



# New insights into the persistence of *Listeria monocytogenes* utilising high throughput proteomics

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## Why is *Listeria monocytogenes* of interest?

- extant economic cost
- outbreaks are inherently unpredictable
- reported rises of listeriosis rates in the EU

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### 11 deaths confirmed, six under investigation in listeriosis outbreak

Eleven deaths have now been linked to the outbreak of listeria, Canadian health officials said Sunday.

BY CAIRWEST NEWS SERVICE AUGUST 31, 2008

STORY PHOTOS (1)

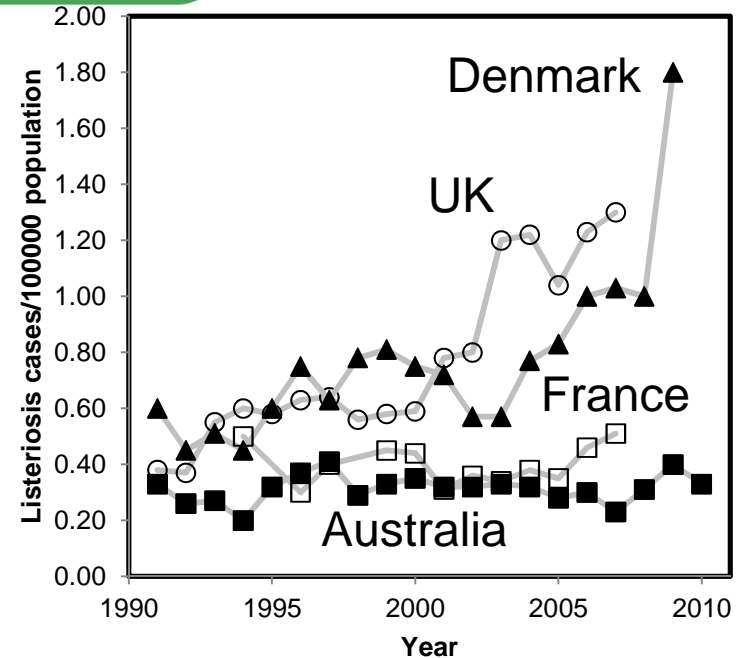


OTTAWA — Eleven deaths have now been linked to the outbreak of listeria, Canadian health officials said Sunday.

At a news conference, officials from Public Health Agency Canada said they have now officially attributed 11 deaths — nine in Ontario, one in B.C. and one in Alberta — to the outbreak

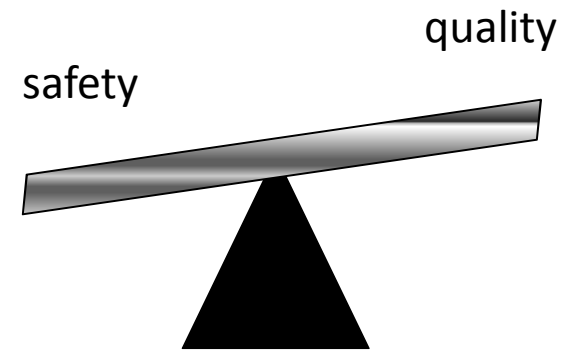
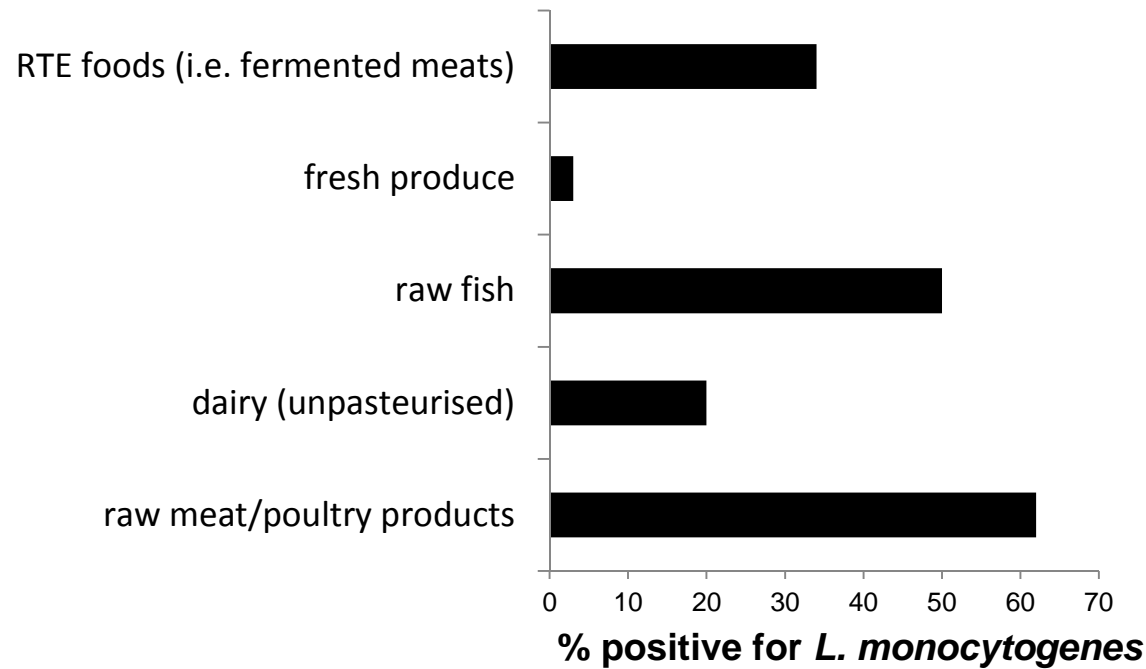
MORE ON THIS STORY

- List of recalled products
- King Bean sandwiches recalled
- Alberta links death to listeria
- More articles on listeriosis

