

The Complete Genome Sequence of *Listeria Monocytogenes* Strain S2542 and Expression of Selected Genes Under High-Pressure Processing

Ilhan Cem Duru (✉ ilhan.duru@helsinki.fi)

University of Helsinki: Helsingin Yliopisto <https://orcid.org/0000-0003-3409-5215>

Florentina Ionela Bucur

Dunarea de Jos University of Galati

Margarita Andreevskaya

University of Helsinki: Helsingin Yliopisto

Anne Ylinen

University of Helsinki: Helsingin Yliopisto

Peter Crauwels

Ulm University: Universitat Ulm

Leontina Grigore-Gurgu

Dunarea de Jos University of Galati

Bahareh Nikparvar

Norwegian University of Science and Technology Faculty of Engineering Science and Technology:
Norges teknisk-naturvitenskapelige universitet Fakultet for ingeniørvitenskap

Tone Mari Rode

Norwegian Institute of Food Fisheries and Aquaculture Research: Nofima AS

Pia Laine

University of Helsinki: Helsingin Yliopisto

Lars Paulin

University of Helsinki: Helsingin Yliopisto

Trond Løvdal

Norwegian Institute of Food Fisheries and Aquaculture Research: Nofima AS

Christian U. Riedel

Ulm University: Universitat Ulm

Nadav Bar

Norwegian University of Science and Technology Faculty of Engineering Science and Technology:
Norges teknisk-naturvitenskapelige universitet Fakultet for ingeniørvitenskap

Daniela Borda

Dunarea de Jos University of Galati

Anca Ioana Nicolau

Dunarea de Jos University of Galati

Petri Auvinen

University of Helsinki: Helsingin Yliopisto

Research note

Keywords: high-pressure processing, genome comparison, Listeria, food safety, gene expression, stress recovery, foodborne pathogen

Posted Date: March 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-322261/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Research Notes on April 15th, 2021. See the published version at <https://doi.org/10.1186/s13104-021-05555-2>.

Abstract

Objectives

The study aims to generate the whole genome sequence of *L. monocytogenes* strain S2542 and to compare it to the genomes of strains RO15 and ScottA. In addition, we aimed to compare gene expression profiles of *L. monocytogenes* strains S2542, ScottA and RO15 after high-pressure processing (HPP) using ddPCR.

Results

The whole genome sequence of *L. monocytogenes* S2542 indicates that this strain belongs to serotype 4b, in contrast to the previously reported serotype 1/2a. Strain S2542 appears to be more susceptible to the treatment at 400 MPa compared to RO15 and ScottA strains. In contrast to RO15 and ScottA strains, viable cell counts of strain S2542 were below the limit of detection after HPP (400 MPa/8 min) when stored at 8 °C for 24 and 48 h. The transcriptional response of all three strains to HPP was not significantly different.

Introduction

Listeriamonocytogenes is a Gram-positive foodborne bacterium that can cause severe infections in humans. Listeriosis, the associated disease, particularly affects individuals with compromised immune systems [1] and may lead to hospitalization and mortality rates of 20-30% [2]. Humans are generally infected following consumption of contaminated ready-to-eat (RTE) food products that do not undergo thermal treatment during the manufacturing process or are contaminated post-thermal treatment. *L. monocytogenes* can thrive in a range of inhospitable environmental conditions including low temperatures thus causing significant challenge to the food industry [3, 4, 5, 6].

Recently, we have studied the transcriptional response of *L. monocytogenes* strains RO15 and ScottA to HPP by RNA-seq [7, 8]. We observed that our previous gene expression results [8] are negatively correlated with the results of a previous study on HPP-induced changes in gene expression of *L. monocytogenes* strain S2542 [9]. Thus, our aim was to make a draft assembly of the genome of the strain S2542 (obtained from Tasmanian Institute of Agricultural Research) and to compare the genome sequences of these three strains.

Based on the conflicting results regarding the HPP-induced changes in gene expression, we also performed a new set of HPP experiments under the same conditions as in our previous study [8] with all the three strains, and analyzed the transcriptional response of a number of representative genes.

Methods

***L. monocytogenes* strain S2542 DNA isolation, library preparation, sequencing, assembly, and comparative genomics**

Genomic DNA of *L. monocytogenes* strain S2542 was isolated as described previously [7]. A library for sequencing of the genome was generated using Nextera XT kit according to the instructions from the manufacturer (Illumina, San Diego, CA, USA). The obtained library was paired-end sequenced on a MiSeq platform using a 600 cycle sequencing kit v3 (Illumina).

