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EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes

Development of a set of *Listeria monocytogenes* strains for conducting challenge tests

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TABLE OF CONTENT

1 INTRODUCTION

1.1 CONTEXT

One of the recommendations of the EURL *Listeria monocytogenes (Lm)* Technical Guidance Document on shelf-life studies for *L. monocytogenes* in ready-to-eat foods (Afssa, 2008) dealt with the choice of strains to conduct microbial challenge tests assessing growth potential. It was noticed that they should be performed with a mixture of at least 3 strains to account for variations in growth or survival among strains. It was recommended that one of the strains must be chosen from reference strains; the others must be chosen between strains isolated from the same or similar food products.

A major outcome of the enquiry launched in April 2010 by EURL *Lm* on the need to revise the EURL *Lm* Technical Guidance Document was related to the choice of the strains. EURL *Lm* had thus settled a working group of volunteering National Reference Laboratories (NRLs) to share knowledge and technical approaches to deal with strain variability, as it may be difficult to have available in any lab well characterised strains (e.g. origin or growth).

Strain variability of the behaviour of foodborne bacterial pathogens is found, whatever the species considered (Lianou and Koutsoumanis, 2013). Yet, the question of its consequence on the results of challenge tests could be raised. A short review was proposed on the most interesting works during the last years on intraspecific variability. This review would serve as a basis for discussion in the working group.

Growth rates and growth limits of *L. monocytogenes* in various media, foods and conditions had been illustrated in numerous papers. We focused the review on studies which included a large number of strains in their experiments.

Growth rate of a strain at a determined temperature only depends on environmental conditions (medium or food). Lag-time is dependent on the last two factors but also on the conditions preceding contamination of that medium/food or on the initial number of bacteria (Guillier and Augustin 2006). Thus, apparent lag-time differences between strains may reflect other factor(s) than strain variability. For this reason, strain variability was only assessed on growth rate.

1.2 GROWTH COMPARISON

Studies of growth comparison may be conducted on growth rate or growth limit (growth/no growth) (Figure 1). When considering growth rates, attention was paid to their relative values for one or several common conditions. When considering growth limit, the focus was on the value of the considered environmental factor for which the growth rate was zero.

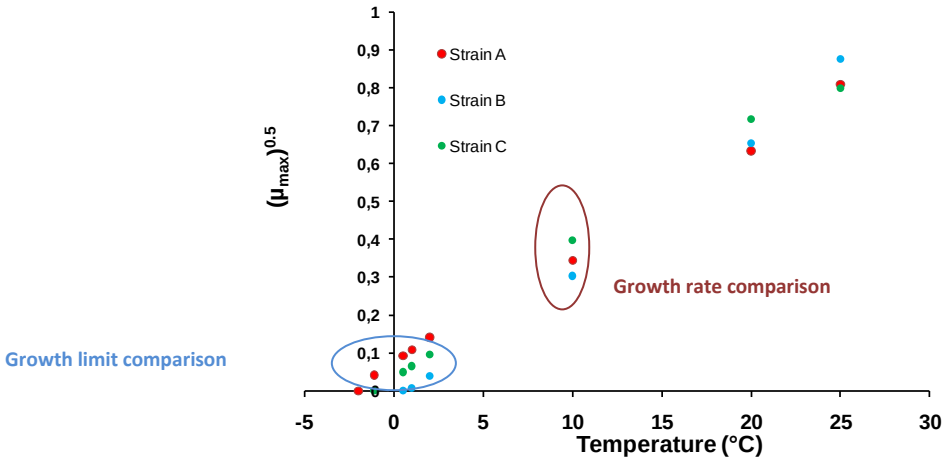

Figure 1. Growth comparison versus the temperature.

1.2.1 STRAIN VARIABILITY AT LOW TEMPERATURE

Numerous studies compared growth of different *L. monocytogenes* strains at low temperature (Barbosa et al., 1994; Begot et al., 1997; Junttila et al., 1988; Lianou et al., 2006; Nufer et al., 2007; Pal et al., 2008b; Walker et al., 1990).

The first fact which emerged from these studies was that differences between strains were more pronounced at low temperature (Begot et al., 1997; Lianou et al., 2006; Nufer et al., 2007) especially in unfavourable growth conditions (Arguedas-Villa et al., 2010).

In Table 1, the factors (serotypes, strain's origin, etc.), identified as significant or not, are summarized.

Table 1. Factors affecting strains variability toward growth at low temperature

Reference	Significant factor(s)	Not significant factor(s)	Comments
(Begot et al., 1997)	Origin	Serotype	
(Pal et al., 2008a)		Serotype	
(Junttila et al., 1988)		Origin	All strains are isolated from animals
(Vialette et al., 2003)	Origin		At 10°C not at 20°C
(Arguedas-Villa et al., 2010)	Genetic lineage	Origin	
(Lianou et al., 2006)		Serotype	4b versus other

Many minimal growth temperatures of *L. monocytogenes* were reported in the literature. They were based on:

- Expert claim: *e.g.* -2°C (Afssa, 2006), -1.5°C (NZFSA, 2001) ;
- Modeling by fitting different secondary models to growth rates data of various strains considered together or alone. -3.5°C (Cornu et al., 2006), -2.83°C (Mejlholm et al., 2010), -2.47°C (Pouillot et al., 2003), -2.7°C (Cornu et al., 2006), -1.72°C (Augustin et al., 2005), -1.7°C (Membré et al., 2005), -1.6°C (Tienungoon et al., 2000), -1.03°C (Mataragas et al., 2006), 0.4°C (Tienungoon et al., 2000), 0.9°C (Cornu et al., 2006);
- Growth monitoring: -0.4°C, -0.1 (Walker et al., 1990), 1.7°C (Junttila et al., 1988).

It was worth to notice the finding of the meta-analysis of growth data in different food types or growth media of Augustin *et al.* (Augustin et al., 2005); they observed a greater variability according to the study considered rather than according to the *L. monocytogenes* strains in use. They characterized the dispersion of T_{min} (Figure 2). The mean and standard deviation of T_{min} were -1.47 and 2.55 respectively. This dispersion reflected both variability and uncertainty. Similar results were obtained by Pouillot *et al.* (Pouillot et al., 2003).

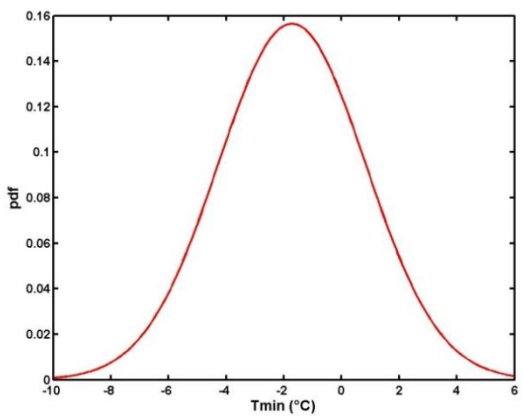

Figure 2. Dispersion of T_{min} of *L. monocytogenes* (biological variability and the uncertainty) (Augustin et al., 2005).

