Data Article

Proteomic dataset of *Listeria monocytogenes* exposed to sublethal concentrations of free and nanoencapsulated nisin

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The cellular proteins of *L. monocytogenes* exposed to free and liposome-encapsulated nisin at sublethal concentration were hydrolyzed by trypsin and examined by tandem mass spectrometry (MS/MS) to obtain proteomic data. In the present study, we use the STRING v11.05 database analyze the interactions among the 78 upregulated proteins from *L. monocytogenes* obtained after treatment with sublethal concentrations of free and nanoliposome-encapsulated nisin. As result, from the upregulated proteins by free nisin was determined a network with 140 edges with two relevant nodes, containing ribosomal proteins and transmembrane transport proteins (SecD and ABC transport system). These two sets of proteins present biological connection as a group, with strong interactions and are related to detoxification and other *Listeria* response mechanisms. In addition, a high amount of membrane proteins was identified in the free nisin treatment. On the other hand, in the interaction analysis of up-regulated proteins by liposome-loaded nisin, was found 156 edges with a single protein network, the same observed in

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free nisin, related to ribosomal proteins. Therefore, according with this analysis, the encapsulation of nisin into liposomes cause upregulation of ribosomal and decrease of *L. monocytogenes* response proteins as compared with free nisin.

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### Specifications Table

| Subject | Biological Sciences: Omics: Proteomics |
|---------|---------------------------------------|
| Specific subject area | Proteomics data of *Listeria monocytogenes* |
| Type of data | Tables and figures |
| How the data were acquired | Liquid chromatography-tandem mass spectrometric (LC-MS/MS) analysis, using a LTQ Orbitrap Velos mass spectrometer connected to the EASY-nLC system through a Proxeon nanoelectrospray ion source |
| Data format | Raw data and analyzed |
| Description of data collection | LC-MS/MS based proteomic profiling of total protein of *Listeria* cells after three treatments: sublethal concentration of free nisin, sublethal concentration of nisin encapsulated in nanoliposomes and unloaded liposomes |
| Data source location | Institution: Universidade Federal do Rio Grande do Sul |
| | City/Town/Region: Porto Alegre/RS |
| | Country: Brazil |
| Data accessibility | Repository name: MassIVE |
| | Data identification number: MSV000089076 |
| | Direct URL to data: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=451961119585408bacabbc136f28d8fb |
| Related research article | C.M.B. Pinilla, P. Stincone, A. Brandelli, Proteomic analysis reveals differential responses of *Listeria monocytogenes* to free and nanoencapsulated nisin, Int. J. Food Microbiol. 346 (2021) 109170. doi:10.1016/j.ijfoodmicro.2021.109170 |

### Value of the Data

- This dataset contains unique information on proteome of *L. monocytogenes* exposed to nanostructured antimicrobial peptide nisin.
- The data may be valuable for scientists of different fields, including microbiology, protein science, food science, and nanotechnology.
- The data can be useful to understand the effect of natural antimicrobials on pathogenic bacteria at molecular level.
- The analysis of data may be used for development of innovative strategies to combat pathogenic bacteria.

### 1. Data Description

The proteomics data analyzed in this article is related to our previous research article titled “Proteomic analysis reveals differential responses of *Listeria monocytogenes* to free and nanoencapsulated nisin” [1]. The data of this article includes the set proteins identified using UniProt, with VIP (Variable Importance in Projection) score ≥1.0, obtained from of *L. monocytogenes* ATCC 7644 cells incubated for 1 h with sublethal concentrations of either free or liposome-encapsulated nisin. The set of proteins showing upregulation as compared with the control cells are summarized in Table 1. This set of proteins denotes the global mechanism, in terms of protein expression and triggered by *L. monocytogenes* cells after treatment with free and nanoencapsulated nisin. These two groups of proteins were selected to explore the interactions among
Table 1
Upregulated protein/peptide reports of *Listeria monocytogenes* ATCC 7644 treated by sub-lethal concentration of free nisin (Nis) or liposome-encapsulated nisin (LNis) for 1 h.

