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Proteomics for depicting the secreted protein patterns of *Lactiplantibacillus plantarum* strains isolated from different food matrices

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ABSTRACT

Lactiplantibacillus plantarum (formerly known as *Lactobacillus plantarum*), a bacterial species isolated from a wide variety of ecological niches, plays an important role in food industries, being used as a starter culture of food fermentations and contributing to improve flavor, texture, sensorial qualities, and shelf life. It also improves the nutritional and functional properties of fermented foods through the biosynthesis of bioactive compounds and several strains exhibit probiotic features. In recent years, this bacterial species has also shown great potential for controlling plant pathogenic fungi. Shotgun proteomic approach and label free quantitative analyses were used to realize a detailed catalog of the proteins secreted by five *L. plantarum* strains isolated from vegetable foods or dairy products and exhibiting specific function features. In particular, these strains showed *in vitro* inhibitory activity against bacteria and molds. Results led to identify 602 proteins differently present in the secretome of the analyzed strains. For instance, proteins of application interest such as bacteriocins, glucansucrase, glycoside hydrolase and chitin binding proteins were differentially secreted in the five strains.

The information gathered in this study is a valuable contribution to increasing knowledge in relation to one of the most important bacterial species used as probiotic, for biocontrol of plant pathogenic bacteria and fungi and in different food production worldwide.

1. Introduction

Lactiplantibacillus plantarum (formerly known as *Lactobacillus plantarum*) is a facultative heterofermentative species belonging to the lactic acid bacteria (LAB) group and is found in a wide variety of ecological niches, from plants to gastro-intestinal tracts of human and animals and in different fermented foods such as dairy products, fruits, vegetables, meat, fish, etc. (Yilmaz et al., 2022; Zheng et al., 2020). *L. plantarum* has one of the largest genomes known among the LAB (3.3 Mb) (Carpi et al., 2022), and exhibits strong adaptability, high versatility and enormous diversity in phenotypic properties and metabolic capacity (Siezen et al., 2010). A typical nomadic lifestyle has been described for the species that is capable to maintain and employ a 'universal' set of genes to grow efficiently in many different environments (Martino et al., 2016).

L. plantarum has a Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA) and Generally Recognized as Safe (GRAS) status from the US Food and Drug Administration (USFDA) (EFSA Panel on Biological Hazards (BIOHAZ)Koutsoumanis et al., 2024). Interestingly, this species plays an important role in food industries, being used as a starter culture of food fermentations (i.e. olives, sourdough, vegetables, fruits, meat and milk products, and wine), thus contributing to improve flavor, texture, sensorial qualities, and shelf life. It also improves the nutritional and functional properties of fermented foods through the biosynthesis of bioactive compounds. Moreover, *L. plantarum* could contribute to enhancing food safety and preventing food spoilage (Li et al., 2023; Quattrini et al., 2018). Its antimicrobial properties are linked to the production of several compounds such as organic acids, hydrogen peroxide and diacetyl and, on the other hand, to the biosynthesis of bacteriocins (Goel & Halami, 2023; Russo et al., 2017).

In recent years, this bacterial species has also shown great potential for controlling plant pathogenic fungi (Gwiazdowski et al., 2024). Finally, several strains act as probiotics exhibiting the ability to modulate the immune system, to lower the cholesterol blood level and having

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excellent antioxidant properties (Fidanza et al., 2021; Rocchetti et al., 2021).

The analysis of secretome of *L. plantarum* strains could provide clues on the molecular basis of specific functional features of this species as extracellular proteins constitute the first-line of contact between bacteria and environment and/or host and are directly involved in processes responsible for beneficial health effects and food technological traits. Proteomics is the key tool for the identification of secreted proteins and has been fundamental for the characterization of the so-called "moonlighting proteins" located on the cell surface or in the secretome. These are cytoplasmic housekeeping proteins (metabolic enzymes, molecular chaperones, etc.) that display diverse biological functions in different cellular locations, and lack any extra-cytoplasmic sorting sequence or binding domain, thus non-canonical secretion pathways have been hypothesized (Jeffery, 2019). Despite the importance and the huge potential of this species, up to now, a few proteomic studies were focused on the analysis of *L. plantarum* secretomes leading to the identification of a very low number of proteins. As matter of fact, Pessione et al. (2015) identified seven proteins in the extracellular proteome of *L. plantarum* S11T3E, while Zhu et al. (2011) identified 22 proteins of *L plantarum* CMCC-P002, although the predicted secretome of this species can encompass hundreds of proteins (Boekhorst et al., 2006; Huang et al., 2020).