Quality trimming and nextera adapter removal was done using Cutadapt v1.14 [10] with -q 25 and -m 50 options. Trimmed reads were assembled using SPades v3.13.0 [11] with default options. Prokka v1.13.3 [12] was used for functional annotation. A secondary functional annotation was done using PANNZER2 [13]. Serotype of the strain S2542 was predicted by aligning the serotype marker primers [14] to the genome using EMBOSS primersearch v 6.6.0 [15]. Multilocus sequence typing based on 7 loci (MLST), clonal complex (CC), and lineage was predicted by uploading the genome to BIGSdb-Lm webserver (<https://bigsdb.pasteur.fr/listeria/listeria.html>) [16]. Multiple genome alignment for strains RO15, ScottA and S2542 was performed using Mauve v2.4.0 [17]. Blast Average Nucleotide Identity (ANIb) analysis between strains was done using JSpeciesWS webtool [18]. Core genome alignment was done using Roary v3.12.0 [19] with the “-mafft” option for the same strains that we used previously [7]. Phylogenetic tree was created from the alignment using FastTree v2.1.11 [20].

HPP experiments

HPP experiments were conducted in order to compare the gene expression profiles of RO15, ScottA and S2542 strains. The strains were cultivated as described previously [8] and pressurized at 400 MPa, 8°C for 8 min. Viability of *L. monocytogenes* cells was determined after storage of the samples at 8°C for 0 min, 24 h and 48 h by performing serial decimal dilutions of both treated samples and corresponding controls in phosphate buffered-saline solution (PBS; Sigma Aldrich, St. Louis, SUA; pH 7.4) and plating dilutions on BHI (brain heart infusion) agar plate. Colony forming units were counted after incubation of the plates at 37°C for 48 h.

RNA extraction

RNA was extracted from HPP-treated samples and the controls with NucleoSpin RNA kit (Macherey-Nagel, Düren, Germany) as described previously [7].

ddPCR

ddPCR was used to compare gene expression levels between strains RO15, ScottA, and S2542. Three replicate samples for each treatment and strain, and their corresponding control samples were analyzed. Expression levels of seven genes (*recG*, *fusA*, *clpE*, *hly*, *agrB*, *ftsE*, and *mscL*) were quantified using strains

RO15, ScottA, and S2542 samples treated with 400 MPa and recovered for 0 h or 24 h. Primers (Table S1) were designed using Primer3Plus [21] and manufactured by Integrated DNA Technologies. The protocol used, including gDNA removal and RT-PCR steps, was performed as described previously [22]. To be able to compare expression levels of different samples, expression of the target genes (cDNA copies/ μ l) was normalized using concentrations of two stably expressed genes (*recG* and *fusA*). To allow comparison to our previously published RNA-Seq data, the results were expressed as \log_2 (gene concentration in treated sample/gene concentration in control sample) values.

Results And Discussion

Genome assembly of *L. monocytogenes* strain S2542

We sequenced and assembled the genome of strain S2542 and compared it with the genomes of the previously studied strains; RO15 and ScottA. Assembly and annotation resulted in a 2.9 Mbp genome consisting of 14 contigs (N50=477482 bp) with 2839 predicted coding sequence (CDS). GC-content of strain S2542 was 37.9%, which is identical to the GC-content of ScottA. Based on Blast Average Nucleotide Identity (ANIb), the genome of strain S2542 was more similar to ScottA than to RO15 (ANIb scores S2542/ScottA: 99.97, S2542/RO15: 94.55). Genome alignment of strains RO15, ScottA, and S2542 indicated high similarities between the genomes. Ortholog gene prediction between three strains revealed that 31 genes were present in S2542 but absent in strains ScottA and RO15 (Table S1). Most of the genes specific to strain S2542 were annotated to encode hypothetical proteins (Table S1). In total, 49 genes were found in both ScottA and RO15 but not in S2542, and 47 of these were prophage genes (Table S1). Thus, the differences in genome sequence most likely do not explain the differences in gene expression response to HPP described in our previous study on RO15 and ScottA and study presented for strain S2542 [8,9].

Based on PHASTER prophage prediction, two small regions in S2542 (Contig1:183471-206364: 22.8 kb; Contig2: 99648-114057: 14.4 kb) were annotated as a prophage with a low confidence score. Homolog of these regions were seen in both strain RO15 and ScottA. We did not observe CRISPR-Cas or anti-CRISPR genes within the strain S2542 genome.

