

Development and validation of a multiplex real-time PCR assay with Taqman® technology for detection of *Escherichia coli* O80:H2

EURL STEC WorkShop 18-19/10/2021

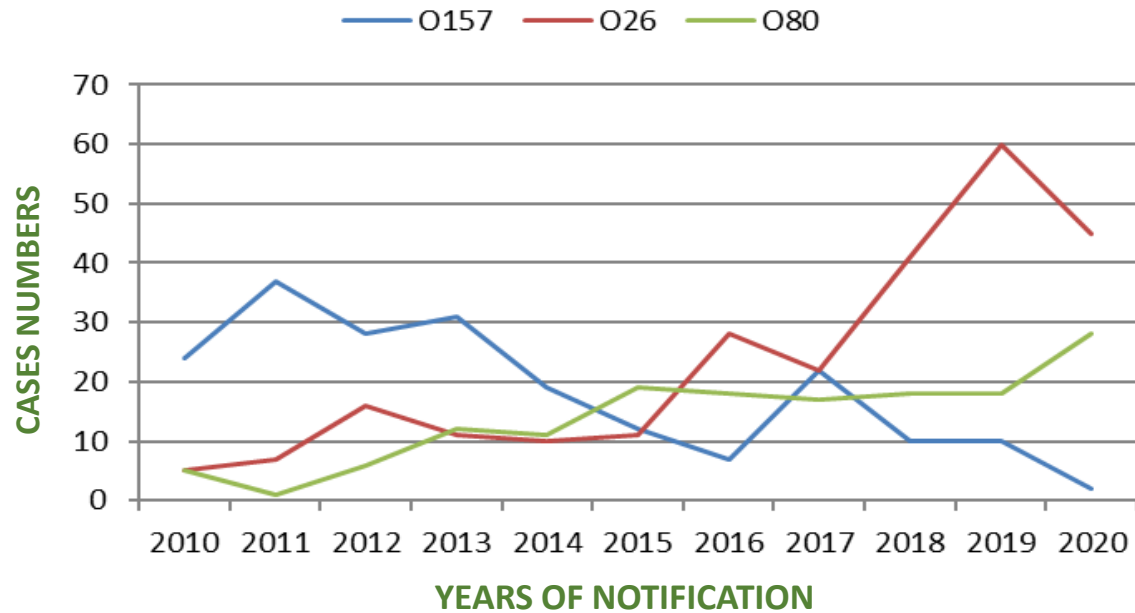
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Associated French CNR

Emergence of O80 STEC strains in France and Europe



Gabrielle JONES Santé publique France 2021

Table 1: STEC serogroup distribution for human cases associated with HUS, BD or hospitalisation (TESSy data, 2012–2017)

Serogroup	Number of HUS cases	%	Serogroup	Number of hospitalised cases	%	Serogroup	Number of BD cases	%
157	634	38.4	157	2,753	60.4	157	4,245	71.6
26	403	24.4	26	705	15.5	26	582	9.8
111	85	5.1	145	137	3.0	103	162	2.7
80	74	4.5	103	107	2.4	145	159	2.7
145	68	4.1	111	97	2.1	91	64	1.1
55	48	2.9	146	51	1.1	146	51	0.9
121	44	2.7	91	33	0.7	111	49	0.8
103	42	2.5	55	32	0.7	128	32	0.5
91	17	1.0	5	26	0.6	5	28	0.5
104	6	0.4	174	21	0.5	55	27	0.5
Other	232	14.0	Other	598	13.1	Other	532	9.0
Total	1,653	100.0	Total	4,560	100.0	Total	5,931	100.0