| Uniprot accession | Gene name | Annotation | Treatment | Description |
|-------------------|-----------|------------|-----------|-------------|
| Q8YA70            | lmo0289   | lmo0289    | Nis / LNis| Annotation not available |
| Q8YB28            | lmo1090   | lmo1090    | Nis / LNis| Annotation not available |
| Q8Y615            | lmo1887   | lmo1887    | Nis / LNis| Hypothetical protein; belongs to the methyltransferase superfamily |
| Q8Y437            | lmo2636   | lmo2636    | Nis/ LNis | Hypothetical protein; flavin transferase that catalyzes the transfer of the FMN moiety of FAD and its covalent binding to the hydroxyl group of a threonine residue in a target flavoprotein |
| Q8Y7A4            | lmo1384   | lmo1384    | LNis      | Hypothetical protein; belongs to the UPF0176 family |
| Q8Y7C5            | lmo1360   | folD       | LNis      | Methenyltetrahydrofolate cyclohydrolase; catalyzes the oxidation of 5,10-methylenetetrahydrofolate and then the hydrolysis to 10-formyltetrahydrofolate |
| Q8YAC0            | lmo0226   | folK       | Nis       | 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase; involved in the biosynthesis of tetrahydrofolate from GTP |
| Q8YA71            | lmo0288   | lmo0288    | Nis       | Annotation not available |
| Q8YA0             | lmo0135   | lmo0135    | Nis       | Annotation not available |
| Q8Y6B7            | lmo1774   | purK       | Nis       | Phosphoribosylaminomimidazole carboxylase ATPase subunit; catalyzes the ATP-dependent conversion of 5-aminoimidazole ribonucleotide and HCO$_3^-$ to N5-carboxyaminomimidazole ribonucleotide |
| Q8YB4             | lmo0842   | lmo0842    | Nis       | Annotation not available |
| Q2CZ4             | lmo1028   | lmo1028    | LNis      | Hypothetical protein; belongs to the UPF0356 family |
| Q8YAR2            | lmo0653   | rplL       | LNis      | 50S ribosomal protein L9; binds to the 23S rRNA |
| Q8Y8E7            | lmo0957   | nagB       | LNis      | Glucosamine-6-phosphate isomerase; catalyzes the reversible isomerization-deamination of glucosamine 6-phosphate to form fructose 6-phosphate and ammonium ion |
| Q8Y6S4            | lmo1609   | lmo1609    | LNis      | Annotation not available |
| P0A4L3            | lmo1233   | trxA       | LNis      | Component of the thioredoxin-thioredoxin reductase system |
| Q8Y626            | lmo1874   | thyA       | LNis      | Thymidylate synthase; catalyzes the reductive methylation of 2′-deoxyuridine-5′-monophosphate (dUMP) to 2′-deoxythymidine-5′-monophosphate (dTMP) while utilizing 5,10-methylenetetrahydrofolate (mTHF) as the methyl donor and reductant in the reaction, yielding dihydrofolate (DHF) as a by-product |
| P65110            | lmo2610   | infA       | LNis      | Translation initiation factor IF-1; one of the essential components for the initiation of protein synthesis |
| Q8Y7A4            | lmo1384   | lmo1384    | LNis      | Hypothetical protein; belongs to the UPF0176 family |
| P66383            | lmo2608   | rpsM       | Nis / LNis| 30S ribosomal protein S13; located at the top of the head of the 30S subunit, contacts several helices of the 16S rRNA |
| Q8Y7B5            | lmo1371   | lmo1371    | LNis      | Dihydrolipoyl dehydrogenase; E3 component of the branched-chain alpha-keto acid dehydrogenase complex; catalyzes the oxidation of dihydrolipoamide to lipoyldeide |
| Q8Y7B6            | lmo1370   | buk        | Nis/ LNis | Butyrate kinase; belongs to the acetylkinase family |
| P33379            | lmo0204   | actA       | Nis/ LNis | Actin-assembly inducing protein precursor; virulence factor required for host cell microfilament interaction |
| P66401            | lmo2619   | rpsZ       | Nis / LNis| 30S ribosomal protein S14; binds 16S rRNA, required for the assembly of 30S particles and may also be responsible for determining the conformation of the 16S rRNA at the A site |
| Q48762            | lmo0234   | lmo0234    | Nis / LNis| Hypothetical protein; RNAse |
| Q8YF7             | lmo2487   | lmo2487    | Nis / LNis| Annotation not available |
| Q48754            | lmo1388   | tcsA       | Nis / LNis| CD4+ T-cell stimulating antigen |
| P66352            | lmo2607   | rpsK       | Nis / LNis| 30S ribosomal protein S11; located on the platform of the 30S subunit, bridges several disparate RNA helices of the 16S rRNA |
| Q8Y701            | lmo1529   | lmo1529    | Nis / LNis| Annotation not available |
| Q8Y69             | lmo1342   | rplU       | Nis / LNis| 50S ribosomal protein L21; this protein binds to 23S rRNA in the presence of protein L20 |
| Q8Y5V6            | lmo1949   | lmo1949    | Nis / LNis| Hypothetical protein; belongs to the pseudouridine synthase RsuA family |

