anses
French agency for food, environmental and occupational health & safety

EURL *Lm*

European Union Reference Laboratory for *Listeria monocytogenes*

APPLICABILITY OF THE STANDARD **EN ISO 11290-1&2** FOR *LM* DETECTION & ENUMERATION **IN PRESENCE OF NEW *LISTERIA* SPECIES**

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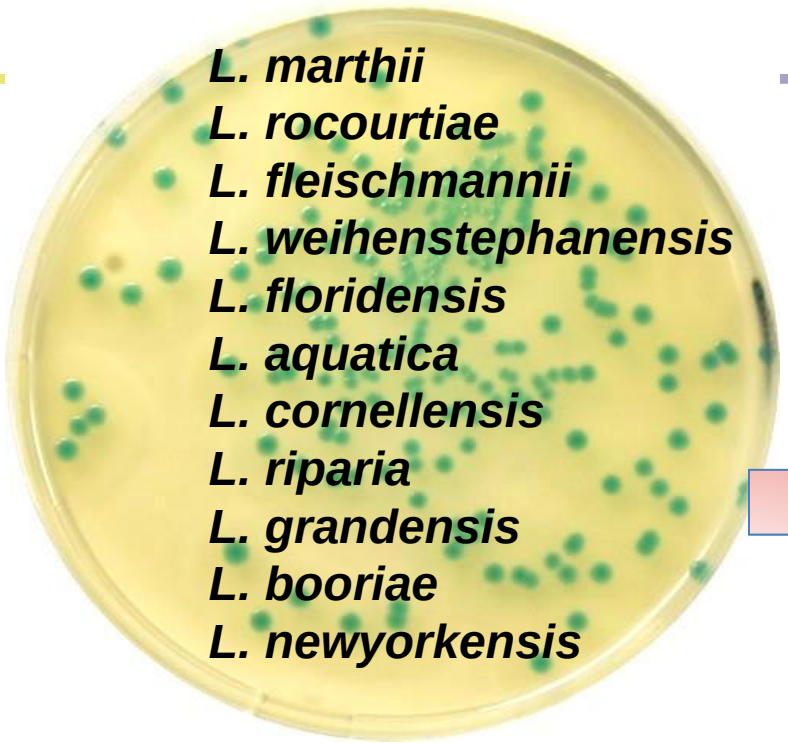
WS Listeria 25-27 march 2015

1. Context and objectives
2. M & M / preliminary results
3. Conclusion

Context

Listeria species

L.monocytogenes
L.innocua
L.ivanovii
L.seeligeri
L.welshimeri
L.grayi



17 species

EN ISO 11290-1&2 under revision (include all other *Listeria* spp)
To check the methods' ability to recover and detect the newly identified *Listeria* species.

- ✓ growth and colony characteristics on commonly used *Listeria* selective isolation agars,
- ✓ reaction to some biochemical tests,
- ✓ growth performance in the selective enrichment broths in the presence or absence of other *Listeria* spp., in particular *L. monocytogenes*

Context

Objective :

- To evaluate the ability of the updated Standard EN ISO 11290-1&2 methods to **detect and identify** newly discovered *Listeria* spp

- To evaluate the impact of strain over-growth during each step of the enrichment process in mixed cultures with *L.monocytogenes* (**false negative results)**

1. Context and objectives
2. M & M / preliminary results
3. Conclusion

1. Inclusivity of the method

- 1.1- Detection of the new Listeria species
- 1.2- Colonies aspect on commonly used *Listeria* selective isolation agars

2. Results to confirmation tests

- 2.1- Results to phenotypic and biochemical tests
- 2.2- Additional confirmation tests for **genus** identification
- 2.3- Additional confirmation tests for **species** identification

3. Growth in selective enrichment broths, and impact on Lm analysis:

- 3.1- Growth rates
- 3.2 - Evolution in co-cultures with Lm
- 3.3- Inhibitory activities

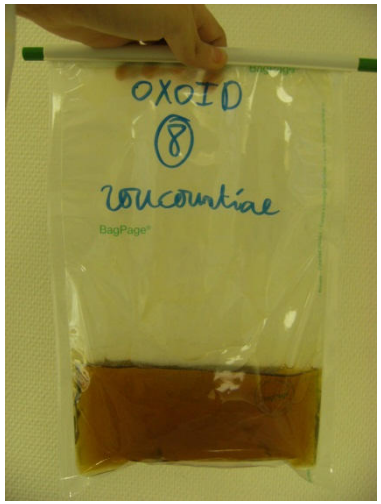
- Preliminary results will be presented
- For each criteria, each species (11) is studied
  - Need 3 repetitions /condition
  - End of the project: end 2015

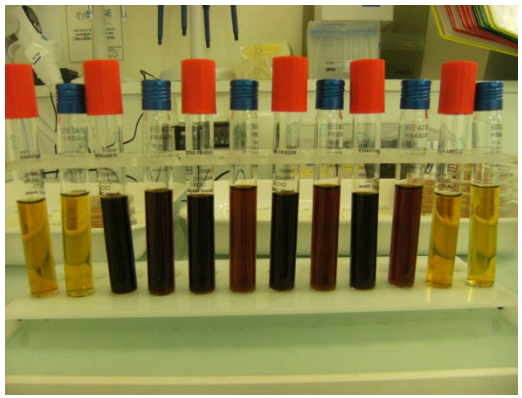
1. Inclusivity of the method

1.1- Detection of the new *Listeria* species

- *L. cornellensis*
- *L. grandensis*
- *L. rocourtiae*
- *L. weihenstephanensis*

