Supporting figures and tables

Table of Contents

Figure S1. An incorrectly predicted pseudogene in strain ScottA.

Protein evidence for a 207-amino acid protein (3 peptides, 17 PSMs) predicted by Prodigal in L. monocytogenes strain ScottA. It starts from an alternative start codon TTG, which codes for leucine in frame -1; the corresponding longer RefSeq protein (282 amino acids) incorrectly annotated as a pseudogene. Both proteins are predicted to encode for phosphosugar binding transcriptional regulators, and both harbor the RpiR-like SIS (sugar isomerase) protein domains (IPR035472).

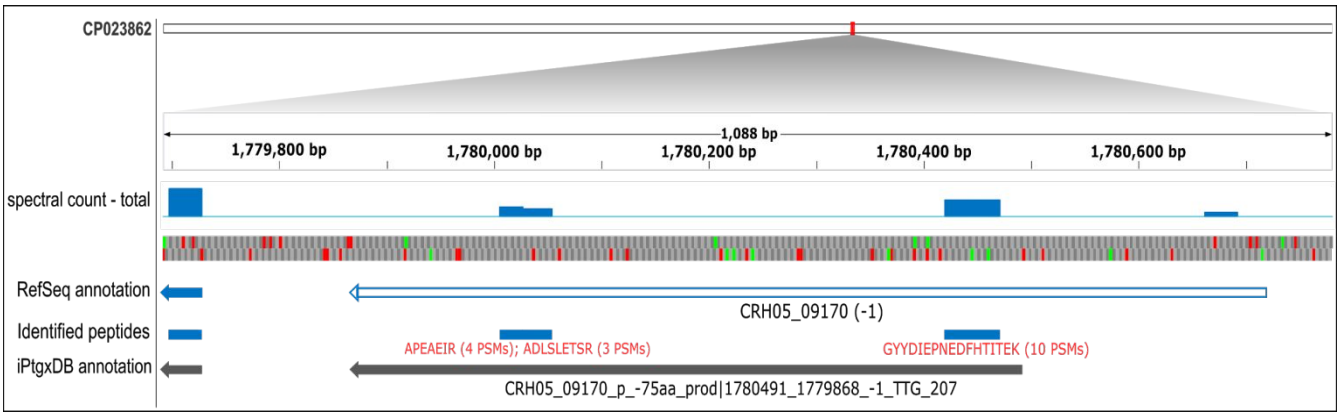
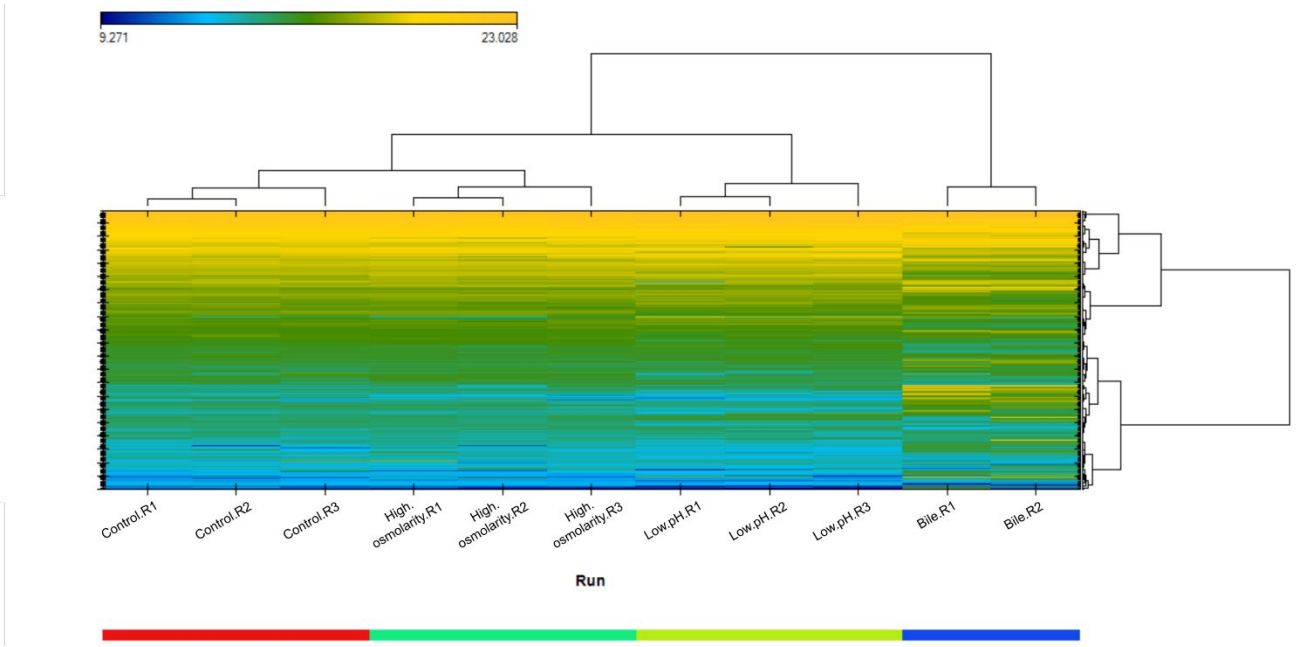


Figure S2. Hierarchical cluster analysis of biological replicates of EGD-e and ScottA.