(continued on next page)
Table 1 (continued)

| Uniprot accession | Gene name | Annotation | Treatment | Description |
|-------------------|-----------|------------|-----------|-------------|
| Q8Y4B8            | lmo2533   | atpF       | Nis / LNis | F_{1},F_{0} ATP synthase; produces ATP from ADP in the presence of a proton or sodium gradient |
| Q8Y6U0            | lmo1592   | thil       | Nis / LNis | Thiamine biosynthesis protein Thil; catalyzes the ATP-dependent transfer of a sulfur to tRNA to produce 4-thioU in position 8 of tRNAs, which functions as a near-UV photosensor |
| Q8YAU3            | lmo0020   |           | Nis / LNis | Annotation not available |
| Q8Y9F0            | lmo0579   |           | Nis / LNis | Annotation not available |
| Q8Y486            | lmo2569   |           | Nis / LNis | Annotation not available |
| Q8Y7L9            | lmo1255   |           | Nis / LNis | Annotation not available |
| Q8Y8C6            | lmo0982   |           | Nis / LNis | Annotation not available |
| Q8Y4U6            | lmo2335   | fruA       | Nis / LNis | FruA protein; sugar transporter, phosphoenolpyruvate-dependent phosphotransferase system |
| Q7AP82            | lmo0685   | lmo0685   | Nis / LNis | Flagellar motor protein MotA; with MotB forms the ion channels that couple flagellar rotation to proton/sodium motive force across the membrane and forms the stator elements of the rotary flagellar machine |
| Q8Y7A1            | lmo1389   | lmo1389   | Nis / LNis | Annotation not available |
| Q8Y7P2            | lmo1231   |           | Nis / LNis | Annotation not available |
| Q8YA9U9           | lmo0014   | qoxB       | Nis / LNis | AA3-600 quinol oxidase subunit 1; belongs to the heme-copper respiratory oxidase family |
| Q8Y670            | lmo1829   | lmo1829   | Nis / LNis | Annotation not available |
| Q8Y8Q5            | lmo0841   |           | Nis / LNis | Calcium-transporting ATPase; catalyzes the hydrolysis of ATP coupled with the transport of calcium |
| Q8Y547            | lmo2229   | lmo2229   | Nis / LNis | Annotation not available |
| Q8Y5U6            | lmo1959   |           | Nis / LNis | Annotation not available |
| Q8Y7M0            | lmo1254   | lmo1254   | Nis / LNis | Annotation not available |
| Q9RTL9            | lmo2528   | rpoB       | Nis / LNis | DNA-directed RNA polymerase subunit beta; DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates |
| P0DJP1            | lmo1469   | rpsU       | Nis / LNis | 30S ribosomal protein S21; belongs to the bacterial ribosomal protein S21 family |
| Q7AP78            | lmo0971   | dltD       | Nis / LNis | DltD protein; involved in lipoteichoic acid biosynthesis phathway |
| Q8YA96            | lmo2529   | rpoC       | Nis / LNis | DNA-directed RNA polymerase subunit beta; DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates |
| Q8Y4A3            | lmo2551   | rho        | Nis / LNis | Transcription termination factor Rho; facilitates transcription termination by a mechanism that involves Rho binding to the nascent RNA, activation of Rho's RNA-dependent ATPase activity, and release of the mRNA from the DNA template |
| Q8Y4A2            | lmo2552   | murZ       | Nis / LNis | UDP-N-acetylglucosamine 1-carboxyvinyltransferase; cell wall formation |
| Q8Y8D4            | lmo0974   | dltA       | Nis / LNis | D-alanine-poly(phosphoribitol) ligase subunit 1; catalyzes the first step in the D-alanylation of lipoteichoic acid (LTA); the activation of D-alanine and its transfer onto the D-alanyl carrier protein (Dcp) DltC |
| Q8YAV6            | lmo0007   | gyrA       | Nis / LNis | DNA gyrase subunit a; type II topoisomerase that negatively supercoils closed circular double-stranded (ds) DNA in an ATP-dependent manner to modulate DNA topology and maintain chromosomes in an unwound state |
| Q8Y5A9            | lmo2159   | lmo2159   | Nis / LNis | Annotation not available |
| Q8Y664            | lmo1836   | pyrKa      | Nis / LNis | Carbamoyl phosphate synthase small subunit; Belongs to the CarA family |
| Q8YAR7            | lmo0047   | lmo0047   | Nis / LNis | Hypothetical protein |
| Q8Y4C9            | lmo2474   | lmo2474   | Nis / LNis | Hypothetical protein; displays ATPase and GTPase activities |
| Q92C24            | lmo1330   | rpoO       | Nis / LNis | 30S ribosomal protein S15; one of the primary rRNA binding proteins, binds directly to 16S rRNA where it helps nucleate assembly of the platform of the 30S subunit by binding and bridging several RNA helices of the 16S rRNA |

