

**Listadapt-
Study of the Genetic
diversity of *Listeria
monocytogenes* from
environment to human**



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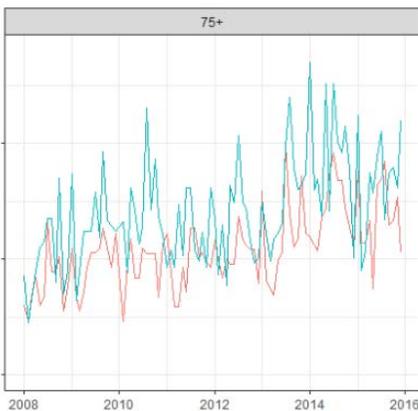
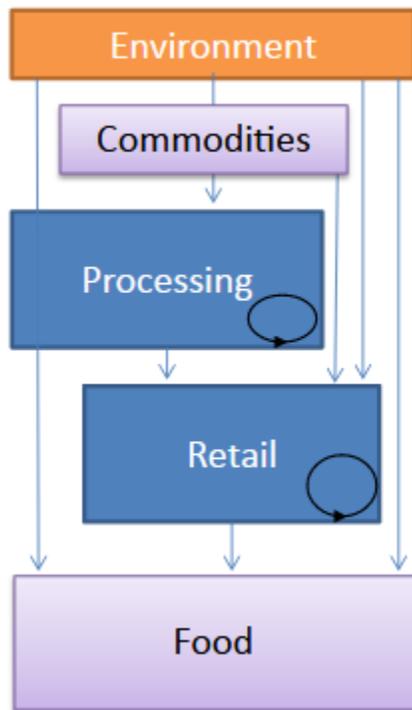
Summary

- Listadapt Project overview
- Listadapt collection
- Methods
- Application on the investigation of CC121 diversity.
- Conclusion

Project overview



Listeria monocytogenes



- Ubiquitous saprophyte & facultative intracellular pathogen
 - ✓ Widely distributed in the environment Soil, water, vegetation
 - ✓ Manufacturing environment persistent strains that have found a harborage site within the facility where it may reside for years
 - ✓ Capable of growing at $T < 4^{\circ} \text{ c}$
- Still a problem for public health
 - ✓ High mortality rate (12,7%) (EFSA 2019)
 - ✓ EFSA's opinion of the increase incidence of listeriosis at EU-level (EFSA 2018)

State of the art

- Performant epidemiological investigation (Moura et al., 2016)
- Knowledge population structure in food (Henri et al. 2016, Maury et al., 2016) , in human (Maury et al., 2016)
- Knowledge on virulence: role of CC (strain-specific virulence difference) (Maury et al., 2016)
- Far less is known on adaptation in environment, farm, industry (despite numerous investigation on persistence in industry)
- Partial knowledge about the genetic factor linked to adaptation to the different ecological niches of *Listeria*

4 objectives of LISTADAPT

LISTADAPT: Adaptive traits of *Listeria monocytogenes* to its diverse ecological niches

1. Decipher genetic traits linked to adaptation in various ecological niches
2. Generate a database of 3000 genomes (mainly farms environment, wild animal, food and human)
3. Develop and implement cutting-edge methodologies (new phenotypic analysis, GWAS)
4. Make available common resources for European research

Listadapt collection



LISTADAPT strain panel:

- European panel Recent : 1990 - 2019
- Farm, soil, cultivated field, pristine water, mud
- Cattle, pig, cow, goat, wild animal
- Food isolates
- Human clinical isolates

Large diversity of animal sources in the collection

Animal pathology



Cow (CH, CZ, FR, EE, HR, IT, LV, SI, SK, UK)
 Sheep (CH, FR, EE, IT, LV, SI, SK, UK)
 Goat (CH, FR, IT, SI, UK)
 Pig (LV)
 Horse (LV)
 Fox (SI)

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Small animals



Slug (NO)



Snail (IT)



Crayfish (IT)



Hedgehog (UK)



Chicken, Birds (FI, SE, NL, IT)



Gerbil (CZ)

Healthy animals



Wild boar (DE, IT, SE)



Sheep (NL, SI, ES, IT)



Mouflon (CZ, SE)



Goat (CH, DE, FR, SI, NL)



Deer (DE, NO, IT, SE)



Badger (IT)



Pig (IT, CZ, FR)



Wolf (IT)



Moose (SE)



Cow (CZ, CH, FI, IT, SI)



Fox (IT, SI)



Bear (IT)

Not classified



Dolphin (IT)