In this light, a shotgun proteomic approach was applied to realize a detailed catalog of the secretome of five *L. plantarum* strains isolated from vegetable foods or dairy products and exhibiting specific function features. All the strains were chosen because *in vitro* they had shown inhibitory activity against bacteria and molds. In particular, the strain ITEM 17215, isolated from wheat bran, showed a strong inhibitory activity towards *Penicillium roqueforti*, *Mucor circinelloides* and mycotoxinogenic molds associated with cereal grains as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium verticillioides*. Moreover, a moderate reduction of the bioavailability of aflatoxin AFB1 was reported. This strain also showed the ability to degrade phytate and utilize FOS (Quattrini et al., 2018). In addition, antifungal and antibacterial activity against yeasts (*Rhodotorula glutinis* and *Candida pelliculosa*), molds (*Penicillium digitatum*, *Aspergillus niger*, *Fusarium oxysporum*, and *Rhizopus oryzae*), and pathogenic bacteria was also reported for *L. plantarum* S61. Interestingly, the protein fraction of the cell free supernatant also exhibited antifungal activity, thus suggesting significant effectiveness of this strain as bio-preservative agent (Abouloifa et al., 2020, 2021, 2022). All the strains effectively inhibited *Fusarium verticillioides* and *Botrytis cinerea* in *in vitro* test (data not shown). Proteomic results highlighted specific features of the secreted protein patterns in the different strains. Secreted proteins were actually involved in the principal cell biological processes including carbohydrate metabolism, proteolysis and peptidoglycan biosynthetic processes.

2. Materials and methods

2.1. Bacterial strains, growth conditions and secretome extraction

Five strains of *L. plantarum* were used in this study: *L. plantarum* ITEM 17215 (isolated from wheat bran), *L. plantarum* ITEM 17218 (isolated from raw milk cheese, Formagèla Val Seriana) and *L. plantarum* ITEM 18335 (isolated from raw milk cheese, Valtellina Casera), present in the Agro-Food Microbial Culture Collection, CNR, Bari, Italy; *L. plantarum* S61 (isolated from fermented green olives, Abouloifa et al., 2020) and *L. plantarum* DSM 20174 (isolated from pickled cabbage) and provided by DSM (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany).

L. plantarum strains, maintained as frozen stock at −80°C in Man, Rogosa and Sharpe broth (MRS) (Thermo Fisher Scientific, Waltham, MA, USA) and 20% (v/v) glycerol (Carlo Erba, Milan, IT) were subcultured in MRS broth, pH 6.8, at 28°C for 24 h. These cultures were used to inoculate 10 mL of MRS test tubes (1% v/v, pH 6.8) that were incubated at 28°C for 14 h (incubation time corresponding to early stationary growth phase). Bacterial cells were harvested by centrifugation (7500 g, 10 min, 4°C), the supernatants were recovered and a protease inhibitor cocktail (1/100 v/v, Sigma, St. Louis, MO, USA) was added to prevent proteolysis. Supernatants were filtered through 0.22 μm nitrocellulose membranes (Merck, Darmstadt, Germany) and secreted proteins were precipitated with TCA (final concentration 10%) by incubating for 2 h at 4°C. The protein pellets were recovered by centrifugation (16000 g for 15 min at 4°C), washed twice with 1 mL of 80% acetone and solubilized in 0.1 mol/L Tris-HCL, 8 mol/L urea, pH 8.5. Protein concentration was measured by a Bradford assay (BioRad, Hercules, CA, USA).

2.2. Sample preparation for proteomic analyses

Reduction of protein samples (20 μg) was carried out 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 min at 37°C and alkylation was carried out in the same buffer with 6 μL of 0.2 mol/L iodacetamide for 30 min at RT in the dark. The alkylation reaction was quenched with 6 μL of 0.2 mol/L dithiotreitol. Finally, protein samples were diluted to 1 mol/L urea by the addition of 0.1 mol/L Tris-HCl, pH 8.5 and digested with 0.2 μg of sequencing grade modified trypsin (1:100 w/w) (Promega, Madison, WI, USA). The digestion was carried out at 37°C for 18 h. The obtained peptide mixtures were desalted by solid phase extraction using C18 ZipTip columns (Merck) conditioned with acetonitrile (ACN) and rinsed with 0.1% formic acid (FA); peptides were 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.

