Identification and metagenetic characterisation of *Listeria monocytogenes*-harbouring communities present in food-related industrial environments.

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Abstract

The main aim of this study was to localise, identify and characterise the *Listeria monocytogenes*-harbouring bacterial communities present in food related premises via 16S rRNA gene metagenetic analysis. With this scope, 319 environmental samples coming from a wide variety of surfaces of fish (n = 120), meat (n = 80) and dairy industries (n = 119), were firstly analysed following ISO 11290-1 and ISO 11290-2 norms. Direct *L. monocytogenes* quantification was possible in 9 samples (2.8 %) with values between 2.00 and 3.97 log CFU/cm^2. After enrichment, an overall *L. monocytogenes* incidence of 12.54 % (n = 40) was obtained, being samples from meat industry the most contaminated. Molecular serotyping assays showed that most of the isolates belonged to 1/2b-3b-7 subgroup, followed by 1/2a-3a and 1/2c-3c. These results combined with *Asc*I and *Apa*I PFGE macrorestriction patterns, yielded 7 different *L. monocytogenes* clusters. Nevertheless, no clear ecological relationships could be stablished. High amounts of *L. monocytogenes*-associated psychrotrophic microbiota were obtained in all cases with values above 9 log CFU/cm^2 in some cases. Metagenetic analysis of one representative sample per each food industry type (fish, meat, dairy) demonstrated that Actinobacteria (53.16 %) was mostly present in the meat sample whereas Proteobacteria was the most representative phylum in dairy (69.58 %) and fish (97.11 %) samples. Subsequent operational taxonomic units (OTUs) analysis, showed a wide variety of taxa associated with *L. monocytogenes* such as spoilage-associated genera (e.g. *Psyschromonas* or *Shewanella*), lactic acid bacteria genera (e.g. *Lactococcus* or *Lactobacillus*) or pathogenic species such as *Yersinia enterocolitica*. It was thus demonstrated, that *L. monocytogenes* is capable to both survive with different bacteria in different ecological niches, highlighting once more the need for proper surveillance schedules so as to guarantee the safety of the food products.

1. Introduction

According to the latest report of the European Food Safety Agency (EFSA), *Listeria monocytogenes* infections raised a 9.3 % compared to previous data. This represents a ratio of 0.47 cases per 100000 population with a clear overall increasing trend since 2008 (EFSA & ECDC, 2017), thus posing a serious thread for global health. The routes of transmission to humans are mainly due to consumption of contaminated foodstuffs such as soft cheeses, cold-smoked fish and other ready-to-eat (RTE) products. Even though the strategies to prevent the infection and spreading of *L. monocytogenes* among population are increasing, several outbreaks are still reported over the last years. As an example in Europe, two unrelated outbreaks have been reported in Spain between 2013-2014 affecting 35 people, one of them linked to the consumption of contaminated *foie gras* (Pérez-Trallero, Zigorraga, Artieda, Alkorta, & Marimón, 2014) or the outbreak in 2014 in Denmark due to contaminated RTE meat with a fatality rate of 41.46 % (Anonymous, 2015). More recently in 2017, a multistate *L. monocytogenes* outbreak has been reported in Europe for which the European Centre for Disease Prevention and Control (ECDC) have constituted a special investigation committee (ECDC, 2017).

This pathogen has a remarkable capability to survive for long periods in the food industry, adhered to food contact and non-food contact surfaces (Carpentier & Cerf, 2011). Moreover, it can be found associated with other bacteria forming the so-called multispecies biofilms, which might confer an increased protection against antimicrobials and other stresses. Regarding this, it has been reported that *L. monocytogenes*-carrying polymicrobial biofilms, can be found among industrial surfaces even after hygienisation procedures (Carpentier & Chassaing, 2004; Rodríguez-López, Saá-Ibusquiza, Mosquera-Fernández, & López-Cabo, 2015). Additionally, it has been reported that in mixed biofilms, *L. monocytogenes* survival rate after sanitation is higher compared to monospecies biofilms (Kostaki,

Chorianopoulos, Braxou, Nychas, & Giaouris, 2012; Saá Ibusquiza, Herrera, Vázquez-Sánchez, & Cabo, 2012). Considering the above mentioned data, it seems obvious that getting further insight into the knowledge of bacterial communities present in industrial premises is an important issue to take into account when developing sanitation protocols.

Previous studies have reported the bacterial composition in environmental samples obtained from surfaces of fish (Bagge-Ravn et al., 2003; Langsrud, Moen, Møretrø, Løype, & Heir, 2016), meat (Marouani-Gadri, Augier, & Carpentier, 2009; Møretrø, Langsrud, & Heir, 2013) and dairy industries (Cleto, Matos, Kluskens, & Vieira, 2012; Langsrud, Seifert, & Møretrø, 2006; P. Teixeira, Lopes, Azeredo, Oliveira, & Vieira, 2005). However, the majority of them still use classical culture-dependent techniques as a sole method to characterise the microbiota present in such samples. These approaches can give in a relatively easy way an approximation of the actual composition of the sample since they rely on culturable bacteria, without considering the viable but non-culturable (VBNC) cells.

In the recent years, the culture-independent approaches such as high-throughput sequencing (HTS) and 16S rRNA gene metagenetic analysis, have been postulated as new, sensitive and efficient methods for ecological studies in the field of food microbiology (Cardinali, Corte, & Robert, 2017; Ercolini, 2013; Ferrocino & Cocolin, 2017). Using this approach, Stellato et al. (2016) characterised in-depth the microbiota present in meat, environment and operators of two different meat processing environments. Results demonstrated that the main microbiota was shared in 80 % of the cases and stablished a direct link between the contamination present in meat and the microbiota present in the processing environment. In a similar way, a study conducted by Calasso et al. (2016) described the microbiota present in Caciotta and Caciocavallo pugliese cheese plants demonstrating how starter cultures such as *Lactococcus lactis* or

Streptococcus thermophilus are able to colonise different surfaces from the cheese plant and suggesting that these colonisers could act as direct sources of starter reinoculations.

Despite the aforementioned studies, to the best of the authors' knowledge, no culture-independent studies regarding *L. monocytogenes*-carrying communities, characterising the microbiota associated to this pathogen in food industry environmental samples, have been carried out up to now. Therefore, this study was aimed to characterise potential *L. monocytogenes* contamination spots present in a variety of surfaces belonging to fish, meat and dairy industries, including detection of the pathogen following enrichment, direct quantification, where possible. Furthermore, representative *L. monocytogenes*-positive samples from each sector were used for a complete description of the bacterial community present using an HTS approach based on 16S rRNA gene metagenetic analysis.

2. Material and methods

2.1. Sampling

Eight different premises belonging to fish, meat and dairy industry were surveyed by an external company (Deinal Soluciones Agroalimentarias, S.L., Lugo, Spain) between February and July 2017 at the end of production activity and before any cleaning and disinfection procedure was performed (Carpentier & Barre, 2012) (Table 1). In each survey, 40 samples were collected except for survey no. 2 where 39 were obtained instead, yielding a total of 319 different samples of various food contact and non-food contact surfaces. For a detailed list of all samples, the reader is referred to Supplementary Table 1. In all surveys, samples were collected in the same day so as to avoid variations in the microbiota derived from the normal activity of the processing plant. Surveys were carried out by an external company due to privacy policies of the premises.

A square of 100 cm^2 was sampled using sterile sponges moistened with 10 ml of LPT Neutralising broth (composition per litre: 0.7 g soy lecithin, 5 g NaCl, 1 g $Na_2S_2O_3$, 2.5 g $NaHSO_3$, 1 g $HSCH_2COONa$, 5 g Yeast Extract, 1 g L-histidine, 5 ml Tween 80, pH 7.6 ± 0.2). Once in the laboratory, sponges were introduced in a stomacher bag together with 90 ml of Fraser Broth Base (Oxoid, Hampshire, England). Following this, they were digested with a stomacher masticator (IUL Instruments, Barcelona, Spain) for 1 min. The resulting product was divided into three different aliquots: the first one (60 ml) was used for detection and quantification of *L. monocytogenes*, the second one (10 ml) was prepared for HTS analysis by mixing the suspension with 50 % glycerol 1:1 (v/v) and kept at – 80 ºC until needed, and a third one (30 ml) was used for quantification of total psychrotrophic microbiota.

149 *2.2. Detection of L. monocytogenes*

150 An aliquot of 50 ml from the first fraction of the stomacher suspension prepared in
151 section 2.1., was used for enrichment and agar plating for *L. monocytogenes* detection
152 following the ISO 11290-1 norm (ISO, 1996) with slight modifications. Firstly, the
153 corresponding amount (800 µl) of Half-Fraser Selective Supplement (Oxoid,
154 Hampshire, England) was added to the aliquot. Following this, 3 replicas of 5 ml each
155 were prepared from this suspension and incubated at 30 ºC for 24 h for primary
156 enrichment. Then, 0.1 ml of each aliquot was transferred to a new tube containing 10 ml
157 of Fraser Broth (Fraser Broth Base with Fraser Selective supplement; Oxoid,
158 Hampshire, England), and incubated at 37 ºC for 48 h for secondary enrichment.
159 Finally, from each tube of the primary and secondary enrichments (independently of the
160 medium colour), a loop was struck onto an ALOA plate and incubated at 37 ºC for 24 h.
161 A presumptive *L. monocytogenes* colony of each strike was recovered and subcultured
162 in TSYEA plates for confirmation as described below.

163 In all cases, this procedure was interrupted if a plate of a given sample turned out to be
164 positive in the quantification assay.

165

166 *2.3. Direct quantification of L. monocytogenes present in samples*

167 For this part of the sampling, an aliquot of 10 ml from the first fraction of the stomacher
168 suspension obtained in section 2.1., was used for *L. monocytogenes* direct quantification
169 following the method described in the ISO 11290-2 norm (ISO, 1998, 2004) with slight
170 modifications. Firstly, the aliquot was incubated at 20 ºC for 1 h to favour revivification
171 of damaged cells. Following this, two different ALOA plates (Scharlab, Barcelona,
172 Spain) were each one inoculated with 500 µl of the aliquot and allowed to partially air
173 dry in the flow cabinet during 30 min, so as to facilitate the ulterior spreading. Plates

were then incubated at 37 ºC for 24 h. After this, plates were examined for typical *L. monocytogenes* colony morphology (blue with a clear halo around). In case colonies were small or absent, plates were incubated for further 24 h. From those positive plates, five presumptive *L. monocytogenes* colonies were picked and struck onto a Tryptone Soya Yeast Extract Agar (TSYEA; TSA supplemented with 0.6 % yeast extract (Cultimed, Barcelona, Spain)) for *L. monocytogenes* confirmation as described below. In those plates with less than five colonies present, all of them were picked. In either case, TSYEA plates were incubated at 37 ºC for 24 h. Isolates recovered, were subcultured in TSA to ensure purity and cultured in TSB at 37 ºC for species confirmation as described below. Results were expressed in log CFU/cm^2

2.4. L. monocytogenes confirmation

Confirmation of *Listeria monocytogenes* was carried out via 16S rRNA gene amplification and sequencing. Firstly, genomic DNA was extracted as described elsewhere (Vázquez-Sánchez, López-Cabo, Saá-Ibusquiza, & Rodríguez-Herrera, 2012). 16S rRNA gene amplification with primers 27FYM and 1492R' was performed as previously described (Weisburg, Barns, Pelletier, & Lane, 1991) using a MyCycler^{TM} Thermal Cycler (Bio-Rad, Hercules, CA). Amplicon size was checked in a 1.5 % agarose gel stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology, Sangdaewon-dong, South Korea) using Hyperladder 50 bp (Bioline, Singapore) as a molecular marker and visualised in a GelDoc 2000 Apparatus equipped with Quantity One software (Bio-Rad, Hercules, CA). PCR products were purified using a GeneJet gel extraction kit (Thermo Scientific, Vilnius, Lithuania) and sequencing was performed at Centro de Apoyo Científico y Tecnológico a la Investigación (CACTI; Vigo, Spain) using an AB 3130 Genetic Analyser (Applied

biosystems, Foster City, CA). Chromatograms were processed and double analysed with Nucleotide-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and RPD (http://rdp.cme.msu.edu/) algorithms.