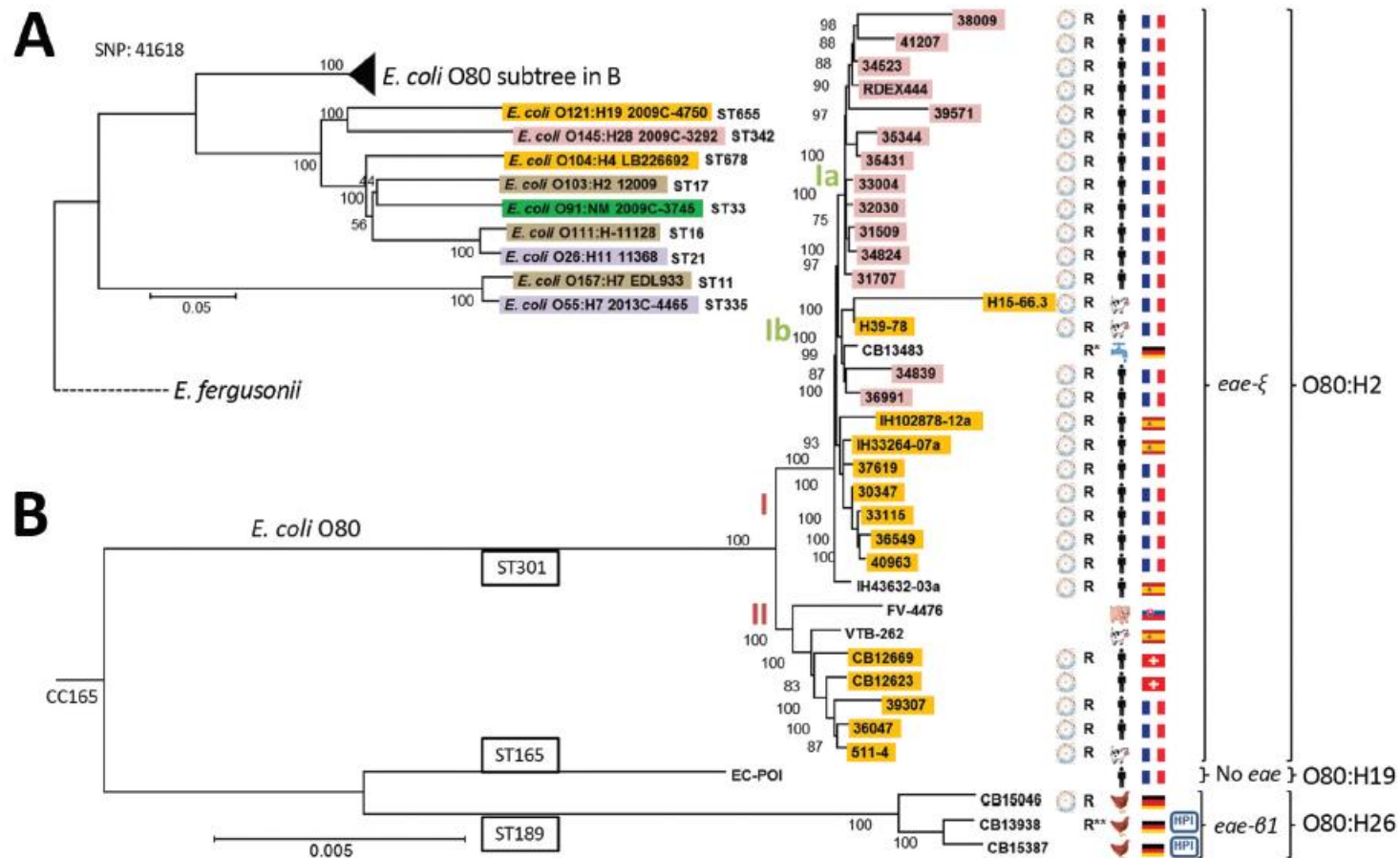
EFSA, zoonoses 2020

O80:H2 : an hybrid pathotype ST301

EHEC-associated genes
Stx2, eae ξ, ehxA

+

ExPEC-associated genes



A Cointe and al, 2018

PRINCIPLE OF MULTIPLEXE O80 PCR

**100% O80:H2 STEC ST301
(137/137) Mel -**

**Non O80 STEC : 95% (333/352) : Mel (+)
5 % (19/352) : Mel (-)**

