

Characterization of *Clostridium perfringens* strains for the investigation of food poisoning outbreaks in France

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**Foodborne pathogens &
whole genome sequencing**

JOINT CONFERENCE 26-28 March 2019

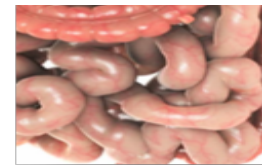
Risks associated with *Clostridium perfringens*

- *C. perfringens* : Gram+, rod-shaped and anaerobe (wiegel et al. 2006)



spore-forming

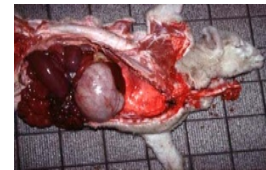
- Ubiquitous (McClane et al. 2001)



- Pathologies



Gas gangrene

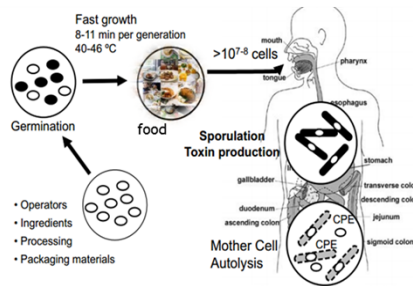


Enterotoxemia



Necrotic enteritis

Causative agent of human food poisoning outbreaks (McClane et al. 2013)



Abdominal pain



Nausea



Diarrhea

8 - 12 h

- ✓ Caused by enterotoxin (CPE) producing strains (McClane et al. 2013)

Classification and toxins of *C. perfringens*

- Current classification : 7 toxinotypes

Toxinotype	α -toxin (<i>plc</i> or <i>cpa</i>)	β -toxin (<i>cpb</i>)	ϵ -toxin (<i>etx</i>)	ι -toxin (<i>iap</i> and <i>ibp</i>)	CPE (<i>cpe</i>)	NetB (<i>netB</i>)
A	+	-	-	-	-	-
B	+	+	+	-	-	-
C	+	+	-	-	±	-
D	+	-	+	-	±	-
E	+	-	-	+	±	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

(Rood et al. 2018)



Toxinotype F is associated to FPO

- 22 virulence factors are now described in the scientific literature (Mathiew et al., 2013 ; Li et al., 2013 ; Gohari et al., 2015)

Health impact of *C. perfringens* FPO

NATIONAL

top 4 causes of bacterial FPO

Pathogen	Confirmed	Suspected	Total
	295	779	1310
<i>Salmonella</i>	89	32	121
<i>B. cereus</i>	47	217	264
<i>C. perfringens</i>	34	49	83
<i>S. cereus</i>	19	308	327
Others*	106	173	279
Undetermined	/	/	236

* histamine, virus, *Shigella*, *Campylobacter*, autres...



EUROPEAN

(EFSA, 2018)

- 108 reported FPO { 38 confirmed, 70 suspected
- 2,324 people
- 27 hospitalizations