2.5. Quantification of psychrotrophic bacteria

From the stomacher suspension (see section 2.1.), decimal dilutions (up to $1:10^6$) were made in buffered peptone water (BPW; Cultimed, Barcelona, Spain) and 100 µl of each dilution was directly spread onto Tryptone Soy Agar (TSA; Cultimed, Barcelona, Spain) plates and incubated at 17 ºC for 72 h for quantification of psychrotrophic microbiota. Results were expressed in log CFU/cm^2.

2.6. Isolates maintenance

In all cases, stock cultures of all *L. monocytogenes* isolates obtained were made in Brain-Heart Infusion Broth (BHI; Scharlab, Barcelona, Spain) containing 50 % glycerol 1:1 (v/v) mixed and kept at – 80ºC. Working cultures were made in the same medium as stock cultures and maintained at – 20 ºC until needed.

2.7. L. monocytogenes subtyping

2.7.1. Pulsed-field gel electrophoresis

The resulting isolates were subtyped using Pulsed-field gel electrophoresis (PFGE) using *Asc*I and *Apa*I restriction endonucleases as described by the PulseNet protocol (PulseNet, 2013). After electrophoresis, gels were stained for 30 min with 1X GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA) in 0.5 X TBE buffer (Panreac

222 Applichem, Barcelona, Spain) and visualised in a GelDoc 2000 Apparatus equipped
223 with Quantity one software (Bio-Rad, Hercules, CA).

224 Similarity factors based on Dice coefficient, UPGMA system cluster analysis and strain
225 dendrograms (tolerance 1 %, optimisation 0.5 %) were constructed using BioNumerics
226 7 software (Applied Maths NV, Belgium).

227

228 *2.7.2. Serotyping*

229 A multiplex-PCR method proposed by Doumith et al., 2004 was used in order to
230 differentiate the major serovars (1/2a, 1/2b, 1/2c and 4b) in *L. monocytogenes* isolates.

231 Amplicons were resolved in a 1.5 % agarose gel stained with RedSafe (iNtRON) using
232 Hyperladder 50 bp (Bioline) as a molecular marker. Bands were then visualised using a
233 GelDoc 2000 Apparatus with Quantity One software (Bio-Rad).

234

235 *2.8. Metagenetic analysis of representative L. monocytogenes-positive samples*

236 Once all the *L. monocytogenes*-positive samples were determined, a representative
237 sample from each of the sectors was further processed recovering the – 80 ºC aliquot
238 obtained in section 2.1., in order to determine the bacterial composition using a
239 metagenetic approach via 16S rRNA gene amplification, sequencing and analysis.

240

241 *2.8.1. Library construction and quantification*

242 Firstly, DNA was extracted using a PureLink^{TM} Microbiome DNA Purification Kit
243 (Life Technologies) following the manufacturer's instructions. After extraction, the 16S
244 rRNA gene was amplified with 16S Ion Metagenomics Kit (Life Technologies) which is

245 designed for rapid analyses of polybacterial samples using Ion Torrent sequencing
246 technology. This kit uses two primer pools that selectively amplify seven hypervariable
247 regions of bacterial 16S rRNA gene (V2, V4, V8 and V3, V6-7, V9). Each reaction
248 contained 2 ng of template DNA. For each sample, all amplicons were equally
249 combined and quantified using the Agilent 2100 Bioanalyser. Libraries were carried out
250 using the Ion Plus Library Kit for AB Library Builder System (ThermoFisher Scientific)
251 following the library preparation protocol for short amplicons. Between 150 and 400 ng
252 of amplified DNA were used for library preparation and each sample was barcoded
253 using the P1 adapter and the barcoded A adapters provided in the Ion Xpress Barcode
254 Adapter Kit (Life technologies). The resulting DNA libraries were subsequently
255 quantified by qPCR using the Ion Library Quantitation Kit (ThermoFisher Scientific)
256 following manufacturer's instructions, to calculate the dilution factor of each library for
257 a final concentration of 10 pmol. After individual quantitation, the barcoded libraries
258 were pooled in equimolar amounts (10 µL each) to ensure equal representation of each
259 sample in the sequencing run.

260

261 *2.8.2. Template preparation and sequencing*

262 Emulsion PCR was performed using the Ion PGM Hi-Q view OT2 kit that is suitable for
263 reads up to 400 bp in length in accordance with the manufacturer's standard protocol
264 using the OneTouch 2 and Ion One Touch ES instruments (ThermoFisher Scientific)
265 following the instructions of the manufacturers. Finally, sequencing was performed on
266 the Ion Personal Genome Machine (PGM) using the Ion Hi-Q Sequencing view Kit
267 (400 bp) and 316 V2 BC chips.

268

269

2.8.3. Data analysis

Base calling and run demultiplexing were performed by Torrent Suite Software version 5.2.2 (ThermoFisher Scientific) with default parameters. FileExporter version 5.2.0.0 (ThermoFisher Scientific) was used to generate BAM files for each sample. Mean read length ranged between 236 bp to 241 bp for all samples. The BAM files were analysed using the 16S Metagenomics workflow v5.4 of the Ion Reporter Software. This enables the rapid identification of microbes present in complex polybacterial research samples using both curated Greengenes (DeSantis et al., 2006) and premium curated MicroSEQ® ID 16S rRNA (Life Technologies) reference databases.

3. Results and discussion

3.1. Detection and quantification of Listeria monocytogenes in environmental samples from three different food industry sectors

In this study, surfaces sampled were from a variety of food-related premises (Supplementary Table 1). Dairy industry was focused on cheese-producing factories, samples from meat sector belonged to slaughterhouses and those from fish industry were from fish markets and cold-drying rooms obtaining a total of 319 samples that were processed in order to detect and quantify *L. monocytogenes*.

Quantification using direct ALOA plating following the method ISO 11290-2 rendered 13 positive samples. Among these, 4 were finally discarded due to lack of growth or typical halo surrounding colonies in subsequent cultures in ALOA plates giving a final result of 9 positive samples for direct quantification with values between 2.00 and 3.97 log CFU/cm^2 (Table 2). All confirmation assays of presumptive *L. monocytogenes* using 16S rRNA gene sequencing turned out to be positive and were used as validation of these ALOA plate counts.

After enrichment and subsequent plating on ALOA plates, presumptive *L. monocytogenes* was detected in 41 of the 319 samples, including those samples but one (sample no. 88) that were already positive in the abovementioned quantification. These isolates were again further analysed by 16S rRNA gene sequencing allowing detecting a unique false positive identified as *Enterococcus faecium* (sample no. 71). This lack of accuracy of selective methods has been previously described (Rodríguez-López et al., 2015; Serraino et al., 2011) and empathises the need to include gene-based techniques in routinely assays in food safety. Thus, the overall incidence of *L. monocytogenes* among samples was of 12.54 % (n = 40) almost three-fold higher when compared to results obtained previously in a similar survey performed in our laboratory (Rodríguez-

López et al., 2015). Results showed the highest incidence among samples coming from meat industry (n = 29; 36,25 %), followed by those from fish (n = 9; 7.20 %) and dairy industries (n = 2; 1.68 %) (Table 1).

The high incidence of *L. monocytogenes* obtained in samples of meat industry can be derived from the origin of the samples, i.e. slaughterhouses, and the location of the sampling points. Even though they were not directly in contact with food products, they were constantly contaminated with organic matter coming from skins, viscera and other animal wastes potentially loaded with *L. monocytogenes*, subsequently stablishing potential hotspots of cross-contamination. Similarly, previous authors demonstrated that the occurrence of contact with foodstuffs and the level of contamination with *L. monocytogenes* is not directly related (Sala, Morar, Irziu, Nichita, & Imre, 2016). The fact that the surveyed premises had cold environments for processed meat products could have also favoured the presence of this pathogen as previously reported (Luo et al., 2017).

In concordance with the outcomes obtained in this work, a recent survey conducted by Muhterem-Uyar et al., (2015) showed overall incidences of *L. monocytogenes* of 32% in meat processing environments, being especially relevant in buildings where slaughter and processing were performed together. Similarly, Sala et al., (2016) found an incidence of *L. monocytogenes* of a 25.8% in environmental samples of a pig abattoir with a higher recovery rates in non-food contact surfaces.

In fish industry, it has been reported that cold, moistened environments greatly influence the presence and persistence of *L. monocytogenes* especially in storage chambers (Pagadala et al., 2012). This could have been the case of survey 5 (fish market) in which most of the *L. monocytogenes*-positive samples were detected in those coming from cold chambers for fresh product storage (Supplementary Table 1), being

specially relevant the load of *L. monocytogenes* in sample no. 168 (Table 2). In this case, *L. monocytogenes* was mainly obtained from non-food contact surfaces, fact that has been previously reported previously (Langsrud et al., 2016), even though other studies indicate that the direct contact with raw material is key to maintain *L. monocytogenes* in a particular scenario (Pagadala et al., 2012).

Sample no. 225 was the only food contact surface positive for *L. monocytogenes* in fish industry and an area with operator direct access. Since anthropogenic factors can enhance pathogen transmission (Greig, Todd, Bartleson, & Michaels, 2007), this area should be especially taken into account to avoid *L. monocytogenes* spreading that could pose a thread for the final product or even an eventual outbreak.

Contrarily to fish and meat industries, incidence of *L. monocytogenes* in dairy industries obtained in this study was remarkably lower. Similar results were obtained in a study performed by Moreno-Enriquez et al., (2007) where no *L. monocytogenes* was detected in environmental samples of *queso fresco* producers in Santa Ana. However, other authors have showed higher incidences and prevalence of *L. monocytogenes* on food contact surfaces (Ibba et al., 2013) and on non-food contact surfaces such as drains and floors (Kabuki, Kuaye, Wiedmann, & Boor, 2004).

In this study, factories surveyed associated to dairy premises were either cheese producers (surveys 1 and 2) or a milk farm (survey 8). In such environments, it is normal to encounter lactic acid bacteria (LAB) among the resident microbiota. This fact, could have deleterious effects on the survival of *L. monocytogenes* as previously reported (Brillet, Pilet, Prevost, Bouttefroy, & Leroi, 2004; Campos, Mazzotta, & Montville, 1997; Koo, Eggleton, O'Bryan, Crandall, & Ricke, 2012).