Difficulty to detect them after the 1st and/or 2nd enrichment





Preliminary results : 2 other repetitions

- ***1.2- Colonies aspect on commonly used* Listeria *selective isolation agars***

ALOA

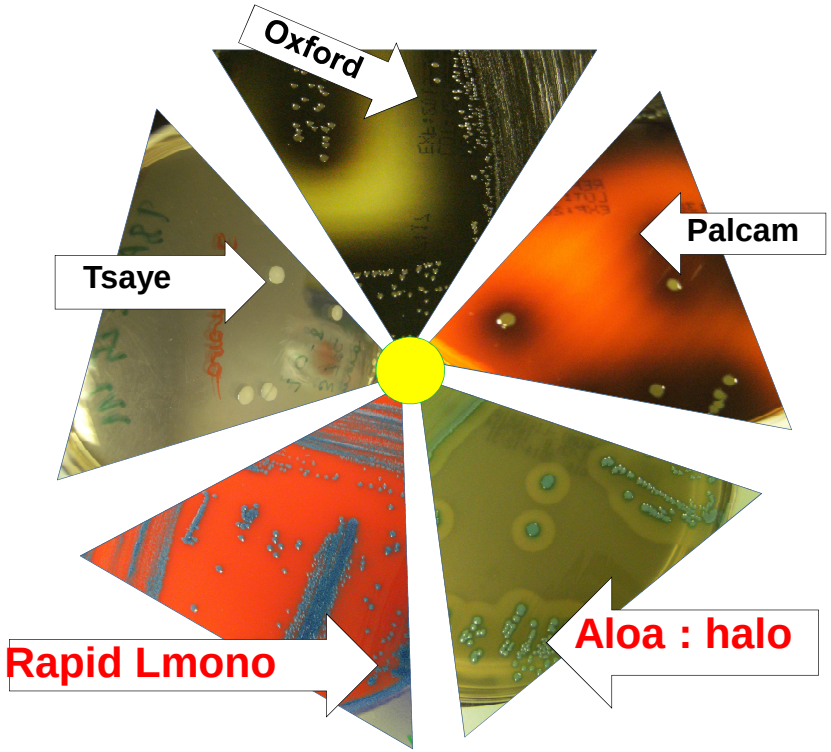
TSAYE **Rapid L mono**

Oxford **Palcam**

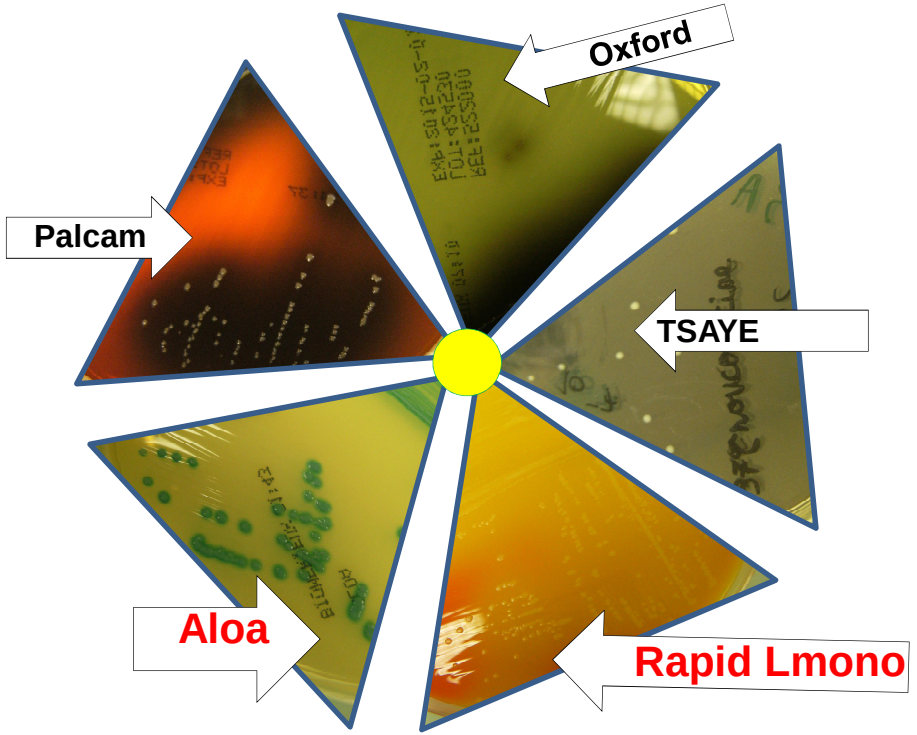
Aspect of colonies:

- **At 30/37° C**
- **After 24/48/72 h of incubation**

L. monocytogenes



L. rocourtiae



Colony aspect on commonly used *Listeria* selective isolation agars

Montage image: Béatrice Tésolin

Satisfactory aspect (in accordance with genus *Listeria* and ≠ species *monocytogenes*)