1.2.2 STRAIN VARIABILITY AT LOW PH

Many minimal growth pH of *L. monocytogenes* were reported in the literature. They were based on:

- Expert claim: *e.g.* 4.6 (Afssa, 2006), 4.4 (NZFSA, 2001) ;
- Modeling by fitting different secondary models to growth rates data of various strains considered together or alone: 4.26 (Augustin et al., 2005), 4.97 (Mejlholm et al., 2010);
- Growth monitoring: 4.4 (van der Veen et al., 2008), 4.1 (Shabala et al., 2008).

These two last studies screened growth limit of more than one hundred strains of various origins. It was quite surprising to observe the shift of the percentage of strains able to grow at the minimal growth pH between both (Figure 3). A methodological bias could explain this shift.

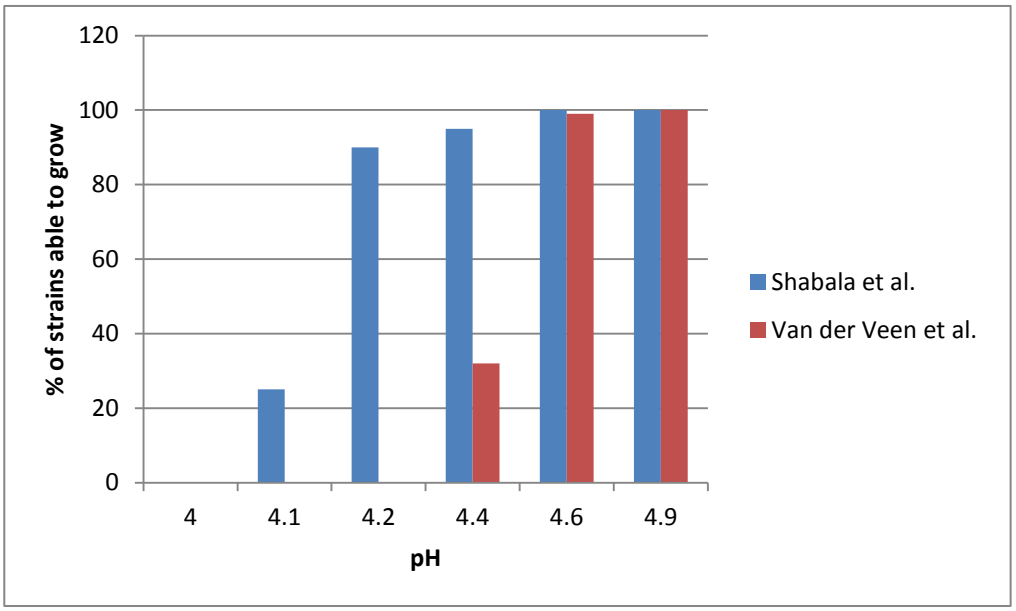

Figure 3. Percentages of strains able to growth at various pH

The influence of serotype or other factors likely to explain differences between strains was not clear (Table 2).

Table 2. Factors affecting strains variability toward growth at low pH

Reference	Significant factor(s)	Not significant factor(s)	Comments
(Shabala et al., 2008)		Serotype, Origin, Growth limit for NaCl	
(van der Veen et al., 2008)	Serotype, Origin, gene presence		
(Cotter et al., 2005)	Serotype, gene presence		

1.2.3 STRAIN VARIABILITY AT LOW WATER ACTIVITY

As for pH and temperature, we collected reported minimal water activity (a_w) for growth (Table 3):

- Expert claim: 0.92 to 0.93 (=11.5 % NaCl) (Afssa, 2006), 0.92 (NZFSA, 2001);
- Modeling by fitting different secondary models to growth rates data of various strains considered together or alone: 0.913 (Augustin et al., 2005), 0.923 (Mejlholm et al., 2010);
- Growth monitoring: 0.92 (van der Veen et al., 2008), 0.903 (Shabala et al., 2008).

Table 3. Factors affecting strains variability toward growth at low a_w

Reference	Significant factor(s)	Not significant factor(s)	Comments
(Shabala et al., 2008)		Serotype, Origin, Growth limit for pH	
(van der Veen et al., 2008)	Serotype, Origin, gene presence		

To constitute this set, some strains were collected from Belgium, France, Switzerland and the United States.

2 CONSTITUTION OF A SET OF *LISTERIA MONOCYTOGENES* STRAINS FOR CONDUCTING CHALLENGE TEST

2.1 CRITERIA FOR THE SELECTION OF THE STRAINS

The objective of EURL for *L. monocytogenes* was to choose efficient strains, i.e. which grow faster and/or in harsher conditions than others. The conditions retained were temperature, pH and water activity. The origin and the genoserotype of the strains were used to classify the strains. The target was to obtain 24 strains (Figure 4).

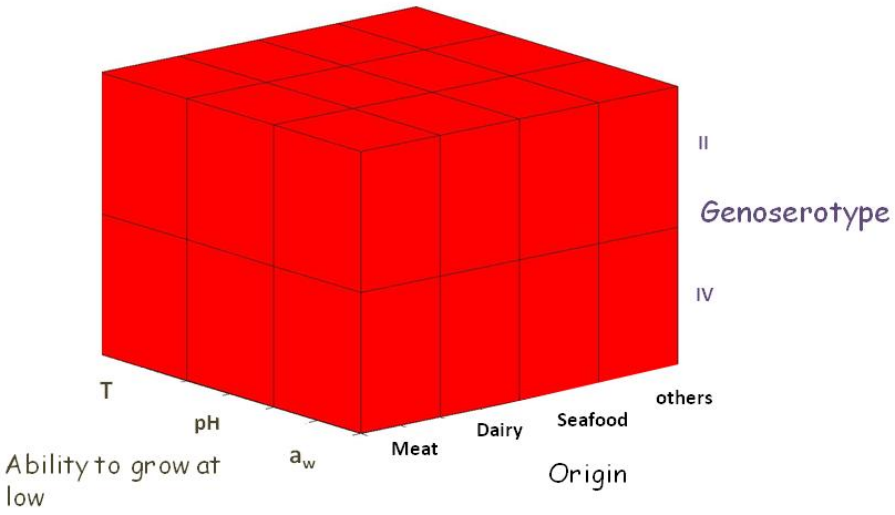

Figure 4. Representation of 24 factors combination.

