, Gap, Fba, and Pgi)—were more abundant (Table S2, sheet quantitative core).

One of the proteins more prevalent in V strains, glyceraldehyde-3-phosphate dehydrogenase (Gap), has already been found on the surface of various strains of L. plantarum and has been shown to bind fibronectin, plasminogen, and human colonic mucin (Glenting et al., 2013; Kinoshita et al., 2008; Saad et al., 2009). Furthermore, Gap mediates the adherence of highly adhesive strains of L. plantarum to intestinal epithelial HT-29 cells and can enhance adhesion in strains with weak adhesion ability, according to Wang et al. (2018). More recently, it has been determined that the extracellular gap of L. plantarum JCM 1149 plays a critical role in the up-regulation of cytokine production and immunomodulation-linked gene expression, thereby validating the moonlighting protein's pivotal role in L. plantarum probiotic functionality (Kudo et al., 2023).

Furthermore, we discovered isoforms of phosphoglycerate mutase and glucosamine-6-phosphate deaminase among the proteins that were only detected on the surface of strains from vegetable matrices. According to Pérez Montoro et al. (2018), a highly adhesive strain of L. pentosus isolated from naturally fermented green table olives had a higher level of these proteins on its surface than less adhesive strains isolated from the same matrix.

When combined, these results may indicate that strains of L. plantarum from vegetable matrices have a higher capacity for adhesion.

Our findings also verified that the L. plantarum strains' surfaces included eight peptidoglycan hydrolases (PGHs) at the protein level. Out of the 16 putative PGHs identified by the investigation of the L. plantarum WCFS1 genome, as retrieved by MAFFT analyses (Table S3), these extracellular proteins share at least 96% sequence identity with them (Kleerebezem et al., 2010; Rolain et al., 2012). PGHs perform a variety of tasks, such as autolysis, peptidoglycan turnover during growth, and daughter cell separation during cell division. They also play a role in adhesion, biofilm formation, and immunomodulation; in fact, they trigger the release of muramyl peptides that are known to interact with immune system receptors through host autolysis and cell-wall turnover (Kleerebezem et al., 2010; Rolain et al., 2012).

Interestingly, of those proteins, peptidoglycan-binding protein (A0A837NMF0) and gamma-D-glutamyl-meso-diaminopimelate peptidase (A0A837NG43) were more abundant in D strains than in V strains, while the glycoside hydrolase family 25 (A0AAE2LME7) was only found on the surface of V strains. The γ-D-Glu-mDAP muropeptidase Lp_3421, which has 96% of its sequence identity with A0A837NG43, is a key PGH in L. plantarum cell morphogenesis and is necessary for appropriate growth and cell cycle progression, as recently shown by Duchene et al. (2019). Given the compelling evidence that PGHs play a significant role in host-microorganism interactions and ecological niche adaptation (Kleerebezem et al., 2010), the unique PGH repertoire of these strains may be considered when assessing their probiotic and biotechnological qualities.

It's important to highlight that only 26 proteins were explicitly found in the proteosurfaceome of dairy strains. The foldase protein PrsA, a membrane-anchored lipoprotein that is essential for the post-translocational folding of secreted proteins and aids in reaching the correct shape for functional activity, was found in significant concentrations in D strains. In response to specific stressors, L. plantarum Ym1 also overexpressed this protein (Petrov et al., 2021).

3.2. Specific features of the proteosurfaceome of strains isolated from vegetables matrices

The two strains showed a very diverse catalog of surface proteins, and the proteosurfaceome of V strains was very complicated. Indeed, 81 proteins were found on the surface of L. plantarum DSM 20174, and up to 123 proteins were particularly identified in L. plantarum S61 (Fig. 2).

