EURL for *Lm*

European Union Reference
Laboratory for
Listeria monocytogenes

Anses/EURL *Listeria monocytogenes* research projects based on WGS:

Genomics of *Listeria monocytogenes*

Sophie ROUSSEL, SEL Unit

Anses PhD project :
Listeria team /DTU-Food (Franck Aarestrup's team; René Hendriksen)

PhD student : Clementine Henri (December 2013-December 2016)

In close collaboration with :
-Ifip (The French Pork Institute ; Carole Feurer)
-PHE (UK NRL for *Lm* ; Corinne Amar)
-INRA Loire Valley Centre (France ; Sylvie Roche)

Aims

1. To compare the genetic diversity of food strains in France

2. To identify the genetic factors that may explain the virulence differences observed within the strain populations. To do a "Stratification" of the virulence ??

   Some CC specific of hypovirulent strains ?

3. To assess cutting-edge technology such as NGS as a molecular typing tool; to compare it to the standard typing methods (PFGE, MLST).
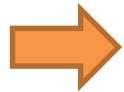
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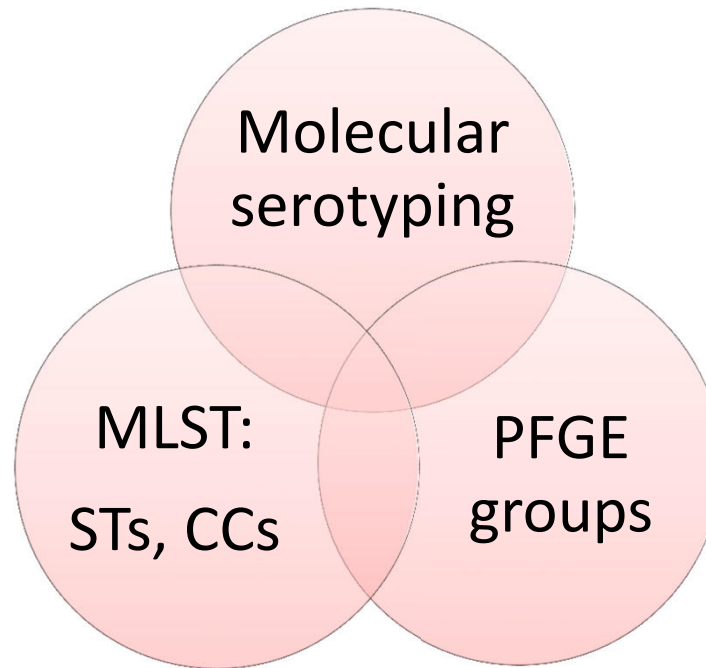
Aims

To understand the distribution of clones
in the different food sectors
Master student : Diane Plouchart (February 2014-July 2014)

➡ To structure large food strain populations using both PFGE and MLST
to assess the prevalence and distribution of CC among the most PFGE groups

Molecular
serotyping

MLST:
STs, CCs

PFGE
groups

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Methodology used for the PhD Project