Based on genome sequence analysis using BIGSdb [16], multilocus sequence typing (based on seven loci) assigned strain S2542 to ST-145. In addition, based on genome sequence, the clonal complex (CC) of strain S2542 was CC2 and it belongs to the Lineage I, as ScottA. Serotype prediction based on the genome suggested that strain S2542 belongs to PCR-serogroup 4b. By contrast S2542 was reported to belong to serogroup 1/2a based on an antigen test [9]. Pan-genome analysis showed that strain S2542 harboured similar genes as serotype 4b strains (Figure 1a). Core genome alignment tree visualization also supported assignment of S2542 to serotype 4b, since two clear clusters were observed for serotype 4b strains (F2365, ScottA, S2542) and serotype 1/2a strains (2HF33, RO4, RO15, MB5, C7, EGD-e, AB199, AB120) (Figure 1a.)

Viable cell count after HPP

To assess cell recovery capacity after HPP, all three strains were treated at 400 MPa and 8°C for 8 min and colony forming units (CFU) were enumerated immediately after treatment and after storage for 24 and 48 h at 8 °C (Figure 1b). For all three strains CFU/ml were higher for the samples plated immediately after HPP (t = 0 min) compared to the samples stored for 24 and 48 h. Also, the reduction in CFU/ml at t = 0 min after HPP compared to untreated controls was significantly higher for S2542 than for ScottA and RO15. Moreover, after 24h and 48 of storage no viable cells could be detected for S2542, i.e. CFU/ml for all replicates of the high pressure treated samples were below the limit of detection, whereas for ScottA and RO15 CFU were detected at least for some of the replicates at these timepoints.

HPP-induced changes in expression of representative genes

In order to investigate the contradictory results of HPP-induced gene expression between the Bowman et al. [9] study and our previous results [8], a number of genes that showed strong negative correlation between the two studies were selected and their expression levels were analysed by ddPCR approach. In contrast, we did not observe any difference in the HPP-induced expression changes of the selected genes between these strains. Furthermore, gene expression changes at 400 MPa after 24 h for strains ScottA and S2542 were significantly correlated (Pearson Correlation; $r = 0.97$) (Table 1). This indicates that the gene expression responses of strains S2542 and ScottA are similar in these experimental conditions. No significant differences were observed between RO15 and S2542 (Table 1). Thus, expression profiles of the selected genes in response to HPP appear to be not significantly different between the strains. The contradictory results between our previous RNA-seq study [8] and the microarray and qPCR data reported by Bowman et al. [9] are difficult to explain, but could be potentially explained by the different experimental conditions (8°C-8 min HPP exposure in Duru et al [8] and 15°C-5 min HPP exposure in Bowman et al [9]) or difference of the growth of the bacteria during the experiments.

Conclusion

In conclusion, we provide the whole draft genome sequence of *Listeriamonocytogenes* strain S2542. The genome sequence revealed that strain S2542 belongs to serotype 4b, although it has previously been reported to belong to serotype 1/2a. Analysis of viable cell count at different timepoints after HPP suggests that S2542 is more sensitive to HPP than ScottA or RO15. Previous results of two studies on HPP-induced gene expression in S2542 on the one hand and RO15 and ScottA on the other hand were in disagreement [8, 9]. Here, we conducted transcriptional analysis comparing the HPP-induced changes of all three strains directly. The results suggest that despite differences in sensitivity and recovery from HPP, none of the strains respond significantly differently to HPP, at least under the conditions tested and for the genes analyzed.

Limitations

In the present study, the transcriptional response of five different genes were compared between three different strains of *L. monocytogenes*. Although genes were carefully selected and representative for two previous studies, it is possible that the global gene expression response of S2542 may still be different than that of RO15 and/or ScottA.

Abbreviations

HPP: high pressure processing, ddPCR: digital droplet PCR, MLST: Multilocus sequence typing, CC: Clonal complex

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All sequencing data and assembled genome have been deposited in the European Nucleotide Archive (ENA) under accession code PRJEB42816. For pan-genome analysis, we publicly available genome assemblies of *L. monocytogenes* strain ScottA