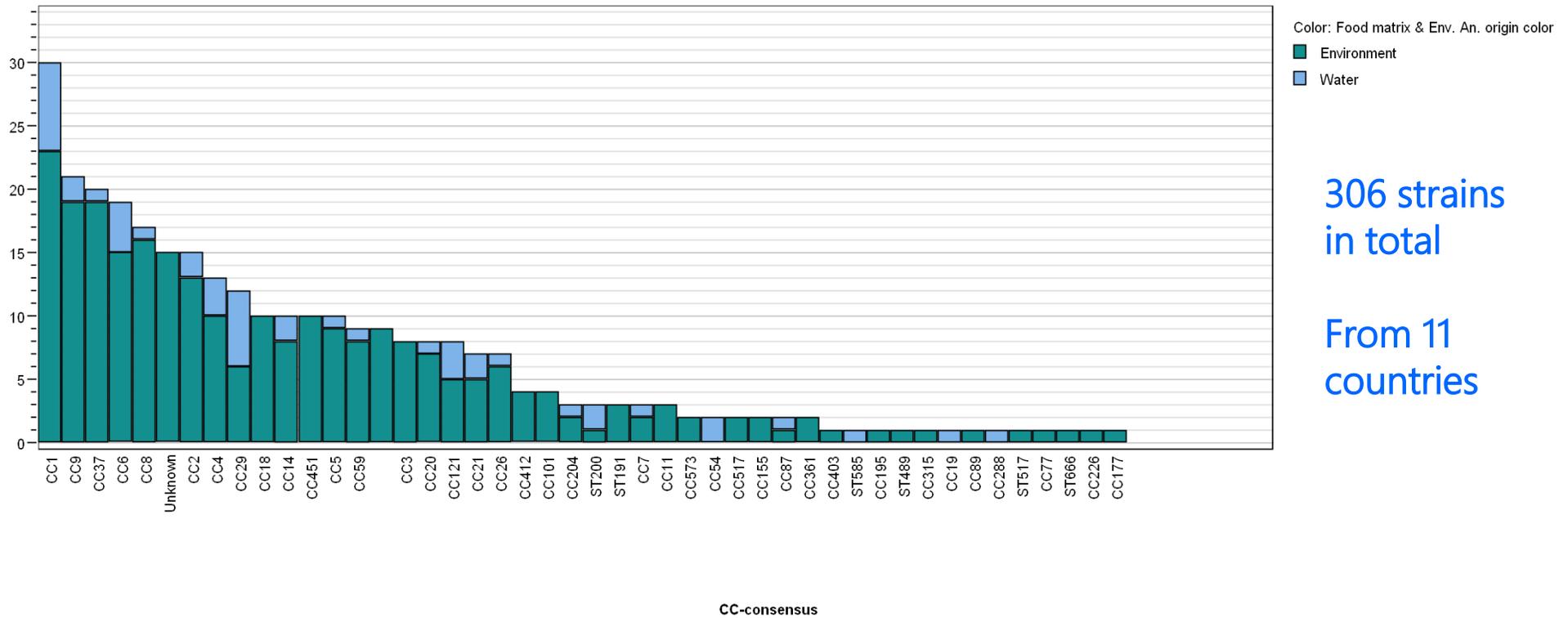
Soil, farm environment



2245 strains

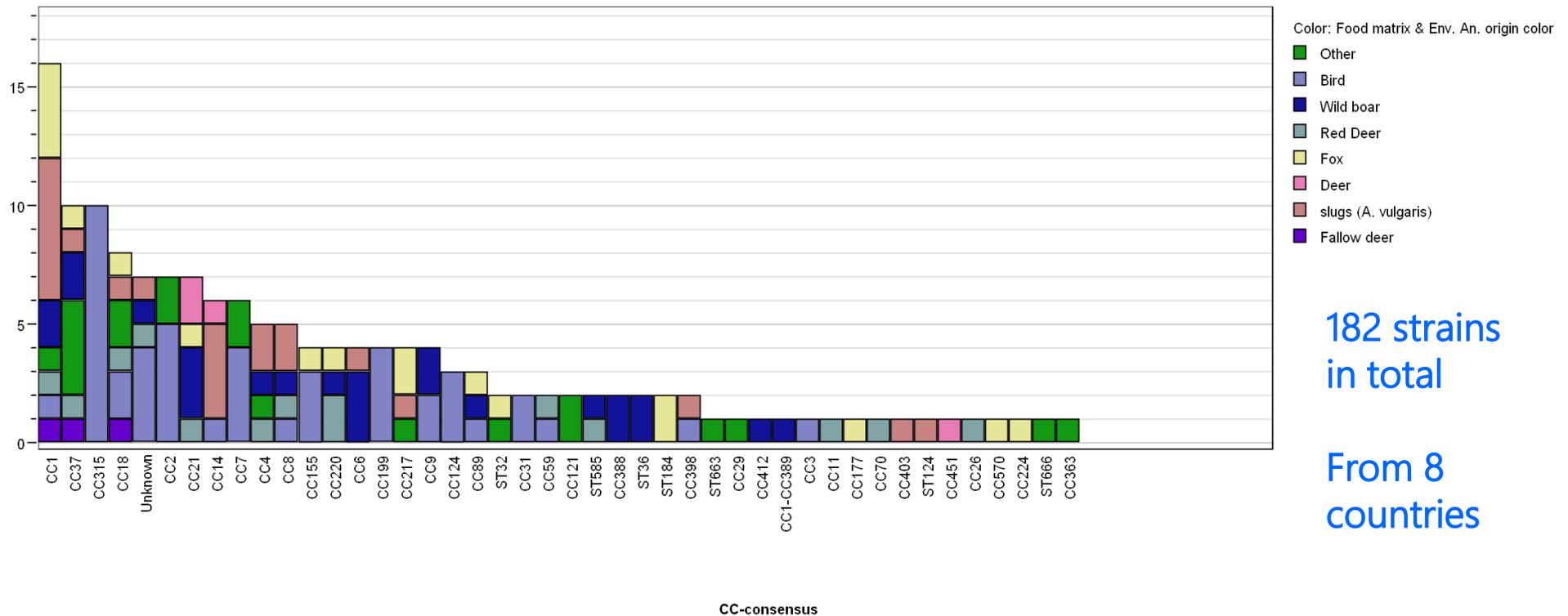
- Environment (306 strains)
 - ✓ Soil (257 str.)
 - ✓ Water (49 str.)
- Wild animals (182 strains)
 - ✓ Venison (91 str.)
 - ✓ Small animal (91 str.)
- Farm animals (777 strains)
 - ✓ Ruminants (669 str.) (pathogenics: 425 str.)
 - ✓ Poultry (6 str.)
 - ✓ Swine (102 str.)
- Animal feed (39 strains)
- Food (685 strains)
 - ✓ Fish (303 str.)
 - ✓ Meat (231 str.)
 - ✓ Dairy product (113 str.)
 - ✓ Vegetables (26 str.)
 - ✓ Composite dishes (13 str.)
- Humans (256 strains)

Environment



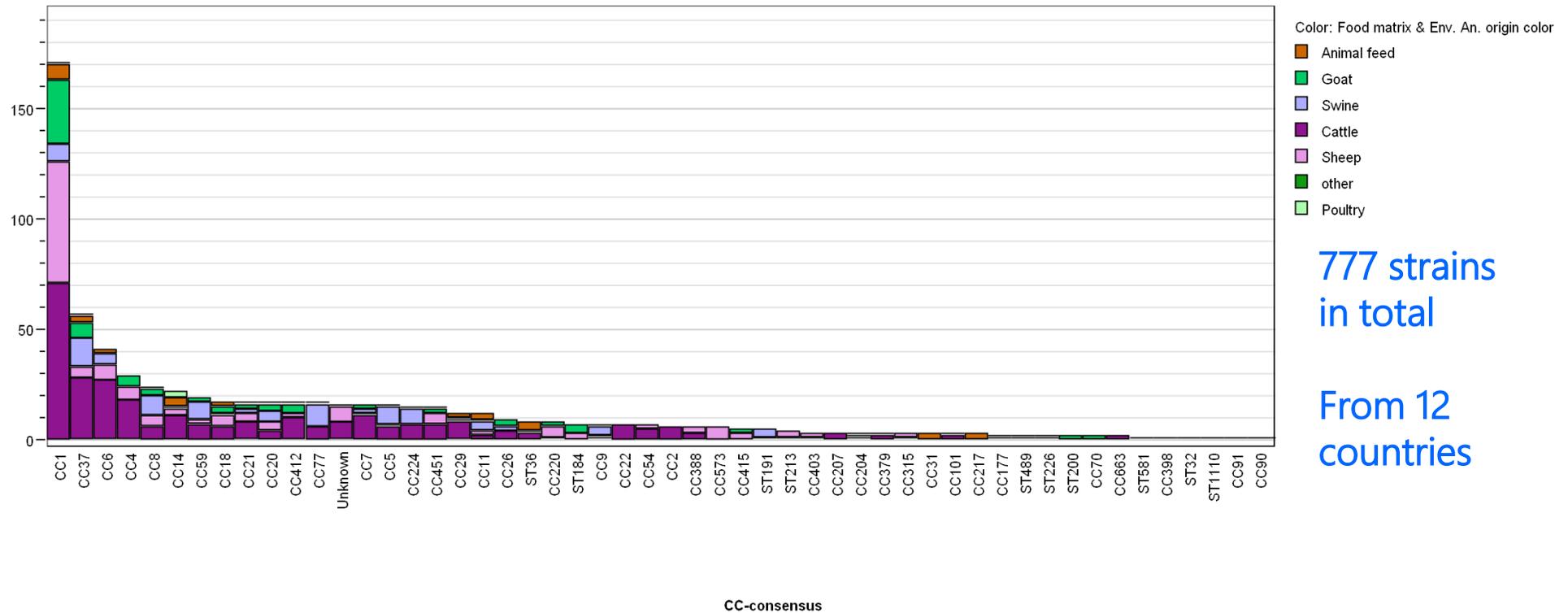
- Cultivated fields, forest, river pond, mud, park, compost and water
- Far from contamination by human or food Lm
- Predominance of the CC1, CC9, CC37, CC6, CC8, CC2, CC4 and CC29

Wild animals



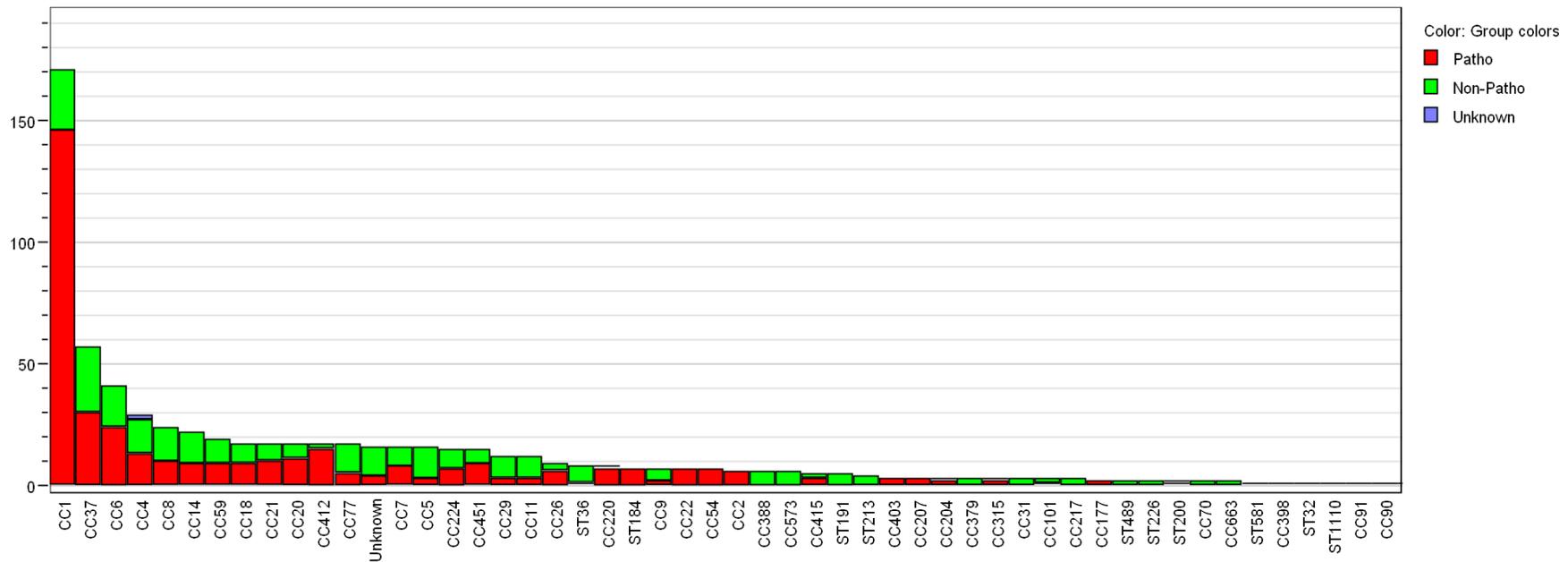
- Mostly from feces sampling
- Other : moose, wolf, bear, crayfish, gerbil, hedgehog, dolphin
- Predominance of the CC1; CC37; CC315; CC18; CC21; CC14; CC7; CC4 and CC8

Farming animals



- Pathogenic & asymptomatics strains
- Strains from farming environment
- Predominance of the CC1, CC37, CC6, CC4, CC8, CC59, CC77, CC412 and CC20

