D-JRP7-3.6 / Collection of data on survival of *Listeria monocytogenes* in soil microcosms.

JRP7 - LISTADAPT

V0.1 27/11/2019

Responsible Partner: IINRA Dijon France,

UMR1347 Agroécologie MERS Team

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Project report ListAdapt

INRA Dijon France, UMR1347
Agroécologie MERS Team

Eliette Ascensio, Géraldine Depret, Laurent Gal, Alain Hartmann, Dominique Garmyn, Pascal Piveteau.

WP 1: Constitution of a strain collection representative of the different reservoirs of Listeria monocytogenes

Abstract:

As part of the WP1, our team investigated a large sampling collection for the detection and the isolation of *Listeria* in the environmental reservoir.

Thanks to an optimized protocol for the detection of *Listeria* in environmental samples with a new real-time PCR assay (*note:* the final draft of a methodological paper is being written for submission to a peer-reviewed international journal), we analyzed more than 1150 samples from various types (soils, water, wastewater, agricultural inputs, wild life samples…).

These samples came from different sources:

- French nationwide soil quality monitoring network (INRA, RMQS) that provided us fresh soils samples from all the French territory

- Farm monitoring: 2 longitudinal studies (organic farming vegetables + cattle) with monthly sampling
- Broad sampling: one-time soil sampling performed by ourselves and by other members of the consortium.

We were able to detect and isolate 98 *Listeria* strains of which 19 *L. monocytogenes* isolates (See Table 1). We chose to sequence *Lm* but also other *Listeria* especially the ones found in the same samplings as *Lm*.

In addition to these strains, 26 other *Lm* soil isolates from previous sampling sessions performed by our team were added for the sequencing task (See Table 2). (for more information please see also Supplemental Data 1.)

Material and Methods:

- ➔ **Specific enrichment.** 10 grams of each environmental sample were weighed in 180 mL pots. Processed samples were suspended in 90 mL of Difco^{TM} Listeria Enrichment Broth modified. After 24h of incubation at 30°C, the first enrichments were vortexed. One mL aliquots were used for DNA preparation by boiling. And other aliquots of 1 mL were transferred in 9 mL of Difco^{TM} UVM Modified Listeria Enrichment Broth for the second enrichment. After 48h of incubation at 30°C, the second enrichments were vortexed and 1 mL aliquots were also used for the DNA preparation by boiling.
- ➔ **DNA extraction.** Bacterial DNA was prepared by boiling. One millilitre of the samples was centrifuged at 5800 x g for 3 minutes. The supernatant was eliminated and the pellet was resuspended in 1 mL of sterile water and centrifuged at 5800 x g for 3 minutes. The supernatant was eliminated and the pellet was resuspended in 1 mL of sterile water. An aliquot of 500 µL was used and subjected to boiling at 100°C in a water bath for 10 minutes, cooled at room temperature and

then centrifugated at 5800 x g 10 seconds before it was stored at -20°C. Boiled enrichments were ten-fold diluted (1:10) and the dilutions were used as template for real-time PCR.

- **Duplex real-time PCR detection of *Listeria* sp. and *Listeria monocytogenes*.** SYBR®Green real-time PCR assays were performed on ABI StepOne^{TM} Real-time thermocycler (Applied Biosystems^{TM}, Thermo Fisher Scientific, France) with the following temperature program: 95°C for 5 minutes and 35 cycles at 95°C 15 seconds and 60°C for 1 minutes. Melt curves were generated with temperature increments of 0.1°C per cycle from 60 to 95°C. DNA was amplified in a 22 µL PCR mix containing 11 µL of Takyon SYBR®Green Low ROX 2X (Eurogentec, Belgium), 0.44 µL of each primer, 2 µL of template DNA diluted (1:10) and 7.24µL of PCR grade water. For detection of *Listeria* from environmental samples, 0.5 µL of T4 Gene 32 Protein (Sigma Aldrich) was added to the PCR mix.

  Two different primers pairs were used in duplex. One pair specific to *L. monocytogenes* was designed to target *inlA*. The second pair of primers is detecting all bacteria from the genus *Listeria* by targeting *prs2*.

- **Isolation of *Listeria* sp.** Positive samples for the presence of *Listeria* were spread on RAPID'L.Mono plates (BioRAD, France). This medium specifically detects the phospholipase of *L. monocytogenes* and its inability to metabolize xylose. After 24h incubation at 37°C, *L. monocytogenes* forms characteristic blue colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. *L. ivanovii* presents blue-green colonies with a yellow halo (xylose positive character). Moreover, the selective solution in the medium permits inhibition of most interfering flora.

Results:

Our procedure of detection by real-time PCR was used on a large scale of more than 1150 environmental samples. These samples were collected in different geographical areas in Austria, France, Slovenia and Sweden. Most samples were soil (~85%), but mud, sediments, water, silage, faeces, burrows and badgers' latrines were also processed. Among all these samples, members of the genus *Listeria* were detected in 98 samples (8.5 %), including 19 *L. monocytogenes* positive samples (1.7 %). The non-*L. monocytogenes* isolates were identified as *L. innocua* (54, 4.7 %), *L. welshimeri* (21, 1.8 %), *L. ivanovii* (2, 0.2 %) and *L. seeligeri* (2, 0.2 %). Details on positive samples are included Table 1. A total of 26 other soil isolates from the strain collection of UMR1347 were included in the sequencing effort for environmental isolates. Twenty six other isolates already sequenced were also included.

Table 1: *Listeria* isolates detected during sampling sessions.

Strains	**Species**	**Origin**	**Date**	**Samples**	**Sequencing status**
12341	*L. monocytogenes*	Austria	May 2018	Soil near a little farm, pasture area for does	☑
12342	*L. monocytogenes*	France	May 2018	Meadow	☑
12345	*L. monocytogenes*	France	June 2018	Badger's burrow	☑
12348	*L. monocytogenes*	France	-	Soil	☑
12350	*L. monocytogenes*	France	March 2018	Soil	☑
12351	*L. monocytogenes*	France	March 2018	Sediments	☑
12353	*L. monocytogenes*	France	March 2018	Latrines for badgers	☑
12354	*L. monocytogenes*	France	March 2018	Badger's burrow	☑
12356	*L. monocytogenes*	France	March 2018	Latrines for badgers	☑
12439	*L. monocytogenes*	Sweden	September 2018	Grass field	☑
12461	*L. monocytogenes*	France	October 2018	Water from a well	In progress
12464	*L. monocytogenes*	France	October 2018	Water from a creek	☑
12468	*L. monocytogenes*	Slovenia	October 2018	Pond mud	☑
12469	*L. monocytogenes*	Slovenia	October 2018	Water from pond mud meadows cows	☑
12727	*L. monocytogenes*	Slovenia	October 2018	Pond mud meadows cows	☑
12733	*L. monocytogenes*	France	December 2018	Sludge from a waste treatment plant	☑
13222	*L. monocytogenes*	France	April 2019	Soil from peas crop	In progress
12128	*L. monocytogenes*	France	July 2019	Water from a creek	In progress
12129	*L. monocytogenes*	France	-	Soil	In progress
12338	*L. innocua*	France	May 2018	Soil from spinach crop	☑
12343	*L. innocua*	France	June 2018	Water from a waste treatment plant	☑
12344	*L. innocua*	Austria	May 2018	Corn field	☑
12347	*L. innocua*	France	July 2018	Rabbit feces in a grass field	☑