2.3. LC-MS/MS analysis

Tryptic peptide mixtures were submitted to LC-MS/MS analysis using a Q-Exactive™ mass spectrometer (Thermo Fisher Scientific) interfaced with an UltiMate 3000 RSLCnano LC system (Thermo Fisher Scientific). Two biological replicates were analyzed for each bacterial strain and three technical replicates were acquired for each biological replicate. A detailed description of the experimental conditions of analysis was reported in Supplementary Information S1.

Mass spectra were acquired in the m/z range 350–1600. Data acquisition was performed in a data dependent mode Full MS/ddMS2, enabling the acquisition of MS/MS spectra for the ten most intense precursor ions (top ten) and dynamic exclusion of 10 s. Resolution was set to 70000 for MS spectra acquisition and 17500 for MS/MS spectra acquisition.

2.4. Protein identification and label-free quantification analysis

The Proteome Discoverer (PD) search engine platform (version 2.4.1.15, Thermo Fisher Scientific) was used for processing the MS raw files and to achieve protein identification and quantification. A detailed description of the processing parameters was reported in Supplementary Information S1.

The Perseus software (version 1.6.0.7) was used for processing the PD data (Tyanova et al., 2016). Contaminants and reverse hits were removed from the dataset. After a log_2-transformation of the abundance values, only proteins identified by means of a number of peptides ≥ 2 and valid abundance values in three replicates of at least one strain were considered reliably identified. Proteins selected with these criteria in only one strain will be considered specifically expressed by that strain.

The heatmap was built with Perseus software, reporting the abundance values for each protein in the six replicates of each strain.

Quantitative analysis was performed by considering the ten possible strain comparisons. In particular, Perseus – multi sample test option and post-hoc Tukey's HSD test were applied to detect statistically significant differences (p value < 0.05) in protein abundance values between

different strain couples. Protein Fold Change Values (FCV) were calculated as the difference of $\log_2$ of mean protein abundance values between each possible couple of strains, and a FCV $\geq \pm 2$ was selected as significant difference.

2.5. Bioinformatics and functional analyses

Identified proteins were analyzed using the SignalP 5.0 server (http://www.cbs.dtu.dk/services/SignalP) that predicts the presence and location of signal peptide cleavage sites in amino acid sequences for translocation across cell membranes (Almagro Armenteros et al., 2019). Functional classification of secreted proteins was retrieved from UniProtKB according to Gene Ontology annotations. The MoonProt database (http://www.moonlightingproteins.org/) was used to obtain putative moonlighting features of the identified proteins (Chen et al., 2021). The Venn diagram was built using the web-based tool InteractiVenn (http://www.interactivenn.net/) (Heberle et al., 2015). Principal Component Analysis (PCA) was carried out using the Tanagra Data Mining software (version 1.4) (https://tanagra.software.informer.com/1.4/).

3. Results and discussion

3.1. Catalog of proteins secreted by the five L. plantarum strains

The five strains of *L. plantarum* under study are sourced from two distinct ecological niches, such as vegetables and dairy products. The choice to consider two different origins was made to investigate the adaptation to different substrates and environmental conditions. In fact, it has been previously reported that strains deriving from the same food niche cluster together according to phenotypical traits (Siezen et al., 2010), but little information is still available with regard to the biological processes involved in the different evolutionary processes.