Bizot et al., 2021

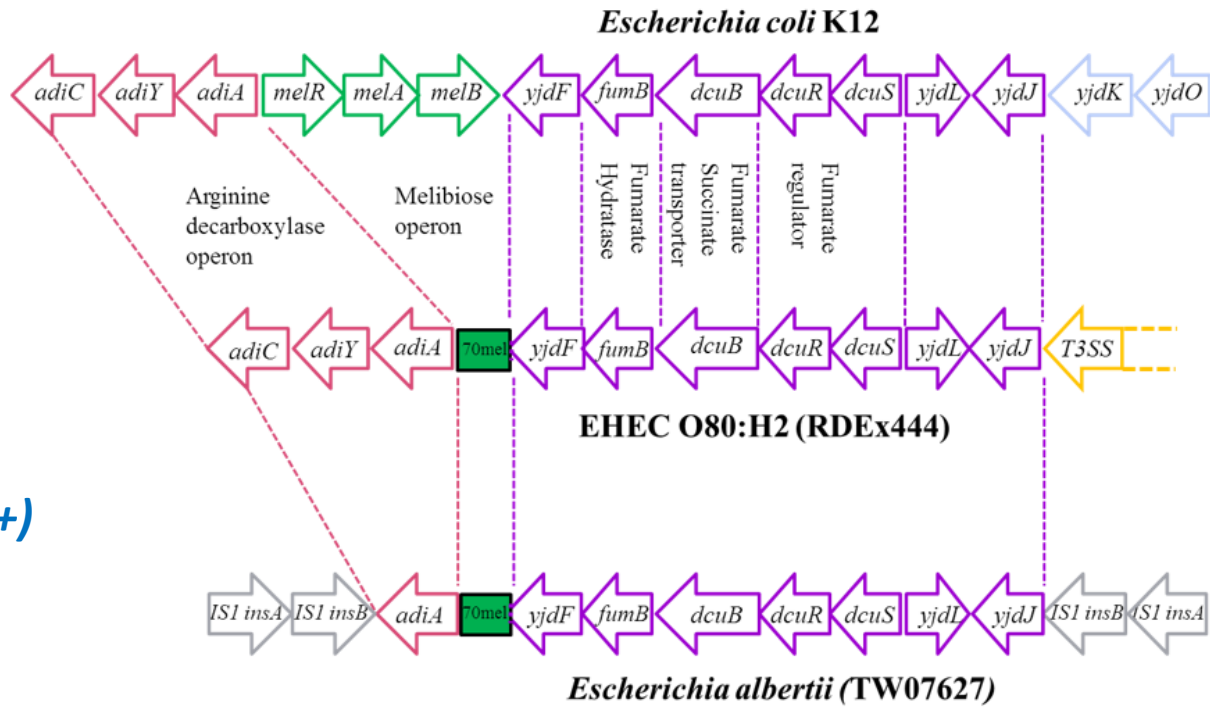
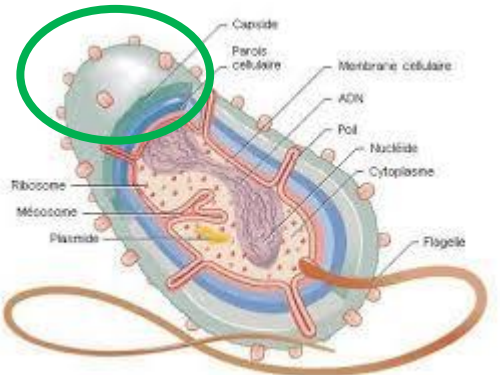
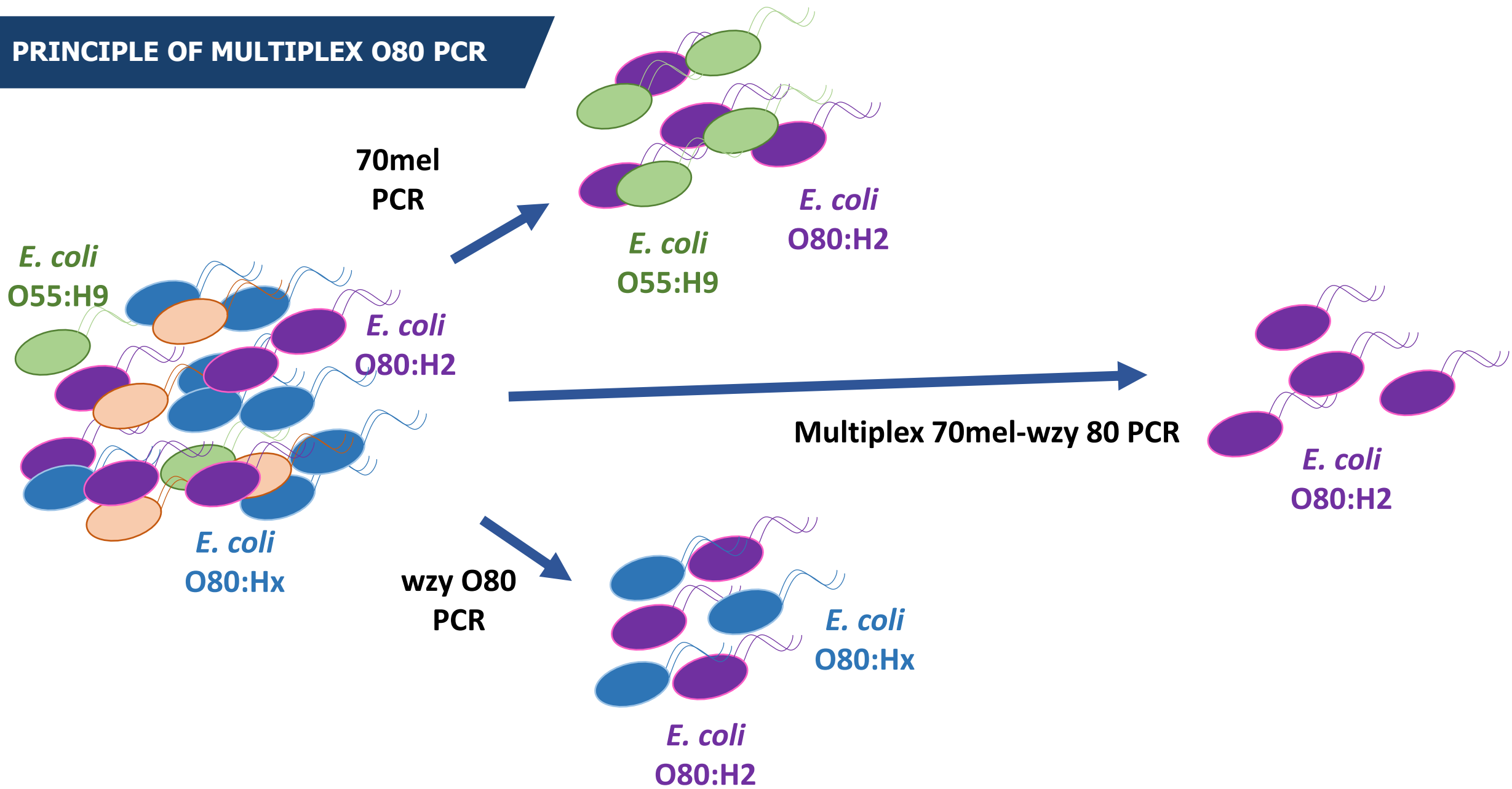


Figure 1. Genetic organization comparison of the melibiose operon and its flanking regions between *Escherichia coli* K12, a representative of O80:H2 EHEC clone (RDEx444) and *Escherichia albertii*.