12357	*L. innocua*	France	March 2018	Latrines for badgers	☑
12380	*L. innocua*	France	August 2018	Water from a waste treatment plant	☑
12441	*L. innocua*	France	October 2018	Water from a waste treatment plant	☑
12466	*L. innocua*	Slovenia	October 2018	Remain of corn silage at farm	☑
12467	*L. innocua*	Slovenia	October 2018	Waste silage	☑
12470	*L. innocua*	Slovenia	October 2018	Pile of manure	☑
12471	*L. innocua*	Slovenia	October 2018	Horse manure worms near the meadow	☑
12472	*L. innocua*	Slovenia	October 2018	Mud from a dry pond	☑
12473	*L. innocua*	Slovenia	October 2018	Agricultural land feces	☑
12474	*L. innocua*	Slovenia	October 2018	Stable at farm, feces on the floor	☑
12722	*L. innocua*	Slovenia	October 2018	Cultivated meadow soil	☑
12724	*L. innocua*	Slovenia	October 2018	Waste material from the surroundings of a barn	☑
12725	*L. innocua*	Slovenia	October 2018	Soil from a pasture in the proximity of the barn	☑
12730	*L. innocua*	France	December 2018	Sludge from a waste treatment plant	☑
12731	*L. innocua*	France	December 2018	Sludge from a waste treatment plant	☑
12732	*L. innocua*	France	December 2018	Sludge from a waste treatment plant	☑
12734	*L. innocua*	France	December 2018	Sludge from a waste treatment plant	☑
12735	*L. innocua*	France	January 2019	Water from a well	☑
12736	*L. innocua*	France	January 2019	Cow litter at the farm	☑
12737	*L. innocua*	France	January 2019	Cow litter at the farm	☑
12738	*L. innocua*	France	January 2019	Water from a creek	☑
12741	*L. innocua*	France	February 2019	Breeding ground for salad culture	☑
12744	*L. innocua*	France	March 2019	Water from a creek	☑
12745	*L. innocua*	France	March 2019	Cow litter at the farm	☑
12746	*L. innocua*	France	-	Soil	☑
13208	*L. innocua*	France	April 2019	Digestate	NO
13209	*L. innocua*	France	April 2019	Digestate	NO
13210	*L. innocua*	France	April 2019	Digestate	NO
13211	*L. innocua*	France	April 2019	Cow manure	NO
13212	*L. innocua*	France	April 2019	Cow manure	NO
13214	*L. innocua*	France	April 2019	Post-digestate	NO
13216	*L. innocua*	France	April 2019	Cow manure	NO
13217	*L. innocua*	France	April 2019	Cow manure	NO
13218	*L. innocua*	France	April 2019	Cow manure	NO
13219	*L. innocua*	France	April 2019	Cow manure	NO
13221	*L. innocua*	France	April 2019	Breeding ground for peas culture	NO
13223	*L. innocua*	France	April 2019	Breeding ground for parsley cultre	NO
13224	*L. innocua*	France	April 2019	Pig manure	NO
13225	*L. innocua*	France	April 2019	Pig manure	NO
13226	*L. innocua*	France	April 2019	Digestate	NO
13227	*L. innocua*	France	April 2019	Digestate	NO
13228	*L. innocua*	France	April 2019	Digestate	NO
13280	*L. innocua*	France	April 2019	Breeding ground for basil culture	NO
13281	*L. innocua*	France	April 2019	Breeding ground for salad culture	NO

13282	*L. innocua*	France	June 2019	Digestate	NO
13283	*L. innocua*	France	June 2019	Digestate	NO
13284	*L. innocua*	France	June 2019	Digestate	NO
13285	*L. innocua*	France	June 2019	Post-digestate	NO
13286	*L. innocua*	France	June 2019	Poultry manure	NO
12127	*L. innocua*	France	July 2019	Soil from a salad field	NO
12352	*L. ivanovii*	France	March 2018	Badger's burrow	☑
12355	*L. ivanovii*	France	March 2018	Badger's burrow	☑
12346	*L. seeligeri*	France	June 2018	Badger's burrow	☑
12381	*L. seeligeri*	Sweden	August 2018	Grass field	☑
12440	*L. welshimeri*	France	September 2018	Cow pasture	☑
12442	*L. welshimeri*	France	October 2018	Breeding ground for salad culture	☑
12462	*L. welshimeri*	France	October 2018	Feces cow	☑
12463	*L. welshimeri*	France	-	Soil	☑
12465	*L. welshimeri*	France	October 2018	Water from a creek	☑
12475	*L. welshimeri*	Slovenia	October 2018	Pile of manure	☑
12476	*L. welshimeri*	Slovenia	October 2018	Soil from a field with tal grass	☑
12720	*L. welshimeri*	Slovenia	October 2018	Soil from a pasture	☑
12721	*L. welshimeri*	Slovenia	October 2018	Mud sample and water plants	☑
12723	*L. welshimeri*	Slovenia	October 2018	Feces, grass and silage remains	☑
12726	*L. welshimeri*	Slovenia	October 2018	Pond mud and aquatic plants	☑
12728	*L. welshimeri*	France	November 2018	Badger's burrow	☑
12729	*L. welshimeri*	France	November 2018	Badger's burrow	☑
12739	*L. welshimeri*	France	-	Soil	☑
12740	*L. welshimeri*	France	February 2019	Soil from basil field	☑
12742	*L. welshimeri*	France	-	Soil	☑
12743	*L. welshimeri*	France	-	Soil	☑
13207	*L. welshimeri*	France	April 2019	Sediments	NO
13213	*L. welshimeri*	France	April 2019	Post-digestate	NO
13215	*L. welshimeri*	France	April 2019	Water from a creek	NO
12126	*L. welshimeri*	France	-	Soil	NO

Table 2 : Additionnal *Lm* isolates from previous samplings for the sequencing procedure.