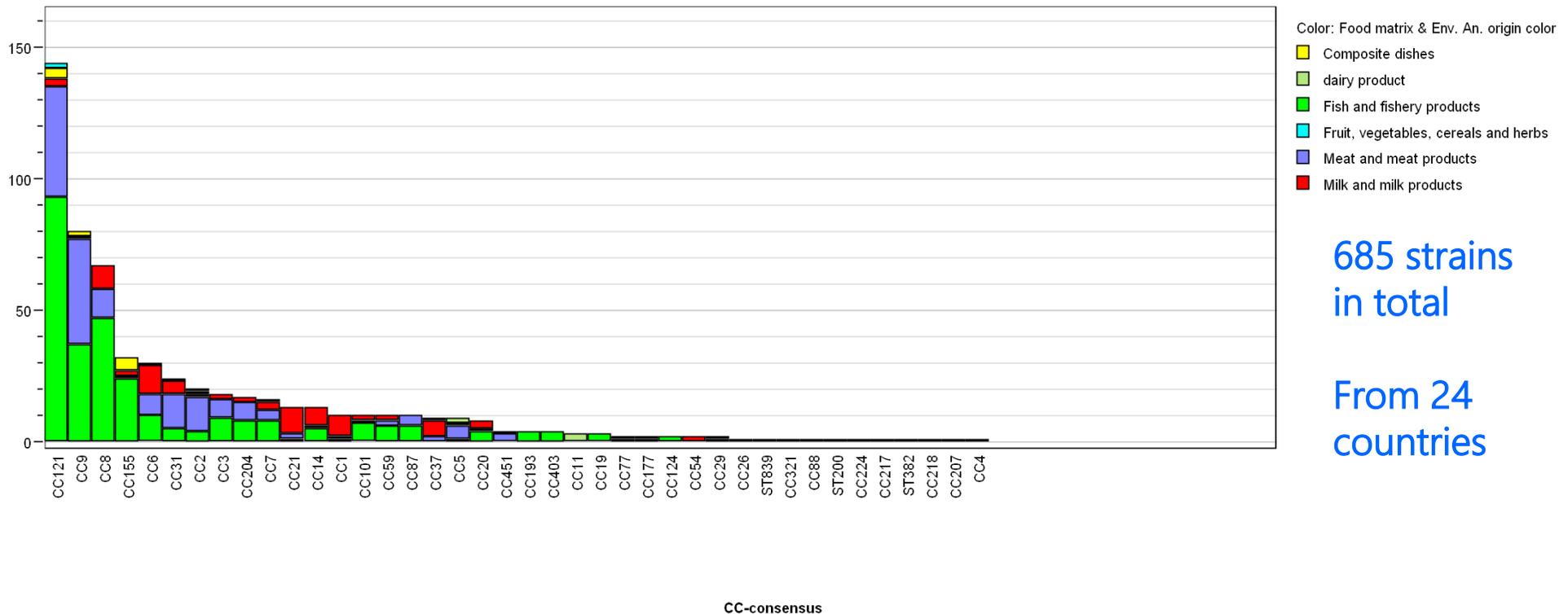
Farming animals patho vs non-patho



CC-consensus

- Pathogenic strains mainly associated with ruminants (90% of the 469 strains)
- Representativity assessed by Bayesian tests
 - ✓ Clinical associated: CC1
 - ✓ Asymptomatic associated: CC5, CC14, CC29
 - ✓ Farming environment associated: CC8

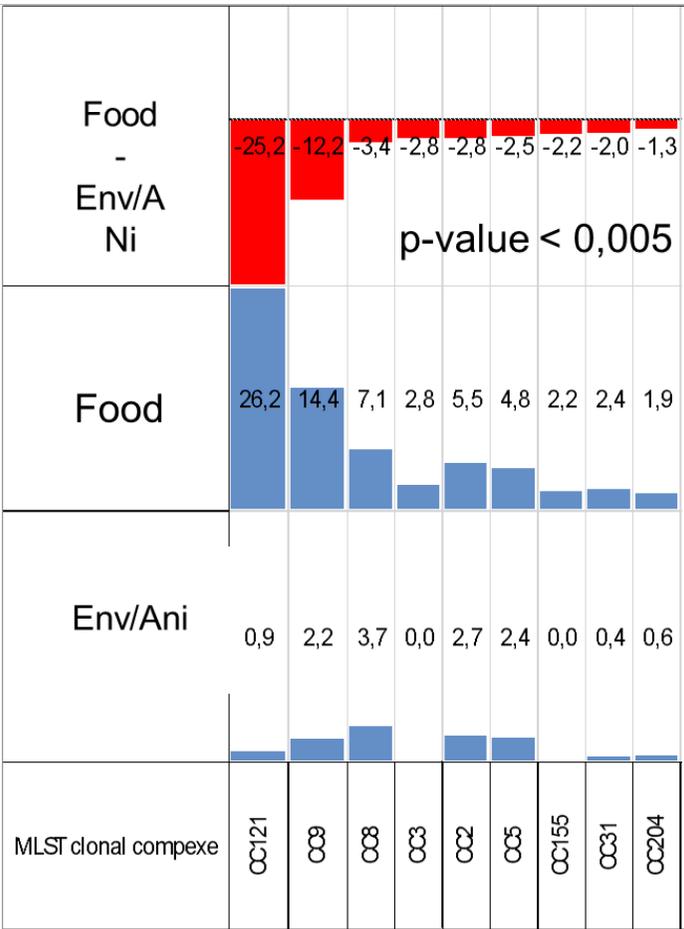
Food strains



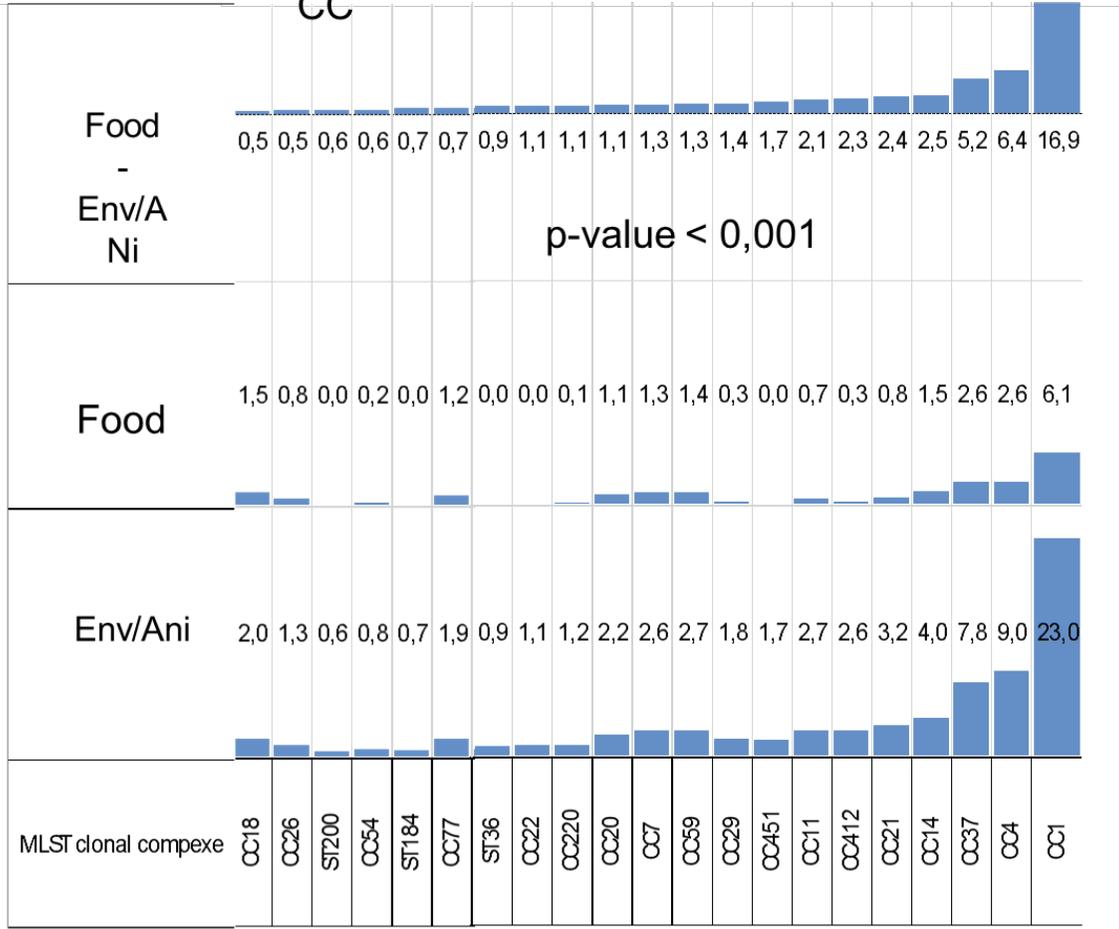
- Majority of strain from smoked fish
- 2nd most important RTE: delicatessen
- Predominance of the CC121, CC9, CC8, CC155, CC6, CC155, CC6, CC31 and CC2