Shotgun proteomics was applied to depict a detailed picture of the proteins secreted by the five *L. plantarum* strains. Processing of mass spectrometric data led to define a dataset of 602 proteins reliable identified in at least one strain (Table S1 sheet Raw data). Bioinformatics processing of the protein sequences included in this dataset using the SignalP tool highlighted that 122 proteins were predicted to contain a signal peptide for translocation across the cell membrane. In addition, 47 proteins had transmembrane domains and other 26 proteins were annotated in the MoonProt database as moonlighting proteins of Gram positive bacteria and, among these, enolase, glyceraldehyde 3-P-dehydrogenase, chaperonin GroEL, elongation factor Tu, glucose-6-phosphate isomerase and glutamine synthetase have been previously detected on the cell surface of LAB, as annotated in MoonProt. These proteins were reported to act as mucin, plasminogen, fibronectin, and laminin binding proteins, thus being involved in adhesion processes. Enolase, glyceraldehyde 3-P dehydrogenase, chaperonin GroEL and elongation factor Tu have been identified in both exoproteome and surface proteins of the probiotic *Lactobacillus acidophilus* NCFM (Celebioglu & Svensson, 2017). In particular, Glenting et al. (2013) demonstrated that glyceraldehyde 3-P dehydrogenase and enolase of *L. plantarum* 299v showed a highly specific binding to plasminogen and fibronectin and could be involved in binding to intestinal epithelial cells. Glucose-6-phosphate isomerase and glutamine synthetase were identified as adhesive moonlighting proteins of *Lactobacillus crispatus* ST1 able to bind plasminogen (Kainulainen et al., 2012). Likewise, Liu et al., (2024) proved that moonlighting adhesins secreted by *L. plantarum* LP-PO23 effectively contributed to the adhesion and colonization mechanism of the *Lactobacillus* strain to intestinal epithelial cells of *Paralichthys olivaceus*; glyceraldehyde 3-P-dehydrogenase exhibiting the most adhesion ability.

Eight ribosomal proteins were also classified as moonlighting in the Gram negative species *Escherichia coli* (http://www.moonlightingproteins.org/ accessed on June 2023). (Table S1 sheet Functional classification).

Several identified proteins (38) were reported in the list of the 'Probiotic marker genes' (PMG) in the *L. plantarum* pan-genome drawn up by Carpi et al. (2022), including nine moonlighting proteins and six extracellular proteins (three cell membrane and three having a signal peptide) (Table S1 sheet PMG). These 38 proteins are reported to be mainly involved in the ability to resist to stress conditions (acid stress and/or bile resistance) and in adhesion, relevant features in bacteria with putative probiotic potential (Carpi et al., 2022). These proteins are present in most of the five analyzed strains, and a putative moonlighting function could be hypnotized for them.

The heatmap obtained from the quantitative proteomic data showed that the five strains were grouped in two clusters: one that included only *L. plantarum* ITEM 17215 (isolated from wheat bran) and the other that included the other four strains. In particular, *L. plantarum* ITEM 18335 (isolated from raw milk cheese), S61 (isolated from fermented green olives) and ITEM 17218 (isolated from raw milk cheese) constituted a sub-cluster while *L. plantarum* DSM 20174 (isolated from pickled cabbage) constituted a further sub-cluster. (Fig. 1). This heatmap clearly highlighted that the secretome of strains ITEM 17215 and DSM 20174 exhibited peculiar features.

Interestingly, this heatmap also highlighted that ITEM 18335 and S61 showed a higher similarity in their secretomes compared to the strain ITEM 17218, thus suggesting that, under the experimental conditions used for the bacterial growth, the habitat of origin could not be the principal factor driving the secretome composition, in line with the already reported nomadic lifestyle of *L. plantarum* (Martino et al., 2016).

The distribution of the identified proteins in the different strains is

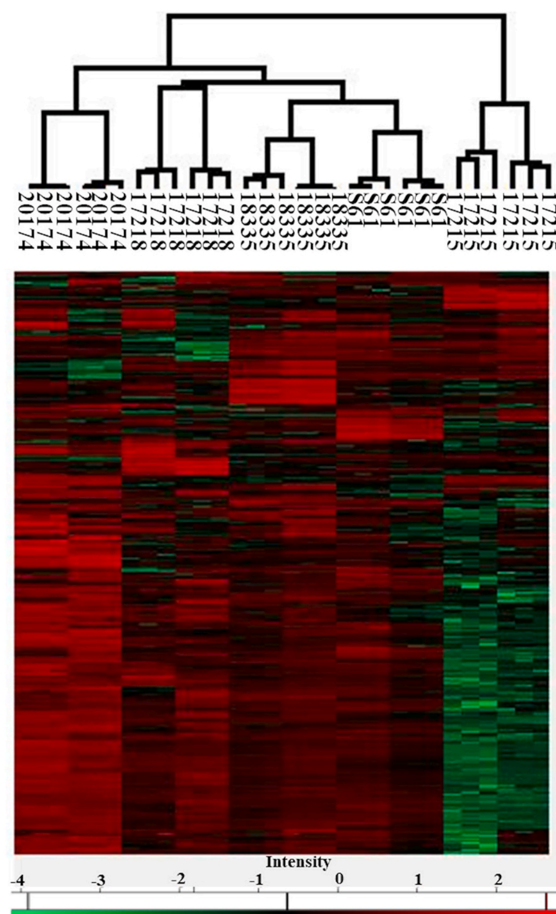

Fig. 1. Heatmap obtained reporting protein abundance values of each protein in technical and biological replicates of each strain. The green and red color ranges refer to the lower and higher abundance, respectively.