Strains	**Species**	**Origin**	**Date**	**Samples**	**Sequencing**
5929	*L. monocytogenes*	France	-	-	☑
5945	*L. monocytogenes*	-	November 2006	Compost	☑
5948	*L. monocytogenes*	-	November 2007	Compost	☑
6579	*L. monocytogenes*	France	December 2008	Soil	☑
6701	*L. monocytogenes*	France	-	Manure	☑
7145	*L. monocytogenes*	France	July 2011	Soil	☑
7147	*L. monocytogenes*	France	July 2012	Soil	☑
7151	*L. monocytogenes*	France	July 2011	Soil	☑

7158	*L. monocytogenes*	France	July 2012	Soil	☑
7164	*L. monocytogenes*	France	July 2013	Soil	☑
7167	*L. monocytogenes*	France	July 2014	Soil	☑
10223	*L. monocytogenes*	France	April 2012	-	☑
10257	*L. monocytogenes*	France	-	Mud	☑
10259	*L. monocytogenes*	France	-	Soil	☑
10281	*L. monocytogenes*	France	-	Fresh soil improver	☑
10320	*L. monocytogenes*	France	October 2013	Compost	☑
10321	*L. monocytogenes*	France	October 2013	Compost	☑
10322	*L. monocytogenes*	France	October 2013	Compost	☑
12319	*L. monocytogenes*	France	March 2018	-	☑
12322	*L. monocytogenes*	France	March 2018	-	☑
12333	*L. monocytogenes*	France	March 2018	-	☑
M15B	*L. monocytogenes*	-	-	-	☑
R10B	*L. monocytogenes*	-	-	-	☑
R1B	*L. monocytogenes*	-	-	-	☑
R2B	*L. monocytogenes*	-	-	-	☑
R5A	*L. monocytogenes*	-	-	-	☑

WP 3: Phenotypic characterization of *Listeria monocytogenes* strains

Abstract:

In the WP3, our team explored survival and persistence of *Lm* strains in soil. These strains were selected by the coordinator’s lab in order to be representative of the diversity of the Listadapt strains collection.

The survival tests were performed in triplicate in a microtiter plate assay according to the protocol described in Supplemental Data 1.

We observe a wide range of survival rates with no correlation between the origin of the isolate and its phenotype.

GWAS on the whole set of data was inconclusive indicating than there is not a single mechanism of adaptation to survival in soil but several that are specific to clonal complexes. When conducted on isolates from the same Clonal Complex, presence/absence of genes statistically significant were identified.

First results were presented in a poster session at ISOPOL 2019 in Toronto (see Poster below)

Material and Methods:

➔ **Preparation of the microtiter plate.** Add 0.5 g of soil in the wells of the first lane of the 24 weels microtiter plate. Incubate 24h at 25°C before inoculation.

➔ **Preparation of the inoculum.**

Day 1: From frozen stock, inoculate 10 mL TSB and incubate 24h at 25°C.

Day 2: Sub-culture in 10 mL TSB (1% inoculation) and incubate 16h at 25°C.

Day 3: Pellet cells and suspend in sterile distilled water. Mesure OD to estimate the population density. Soil will be inoculated at 10^6 CFU/g dry soil. The total volume of inoculum must be calculated in order to reach a final water content of 60% of the Water Holding Capacity of the soil. Incubate at 25°C for 36h.

➔ **Quantification of the population of Lm.**

Day 5: Add 2 mL of Trypton Salt (TS) in the wells filled with soil. With the multichannel automatic pipet, fill the remaining wells with 900 µL of TS. Agitate on the rotary shaker for 10 min at 350 rpm. With the multichannel automatic pipet, proceed with serial dilutions and spot inoculate (10 µL) on Rapid'L.mono agar plates. Incubate plates at 37°C for 24h.

Day 6: Read the results.

Results:

As shown Figure 1, the ability of *L. monocytogenes* to survive in soil was strain dependent. Survival ranged from zero to 22%.

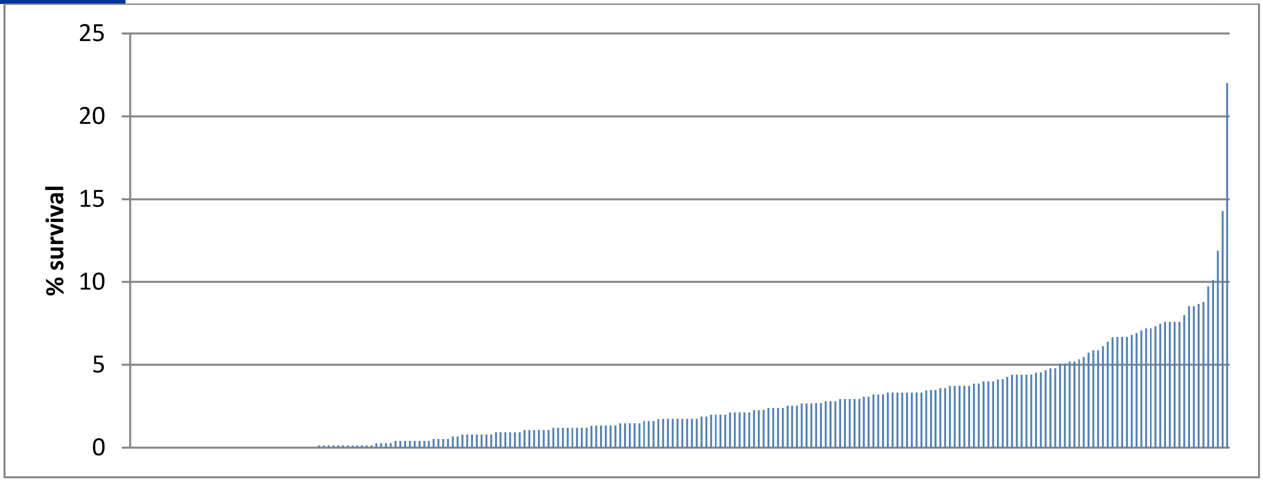

Figure 1. Soil survival phenotype of 230 isolates of *Listeria monocytogenes*.

Ascending Hierarchical Clustering clearly identified 3 groups of phenotypes (Figure 2), possibly indicating that some isolates may be better competitors in complex habitats such as soil. Further analysis was performed to investigate correlation between the phenotypes and the characteristics of the genomes of these isolates.