Notable proteins found in the proteo-surfaceome of L. plantarum S61 include sucrose-6-phosphate hydrolase (A0A166D1C7) and protein-N(pi)-phosphohistidine-sucrose phosphotransferase (A0A151G6C1), which are known to be involved in the absorption and degradation of sucrose. The capacity of L. plantarum WCFS1 to use short-chain fructooligosaccharides as a source of carbohydrates, which are abundant in plant meals, has been linked to an upregulation in the production of these enzymes (Saulnier et al., 2007). The capacity of L. plantarum S61 to ferment fructooligosaccharides, as previously reported by Abouloifa et al. (2020b), is further supported by these results and is a crucial aspect of its probiotic activity.

Furthermore, L. plantarum S61 has the proteins fructose permease IID component (A0A151G8Y6), PTS system mannose-specific EIIAB component (A0A837P489), and mannitol-specific phosphotransferase enzyme IIA component (A0A2S3U0X0) that participate in the transport of carbohydrates across cell membranes. Compared to strains isolated from other plant foods, those isolated from

fermented olives have a greater ability to use raffinose and galactose as sources of carbohydrates. This may be because the main sugars in olive fruits and leaves were mannitol, glucose, fructose, and galactose (Go´mez-Gonza´lez et al., 2010; Yu et al., 2021).

It is noteworthy that one of L. plantarum S61's most intriguing technological traits is its reported high beta-glucosidase activity (Abouloifa et al., 2020a). Since the 6-phospho-beta-glucosidases were specifically found on the surface of L. plantarum S61, our proteomic data closely matched these findings. It is intriguing to note that this class of proteins may have a role in the bio-conversion of olive phenolic glucosides (Muṇoz et al., 2024).

Additionally, a dataset of 452 proteins found in both V strains was subjected to a quantitative proteomic analysis. The findings indicated that, in comparison to L. plantarum DSM 20174, 38 proteins were more abundant and 19 proteins were less numerous in L. plantarum S61. L. plantarum S61 mostly contained larger amounts of proteins with molecular activities such transferase activity and transmembrane transporter activity, as well as proteins involved in the biological processes of proteolysis and peptidoglycan production (Table S2, sheet S61 versus 20,174).

4. Conclusions

Proteomics has made significant contributions in recent decades to the study of the pattern of cell-surface proteins that serve as the initial point of interaction between bacteria and their host or environment. These proteins are directly involved in a number of molecular pathways that have positive health consequences. Future research is required to determine whether these characteristics are reproducibly unique of other strains with similar origins, but our proteomic analysis produced a detailed picture of the proteins found on the surface of various L. plantarum strains, highlighting variations that may be connected to their habitat of origin.

These strains, especially L. plantarum S61, may have a substantial probiotic and biotechnological potential based on their more varied surface protein pattern and their roles in adhesion and prebiotic degradation. These strains were isolated from vegetable matrices.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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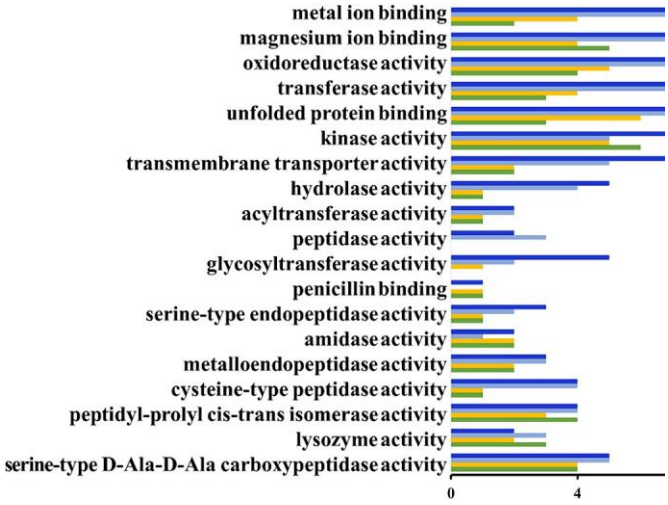

Fig. 3. Histogram reporting the number of proteins exhibiting selected molecular functions identified on the surface of strains of dairy or vegetable origin.