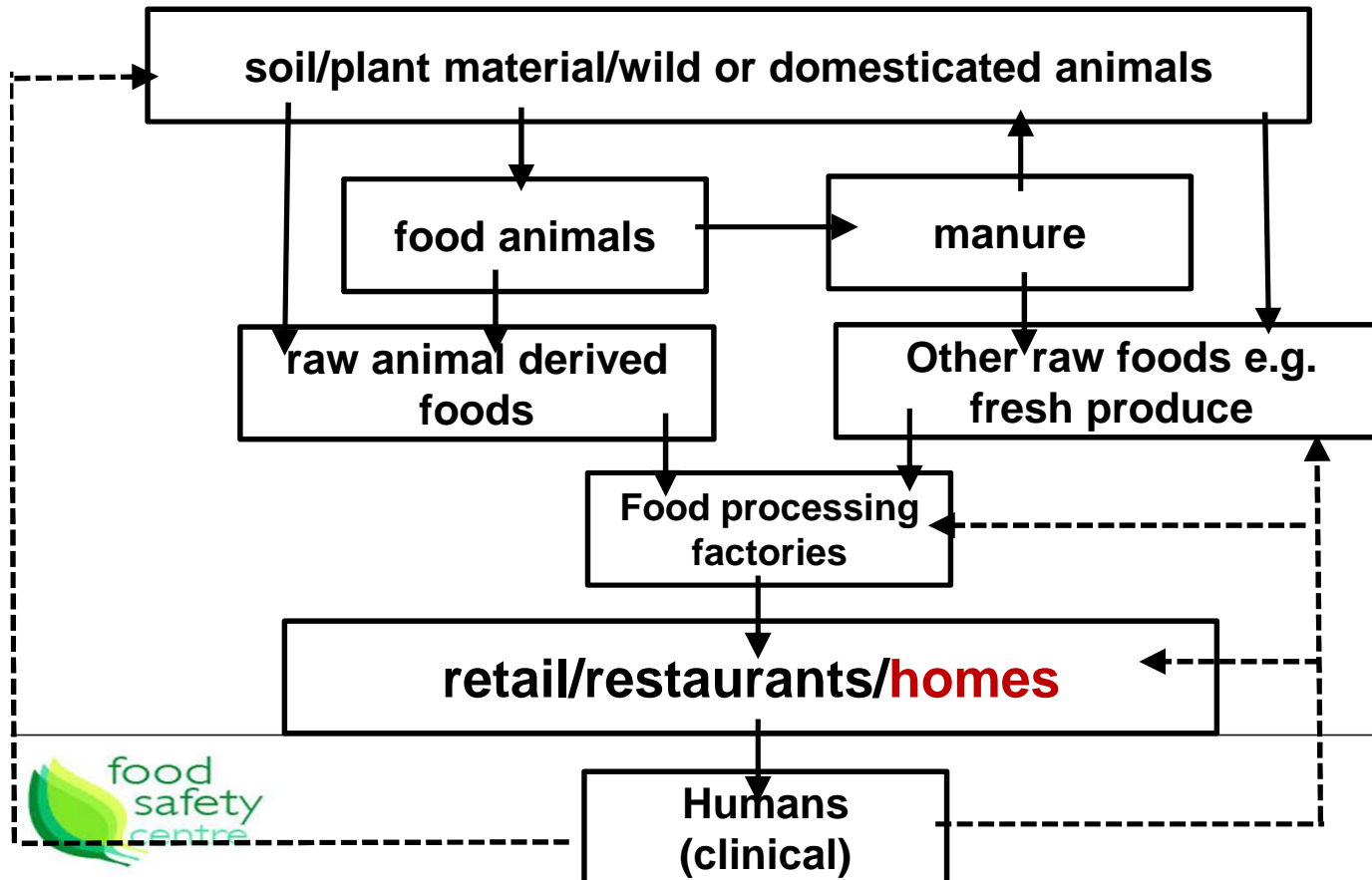


Economic benefit in **keeping** food safe from *L. monocytogenes* estimate >\$2.3-23 billion (USA) (Ivanek et al. 2004)

## A compromise between safety and quality of “high risk” foods



Where are *L. monocytogenes* found?  
 Do virulent strains recycle back to the environment?  
 Do they live in your fridge?



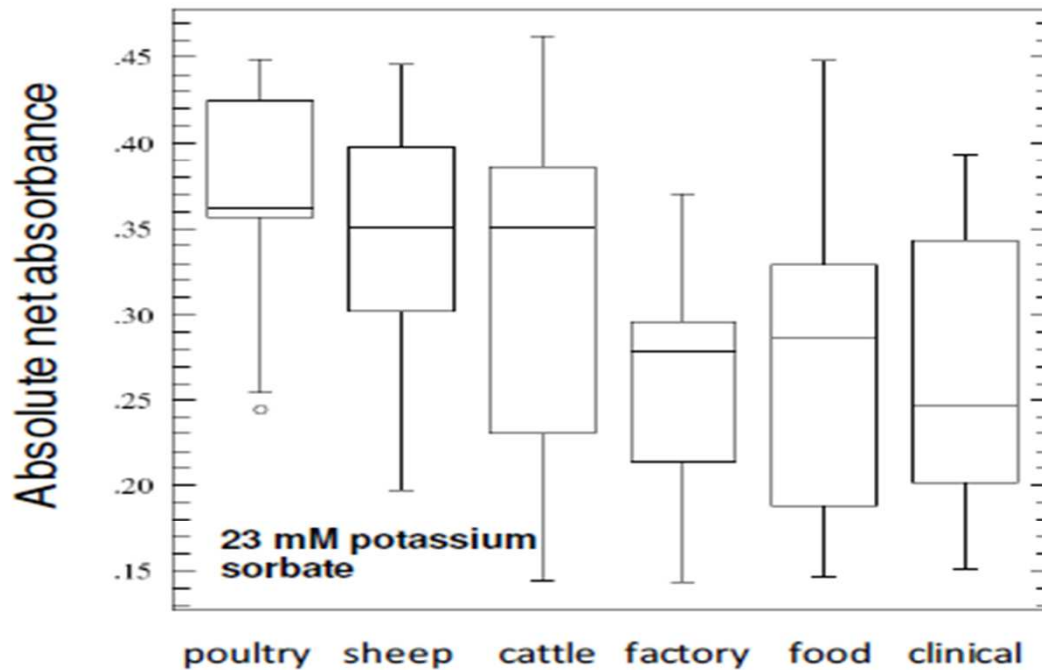
## Current and future risk factors for listeriosis?