The first step was to identify efficient strains from previously published studies. Strains were considered efficient when they have a higher maximum growth rate for one condition or several, among the strains tested. In case no efficient strain was identified, the EURL *Lm* set of strains was used to select one efficient strain within at least 10 strains of given genoserotype and origin (36 strains were studied).

2.2 CHARACTERISATION OF THE SET

The aim was to confirm the ability of the selected set to grow in harsh conditions, 8°C, pH = 5 or a_w = 0.95.

Bioscreen C was used to estimate the maximal growth rate (μ_{max}) of strains. The optical density (OD) with "wide-band filter" was measured every 30 min until the end of growth. Three media were tested (standard TSBYe, TSBYe at pH = 5, TSBYe with a_w = 0.95). The method of "Times To Detection" (TTD) using multiple initial inocula was chosen to determine μ_{max} (Cuppers and Smelt 1993).

Each medium was inoculated with a standardised inoculum (≈10^6 cfu.ml^{-1}) and then, four consecutive five-fold dilutions were performed (1/5 to 1/625) with the same medium in order to obtain 5 different inoculums levels. For each inoculum level, 3 microplate wells were filled in with 300 µl of suspensions and incubated at the appropriate temperature (20°C or 8°C) with mild and discontinuous agitation (30 s every 10 min).

Regressions in the linear phase of turbidimetry curves were performed and TTD (t_d) was determined, as shown on Figure 5A. Then, growth rates were calculated according to linear regression as following: $(D) = \mu_{max} \cdot t_d - b$, with D: dilution factor. Fifteen values of t_d were used to estimate μ_{max} as shown on Figure 5B. Thirteen strains were followed by experiment including a reference strain used to assess inter-experimental variability.

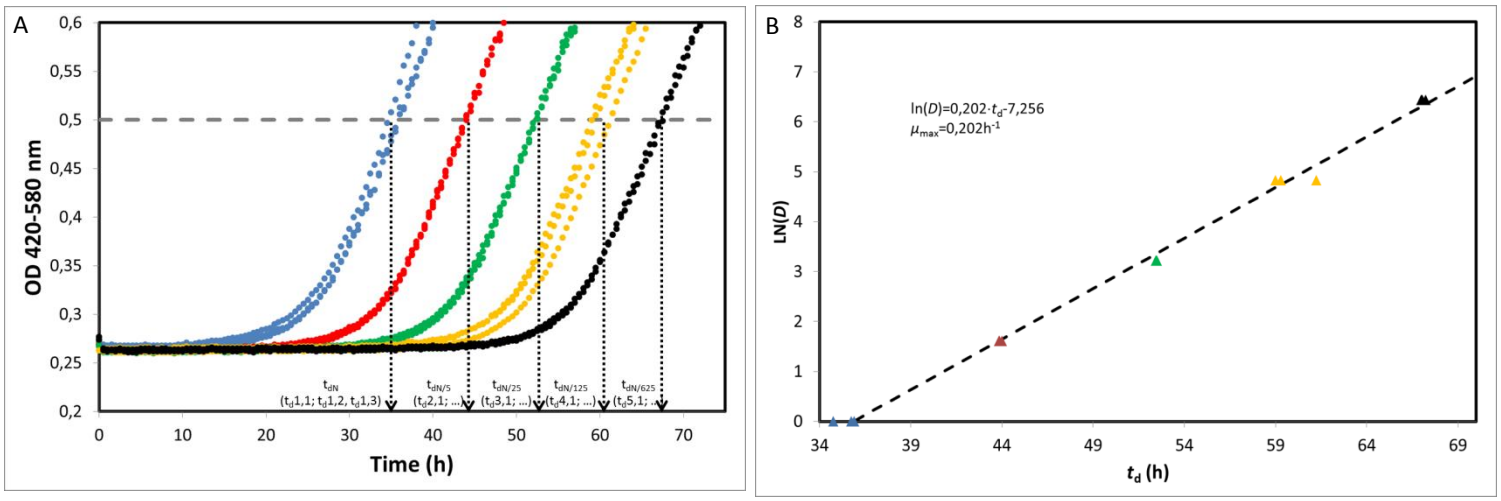

Figure 5(A) Observed optical density incubation time plot (•) for a *Listeria monocytogenes* strain incubated at 20°C in TSBYe at pH 5 with initial inocula of (from left to right) N, N/5, N/25, N/125 and N/625 respectively. (B) Growth rate determination using linear regression.

Obtained μ_{max} for all selected strains are shown in Table 4.

Table 4. Obtained μ_{max} for all selected strains

Name of Lm strain	Origin	Genoserotype	Tested condition	Mean μ_{max}	Standard deviation
12MOB085LM	Meat	IV	20°C	0.458	0.013
			8°C	0.090	0.003
			pH 5	0.139	0.008
			a_w 0.95	0.137	0.129
12MOB089LM	Meat	IV	20°C	0.460	0.007
			8°C	0.092	0.004
			pH 5	0.194	0.004
			a_w 0.95	0.126	0.119
12MOB045LM	Meat	II	20°C	0.446	0.011
			8°C	0.092	0.000
			pH 5	0.184	0.010
			a_w 0.95	0.137	0.170
12MOB046LM	Meat	II	20°C	0.428	0.006
			8°C	0.089	0.000
			pH 5	0.176	0.010
			a_w 0.95	0.132	0.090
12MOB047LM	Other	II	20°C	0.441	0.006
			8°C	0.089	0.000
			pH 5	0.184	0.010