Declared by only 7 EU Member States

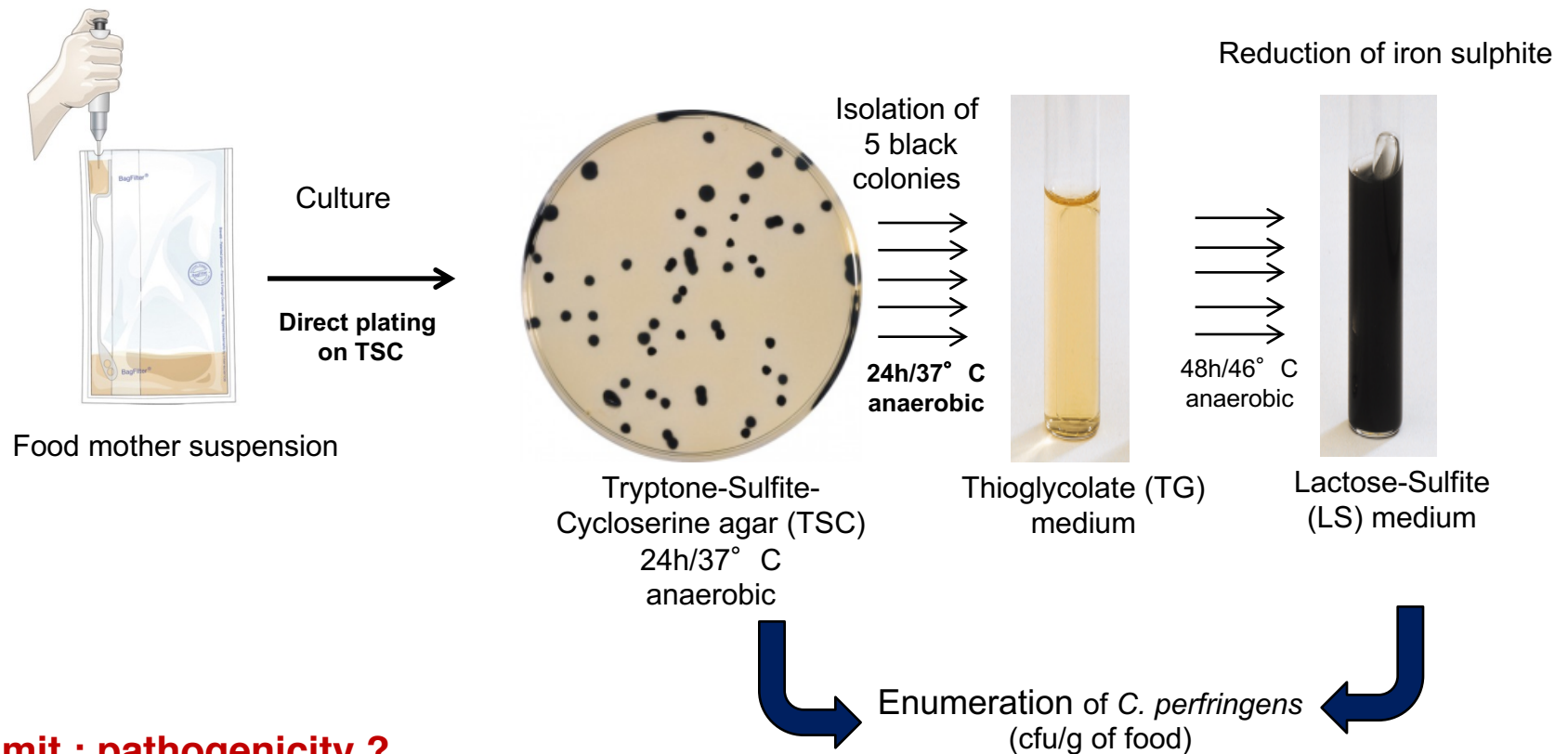
1,243 people, 38 hospitalizations (Santé publique France, 2019)

implicated foods



Official method for detection and enumeration of *C. perfringens* in FPO analysis

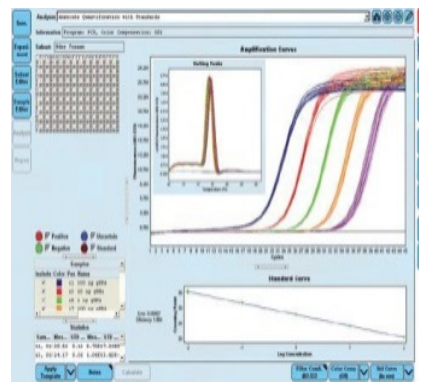
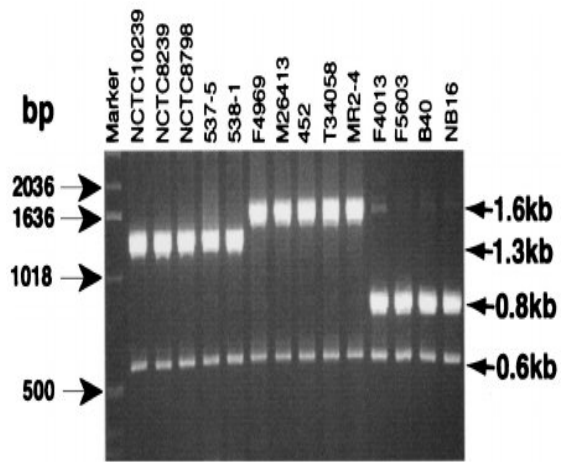
➤ NF EN ISO 7937 standard: horizontal method for the enumeration of *C. perfringens*



➤ **Limit : pathogenicity ?**

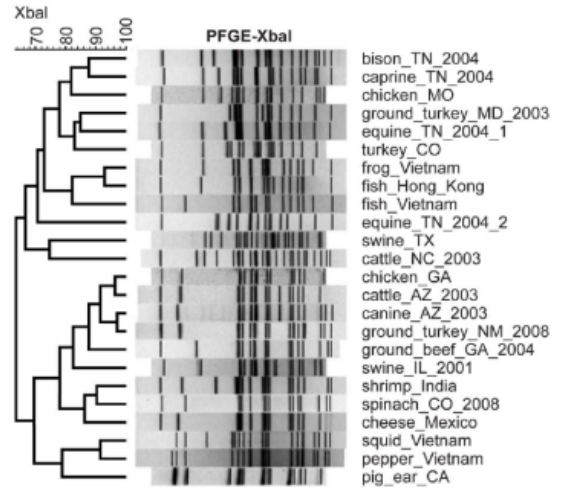
Molecular typing methods in use for *C. perfringens*