Genetic diversity food vs animal & environment

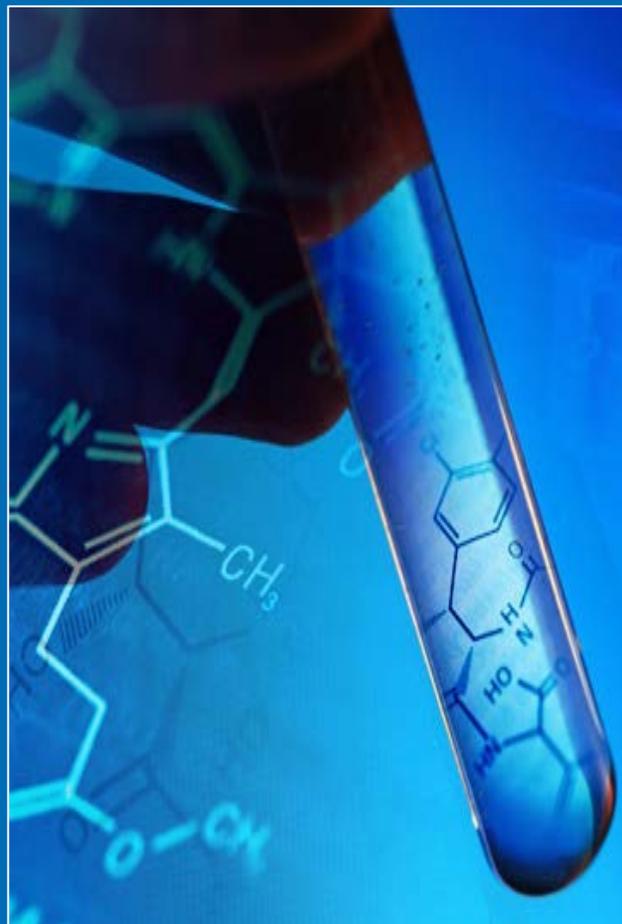
Food associated CC



Environment - Animal associated CC



Methods

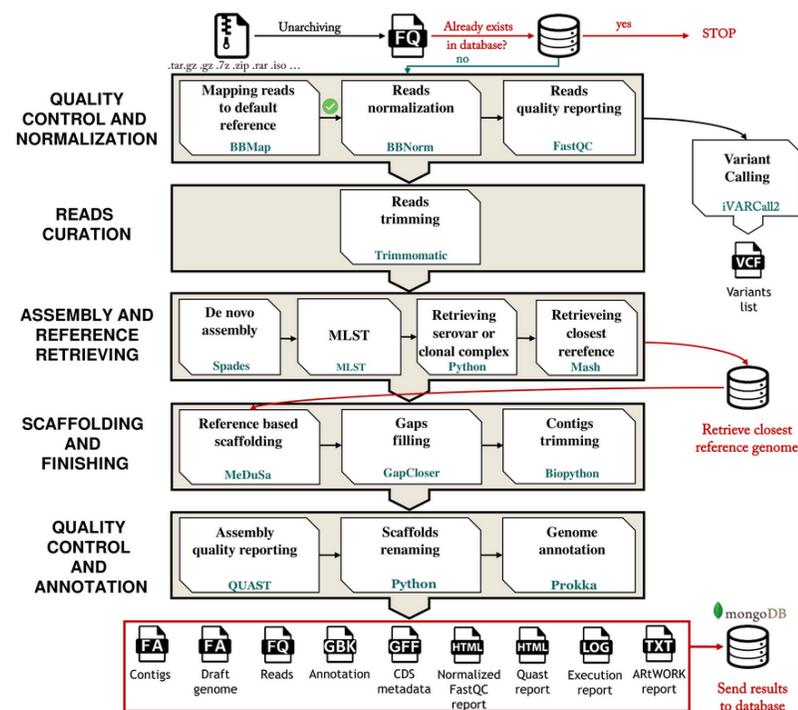
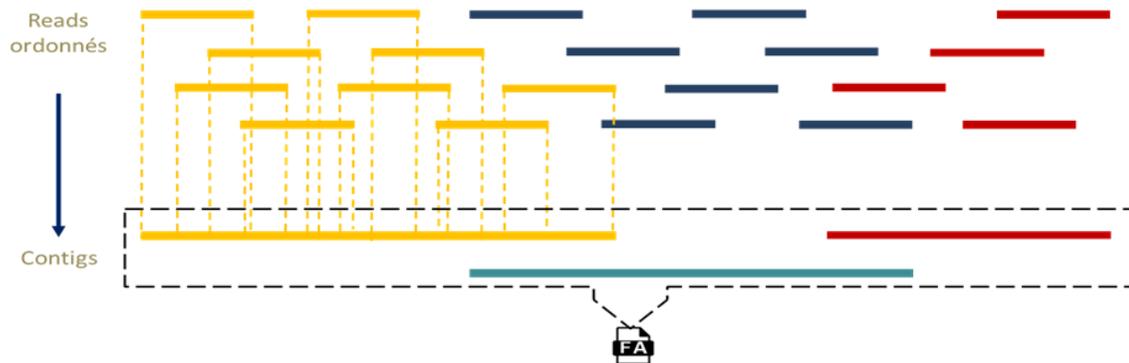


ARTWORK a pipeline for genome assembly and annotation

- Quality assessment
- Trimming
- De novo assembly
- Genome annotation

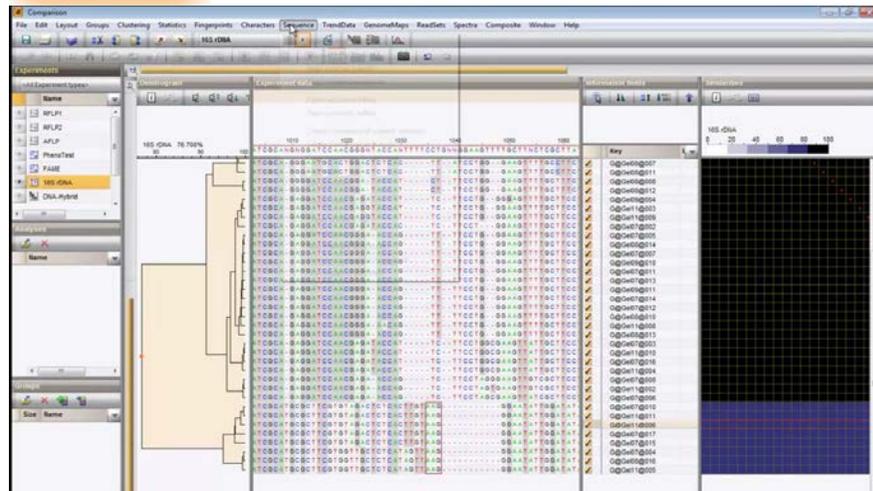


De Novo assembly



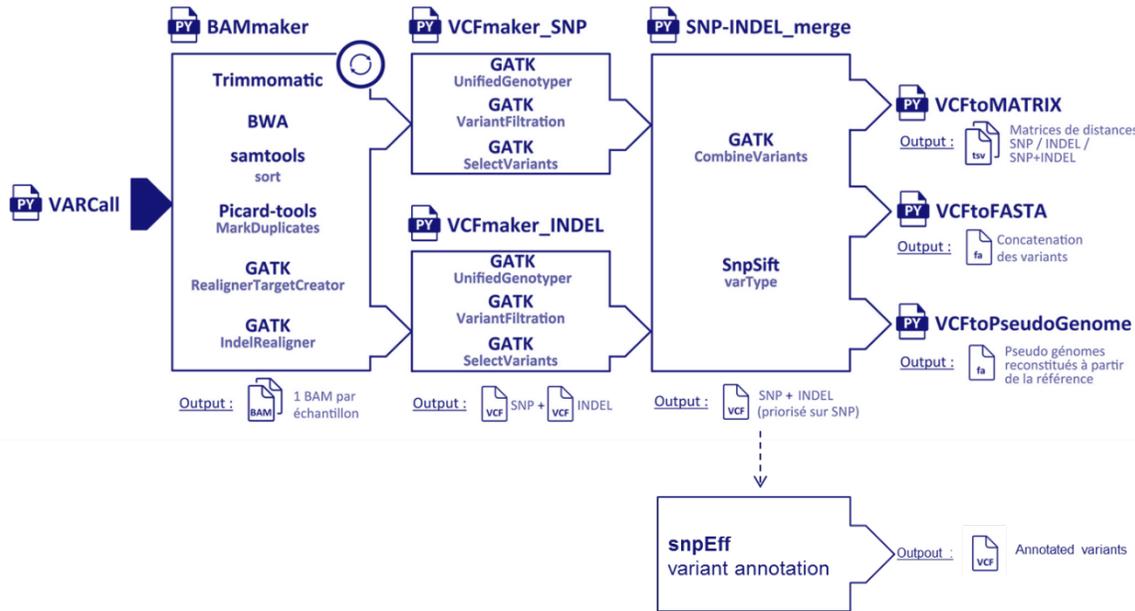
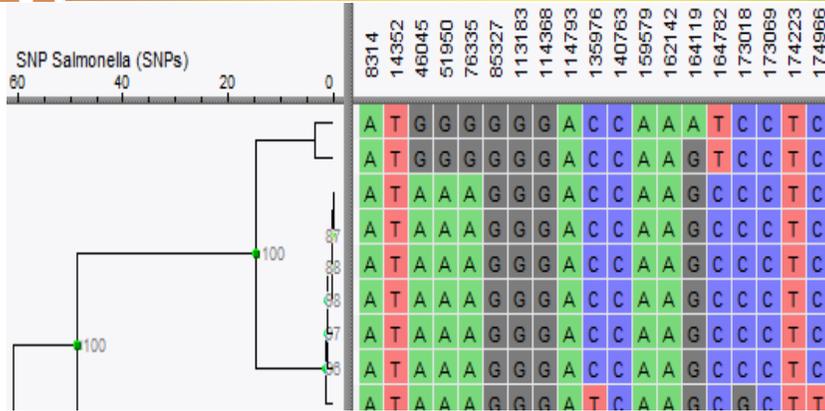
<https://github.com/afelten-Anses/ARTWORK>

Database LISTADAPT - Bionumerics



- BioNumerics version 7.6.3
- Storage of epidemiological data alongside experimental data
- MLST typing
- Allele calling from assembly only:
 - ✓ Default settings, including 85% minimum identity to reference allele
 - ✓ Exception: do not assign allele when multiple calls are made (instead of allele with lowest ID)
- cgMLST
 - ✓ Listeria: Pasteur scheme (Moura et al. 2016) with 1748 loci

SNP prediction and phylogeny



(Felten et al., 2017)

<https://github.com/afelten-Anses/VARtools/tree/master/iVARCall2>

- SNP prediction iVarcall2
- Iqtree (V. 1.6.9)
 - ✓ Fast accurate phylogenomic algorithm
 - ✓ Phylogenetic inference by maximum likelihood
- Outcome:
 - ✓ Global genetic diversity of listeria in Europe
 - ✓ Diversity into specific CC
 - ✓ Diversity in group of interest

Phenotypic characterization

- 200 strains : 100 from environment 100 from foods
 - ✓ Antibiotics and biocides resistance
 - ✓ Adhesion and biofilm formation
 - ✓ Survival and persistence in soil microcosm
 - ✓ Survival in synthetic stomach acid at 37° C
 - ✓ Growth potential at cold storage temperature, low pH,...