			a_w 0.95	0.123	0.119
12MOB048LM	Other	II	20°C	0.428	0.004
			8°C	0.089	0.000
			pH 5	0.167	0.006
			a_w 0.95	0.138	0.133
12MOB049LM	Other	II	20°C	0.438	0.034
			8°C	0.090	0.001
			pH 5	0.170	0.010
			a_w 0.95	0.113	0.108
12MOB050LM	Other	IV	20°C	0.447	0.019
			8°C	0.090	0.001
			pH 5	0.156	0.019
			a_w 0.95	0.134	0.206
12MOB051LM	Other	II	20°C	0.440	0.008
			8°C	0.090	0.003
			pH 5	0.170	0.005
			a_w 0.95	0.120	0.219
12MOB052LM	Other	IV	20°C	0.429	0.010
			8°C	0.091	0.001
			pH 5	0.135	0.000
			a_w 0.95	0.131	0.243
12MOB053LM	Dairy	IV	20°C	0.435	0.014
			8°C	0.088	0.003
			pH 5	0.172	0.018
			a_w 0.95	0.153	0.145
12MOB096LM	Dairy	IV	20°C	0.448	0.020
			8°C	0.096	0.002
			pH 5	0.174	0.009
			a_w 0.95	0.137	0.172
12MOB097LM	Dairy	IV	20°C	0.438	0.018
			8°C	0.092	0.003
			pH 5	0.173	0.014
			a_w 0.95	0.126	0.196
12MOB118LM	Dairy	II	20°C	0.436	0.018
			8°C	0.093	0.007
			pH 5	0.173	0.025
			a_w 0.95	0.132	0.123
12MOB098LM	Dairy	II	20°C	0.445	0.016
			8°C	0.094	0.002
			pH 5	0.174	0.006
			a_w 0.95	0.129	0.154
12MOB099LM	Seafood	II	20°C	0.439	0.012
			8°C	0.094	0.001
			pH 5	0.147	0.012
			a_w 0.95	0.124	0.118
12MOB100LM	Seafood	II	20°C	0.419	0.011
			8°C	0.088	0.001
			pH 5	0.160	0.031
			a_w 0.95	0.136	0.213
12MOB101LM	Seafood	II	20°C	0.434	0.008
			8°C	0.092	0.003
			pH 5	0.168	0.010
			a_w 0.95	0.150	0.188
12MOB102LM	Seafood	IV	20°C	0.434	0.009
			8°C	0.092	0.001

			pH 5	0.161	0.012
			a_w 0.95	0.135	0.170
12MOB103LM	Seafood	IV	20°C	0.458	0.019
			8°C	0.090	0.001
			pH 5	0.165	0.006
			a_w 0.95	0.169	0.216
12MOB104LM	Seafood	IV	20°C	0.458	0.015
			8°C	0.089	0.005
			pH 5	0.175	0.007
			a_w 0.95	0.119	0.080
12MOB105LM	Dairy	IV	20°C	0.448	0.021
			8°C	0.094	0.003
			pH 5	0.168	0.016
			a_w 0.95	0.133	0.132
12MOB106LM	Dairy	IV	20°C	0.449	0.015
			8°C	0.093	0.002
			pH 5	0.161	0.007
			a_w 0.95	0.140	0.178
12MOB107LM	Seafood	IV	20°C	0.450	0.023
			8°C	0.090	0.002
			pH 5	0.152	0.011
			a_w 0.95	0.114	0.109
12MOB079LM	Dairy	II	20°C	0.444	0.009
			8°C	0.093	0.001
			pH 5	0.163	0.017
			a_w 0.95	0.121	0.120
12MOB119LM	Dairy	II	20°C	0.432	0.012
			8°C	0.093	0.002
			pH 5	0.169	0.010
			a_w 0.95	0.132	0.166
12MOB120LM	Dairy	II	20°C	0.438	0.019
			8°C	0.092	0.002
			pH 5	0.160	0.006
			a_w 0.95	0.125	0.161
12MOB112LM	Meat	IV	20°C	0.443	0.018
			8°C	0.089	0.003
			pH 5	0.151	0.016
			a_w 0.95	0.115	0.107
12MOB113LM	Meat	IV	20°C	0.451	0.015
			8°C	0.089	0.002
			pH 5	0.152	0.008
			a_w 0.95	0.115	0.105
12MOB114LM	Meat	IV	20°C	0.439	0.021
			8°C	0.090	0.005
			pH 5	0.130	0.008
			a_w 0.95	0.106	0.097

A multi-way ANOVA and multiple comparison tests with the Matlab software were performed:

- The origin of strains from the set had a significant impact on growth rate:
  - At low pH, strains of meat origin had a higher growth rate than the others (Figure 6A).
  - At low temperature, strains of dairy origin had the highest growth rate (Figure 6B).
  - At low a_w, seafood strains were the fastest strains (Figure 6C).
- Genoserotype influenced the growth rate variability strains:
  - At low pH and low temperature, genoserotype had no impact (Figures 6D and E).
  - At low a_w, genoserotype IV strains had significantly higher growth rate than genoserotype II (Figure 6F).

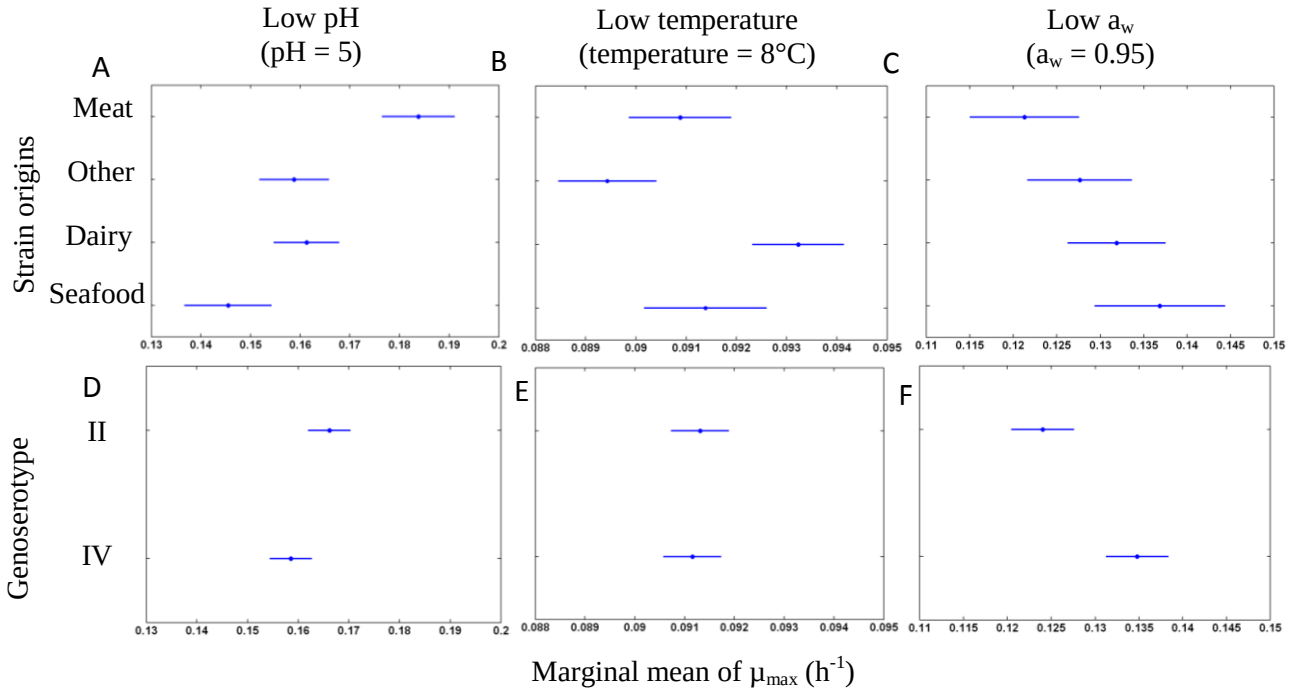

Figure 6: Population marginal means with standard error of growth rates of strains of different genoserotypes and different origins. (Two marginal means are significantly different if their intervals are disjoint)

It can be suggested that:

- one strain required in a challenge test performed at low pH would belong to the category “Meat strains”,
- one strain required in a challenge test performed at low temperature would belong to the category “Dairy strains”,
- one strain required in a challenge test performed at low water activity would belong to the category “Seafood strains” and or “Genoserotype IV”.

