Annexes

Population genetic structure and diversity

- Great diversity of strains : genetic make-up / virulence level

- Few clonal groups ="major clones" : majority of outbreaks in France and in the word (Ragon et al., 2008, Plos Pathogens ; Chenal-Francisque, V. et al., 2011 JCM; 2013 JCM ; Cantinelli et al., 2013, JCM)

- Sporadic human cases in France and in the world : mostly caused by isolates that belong to these clones (Ragon et al., 2008, Plos Pathogens ; Haase et al. 2011 Environ Microbiol; Chenal-Francisque, V. et al., 2011 JCM; 2013 JCM ; Cantinelli et al., 2013, JCM)

Population genetic structure and diversity

- Limited data on these clones:

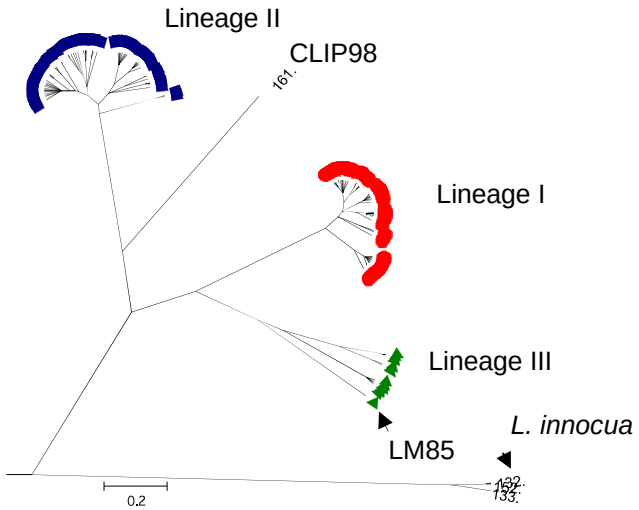
  Lack of information on

  - ➡ Diversity according to the sources (human,animals,food)?
  - ➡ Distribution according to the different food sectors ?

Diversity and evolution of serotypes within lineages

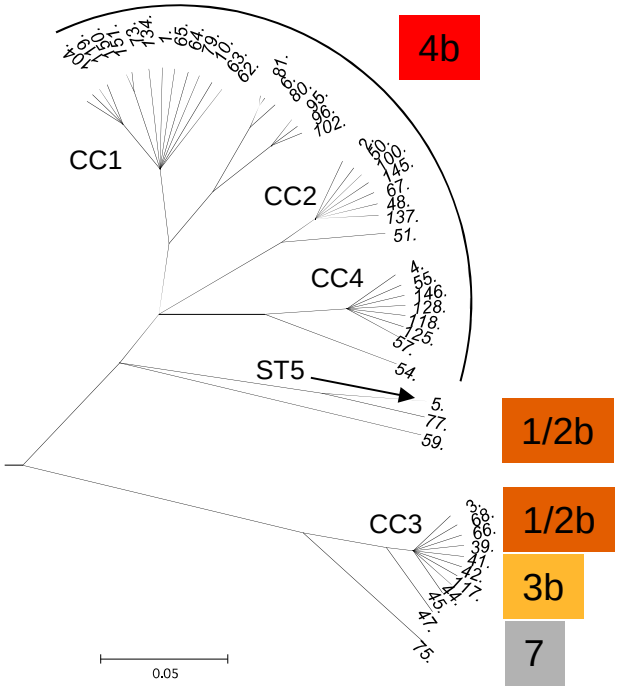
(Brisse, EURL workshop, Maisons-Alfort, 2012)