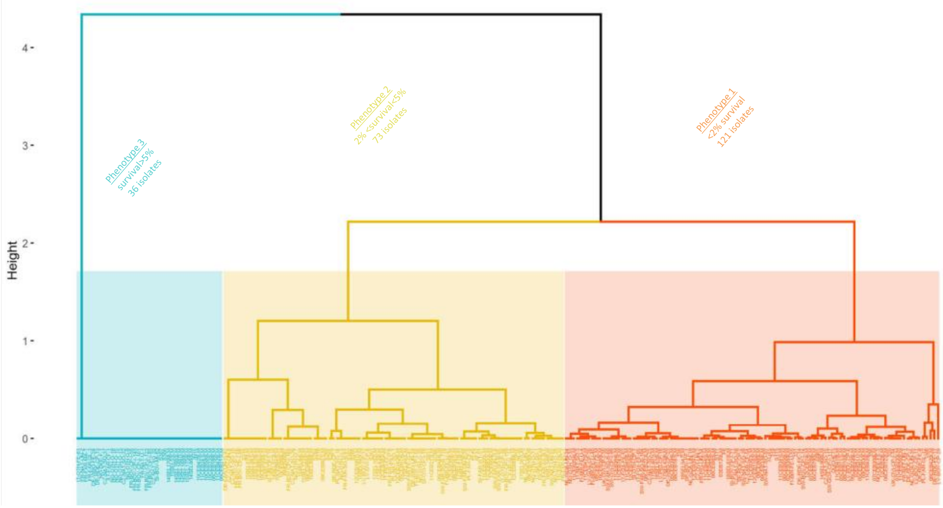

Figure 2. Ascending Hierarchical Clustering of soil survival data.

Investigation of genome characteristics underlying fitness of *Listeria monocytogenes* in soil

Ascensio-Schultz E, Gal L, Garmyn D, Szymczak B, Karpiskova R, Pietzka A, Ruppitsch W, Boysen M, Pomilio F, Torresi M, Camma C, Dipasquale A, Mateja P, Skjerdal T, Sevellec, Y., Felix B, Guillier L, Piveteau P.

The One Health European Joint Programme (OHEJP) is an EU initiative to reinforce collaboration between European institutes involved in the fields of emerging threats, antimicrobial resistance and foodborne zoonoses. Listadapt is a Joint Research Project funded by OHEJP. Acclaimed food, veterinary and medical laboratories and institutes dealing with *Listeria monocytogenes* hazards across Europe joined efforts to gain insight into the ecology and biology of *Listeria monocytogenes*. A unique collection of outdoors, animals and food isolates was built and over 4000 genomes were sequenced. In order to gain insight in the specific adaptation, evolution and genetic make-up of *Listeria monocytogenes* strains driving fitness in specific outdoor environmental niches, a selection of 219 isolates representative of various habitats (outdoors, animals, food) and covering the genomic diversity of the collection was screened for soil survival and the results were integrated in a Genome Wide Association Study.

Hierarchical clustering identified three groups of strains differing in their ability to survive in soil. With a majority of isolates, a sharp decline was observed and less than 2% of the initial population was detected after 36 h of incubation in soil while a minority of isolates had survival rates of over 5%. GWAS was applied to these phenotypical results in order to identify genetic features directly connected to the enhanced fitness of some isolates in soil.

Investigation of genome characteristics underlying fitness of *Listeria monocytogenes* in soil

Ascencio-Schultz[1] E, Gal[1] L, Garmyn[1] D, Szymczak[2] B, Karpiskova[3] R, Pietzka[4] A, Ruppitsch[4] W, Boysen[5] M, Pomilio[6] F, Torresi[6] M, Camma[6] C, Dipasquale[6] A, Pate[7] M, Skjerdal[8] T, Sevellec[9] Y., Felix[9] B, Guillier[9] L, Piveteau[1] P.

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Introduction

Soil is a central habitat in the transmission routes of *L. monocytogenes* from the farm environment to foodstuff. Little information is available on the genetic feautures underlying its fitness in this complex habitat. The aim of this study was to investigate genome characteristics linked to fitness in soil. Soil survival was assessed in a large collection of strains isolated from soil, water, animals and foodstuff. Genome Wide Association Study (GWAS) was performed in order to identify genes connected to soil fitness.

Materials and Methods

230 strains of *Listeria monocytogenes* were collected. All strains were sequenced and grouped in Clonal Complexes. Soil survival was assessed in quadruplicate :

1. inoculation of soil microcosms (10^6 CFU/g) 2. incubation 36 h at 25°C 3. numeration on Rapid'L mono.

The pan-genome was extracted with the sofware Roary V. 3.12.0 and Genome Wide Association Study (GWAS) was performed with Scoary V. 1.6.16 (1, 2) to extract the matrix of presence/absence of genes in the phenotypes clustered in three groups (phenotype 1, 2 and 3) after Ascending Hierarchical Clustering. The genes with significant association ($p < 0.05$) were reported.

Results

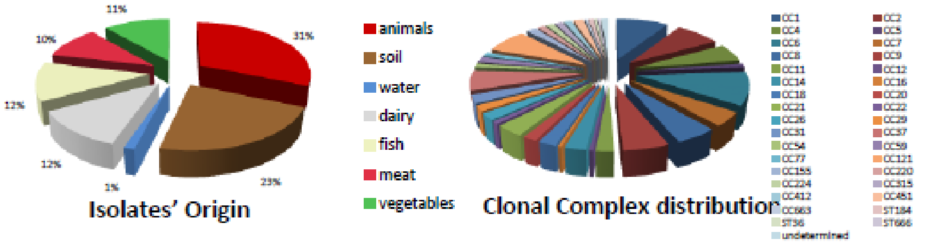


Figure 1. Origin and phylogenetic distribution of the collection of isolates of *L. monocytogenes*.

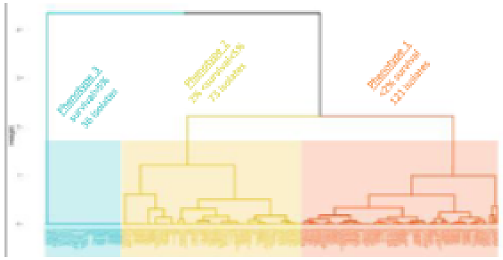


Figure 2. Ascending Hierarchical Clustering of the survival data.

3 groups were identified and further used for GWAS.

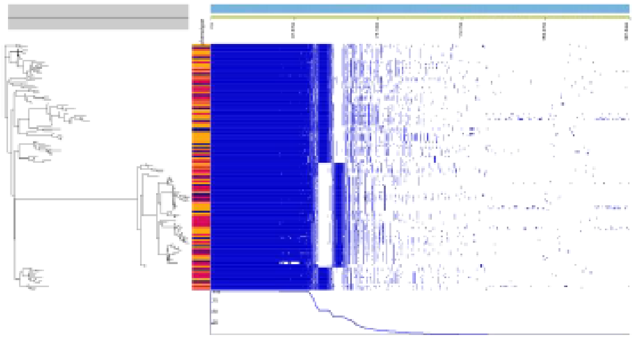


Figure 3. Representation of the pan-genome (3) of the collection according to the phylogenetic position of the isolate. Annotation of the survival potential is also given.

The presence and absence of core and accessory genes is consistent with the phylogenetic tree, discriminating lineage 1 from lineage 2 isolates.