(https://www.ncbi.nlm.nih.gov/assembly/GCF_000212455.1), strain RO15

(https://www.ncbi.nlm.nih.gov/assembly/GCF_902827145.1), strain EGDe

(https://www.ncbi.nlm.nih.gov/assembly/GCF_000196035.1), strain 2HF33

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902838845.1), strain C7

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902837585.1), strain MB5

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902838455.1), strain RO4

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902833935.1), strain AB120

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902837535.1), strain AB199

(https://www.ncbi.nlm.nih.gov/assembly/GCA_902837525.1), and strain F2365

(https://www.ncbi.nlm.nih.gov/assembly/GCF_000008285.1) from NCBI database.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported within the ERA-IB2 consortium “SafeFood” (ID: ERA-IB-16- 247 014) by grants from Academy of Finland to PA (307856 and 311717), by a grant of the Executive Agency for Higher Education, Research, Development and Innovation Funding in Romania to AIN (International and European Cooperation – 250 Subprogramme 3.2 – Horizon 2020 – Contract No. 15/2017) and by a grant of the German Ministry for Education and Research to CUR (Grant No: 031B0268). ICD supported by the MBDP doctoral program. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Authors' contributions

PA, AIN, DB, TL, CUR, and NB conceived and designed the study. TMR, LG, TL, FIB, LGG, BN, AIN, and DB collected the samples and performed the pressure treatments and viable cell count. ICD, MA, and PL performed the bioinformatics analyses. AY performed RNA extraction and ddPCR. LP organized NGS assays. PC extracted DNA. ICD and PA drafted the manuscript. All authors have read, commented, and approved the final manuscript.

Acknowledgements

We want to thank Kirsi Lipponen, Eeva-Marja Turkki and Paula Collin for performing the NGS procedures for this project. We wish to acknowledge CSC – IT Center for Science, Finland, for computational resources.

References

1. EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, et al. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA J.* 2018;16:e05134.
2. Bucur FI, Grigore-Gurgu L, Crauwels P, Riedel CU, Nicolau AI. Resistance of *Listeria monocytogenes* to Stress Conditions Encountered in Food and Food Processing Environments. *Front Microbiol.* 2018;9. doi:10.3389/fmicb.2018.02700.
3. Gahan CGM, Hill C. *Listeria monocytogenes*: survival and adaptation in the gastrointestinal tract. *Front Cell Infect Microbiol.* 2014;4. doi:10.3389/fcimb.2014.00009.
4. NicAogáin K, O'Byrne CP. The Role of Stress and Stress Adaptations in Determining the Fate of the Bacterial Pathogen *Listeria monocytogenes* in the Food Chain. *Front Microbiol.* 2016;7. doi:10.3389/fmicb.2016.01865.
5. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments.

- Food Control. 2017;75:1–13.
6. Chan YC, Wiedmann M. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. *Crit Rev Food Sci Nutr*. 2009;49:237–53.
 7. Duru IC, Andreevskaya M, Laine P, Rode TM, Ylinen A, Løvdal T, et al. Genomic characterization of the most barotolerant *Listeria monocytogenes* RO15 strain compared to reference strains used to evaluate food high pressure processing. *BMC Genomics*. 2020;21:455.
 8. Duru IC, Bucur FI, Andreevskaya M, Nikparvar B, Ylinen A, Grigore-Gurgu L, et al. High-pressure processing-induced transcriptome response during recovery of *Listeria monocytogenes*. *BMC Genomics*. 2021;22:117.
 9. Bowman JP, Bittencourt CR, Ross T. Differential gene expression of *Listeria monocytogenes* during high hydrostatic pressure processing. *Microbiology*. 2008;154 Pt 2:462–75.
 10. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*. 2011;17:10–2.
 11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 2012;19:455–77.
 12. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30:2068–9.
 13. Törönen P, Medlar A, Holm L. PANNZER2: a rapid functional annotation web server. *Nucleic Acids Res*. 2018;46:W84–8.
 14. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the Major *Listeria monocytogenes* Serovars by Multiplex PCR. *J Clin Microbiol*. 2004;42:3819–22.
 15. Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet*. 2000;16:276–7.
 16. Moura A, Criscuolo A, Pouseele H, Maury MM, Leclercq A, Tarr C, et al. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nat Microbiol*. 2016;2:1–10.
 17. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS ONE*. 2010;5:e111147.
 18. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics*. 2016;32:929–31.
 19. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics*. 2015;31:3691–3.
 20. Price MN, Dehal PS, Arkin AP. FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLOS ONE*. 2010;5:e9490.
 21. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Res*. 2007;35 Web Server issue:W71-74.
 22. Andreevskaya M, Jääskeläinen E, Johansson P, Ylinen A, Paulin L, Björkroth J, et al. Food Spoilage-Associated *Leuconostoc*, *Lactococcus*, and *Lactobacillus* Species Display Different Survival