Preliminary results : 2 other repetitions

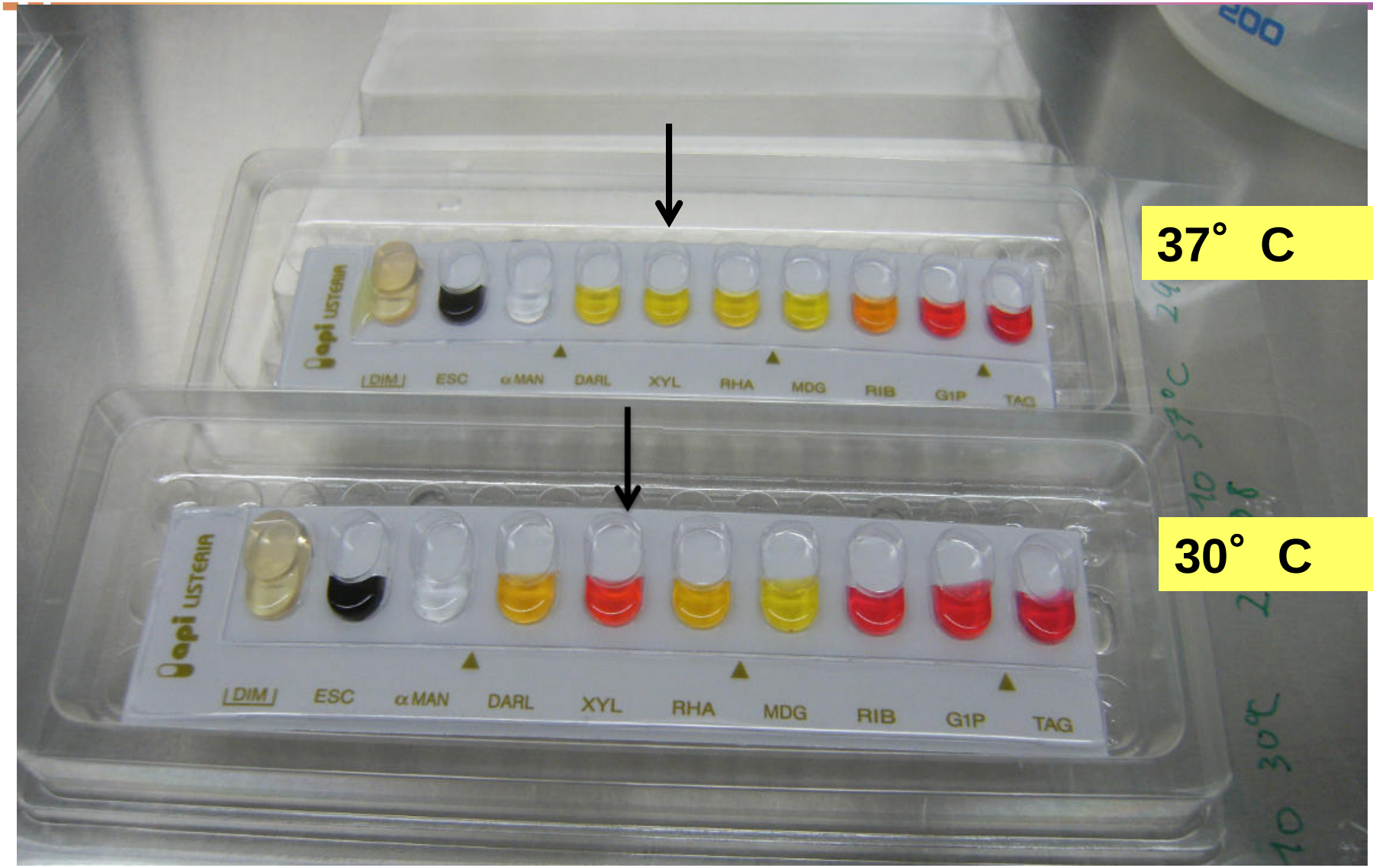
2. Results of confirmation tests

2.1- Results of phenotypic and biochemical tests

Confirmation test	Results obtained with new species
Gram	+
Catalase	+
Motility	nd
Hemolysis	-
CAMP	-
VP	nd
Xyl / Rham	+/- (T°C)

Preliminary results : 2 other repetitions

Confirmation test satisfactory (Motility, VP?)

