



Data Article

Proteomic data of *Listeria monocytogenes*

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ABSTRACT

Listeria monocytogenes is an important food-borne pathogen that is responsible for contamination of a variety of food products. It causes listeriosis which is one of the most severe and serious foodborne diseases, whose main severe clinical forms include septicaemia, meningitis, and neonatal infections. Also because of its ability to adhere to industrial surfaces, it is difficult for them to come up against this danger. Although there are researches in *L. monocytogenes* proteome, most of them are based on the study of a single strain, yet little is known about the proteome of more of the microbe's strains. By studying the proteome of the microorganism with state-of-the-art mass spectrometry technology in terms of proteomics, we will be able to have an overall picture of the pathogenicity of the bacterium, as this is largely based on its ability to adapt to certain environments and to project its toxicity in them. To address that we provide a dataset of 2227 different proteins from 4 strains of *L. monocytogenes*, grouped by their molecular function and their biological process. The identified proteins confirm the complex molecular mechanisms of the bacterium.

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Specifications Table

Subject	Omics: Proteomics
Specific subject area	4 strains of <i>L. monocytogenes</i>
Type of data	Table, Figure
Data collection	1D-nanoLC-MS/MS, bottom-up proteomics NanoElute 2 liquid chromatography system equipped with a timsTOF Ultra2 mass spectrometer (Bruker Daltonik GmbH). <i>Listeria monocytogenes</i> Uniprot reference proteome FASTA
Data source location	Institution: Agricultural University of Athens City/Town/Region: Athens Country: Greece
Data accessibility	https://www.ebi.ac.uk/pride/archive/projects/PXD075511
Related research article	None

1. Value of the Data

- A new report on *L. monocytogenes* proteome based on leading edge technology.
- The proteomic data of 4 strains of 2 different serotypes provide us a sight of the molecular mechanisms of the microorganism.
- The analysis of data may produce new ways of dealing against the microorganism in industrial level.

2. Background

Over the past decades *Listeria monocytogenes* has become one of the most serious food-borne pathogens. According to EFSA, in 2022 European members reported 2738 cases of listeriosis which caused 1330 hospitalisations and 286 deaths, being the highest number of confirmed cases since 2007 [1]. There is a variety of potential implicated food vehicles such as meats, fish products, dairy products and vegetables. Also *L. monocytogenes* has a high risk association with ready-to-eat (RTE) products [2].

Proteomics is a powerful technology in order to reveal the mechanisms that bacteria use for many of their different functions allowing us to develop more effective ways to come up against them. Data-Independent Acquisition (DIA) Proteomics help us in this direction by their ability to identify numerous proteins through analysis with the technological edge of LC-MS/MS setups. Our study provide a full dataset of *L. monocytogenes* proteins from 4 selected strains giving by this way a general picture of the bacterium's proteome.

3. Data Description

L. monocytogenes has been recognized as one of the most important bacteria in the food industry [3]. 4 strains of *L. monocytogenes* from our laboratory analyzed 3 times each (biological replicates) with LC-MS/MS. In particular the analyzed strains are: C5 (serotype 4b, dairy farm environment), 6179 (serotype 1/2a, farm house cheese), ScottA (serotype 4b, human isolate) [4] and EGDe (serotype 1/2a, human isolate) [5]. A total of 2,227 proteins were identified across the analyzed samples. In Supplementary Table 1 the identified proteins are listed along with their accession numbers and corresponding descriptions based on the Swiss-Prot/UniProtKB database. Data for protein classification (Fig. 1) and categorization (Fig. 2) obtained from Swiss-Prot/UniProtKB database [6]. Proteins' classification according to their molecular function represented in Fig. 1, whereas their categorization according to their biological process represented in Fig. 2. The figures visualize the proteins according to the GeneOntology (GO) system [6]. It is noted that 885 identified proteins have no citation for their molecular function in the keywords of the database and also 1351 proteins have no citation for their biological process.

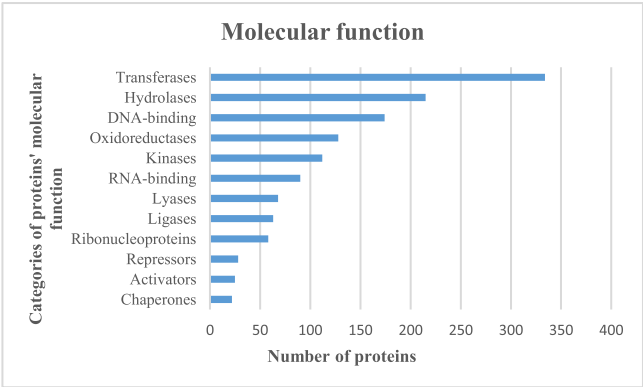


Fig. 1. *Listeria monocytogenes* proteins' molecular function.

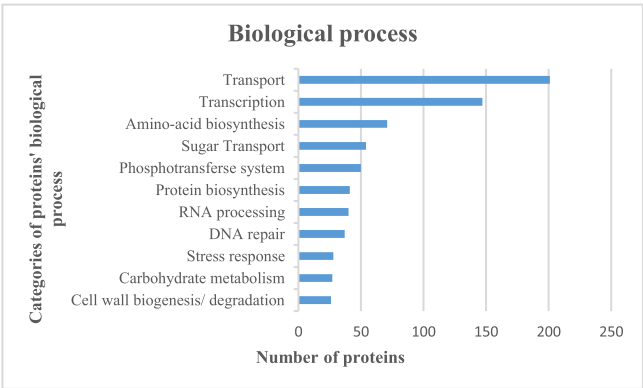


Fig. 2. *Listeria monocytogenes* proteins' biological process.