Phenotypic selection – food strains

Food strains selection

- 100 strains from the ANSES collection
- From 2002 to 2017
- From 4 categories of RTE food (fish; meat; dairy and vegetables)
- Selected from the top 6 CC isolated in each categories

List of strain by food source and clonal complex							
	Lineage	Clonal complex MLST	S train 1	S train 2	S train 3	S train 4	additional S train
Food - RTE Fish	II	CC 121	10CEB368LM	10CEB588LM	11CEB475LM	15SEL1759LM	08CEB397LM
	II	CC 8	11CEB741LM	12CEB74LM	15SEL53LM	15SEL64LM	
	II	CC 9	11CEB429LM	11CEB445LM	08CEB172LM	03EB120LM	SO96
	II	CC 155	11CEB426LM	08CEB181LM	15SEL880LM	15SEL881LM	
	I	CC 6	05CEB394LM	05CEB671LM	05CEB795LM	06CEB188LM	
	I	CC 2	08CEB85LM	10CEB68LM	11CEB241LM	02EB477LM	
Food - RTE Dairy	I	CC 6	05CEB256LM	07CEB308LM	07CEB768LM	12CEB898LM	
	II	CC 21	09CEB338LM	10CEB645LM	09CEB601LM	11CEB260LM	
	I	CC 1	02CEB369LM	05CEB711LM	AF38	IN15	AF32
	II	CC 37	17SEL438LM	14SEL1687LM	12CEB855LM	14SEL1688LM	
	II	CC 7	17SEL102LM	17SEL420LM	17SEL567LM	17SEL401LM	
	I	CC 4	06CEB420LM	07CEB235LM	09CEB160LM	17SEL419LM	
Food - RTE Meat and meat products	II	CC 121	12CEB397LM	11CEB213LM	10CEB428LM	11CEB341LM	
	II	CC 9	14SEL860LM	12CEB857LM	11CEB418LM	09CEB866LM	
	I	CC 2	12CEB366LM	12CEB368LM	14SEL872LM	14SEL906LM	
	II	CC 8	10CEB335LM	11CEB179LM	11CEB458LM	15SEL670LM	
	I	CC 6	12CEB383LM	14SEL1683LM	06CEB444LM	12CEB650LM	
	I	CC 1	14SEL873LM	AF40	15SEL676LM	14SEL1700LM	
Food - Vegetables	I	CC 1	06CEB656LM	06CEB122LM	17SEL493LM	Missing	
	II	CC 121	08CEB109LM	12CEB1182LM	11CEB364LM	08CEB60LM	
	I	CC 2	12CEB1097LM	05CEB674LM	15SEL845LM	Missing	
		CC 31	11CEB363LM	12CEB28LM	11CEB446LM	11CEB354LM	
	II	CC 37	11CEB205LM	12CEB09LM	10CEB580LM	12CEB1426LM	
	I	CC 6	06CEB211LM	09CEB30LM	08CEB223LM	15SEL1228LM	

Phenotypic selection – environmental strains

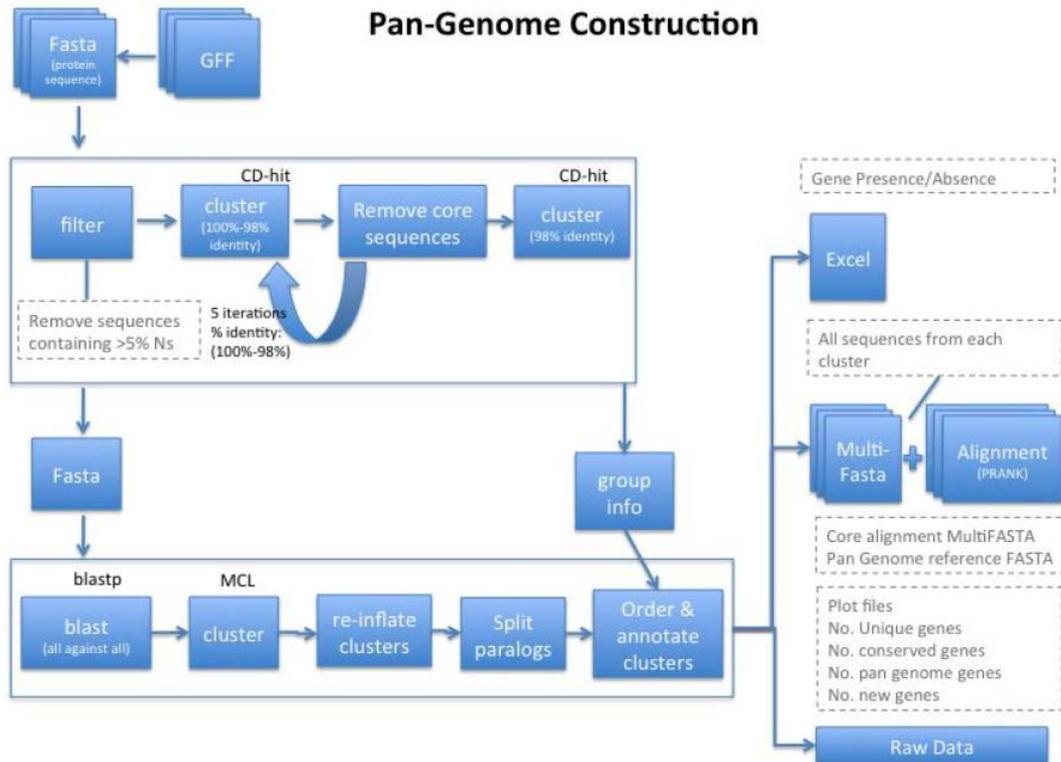
List of strain by food source and clonal complex

Clonal Complex	Strain 1	Strain 2	Strain 3	Strain 4
CC1	IT-FOX-FE-63	NO-OTH-O-15	SW-FAR-UN-B-20	CZ-NAT-SO-22
CC2	SI-BOV-MI-62	IT-NAT-SO-3	PL-FAR-GL-14	LV-BOV-CP-11
CC4	NO-OTH-O-13	DE-RDE-FE-17	AT-FAR-CF-1	16SEL712LM
CC6	NO-OTH-O-7	FR-FAR-MA-1	SW-FAR-SO-B-104	FR-BOV-CP-I-159
CC7	SW-FAR-SO-B-110	CZ-OTH-UN-8	NO-OTH-O-31	FR-NAT-UN-2
CC8	NO-OTH-O-19	DE-FAR-UN-G-42	LV-BOV-CP-6	FR-NAT-U-1
CC9	FR-BOV-CP-I-143	CZ-OVI-UN-2	LV-GOA-CP-26	NO-OTH-O-33
CC315	SI-OVI-CP-I-18	FI-BIR-FE-U-40	FI-BOV-CP-I-58	
CC37	SW-FAR-SO-B-65	CZ-NAT-SO-32	FR-BOV-CP-I-160	NO-OTH-O-5
CC121	IT-OTH-CP-36	DE-WBO-UN-34	CZ-FAR-CF-54	SI-FAR-WT-52
CC11	SW-FAR-UN-O-8	CZ-NAT-SO-37	DE-RDE-CP-13	FR-PIG-UN-34
CC14-ST14	LMNC 244	16SEL1404LM	FI-BIR-FE-U-55	
CC14-ST91	LV-BOV-CP-17	NO-OTH-O-20	SE-RDE-CP-2	
CC21	CZ-NAT-SO-36	DE-RDE-CP-7	NO-DEE-F-1	16SEL589LM
CC29	PL-NAT-SO-1	SW-FAR-SO-B-60	LV-PIG-CP-18	IT-OTH-FE-61
CC412	LMNC 234	DE-WBO-CP-23	FR-BOV-CP-I-83	
CC451	CZ-NAT-SO-23	SE-RDE-CP-7	LV-BOV-CP-29	NO-DEE-F-3
CC5	FR-FAR-AS-B-5	SW-NAT-SO-97	PL-NAT-SO-18	SP-BOV-FE-1
CC59	FI-BIR-FE-U-37	PL-FAR-GL-12	DE-RDE-FE-14	FR-BOV-CP-I-101
CC20	CZ-NAT-SO-15	LV-OVI-CP-8	PL-FAR-CF-9	UK-OVI-CP-I-30
CC18	FR-NAT-UN-1	CZ-NAT-SO-38	NO-OTH-O-8	SW-FAR-FE-G-42
CC77	16SEL589LM	FR-FAR-AS-P-8	NL-GOA-UN-19	
CC220	NL-GOA-UN-34	UK-OVI-CP-I-34	DE-WBO-FE-24	
CC224	SI-UN-CP-I-16	16SEL662LM	NL-GOA-UN-35	
CC26	NL-GOA-UN-2	FR-FAR-GL-3	DE-RDE-FE-8	SW-NAT-UN-55
CC22	SI-BOV-CP-I-71	FR-BOV-CP-I-55		
ST36	IT-PIG-FE-22	SW-GOA-CP-I-117		
CC54	IT-NAT-SO-1	16SEL594LM	LMNC075	
ST184	16SEL533LM	2019-TE-1425-1-1		