L. monocytogenes

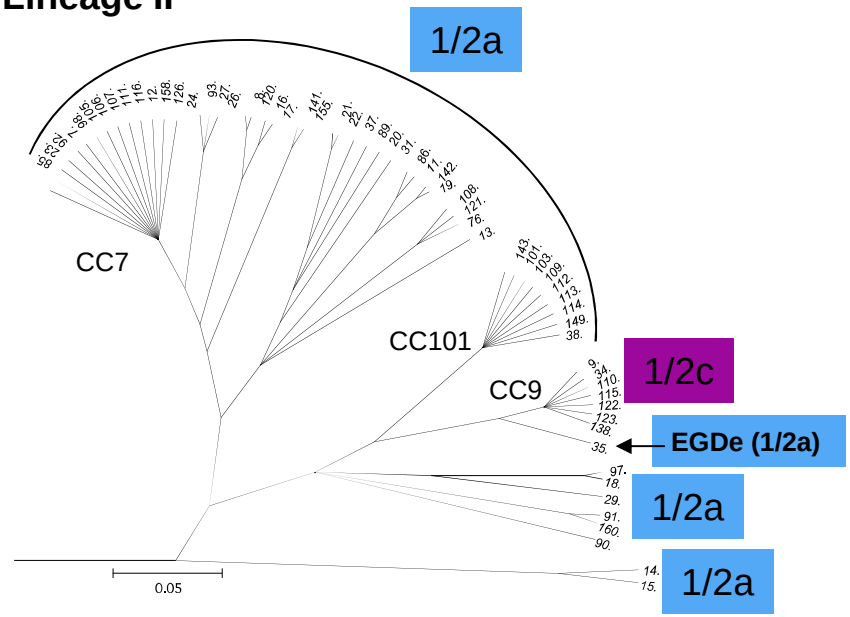


Ragon et al.,
PLoS Pathogens 2008

Lineage I



Lineage II



- Lineages and serotypes are heterogeneous
- 4b monophyletic, evolved from 1/2b
- 1/2c recently derived from 1/2a
- EGDe: '1/2a with 1/2c genome'

Molecular serotyping

- The first-line approach
- The European standard for typing tool : (Doumith et al., 2004)

Limitations:

-Strains such as 1/2a EGDe strain : not grouped into the expected molecular serogroup : IIc instead of IIa (Doumith et al., 2004)

-The variant profile of serogroup IVb, characterized by the amplification of a supplementary gene fragment (lmo0737 gene fragment). Graves LM et al 2007 ; De Vasconcelos et al., 2008 ; Huang B et al., JCM 2011 ;Leclercq et al., 2011

2013 Whole genome deposited at GenBank (5 strains)

➡ Fine genomic characterization /pan-genomic microarray.: Laksanalamai et al., 2014, Plos one ;

Crucial to develop a new phylogenic scheme

PFGE

- The current international standard for subtyping (Graves & Swaminathan, 2001)

- Still useful tool to investigate outbreaks

limitations :

- Explore only a limited area of the bacterial genome.
- It Does not show the relatedness between strains.
- DNA methylation can make restriction sites invisible to enzymes

Anses/EURL research program

3 Aims :

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

- To define another typing new phylogenic scheme;

- To identify the genetic factors that may explain the virulence differences observed within the strain populations.

Outlines

1. To compare the genetic diversity of animal, food and clinical strains in Europe

2. Identify genomic determinants underlying virulence. Novel diagnostic Targets/signatures

3. Transform bacterial with the identified factors to determine whether these factors are involved in strain virulence

4. Screen markers of interest on a large strain population

5. Develop rapid molecular tests to detect potentially pathogenic strains.

Outlines

1. Compare the genetic diversity of animal, food and clinical strains in Europe

2. To find and to compare genomic determinants underlying virulence factors = Novel diagnostic targets/signatures

3. Transform bacterial with the identified factors to determine whether these factors are involved in strain virulence

4. Screen markers of interest on a large strain population

5. Develop rapid molecular tests to detect potentially pathogenic strains.

Outlines

1. Compare the genetic diversity of animal, food and clinical strains in Europe

2. Identify genomic determinants underlying virulence. Novel diagnostic Targets/signatures

3. To transform bacterial with the identified factors to confirm or not whether these factors are involved in strain virulence