somatic antigen O

PRINCIPLE OF MULTIPLEX O80 PCR



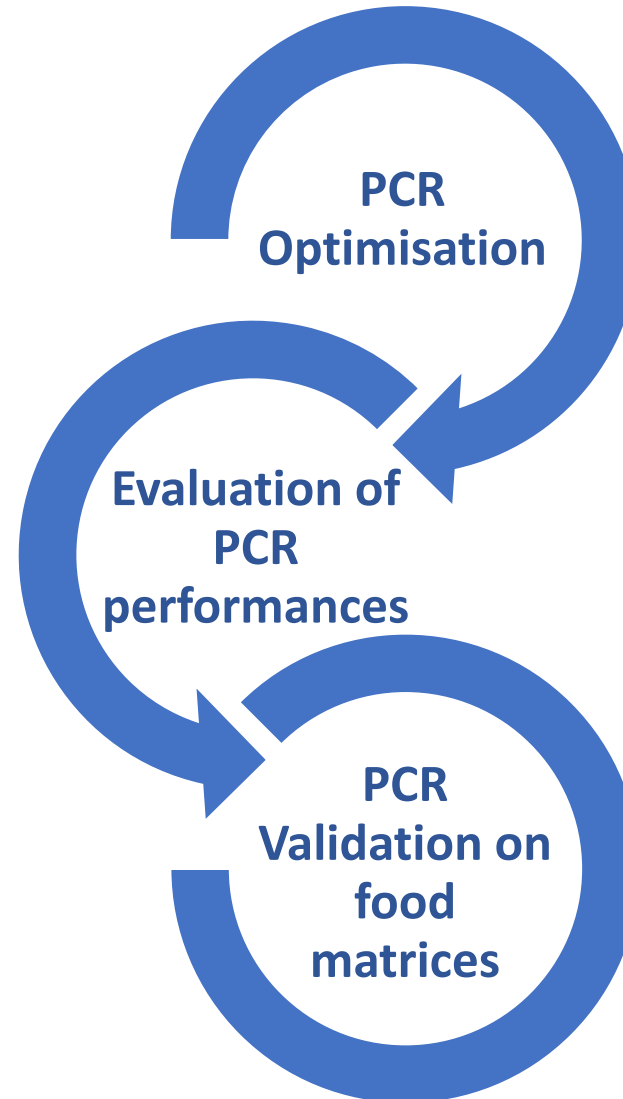
Point
1

The aim of this study was to develop a multiplex RT-PCR assay with Taqman[®] technology for the detection of *E. coli* O80:H2 in different food samples

Point
2

The method had to be specific, sensitive for *E. coli* O80:H2, rapid, robust

Multiplex real-time PCR O80:H2 development



Primers and probes design

✓ Work bases:

- **wzyO80**: Targets the wall lipoprotein characteristic of serogroup O80.
Igushi et al 2015

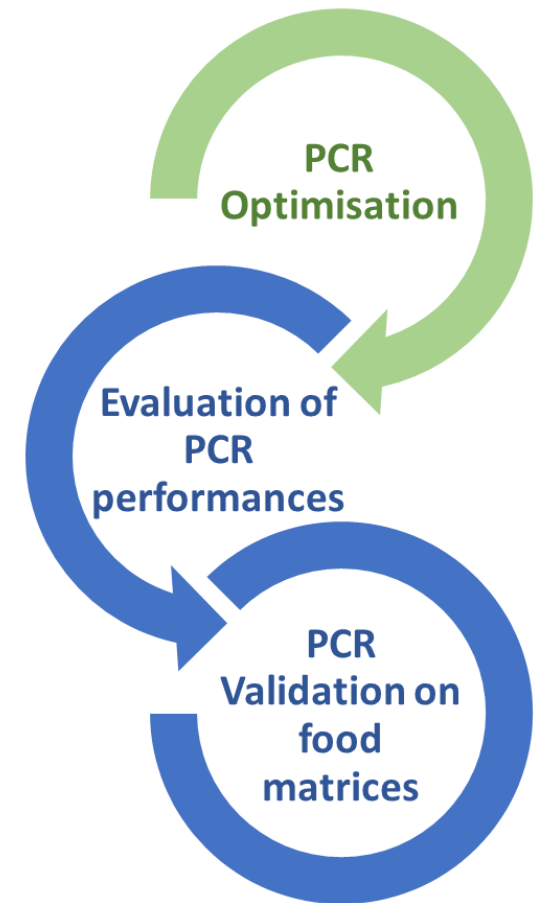
O80	wzy	Og80-PCR_F	TGGTGTTGATTCCACTAGCGT
		Og80-PCR_R	CGAGAGTACCTGGTCCCAA

- **70mel**: Deletion of melebiose operon with insertion of a 70pb sequence characteristic of ST301 (O80:H2, O55:H9....) *Bizot et al. 2021*

O80:H2 Mel	ΔMEL-F	AGCGCCAGAAGTGTTCAATCT
	ΔMEL-R	TCAGGCTGTAAAGCGCATGA

✓ Design Primers and probes => [genscript.com](https://www.genscript.com) platform

Target gene		Forward primer, reverse primer and probe sequences (5'-3')	GenBank accession number
70mel	Forward primer	AGCGCCAGAAGTGTTCAATCT	/
	Reverse primer	ATCGTCTGTAGCCGGGAAGC	
	Probe	CGCAACCCGCCCGGCAGCCA	
wzyO80	Forward primer	TGGTGTTGATTCCACTAGCGT	AB812032
	Reverse primer	AGCCATCCTGATTGGCGTGA	
	Probe	CGGCAGCAGCAACCAAGGATGATGCC	