Discrepancies in the level of incidence between the outcomes presented in this article and previously reported data, could have also been derived from the sampling method

(Gómez, Ariño, Carramiñana, Rota, & Yangüela, 2012; Keeratipibul et al., 2017), the time of collection and the surface surveyed (Rodríguez-López et al., 2015), and the number of samples. Moreover, in dairy industry, the sort of dairy industry surveyed (i.e. farm, cheese producer, etc.) could have influenced the results. This makes comparison among different datasets published somehow difficult and highlights once more the need to standardise the sampling methods for surfaces in the food industry.

3.2. Subtyping of L. monocytogenes isolates

For subtyping assays, 51 different colonies were processed: three different colonies from each sample isolated from the quantification phase (where possible) were used, whereas in those samples positive in the detection phase, just one colony was isolated.

3.2.1. Pulsotyping

All the obtained isolates, were subtyped by PFGE with enzymes *Asc*I and *Apa*I (PulseNet, 2013). This was intended to observe the molecular relationships based on the "3-band rule" (Tenover et al., 1995) and establish ecological patterns among the different isolates. Dendrograms were subsequently produced based on the composite fingerprint analysis using a UPGMA clustering using Dice correlation index.

Considering a 70 % similarity as a cut-off threshold, isolates were clustered in 7 different clusters as depicted in Fig. 1. These clusters were further divided in different pulsotypes considering equal those with a similarity ≥ 99 %, closely related those with a similarity ≥ 97 % and unrelated those whose similarity < 97 %. These percentages were empirically determined by comparing the band patterns obtained in the PFGE gels (Tenover et al., 1995). Outcomes showed an overall result of 29 different pulsotypes

391 yielding a discrimination index (D.I.) of 0.979 (Hunter & Gaston, 1988). As observed in
392 the dendrogram, the same pulsotype was recovered in different sampling points within
393 the same survey (e.g. isolates 102, 103 and 104, and isolates 162 and 166), showing a
394 clonal expansion inside the premises as observed previously (Rodríguez-López et al.,
395 2015).

396 Generally, no relationship between the source of isolation (i.e. the food industry sector
397 from it was isolated) and the molecular pattern obtained in the PFGE analysis was
398 observed. This highlighted once again the outstanding capacity of this pathogen to adapt
399 to different ecological niches regardless of the industry sector (Gianfranceschi, Gattuso,
400 Tartaro, & Aureli, 2003; Valderrama & Cutter, 2013) and that its environmental
401 distribution seems to be of a multifactorial nature (Pagadala et al., 2012).

402 Additionally, in one of the clusters there were three isolates that conformed one
403 pulsotype but were obtained from two different surveys (isolate 112 came from survey 3
404 whilst isolates 140 and 134 came from survey 4, Fig. 1), indicating an intra- and inter-
405 premise clonal expansion. The fact that the same pulsotype was present in surveys 3 and
406 4, could be explained because of the fact that they are a bovine and porcine abattoir, and
407 a quartering and processing facility, close to each other (25 Km distance,
408 approximately). Besides, premise 4 imports animals from 3 occasionally, it is not
409 strange to think that animals harbouring a particular *L. monocytogenes* pulsotype, could
410 have ended in both places. This is of a special relevance since clearly demonstrates how
411 the anthropogenic factor plays an important role in the dissemination of bacterial clones.

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413

414

3.2.2. Serotyping

Results of multiplex PCR, showed three different serogroups among the pulsotypes obtained (Fig. 1). Most of them belonged to subgroup 1/2b-3b-7 (48.28 %), followed by 1/2a-3a (27.59 %) and 1/2c-3c (24.14%). This contrasted with information published previously stating that, in industry, serotype 3a and especially 1/2a are mostly present (Kramarenko et al., 2013; Lambertz, Ivarsson, Lopez-Valladares, Sidstedt, & Lindqvist, 2013; Leong, Alvarez-Ordóñez, & Jordan, 2014; Oliveira et al., 2018; W. Wang et al., 2017). No isolates belonging to the outbreak-associated serovar (4b) (Burall, Grim, & Datta, 2017) were obtained. Nevertheless, recent studies have demonstrated that there is a shift in the human listeriosis-associated serovars, being those belonging to 1/2 serotypes mostly responsible among clinical cases (Mammina et al., 2009; Pontello et al., 2012; Y. Wang et al., 2015). The causes of this shift are under investigation but this prevalence could be due to the biofilm-forming ability associated to certain serotypes (Kadam et al., 2013), although this fact still remains unclear (Borucki, Peppin, White, Loge, & Call, 2003; Doijad et al., 2015). Since it is reported that *L. monocytogenes* 1/2 members are generally better adapted to food environments (Vázquez-Boland et al., 2001) and can cause illness in humans (Nelson et al., 2004), these results should be taken into consideration to improve the current sanitisation practises in the premises surveyed to avoid an eventual contamination of the final product.

3.3. Quantification of psychrotrophic microbiota associated with samples surveyed

Generally, high amounts of psychrotrophic microbiota were detected in almost all samples (Fig. 2). Mean values were the highest in fish industry samples (7.87 ± 1.34 log CFU/cm^2), followed by those from dairy and meat industries (6.97 ± 2.41 and 6.97 ±

1.05 log CFU/cm^2, respectively). For a detailed list of psychrotrophs quantification, the reader is referred to Supplementary Table 2.

In *L. monocytogenes*-positive samples, psychrotrophic microbiota detected also showed maximum values in samples coming from fish industry (Fig. 2). Specifically, outcomes ranged from 7.95 ± 0.28 to 9.34 ± 0.01 log CFU/cm^2. Following these values, were those coming from samples of meat industry ranging from 5.58 ± 0.02 to 8.97 ± 0.01 log CFU/cm^2, whereas in samples from dairy environments, the yield of associated psychrotrophs ranged from 5.72 ± 0.38 to 7.72 ± 0.19 log CFU/cm^2 (Fig. 2). Of note, since only two *L. monocytogenes* positive samples were obtained from dairy industry samples, it was not possible to establish statistical significance between the psychrotrophs counts between these samples and those obtained in fish and meat industry samples.

3.4. Metagenetic analysis of representative samples

Three representative samples, one per each food industry type (fish, meat, dairy), were chosen for metagenetic analysis based on the high amount of *L. monocytogenes* present in the quantification phase (samples no. 96 and 168) or due to the quantity of psychrotrophic bacteria detected (sample no. 9).

3.4.1. Analyses, rarefaction curves and diversity indexes

For detailed data regarding metagenetic analysis performed with Ion Reporter Software v. 5.6 (ThermoFisher Scientific) using Metagenomics 16S w1.1 workflow the reader is referred to Supplementary Table 3. Broadly, 594604, 644047 and 698222 valid reads (i.e. with a number of copies detected > 10) were obtained in dairy, meat and fish

industry samples, respectively. Among these, the percentage of mapped sequences using the curated MicroSEQ® reference library v.2013.1 (ThermoFisher Scientific) and the curated Greengenes database v13.5 (http://geengenes.lbl.gov) was 78.18, 77.86 and 86.10 % for dairy, meat and fish industry samples, respectively.

Rarefaction curves were constructed based on the percentage of identity of the sequences mapped further classified into the different OTUs (Fig. 3). For this, an identity > 95 % was used for families and > 97 % was preferred for genera. As depicted in Fig. 3 fish industry sample presented the lowest number of OTUs in both taxonomical levels and was further confirmed by the Shannon-Wiener and Simpson's diversity indexes (Table 3).

In meat and dairy industry samples, richness levels were different depending on the taxon considered, being higher for the dairy sample in the family taxon compared with meat sample whereas in genus taxon the situation was the opposite (Fig. 3). Alpha diversity indexes directly corroborated these results in genera (Table 3). The salty environment present could have driven the subsequent reduced OTUs variability in fish sample, thus acting as a selective agent on the community (Yang, Ma, Jiang, Wu, & Dong, 2016). However, for family taxon both indicators were higher in meat sample even though family richness was higher in dairy sample (Fig. 3, Table 3). This fact was due to the number of reads (higher in meat sample) and the random distribution of them into the different OTUs regardless the absolute number of the latter, and indicated that even though the dairy sample was richer in families, meat sample was more diverse.

3.4.2. Taxonomic diversity

As depicted in Fig. 4, Proteobacteria was the most representative phylum in the samples of the fish and dairy industries accounting for the 97.11 and the 69.58 % of the 16S

rRNA gene fragments, respectively whereas in the meat industry the dominant phylum was Actinobacteria representing the 53.16 % of the total mapped reads of the sample. Other representative phyla were Firmicutes in the dairy sample (22.09 %), and Proteobacteria (29.37 %) and Bacterioidetes (16.85 %) in the meat sample.

Overall, Gram-negative species were mostly detected in samples of meat and fish industries, whereas Gram-positives were found at a higher proportion in the dairy industry (Fig. 5). Curiously, no *L. monocytogenes* was detected via 16S rRNA gene sequencing because the quantity of valid sequences remained below the level of detection (i.e. at least ten valid reads in the metagenetic analysis). This indicates that even this pathogen was present, the levels of contamination among the surveyed surfaces were very low.

As aforementioned, the presence of Proteobacteria made up the largest division in fish industry sample (Fig. 4) being in agreement with previous studies reporting that this phylum constitutes the major component of the microbiome of surfaces in fish processing premises (Bagge-Ravn et al., 2003; Langsrud et al., 2016). Focusing in this phylum, most of the members in the fish industry sample belonged to the family Pseudoalteromonadaceae (56.45 % of total mapped sequences, Fig. 5A), which is logical since this sample was from a food contact surface and members of this family are commonly associated as part of the commensal microbiota of fish skin (Lokesh & Kiron, 2016). Following this, Moraxellaceae (15.88 %) and the Vibrionaceae (13.84 %) were the most abundant (Fig. 5A), being especially relevant the presence of the genera *Psychrobacter* and *Photobacterium* (Fig. 5B) as bacteria commonly associated with marine and coastal environments (Gomez-Gil et al., 2014; L. M. Teixeira & Merquior, 2014).

512 On the other hand, results show that, despite Proteobacteria was also the most
513 representative phylum in dairy sample, the family distribution varied being
514 Moraxellaceae (38.05 %, Fig. 5A) and particularly the genus *Acinetobacter* (34.82 %,
515 Fig. 5B) the most representative members. This was in concordance with previous
516 published data (Langsrud et al., 2006). Pseudomonadaceae (11.71 %) and
517 Enterobacteriaceae (9.64 %) were also significantly represented in this sample (Fig 5A).
518 Amongst them, *Pseudomonas* and *Yersinia* represented the major part of the total
519 mapped samples (11.70 and 6.60 %, respectively). These genera have been found
520 previously in dairy industry environments and are associated to spoilage phenomena
521 due to enzyme secretion (Machado et al., 2017) and the latter also to foodborne
522 outbreaks linked to contaminated dairy products consumption (De Lamo-Castellví,
523 Roig-Sagués, Capellas, Hernández-Herrero, & Guamis, 2005). Furthermore, a high
524 proportion of Gram-positives, i.e. phylum Firmicutes, was also significantly represented
525 in this sample (22.09 %, Fig. 5) with a high proportion of the genera *Lactococcus* (12.68
526 %), *Carnobacterium* (3.32 %) and *Lactobacillus* (2.68 %), widely used as starter
527 cultures in cheese-producing premises (Møretrø et al., 2017).