However, the analysis was not discriminant regarding the phenotype. Indeed, low sensitivity or specificity (<50%) were recorded in the matrix.

Then, the analysis was performed within CCs.

Table 1. Summary of the GWAS Scoary results for CC6 isolates

	Absence	presence
Phenotype 3	5 phage-related genes	*inlH* variant
Phenotype 2	*clpC, ctsR*	4 genes coding hypothetical proteins
Phenotype 1	-	6 phage-related genes

Conclusions

- ✓ The fitness of *L. monocytogenes* in soil showed large intra-specific diversity.
- ✓ No direct correlation between the origin and the phenotype could be found.
- ✓ GWAS on the whole set of data was inconclusive indicating than there is not a single mechanism of adaptation to survival in soil but several that are specific to clonal complexes
- ✓ When conducted on isolates from the same Clonal Complex, presence/absence of genes statistically significant were identified.
- ✓ For example within CC6 the analysis suggests that phage insertion affects fitness in soil while surface protein variants may have an adaptive advantage in soil.

References: 1. Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T., ... & Parkhill, J. (2015). Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics, 31(22), 3691-3693.
2. Brynildsrud, O., Bohlin, J., Scheffer, L., & Eldholm, V. (2016). Rapid scoring of genes in microbial pan-genome-wide association studies with Scoary. Genome biology, 17(1), 238.
3. Hadfield, J., Croucher, N. J., Goater, R. J., Abudahab, K., Aanensen, D. M., & Harris, S. R. (2017). Phandango: an interactive viewer for bacterial population genomics. Bioinformatics, 34(2), 292-293.

Listadapt
https://onehealthejp.eu/jrp-listadapt/
H2020 Grant Agreement No 773830

Conclusion

Our results confirmed that bacteria from the genus Listeria can be found in soil. However, the percentage of detection is rather low. Moreover, less than 2% of the samples were positive for the presence of *Listeria monocytogenes*. These figures are in agreement with the data from the literature.

Strong phenotypic heterogeneity was observed regarding soil survival but direct correlations with the origin of the strain nor lineage could not be found. Genome Wide Association Study was inconclusive when tested on the whole panel of strains. This is a consequence of the large intraspecific biodiversity of the species *Listeria monocytogenes*. However, when GWAS was performed on closely related strains, significant correlations could be evidenced and putative mechanisms important for soil survival were highlighted.

Suppl. Data 1: Presentation EJP April 2019 Maisons-Alfort

INRA
SCIENCE & IMPACT

Collection of *Listeria monocytogenes* from environmental samples

LISTADAPT project restitution meeting

Eliette ASCENSIO

10 / 04 / 2019

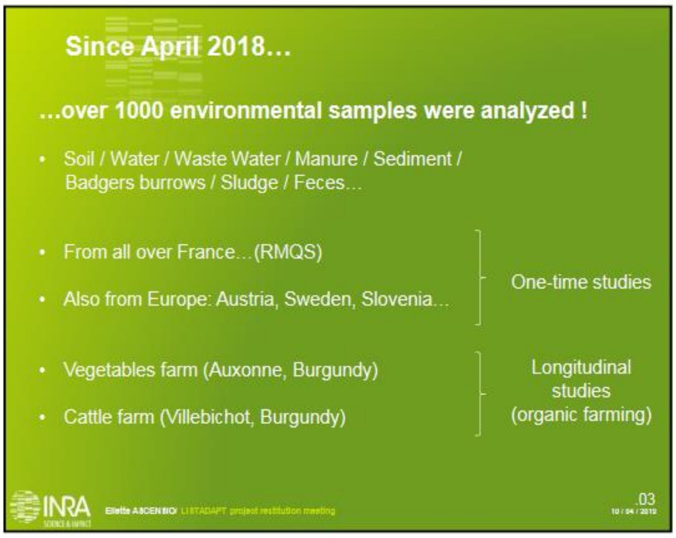
1

Environmental samples

Eliette ASCENSIO/ LISTADAPT project restitution meeting

.02
10 / 04 / 2019

2



Since April 2018…

…over 1000 environmental samples were analyzed !

- Soil / Water / Waste Water / Manure / Sediment / Badgers burrows / Sludge / Feces…
- From all over France…(RMQS)
- Also from Europe: Austria, Sweden, Slovenia…

One-time studies

- Vegetables farm (Auxonne, Burgundy)
- Cattle farm (Villebichot, Burgundy)

Longitudinal studies (organic farming)

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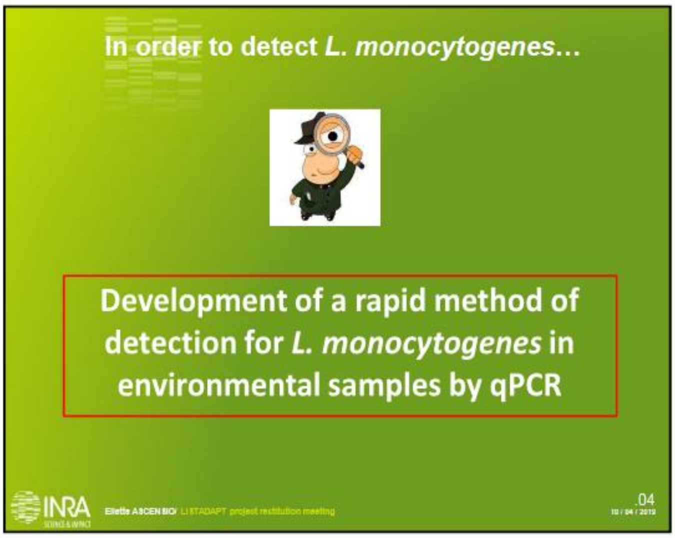

In order to detect *L. monocytogenes*…

Development of a rapid method of detection for *L. monocytogenes* in environmental samples by qPCR