Environmental strains selection

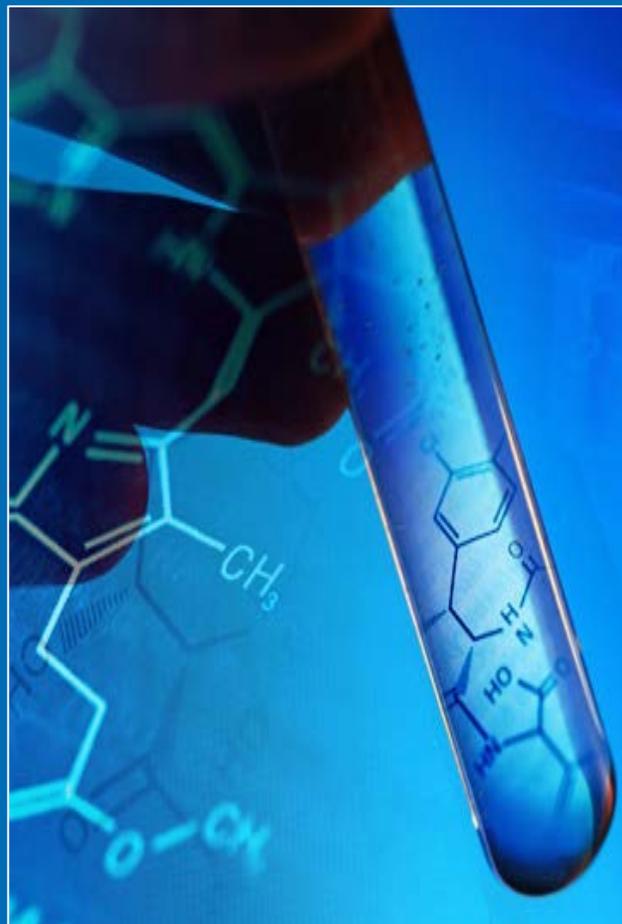
- 100 strains from Europa
- Selected from environment dominant CC and environment strain from food
- For each CC, one strain from each available country
- From 2002 to 2017

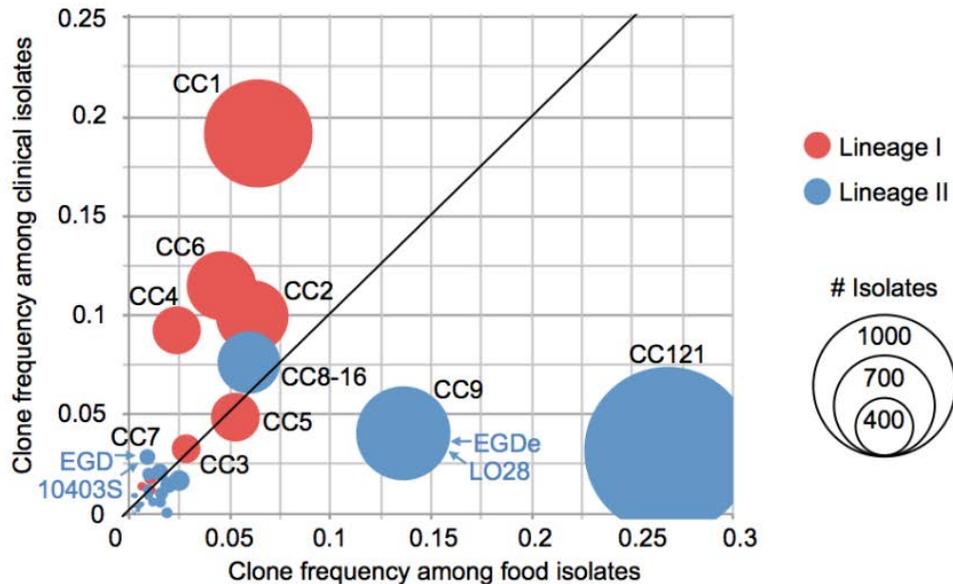
GWAS- ROARY & SCOARY



- ROARY (V. 3.12.0)
 - ✓ Correlate genome annotation with sequence
 - ✓ Gene presence absence matrix
 - ✓ Determination of the core and pan genome
 - ✓ Differentiation of variants of a same gene
- Scoary (V. 1.6.16)
 - ✓ From the pan genome, calculate the association of a gene to a phenotype.
- Phage prediction
 - ✓ Phaster (Arndt, 2016)
- Plasmid prediction
 - ✓ Plasmid spades (V. 3.13.0)

Application on the investigation of CC121 diversity.





(Maury et al., 2016)

- Predominant in food
- Rarely identified in human or in the environment
- Hypovirulent
- Show important persistence capacity in food processing facilities



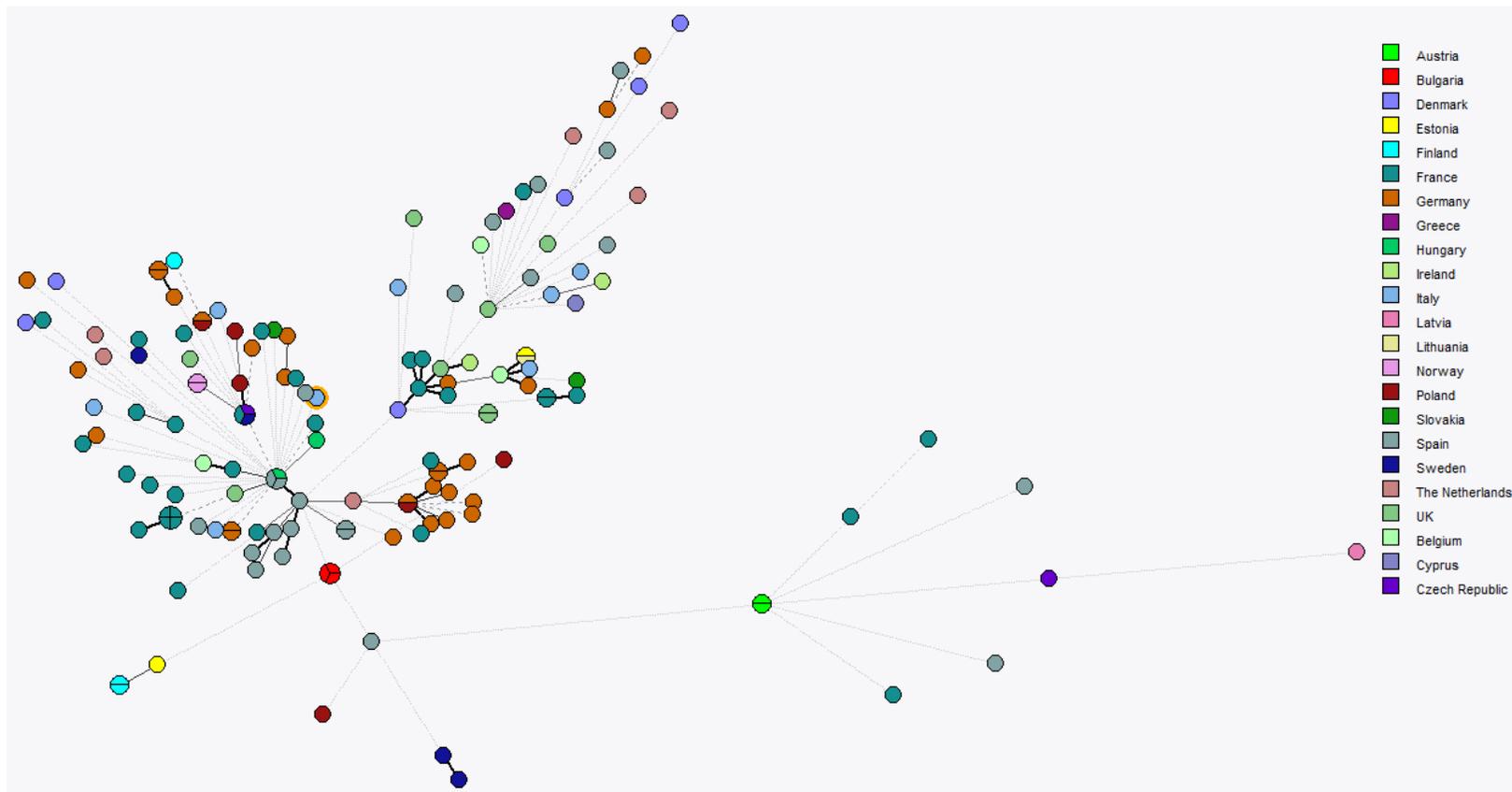
- Found near Orbetello
- Death probably caused by encephalitis caused by listeria
- Strain isolated from the brain belong to CC121

Environmental strains

- 6 strains from 5 countries from 2014-2018
- 3 strains from animals
- 1 strain from soil
- 1 strain from vegetable from the field
- 1 strain from water