4. Screen markers of interest on a large strain population

5. Develop rapid molecular tests to detect potentially pathogenic strains.

Outlines

1. Compare the genetic diversity of animal, food and clinical strains in Europe

2. Identify genomic determinants underlying virulence. Novel diagnostic Targets/signatures

3. Transform bacterial with the identified factors to determine whether these factors are involved in strain virulence

4. To screen markers of interest on a large strain population

5. Develop rapid molecular tests to detect potentially pathogenic strains.

Outlines

1. Compare the genetic diversity of animal, food and clinical strains in Europe

2. Identify genomic determinants underlying virulence. Novel diagnostic Targets/signatures

3. Transform bacterial with the identified factors to determine whether these factors are involved in strain virulence

4. Screen markers of interest on a large strain population

5. To develop rapid molecular tests to detect potentially pathogenic strains.

anses
French agency for food, environmental and occupational health safety

EURL for *Lm*

European Union Reference Laboratory for *Listeria monocytogenes*

Research project EFSA/BIOCONTAM/2014/01

Comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS) analysis

Background

- Scientific opinion of the BIOHAZ Panel (2008):
  - Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness

  http://www.efsa.europa.eu/en/efsajournal/pub/599.htm
- Scientific reports of EFSA:
  - Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates (2013)

  http://www.efsa.europa.eu/en/efsajournal/pub/3241.htm
  - Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part B: analysis of factors related to prevalence and exploring compliance (2014)

  http://www.efsa.europa.eu/en/efsajournal/pub/3810.htm

Background

- External scientific reports:
  - Statistical analysis of the *Listeria monocytogenes* EU-wide baseline survey in certain ready-to-eat foods Part A: *Listeria monocytogenes* prevalence estimates (2013)

    http://www.efsa.europa.eu/en/supporting/pub/441e.htm
  - Statistical analysis of the *Listeria Monocytogenes* EU-wide baseline survey in certain ready-to-eat foods Part B: analysis of factors related to the prevalence of *Listeria Monocytogenes*, predictive models for the microbial growth and for compliance with food safety criteria (2014)

    http://www.efsa.europa.eu/en/supporting/pub/606e.htm
- Scientific colloquium report (to be published by January 2015):
  - Use of WGs of food-borne pathogens for public health protection

Closing gaps for performing a risk assessment on *L. monocytogenes* in ready-to-eat (RTE) foods (Launching tender with 3 activities - 2014)

Objectives

- To carry out the molecular characterisation of a selection of *L. monocytogenes* isolates from different sources, i.e. RTE foods, compartments along the food chain (e.g. food producing animals, food processing environment), and humans employing WGS analysis

- To analyse the WGS typing data of the selected *L. monocytogenes* isolates with three goals:

  – To explore the genetic diversity of *L. monocytogenes* within and between the different sources and human origin;

Objectives

- To assess the epidemiological relationship of *L. monocytogenes* from the different sources and of human origin considering the genomic information and the metadata available for each isolate;
- To identify the presence of putative markers conferring the potential to survive/multiply in the food chain and/or cause disease in humans (e.g. virulence and antimicrobial resistance).

- To perform a retrospective analysis of outbreak strains (i.e. using a subset of epidemiologically linked human and food isolates) to investigate the suitability of WGS as a tool in outbreak investigations

EFSA contacts

- Scientific - main contact point:

MariaTeresa.DASILVAFELICIO@efsa.europa.eu

- Additional team members following this project:

Winy.MESSENS@efsa.europa.eu

Ernesto.LIEBANACRIADO@efsa.europa.eu

Four partners

- SSI, DK-NPHL, contractor for ECDC : Coordinator (Eva Moller-Nielsen, Jonas T Larsson)
- PHE , UK NRL-NPHL (Corinne Amar, Kathie Grant, Tim Dallman)
- Aberdeen University, UK (K Forbes ; N. Strachan)
- Anses (EURL Listeria monocytogenes), FR (L. Guillier, B. Félix, S. Roussel)

Milestones

- About 1000 human and food strains selected (19 December 2014) (deliverable 1)

- The whole panel : sequenced at PHE from March 2015