Following this screening, a proposal of a set of *Listeria monocytogenes* strains was made (Table 5).

Table 5. Proposed set of *Listeria monocytogenes* strains

Condition TEMPERATURE (temperature = 8°C)				
Genoserotype/Origin	Meat	Fish	Dairy	Other
II	12MOB045LM	12MOB099LM	12MOB098LM	12MOB049LM
IV	12MOB085LM	12MOB102LM	12MOB096LM	12MOB052LM
Condition pH(pH = 5)				
II	12MOB045LM	12MOB101LM	12MOB118LM	12MOB051LM
IV	12MOB112LM	12MOB103LM	12MOB053LM	12MOB050LM
Condition a_w (a_w = 0.95)				
II	12MOB045LM	12MOB101LM	12MOB098LM	12MOB04LM0
IV	12MOB085LM	12MOB103LM	12MOB053LM	12MOB050LM

2.3 COMPARISON OF THE SET OF *LISTERIA MONOCYTOGENES* STRAINS TO STANDARD *LISTERIA MONOCYTOGENES* STRAINS

Listeria monocytogenes strains from EURL *Lm* collection were chosen according to their origin and genoserotype (Table 6).

Table 6. *Listeria monocytogenes* strains selected from EURL *Lm* collection

Genoserotype/Origin	Meat	Fish	Dairy	Other
II	12MOB072LM	TQA258	TQA157	12MOB076LM
IV	10MQER026LM	12MOB091LM	TQA158	12MOB068LM

These *Listeria monocytogenes* strains were compared to the equivalent *Listeria monocytogenes* strains from the possible set of *Listeria monocytogenes* strains by Bioscreen (Figures 7, 8 and 9, the symbol blue square represents the *Listeria monocytogenes* strains set and the symbol red triangle the standard *Listeria monocytogenes* strains described in the table 6).

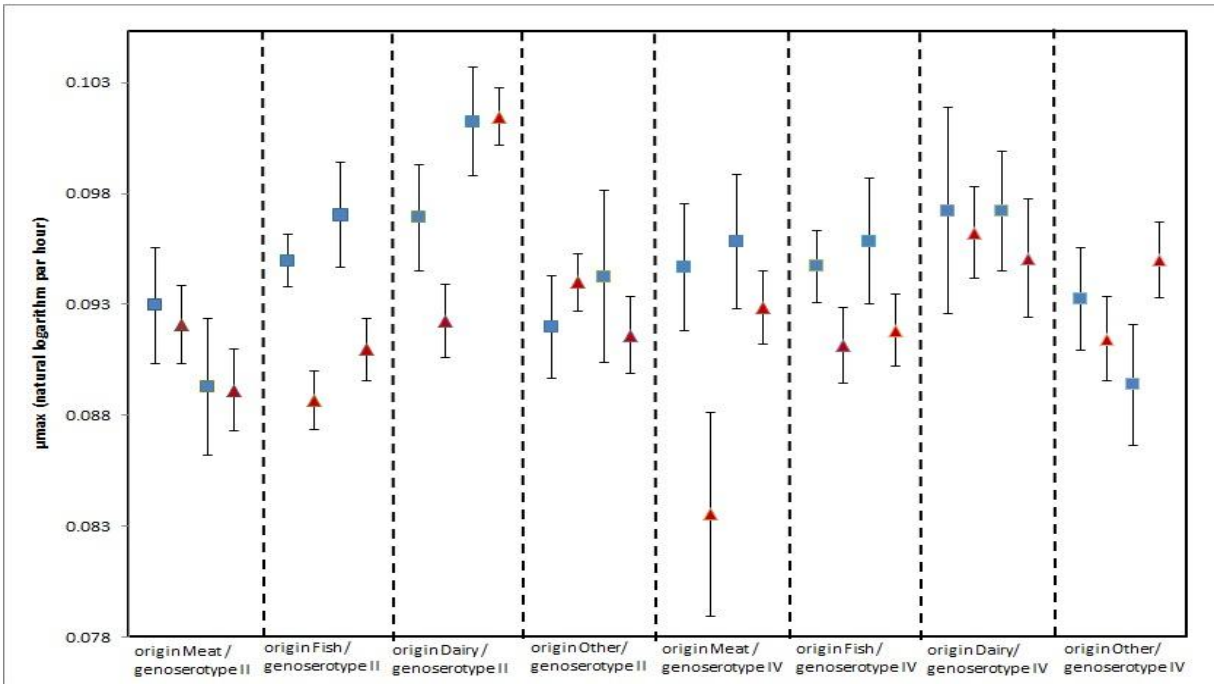

Figure 7: Comparison of the *Listeria monocytogenes* strains set and the standard *Listeria monocytogenes* strains for the different origins and genoserotypes in a non-selective broth at 8°C