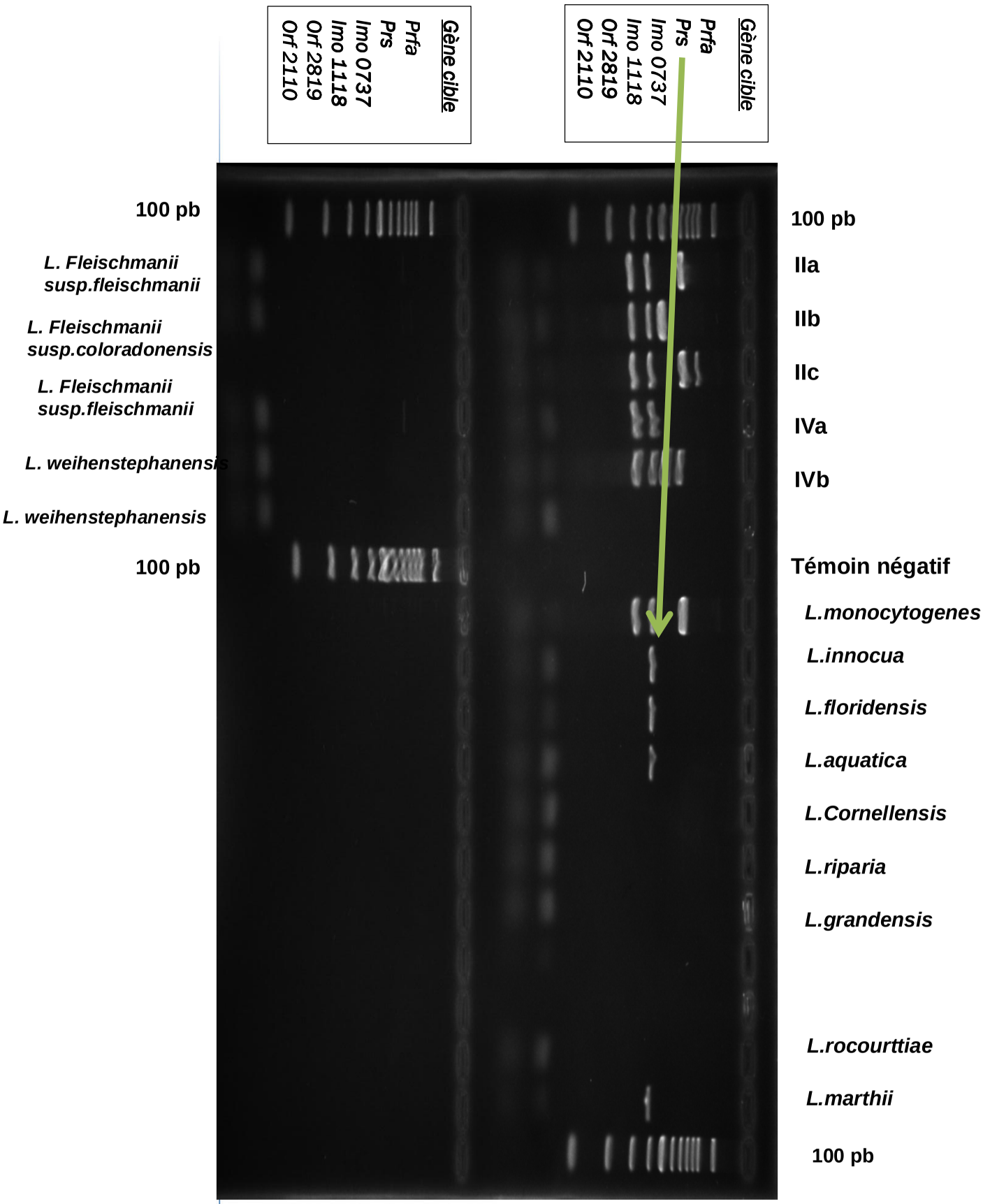


Api Listeria : *L.fleischmannii* incubated at 37° C and 30° C

- **2.2- Additional confirmation tests for genus identification:**

EURL Molecular serotyping scheme :
prs gene (specific of *Listeria* genus) :

Out of 13 strains tested : for 9 strains : no amplification


Gène cible
Prfa
Prs
Imo 0737
Imo 1118
Orf 2819
Orf 2110
Gène cible
Prfa
Prs
Imo 0737
Imo 1118
Orf 2819
Orf 2110
100 pb
L. Fleischmanii susp.fleischmanii
L. Fleischmanii susp.coloradonensis
L. Fleischmanii susp.fleischmanii
L. weihenstephanensis
L. weihenstephanensis
100 pb
100 pb
IIa
IIb
IIc
IVa
IVb
Témoin négatif
L.monocytogenes
L.innocua
L.floridensis
L.aquatica
L.Cornellensis
L.riparia
L.grandensis
L.rocourttiae
L.marthii
100 pb

- **2.3- Additional confirmation tests for species identification:**

-Select other genes specific of the *Listeria* genus available in the literature (*iap* gene or others ?).

3. Growth in selective enrichment broths, and impact on Lm analysis:

- ***3.1- Growth rates:***

Growth curves for each strain will be determined in Half Fraser and Fraser broths.

- ***3.2 - Evolution in co-cultures with Lm:***

To check the evolution of relative proportion of any given species over the enrichment process.

- ***3.3- Inhibitory activities:***

The new *Listeria* spp. will be screened for the production of inhibitory activities. BHI & Half Fraser broth cultures will be filter sterilized and tested for inhibitory activity by drop testing. Cross-testing will be carried out against a standard test panel consisting of a variety of *Lm* and of *Listeria* spp.