- Aging populations
- Increased consumption of minimally processed (RTE) foods & foods with long shelf lives (fermented products)
- Changes to regulatory policies e.g. allowance of raw milk products
- Changes to virulence
- Selection of strains with heightened tolerance to “intervention” i.e. persistent strains



Strains exhibit differential tolerance to food preservatives  
Including acidulants and membrane disturbing compounds

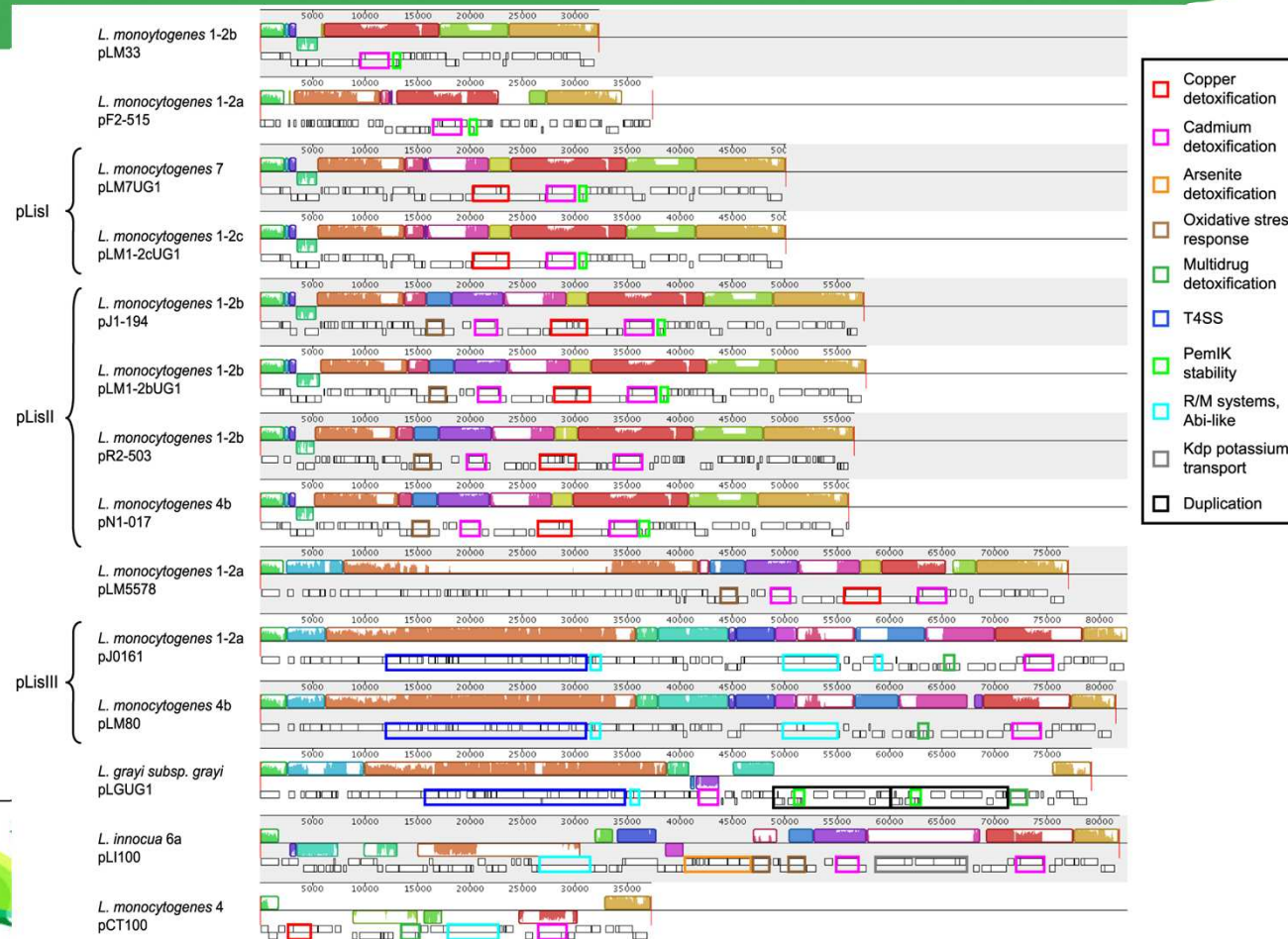


Strain-dependent factors are involved?  
i.e. plasmids, chromosomal insertions

# Plasmids are common in *L. monocytogenes*



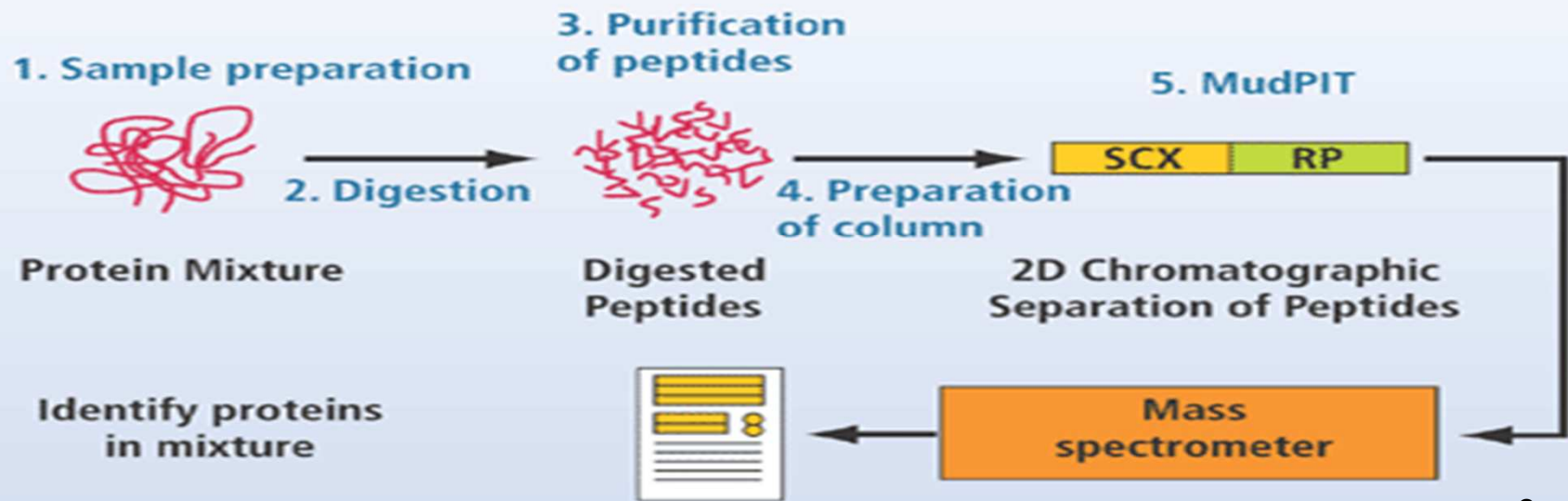
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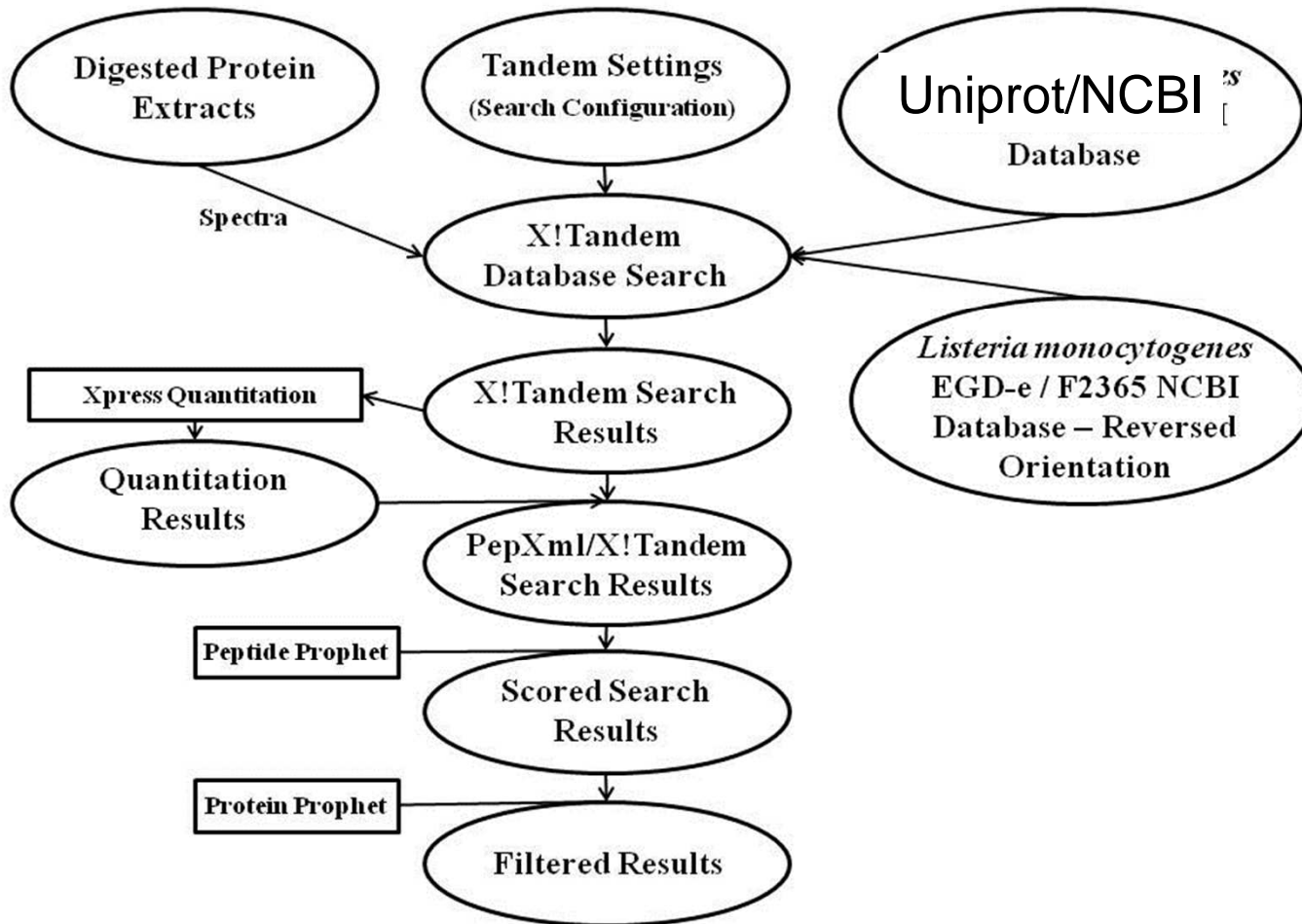


“Bottom-up” (empirical) studies connecting proteins with phenotypes using new “omics” technology