PCR-based typing method targeting major toxins encoding genes



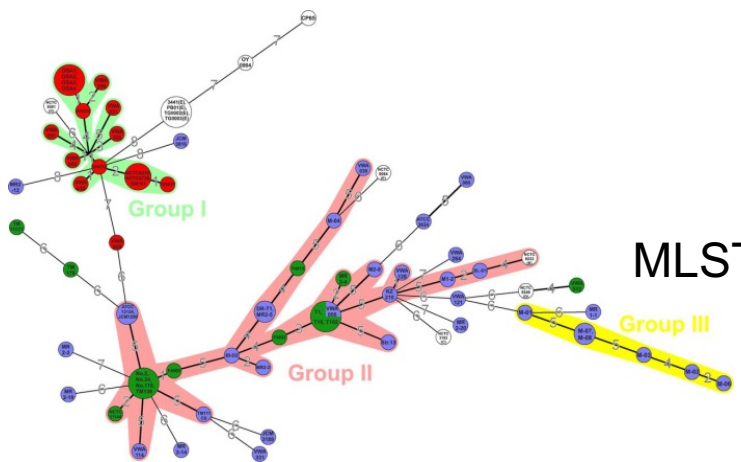
(Albini et al., 2008 ; Baums et al., 2004; Miyamoto et al., 2004)

PFGE



(Maslanka et al., 1999 ; Schalch et al., 2003)

(Xiao et al., 2012)



MLST (8 housekeeping genes)

Lack of a standard typing method for *C. perfringens*

Recent studies used WGS for analysis of the genetic diversity of *C. perfringens* species



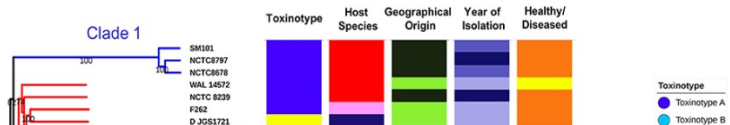
Front. Microbiol. 2017; 8: 2485.
Published online 2017 Dec 12. doi: 10.3389/fmicb.2017.02485

PMCID: PMC5733095
PMID: 29312194

Probing Genomic Aspects of the Multi-Host Pathogen *Clostridium perfringens* Reveals Significant Pangenome Diversity, and a Diverse Array of Virulence Factors

Raymond Kiu,^{1,2,†} Shabbonam Caim,^{1,†} Sarah Alexander,³ Purnima Pachori,⁴ and Lindsay J. Hall^{1,*}

† Author information † Article notes † Copyright and License information † Disclaimer



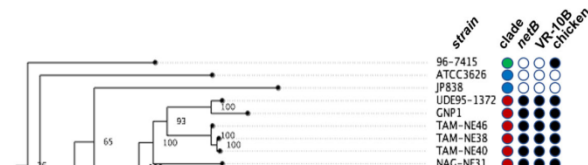
Lacey et al. BMC Genomics (2018) 19:379
https://doi.org/10.1186/s12864-018-4771-1

BMC Genomics

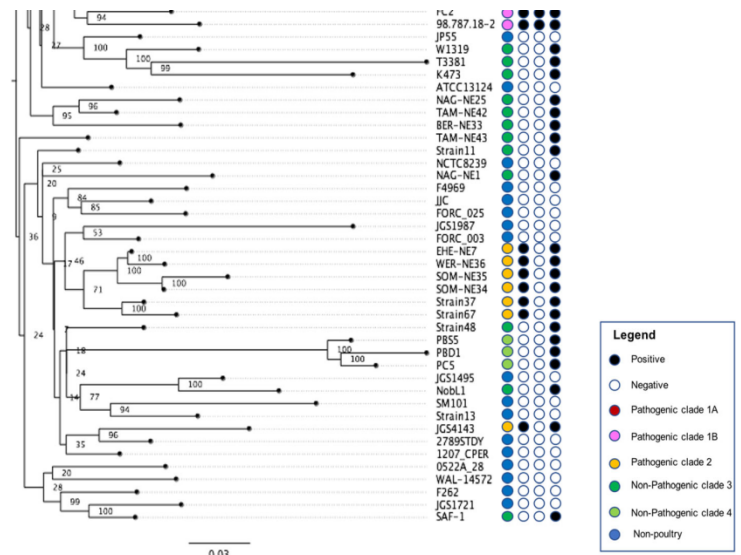
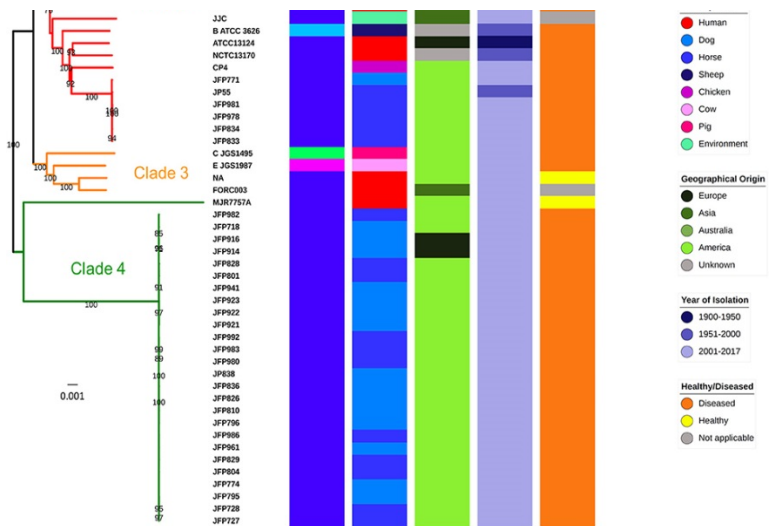
RESEARCH ARTICLE Open Access