Food strains

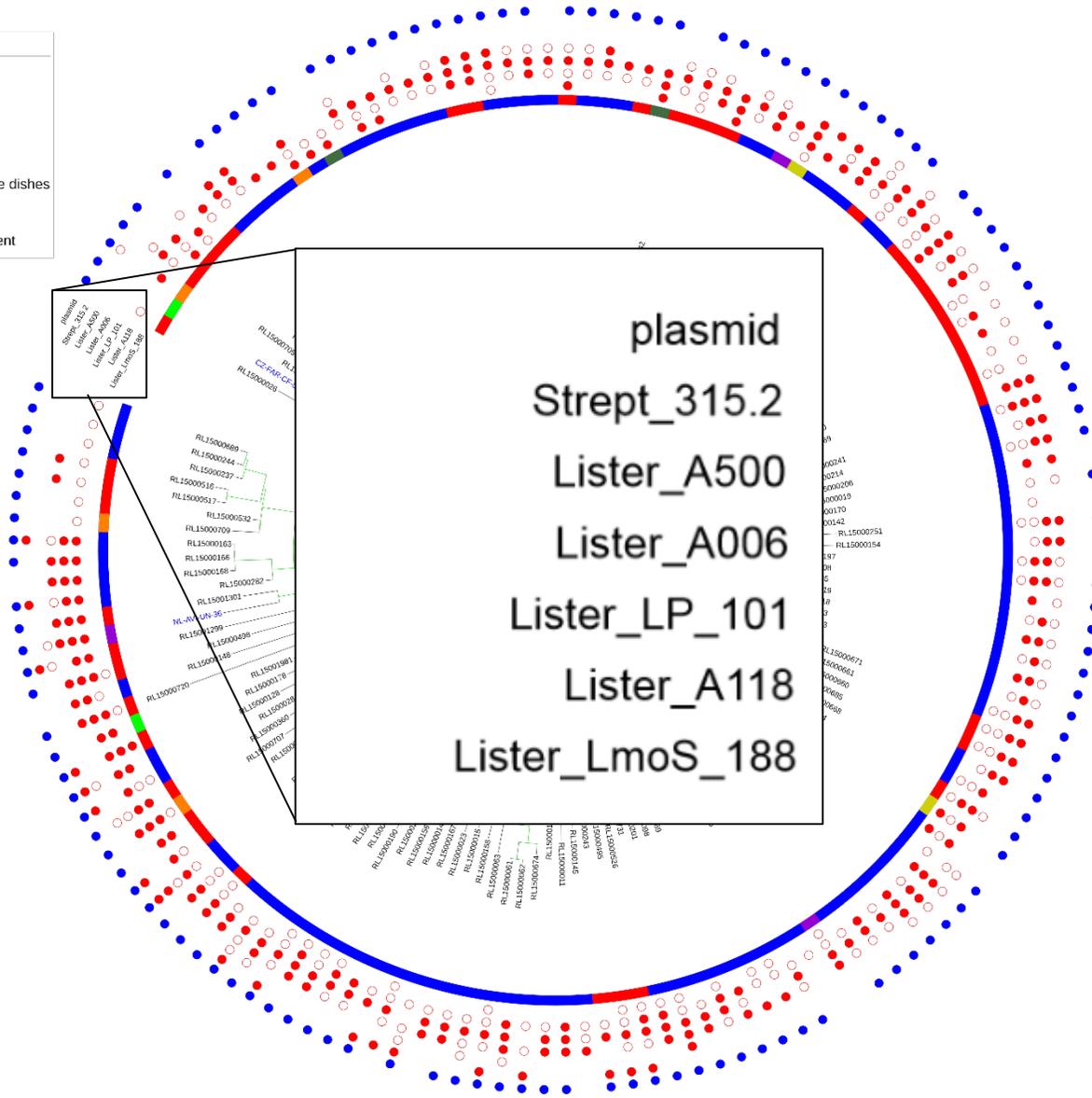
- 142 strains from 24 countries from 2010 to 2012
- 93 strains from fish products
- 42 strains from meat
- 4 strains from composite dishes
- 3 strains from milk
- 1 strain from vegetable



- 2 distinct cluster distant from more than 30 allelic variations

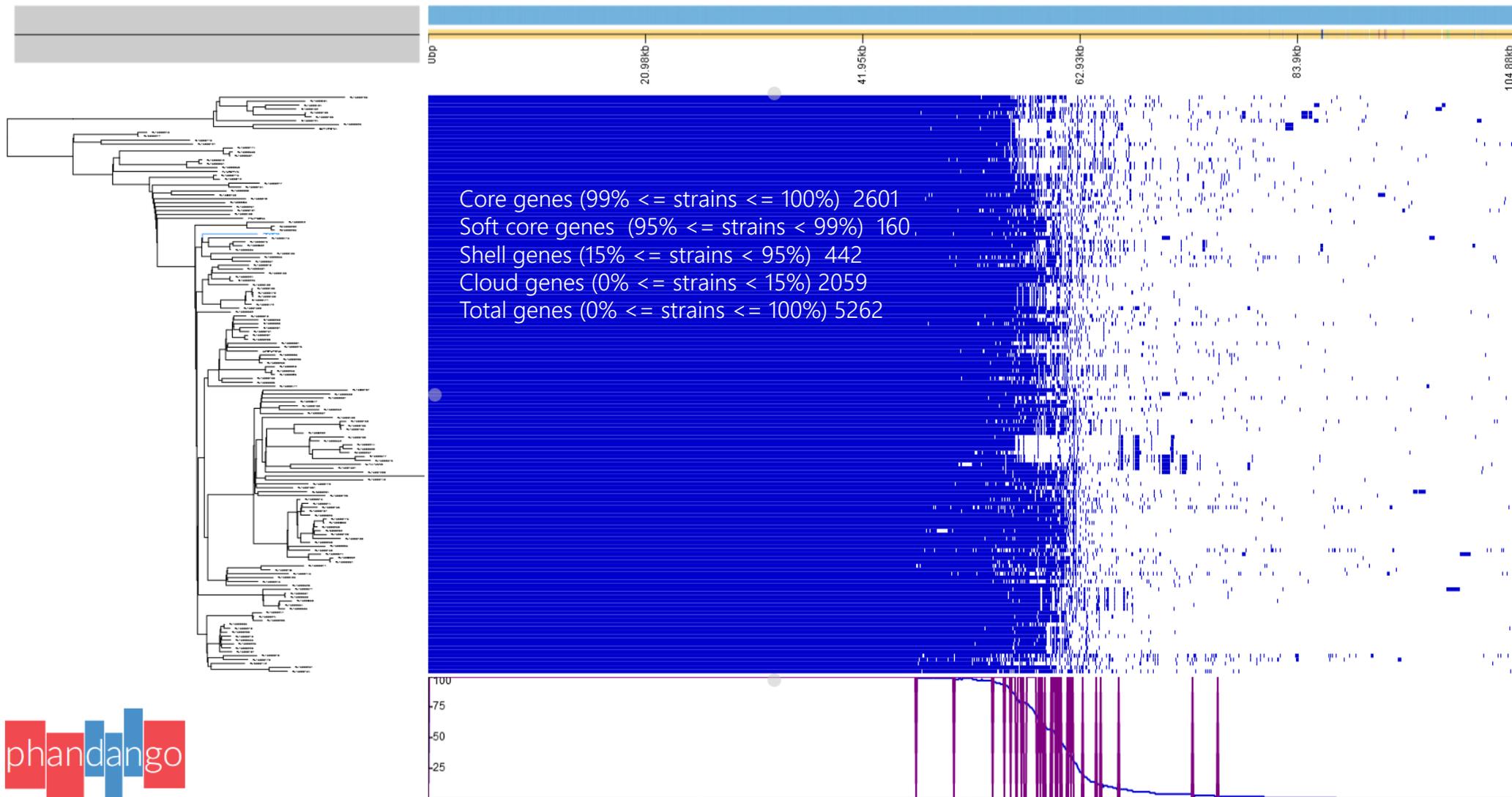
Phylogeny – Phage and plasmids

Tree scale: 0.00001



- 16 phages in total
- 6 phages are present in more than 10 genomes
- A118 present in all genome except one
- P-101 and A-006 present in more than 100 genomes
- Presence of the plasmid Plm6179
 - ✓ clpL heat stress resistance
 - ✓ cadA, cadC cadmium tolerance & resistance to bacteriophage

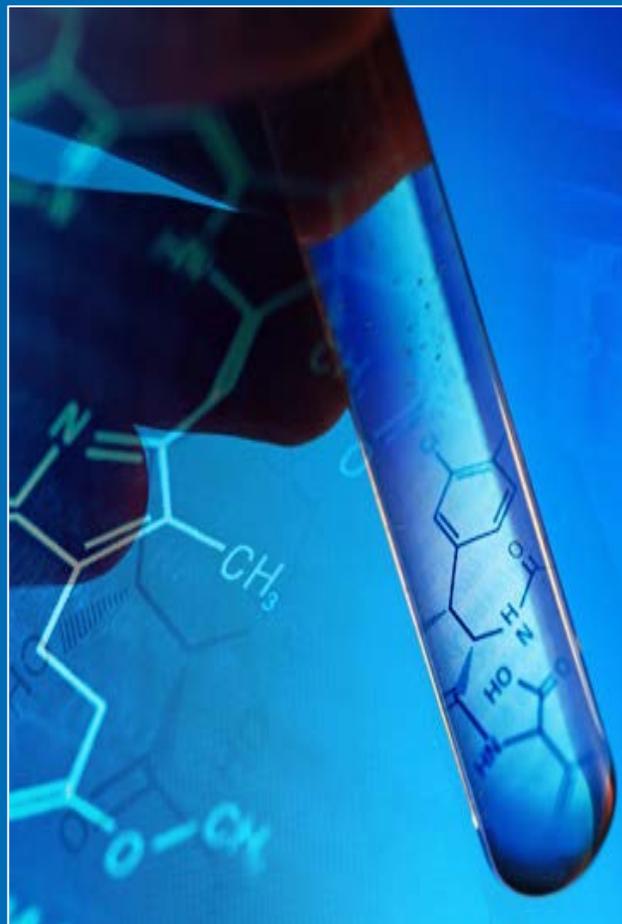
GWAS - ROARY



- Most pan-genomics variation concern phages or plasmid related genes

- No significant differences between the different food sources
- Cluster 1 and 2 can be distinguished by variation of the *inlC* and *inlH* gene (p value > 1×10^{-5})
 - ✓ *InlC* promote cell to cell spread and reduce immune response to listeriosis
 - ✓ *InlH* reduce the inflammatory response to systemic invasion (personnic et al. 2010)
- Dolphin strain:
 - ✓ Absence of the genes *inlJ_4* corresponding to a internalin
 - ✓ Presence of the putative ABC transporter *yhel*
- All CC121 strains presented a *truncated inlA* which induce impaired invasion capability and increased in biofilm formation (Franciosa et al., 2009)
- Deletion in the *actA* gene controlling intracellular mobility

Conclusion and perspectives.