(A) Unsupervised clustering reveals a high similarity for EGD-e biological replicates across all four conditions. (B) Unsupervised clustering reveals a high similarity for ScottA biological replicates across all four conditions.

A. EGD-e



B. ScottA

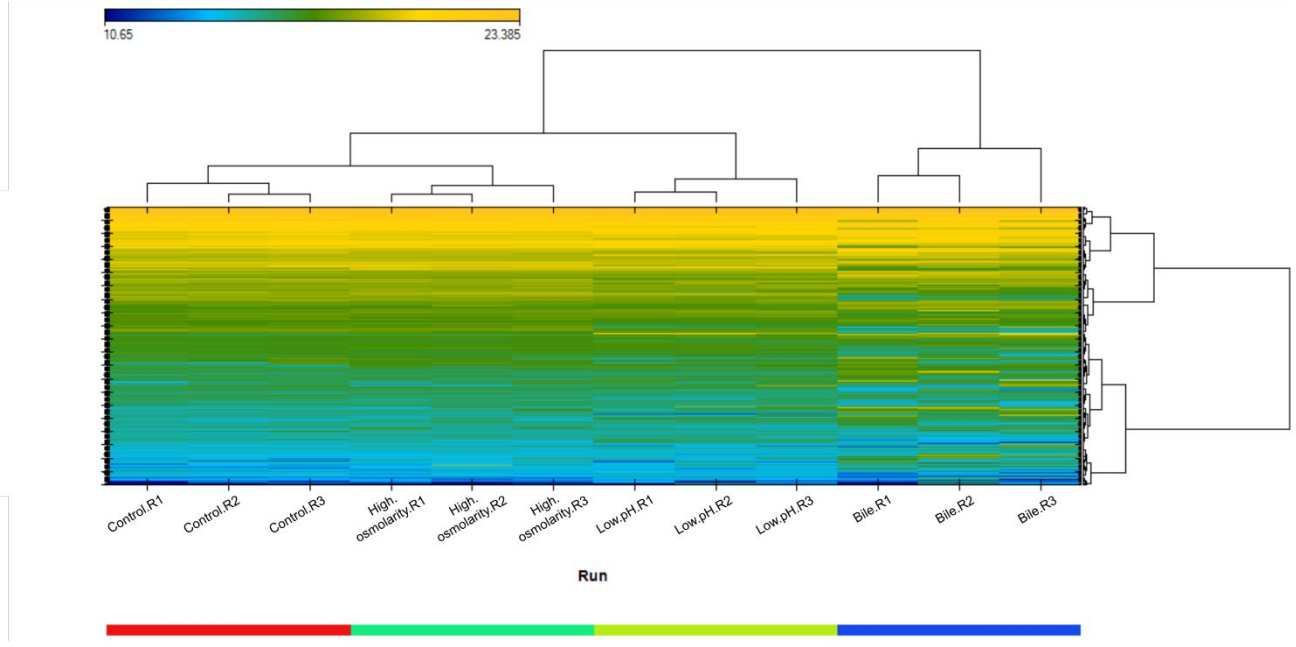


Table S1. Bacterial strains.

Strain no.	Name	Isolate type	Serotype
NF-L101	EGD-e	Virulent laboratory strain (guinea pig)*	1/2a
NF-L725	ScottA	Virulent laboratory strain from a clinical isolate (foodborne)**	4b

Source of strains: * Mackaness GB. The immunological basis of acquired cellular resistance. J Exp Med. 1964; 120:105-120. Glaser P, et al. Comparative genomics of *Listeria* species. Science. 2001;294: 849–852. ** Fleming DW, et al. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med. 1985;312: 404-407. Briers Y, et al. Genome sequence of *Listeria monocytogenes* ScottA, a clinical isolate from a food-borne listeriosis outbreak. J Bacteriol. 2011;193: 4284–4285.

Table S2. Overview of genome properties of strains EGD-e and ScottA.