Whole genome analysis reveals the diversity and evolutionary relationships between necrotic enteritis-causing strains of *Clostridium perfringens*

Jake A. Lacey^{1,2,3}, Theodore R. Allnut^{1,4}, Ben Vozina^{1,2,3,5}, Thi Thu Hao Van⁶, Thomas Stent^{1,3}, Xiaoyan Han¹, Julian I. Rood^{1,3}, Ben Wade^{1,4}, Anthony L. Keyburn^{1,2,3,6}, Torsten Seemann^{1,2}, Honglei Chen¹, Volker Hanning¹, Priscilla A. Johansen¹, Dena Lyas¹ and Robert J. Moore^{1,2,3,6*}



➤ Interesting approach to compare *C. perfringens* strains involved in FPO



An original collection of 141 Cp strains isolated from FPO related food samples

FBO	Year	Food vehicle	Human cases (n)	cfu/g	Recovered strain (n)	Other identified FBO bacteria	cfu/g
2207	2013	Vegetable	130	3.7E+06	1	-	-
2253	2013	Poultry	160	6.0E+06	1	-	-
435	2014	Other	22	3.0E+02	1	-	-
1601	2014	Other	15	3.5E+05	1	BC	2.7E+04
1622	2014	Other	21	1.1E+04	1	-	-
2370	2014	Pork	27	2.6E+03	1	-	-
529	2015	Vegetable	6	3.0E+01	2	BC	1.5E+03
538	2015	Poultry	38	1.5E+04	7	-	-
2291	2015	Pork	118	4.0E+06	5	-	-
2318	2015	Poultry	34	1.1E+03	1	-	-
2540	2015	Vegetable	9	4.0E+02	2	BC	8.0E+03
2606	2015	Vegetable	9	3.7E+02	5	BC	1.0E+05
0	2015	NK	5	NK	5	NK	NK
2727	2015	Pork	7	3.7E+03	3	-	-
2773	2015	Poultry	50	1.5E+07	5	-	-
2774	2015	Poultry	50	1.5E+07	5	-	-
2987	2015	Poultry	39	3.6E+04	8	-	-
2988	2015	Poultry	39	1.4E+02	4	BC	1.4E+05
3803	2015	Pork	4	1.3E+04	4	SCP	4.0E+02
3958	2015	Pork	NK	1.6E+02	5	-	-
3863	2015	Vegetable	20	2.2E+02	5	-	-
4068	2015	Other	2	4.0E+04	2	-	-
4092	2015	Beef	30	1.2E+02	4	-	-
4115	2015	Vegetable	2	3.6E+01	2	-	-
4127	2015	Pork	26	5.8E+05	4	-	-
4138	2016	Other	1	6.0E+02	3	-	-
370	2016	Vegetable	8	4.0E+01	1	BC	1.0E+03
490	2016	Poultry	40	7.0E+01	5	-	-
553	2016	NK	4	8.0E+02	1	-	-
1781	2016	Vegetable	28	1.1E+05	5	-	-
1782	2016	Vegetable	28	4.0E+01	1	BC	5.5E+04
1923	2016	Vegetable	13	8.4E+04	5	-	-
3199	2016	Vegetable	5	4.0E+01	1	-	-
3566	2016	Vegetable	51	2.4E+06	5	-	-
4286	2016	Vegetable	2	4.0E+01	1	BC	4.0E+03
4430	2016	Beef	2	1.1E+03	5	-	-
4493	2017	Vegetable	2	3.6E+02	4	BC	1.5E+04
4755	2017	Vegetable	120	9.3E+02	5	-	-
274	2017	Beef	2	4.0E+02	1	-	-
759	2017	Beef	31	4.9E+06	5	-	-
762	2017	Beef	31	7.0E+01	4	-	-
1270	2017	Beef	41	1.9E+03	5	-	-