1. Context and objectives
2. M & M / preliminary results
3. Conclusion

➢ Overview of the preliminary results

➢ Detection of new species :

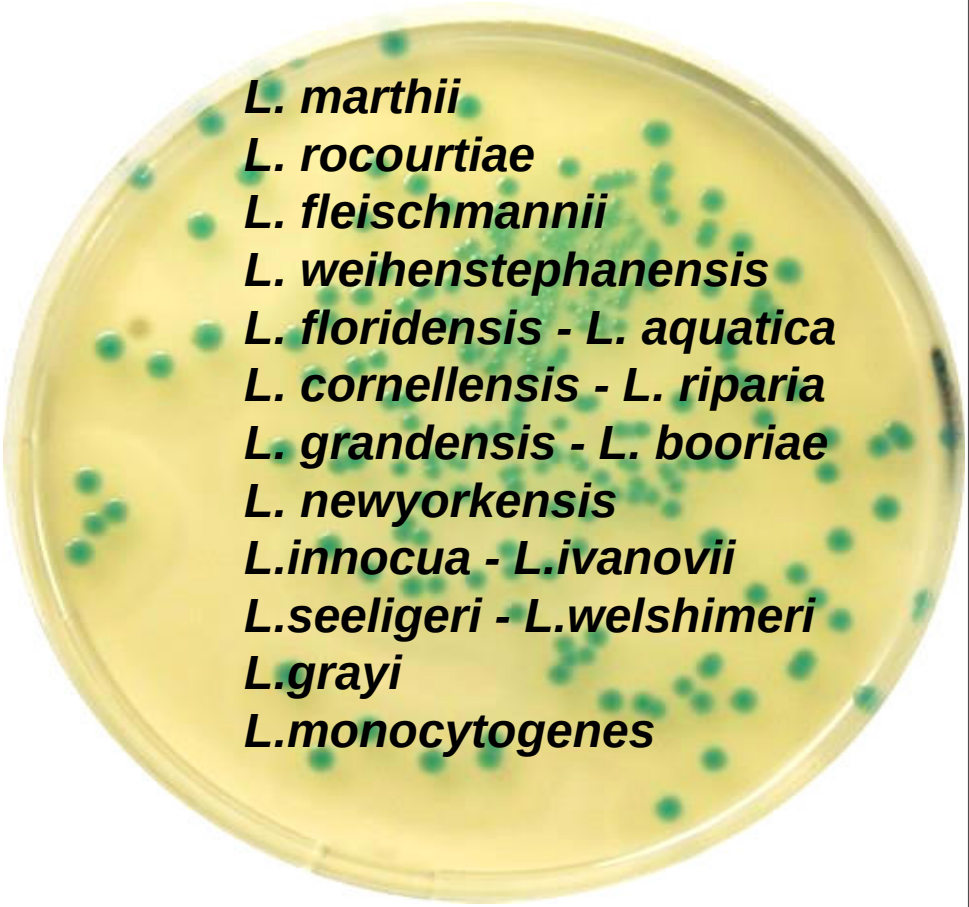
- -/+ detection after enrichment
- Aspect on selective agar satisfactory (no false + results with *Lm*)
- At 1rst sight, if *Lm* + other L. *spp*> detection of *Lm* correct (no false – results with *Lm*)

- That was just a preliminary conclusion !

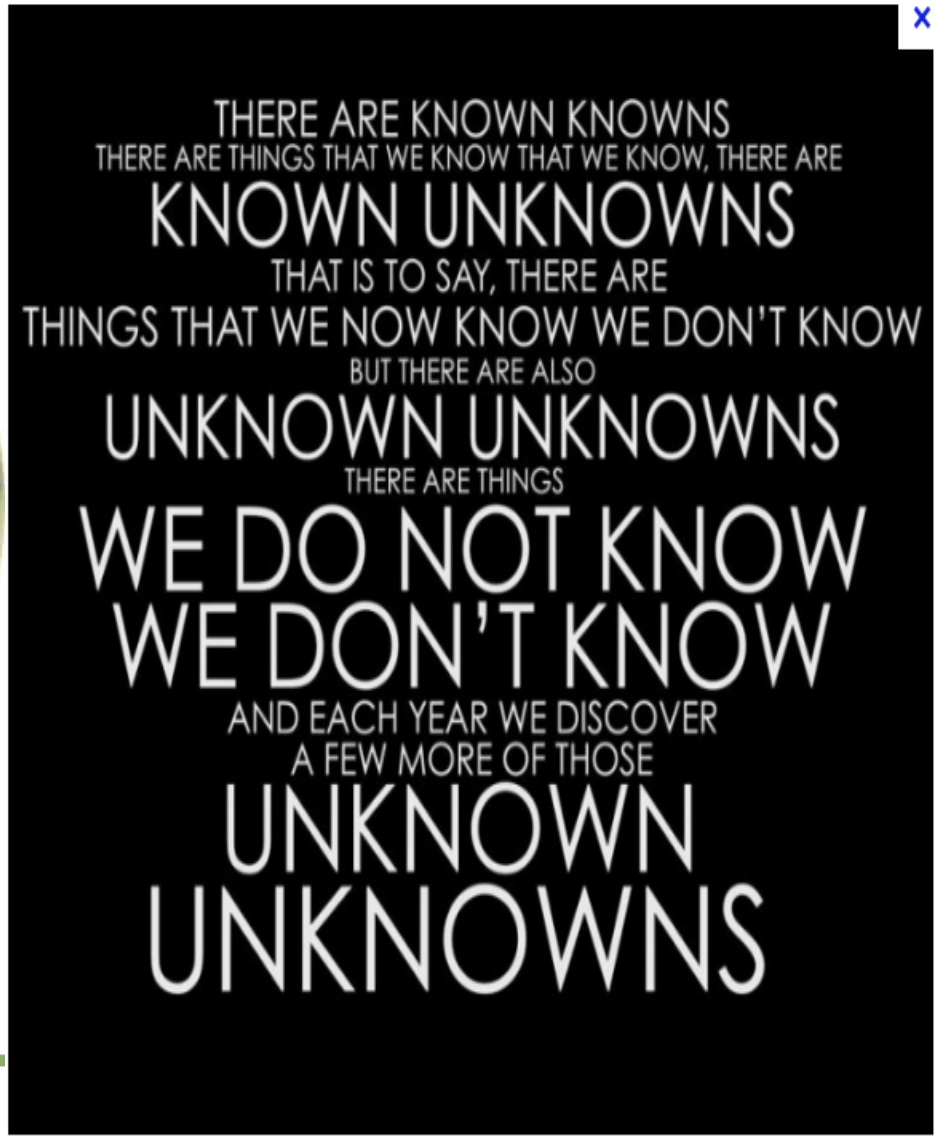
- Many experiments to be further conducted (detection) and others to start