Conclusion

- Comprehensive investigation of listeria genomic diversity from in Europe
- Will allow to understand the diversity of *Listeria* from the environment to human
- Determine the differences between *Listeria* strains from different niches or host
- Will proves extremely valuable for future source attribution studies and outbreaks investigation

- Use of GWAS investigation to determine genomic characteristics specific of niches, host.
- Determination of genetic feature which leads to persistence in food processing facilities
- Investigation of the variation of key virulence factor and their impact on the adaptation and the virulence of *Listeria*



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Aurélie Leroux

LISTADPT consortium partners

AGES (AT)

VRI (CZ)

INRA (FR)

NVI (NO)

ANSES (FR)

IZSAM (IT)

SVA (SE)

External partners

VetSuisse (CH)

Munich University (DE)

Freie Universität of Berlin IHMT (DE)

VetLab (EE)

NEIKER (ES)

University of Helsinki (FI)

French Institute for Pig Industry (FR)

Veterinary faculty of Zagreb (HR)

BIOR (LT)

VWA (NL)

Szczecin University (PL)

INIAV (PT)

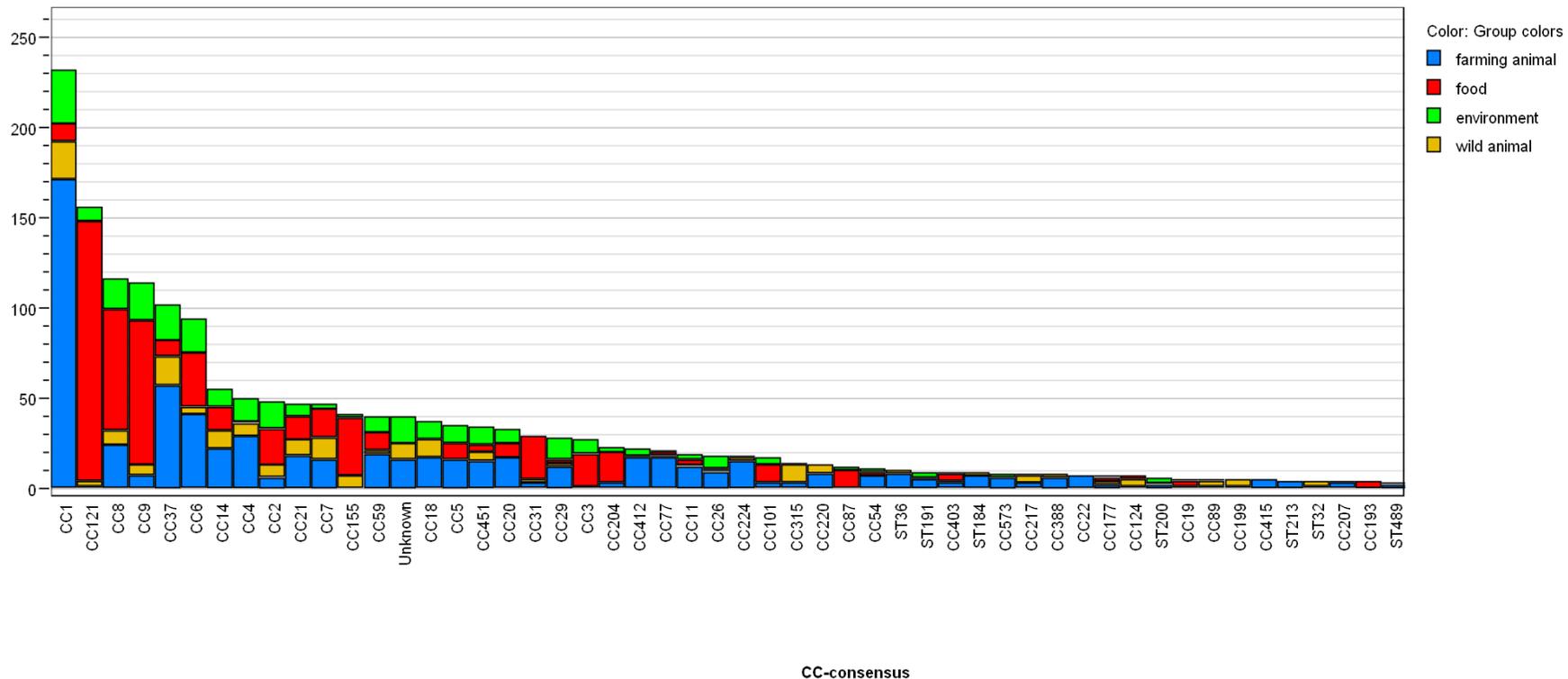
Veterinary faculty of Ljubjana (SI)

Public Health England (UK)

State Veterinary and Food Institute (SL)

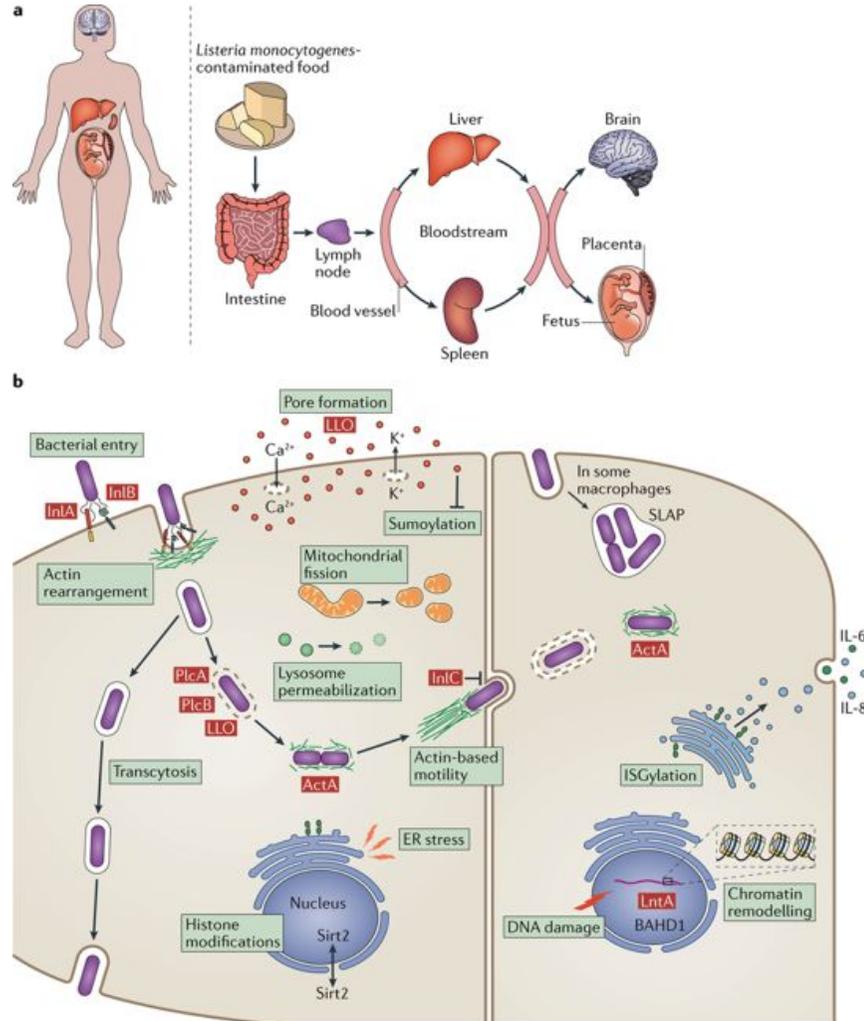


Occurrence of the CC groups by categories



- Food predominant: CC121; CC8; CC9; CC155; CC31; CC3; CC204; CC101; CC87
- Animal predominant: CC1; CC37; CC14; CC4; CC18; CC451; CC29; CC412; CC77
- Environment predominant:

Virulence factor of *Listeria*



Nature Reviews | Microbiology

(Radoshevich et al. 2017)

- *Adhesion*
 - ✓ InlJ
- *Invasion*
 - ✓ InlA
 - ✓ InlB
- *Intracellular survival*
 - ✓ PlcA
 - ✓ PlcB
- *Multiplication*
 - ✓ LLO
- *Intercellular infection*
 - ✓ ActA
 - ✓ InlC