528 Additionally, results obtained in this study demonstrated that meat sample was the most
529 diverse in terms of family and genera distribution compared with the two previous
530 samples (Figs. 5A, B). Among the Actinobateria phylum, the most represented genera
531 in the sample were *Rhodococcus* (F. Nocardiaceae, 22.89 %) and *Microbacterium* (F.
532 Microbacteriaceae, 13.11 %) as portrayed in Figs. 5A, B. These results are in the same
533 line of previously published data, depicting these as commonly present genera in food
534 related premises (Møretrø & Langsrud, 2017; Ohta et al., 2013) although other authors
535 have pointed out *Enterobacter* or LAB (e.g. *Leuconostoc* or *Streptococcus*) as the main
536 genera present (Hultman, Rahkila, Ali, Rousu, & Björkroth, 2015; Møretrø et al., 2013).
537 Observed discrepancies in the composition of the microbiome characterised in this

study and those reported previously, could also be attributed to modifications of microbiota from the incoming raw material affecting the relative abundances (Bagge-Ravn et al., 2003).

3.4.3. Comparison of microbiome composition

In order to compare the samples analysed, a Venn diagram with all the OTUs obtained was constructed at a genus level considering a cut-off threshold of > 97 % identity (Fig. 6).

As observed, 8 different OTUs were shared in all three sectors analysed. Among them, the genus *Acinetobacter* presented one species in all three samples (*Acinetobacter lwofii*) although in all cases, the abundance regarding the total of mapped reads was below 1 % (results not shown). It is noticeable that fish sample, with respect to meat and dairy samples, shared the least of OTUs detected (Fig. 6). This could be the consequence of the pressure that the halophile environment poses in this particular environment, which is eventually translated in the selection of a determinate sort of genera (Yang et al., 2016).

Most of the genera obtained comprised species commonly found as part of the normal microbiome in food processing environments. However, is important to take into account some of the genera due to their potential relevance in various aspects affecting the food quality features. For example, *Shewanella putrefaciens* accounted to the 2.05 % of the mapped sequences in dairy sample. This bacterium has been typically associated with spoilage in fish (Jørgensen & Huss, 1989). Besides, it has been reported that the biofilm-forming capacity of this bacterium on stainless steel is diminished due to the presence of milk proteins (Bagge, Hjelm, Johansen, Huber, & Gram, 2001), so, it

562 could be the case that, the presence of other commensal bacteria could have aided *S.*
563 *putrefaciens* to overcome such negative effects.

564 Another example to take into consideration is the presence of *Yersina enterocolitica* in
565 dairy sample, which made up the 4.1 % of mapped reads in the sample. This
566 microorganism is considered a foodborne pathogen of serious concern able to survive at
567 low temperatures (Ye, Ling, Han, & Wu, 2014) and even to endure high pressure
568 conditions (De Lamo-Castellví et al., 2005) highlighting the necessity to improve
569 sanitation procedures in this particular area.

570 Other microorganisms such as *Psychromonas arctica* accounted for the 4.12 % of
571 mapped sequences in fish sample. The genus *Psycrhomonas*, despite not pathogen, has
572 been associated with fish spoilage (Møretrø, Moen, Heir, Hansen, & Langsrud, 2016).
573 Moreover, it has been reported to be able to form biofilms at low temperatures
574 (Groudieva, Grote, & Antranikian, 2003) which gives it better competitiveness in
575 addition to its adaptation to grow at low temperatures (Kergourlay, Taminiau, Daube, &
576 Champomier Vergès, 2015). The matrix of such biofilms could entrap other
577 microorganisms (Chmielewski & Frank, 2003), including pathogens, protecting them
578 from low temperatures aiding them to survive in such conditions.

579 Hence, metagenetic analysis revealed not only the presence of spoilage or pathogenic
580 microorganisms, but also bacteria that, although not being pathogenic, could somehow
581 enhance the survival of the latter and thus the possibility of their spreading and food
582 contamination. Moreover, this sort of analysis indicates not only the currently viable
583 organisms in a determinate time, but also allows the identification of non-viable and
584 death cells, giving an overall idea of the ecology and the diversity of population along
585 time in a given setting.

586

4. Conclusions

The results obtained in this work demonstrated that despite the hygienisation systems applied, *L. monocytogenes* is able to survive adhered to a variety of surfaces and environments in dairy, fish and meat industries. Moreover, plate counts and 16s rRNA gene metagenetic analysis demonstrated that this pathogen is able to cohabit with different microorganisms as previously demonstrated (Langsrud et al., 2016; Rodríguez-López et al., 2015). Thus, in order to assure the microbiological safety of the final food products, the information provided in this piece of work should be taken into account for the design of novel sanitisation systems in the food industry, as well as to take necessary corrective measures so as to improve the application of the already existing ones.

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610 **References**

611 Anonymous. (2015). Annual Report on Zoonoses in Denmark 2014. Retrieved January
612 20, 2018, from http://www.food.dtu.dk/english/-
613 /media/Institutter/Foedevareinstituttet/Publikationer/Pub-2015/Annual-Report-
614 2014-final.ashx?la=da

615 Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J. N., Johansen, C., & Gram, L.
616 (2003). The microbial ecology of processing equipment in different fish
617 industries—analysis of the microflora during processing and following cleaning
618 and disinfection. *International Journal of Food Microbiology*, *87*(3), 239–250.
619 http://doi.org/10.1016/S0168-1605(03)00067-9

620 Bagge, D., Hjelm, M., Johansen, C., Huber, I., & Gram, L. (2001). Shewanella
621 putrefaciens Adhesion and Biofilm Formation on Food Processing Surfaces.
622 *Applied and Environmental Microbiology*, *67*(5), 2319–2325.
623 http://doi.org/10.1128/AEM.67.5.2319-2325.2001

624 Borucki, M. K., Peppin, J. D., White, D., Loge, F., & Call, D. R. (2003). Variation in
625 biofilm formation among strains of Listeria monocytogenes. *Applied and*
626 *Environmental Microbiology*, *69*(12), 7336–7342.
627 http://doi.org/10.1128/AEM.69.12.7336

628 Brillet, a, Pilet, M.-F., Prevost, H., Bouttefroy, a, & Leroi, F. (2004). Biodiversity of
629 Listeria monocytogenes sensitivity to bacteriocin-producing Carnobacterium
630 strains and application in sterile cold-smoked salmon. *Journal of Applied*
631 *Microbiology*, *97*(5), 1029–37. http://doi.org/10.1111/j.1365-2672.2004.02383.x

632 Burall, L. S., Grim, C. J., & Datta, A. R. (2017). A clade of Listeria monocytogenes
633 serotype 4b variant strains linked to recent listeriosis outbreaks associated with

produce from a defined geographic region in the US. *PLoS ONE*, *12*(5), e0176912. http://doi.org/10.1371/journal.pone.0176912

Calasso, M., Ercolini, D., Mancini, L., Stellato, G., Minervini, F., Di Cagno, R., … Gobbetti, M. (2016). Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at the same dairy plant. *Food Microbiology*, *54*, 115–126. http://doi.org/10.1016/j.fm.2015.10.008

Campos, C. A., Mazzotta, A. S., & Montville, T. J. (1997). Inhibition of Listeria monocytogenes by Carnobacterium piscicola in vacuum-packaged cooked chicken at refrigeration temperatures. *Journal of Food Safety*, *17*(3), 151–160. http://doi.org/10.1111/j.1745-4565.1997.tb00184.x

Cardinali, G., Corte, L., & Robert, V. (2017). Next Generation Sequencing: problems and opportunities for next generation studies of microbial communities in food and food industry. *Current Opinion in Food Science*, *17*, 62–67. http://doi.org/10.1016/j.cofs.2017.09.009

Carpentier, B., & Barre, L. (2012). Guidelines on sampling the food processing area and equipment for the detection of Listeria monocytogenes. Retrieved January 3, 2018, from https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_guidelines_on_sampling.pdf

Carpentier, B., & Cerf, O. (2011). Review - Persistence of Listeria monocytogenes in food industry equipment and premises. *International Journal of Food Microbiology*, *145*(1), 1–8. http://doi.org/10.1016/j.ijfoodmicro.2011.01.005

Carpentier, B., & Chassaing, D. (2004). Interactions in biofilms between Listeria monocytogenes and resident microorganisms from food industry premises.

658 *International Journal of Food Microbiology*, *97*(2), 111–22.
659 http://doi.org/10.1016/j.ijfoodmicro.2004.03.031

660 Chmielewski, R. A. N., & Frank, J. F. (2003). Biofilm Formation and Control in Food
661 Processing Facilities. *Comprehensive Reviews in Food Science and Food Safety*,
662 *2*(1), 22–32. http://doi.org/10.1111/j.1541-4337.2003.tb00012.x

663 Cleto, S., Matos, S., Kluskens, L., & Vieira, M. J. (2012). Characterization of
664 contaminants from a sanitized milk processing plant. *PLoS ONE*, *7*(6), e40189.
665 http://doi.org/10.1371/journal.pone.0040189

666 De Lamo-Castellví, S., Roig-Sagués, A. X., Capellas, M., Hernández-Herrero, M., &
667 Guamis, B. (2005). Survival and growth of Yersinia enterocolitica strains
668 inoculated in skimmed milk treated with high hydrostatic pressure. *International*
669 *Journal of Food Microbiology*, *102*, 337–342.
670 http://doi.org/10.1016/j.ijfoodmicro.2004.11.025

671 DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., …
672 Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database
673 and workbench compatible with ARB. *Applied and Environmental Microbiology*,
674 *72*(7), 5069–5072. http://doi.org/10.1128/AEM.03006-05

675 Doijad, S. P., Barbuddhe, S. B., Garg, S., Poharkar, K. V, Kalorey, D. R., Kurkure, N.
676 V., … Chakraborty, T. (2015). Biofilm-forming abilities of listeria monocytogenes
677 serotypes isolated from different sources. *PLoS ONE*, *10*(9), e0137046.
678 http://doi.org/10.1371/journal.pone.0137046

679 Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004).
680 Differentiation of the major Listeria monocytogenes serovars by multiplex PCR.
681 *Journal of Clinical Microbiology*, *42*(8), 3819–22.

682 http://doi.org/10.1128/JCM.42.8.3819-3822.2004

683 ECDC. (2017). Multi-country outbreak of Listeria monocytogenes PCR serogroup IVb
684 ST6, (6 December 2017), Stockholm: ECDC;2017.

685 EFSA, & ECDC. (2017). The European Union summary report on trends and sources of
686 zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*,
687 *15*(12), 5077. http://doi.org/10.2903/j.efsa.2017.5077

688 Ercolini, D. (2013). High-throughput sequencing and metagenomics: Moving forward
689 in the culture-independent analysis of food microbial ecology. *Applied and*
690 *Environmental Microbiology*, *79*(10), 3148–3155.
691 http://doi.org/10.1128/AEM.00256-13

692 Ferrocino, I., & Cocolin, L. (2017). Current perspectives in food-based studies
693 exploiting multi-omics approaches. *Current Opinion in Food Science*, *13*, 10–15.
694 http://doi.org/10.1016/j.cofs.2017.01.002

695 Gianfranceschi, M., Gattuso, A., Tartaro, S., & Aureli, P. (2003). Incidence of Listeria
696 monocytogenes in food and environmental samples in Italy between 1990 and
697 1999: Serotype distribution in food, environmental and clinical samples. *European*
698 *Journal of Epidemiology*, *18*(10), 1001–1006.
699 http://doi.org/10.1023/A:1025849532417

700 Gomez-Gil, B., Thompson, C. C., Matsumura, Y., Sawabe, T., Iida, T., Christen, R., …
701 Sawabe, T. (2014). The famlily vibrionaceae. In M. Dworkin, S. Falkow, E.
702 Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes:*
703 *Gammaproteobacteria* (4th ed., Vol. 9783642389, pp. 659–747). New York, NY:
704 Springer. http://doi.org/10.1007/978-3-642-38922-1_225

705 Gómez, D., Ariño, A., Carramiñana, J. J., Rota, C., & Yangüela, J. (2012). Comparison

706 of sampling procedures for recovery of Listeria monocytogenes from stainless steel
707 food contact surfaces. *J. Food Prot.*, *75*(6), 1077–82. http://doi.org/10.4315/0362-
708 028X.JFP-11-421

709 Greig, J. D., Todd, E. C. D., Bartleson, C. A., & Michaels, B. S. (2007). Outbreaks
710 Where Food Workers Have Been Implicated in the Spread of Foodborne Disease.
711 Part 1. Description of the problem, Methods and Agents involved. *Journal of Food*
712 *Protection*, *70*(7), 1752–1761.