To **characterize** the species of *Listeria* recently identified

Thank you



L. marthii
L. rocourtiae
L. fleischmannii
L. weihenstephanensis
L. floridensis - L. aquatica
L. cornellensis - L. riparia
L. grandensis - L. booriae
L. newyorkensis
L.innocua - L.ivanovii
L.seeligeri - L.welshimeri
L.grayi
L.monocytogenes



	N° d'identification	Espèce	N° échantillon	Référence	Isolée de	Littérature	Génome
5	DSM 26687	L. floridensis	15.USEL.003	BEI NR-42632,	eau	http://www.ncbi.nlm.nih.gov/pubmed/24599893	http://www.ncbi.nlm.nih.gov/nuccore/JX961636
6	DSM 26686	L. aquatica	15.USEL.004	BEI NR-42633, FSL S10-1188	eau	http://www.ncbi.nlm.nih.gov/pubme	http://www.ncbi.nlm.nih.gov/nuccore/JX961637
7	DSM 26689	L. cornellensis	15.USEL.005	BEI NR-42630, FSL F6-0969, FSL F6-969, TTU	eau	http://www.ncbi.nlm.nih.gov/pubmed/24599893	http://www.ncbi.nlm.nih.gov/nuccore/JX961634
8	DSM 26685	L. riparia	15.USEL.006	BEI NR-42634, FSL S10-1204	eau	http://www.ncbi.nlm.nih.gov/pubmed/24599893	http://www.ncbi.nlm.nih.gov/nuccore/JX961638
9	DSM 26688	L. grandensis	15.USEL.007	FSL F6-0971, FSL F6-971, TTU A1-212	eau	http://www.ncbi.nlm.nih.gov/pubmed/24599893	http://www.ncbi.nlm.nih.gov/nuccore/JX961635
10	DSM 22097	L. rocourtiae	15.USEL.008	Allerberger 700284/02, CLIP 2007/00563	laitue	http://www.ncbi.nlm.nih.gov/pubmed/19915117	http://www.ncbi.nlm.nih.gov/nuccore/FJ557241
11	DSM 23813	L. marthii	15.USEL.009	FSL S4-120	sol	http://www.ncbi.nlm.nih.gov/pubmed/19667380	http://www.ncbi.nlm.nih.gov/nuccore/EU545982
12	DSM 24998	L. fleischmanii subsp. fleischmanii	15.USEL.010	LU2006	fromage	http://www.ncbi.nlm.nih.gov/pubmed/22523164 http://www.ncbi.nlm.nih.gov/pubmed/23524352	
13	DSM 25391	L. fleischmanii subsp. coloradonensis	15.USEL.011	TTU M1-001	sol de pâturage pour le bétail	http://www.ncbi.nlm.nih.gov/pubmed/23524352	http://www.ncbi.nlm.nih.gov/nuccore/JQ287762
14	DSM 25003	L. fleischmanii subsp. fleischmanii	15.USEL.012	81384-1, FAM 21875, LU2006-3	affinage du fromage (cave)	http://www.ncbi.nlm.nih.gov/pubmed/22523164	http://www.ncbi.nlm.nih.gov/nuccore/JN093103
15	DSM 24698	L. weihenstephanensis	15.USEL.013	WS 4560	usine d'eau *Lemna minuta* à partir d'un étang	http://www.ncbi.nlm.nih.gov/pubmed/22544790	http://www.ncbi.nlm.nih.gov/nuccore/FR850019
16	DSM 24699	L. weihenstephanensis	15.USEL.014	WS 4615	usine d'eau *Lemna minuta* à partir d'un étang	http://www.ncbi.nlm.nih.gov/pubmed/22544790	http://www.ncbi.nlm.nih.gov/nuccore/FR850020
17	DSM 28860	L. booriae	15.USEL.015	FSL A5-0281	surface non en contact avec les aliments dans une usine de transformation de produits laitiers	http://ijs.sgmjournals.org/content/65/Pt_1/286.abstract	http://www.ncbi.nlm.nih.gov/nuccore/JNFA00000000
	DSM 28861	L. newyorkensis	15.USEL.016	FSL M6-0635	surface non en contact avec les aliments dans une usine de	http://ijs.sgmjournals.org/content/65/Pt_1/286.abstra	http://www.ncbi.nlm.nih.gov/nuccore/JNFB00000