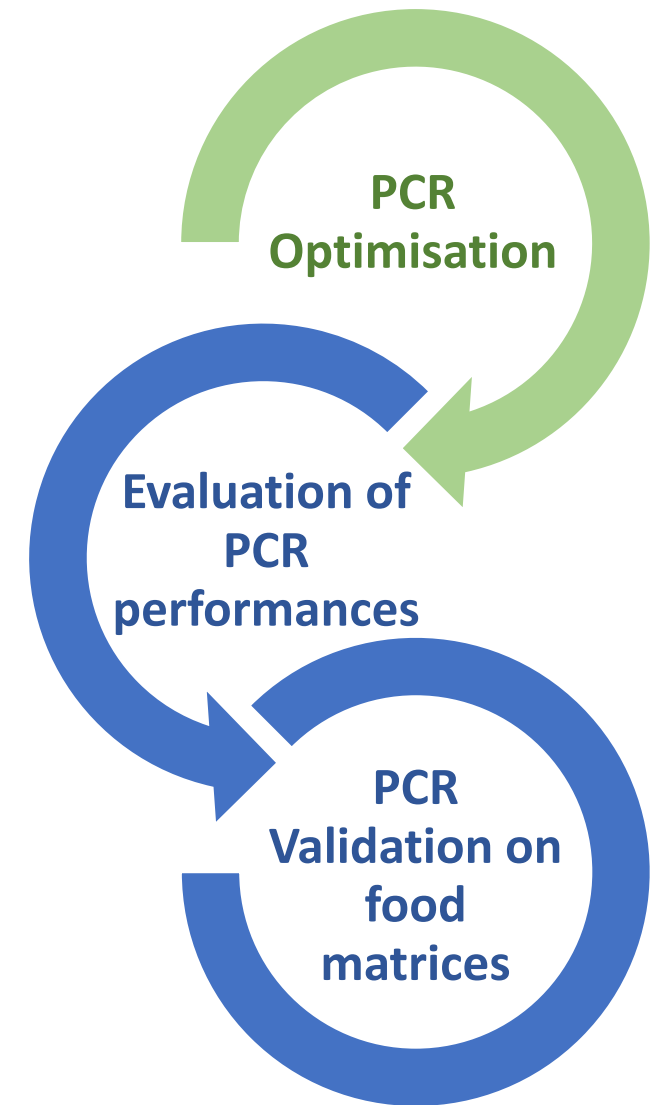
In silico
tests on
NCBI

PCR Multiplex optimisation

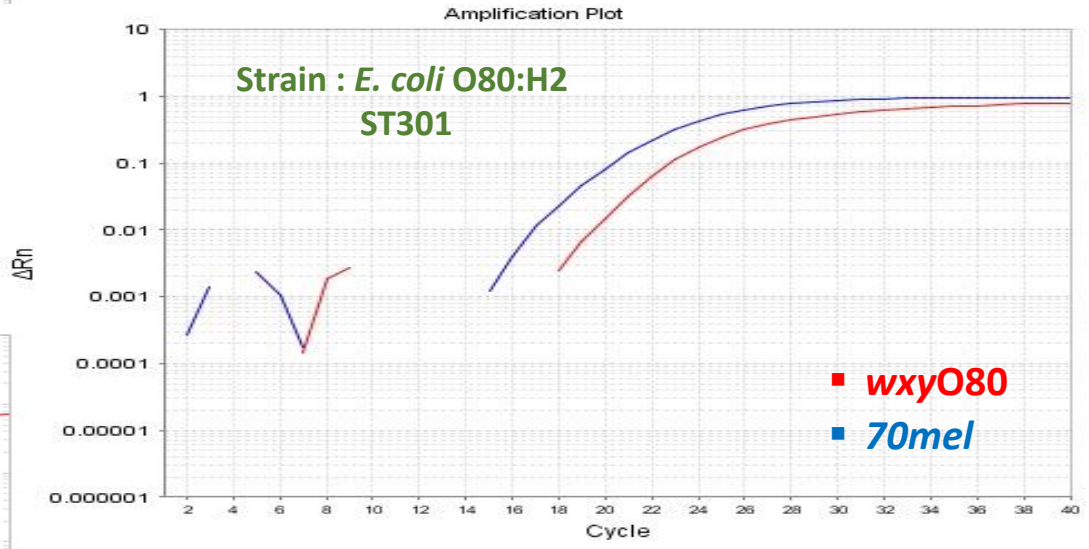
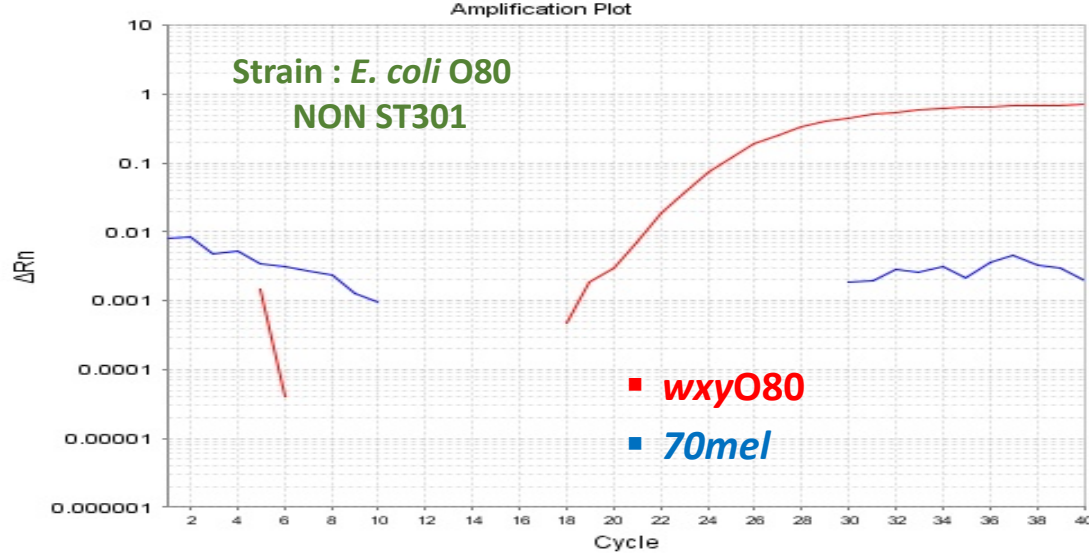
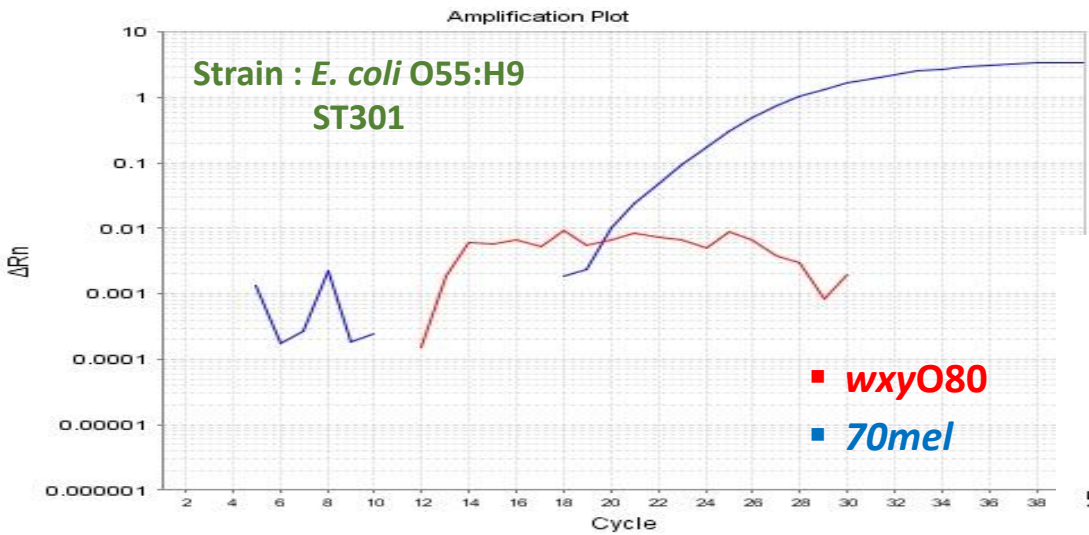
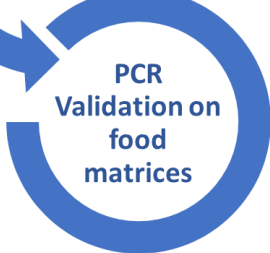
Mixe PCR Taqman x1	
Taqman environnemental 2.0 Master mixe	1X
Primer wxyO80 Fwd	500 nM
Primer wxyO80 Rev	500 nM
Primer 70mel Fwd	500 nM
Primer 70mel Rev	500 nM
Probe wxyO80	200 nM
Probe 70mel	200 nM
DNA	5µL
Water to final volume	20µL