Objectives

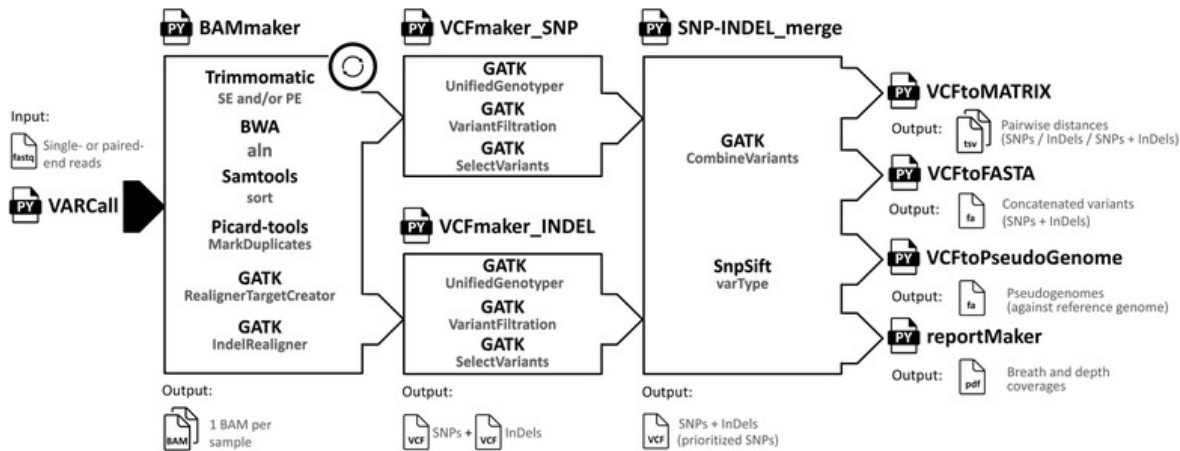
Explore the genetic diversity of FPO associated *Cp* strains

- ✓ Diversity of virulence gene profiles
- ✓ Genetic Relationship of FPO associated *C. perfringens* isolates
- ✓ Plasmids carriage of FPO isolates
- ✓ Relationship between genetic clades and Food vehicles

Genomic analysis of the collection



- DNA extraction of 141 isolates
- Sequencing (NextSeq) at ICM <https://icm-institute.org>
- **In home pipeline for QC, assembly and SNP calling**
- ✓ ARTWork (Durimel et al., 2017)
- ✓ iVARCall (Felten et al., 2017)



GAMeR genomic database v 2.00

Strain ID	Project	Center	Sample ID	Sample ID	Normalized read #1	Normalized read #2	Variant	Assembly	Assembly quality	CRK	ATCC root
SI-297	SI-297	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-298	SI-298	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-299	SI-299	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-300	SI-300	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-301	SI-301	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-302	SI-302	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-303	SI-303	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-304	SI-304	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-305	SI-305	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-306	SI-306	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-307	SI-307	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-308	SI-308	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-309	SI-309	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta
SI-310	SI-310	ICM	SI	SI	SI	SI	gATC	Fastx	Good	OK	Ta

Storage of WGS data analyses and traceability

- 24 virulence genes identified in the literature (Revitt-Mills et al., 2015)

detection by blast approach : **Identity of 90 % and Coverage of 85 %**

Toxinic profiles of 42 *C. perfringens* FPO

Toxin-gene profile	Number of strains	Genes detected								
		<i>cpb2</i>	<i>ia</i>	<i>ib</i>	<i>cpe</i>	<i>pfoA</i>	<i>lam</i>	<i>nagH</i>	<i>nanI</i>	<i>nanJ</i>
I	1	-	+	+	+	+	-	+	+	+
II	9	+	-	-	+	+	-	+	+	+
III	1	-	-	-	+	+	-	+	+	+
IV	12	-	-	-	+	-	-	+	-	+
V	24	-	-	-	+	-	-	+	-	-
VI	4	-	-	-	+	-	-	-	-	+
VII	29	-	-	-	+	-	-	-	-	-
VIII	7	+	-	-	-	+	-	+	+	+
IX	1	+	-	-	-	+	-	-	+	-
X	40	-	-	-	-	+	-	+	+	+
XI	5	-	-	-	-	-	-	+	+	+
XII	1	-	-	-	-	+	-	-	+	-
XIII	5	-	-	-	-	-	-	+	-	-
XIV	1	+	-	-	-	+	+	+	+	+
XV	1	-	-	-	-	-	-	-	-	-

- 4 genes detected in all strains:

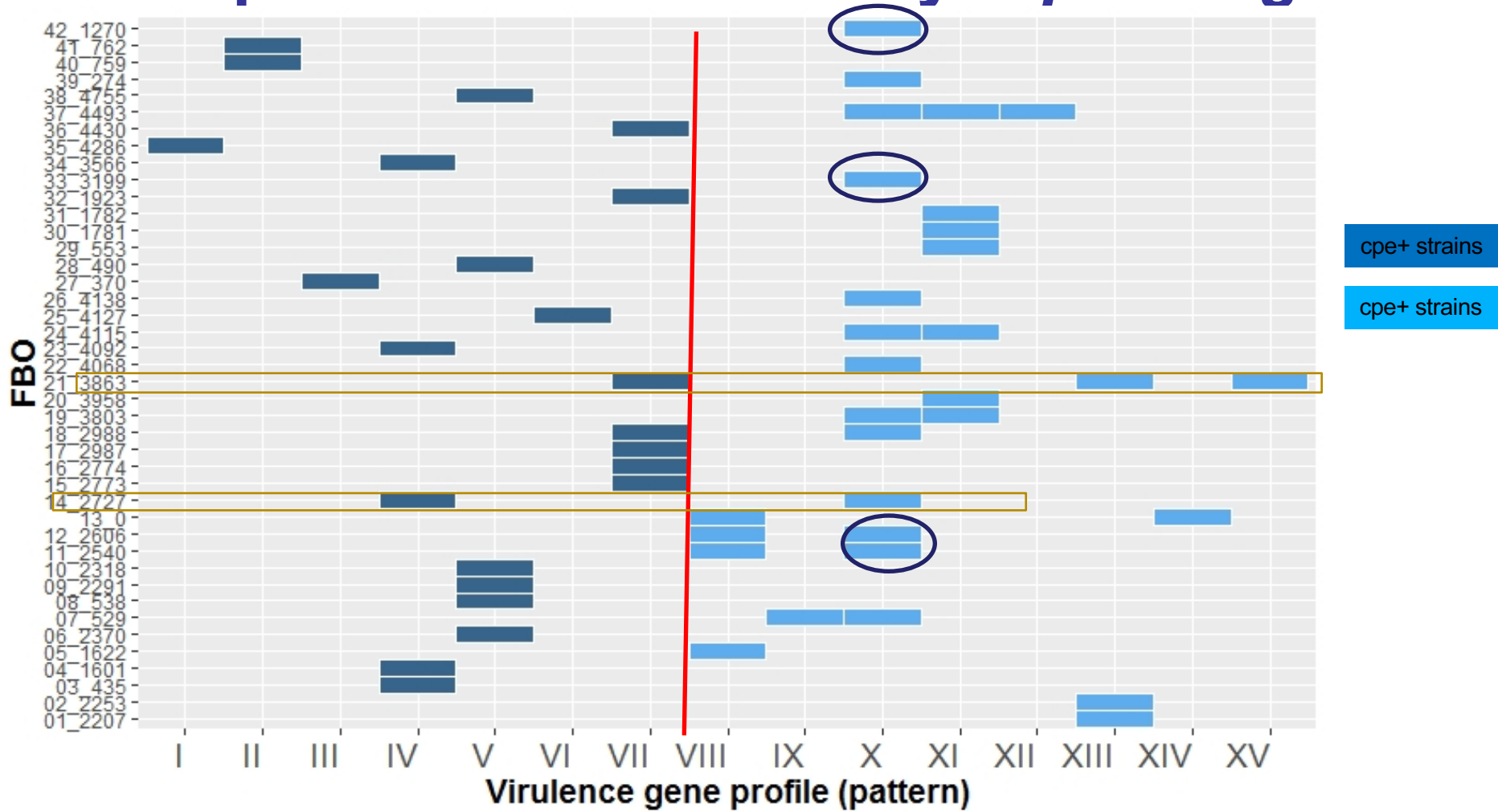
cpa, *colA*, *nanH* and *cadA*

- 11 genes not detected: *cpb*,

etx, *netBEFG*, *becAB*, *tpeL*, *cpd*, and *ureABC*

- 15 profiles identified
- More discriminant than current classification system
- F: 1 toxinotype vs 7
- 55% *cpe*+ and 45% *cpe*-

Some unexpected characteristics of food samples contamination by *C. perfringens*



Heterogeneous contamination of food samples,
Contamination by only *cpe-* *C. perfringens*