713 Groudieva, T., Grote, R., & Antranikian, G. (2003). Psychromonas arctica sp. nov., a
714 novel psychrotolerant, biofilm-forming bacterium isolated from Spitzbergen.
715 *International Journal of Systematic and Evolutionary Microbiology*, *53*, 539–545.
716 http://doi.org/10.1099/ijs.0.02182-0

717 Hultman, J., Rahkila, R., Ali, J., Rousu, J., & Björkroth, K. J. (2015). Meat processing
718 plant microbiome and contamination patterns of cold-tolerant bacteria causing food
719 safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages.
720 *Applied and Environmental Microbiology*, *81*(20), 7088–7097.
721 http://doi.org/10.1128/AEM.02228-15

722 Hunter, P. R., & Gaston, M. A. (1988). Numerical index of the discriminatory ability of
723 typing systems : an application of Simpson ' s index of Numerical Index of the
724 Discriminatory Ability of Typing Systems : an Application of Simpson ' s Index of
725 Diversity. *Journal of Clinical Microbiology*, *26*(11), 2465–2466.
726 http://doi.org/0095-1137/88/112465-02$02.00/0

727 Ibba, M., Cossu, F., Spanu, V., Virdis, S., Spanu, C., Scarano, C., & De Santis, E. P. L.
728 (2013). Listeria monocytogenes contamination in dairy plants: evaluation of
729 Listeria monocytogenes environmental contamination in two cheese-making plants
730 using sheeps milk. *Italian Journal of Food Safety*, *2*(2), 109–112.

731 http://doi.org/10.4081/ijfs.2013.e31

732 ISO. (1996). ISO 11290-1 Microbiology of food and animal feeding stuffs - Horizontal
733 method for the detection and enumeration of Listeria monocytogenes - Part 1:
734 Detection method.

735 ISO. (1998). ISO 11290-2:1998 Microbiology of food and animal feeding stuffs -
736 Horizontal method for the detection and enumeration of Listeria monocytogenes -
737 Part 2: Enumeration method.

738 ISO. (2004). ISO 11290-2:1998/AM1:2004 Microbiology of food and animal feeding
739 stuffs - Horizontal method for the detection and enumeration of Listeria
740 monocytogenes - Part 2: Enumeration method - Amendment 1: Modification of the
741 enumeration medium.

742 Jørgensen, B. R., & Huss, H. H. (1989). Growth and activity of Shewanella putrefaciens
743 isolated from spoiling fish. *International Journal of Food Microbiology*, *9*, 51–62.
744 http://doi.org/10.1016/0168-1605(89)90037-8

745 Kabuki, D. Y., Kuaye, A. Y., Wiedmann, M., & Boor, K. J. (2004). Molecular
746 Subtyping and Tracking of Listeria monocytogenes in Latin-Style Fresh-Cheese
747 Processing Plants. *Journal of Dairy Science*, *87*, 2803–2812.

748 Kadam, S. R., den Besten, H. M. W., van der Veen, S., Zwietering, M. H., Moezelaar,
749 R., & Abee, T. (2013). Diversity assessment of Listeria monocytogenes biofilm
750 formation: Impact of growth condition, serotype and strain origin. *International*
751 *Journal of Food Microbiology*, *165*, 259–264.
752 http://doi.org/10.1016/j.ijfoodmicro.2013.05.025

753 Keeratipibul, S., Laovittayanurak, T., Pornruangsarp, O., Chaturongkasumrit, Y.,
754 Takahashi, H., & Techaruvichit, P. (2017). Effect of swabbing techniques on the

efficiency of bacterial recovery from food contact surfaces. *Food Control*, *77*, 139–144. http://doi.org/10.1016/j.foodcont.2017.02.013

Kergourlay, G., Taminiau, B., Daube, G., & Champomier Vergès, M. C. (2015). Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology*, *213*, 31–39. http://doi.org/10.1016/j.ijfoodmicro.2015.09.010

Koo, O. K., Eggleton, M., O'Bryan, C. A., Crandall, P. G., & Ricke, S. C. (2012). Antimicrobial activity of lactic acid bacteria against Listeria monocytogenes on frankfurters formulated with and without lactate/diacetate. *Meat Science*, *92*(4), 533–537. http://doi.org/10.1016/j.meatsci.2012.05.023

Kostaki, M., Chorianopoulos, N., Braxou, E., Nychas, G.-J., & Giaouris, E. (2012). Differential biofilm formation and chemical disinfection resistance of sessile cells of listeria monocytogenes strains under monospecies and dual-species (with Salmonella enterica) conditions. *Applied and Environmental Microbiology*, *78*(8), 2586–95. http://doi.org/10.1128/AEM.07099-11

Kramarenko, T., Roasto, M., Meremäe, K., Kuningas, M., Põltsama, P., & Elias, T. (2013). Listeria monocytogenes prevalence and serotype diversity in various foods. *Food Control*, *30*, 24–29. http://doi.org/10.1016/j.foodcont.2012.06.047

Lambertz, S. T., Ivarsson, S., Lopez-Valladares, G., Sidstedt, M., & Lindqvist, R. (2013). Subtyping of Listeria monocytogenes isolates recovered from retail ready-to-eat foods, processing plants and listeriosis patients in Sweden 2010. *International Journal of Food Microbiology*, *166*(1), 186–192. http://doi.org/10.1016/j.ijfoodmicro.2013.06.008

Langsrud, S., Moen, B., Møretrø, T., Løype, M., & Heir, E. (2016). Microbial dynamics

779 in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in
780 salmon-processing plants. *Journal of Applied Microbiology*, *120*(2), 366–378.
781 http://doi.org/10.1111/jam.13013

782 Langsrud, S., Seifert, L., & Møretrø, T. (2006). Characterization of the microbial flora
783 in disinfecting footbaths with hypochlorite. *Journal of Food Protection*, *69*(9),
784 2193–2198.

785 Leong, D., Alvarez-Ordóñez, A., & Jordan, K. (2014). Monitoring occurrence and
786 persistence of Listeria monocytogenes in foods and food processing environments
787 in the Republic of Ireland. *Frontiers in Microbiology*, *5*, 436.
788 http://doi.org/10.3389/fmicb.2014.00436

789 Lokesh, J., & Kiron, V. (2016). Transition from freshwater to seawater reshapes the
790 skin-associated microbiota of Atlantic salmon. *Scientific Reports*, *6*, 19707.
791 http://doi.org/10.1038/srep19707

792 Luo, L., Zhang, Z., Wang, H., Wang, P., Lan, R., Deng, J., … Ye, C. (2017). A 12-
793 month longitudinal study of Listeria monocytogenes contamination and persistence
794 in pork retail markets in China. *Food Control*, *76*, 66–73.
795 http://doi.org/10.1016/j.foodcont.2016.12.037

796 Machado, S. G., Baglinière, F., Marchand, S., Coillie, E. Van, Vanetti, M. C. D., Block,
797 J. De, & Heyndrickx, M. (2017). The biodiversity of the microbiota producing
798 heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy
799 products. *Frontiers in Microbiology*, *8*, 302.
800 http://doi.org/10.3389/fmicb.2017.00302

801 Mammina, C., Aleo, A., Romani, C., Pellissier, N., Nicoletti, P., Pecile, P., … Pontello,
802 M. M. (2009). Characterization of Listeria monocytogenes isolates from human

listeriosis cases in Italy. *Journal of Clinical Microbiology*, *47*(9), 2925–2930. http://doi.org/10.1128/JCM.00102-09

Marouani-Gadri, N., Augier, G., & Carpentier, B. (2009). Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection - Influence of isolated strains on biofilm formation by Sakaï and EDL 933 E. coli O157:H7. *International Journal of Food Microbiology*, *133*, 62–67. http://doi.org/10.1016/j.ijfoodmicro.2009.04.028

Moreno-Enriquez, R. I., Garcia-Galaz, A., Acedo-Felix, E., Gonzalez-Rios, I. H., Call, J. E., Luchansky, J. B., & Diaz-Cinco, M. E. (2007). Prevalence, types, and geographical distribution of Listeria monocytogenes from a survey of retail Queso Fresco and associated cheese processing plants and dairy farms in Sonora, Mexico. *Journal of Food Protection*, *70*(11), 2596–2601.

Møretrø, T., & Langsrud, S. (2017). Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. *Comprehensive Reviews in Food Science and Food Safety*, *16*, 1022–1041. http://doi.org/10.1111/1541-4337.12283

Møretrø, T., Langsrud, S., & Heir, E. (2013). Bacteria on Meat Abattoir Process Surfaces after Sanitation: Characterisation of Survival Properties of Listeria monocytogenes and the Commensal Bacterial Flora. *Advances in Microbiology*, *3*, 255–264. http://doi.org/10.4236/aim.2013.33037

Møretrø, T., Moen, B., Heir, E., Hansen, A., & Langsrud, S. (2016). Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology*, *237*, 98–108. http://doi.org/10.1016/j.ijfoodmicro.2016.08.016

827 Møretrø, T., Schirmer, B. C. T., Heir, E., Fagerlund, A., Hjemli, P., & Langsrud, S.
828 (2017). Tolerance to quaternary ammonium compound disinfectants may enhance
829 growth of Listeria monocytogenes in the food industry. *International Journal of*
830 *Food Microbiology*, *241*, 215–224.
831 http://doi.org/10.1016/j.ijfoodmicro.2016.10.025

832 Muhterem-Uyar, M., Dalmasso, M., Bolocan, A. S., Hernandez, M., Kapetanakou, A.
833 E., Kuchta, T., … Wagner, M. (2015). Environmental sampling for Listeria
834 monocytogenes control in food processing facilities reveals three contamination
835 scenarios. *Food Control*, *51*, 94–107.
836 http://doi.org/10.1016/j.foodcont.2014.10.042

837 Nelson, K. E., Fouts, D. E., Mongodin, E. F., Ravel, J., DeBoy, R. T., Kolonay, J. F., …
838 Fraser, C. M. (2004). Whole genome comparisons of serotype 4b and 1/2a strains
839 of the food-borne pathogen Listeria monocytogenes reveal new insights into the
840 core genome components of this species. *Nucleic Acids Research*, *32*(8), 2386–
841 2395. http://doi.org/10.1093/nar/gkh562