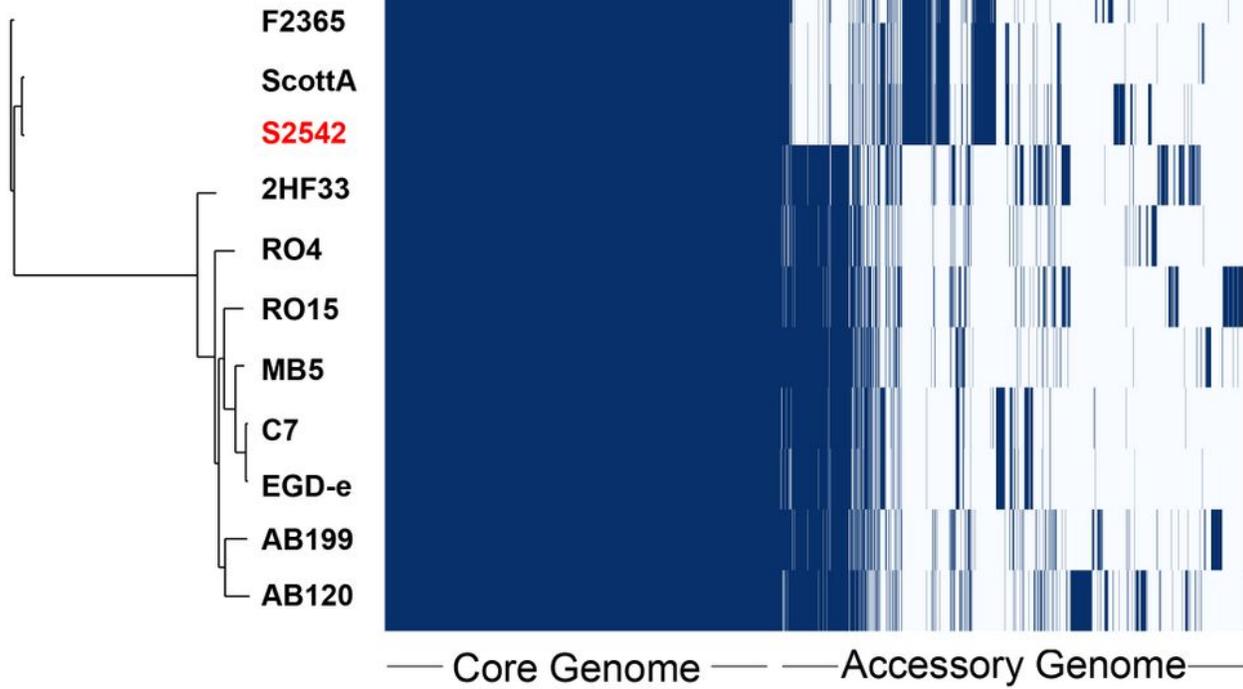
Tables

Table 1. Comparison of two studies [8,9] and ddPCR-based expression changes for strain R015, ScottA and S2542. a) \log_2 fold change results of the two different studies [8,9] with samples treated at 400 MPa and correlation of the RNAseq results to the DNA microarray results. b) ddPCR \log_2 fold change results at 0 min after 400 MPa treatment comparison with data from strains R015, ScottA and S2542 showing the correlation of the gene expression between them. c) ddPCR \log_2 fold change results at 24 hours after 400 MPa treatment with data from strains R015, ScottA, and S2542 showing the correlation of the gene expression between them.

a) log₂ fold change of the indicated gene after HPP at 400 MPa as determined by RNA-seq for RO15 and ScottA (Duru et al. [8]) or microarray for S2542 (Bowman et al. [9])			
Gene	RO15 (RNA-seq, Duru et al. [8]) log ₂ fold change	ScottA (RNA-seq, Duru et al. [8]) log ₂ fold change	S2542 (microarray, Bowman et al [9]) log ₂ fold change
agrB	-0.35	-0.15	2.84
hly	0.89	2.57	-4.34
ftsE	-1.11	-0.66	2.71
clpE	2.26	2.27	-3.93
mscL	0.24	0.33	1.04
Pearson correlation with S2542	-0.87	-0.99	
b) log₂ fold change of the indicated gene immediately after HPP at 400 MPa as determined by ddPCR for all three strains (This study)			
Gene/Strain	RO15	ScottA	S2542
agrB	-0.10	-0.16	-0.32
hly	-0.02	0.34	0.36
ftsE	-0.30	-0.06	0.20
clpE	0.27	0.27	0.57
mscL	0.01	-0.73	0.83
Pearson correlation with S2542	0.49	-0.24	
c) log₂ fold change of the indicated gene immediately after HPP at 400 MPa and storage for 24 hours as determined by ddPCR for all three strains (This study)			
Gene/Strain	RO15	ScottA	S2542
agrB	0.36	0.09	0.59
hly	-0.06	0.08	0.45
ftsE	0.02	0.60	1.78
clpE	0.28	0.21	0.35
mscL	0.57	0.82	2.30
Pearson correlation with	0.38	0.97	