Feuil1 Feuil2 Feuil3

Prêt

LM DETECTION & ENUMERATION IN PRESENCE OF NEW *LISTERIA* SPECIES

	Culture en BHI 24h (6h + 18h)								
Semaine 48	NOM	30°C		37°C			30°C		37°C
		-7	-8	-5	-6	-7	-8	UFC/ml 30°C	Calcul dilution pour 100UFC/ml	UFC/ml 37°C
1	MONO	128	18			115	10	1,44E+09	0,1ml 10-6	1,24E+09
2	innocua	145	9			70	10	1,53E+09	0,1ml 10-6	7,91E+08
3	floridensis	166	17			115	7	1,81E+09	0,1ml 10-6	1,21E+09
4	aquatica	214	16			117	17	2,29E+09	0,05 ml 10-6	1,32E+09
5	cornellensis	99	11	117	35			1,09E+09	0,1ml 10-6	1,49E+07
6	riparia	111	11			83	5	1,21E+09	0,1ml 10-6	8,75E+08
7	grandesis	111	10	124	0			1,20E+09	0,1ml 10-6	1,24E+07
8	rocourtiae	109	7		171	22	2	1,15E+09	0,1ml 10-6	1,91E+08
9	marthii	138	18			72	7	1,54E+09	0,1ml 10-6	7,84E+08
10	fleischmanii	207	18			155	18	2,23E+09	0,05 ml 10-6	1,71E+09
11	coloradonensis	234	35			152	14	2,66E+09	0,05 ml 10-6	1,65E+09
12	fleischmanii	179	20		172	16		1,97E+09	0,1ml 10-6	1,87E+08
13	weihenstephanensis	67	4		210	28	4	7,06E+08	0,2ml 10-6	2,35E+08
14	weihenstephanensis	52	2		177	2		0,00E+00	0,2ml 10-6	0,00E+00

B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
NOM	30°C										CODE	identification/ choix suivant	37°C									
	DIM(1)	ESC (2)	MAN(4)	DARL(1)	XYL (2)	RHA (4)	MDG (1)	RIB(2)	G1P (4)	TAG (1)			DIM(1)	ESC (2)	MAN(4)	DARL(1)	XYL (2)	RHA (4)	MDG (1)	RIB(2)	G1P (4)	TAG (1)
MONO	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	6510	Bonne mono 98,6% innocua 1,4%	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)
innocua	(+)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	7510	Bonne innocua 99,6% mono 0,1%	(+)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)
floridensis	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	2610	Profil inacceptable iva, wel, mono, seeli,ino	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)
aquatica	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	6601	Profil inacceptable welshimeri	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(+)
cornellensis	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	2210	Profil acceptable seeli 82,3%, iva 17,6%, wel 0,1%	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)
riparia	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	2610	Profil inacceptable iva, wel, mono, seeli,inno	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)
grandensis	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	2210	Profil inacceptable seeli 82,3%, iva 17,6%, wel 0,1%	(-)	(+)	(-)	(-)	(+)	(+)	(+/-)	(-)	(-)	(-)
rocourtiae	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	2630	Profil inacceptable iva, grayi	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)
marthii	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	6110	bonne mono 80,2%, inno 19,4%, grayi 0,2%	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)
fleischmanii	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	2510	bonne mono 98,5%, inno 1,4%	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)
coloradonensis	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	2510	bonne mono 98,5%, inno 1,4%	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)
fleischmanii	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	2510	bonne mono 98,5%, inno 1,4%	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)
weihenstephanensis	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	2710	acceptable iva 59,2%, wel 28,1%, seeli 6,5%	(-)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
weihenstephanensis	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	2210	Profil inacceptable seeli 82,3%, iva 17,6%, wel 0,1%	(-)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)