(continued on next page)
| Uniprot accession | Gene name | Annotation | Treatment | Description |
|-------------------|-----------|------------|-----------|-------------|
| P66103            | lmo2656   | rpsL       | Nis / LNis | 3OS ribosomal protein S12; with S4 and S5 plays an important role in translational accuracy |
| P0A3L1            | lmo1785   | infC       | Nis / LNis | Translation initiation factor if-3; IF-3 binds to the 30S ribosomal subunit and shifts the equilibrium between 70S ribosomes and their 50S and 30S subunits in favor of the free subunits, thus enhancing the availability of 30S subunits on which protein synthesis initiation begins |
| Q8Y776            | lmo1420   | murB       | Nis / LNis | UDP-N-acetylenolpyruvoylglucosamine reductase; cell wall formation |
| Q92EH3            | lmo0484   | isdG       | Nis / LNis | Heme-degrading monoxygenase IsdG; allows bacterial pathogens to use the host heme as an iron source |
| Q8Y6Z6            | lmo1534   | ldh2       | Nis / LNis | L-lactate dehydrogenase; catalyzes the conversion of lactate to pyruvate |
| Q8YAB2            | lmo0238   | cysE       | Nis / LNis | Serine acetyltransferase; involved in the subpathway that synthesizes L-cysteine from L-serine |
| Q8YAD8            | lmo0193   | lmo0193    | Nis / LNis | Hypothetical protein |
| Q8Y8A1            | lmo0278   | lmo2078    | Nis / LNis | Sugar ABC transporter ATP-binding protein; belongs to the ABC transporter superfamily |
| Q8Y843            | lmo1075   | tagH       | Nis / LNis | Teichoic acid ABC transporter ATP-binding protein; part of the ABC transporter complex TagGH involved in teichoic acids export |
| Q8YAM0            | lmo0098   | lmo0098    | Nis / LNis | Annotation not available |
| Q8YAD4            | lmo0198   | glmU       | Nis / LNis | Glucosamine-1-phosphate N-acetyltransferase; catalyzes the last two sequential reactions in the de novo biosynthetic pathway for UDP-N-acetylg glucosamine (UDP-GlcNAc) |
| Q7AP53            | lmo2193   | lmo2193    | Nis / LNis | Peptide ABC transporter ATP-binding protein; belongs to the ABC transporter superfamily |
| Q8Y5T8            | lmo1967   | lmo1967    | Nis / LNis | Toxic ion resistance protein; belongs to the TetA family |
| Q8Y767            | lmo1434   | lmo1434    | Nis / LNis | Hypothetical protein; RNase that has 5′-3′ exonuclease and possibly endonuclease activity |
| Q8Y7C3            | lmo1362   | xseB       | Nis / LNis | Exodeoxyribonuclease VII small subunit; bidirectionally degrades single-stranded DNA into large acid-insoluble oligonucleotides, which are then degraded further into small acid-soluble oligonucleotides |
| Q8Y7B2            | lmo1374   | lmo1374    | Nis / LNis | Annotation not available |
| Q8Y6J3            | lmo1691   | lmo1691    | Nis / LNis | Deoxyuridine triphosphate nucleotidohydrolase; enzyme involved in nucleotide metabolism, produces dUMP, the immediate precursor of thymidine nucleotides and it decreases the intracellular concentration of dUTP so that uracil cannot be incorporated into DNA |
| Q8Y3M5            | lmo2810   | gidA       | Nis / LNis | tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA; NAD-binding protein involved in the addition of a carboxymethylaminomethyl group at the wobble position (U34) of certain tRNAs |
| P66103            | lmo1783   | rpfT       | Nis / LNis | 50S ribosomal protein L20; binds directly to 23S ribosomal RNA and is necessary for the in vitro assembly process of the 50S ribosomal subunit |
| Q8Y4C1            | lmo2529   | atpD       | Nis / LNis | ATP synthase F0F1 subunit beta; produces ATP from ADP in the presence of a proton gradient across the membrane |
| Q8Y5 × 1          | lmo1933   | folE       | Nis / LNis | GTP cyclohydrolase 1; involved in the first step of tetrahydrofolate biosynthesis; catalyzes the formation of formate and 2-amino-4-hydroxy-6-(erythro-1,2,3-trihydroxypropyl) dihydروperidine triphosphate from GTP and water; forms a homopolymer |
| Q8Y6Z1            | lmo1539   | lmo1539    | Nis       | Glycerol transporter; belongs to the MIP/aquaporin |
| Q8Y703            | lmo1527   | secD       | Nis       | Part of the Sec protein translocase complex |
| Q8Y980            | lmo0653   | lmo0653    | Nis       | Hypothetical protein |
| Q8Y839            | lmo1079   | lmo1079    | Nis       | Annotation not available |
| Q8Y8 × 2          | lmo0770   | lmo0770    | Nis       | Annotation not available |
| Q8Y6E9            | lmo0955   | lmo0955    | Nis       | Hypothetical protein |
| Q8Y7A4            | lmo1384   | lmo1384    | LNis      | Hypothetical protein; belongs to the UPF0176 family |
Fig. 1. Protein-protein interaction network of upregulated *Listeria monocytogenes* ATCC 7466 proteins after interaction with free nisin. The proteins are represented by nodes whereas their interactions by edges. The line colors indicate different types of known (pink and light blue), predicted (green, red and blue) and other (yellow, black and gray) interactions. The proteins (identified by its code gene) in red color related cellular nitrogen compounds biosynthesis, blue color proteins related to translation and channel activity, and green color with membrane proteins. The network was constructed with STRING v11.05.