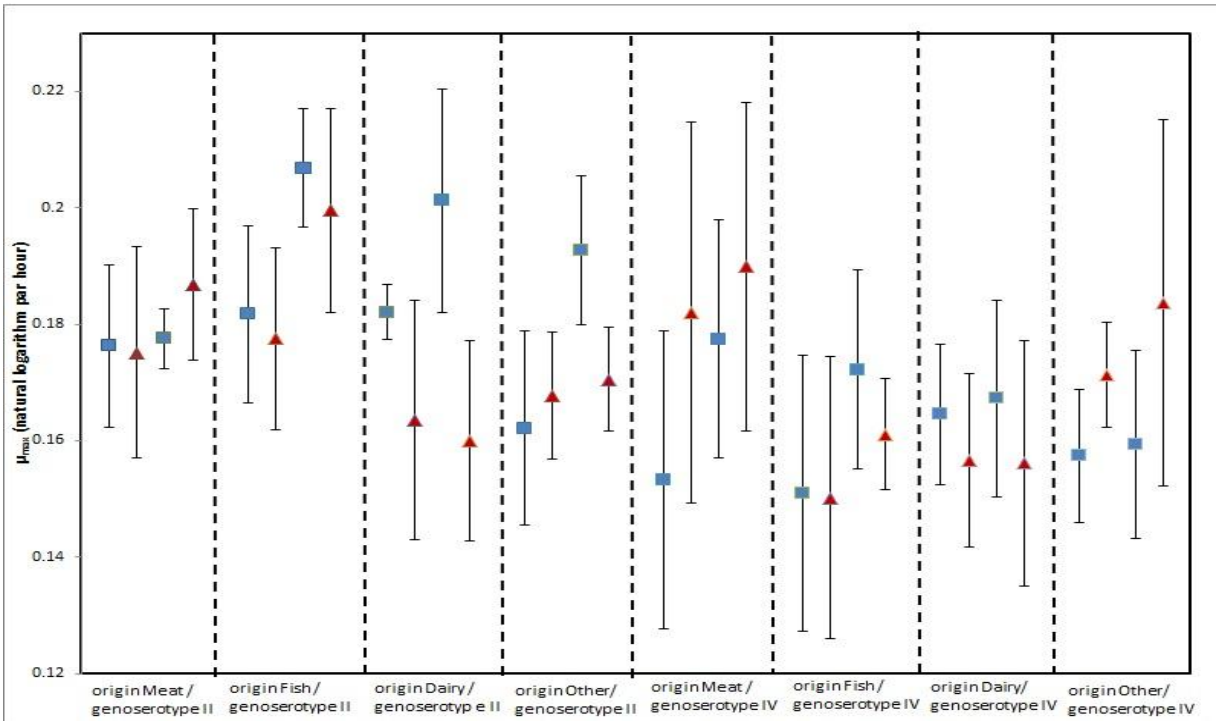

Figure 8: Comparison of the *Listeria monocytogenes* strains set and the standard *Listeria monocytogenes* strains for the different origins and genoserotypes in a non-selective broth with a pH 5

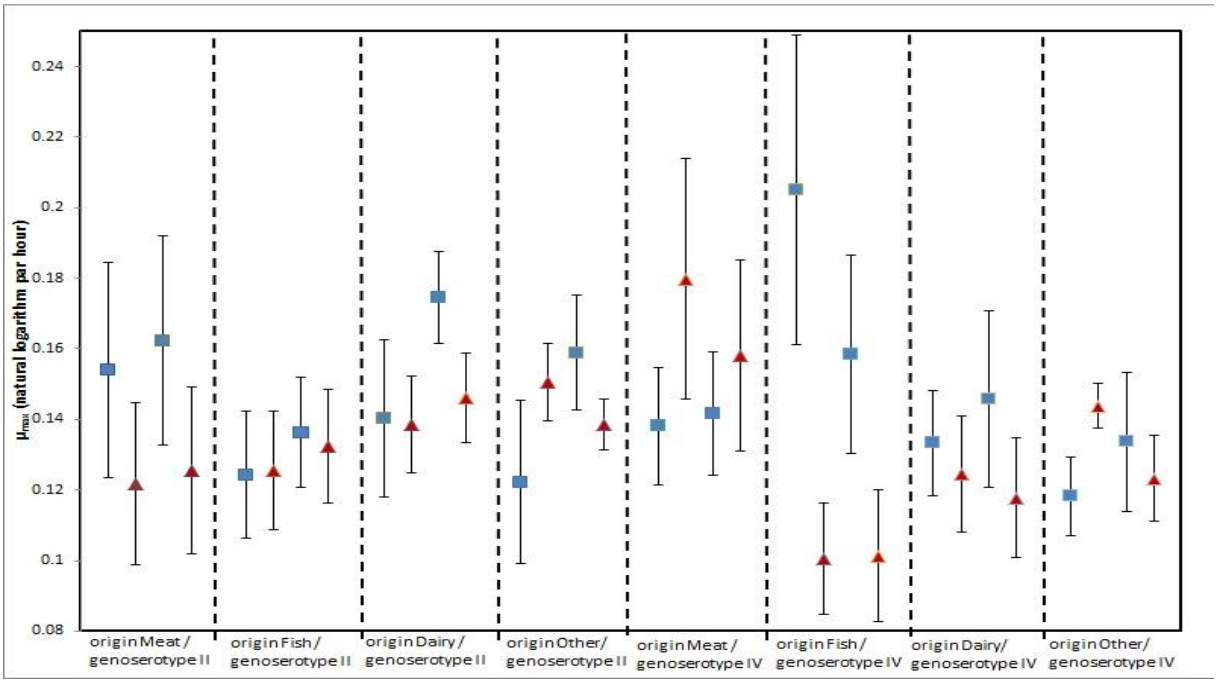

Figure 9: Comparison of the *Listeria monocytogenes* strains set and the standard *Listeria monocytogenes* strains for the different origins and genoserotypes in a non-selective broth with an a_w 0.95

The observed differences were minor, so it was decided to keep the *Listeria monocytogenes* strains chosen in the initial possible *Listeria monocytogenes* strain set. These strains are not considered at the moment as the most efficient in some harsh conditions but these *Listeria monocytogenes* strains are well known according to the growth rate in broth in harsh conditions of temperature, pH or water activity.

Another classification of strains, only according to a given condition, is possible (Table 7).

Table 7. Classification of *Listeria monocytogenes* strains according to different conditions

Classification by decreasing growth rate (in natural logarithm par hour)				
Rank	Low temperature (8°C)	Low pH (pH 5)	Low a_w (0.95)	Mean rank for all tested conditions
1	12MOB103LM (F, IV)	12MOB096LM (D, IV)	12MOB045LM (M, II)	12MOB096LM (D, IV)
2	12MOB053LM (D, IV)	12MOB098LM (D, II)	12MOB096LM (D, IV)	12MOB045LM (M, II)
3	12MOB101LM (F, II)	12MOB099LM (F, II)	12MOB098LM (D, II)	12MOB098LM (D, II)
4	12MOB048LM (O, II)	12MOB118LM (D, II)	12MOB118LM (D, II)	12MOB118LM (D, II)
5	12MOB085LM (M, IV)	12MOB045LM (M, II)	12MOB053LM (D, IV)	12MOB101LM (F, II)
6	12MOB045LM (M, II)	12MOB102LM (F, IV)	12MOB051LM (O, II)	12MOB103LM (F, IV)
7	12MOB094LM (D, IV)	12MOB101LM (F, II)	12MOB049LM (O, II)	12MOB102LM (F, IV)
8	12MOB102LM (F, IV)	12MOB052LM (O, IV)	12MOB101LM (F, II)	12MOB053LM (D, IV)
9	12MOB050LM (O, IV)	12MOB085LM (M, IV)	12MOB048LM (O, II)	12MOB048LM (O, II)
10	12MOB118LM (D, II)	12MOB103LM (F, IV)	12MOB103LM (F, IV)	12MOB099LM (F, II)
11	12MOB052LM (O, IV)	12MOB049LM (O, II)	12MOB102LM (F, IV)	12MOB085LM (M, IV)
12	12MOB098LM (D, II)	12MOB051LM (O, II)	12MOB050LM (O, IV)	12MOB051LM (O, II)