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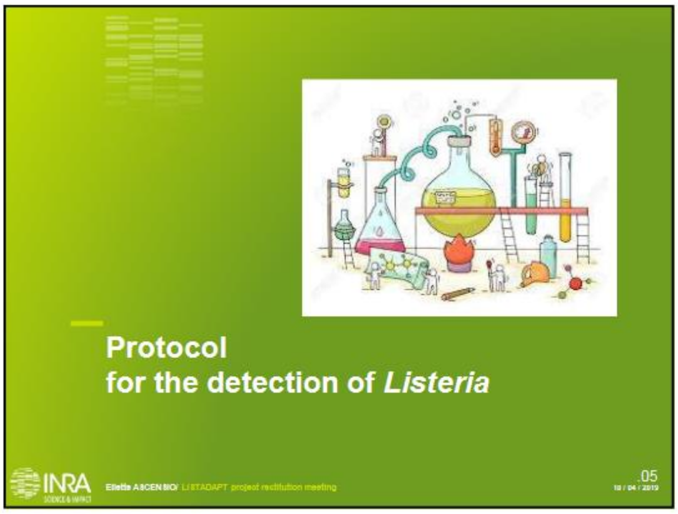
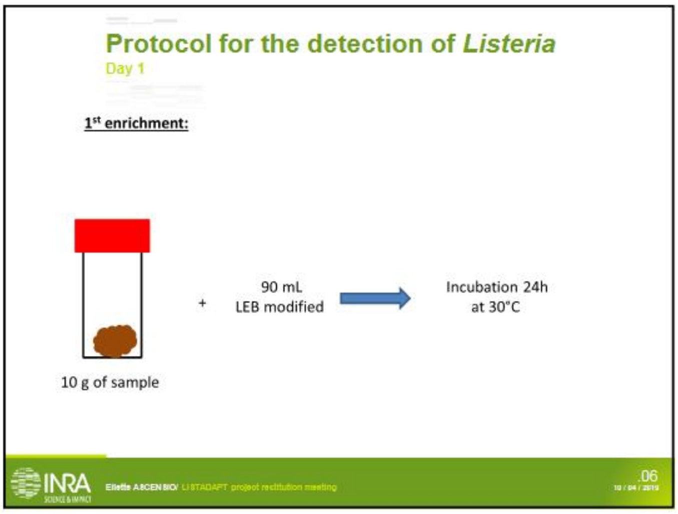


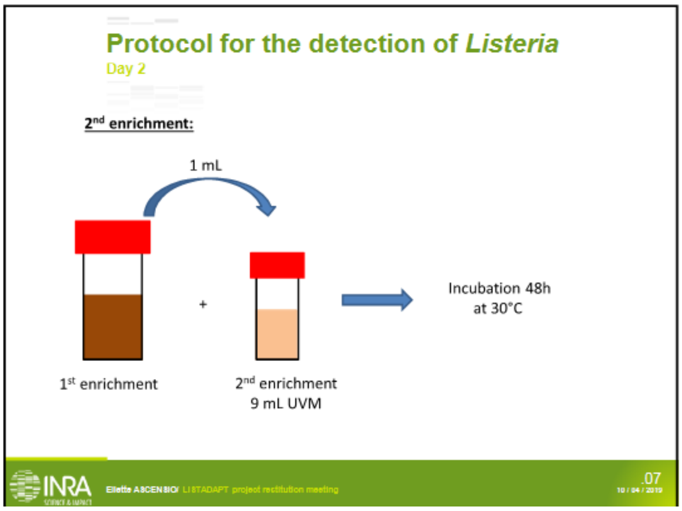
Protocol
for the detection of *Listeria*



Protocol for the detection of *Listeria*

Day 1

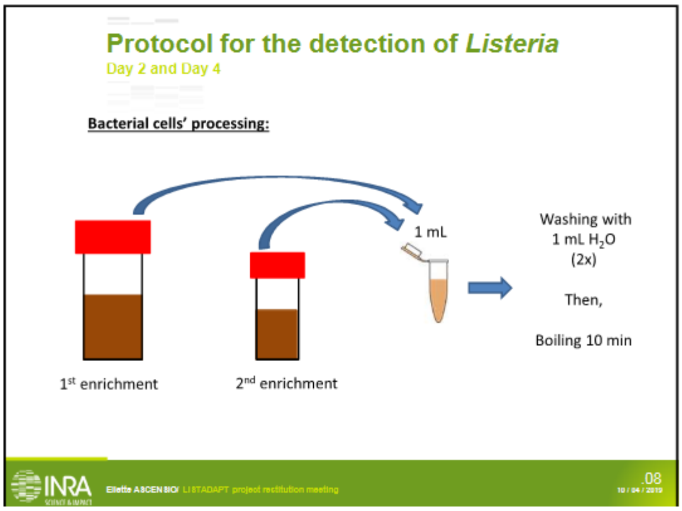
1st enrichment:



Protocol for the detection of *Listeria*

Day 2

2nd enrichment:



Protocol for the detection of *Listeria*

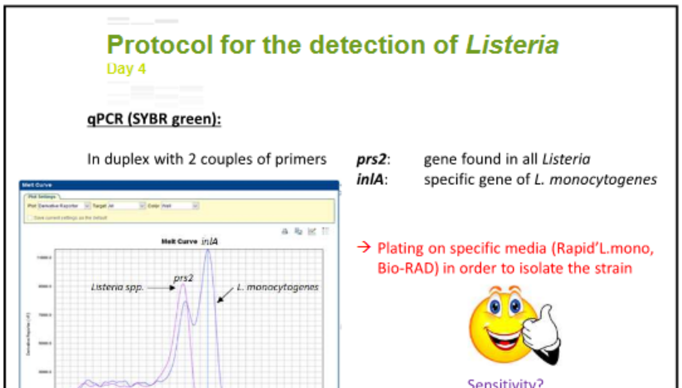
Day 2 and Day 4

Bacterial cells' processing:

Washing with
1 mL H_2O
(2x)

Then,

Boiling 10 min



Protocol for the detection of *Listeria*

Day 4

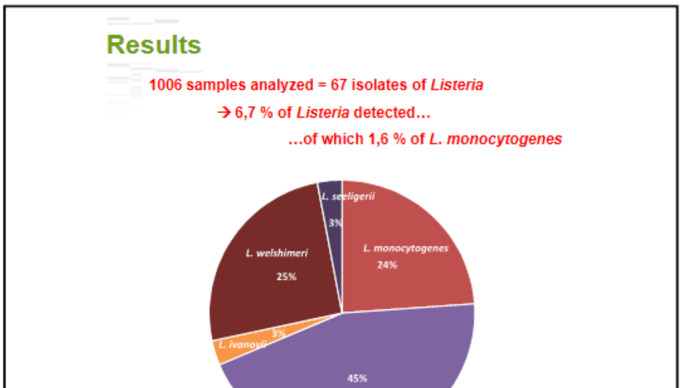
qPCR (SYBR green):

In duplex with 2 couples of primers

prs2: gene found in all *Listeria*
inlA: specific gene of *L. monocytogenes*

→ Plating on specific media (Rapid'L.mono, Bio-RAD) in order to isolate the strain

Sensitivity?