proteins that showed differential expression. An *in silico* analysis was conducted using the free available software STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) version 11.05. For each set of proteins that were upregulated in response to free nisin and/or nanoencapsulated nisin, it was determined the number of protein-protein interactions documented in the database and the network functional enrichment. The complete set of proteins obtained from the STRING enrichment analysis for both free and liposome encapsulated nisin, are showed in the supplementary Table S1 and Table S2, respectively (available in the on-line repository MSV000089076). In addition, a graph linking proteins symbolized by nodes with known interactions with the encoded genes of the identified proteins was assembled for visualization purposes. Different colors were used to evaluate the functional characteristics of proteins that were present in the nodes observed for upregulated proteins in treatments with free nisin (Fig. 1) and nanoencapsulated nisin (Fig. 2). A network with 140 edges with two relevant nodes was obtained with the analysis of proteins upregulated by free nisin, including a great quantity of
Fig. 2. Protein-protein interaction network of upregulated *Listeria monocytogenes* ATCC 7466 proteins after interaction with nisin-loaded liposomes. The proteins are represented by nodes whereas their interactions by edges. The line colors indicate different types of known (pink and pastel blue), predicted (green, red and blue) and others (yellow, black and gray) interactions. The proteins (identified by its code gene) in red color related with cellular nitrogen compounds biosynthesis, blue color with translation and channel activity, and green color with protein-containing complex. The network was constructed with STRING v11.05.