The thermocycler conditions		
	Temperature	Time
pre-incubation	50°C	2 min
	95°C	10 min
Denaturation	95°C	15 s
Hybridation/elongation	60°C	1 min

X40



PCR Multiplex optimisation



PCR performances

Sensitivity : *E. coli* O80:H2 (n=23) => **100 %**

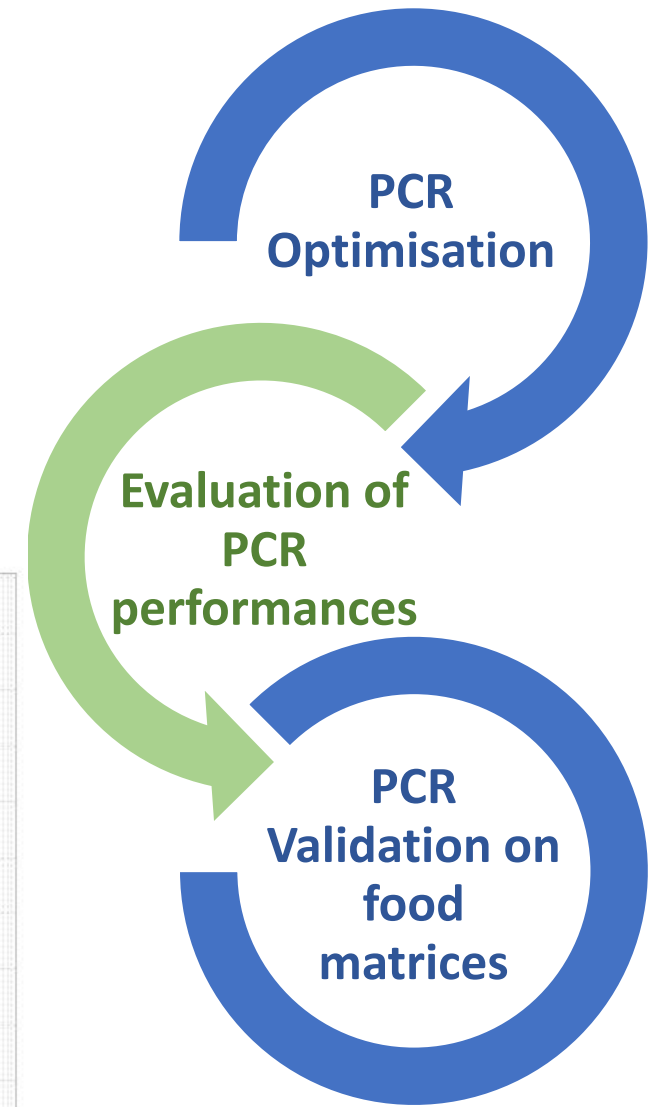
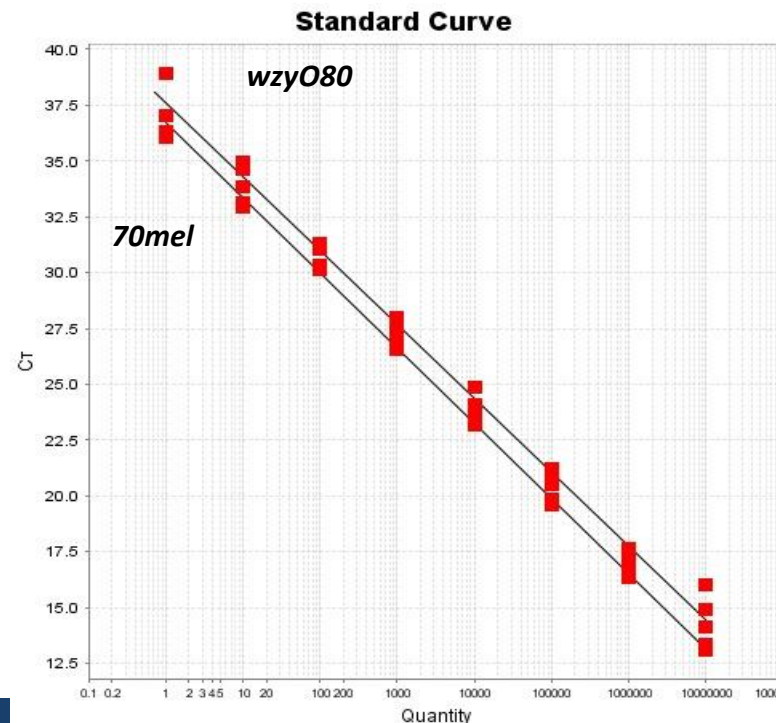
Specificity : Non *E. coli* O80:H2 (n=102) => **100 %**

LD PCR 95 : This assay detected between 5 and 10 copies of the two target gene per reaction with **good efficiency**

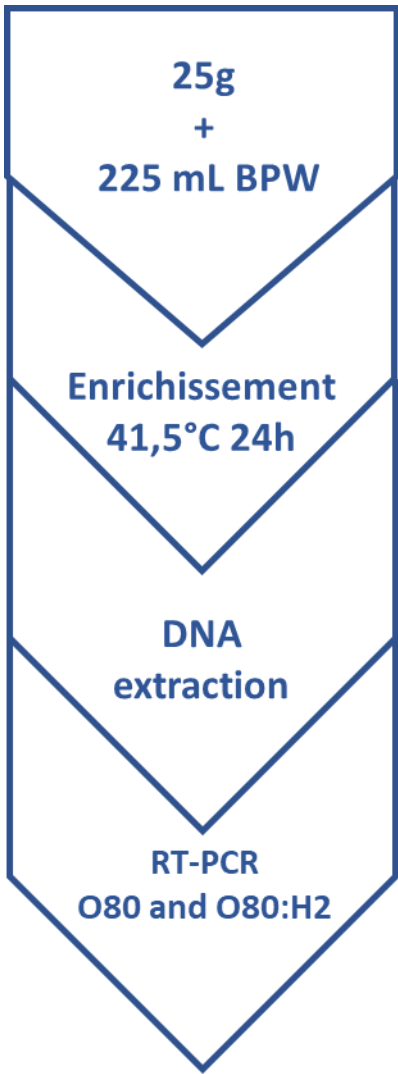
wzyO80 : 100,441 avec $R^2 = 0,993$

70mel : 98,768 avec $R^2 = 0,999$

Strains	Number
<i>E. coli</i> O80	4
<i>E. coli</i> O55:H9 mel –	6
<i>E. coli</i> O55:H9 mel +	2
<i>E. coli</i> STEC Top 5	44
<i>E. coli</i> STEC non Top 5	16
Non <i>E. coli</i>	30
	102