	***L. monocytogenes* EGD-e**	***L. monocytogenes* ScottA**
Genbank accession #	CP023861	CP023862
# Chromosomes	1	1
Size of the chromosome (bp)	2,944,523	3,030,813
G+C content (%)	37.9	37.9
Coverage (PacBio)	260	283
Coverage (Illumina MiSeq)	302	435
Total number of protein-coding genes	2,887	2,979
Number of rRNA operons (16S-23S-5S)	6	6
Number of tRNA genes	67	67
Number of pseudogenes	29	30
Prophages	2 (probable)	2 (intact) + 1 (probable)

Table S3. Overview of core genes and genes specific to ScottA and to EGD-e.

The subsets of core and strain-specific protein-coding genes are listed for both strains (see separate Excel table). Detailed functional annotation is provided. The 14 genes that were missing in the NCBI reference sequence of ScottA (CM001159.1) are highlighted in orange. Strain-specific genes that are represented in the spectral libraries are marked "TRUE" in the column "present in spectral_library".

Table S4. Gene Ontology (GO) categories enriched among the strain-specific and differentially expressed proteins of each strain.

See separate excel table.

Table S5. Master table.

The list of all protein-coding genes in the *de novo* assembled strains, their functional annotations (NCBI and additional functional annotations; see Materials and Methods), proteomics expression evidence obtained using MS-GF+ after filtering for PSM level FDR of 0.05% (protein level FDR 1%), as well as the results of a reciprocal best BLAST hit analyses with the ListiList strain EGD-e (facilitating integration with other datasets) and its corresponding Uniprot accession are shown in a separate Excel file.

Table S6. Summary of annotation clusters in the iPtgxDBs.

A. EGD-e (65,393 proteins)

Annotation source (prefix)	# total CDS	# total clusters	# total new clusters	# total new reductions	# total new extensions	# total clusters	# total ids
RefSeq (refseq)	2,919	2,919	2,919	0	0	2,919	2,919
Prodigal (prod)	2,877	2,877	34	80	84	2,953	3,117
In silico ORFs (orf)	67,980	48,406	45,466	31	18,847	48,419	67,461*

*Excluded were 1,989 proteins smaller than 6 amino acids; 29 entries were annotated as pseudogene by both RefSeq and Prodigal annotation sources and hence excluded from the iPtgxDB; 50 shorter entries had indistinguishable internal start sites and were also excluded from the iPtgxDB.

B. ScottA (67,150 proteins)

Annotation source (prefix)	# total CDS	# total clusters	# total new clusters	# total new reductions	# total new extensions	# total clusters	# total ids
RefSeq (refseq)	3,010	3,010	3,010	0	0	3,010	3,010
Prodigal (prod)	2,970	2,970	48	75	89	3,058	3,222
In silico ORFs (orf)	69,931	49,726	46,677	23	19,433	49,735	69,355*

*Excluded were 2,122 proteins smaller than 6 amino acids; 30 entries were annotated as pseudogene by both RefSeq and Prodigal annotation sources and hence excluded from the iPtgxDB; 53 shorter entries had indistinguishable internal start sites and were also excluded from the iPtgxDB.

Table S7. Spectral evidence for the novelties identified through the proteogenomics search using MS-GF+ of DDA data for *L. monocytogenes* strains EGD-e and ScottA.

Annotated mass spectra and matching scores derived from Proteome Discoverer v2.4 (MS Amanda search engine) for peptides supporting novel proteins and short ORFs are provided. See separate Excel table.

Table S8: Summary information of precursors, peptides, and protein groups identified for EGD-e and ScottA over all conditions using DIA.

	EGD-e	ScottA
Precursors	22,993	25,585
Peptides	16,725	16,635
Modified Peptides	19,002	21,169
Protein Groups	1,708	1,876
Precursor recovery from library [%]	90.8	97.5

Table S9. Summary of library recovery percentage, data completeness, and median CVs for the DIA dataset. Part of this dataset is also shown in Fig 6.

	EGD-e				ScottA			
Condition	**Control**	**Low pH**	**High osmolarity**	**Bile salts**	**Control**	**Low pH**	**High osmolarity**	**Bile salts**
Data completeness	85%	84%	81%	35%	91%	80%	87%	51%
Library recovery	85%	85%	82%	36%	95%	88%	92%	65%
Median CVs	17%	19%	17%	20%	20%	21%	17%	81%

Table S10. List of differentially abundant proteins.

Proteins identified as differentially abundant in the bile, low pH, and high osmolarity conditions for both strains. Strain-specific genes are highlighted in orange. See separate Excel table.

Table S11. Candidate genes for bile resistance and operons for flagellar genes.

List of *Listeria* genes previously shown to be involved in bile resistance and genes that are part of two operons with flagellar-related and motility genes that flank the *flaA* gene (Bécavin C, et al. Comparison of widely used *Listeria monocytogenes* strains EGD, 10403S, and EGD-e highlights genomic variations underlying differences in pathogenicity. MBio. 2014;5: e00969–14). See separate Excel table.