More than five isolates to be characterized

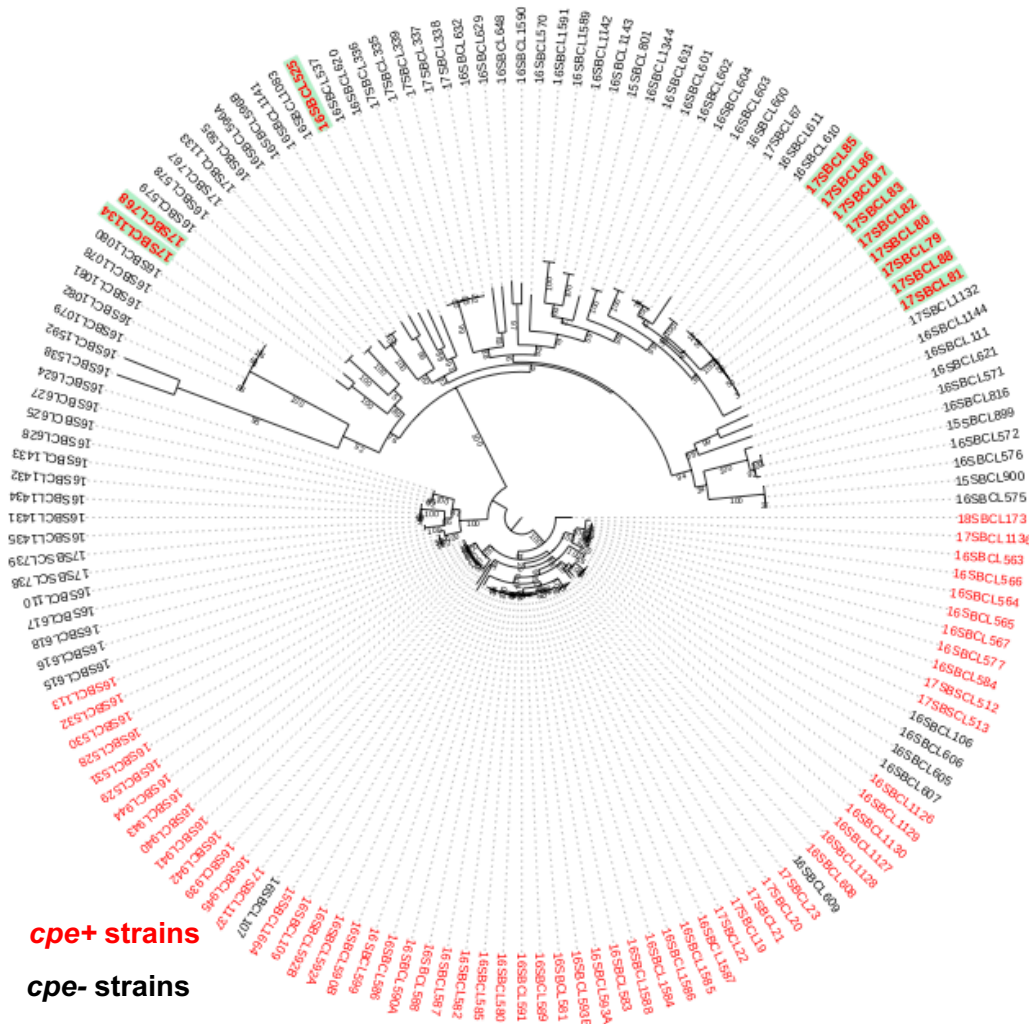
Objectives

Explore the genetic diversity of FPO associated *Cp* strains

- ✓ Diversity of virulence gene profiles
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Phylogenomic analysis of FPO associated *C. perfringens* strains (core genome SNPs)

Tree scale: 0.001



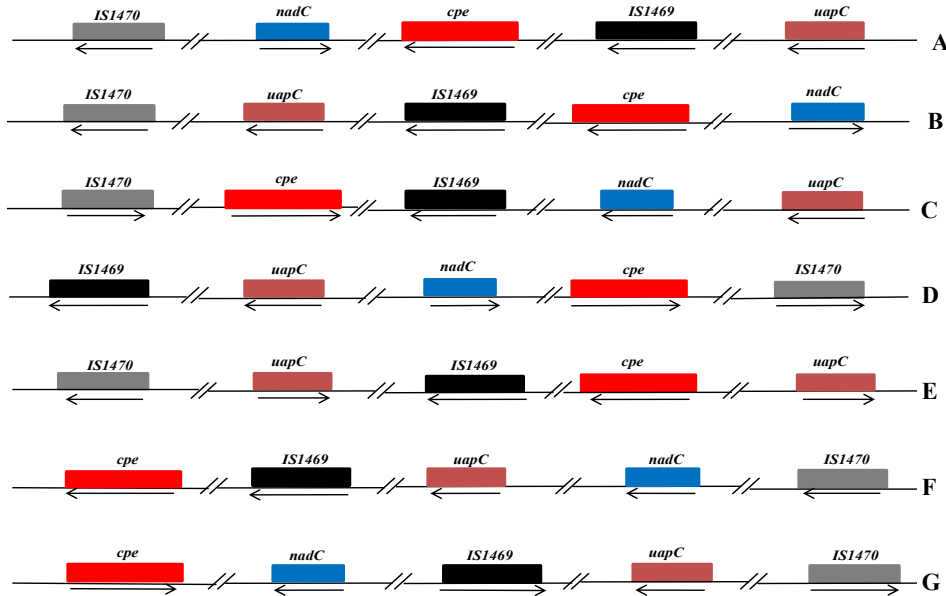
- Two main clades:
 - Clade 1 with 81 strains **CPE+**
 - Clade 2 with 40 strains **CPE-**
- Clade 1 more homogeneous than clade 2 (**8k SNP vs 21k SNP**)
- Different reservoirs for Clade 1 and clade 2 isolates ?
- Why are clade 1 isolates more prone to acquire *cpe* gene ?