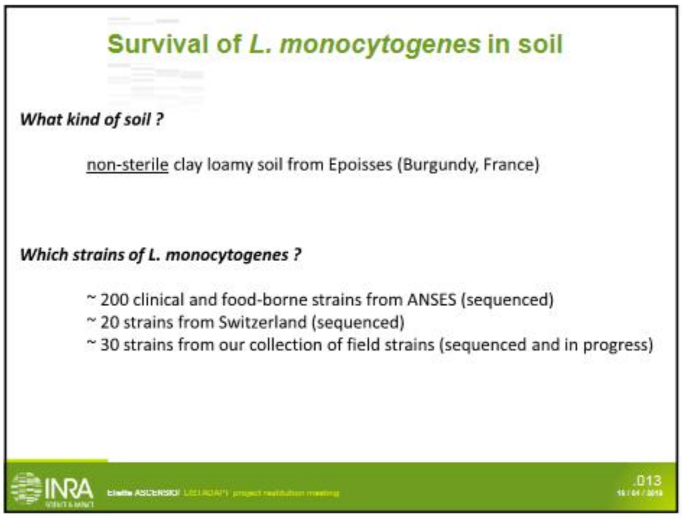


Results

1006 samples analyzed = 67 isolates of *Listeria*

→ 6,7 % of *Listeria* detected…

…of which 1,6 % of *L. monocytogenes*



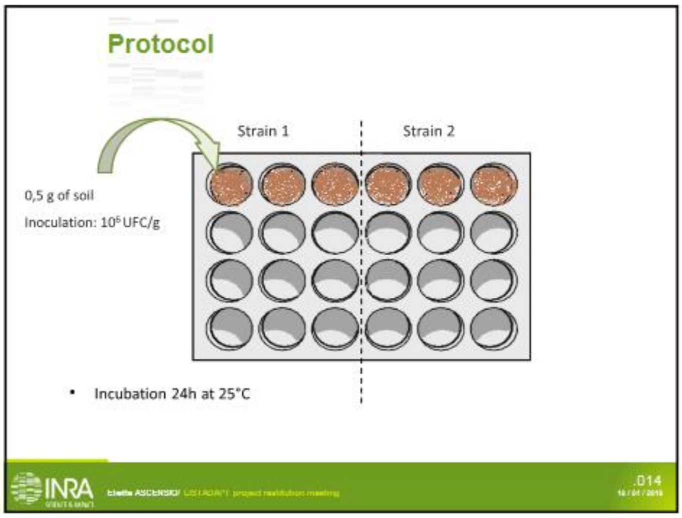
Survival of *L. monocytogenes* in soil

What kind of soil ?

non-sterile clay loamy soil from Epoisses (Burgundy, France)

Which strains of L. monocytogenes ?

~ 200 clinical and food-borne strains from ANSES (sequenced)
~ 20 strains from Switzerland (sequenced)
~ 30 strains from our collection of field strains (sequenced and in progress)

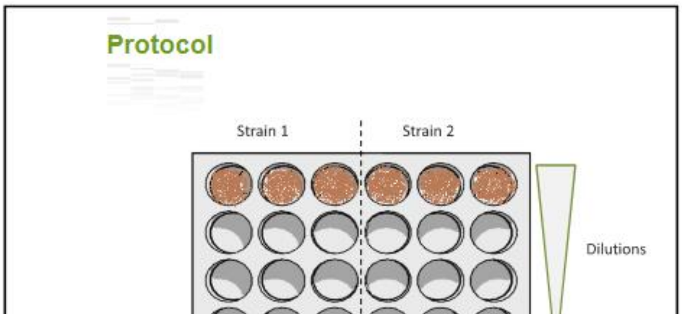


Protocol

Strain 1
Strain 2

0,5 g of soil
Inoculation: 10^6 UFC/g

- Incubation 24h at 25°C

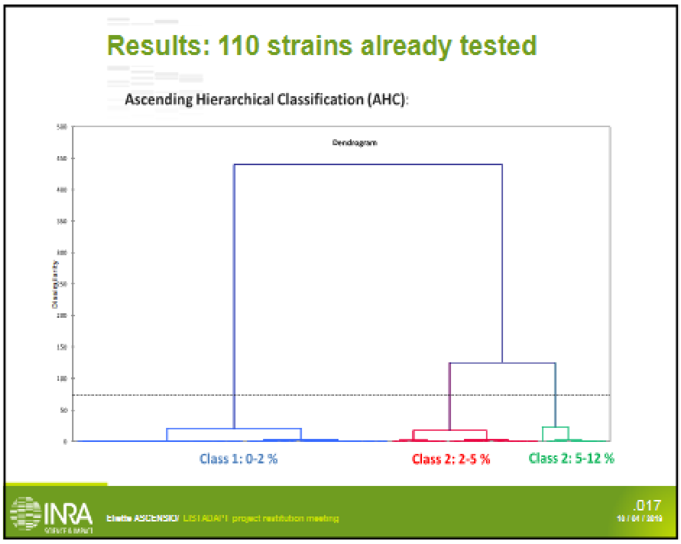


Protocol

Strain 1
Strain 2

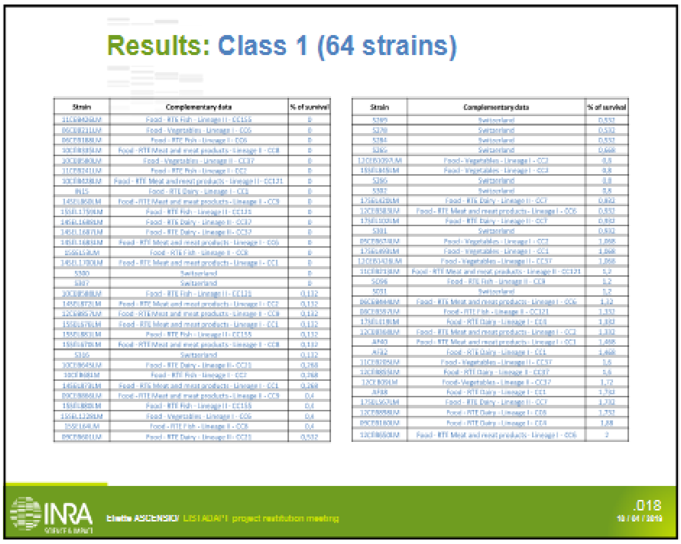
Dilutions

Protocol

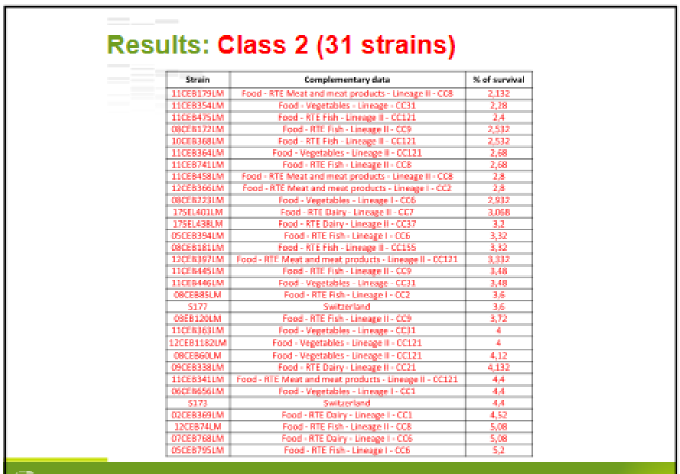


Results: 110 strains already tested

Ascending Hierarchical Classification (AHC):