Brisse 2012

Lineage   Serotype   **MLST**   PFGE   WGS
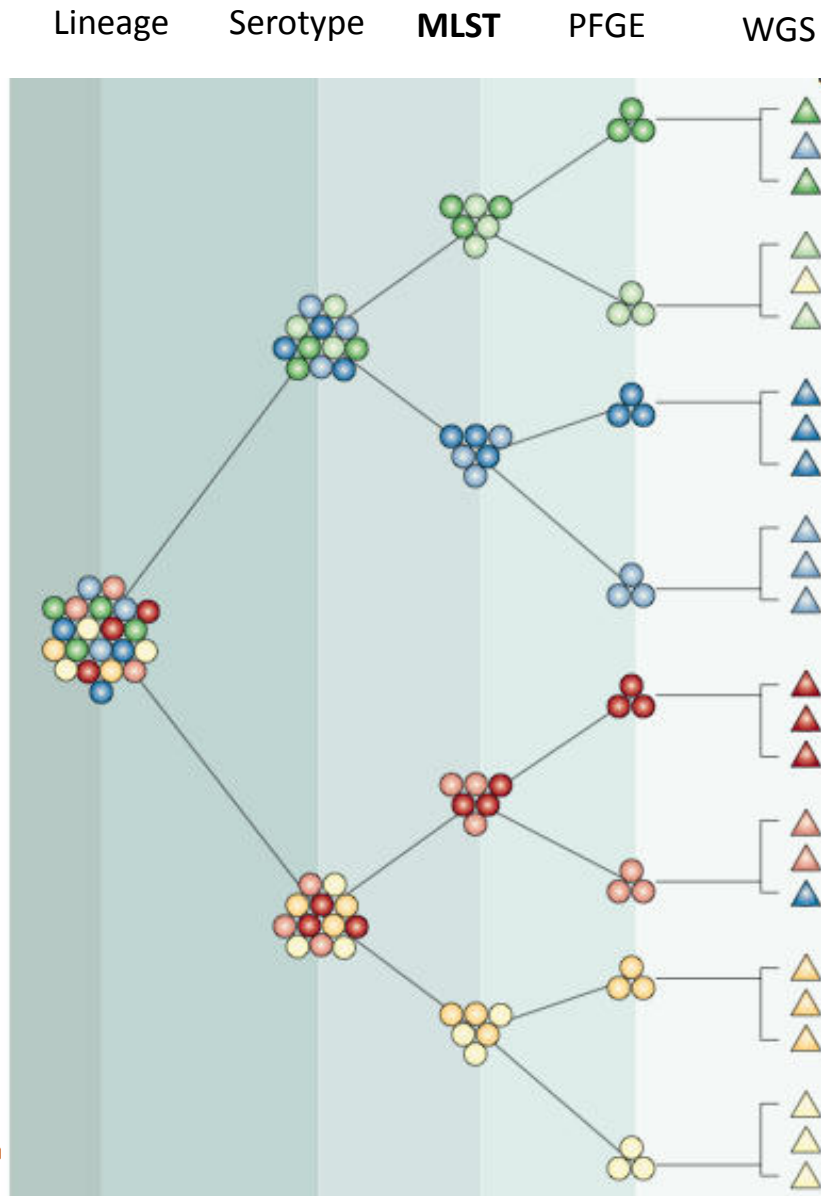
MLST, WGS
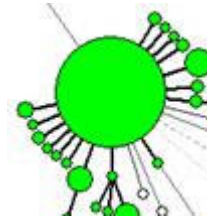
Population biology,
Global epidemiology
Phylogeny

PFGE

• Subtyping
• Local epidemiology
• Outbreak investigation

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PFGE and MLST

MLST

- Clonal population study
- Global epidemiology
- Phylogeny

PFGE

- Micro-epidemiology
- Epidemio - surveillance
- Outbreak investigation
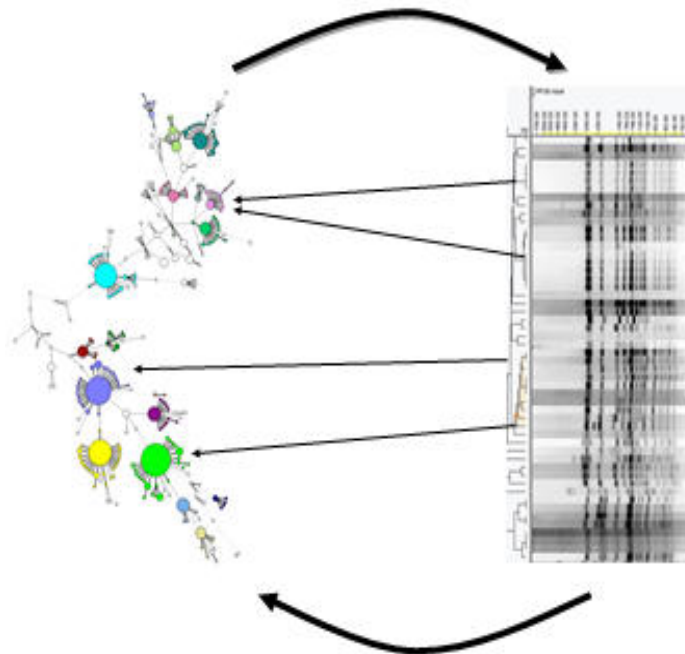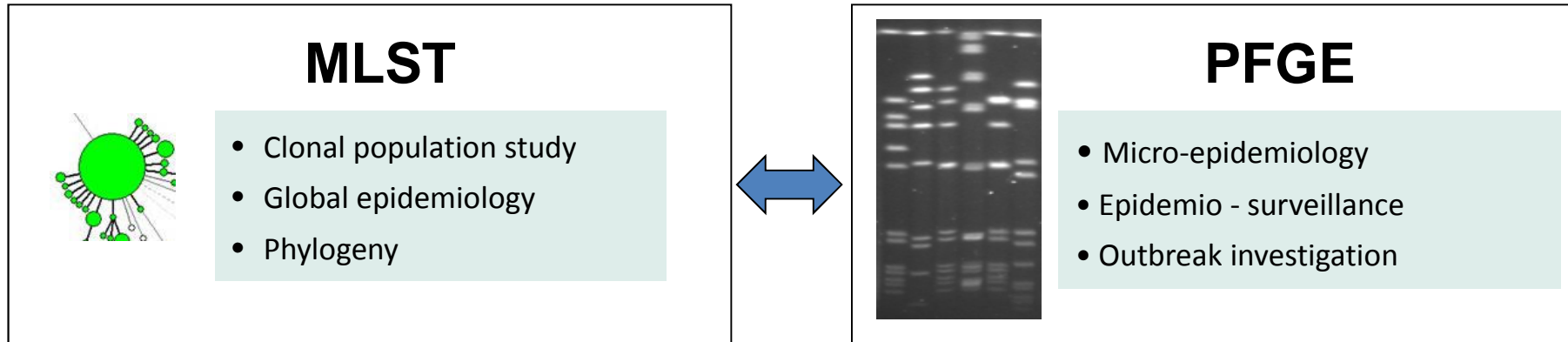
Link PFGE – MLST through dictionary, Brisse 2012