membrane proteins. These protein clusters present biological connection and are related to stress response mechanisms in *L. monocytogenes*. The interaction analysis of upregulated proteins by liposome-loaded nisin showed 156 edges with a single protein network, the same observed in free nisin, related to ribosomal proteins.

2. Experimental Design, Materials and Methods

2.1. Samples

The influence of free and nanoliposome-encapsulated nisin on the proteomic profile of *L. monocytogenes* was investigated using the strain ATCC 7644 (American Type Culture Collection, Manassas, VA, USA). The bacterial strain was retrieved from the stock culture maintained in Brain Heart Infusion (BHI) broth (Kasvi, São José dos Pinhais, Paraná, Brazil) containing 20% (v/v) glycerol for long-standing storage. To acclimatize the strain to the experimental conditions, an aliquot of the culture (0.1 mL) was inoculated into 9.9 mL BHI broth and incubated overnight in a shaker at operating 37°C and 125 rpm. Afterwards, the bacterial cells were cultivated in BHI broth for 24 h at 37°C using a 1% (v/v) inoculum. For the analysis, cells were then cultivated at 37°C until they reached the mid-exponential growth phase (at hour 6 and OD_{600} about 0.4). At this time, either free or liposome-encapsulated nisin were added at 0.3 μg/mL final concentration in separate treatments [1]. The liposomes were prepared by the thin film hydration method using purified phosphatidylcholine (Phospholipon 90G, provided by Lipoid, Ludwigshafen, Germany) as detailed in a previous work [2]. This method result in stable liposomes with entrapment efficiency of nisin superior to 90% [3]. Cells of *L. monocytogenes* incubated under the same
conditions but without any treatment were used as control. The bacterial cells were incubated at 37 °C for 1 h, then harvested by centrifugation at 5000 g at 4 °C for 10 min, and the pellets were washed three times with 2 mL of PBS pH 7.4 and then reserved for protein extraction [4]. Each treatment was performed in triplicate (biological replicates). For the analysis, samples of *L. monocytogenes* treated with free and liposome-encapsulated nisin were compared with control *L. monocytogenes* cultures.

### 2.2. Protein digestion and preparation of peptides

Protein digestion was performed according to standard protocols for complex protein mixtures [5]. In summary, the protocol consisted of the following steps:

(a) Denaturation of extracts containing 100 μg of *L. monocytogenes* proteins using 8 M urea (1:1, v/v) for 30 min;
(b) Reduction of the samples using 5 mM dithiothreitol (DTT, Sigma-Aldrich, St. Louis, MO, USA) during 25 min at 56 °C;
(c) Alkylation with 14 mM iodoacetamide (IAA, Sigma-Aldrich), during 30 min at room temperature in a light protected ambient;
(d) Addition of 5 mM DTT followed by 15 min incubation to eliminate the remaining IAA;
(e) Dilution of the samples with 50 mM ammonium bicarbonate (1:5, v/v) to reach a concentration of 1.6 M urea, containing 1 mM CaCl₂ as a trypsin cofactor;
(f) Addition of trypsin (Sequencing Grade Modified Trypsin, Promega, WI, USA), prepared at 20 μg/mL in 50 mM ammonium bicarbonate, at 1:50 E/S ratio;
(g) Incubation at 37 °C during 16 h for protein digestion;
(h) Addition of 2% (v/v) trifluoroacetic acid to stop the proteolytic reaction.