4. Experimental Design, Materials and Methods

4.1. Sample preparation

Working cultures of 0.1mL came from the stock culture at -20 °C. The strains were initially grown in 10 mL of TSBYE at 30 °C for 24 h, then subcultured for 18 h at 30 °C in fresh 10 mL TSBYE supplemented with 5 × PBS to maintain a stable pH during overnight growth [7]. This growth temperature (30 °C) was chosen because it reflects environmental and food-associated conditions that *L. monocytogenes* faces outside the human host. Under these conditions, the cultures reached an approximate density of 10⁹ cells/mL in static phase. After 3 centrifugations with 10 mL physiological saline each (3,200 × g; 4 °C; 10 min), the cells harvested in a buffer consisting of 1mL Tris-HCl (50 mM pH 7.3) and 10 µL Protease Inhibitors.

Then, the cells were lysed through the ultrasound process (Q125 Sonicator, Qsonica Sonicators-1 minute of ultrasound followed by 1 minute of rest x3 times for each strain). In addition, centrifugation was performed (21,000 × g; 4 °C; 20 min) and 0.2 g of TCA was added to the supernatant obtained, in order to precipitate the proteins. These mixtures were vortexed and incubated on ice for an additional one hour. Then, they were centrifuged for 30 min under the above conditions. After that, the supernatant was discarded and at the end of the process, 1 mL of frozen acetone was added to the samples. This was followed by incubation for 30 min in a water bath with ultrasound, followed by an overnight incubation at -20 °C. The next day, the

samples were centrifuged for 20 min under the above conditions and 250 μL of sample buffer (4% SDS, 100 mM DTT, and 100 mM triethylamine bicarbonate (TEAB), 10 μL Protease Inhibitors) was added to each pellet. Vortex and ultrasound water bath took place in order to solubilize the pellets.

4.2. Peptide generation

30 μL of the protein extract was taken for further analysis. The mixture was subjected to alternating heating and sonication twice. Subsequently, the sample was centrifuged at $17,000 \times g$ for 15 min and the supernatant was treated according to Hughes' single-pot solid-phase enhanced sample preparation (Sp3) method [8] without acidification and including a protein cysteine alkylation step in 100 mM iodoacetamide. Digestion was performed at 1200 rpm and 37 °C using 0.5 μg Trypsin Platinum mixture in 100 mM TEAB buffer.

The next day, magnetic particles were removed, and the peptide sample was further purified using the Sp3 peptide purification kit as previously described [8]. Briefly, 30 μL of the peptide mixture was added to 1.2 mL of acetonitrile (ACN) and they were mixed by inversion for 30 min, then the tubes were placed on a magnetic rack. The supernatant was removed and the beads with the bound peptides were washed twice with 200 μL of 100% ACN. Subsequently, 50 μL of water was added to elute the peptide and magnetic particles. The elution buffer was concentrated using SpeedVac. Finally, the dried peptide was dissolved in buffer A, sonicated for 5 min, and the absorbance was measured at 280 nm using a Nanodrop spectrophotometer to determine the peptide concentration.

4.3. 1-D nanoLC-MS/MS analysis

The peptide content of the samples was then analyzed using a NanoElute 2 liquid chromatography system coupled to a timsTOF Ultra2 mass spectrometer (Bruker Daltonik GmbH). Briefly, peptides were separated on a temperature-controlled (50 °C) Pepsep column (25 cm $\times$ 75 μm , 1.9 μm particle size, Bruker) and ionized using a Captive Spray Ultra-2 ion source. Chromatographic separation was achieved at a constant flow rate of 250 nL/min using a binary mobile phase consisted by two buffers: Buffer A (0.1% formic acid in water) and Buffer B (0.1% formic acid in acetonitrile) Peptide elution from the column was performed using a 30-minute gradient elution as follows.

- An initial ramp from 5% to 23% Buffer B over 18 min.
- An increase to 35% Buffer B over 4 min.
- A wash step reaching 90% Buffer B at 26 min, which was maintained until the end of the analysis.

The mass spectrometer was in dia-PASEF mode, using standard methods for small sample volumes (mobilities 0.64–1.45, cycle time 0.96 s). Complete MS data were acquired in the range of 400–1000 m/z. The ion mobility settings included three isolation windows, all of which were covered in a single TIMS scan. Eight TIMS scans were required to cover all 24 windows.

4.4. Data analysis

The timsTOF raw data were analyzed in the Proteoscape environment using Spectronaut software version 19. The search was performed against the reference *Listeria monocytogenes* database from Uniprot in library-free mode, allowing up to two missed tryptic cleavages. Default settings were applied including oxidation of methionine residues and acetylation of the protein N-termini as variable modifications and carbamidomethylation of cysteine residues as a fixed

modification. N-terminal methionine excision was enabled. The match between runs (MBR) feature was used for all analyses and the output (precursor and protein group) was filtered at 1% FDR. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [9] partner repository with the dataset identifier PXD075511.

Limitations

None.

Ethics Statement

The authors have read and follow the [ethical requirements](#) for publication in Data in Brief and confirming that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Credit Author Statement

Stavros Proikakis: Investigation, Writing – Original Draft; **George Tsangaris:** Conceptualization, Methodology, Validation; **Martina Samiotaki:** Investigation, Formal Analysis, Data Curation, Visualization, Writing – Review and Editing; **Konstantinos Papadimitriou:** Conceptualization, Methodology, Writing – Review and Editing; **Panagiotis Skandamis** Conceptualization, Supervision, Project administration, Funding acquisition.

Data Availability

[Proteomic data of *Listeria monocytogenes* \(Original data\)](#) (PRIDE database).

[Proteomic data of *Listeria monocytogenes* \(Original data\)](#) (PRIDE).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.dib.2026.112881](https://doi.org/10.1016/j.dib.2026.112881).

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