Figures

a) Phylogenetic tree



b)

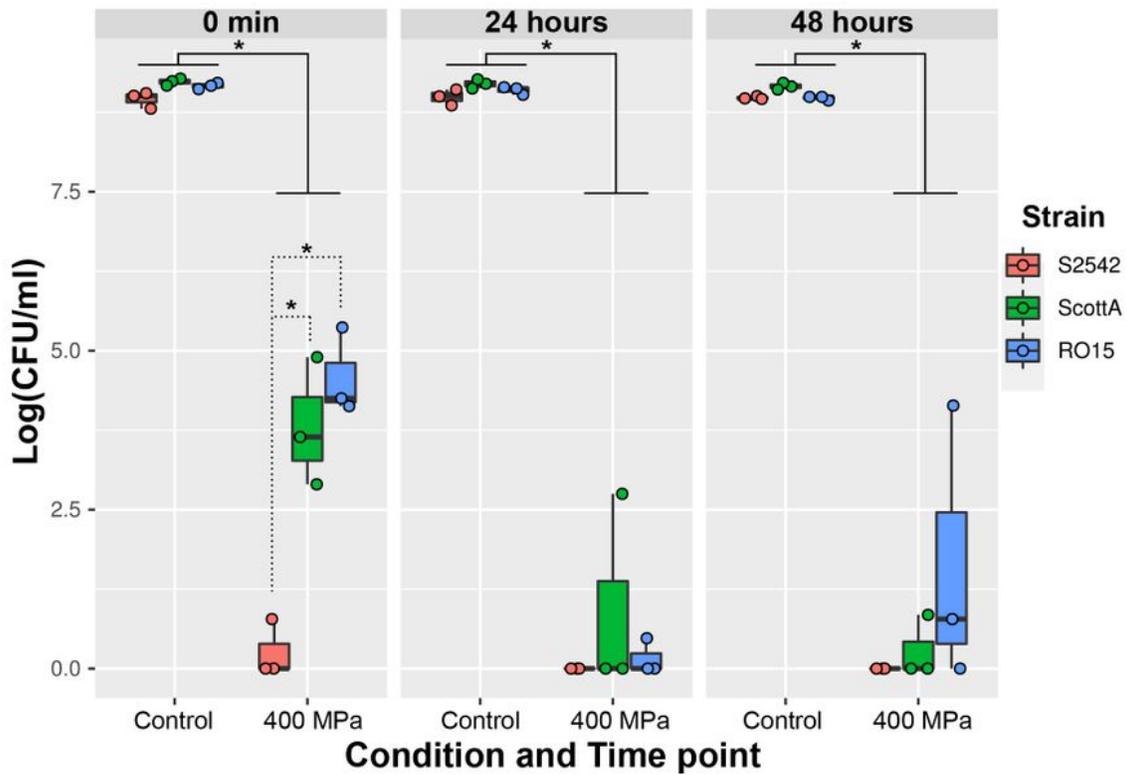


Figure 1

a) Pan-genome visualization of selected strains, b) viability (Log(CFU/ml)) of *L. monocytogenes* strain S2542, ScottA, and RO15 before and after the pressure treatment 400 MPa, 8 min. a) Phylogenetic tree have been calculated using the core genomes of the indicated strains. *L. monocytogenes* strain names were shown at the end of the phylogenetic tree branch. The matrix shows presence (blue) and absence (white) of the core and strain-specific genes. b) Effect of HPP on cell survival. 0 min represents samples plated on agar just after pressure treatment, 24 hours and 48 hours represent samples that were allowed to recover for 24 hours and 48 hours at 8°C and plated on agar. Strain S2542 is presented in red, ScottA in green, and RO15 in blue. For each experimental group individual values of biological triplicates (HPP of independent bacterial cultures) and box-and-whiskers with median and 25th-75th percentile are shown. Data was analysed by pairwise Student's t-test and differences between groups were considered statistically significant at p-values < 0.05 (indicated by *).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AdditionalFile1SupplementaryTableS1.xlsx](#)