Afterwards, samples were centrifuged at 14,000 g for 20 min, and the supernatants were collected and applied to C18 reverse phase Stage Tips for desalination [6]. Stage Tips were previously conditioned with methanol and equilibrated with 0.1% (v/v) formic acid. Samples were loaded and 0.1% (v/v) formic acid was used to wash the salt residues. Peptides were then eluted with 60% (v/v) acetonitrile containing 0.1% (v/v) formic acid and the samples were freeze-dried and stored at −20 °C until LC-MS/MS analysis.

### 2.3. Nano-LC-MS/MS analysis

The dried peptide samples were suspended in 10 μL formic acid (0.1%, v/v). Aliquots of 3 μL were analyzed using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), coupled to EASY-nano-LC system equipped with a Proxeon nanoelectrospray ion source (Proxeon Biosystem, West Palm Beach, FL, USA). The LC-MS/MS parameters were as follows:

- **Column**: 20 cm x 75 μm ID, 5 μm particle size PicoFrit column (New Objective, Littleton, MA, USA).
- **Elution conditions**: 2–90% (v/v) acetonitrile gradient containing 0.1% (v/v) formic acid, eluted at a flow rate of 300 μL/min over 65 min.
- **Instrumental procedures**: set up in the data-dependent acquisition (DDA) mode; nanoelectrospray voltage 2.2 kV; source temperature 275 °C; resolution *r* = 60,000; collision energy of 35% for CID (collision-induced dissociation) fragmentation of most abundant ions with charge ≥2, sequentially isolated to a target value of 5000; dynamic exclusion enabled at size list of 500 peptides, exclusion duration of 60 s and a repetition count of 1.
2.4. Data processing

Raw MS files were processed with the MaxQuant software version v1.3.0.3 [7], and the Andromeda engine was employed to match MS/MS spectra against the Listeria monocytogenes UniProt protein sequence database and contaminant protein sequence (https://www.uniprot.org/). The following parameters were used for MaxQuant: trypsin digestion, with maximum 2 missed cleavages and minimum peptide length of 7; cysteine carbamidomethylation as a fixed modification, while variable modifications were methionine oxidation and acetylation (Protein N-term); mass tolerance for peptides and fragments was set to ±20 ppm and ±0.1 Da; peptide and protein false discovery rate (FDR) cut-off was set to 0.01. The statistical analysis was performed using MetaboAnalyst 3.068. Only proteins with valid intensity values of label-free quantification (LFQ) detected in ≥50% of the samples were considered for analysis. The data was subjected to partial least squares discriminate statistical analysis (PLS-DA), then was established the cutoff value VIP (Variable Importance in Projection) and proteins with VIP score ≥1.0 were considered as upregulated [8]. All the proteins with VIP score ≥1.0 were characterized using UniProt and regrouped as upregulated proteins.

From the treatments, two groups of proteins (free and nanoencapsulated-encapsulated nisin) were selected for examination of the interactions among proteins showing differential expression (upregulation). An in silico analysis was conducted using the free available software STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database version 11.05 [9]. The number of protein-protein interactions registered in the database were determined for the proteins that were differentially over expressed. For visualization purposes, a diagram was assembled linking proteins depicted by nodes with recognized connections with the identified proteins. At the same time, proteins with common gene ontology terms were identified by different colors.

Ethics Statements

This work does not involve human subjects, animal experiments or data collected from social media platforms.

CRediT Author Statement

Cristian Mauricio Barreto Pinilla: Methodology, Software, Writing; Paolo Stincone: Methodology, Data curation, Writing; Adriano Brandelli: Conceptualization, Writing, Editing.

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

MSV000089076 (Original data) (MASSIVE).

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