reported in the Venn Diagram, showing peculiarities of the extracted secretomes (Fig. 2). As many as 483 proteins were identified in *L. plantarum* DSM 20174, 348 proteins in *L. plantarum* ITEM 18335, 313 proteins in *L. plantarum* S61, 259 proteins in *L. plantarum* ITEM 17218 and 145 proteins in *L. plantarum* ITEM 17215. In addition, 147 proteins were specifically present only in *L. plantarum* DSM 20174, 34 proteins in *L. plantarum* ITEM 18335, 22 proteins in *L. plantarum* S61, 22 proteins in *L. plantarum* ITEM 17218 and 7 proteins in *L. plantarum* ITEM 17215. A common core of 84 proteins was present in the secretome of all the strains and, among those, 35 proteins showed a similar abundance level in all the strains while 49 of them were present in different amount (Table S2 sheet Core proteins).

The functional classification of these proteins was retrieved from UniProtKb, according to Gene Ontology annotations. The most representative biological processes for all the strains were carbohydrate metabolism, proteolysis, peptidoglycan metabolism, transmembrane transport, cell division (Fig. 3).

Interestingly, Plantaricin F (plnF, M1HIB0), Plantaricin N (plnN, P71463), Plantaricin J (plnJ, Q1WFE1), and Plantaricin K (plnK, M1H4I6) were identified among the proteins specifically present in *L. plantarum* ITEM 17215 secretome, while a Bacteriocin ABC transporter was detected in S61. These bacteriocins are small, heat stable and basic proteins, classified as class IIb bacteriocins and their antimicrobial activity may depend on the complementary action of two different peptides (Plantaricin J/K) (Goel & Halami, 2023). Bacteriocins have been claimed to have antimicrobial activities against foodborne pathogens (i.e. *Listeria monocytogenes, Staphylococcus aureus*, *Klebsiella, Salmonella*, *Escherichia coli*) and spoilage bacteria (molds), thus exerting different beneficial effects in foods increasing shelf life and reducing the usage of food preservatives. Moreover, many *L. plantarum* strains have been shown to produce bacteriocins that are effective against Gram-negative bacteria, which is a very intriguing feature since this property is relatively unusual in LAB (Echegaray et al., 2023). More interestingly, these proteins may have the ability to inhibit a wide range of clinically pathogenic and multidrug-resistant bacteria, preventing the infections caused by these bacteria in the human body, thus contributing to the probiotic features of these strains (Huang et al., 2021).

Although Carpi et al. (2022), in their pangenome analysis, found that bacteriocin coding genes were present in the genome of all the 130 *L. plantarum* strains included, we detected plantaricins specifically in ITEM 17215. This finding could suggest interesting antimicrobial properties of ITEM 17215 and support its potential application in the agro-food sector. However, we cannot rule out that others analyzed strains, such as S61 that secreted a Bacteriocin ABC transporter, produced bacteriocins that could escape identification due to a very lower amount secreted in the applied experimental conditions. It is worth noting that since plantaricin production is sensitive to environmental factors, the experimental condition applied in our study could be far from the optimum conditions, thus halting bacteriocin production (Goel & Halami, 2023; Leal-Sánchez et al., 2002).

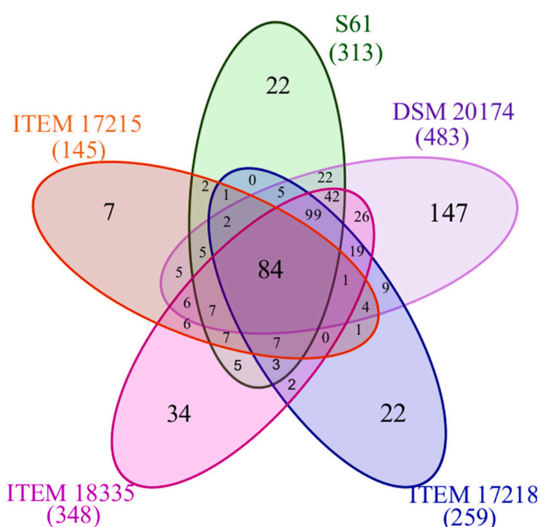

Fig. 2. Venn diagram summarizing proteomic results. Lists including the accession of proteins identified in each strain have been used to build the diagram. The number of secreted proteins present in each strain is reported in bracket.