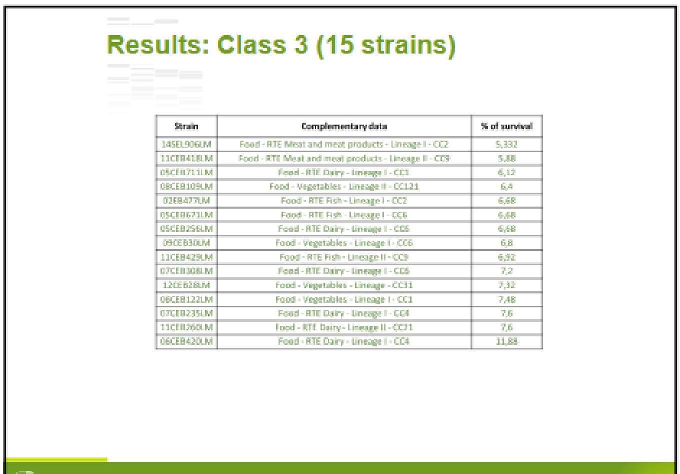


Results: Class 1 (64 strains)



Results: Class 2 (31 strains)

Strain	Complementary data	% of survival
11CEB179LM	Food - RTE Meat and meat products - Lineage II - CC8	2,132
11CEB354LM	Food - Vegetables - Lineage - CC31	2,28
11CEB475LM	Food - RTE Fish - Lineage II - CC121	2,4
08CEB172LM	Food - RTE Fish - Lineage II - CC9	2,512
10CEB368LM	Food - RTE Fish - Lineage II - CC121	2,532
11CEB364LM	Food - Vegetables - Lineage II - CC121	2,68
11CEB741LM	Food - RTE Fish - Lineage II - CC8	2,68
11CEB458LM	Food - RTE Meat and meat products - Lineage II - CC8	2,8
12CEB366LM	Food - RTE Meat and meat products - Lineage I - CC2	2,8
08CEB223LM	Food - Vegetables - Lineage I - CC6	2,932
17SEL401LM	Food - RTE Dairy - Lineage II - CC7	3,068
17SEL438LM	Food - RTE Dairy - Lineage II - CC37	3,2
05CEB394LM	Food - RTE Fish - Lineage I - CC6	3,32
08CEB181LM	Food - RTE Fish - Lineage II - CC155	3,32
12CEB397LM	Food - RTE Meat and meat products - Lineage II - CC121	3,332
11CEB445LM	Food - RTE Fish - Lineage II - CC9	3,48
11CEB446LM	Food - Vegetables - Lineage - CC31	3,48
08CEB885LM	Food - RTE Fish - Lineage I - CC2	3,6
S177	Switzerland	3,6
03EB120LM	Food - RTE Fish - Lineage II - CC9	3,72
11CEB363LM	Food - Vegetables - Lineage - CC31	4
12CEB1162LM	Food - Vegetables - Lineage II - CC121	4
08CEB860LM	Food - Vegetables - Lineage II - CC121	4,12
09CEB338LM	Food - RTE Dairy - Lineage II - CC21	4,132
11CEB341LM	Food - RTE Meat and meat products - Lineage II - CC121	4,4
06CEB656LM	Food - Vegetables - Lineage I - CC1	4,4
S173	Switzerland	4,4
02CEB369LM	Food - RTE Dairy - Lineage I - CC1	4,52
12CEB74LM	Food - RTE Fish - Lineage II - CC8	5,08
07CEB768LM	Food - RTE Dairy - Lineage I - CC6	5,08
05CEB795LM	Food - RTE Fish - Lineage I - CC6	5,2



Results: Class 3 (15 strains)

Strain	Complementary data	% of survival
14SEL906LM	Food - RTE Meat and meat products - Lineage I - CC2	5,332
11CEB418LM	Food - RTE Meat and meat products - Lineage II - CC9	5,88
05CEB711LM	Food - RTE Dairy - Lineage I - CC1	6,12
08CEB109LM	Food - Vegetables - Lineage II - CC121	6,4
02EB477LM	Food - RTE Fish - Lineage I - CC2	6,68
05CEB671LM	Food - RTE Fish - Lineage I - CC6	6,68
05CEB256LM	Food - RTE Dairy - Lineage I - CC6	6,68
09CEB30LM	Food - Vegetables - Lineage I - CC6	6,8
11CEB429LM	Food - RTE Fish - Lineage II - CC9	6,92
07CEB308LM	Food - RTE Dairy - Lineage I - CC6	7,2
12CEB28LM	Food - Vegetables - Lineage - CC31	7,32
06CEB122LM	Food - Vegetables - Lineage I - CC1	7,48
07CEB235LM	Food - RTE Dairy - Lineage I - CC4	7,6
11CEB260LM	Food - RTE Dairy - Lineage II - CC21	7,6
06CEB420LM	Food - RTE Dairy - Lineage I - CC4	11,88

Results

HETEROGENICITY of the survival rates for *L.monocytogenes*

→ Analysis of the sequences
(core genome + accessory genome)

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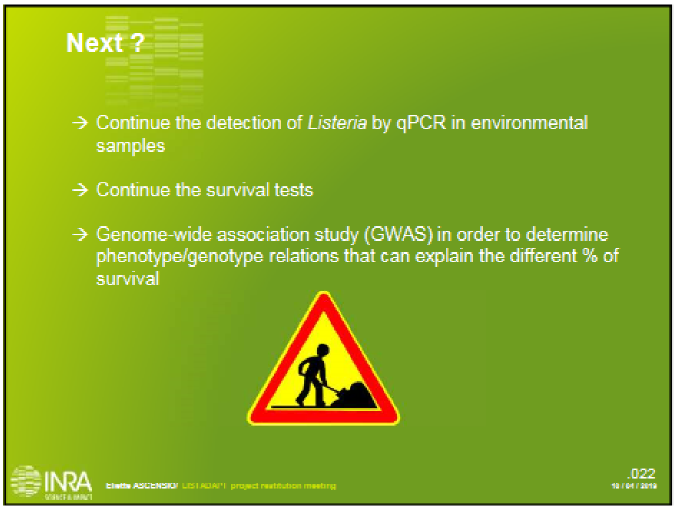

Next ?

→ Continue the detection of *Listeria* by qPCR in environmental samples

→ Continue the survival tests

→ Genome-wide association study (GWAS) in order to determine phenotype/genotype relations that can explain the different % of survival

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