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Scientific background

- Strains isolated from pork chain :

2002-2004 : A French research program (Anses, Ifip, Pasteur Institute)
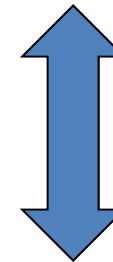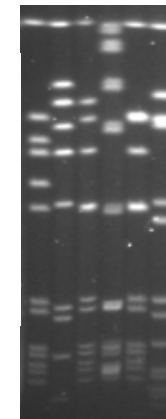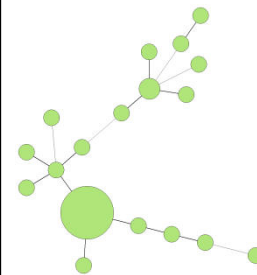
      not a lot of diversity among the PFGE profiles

      78% of strains isolated from pork products : two PFGE groups « a1 » et « a2 » (Hong et al., 2007 et Giovannacci et al. 1999)

- 2012: Roche et al.: "stratification" of virulence of food and clinical strains repartition of hypovirulent strains in some specific genetic groups

- 2010-2012 : French partnership between Pasteur Institute/Anses

Preliminary results :

CC121 is the most frequent Clonal Complex among a large panel of food strains   - CC121 is the clonal complex including the less of clinical strains

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Materials & methods

On a panel of 1907 strains (French NRL database):

- -121 strains isolated from clinical cases

- -284 strains isolated from pork products

- -92 strains for which virulence was evaluated using *in vivo* and *in vitro* tests

Molecular serotyping results

Serogroups	Food products and food processing environment							Clinical cases	Animal	Environnement	n.g.	Total
	Meat and meat products	Milk and milk products	Fruit vegetables, seeds and cereals	Fish and fishery products	Elaborated food products combining several food categories	Eggs	Other categories					
IIa	354	161	42	154	134	3	70	36	18	11	118	1001 53%
IIc	172	11		9	32		27	9	2	1	4	267 14%
IVb	125	75	15	32	37		40	56	9	22	12	423 22%
IIb	61	33	5	26	22		21	18	10	7	2	205 10%
IVa								2	2			4 <1%
Total	712	280	62	221	225	3	158	121	41	41	36	1900

Table 2 : dispatch of the 1907 strains

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Tableau 2 : Répartition des 1907 souches

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Tableau 2 : Répartition des 1907 souches

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PFGE results

Average similarity UPGMA dendrograms UPGMA AscI et ApaI (Dice coefficient, optimization et tolerance set at 1%.)