3.2. Quantitative proteomic analysis

To further compare the five *L. plantarum* strains, a label free quantitative proteomic analysis was performed on a dataset including the 370 proteins present in at least two strains. The results indicated that 301 proteins exhibited different abundance level in at least one of the ten performed comparisons (significant FCV $\geq \pm 2$) (Table S2 sheet Quantitative analysis).

The principal component analysis (PCA) performed on the quantitative proteomic data showed that the ten comparisons clearly clustered in four different groups (Fig. 4). The first two principal components, PC1 and PC2, accounted for 66.5 % of total variance (53.07 % and 13.71 % respectively).

Interestingly, the comparisons between ITEM 17215 and the strains ITEM 17218, ITEM 18335 and S61 were close in the PCA diagram (quadrant 1) while the comparison ITEM 17215 vs DSM 20174 was clearly separated from all the others comparisons (quadrant 4), thus confirming specific peculiarities of these two strains.

In fact, the number of secreted proteins (145) was particularly low in ITEM 17215 and, in the four comparisons between ITEM 17215 and all the other strains, only 7 proteins were more abundant and as many as 92 were less abundant.

Worth to note, among the proteins more abundant in the secretome of ITEM 17215, we identified two extracellular chitin-binding proteins (A0A806J611 and A0A151G249), multifunctional proteins involved in the adhesion process to intestinal mucins and epithelial cells and also acting as an accessory protein essential for chitin degradation, as its binding to polymeric N-acetylglucosamine favors the degradative actions of chitinases (Sanchez et al., 2011). To note, chitinase activity is one of the mechanisms underlying the biocontrol activity of fungi by *L. plantarum*, in addition to the production of organic acids and bioactive peptides. (Zhao et al., 2019). The inhibitory effects of lactobacilli on hyphal morphogenesis was previously reported for strains counteracting *C. albicans* virulence, dairy yeast contaminants, and chitinases have shown promise as biocontrol agents, chiefly against phytophathogenic fungi. Chitin-binding proteins have been supposed also to be a specific colonization factor of *L. plantarum* (Allonsius et al., 2019; Kavková et al., 2021; Raman et al., 2022).

Moreover, the glycoside hydrolase family 25 (A0A151G4Y3), more abundant in the secretome of ITEM 17215, exhibits a lysozyme activity and is involved in peptidoglycan catabolic processes.

Similarly, *L. plantarum* ITEM 18335 produced high level of Glycosyl hydrolase family protein (A0A806J1W3) compared to all the other strains, a protein also belonging to the glycosyl hydrolase 25 family. Wort to note the glycoside hydrolases through autolysis in the host and the cell-wall turnover, drive the release of muramyl-peptides that are known to interact with receptors of the immune system thus being involved in adhesion and immunomodulation, key processes at the base of bacterial probiotic properties (Kleerebezem et al., 2010; Rolain et al., 2012).

The quantitative proteomic analysis further confirmed that strain DSM 20174 produced a particularly rich secretome. In fact, a significant number of proteins were more abundant in DSM 20174 compared to the other strains and as many as 147 proteins were exclusively secreted by DSM 20174 (Table 1, Fig. 2).

On the other hand, the quantitative analysis showed that the ITEM

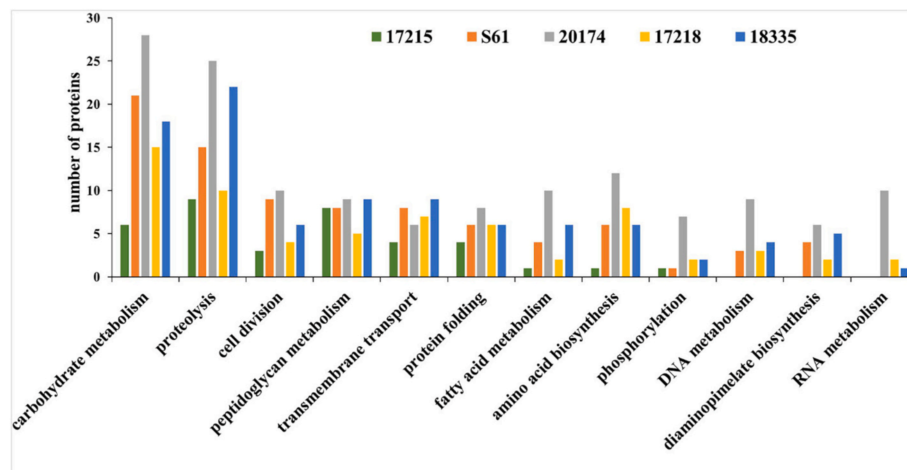

Fig. 3. Histogram reporting the number of secreted proteins belonging to each biological process of the characterized strains (ribosomal proteins were not included in this diagram).