cpe+ strains
cpe- strains



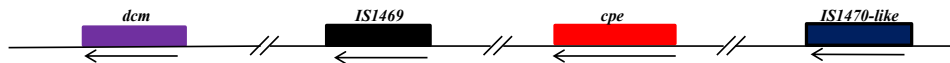
Mosaicity of chromosomal *cpe* gene organization

- Organization of the chromosomal *cpe* locus observed in our collection



- A great diversity for the organization of the *cpe* locus (clade 1)
- Always flanked by IS sequences indicating *cpe* gene acquisition by horizontal transfer in clade 1 strains only

- Organization plasmid *cpe* locus



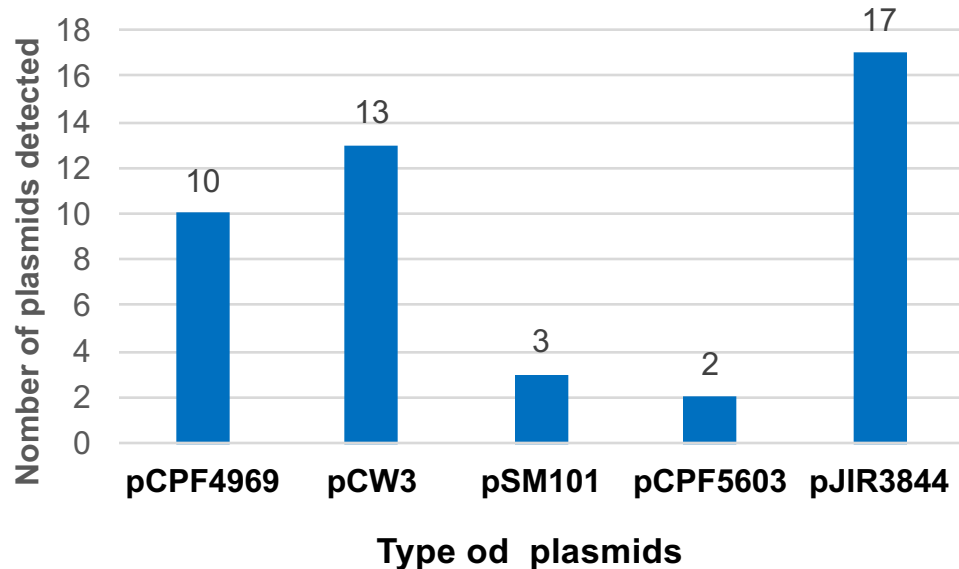
What are the consequences on *cpe* expression?

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Distinct plasmid carriage between clade 1 and clade 2 *C. perfringens* isolates



5 types of known plasmids:

- pJIR3844 (*cpb2*) (Miyamoto et al. 2006)
- pCPF5603 (*cpb2* and *cpe*) (Li et al. 2013)
- pCPF4969 (*cpe*) (Li et al. 2013; Miyamoto et al. 2006)
- pSM101 (Shimizu et al. 2002)
- pCW3 (*tet* resistance gene) (Adam et al. 2018)

- 39 of 45 known plasmids were detected in clade 2 isolates.
- 6 isolates of clade 1 contain plasmids pSM101 and pCW3
- Is pathogenicity in clade 2 linked to the presence of plasmids ?
- Does clade 1 strains carry unknown plasmids ?
- What is the (genetic/environmental) reason for this distinct pattern ?

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Conclusion and perspectives

Genomic analysis provides a new picture of *C. perfringens* associated FPOs

- Are *cpe*+ positive strains the only responsible of enteropathogenicity ? Role of *cpe* isolates in enteropathogenicity ?
- Contaminations of food samples by distinct strains is common : A challenge for FPO investigation
- The ISO method for Cp detection is inadequate to characterize the contamination > Necessity to develop characterization method as complementary method
- Further on-field studies are required for an epidemiological insight on the origin of the two clades
- Enlarge the collection to further explore an eventual food matrices-genomic link
- Impact of genetic organization on CPE production ?



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