### MUDPIT (Multidimensional Protein Identification Technology)





# Peptide identification within complex peptide mix

-Matched against database e.g. X!TANDEM, SEQUEST

-Generate a probability of a correct match “*Protein Prophet*”



starved\_heat\_mudpit.xls [Compatibility Mode] - Microsoft Excel

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E9 65

	A	B	C	D	E	F	G
1	Run	Peptide	Charge	Protein	Total Peptide Count	Max Peptide Prophet	Avg Retention Time
2	<a href="#">data_august/2318</a>	K.IFAVAEAEANHLDEIDVLLGPPQVR.F	3	<a href="#">gil153175990 ref ZP_01931249_1 </a>	442	1.0000	28698.38852
3	<a href="#">data_august/2318</a>	K.GFGFIERENGDDVVFHFSAIQGDGFK.S	3	<a href="#">splP0A355ICSPA_LISMO</a>	208	1.0000	29242.15097
4	<a href="#">data_august/2318</a>	K.IPAIIATSYITGMGMEELDEEILAHLK.-	3	<a href="#">Q7ANU0IQ7ANU0_LISIN</a>	170	1.0000	31708.71186
5	<a href="#">data_august/2318</a>	K.GFGFIEVEGGDDVVFHFSAIIEGEGFK.T	3	<a href="#">CSPB_LISIN</a>	98	1.0000	34557.70603
6	<a href="#">data_august/2318</a>	K.AIEEEFGVTAAPVAVAAAGGAAAEQTEFTVELASAGDS	4	<a href="#">RL7_LISMF</a>	85	1.0000	13007.71643
7	<a href="#">data_august/2316-</a>	K.NSSMGIDLLQTADSALSSMSSILQR.M	3	<a href="#">FLAA_LISIN</a>	75	1.0000	19570.24563
8	<a href="#">data_august/2318</a>	R.IQYGGSVKPENIADYLAESDIDGALVGGASLEPASFLALLI	4	<a href="#">splQ8Y4I3ITPIS1_LISMO</a>	74	1.0000	24421.33521
9	<a href="#">data_august/2318</a>	K.GADITYTEGSDEKEAIEGLTEVLKK.E	3	<a href="#">PTHP_LISIN</a>	65	1.0000	27143.77067
10	<a href="#">data_august/2316-</a>	K.IEQQGAMGTIDEITPAEVSAAADVIIAADK.V	3	<a href="#">Q7AP30IQ7AP30_LISIN</a>	51	1.0000	11242.4235
11	<a href="#">data_august/2318</a>	R.E^NGDDVVFHFSAIQGDGFK.S	2	<a href="#">gil16800469 ref NP_470737_1 </a>	51	1.0000	12286.31193
12	<a href="#">data_august/2318</a>	K.GIPLAVINSVDYGMKK.G	2	<a href="#">gil153175990 ref ZP_01931249_1 </a>	49	1.0000	18806.3286
13	<a href="#">data_august/2318</a>	K.YWASLGDVFNDAFGTAHR.A	3	<a href="#">PGK_LISIN</a>	44	1.0000	25371.72061
14	<a href="#">data_august/2316-</a>	R.DLLTEYEFPGDDIPVIK.G	2	<a href="#">gil11612456 gb AAG39254_1 </a>	40	1.0000	9927.25599
15	<a href="#">data_august/2316-</a>	K.TVVWNGPMGVFELSNFAK.G	2	<a href="#">PGK_LISMF</a>	38	1.0000	20273.6894
16	<a href="#">data_august/2318</a>	K.YAVGQFNINNLEWTQAILK.A	2	<a href="#">gil16801762 ref NP_472030_1 </a>	38	1.0000	21695.20796
17	<a href="#">data_august/2316-</a>	R.DTDKPFMMPVEDVFSITGR.G	3	<a href="#">gil11612456 gb AAG39254_1 </a>	37	1.0000	19430.57834
18	<a href="#">data_august/2318</a>	K.ISEAASMQDGADLIVSTTILPTTYK.I	3	<a href="#">gil16804703 ref NP_466188_1 </a>	35	1.0000	11127.2543
19	<a href="#">data_august/2316-</a>	K.SLDEGQAVTFDVEEGQR.G	2	<a href="#">gil16800469 ref NP_470737_1 </a>	33	1.0000	7520.52092
20	<a href="#">data_august/2316-</a>	K.ILSSVAVNLEEAFQEQLTIK.T	3	<a href="#">gil16799498 ref NP_469766_1 </a>	29	1.0000	32263.16204
21	<a href="#">data_august/2316-</a>	R.IVAALPTIEYILEQNGK.A	2	<a href="#">PGK_LISMF</a>	29	1.0000	20059.7898
22	<a href="#">data_august/2318</a>	R.ENGDDVVFHFSAIQGDGFK.S	2	<a href="#">gil16800469 ref NP_470737_1 </a>	28	1.0000	18972.59999
23	<a href="#">data_august/2316-</a>	K.EFNSDAPFHTVSADSIPEMGLDIGQATIDLFTK.E	3	<a href="#">gil47097455 ref ZP_00235000_1 </a>	27	1.0000	22471.61104
24	<a href="#">data_august/2318</a>	K.TTLTAAITTVLAK.K	2	<a href="#">EFTU_LISW6</a>	27	1.0000	18068.88903

## Persistent strain stress responses determined using MudPIT



**“Persistent” strains** – (factories, food, clinical) organic acid and salt tolerant

**Does the proteome respond dynamically during short term non-growth permissive conditions?**

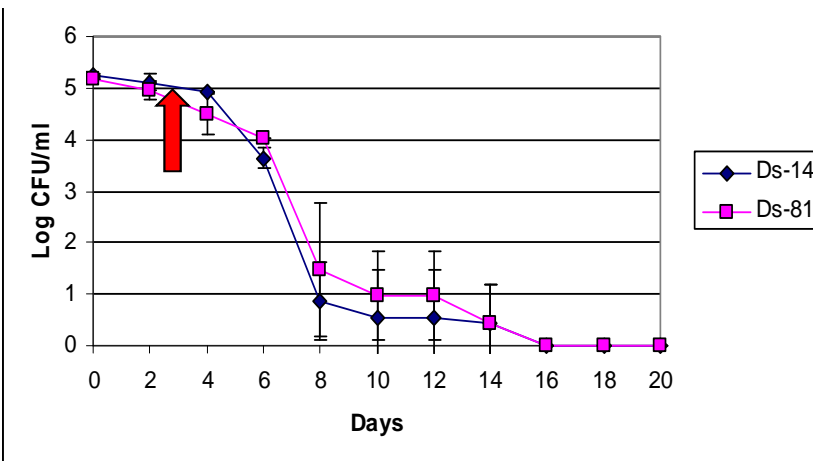
**Are plasmid-related proteins promoted?**