Aspects of colonies on Rapid L mono

And other species

Aspects of colonies on Rapid L mono

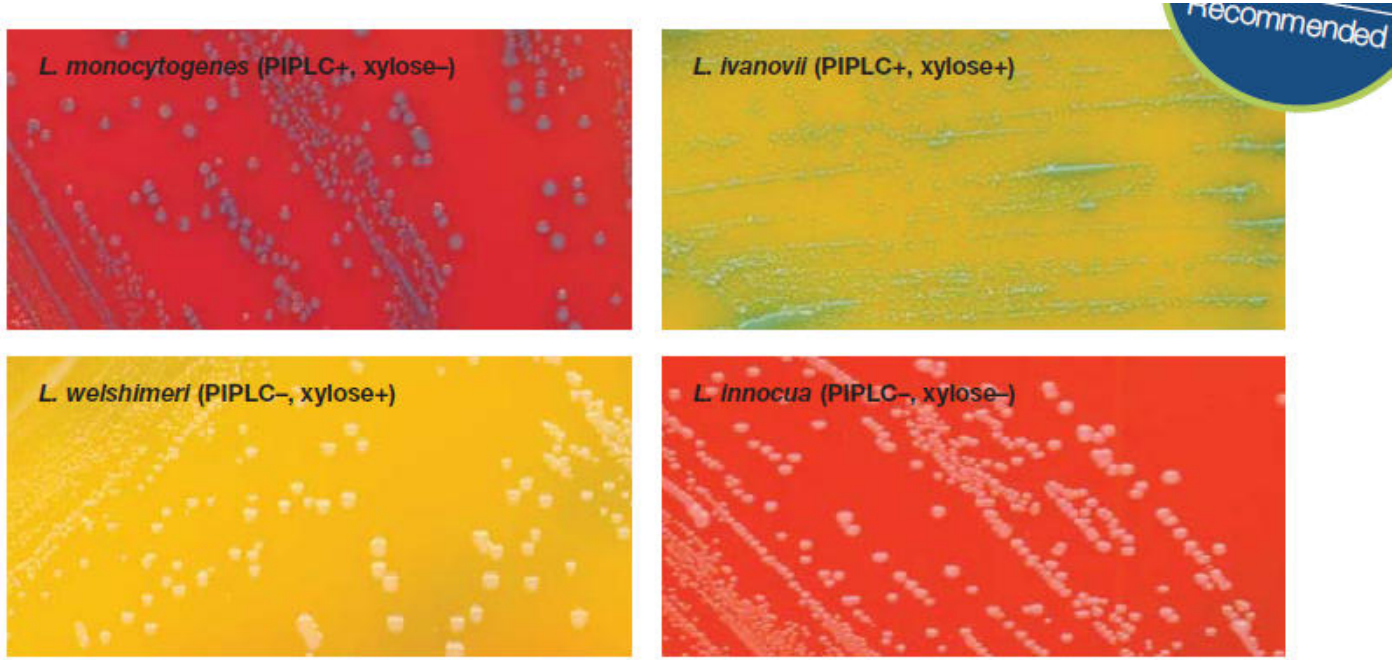


Fig. 1. *Listeria* species on RAPID'*L.Mono* chromogenic medium.

Table E.1 — *Listeria* selective agars (see Reference [6])

	LiCl (g/l)	Polymyxin (P) or Colistin (C) (mg/l)	Acriflavine (mg/l)	Other (mg/l)	Indicator system [a]	References
Agars which do not differentiate *Listeria* spp.						
Harlequin listeria medium	15	C10	2,5	Fosfomycin 5, Cefotetan 1, Cycloheximide 200	CHEg + Fe	Smith et al., 2000
LPM	5	0	0	Moxalactam 20, Glycerine anhydride 10, Phenylethanol 2500	Henry	Lee and McCain, 1986
Oxford	15	C20	5	Fosfomycin 10, Cefotetan 2 Cycloheximide 4009	Ae et Fe	Curtis et al., 1989
Modified Oxford (MOX)	12	C10	0	Ceftazidime 20	Ae et Fe	Cook, 1998
PALCAM	10	P10	5	Ceftazidime 30	Ae + Fe Mann + PR	Van Netten et al., 1989
Agars which differentiate species by haemolysis						
EHA	10	P10	5	Ceftazidime 30	MUG Sheet blood	Cox et al., 1991 b
LMBA	10	P10	0	Ceftazidime 20	Sheep blood	Johansson, 1998
Agars which are specific for pathogenic *Listeria* spp.						
ALOA	10			Cycloheximide, Nalidixic acid	Chrom	Ottaviani et al., 1997
BCM LMPM		*Composition not published*			Chrom	Restaino et al., 1999
Rapid'L.mono		*Composition not published*			Chrom	Foret and Dorey, 1997

[a] Ae aesculin ;
CHEg CHE-glucoside
Chrom Chromogenic substrate
Fe Iron salt

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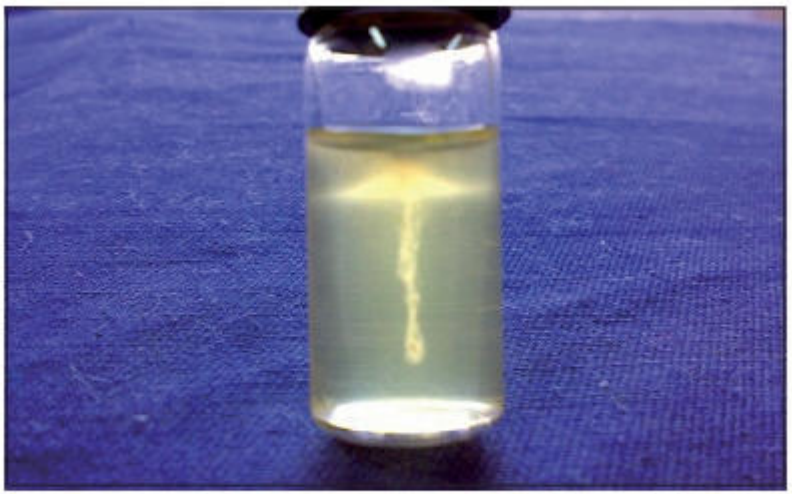

Figure 1: Umbrella Motility in Semisolid Nutrient Agar at Room Temperature