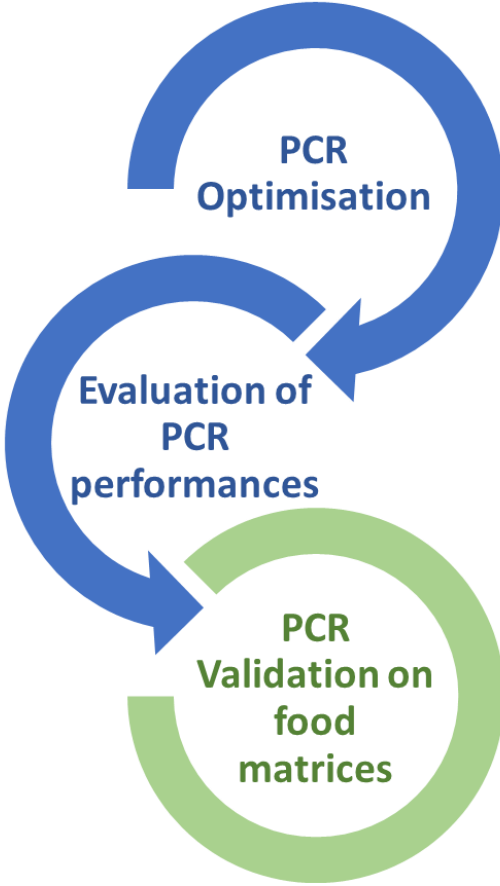
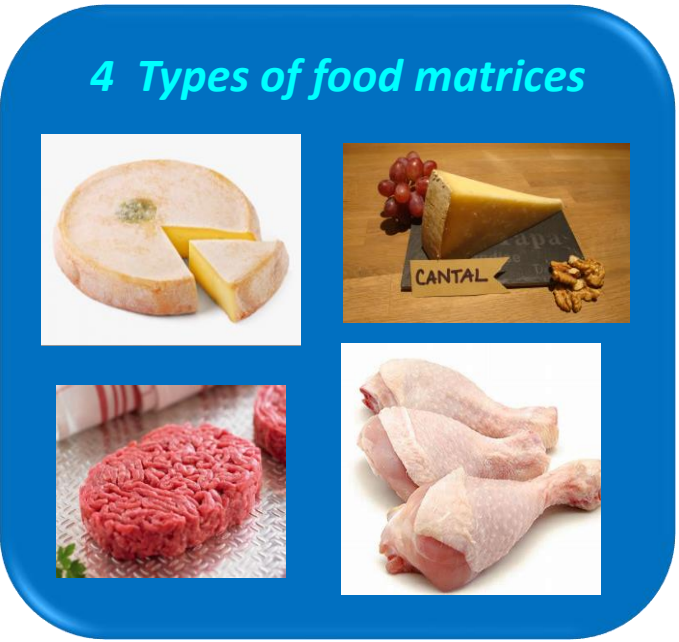


Sensitivity essays in foods



6 contaminations levels
 10^1 CFU/ml \Rightarrow 10^6 CFU/ml

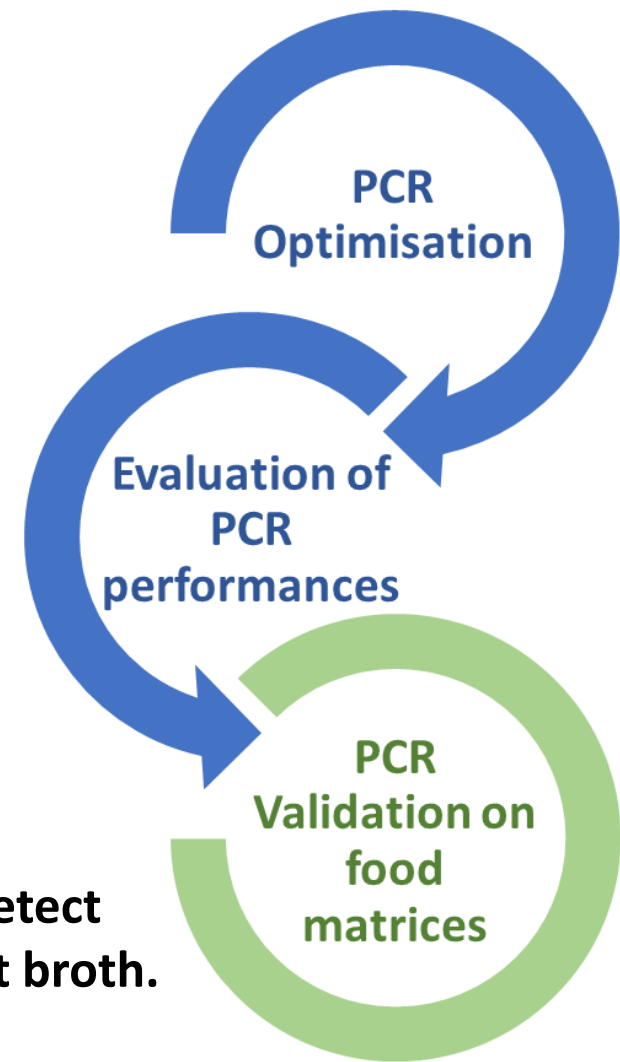
STEC 3 strains
O80:H2



Sensitivity essays in foods

Food	Poultry			Minced beef			Raw milk cheese					
							Cantal			Reblochon		
Concentration (CFU/ ml)	strain 1	strain 2	strain 3	strain 1	strain 2	strain 3	strain 1	strain 2	strain 3	strain 1	strain 2	strain 3
1,00E+01	-	-	-	-	-	-	-	-	-	-	-	-
1,00E+02	-	+	+	+	-	+	+	+	-	+	+	-
1,00E+03	+	+	+	+	+	+	+	+	+	+	+	+
1,00E+04	+	+	+	+	+	+	+	+	+	+	+	+
1,00E+05	+	+	+	+	+	+	+	+	+	+	+	+
1,00E+06	+	+	+	+	+	+	+	+	+	+	+	+

The post enrichment protocol showed that this RT-PCR assay was sensitive to detect *E. coli* O80:H2 at a level of 10²/10³ CFU *E. coli* per ml of cheese or meat enrichment broth.



Further work will be carried out :

- ✓ On the specificity of the multiplex RT-PCR,
- ✓ testing new experimentally and naturally contaminated food matrices

The tool developed will be used to identified for O80 STEC strains:

- ✓ for industrial controls,
- ✓ in the monitoring plans developed by the Ministry of Agriculture,
- ✓ or food alerts and outbreaks,
- ✓ And to help to identify the reservoir, currently unknown, and thus improve the epidemiological knowledge of this emerging clone.

Thank you