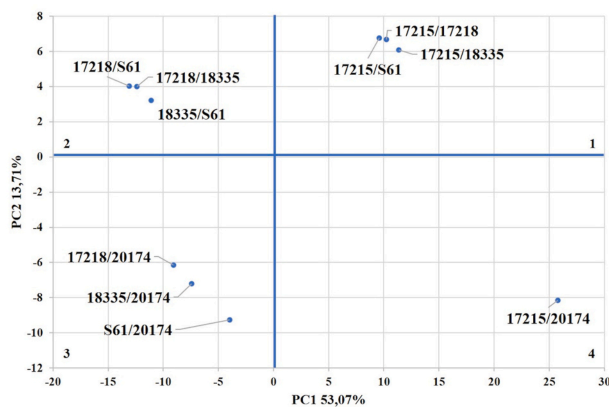

Fig. 4. PCA analysis based on significant Fold Change Values (FCV) calculated for each protein in the strain comparisons. The number of quadrants is indicated.

Table 1
Summary of the quantitative proteomic results. Number of differentially abundant proteins detected in all the ten comparisons is reported.

Comparison	Differentially abundant proteins	Proteins more abundant	Proteins less abundant
17215_17218	169	33	136
17215_18335	188	16	172
17215_S61	175	13	162
17218_18335	45	11	34
17218_S61	47	19	28
18335_S61	26	17	9
17218_20174	85	14	71
18335_20174	87	27	60
S61_20174	118	21	97
17215_20174	244	27	217

17218, ITEM 18335 and S61 produced secretomes with more similar compositions. In fact, the number of proteins with different abundance level is relatively lower. Worth nothing, S61 was isolated from table olives while ITEM 17218 while ITEM 18335 had dairy origin.

Interestingly, *L. plantarum* ITEM 17218 showed the higher level of dextransucrase (A0A806J6C1), a protein that belongs to the glycosyl hydrolase 70 family and is involved in the glucan biosynthetic process. The production of α-glucans, catalyzed by glucansucrases is particularly important for Lactobacilli as dextrans/α-glucans can be used to positively influence the properties of different food products (Lynch et al., 2018). For instance, dextran produced by LAB during sourdough fermentation could improve freshness, crumb structure, mouthfeel and softness of several kinds of baked goods (Lacaze et al., 2007). Moreover, dextrans/α-glucans are knonw to be involved in prebiotic, probiotic, and biological activities (Silva et al., 2019).

The quantitative proteomic data well parallel with peculiar functional features of the analyzed strains.

4. Conclusions

The information gathered is a valuable contribution to increasing knowledge in relation to one of the most important bacterial species used as probiotic, for biocontrol of plant, animals and human pathogens and in different food production worldwide.

Five *L. plantarum* strains were characterized for their subsequent potential use in the agri-food sector. The secretome of the five strains showed important diversities in relation to the proteins detected and their quantity. Proteins of application interest such as bacteriocins, glucansucrase, glycoside hydrolase and chitin binding proteins were differentially secreted in the five strains. Further studies are needed to deepen the mechanism underlying the different activities (i.e. cell density-dependent mechanism, induction factor-dependent, and coculture-dependent mechanism) and to provide the necessary information to choose the strains so that the conditions of application come as close as possible with the conditions of optimal expression of the activities of interest.

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CRediT authorship contribution statement

Maria Fiorella Mazzeo: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Alida Sorrentino:** Writing – review & editing, Validation, Investigation. **Stefano Morandi:**

Writing – review & editing, Resources, Investigation. **Houssam Abouloifa:** Writing – review & editing, Resources, Investigation. **Abdeslam Asehraou:** Writing – review & editing, Resources, Investigation. **Milena Brasca:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Conceptualization. **Rosa Anna Siciliano:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104474.

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