842 Ohta, Y., Ito, T., Mori, K., Nishi, S., Shimane, Y., Mikuni, K., & Hatada, Y. (2013).
843 Microbacterium saccharophilum sp. nov., isolated from a sucrose-refining factory.
844 *International Journal of Systematic and Evolutionary Microbiology*, *63*, 2765–
845 2769. http://doi.org/10.1099/ijs.0.047258-0

846 Oliveira, T. S., Varjão, L. M., da Silva, L. N. N., Pereira, R. de C. L., Hofer, E., Vallim,
847 D. C., & Almeida, R. C. de C. (2018). Listeria monocytogenes at chicken
848 slaughterhouse: Occurrence, genetic relationship among isolates and evaluation of
849 antimicrobial susceptibility. *Food Control*, *88*, 131–138.
850 http://doi.org/10.1016/j.foodcont.2018.01.015

851 Pagadala, S., Parveen, S., Rippen, T., Luchansky, J. B., Call, J. E., Tamplin, M. L., &

852 Porto-Fett, A. C. S. (2012). Prevalence, characterization and sources of Listeria
853 monocytogenes in blue crab (Callinectus sapidus) meat and blue crab processing
854 plants. *Food Microbiology*, *31*(2), 263–70. http://doi.org/10.1016/j.fm.2012.03.015

855 Pérez-Trallero, E., Zigorraga, C., Artieda, J., Alkorta, M., & Marimón, J. M. (2014).
856 Two outbreaks of listeria monocytogenes infection, Northern Spain. *Emerging*
857 *Infectious Diseases*, *20*(12), 2155–2157. http://doi.org/10.3201/eid2012.140993

858 Pontello, M., Guaita, A., Sala, G., Cipolla, M., Gattuso, A., Sonnessa, M., &
859 Gianfranceschi, M. V. (2012). Listeria monocytogenes serotypes in human
860 infections (Italy, 2000-2010). *Annali Dell'Istituto Superiore Di Sanita*, *48*(2), 146–
861 150. http://doi.org/10.4415/ANN_12_02_07

862 PulseNet. (2013). Standard Operating Procedure for PulseNet PFGE of Listeria
863 monocytogenes.

864 Rodríguez-López, P., Saá-Ibusquiza, P., Mosquera-Fernández, M., & López-Cabo, M.
865 (2015). Listeria monocytogenes – carrying consortia in food industry.
866 Composition, subtyping and numerical characterisation of mono-species biofilm
867 dynamics on stainless steel. *International Journal of Food Microbiology*, *206*, 84–
868 95. http://doi.org/10.1016/j.ijfoodmicro.2015.05.003

869 Saá Ibusquiza, P., Herrera, J. J. R., Vázquez-Sánchez, D., & Cabo, M. L. (2012).
870 Adherence kinetics, resistance to benzalkonium chloride and microscopic analysis
871 of mixed biofilms formed by Listeria monocytogenes and Pseudomonas putida.
872 *Food Control*, *25*, 202–210. http://doi.org/10.1016/j.foodcont.2011.10.002

873 Sala, C., Morar, A., Irziu, E., Nichita, I., & Imre, M. (2016). Environmental Occurrence
874 and Antibiotic Susceptibility Profile of Listeria monocytogenes at a
875 Slaughterhouse Raw Processing Plant in Romania. *Journal of Food Protection*,

876 *79*(10), 1794–1797. http://doi.org/10.4315/0362-028X.JFP-16-052

877 Serraino, a, Giacometti, F., Piva, S., Florio, D., Pizzamiglio, V., & Zanoni, R. G.
878 (2011). Isolation of glucosidase and phospholipase positive Bacillus circulans on
879 ALOA medium. *Letters in Applied Microbiology*, *53*(2), 244–6.
880 http://doi.org/10.1111/j.1472-765X.2011.03084.x

881 Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., & Ercolini, D.
882 (2016). Overlap of spoilage-associated microbiota between meat and the meat
883 processing environment in small-scale and large-scale retail distributions. *Applied*
884 *and Environmental Microbiology*, *82*(13), 4045–4054.
885 http://doi.org/10.1128/AEM.00793-16

886 Teixeira, L. M., & Merquior, V. L. C. (2014). The family moraxellaceae. In M.
887 Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The*
888 *Prokaryotes: Gammaproteobacteria* (4th ed., Vol. 9783642389, pp. 443–476).
889 New York, NY: Springer. http://doi.org/10.1007/978-3-642-38922-1_245

890 Teixeira, P., Lopes, Z., Azeredo, J., Oliveira, R., & Vieira, M. J. (2005). Physico-
891 chemical surface characterization of a bacterial population isolated from a milking
892 machine. *Food Microbiology*, *22*, 247–251.
893 http://doi.org/10.1016/j.fm.2004.03.010

894 Tenover, F. C., Arbeit, R. D., Goering, R. V, Mickelsen, P. A., Murray, B. E., Persing,
895 D. H., & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction
896 patterns produced by pulsed- field gel electrophoresis: Criteria for bacterial strain
897 typing. *Journal of Clinical Microbiology*, *33*(9), 2233–2239. http://doi.org/0095-
898 1137/

899 Valderrama, W. B., & Cutter, C. N. (2013). An ecological perspective of Listeria

900 monocytogenes biofilms in food processing facilities. *Critical Reviews in Food*
901 *Science and Nutrition*, *53*(8), 801–17.
902 http://doi.org/10.1080/10408398.2011.561378

903 Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domi, G., González-
904 zorn, B., & Wehland, J. (2001). Listeria Pathogenesis and Molecular Virulence
905 Determinants Listeria Pathogenesis and Molecular Virulence Determinants. *Clin.*
906 *Microbiol. Rev.*, *14*(3), 584–640. http://doi.org/10.1128/CMR.14.3.584

907 Vázquez-Sánchez, D., López-Cabo, M., Saá-Ibusquiza, P., & Rodríguez-Herrera, J. J.
908 (2012). Incidence and characterization of Staphylococcus aureus in fishery
909 products marketed in Galicia (Northwest Spain). *International Journal of Food*
910 *Microbiology*, *157*(2), 286–296. http://doi.org/10.1016/j.ijfoodmicro.2012.05.021

911 Wang, W., Zhou, X., Suo, Y., Deng, X., Cheng, M., Shi, C., & Shi, X. (2017).
912 Prevalence, serotype diversity, biofilm-forming ability and eradication of Listeria
913 monocytogenes isolated from diverse foods in Shanghai, China. *Food Control*, *73*,
914 1068–1073. http://doi.org/10.1016/j.foodcont.2016.10.025

915 Wang, Y., Jiao, Y., Lan, R., Xu, X., Liu, G., Wang, X., … Ye, C. (2015).
916 Characterization of Listeria monocytogenes isolated from human Listeriosis cases
917 in China. *Emerging Microbes & Infections*, *4*, e50.
918 http://doi.org/10.1038/emi.2015.50

919 Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal
920 DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*(2), 697–
921 703.

922 Yang, J., Ma, L., Jiang, H., Wu, G., & Dong, H. (2016). Salinity shapes microbial
923 diversity and community structure in surface sediments of the Qinghai-Tibetan

924 Lakes. *Scientific Reports*, *6*, 25078. http://doi.org/10.1038/srep25078

925 Ye, Y., Ling, N., Han, Y., & Wu, Q. (2014). Detection and prevalence of pathogenic
926 Yersinia enterocolitica in refrigerated and frozen dairy products by duplex PCR
927 and dot hybridization targeting the virF and ail genes. *Journal of Dairy Science*,
928 *97*(11), 6785–6791. http://doi.org/10.3168/jds.2014-8382

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945 **Figure captions**

946 **Figure 1.** Dendrogram corresponding to the UPGMA cluster analysis based on the
947 composite *Asc*I and *Apa*I macrorestriction patterns. Additionally, in each of the clusters
948 obtained, considering a similarity > 70 %, the serogroup and the lineage of the isolates
949 has been included based on the results of the multiplex PCR described by Doumith et
950 al., (2004). The numbers in brackets indicate the number of survey from where they
951 were obtained.

952 **Figure 2.** Quantification (plate count) of psychrotrophic microbiota associated to *L.*
953 *monocytogenes*-positive samples obtained from sponges sampling a surface of 100 cm^2.
954 Error bars = SD.

955 **Figure 3.** Rarefaction curves for 16S rRNA gene metagenetic analysis in *L.*
956 *monocytogenes*-positive representative samples depicting the number of operational
957 taxonomic units (OTUs) obtained in A) families and B) genera regarding the number of
958 reads in the sample. Curve plateaus in the plot indicated an approach to completeness.
959 The lack of extension all the way to the right of the x-axis in some curves indicate that
960 the number of the sequences mapped was lower.

961 **Figure 4.** Phyla distribution based on 16S rRNA gene metagenetic analysis output.

962 **Figure 5.** Family (A) and genera (B) distribution in percentages obtained via 16S rRNA
963 gene high throughput sequencing performed in representative samples. In both cases, in
964 the "others" box the OTUs with an abundance < 1 % were included.

965 **Figure 6.** Venn diagram showing the genera obtained in each sample and the genera
966 shared among samples after 16 rRNA gene metagenetic analysis. Yellow: Dairy
967 industry sample; Blue: Fish industry sample; Red: Meat industry sample.

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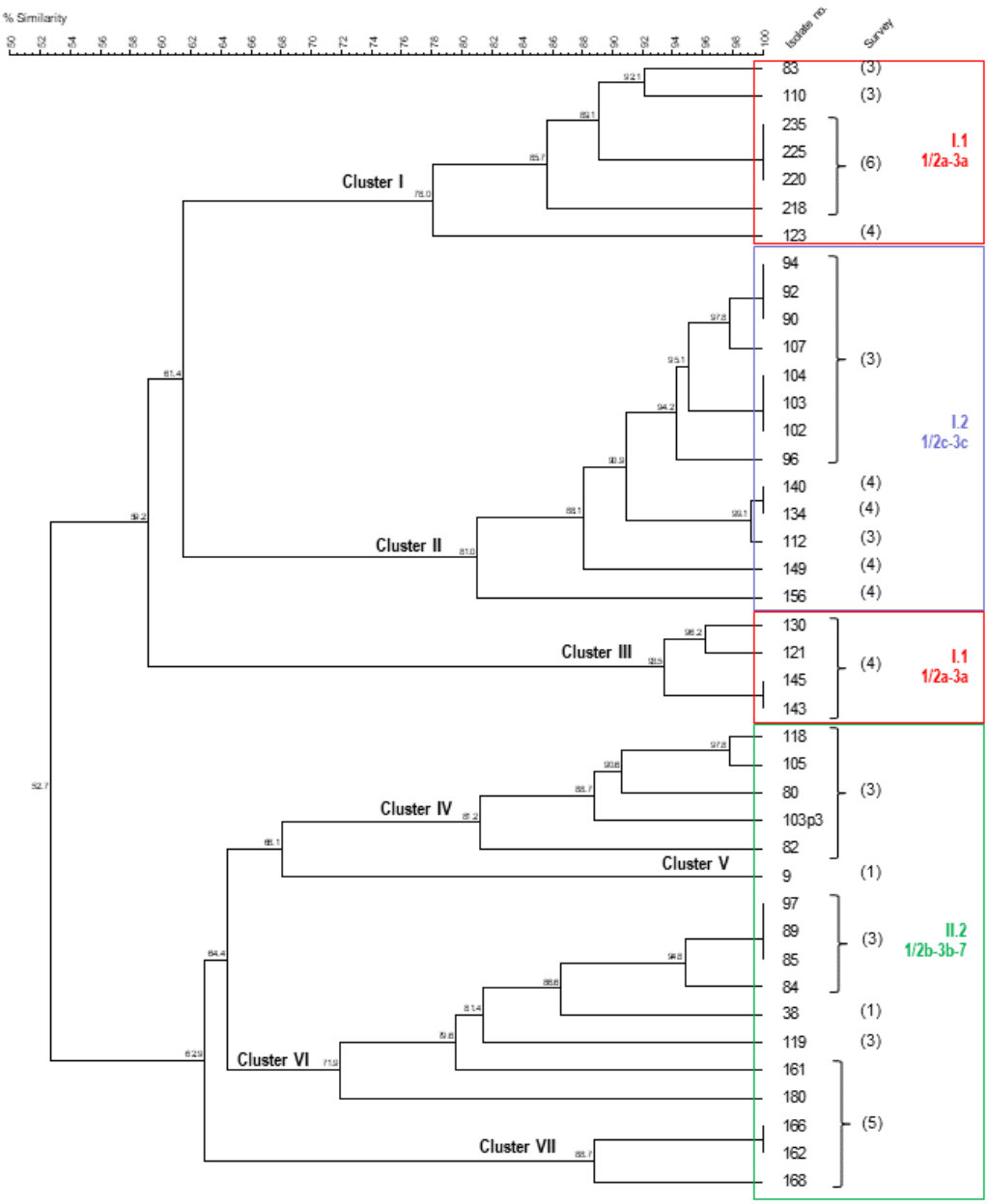
Figure captions for supplementary tables