72 PFGE groups

- 5 groups > 100 strains
- 43 groups < 10 strains

PFGE results

PFGE groups	N=	Serogroups			
		IIa	IIb	IIc	IVb
A	402	400		2	
B	265	19		243	
C	135	135			
D	121		1		119
E	118				117
F	90		1		88
G	84		81		3
H	66		61		4
I	65		2		63
J	47	46		1	

PFGE results

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F	90		1		88
G	84		81		3
H	66		61		4
I	65		2		63
J	47	46		1	

IIa gathered the majority of the strains

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PFGE results

PFGE groups	N=	Serogroups				Source	
		IIa	IIb	IIc	IVb	Food products	Clinical cases
A	402	400		2		393	2
B	265	19		243		246	11
C	135	135				114	15
D	121		1		119	88	19
E	118				117	79	25
F	90		1		88	76	7
G	84		81		3	79	5
H	66		61		4	50	8
I	65		2		63	53	2
J	47	46		1		41	2

PFGE groups of clinical cases + food strains

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PFGE results

PFGE groups	N=	Serogroups				Source		Strain isolated from pork products
		IIa	IIb	IIc	IVb	Food products	Clinical cases	
A	402	400		2		393	2	68 (24%)
B	265	19		243		246	11	60
C	135	135				114	15	27
D	121		1		119	88	19	15
E	118				117	79	25	12
F	90		1		88	76	7	6
G	84		81		3	79	5	17
H	66		61		4	50	8	10
I	65		2		63	53	2	7
J	47	46		1		41	2	10

28 PFGE groups

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PFGE results

PFGE groups	N=	Serogroups				Source		Virulence	
		IIa	IIb	IIc	IVb	Food products	Clinical cases	Hypo-virulente	Virulente
A	402	400		2		393	2		
B	265	19		243		246	11	2	5
C	135	135				114	15		3
D	121		1		119	88	19	4	2
E	118				117	79	25	6	9
F	90		1		88	76	7	2	1
G	84		81		3	79	5		
H	66		61		4	50	8	1	3
I	65		2		63	53	2	2	1
J	47	46		1		41	2	3	2

15 PFGE groups

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MLST and PFGE dictionary

- 49 PFGE groups 49 STs,
- 43 STs carried a unique PFGE type
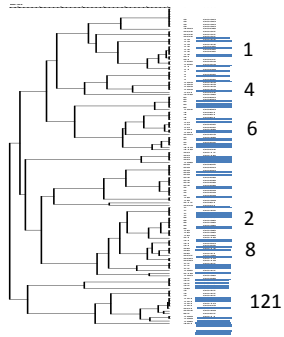
MLST	CC121	CC9
PFGE groups	Group « A » Groupe 10	Group « B » and groups 33, 34, 35

PFGE Groups	A	B
MLST	CC121	CC9

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Results and discussion

- No correlation between the strain origins (food, clinical, etc) and PFGE groups or STs.

- 8 main CCs: **CC121**, CC9, CC1, CC2, CC6, CC8, CC5, CC4

CC121 : clustered few clinical strains

- Dispatch of strains in pork products:
  - **28 PFGE groups**
  - **20STs, 11CCs**

→ Large diversity of strains isolated from pork industry (in the opposite of the results of Hong et al., (2007) and Giovannacci et al. (1999).

- Dispatch of strains for which virulence was evaluated

→ No ST or PFGE groups specific of hypo-virulent strains

- PFGE and MLST

MLST /PFGE

   44 PFGE groups   : 1ST

→ 10 PFGE groups : related to several STs

→ 18 PFGE groups : new ST

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2. To identify the genetic factors that may explain the virulence differences observed within the strain populations. To do a "Stratification" of the virulence ??

Some CC specific of hypovirulent strains ?

Toward the the set up of a French reference strain panel….

- Selection of a food strain panel representative of the genetic diversity observed in France over the 20 past years : (with some strains of virulence degree known, historical strains)

- at less one strain for each PFGE type : 196 strains : WGS (Oxford, DTU)

- Genome comparison on going at DTU

- Sequences will be used for the Compare project . (WPs 4 and 7 : collection of well-described genomes that can be used for testing different analysis approaches for outbreak detection, linking of isolates from sources and reservoirs)

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Toward the set up of a European reference strain panel....

- Are you interested in a collaboration with the EURL to set up a European reference strain panel.???