# Listeria monocytogenes inactivation

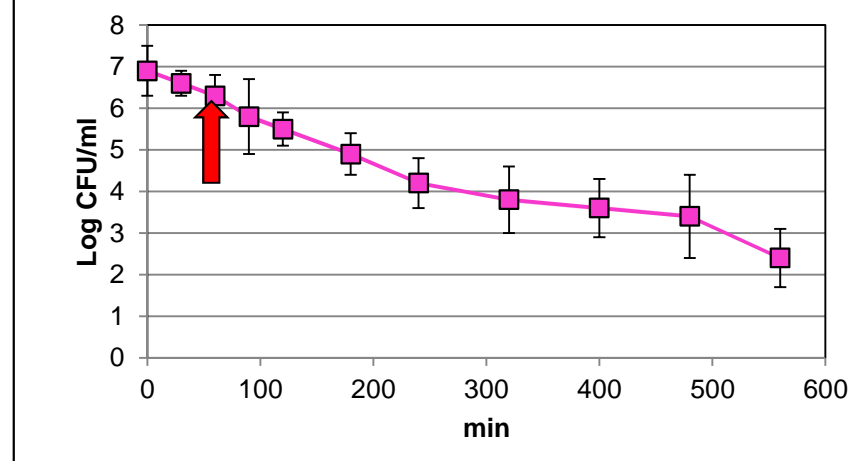


## Persistent food processing plant isolate DS81 (serotype 1/2b)

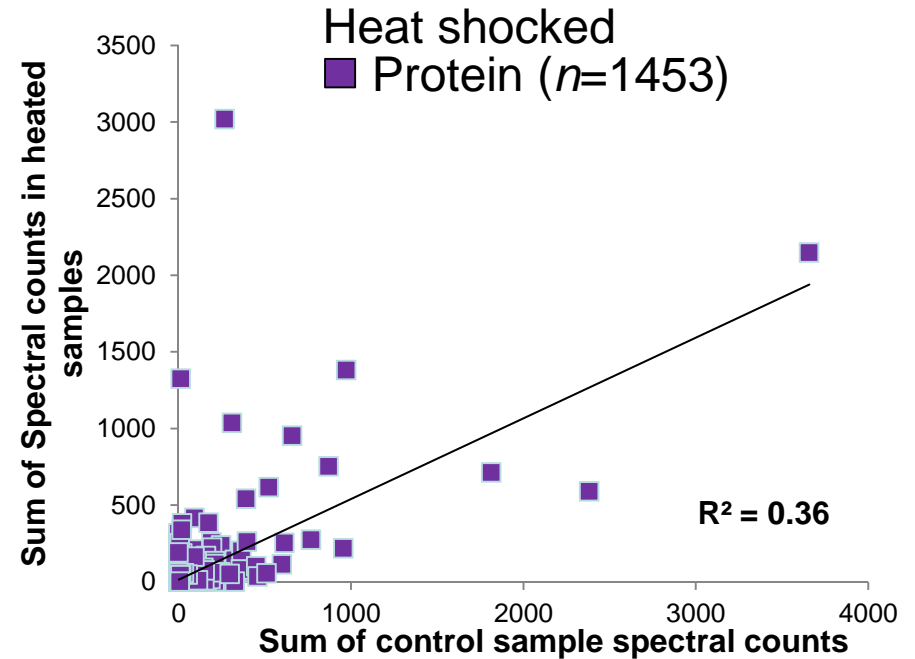
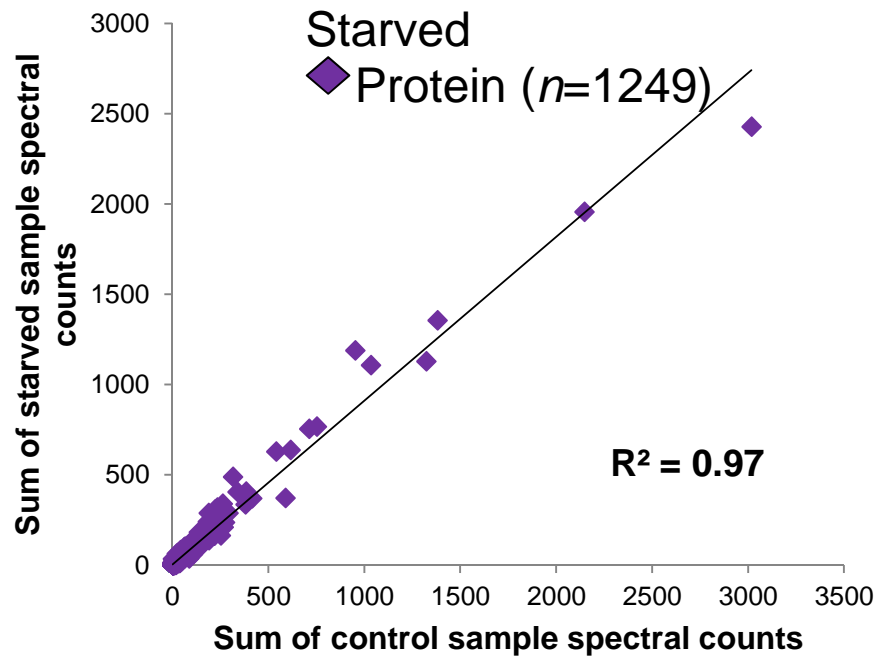
### Starvation in distilled water