Supplementary Table 1. Complete list of samples, including their origins, obtained and analysed in this work.

Supplementary Table 2. Complete list of quantification (plate count) in CFU/cm^2 of psychrotrophic microbiota obtained in each one of the samples obtained in this work.

990 Figure 1



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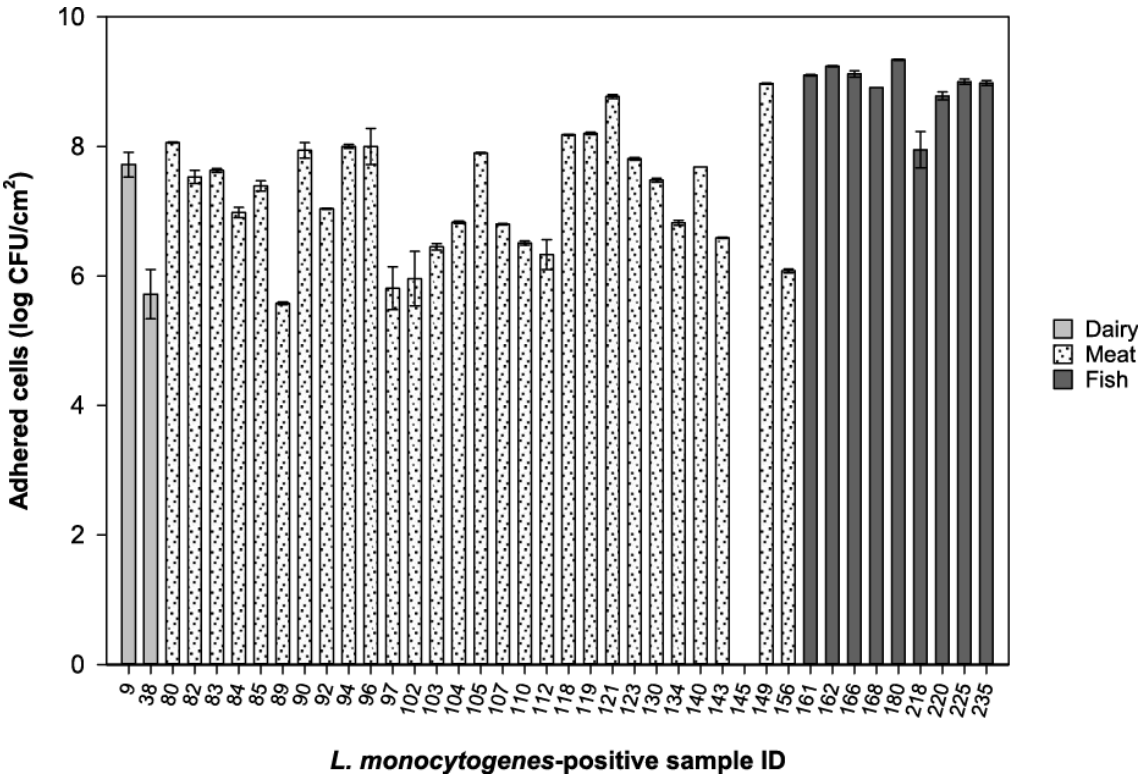
995 Figure 2



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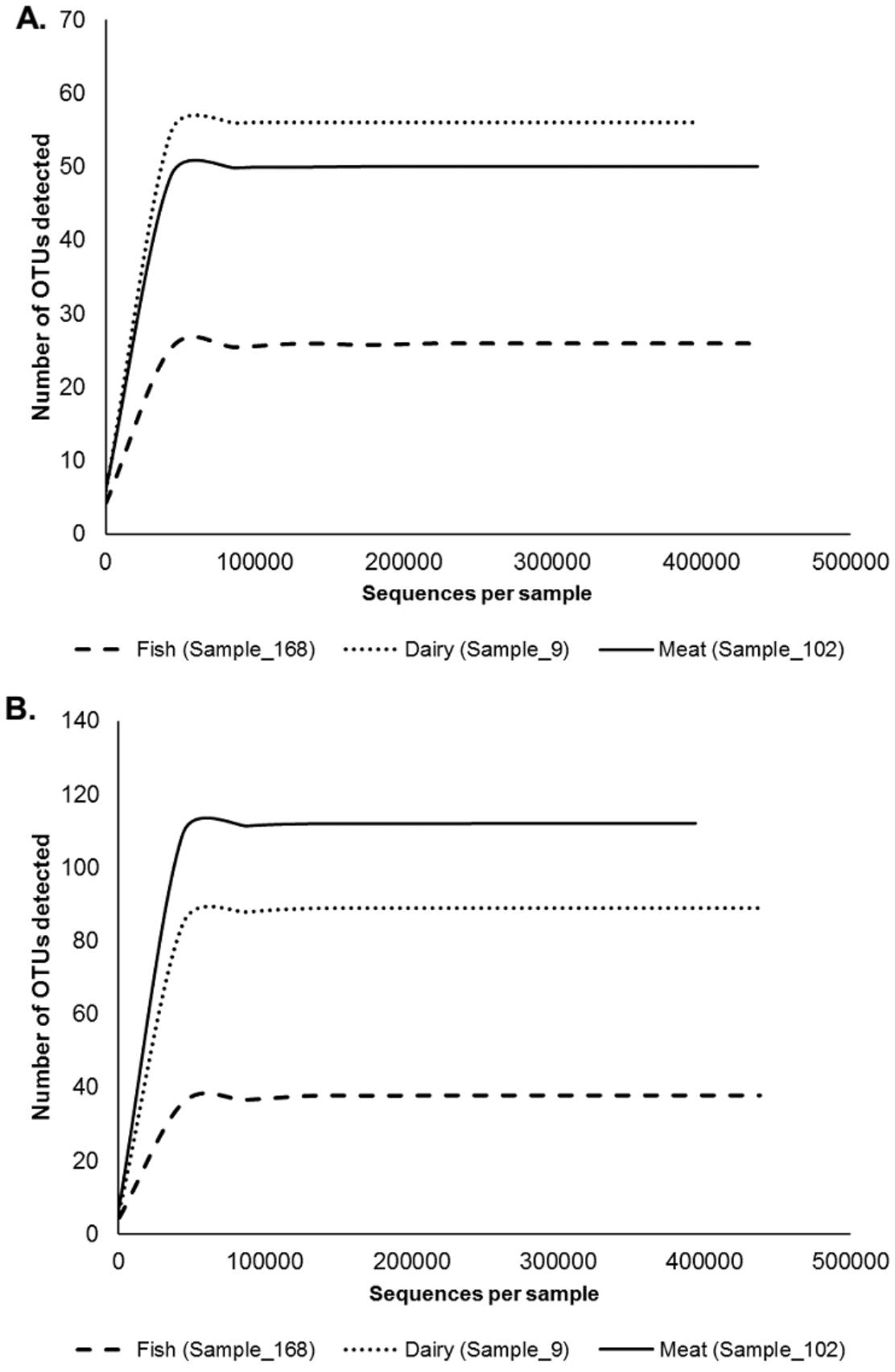
1008 Figure 3



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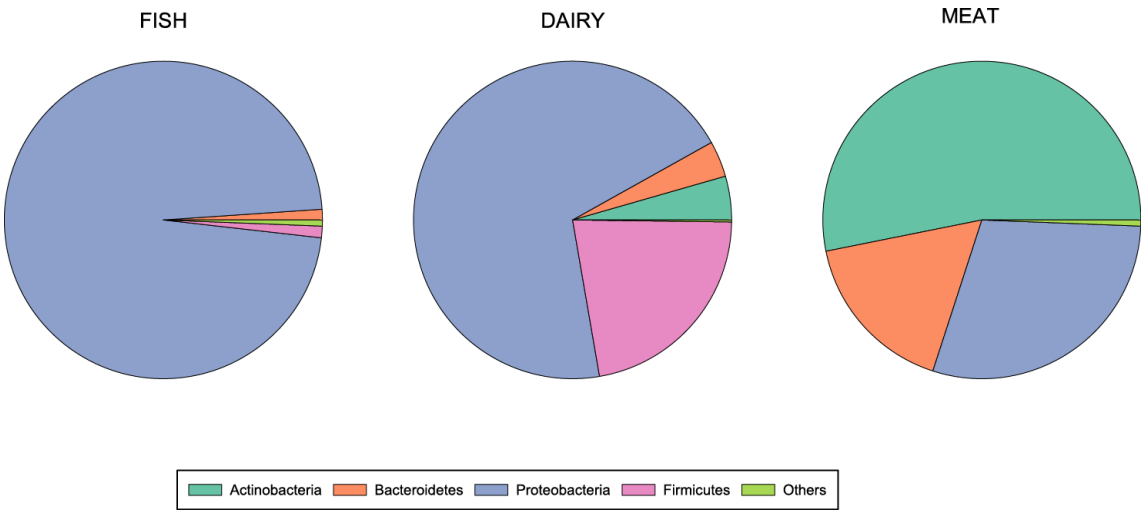
1010 Figure 4