13	12MOB099LM (F, II)	12MOB050LM (O, IV)	12MOB112LM (M, IV)	12MOB050LM (O, IV)
14	12MOB051LM (O, II)	12MOB112LM (M, IV)	12MOB098LM (F, II)	12MOB049LM (O, II)
15	12MOB112LM (M, IV)	12MOB048LM (O, II)	12MOB085LM (M, IV)	12MOB052LM (O, IV)
16	12MOB049LM (O, II)	12MOB053LM (D, IV)	12MOB052LM (O, IV)	12MOB112LM (M, IV)

D: from dairy product F: from fish product M: from meat product O: from product with an unknown origin
II: genoserotype II IV: genoserotype IV

This classification would be used for the choice of *Listeria monocytogenes* strains in the implementation of challenge test assessing maximum growth rate in some food matrices.

There can have other more efficient strains in one or several conditions, not tested in this study.

Note: some experiments had been performed on the set with Bioscreen by coupling 2 harsh conditions (8°C/pH = 5 and 8°C/a_w = 0.95) but the results were not exploitable even if the experiment time was increased (25 days) and the dilutions used were lower (from 1/100 to 1/1600). Bioscreen apparatus may not be suitable to test these drastic conditions.

2.4 CHALLENGE TESTS ASSESSING MAXIMUM GROWTH RATE OF *LISTERIA MONOCYTOGENES* STRAINS IN FOOD MATRICES

Two types of food matrices, French custard and tuna rillettes, were tested with two *Listeria monocytogenes* strains in each case, the strain with the higher growth rate and the strain with the lower growth rate from the mean rank: 12MOB096LM and 12MOB112LM. These 2 food matrices, a milk product and a fish product, are rather simple (no background microflora and easy inoculation method); so the growth of *L. monocytogenes* strains was not disturbed by other factors, such as the presence of the background microflora.

2.4.1 MATERIAL AND METHOD

Three batches were prepared.
The 2 chosen strains, 12MOB112LM and 12MOB096LM, were tested separately.
Each strain was subcultured twice at 37°C during 18h. Then, each one was diluted in physiological water in order to obtain an inoculum at the expected concentration. Each inoculum was enumerated on TSAYe and PALCAM.
The matrix was separated in 3 parts: one for the global contamination with the strain 12MOB096LM, the 2nd for the global contamination with the strain 12MOB112LM and the last part for the uncontaminated test units. Twenty-four test units of 25g were prepared for each set of *L. monocytogenes* contaminated test units and 3 other test units of 25g were prepared, one for the detection of *L. monocytogenes*, one for the enumeration of microflora and the last for physico-chemical measurements. *L. monocytogenes* enumeration was performed on 2 test units per analysis point.
The targeted concentration of the inoculum was 100 cfu/g.

In the case of French custard, the storage conditions were 8°C during 16 days.
In the case of tuna rillettes, the storage conditions were 10°C during 14 days.

2.4.2 RESULTS FOR CHALLENGE TESTS ASSESSING MAXIMUM GROWTH RATE OF *LISTERIA MONOCYTOGENES* STRAINS IN FRENCH CUSTARD

The physico-chemical characteristics are shown in Table 8.

Table 8: Evolution of physico-chemical characteristics of French custard for 3 batches

Measurement at day	0	6	13
pH	6.57±0.01	6.61±0.01	6.56±0.01
a_w	0.978±0.001	0.973±0.002	0.985±0.003

The 2 physico-chemical characteristics measured were stable during the duration of the challenge tests.

The mean concentration of the inoculum from the strain 12MOB096LM was 2.10±0.08 $\log_{10}$ cfu/g. The mean concentration of the inoculum from the strain 12MOB112LM was 1.81±0.25 $\log_{10}$ cfu/g.

Figure 10 shows the mean increase of *L. monocytogenes* for both strains in French custard at 8°C.

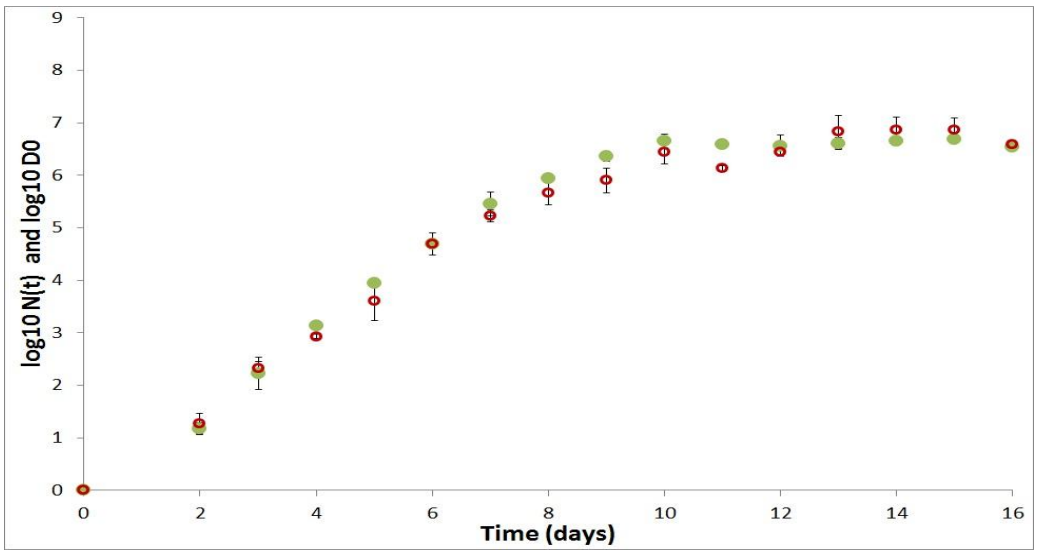

Figure 10: Mean increase of the *L. monocytogenes* strains 12MOB096LM (•) and 12MOB112LM (•)in French custard at 8°C

Table 9 summarises the mean growth parameters for both strains, obtained by using DMFit software.