### Heat inactivation (50°C)

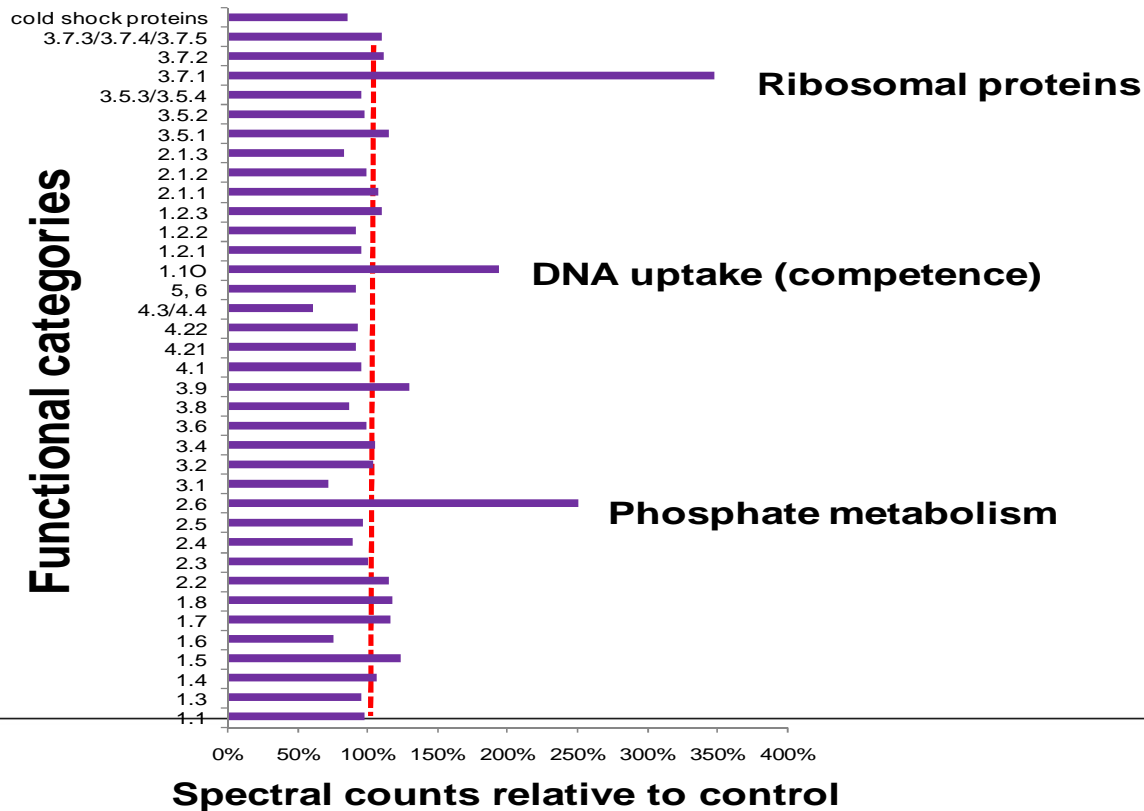


# Comparison between “dying” cells and actively growing cells using MudPIT



# Starvation of *L. monocytogenes* - functional responses

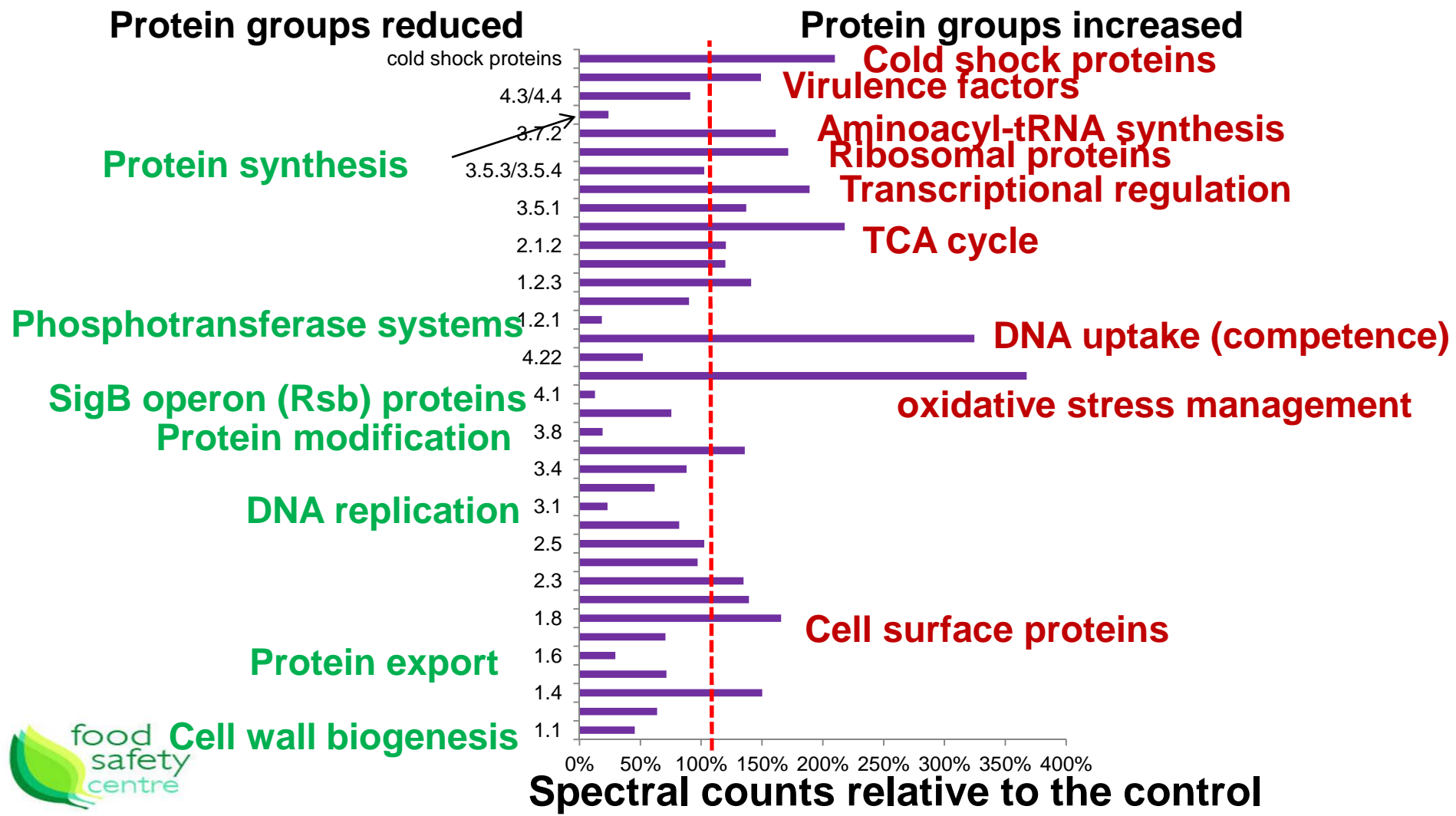
## Summation of spectral counts on the basis of ontology