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1012 Figure 5

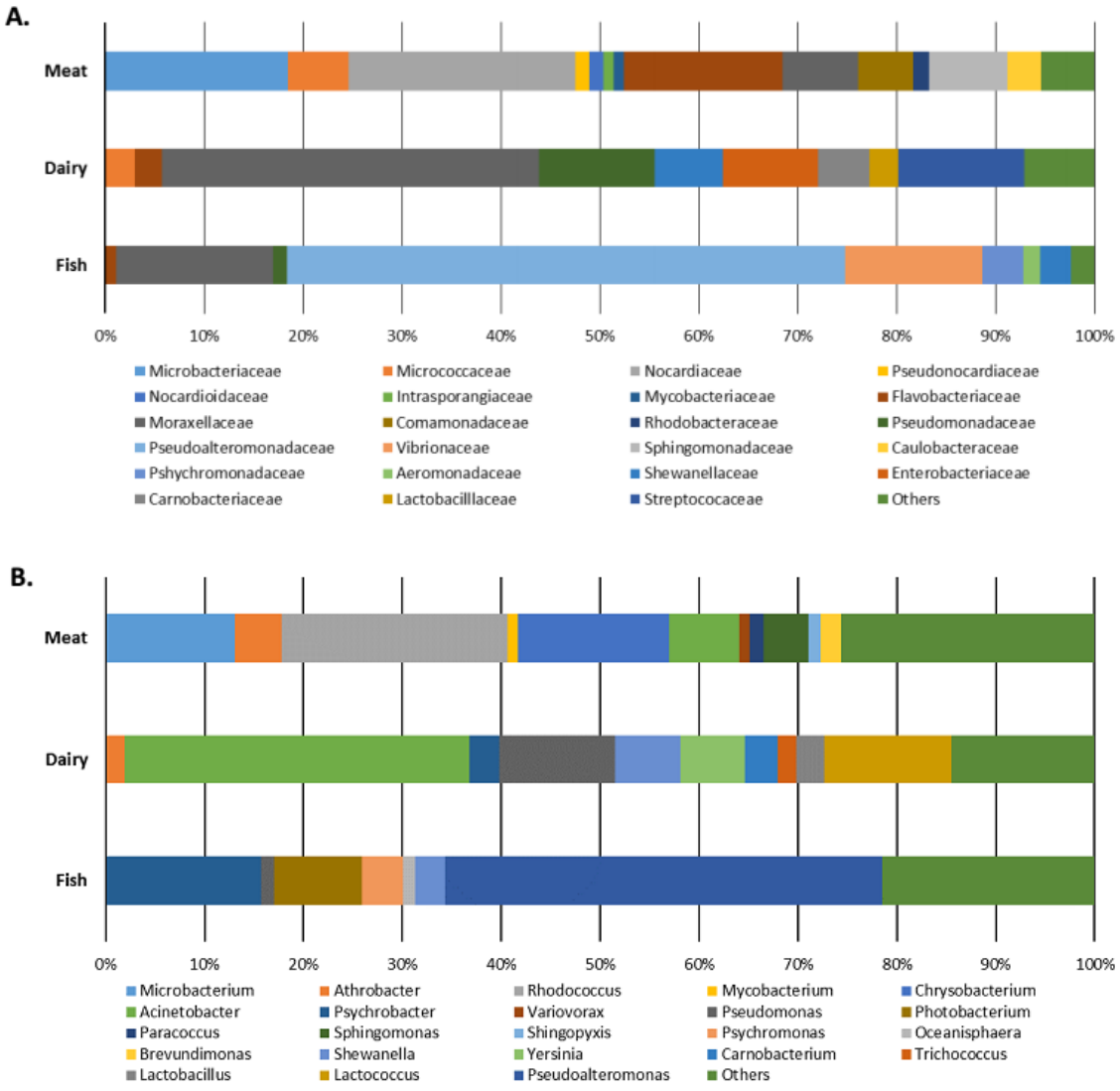

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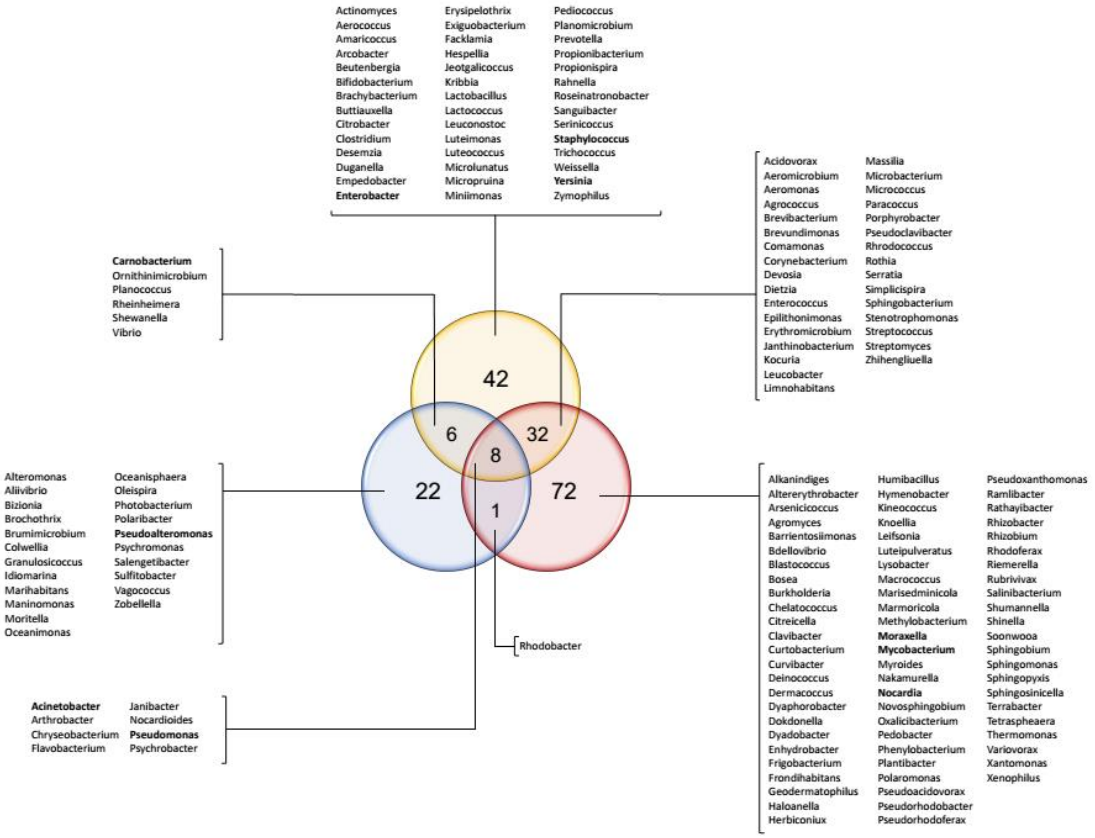
Figure 6


Actinomyces
Aerococcus
Amaricoccus
Arcobacter
Beutenbergia
Bifidobacterium
Brachybacterium
Buttiauxella
Citrobacter
Clostridium
Desemzia
Duganella
Empedobacter
Enterobacter
Erysipelothrix
Exiguobacterium
Facklamia
Hespellia
Jeotgalicoccus
Kribbia
Lactobacillus
Lactococcus
Leuconostoc
Luteimonas
Luteococcus
Microlunatus
Micropruina
Miniimonas
Pediococcus
Planomicrobium
Prevotella
Propionibacterium
Propionispira
Rahnella
Roseinatronobacter
Sanguibacter
Serinicoccus
Staphylococcus
Trichococcus
Weissella
Yersinia
Zymophilus
Carnobacterium
Ornithinimicrobium
Planococcus
Rheinheimera
Shewanella
Vibrio
Acidovorax
Aeromicrobium
Aeromonas
Agrococcus
Brevibacterium
Brevundimonas
Comamonas
Corynebacterium
Devosia
Dietzia
Enterococcus
Epilithonimonas
Erythromicrobium
Janthinobacterium
Kocuria
Leucobacter
Limnohabitans
Massilia
Microbacterium
Micrococcus
Paracoccus
Porphyrobacter
Pseudoclavibacter
Rhodococcus
Rothia
Serratia
Simplicispira
Sphingobacterium
Stenotrophomonas
Streptococcus
Streptomyces
Zhihengliuella
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Rhodobacter
Alteromonas
Aliivibrio
Bizionia
Brochothrix
Brumimicrobium
Colwellia
Granulosicoccus
Idiomarina
Marihabitans
Maninomonas
Moritella
Oceanimonas
Oceanisphaera
Oleispira
Photobacterium
Polaribacter
Pseudoalteromonas
Psychromonas
Salengetibacter
Sulfitobacter
Vagococcus
Zobellella
Acinetobacter
Arthrobacter
Chryseobacterium
Flavobacterium
Janibacter
Nocardioides
Pseudomonas
Psychrobacter
Alkanindiges
Altererythrobacter
Arsenicicoccus
Agromyces
Barrientosiimonas
Bdellovibrio
Blastococcus
Bosea
Burkholderia
Chelatococcus
Citreicella
Clavibacter
Curtobacterium
Curvibacter
Deinococcus
Dermacoccus
Dyaphorobacter
Dokdonella
Dyadobacter
Enhydrobacter
Frigobacterium
Frondihabitans
Geodermatophilus
Haloanella
Herbiconiux
Humibacillus
Hymenobacter
Kineococcus
Knoellia
Leifsonia
Luteipulveratus
Lysobacter
Macrococcus
Marisedminicola
Marmoricola
Methylobacterium
Moraxella
Mycobacterium
Myroides
Nakamurella
Nocardia
Novosphingobium
Oxalicibacterium
Pedobacter
Phenylobacterium
Plantibacter
Polaromonas
Pseudoacidovorax
Pseudorhodobacter
Pseudorhodoferax
Pseudoxanthomonas
Ramlibacter
Rathayibacter
Rhizobacter
Rhizobium
Rhodoferax
Riemerella
Rubrivivax
Salinibacterium
Shumannella
Shinella
Soonwooa
Sphingobium
Sphingomonas
Sphingopyxis
Sphingosinicella
Terrabacter
Tetraspheaera
Thermomonas
Variovorax
Xantomonas
Xenophilus

Table 1. Origins, dates, number of samples and number of *Listeria monocytogenes*-positive samples after enrichment obtained in this study.

Survey	Sector	Sort of factory	Date	n. of samples	*L. monocytogenes* positive samples
1	Dairy	Cheese producer	Feb 2017	40	2
2	Dairy	Cheese producer	Mar 2017	39	0
3	Meat	Abattoir	Apr 2017	40	20
4	Meat	Quartering and processing	Apr 2017	40	9
5	Fish	Fish market	Apr 2017	40	5
6	Fish	Fish processing	May 2017	40	4
7	Dairy	Milk farm	Jun 2017	40	0
8	Fish	Fish processing	Jul 2017	40	0
Total				319	40

Table 2. ALOA-positive samples determined by direct plating following ISO 11290-2 detected in the survey performed. Values of log CFU/cm^2 were considered as valid after 16S rRNA gene sequencing confirmation. (n.a.: not applicable)

Survey	Sector	Sample ID	Sector	Confirmed	log CFU/cm^2
1	Dairy	9	Dairy	-	n.a.
3	Meat	88	Meat	-	n.a.
		96	Meat	+	3.18
		97	Meat	+	2.00
		98	Meat	-	n.a.
		102	Meat	+	3.97
		103	Meat	-	n.a.
		107	Meat	+	2.04
4		121	Meat	+	2.00
		134	Meat	+	2.48
		149	Meat	+	2.00
5		168	Fish	+	3.36
6	Fish	225	Fish	+	2.30

Table 3. Alpha diversity indexes obtained in metagenetic analysis of *L. monocytogenes*-positive representative samples.

Sample ID	Family		Genus	
	Simpson	Shannon-Wiener	Simpson	Shannon-Wiener
Fish_168	0.633	2.078	0.661	2.251
Dairy_9	0.806	3.094	0.815	3.290
Meat_102	0.866	3.423	0.856	3.638