  Strain Selection according to criteria to define together   :
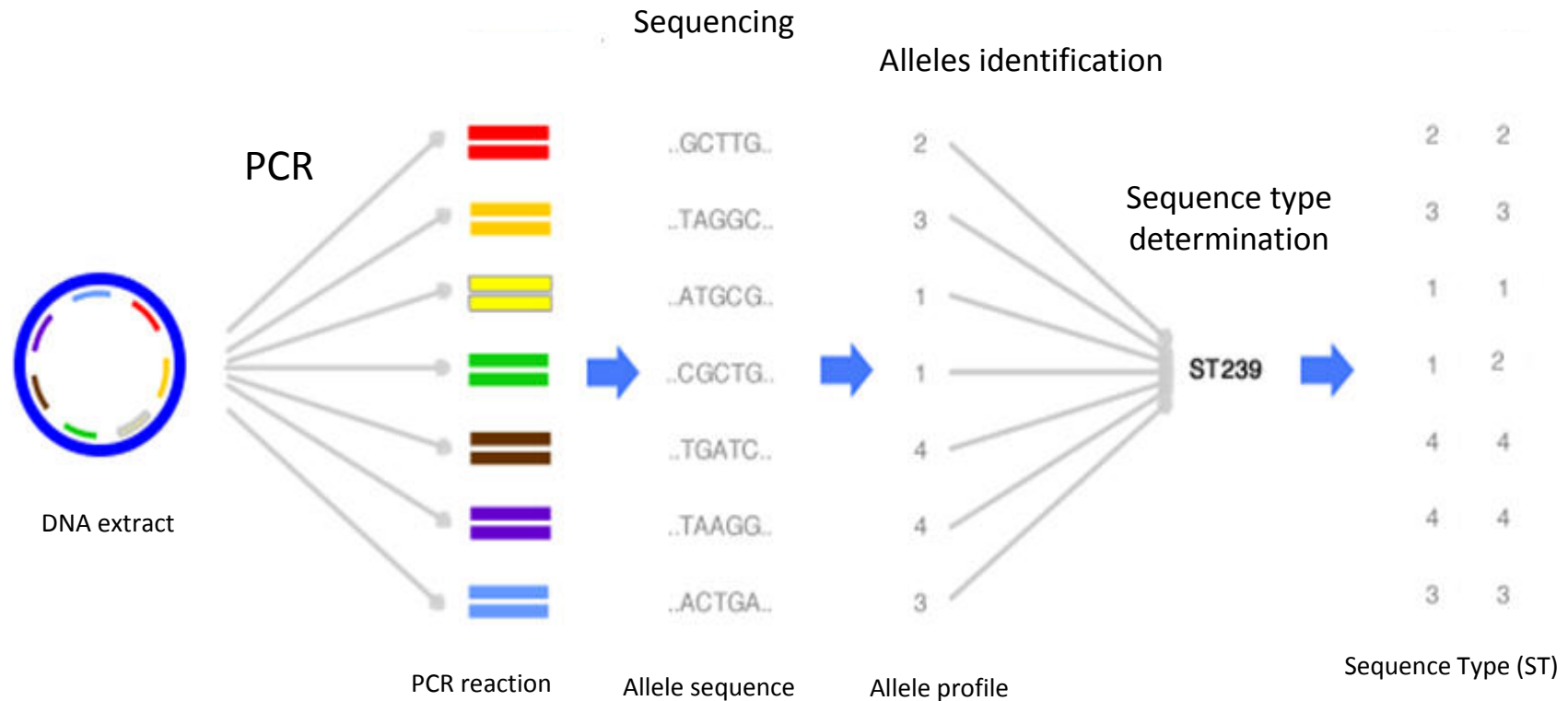
  PFGE profiles, serotypes, metadata
  (Using the EURL Lm DB),

  epidemiologically related outbreak strains, no related strains, metadata, historical strains....)

  Damien : leader of this new EURL project

  Strain sequences could be potentially used for COMPARE or other European projects to initiate together

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MLST : MultiLocus Sequence Typing

Sequencing

Alleles identification

PCR

..GCTTG.. 2

..TAGGC.. 3

Sequence type determination

..ATGCG.. 1

..CGCTG.. 1 → ST239

..TGATC.. 4

..TAAGG.. 4

..ACTGA.. 3

DNA extract

2	2
3	3
1	1
1	2
4	4
4	4
3	3

PCR reaction   Allele sequence   Allele profile

Sequence Type (ST)

7 housekeeping genes (**Ragon et al, 2008 ; Haase et al, 2011**):

abcZ (**transporteur ABC**)
dat (**transférase**)
bglA (**β-glucosidase**)
cat (**catalase**)

dapE (**succinyl diaminopinelate desuccinylase**)
ldh (**lactate déshydrogénase**)
lhkA (**histidine kinase**)

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