## Top protein abundance changes after starvation (relative change, pseudospectral count set at 1.0)



EGD ORF	Homolog	Function	Fold change ( $p < 10^{-5}$ )
lmo2656	RpsL	ribosomal protein S12 membrane-anchored lipoprotein similar to conjugation pheromone cAD1 of <i>Enterococcus</i> spp.	+24.4
lmo2637			+20.7
lmo1393	PqqL	putative Zn-dependent peptidase	+15.8
lmo1218	SpoU	putative 23S rRNA methyltransferase	+13.8
lmo1649		putative secreted protein	+8.1
lmo1530	Tgt	queuine tRNA-ribosyltransferase	+8.0
lmo0640	YdhF	predicted oxidoreductase	+7.3
lmo2389	Ndh	respiratory NADH dehydrogenase	+6.7
lmo2564	PptA	putative keto-enol tautomerase	-13.4
lmo0691	CheY	chemotaxis response regulator	-9.4
lmo0004	YaaA	similar to S4-like RNA binding proteins	-6.3
lmo2426	YffB	putative arsenate reductase (glutaredoxin)	-14.8
lmo0903	YhfA	similar to OsmC family proteins	-10.7
lmo0896	RsbX	negative regulator of sigma-B (phosphoserine phosphatase)	-6.5



## Top protein abundance changes after heat shock



EGD ORF	Homolog	Function	Fold- change
lmo2248		putative regulator, PhoP-like	+132
lmo1664	MetK	<b>S-adenosylmethionine synthetase</b>	+137
lmo1288	LuxS	<b>S-ribosylhomocysteine lyase</b>	+65
lmo0906	Gor	<b>glutathione reductase</b>	+102
lmo2013	IspB	polyprenyl synthetase	+99
lmo1805	Rnc	ribonuclease III	+65
lmo2398	LtrC	<b>phosphatidylglycerophosphatase</b>	+67
lmo1571/ lmo0342	PykA, TktA	<b>6-phosphofructokinase, transketolase</b>	+90, +85
lmo0398, lmo0399		<b>Phosphotransferase system, fructose-specific (?) (IIA and IIB subunits)</b>	-110,-253
lmo1808	FabD	malonyl CoA-acyl carrier protein transacylase	-170
lmo0786	AzoR	putative azoreductase, FMN-dependent	-185
lmo1322	NusA	transcription elongation factor	-110
lmo1657	Tsf	elongation factor EF-Ts	-113
lmo1267	Tig	trigger factor	-107

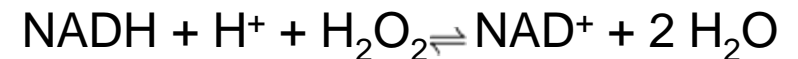
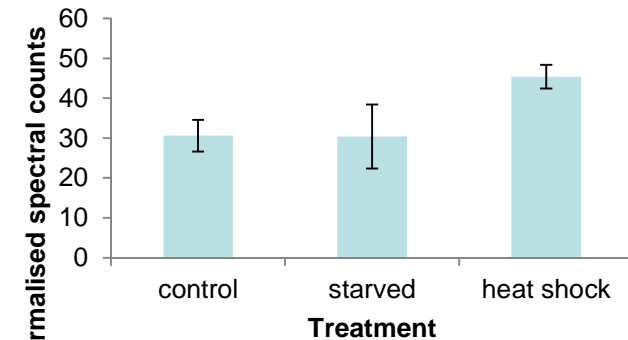


## Evidence for a plasmid coded metal and peroxide resistance proteins in strain DS81



- plasmid replication initiation protein
- TraG/TraD family (conjugation) protein
- *E. faecalis* VanRS-like two component signal transduction system
- multi-copper oxidase
- cation (copper) transport P-type ATPase
- heavy metal translocating P-type ATPase
- putative S-adenosylmethionine-dependent methyltransferase
- **NADH-dependent organic peroxidase\***
- putative transcriptional regulator, TetR family
- transposases/resolvases
- 2 proteins with unknown function

### NADH peroxidase abundance (EC 1.1.11.1)



## Conclusions and Future Prospects



-*Listeria monocytogenes* remains a public health problem even in countries with sophisticated food supply chains

-Label-free MudPIT proteomics approach using spectral counting is a powerful tool to analyse dynamic genome-level responses reasonably cost-effectively.

- MudPIT can detect dynamic changes in bacterial proteomes responding to growth and non-growth permissive stresses.

-Further analyses of strains delineated in typing schemes (i.e. MLST) on the basis of phenotypes (enhanced virulence, enhanced stress tolerance) could better define food safety relevant characteristics in *L. monocytogenes* and other pathogens



# Acknowledgements



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