Table 9: Mean growth parameters for *L. monocytogenes* strains 12MOB096LM and 12MOB112LM at 8°C in French custard

Strains / Growth parameters	μ_{max} (j^{-1})	N_0 (log_{10} cfu/g)	N_{max} (log_{10} cfu/g)
12MOB096LM	0.84±0.02	2.10±0.08	8.78±0.04
12MOB112LM	0.75±0.03	1.81±0.25	8.55±0.08

As observed in broth, strain 12MOB096LM grew faster than strain 12MOB112LM. The initial concentration was about 2 log_{10} cfu/g, as expected. The maximum concentration was lightly higher for strain 12MOB096LM.

By comparison with culture in broth, in the condition that was the closest to the condition in French custard (TSBYe 8°C, pH 7 a_w 0.99), strain 12MOB096LM grew 2.7 times faster in broth than in French custard and strain 12MOB112LM 2.9 times faster. The differences may be due to the impact of the matrix structure and the lower pH in the matrix (mean pH of 6.58).

2.4.3 RESULTS FOR CHALLENGE TESTS ASSESSING MAXIMUM GROWTH RATE OF *LISTERIA MONOCYTOGENES* STRAINS IN TUNA RILLETTES

The physico-chemical characteristics are shown in Table 10.

Table 10: Evolution of physico-chemical characteristics of tuna rillettes for 3 batches

Measurement at day	0	5	13
pH	5.85±0.01	5.83±0.01	5.83±0.00
a_w	0.982±0.001	0.986±0.001	0.978±0.001

The 2 physico-chemical characteristics measured were quite stable during the duration of the challenge tests.

The mean concentration of the inoculum from the strain 12MOB096LM was 1.94±020 log_{10} cfu/g. The mean concentration of the inoculum from the strain 12MOB112LM was 1.55±0.38 log_{10} cfu/g.

Figure 11 shows the mean increase of *L. monocytogenes* for both strains in tuna rillettes at 10°C.

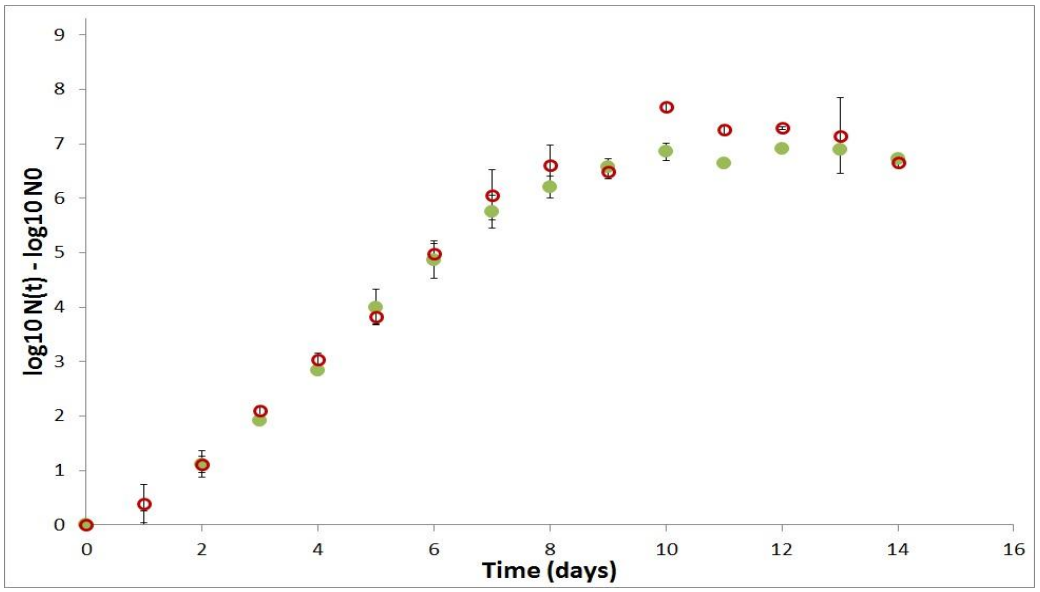

Figure 11 : Increase of the *L. monocytogenes* strains 12MOB096LM (•) and 12MOB112LM (•) for 3 batches in tuna rillettes at 10°C

Table 11 summarises the mean growth parameters for both strains, obtained by using DMFit software.

Table 11: Mean growth parameters for *L. monocytogenes* strains 12MOB096LM and 12MOB112LM at 10°C in tuna rillettes

Strains / Growth parameters	μ_{max} (j^{-1})	N_0 (log_{10} cfu/g)	N_{max} (log_{10} cfu/g)
12MOB096LM	0.96±0.10	1.94±0.20	8.95±0.10
12MOB112LM	1.03±0.04	1.55±0.38	8.74±0.18

As observed in broth, strain 12MOB096LM grew as fast as strain 12MOB112LM. The initial concentration was about 2 log_{10} cfu/g, as expected. The maximum concentration was similar for both strains.

By comparison with culture in broth, in the condition that was the closest to the condition in tuna rillettes (TSBYe 8°C, pH 7 a_w 0.99), strain 12MOB096LM grew 3.4 times faster in broth than in tuna rillettes and strain 12MOB112LM 3.0 times faster. The differences may be due to the impact of the matrix structure and the lower pH in the matrix (mean pH of 5.84).

3 CONCLUSION

According to the current (2nd) version of the EURL Lm "Technical Guidance Document on shelf-life studies for *L. monocytogenes* in ready-to-eat foods", the inoculation of the samples intended to the evaluation of the growth potential when performing challenge tests is made with a mixture of at least 3 strains: a reference strain and strains isolated from the same or a similar strain matrix.

EURL *Lm* constituted a set of strains from various origin (meat, dairy products, fish, ...) and various genoserotypes (II and IV) . Strains were initially selected for their ability to grow rapidly and to grow in harsh conditions of temperature, pH and a_w, according to literature.

This study allowed comparing the growth of selected strains of *Listeria monocytogenes* in broth and in matrix.

In broth, according to the tested conditions, the origin of strains has influenced the growth rate, even though the impact was limited. From a global point of view for all tested conditions, the strain with the mean higher growth rate was 12MOB096LM and the strain with the mean lowest growth rate was 12MOB112LM. But differences were limited.

In the 2 matrices tested (French custard and tuna rillettes), the differences between the two strains were also minor. The same magnitude was observed in the French custard as in broth, with a few higher growth rates for strain 12MOB096LM. In tuna rillettes, both strains grew at the same speed. So challenge tests seemed to corroborate the results obtained in broth.

In both matrices, both strains grew slower than in broth, which may be due to their lower pH.

The set of *Listeria monocytogenes* strains is sent to the NRLs, upon their request.

It is preferable to use well-characterised strains related to their growth (in broth) to perform shelf-life studies than using non-characterised strains coming from food, environment, epidemiology… We recommend to use this *L. monocytogenes* strain set as a landmark in the growth study of wild strains and to estimate the growth performance degree of new tested strains.

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