GROWTH OF MIXED CULTURES OF *LISTERIA* AND *PSEUDOMONAS* SPP. IN BEEF MEAT AND THEIR PREDICTION USING POLYNOMIAL MODELS

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Background

Among the psychrotrophic Gram-negative bacteria that can multiply in meat products, dairy products and vegetables, only a few have an influence on the quality and the shelf-life of these products and can be responsible for considerable financial losses. The principal flora responsible for such spoilage during aerobic storage are the pseudomonads. *Pseudomonas* spp. grew faster than other strains present in meat at temperatures between 2 and 15°C. Several pathogenic bacteria also grow within this temperature range and may cause foodborne outbreaks: *Listeria monocytogenes* has been shown to be responsible for outbreaks of food poisoning in France and in many countries (Rocourt and Bille, 1997).

Objective

To predict the growth of both spoilage *Pseudomonas* spp. and pathogenic *Listeria* in meat products

Methods

A first set of experiments were performed in decontaminated beef meat that was inoculated with *Listeria* or *Pseudomonas* strains. Strains were grown alone or together. The second set of experiments was performed in naturally contaminated meat that was purchased at the supermarket, meats were available in oxygen-permeable bags (experiments B and D) or in vacuum packs (experiments A and C) (Figure 1). The dominant *Pseudomonas* spp. were grown alone or in the presence of inoculated *Listeria.*
Meat was stored under aerobic conditions at low temperatures. Bacterial counts were performed on: Tryptone Soja Agar (Difco, OSI, Maurepas, France) for non selective growth; on Palcam Agar (Merck, Nogent-sur-Marne, France) for *Listeria*; on *Pseudomonas* Agar (Oxoïd, Unipath Ltd, Basingstocke, England) for *Pseudomonas*.
Growth parameters (A, logarithmic increase in the bacterial population, L, lag time, GT, generation time) were calculated by fitting the growth curves using a modified Gompertz equation (Zwietering et al., 1990). The *Listeria* model of Lebert et al. (1998) and the three *Pseudomonas* models of Robles (1999) were used as they took the growth variations into account and calculate a growth response interval.

Results and discussion

Three models for one rapid and one slow growing strain of *P. fragi* and one slow growing strain of *P. fluorescens* were developed in a meat broth and tested in naturally-contaminated meat. TSA and CFC counts were similar indicating that *Pseudomonas* spp. were dominant. Table 1 shows that experimental L were longer than predicted L but that the observed GT were between the GT predicted by *Pfr*162 and *Pfr*K1 model. The *Pseudomonas* models can be used to predict the growth of *Pseudomonas* spp. in meat.

In decontaminated meat inoculated with *Listeria* or *Pseudomonas* strains (Table 2), all the models provided satisfactory predictions. In mixed population experiments (Table 2), generation times for *Listeria* were similar when grown alone or with *P. fragi*, and were predicted satisfactory by the models. A comparison of the growth parameters of *Pfr*162 alone and with *Lm*14 showed that they were similar if account was taken of the confidence intervals. When co-inoculated, the growth of neither organism was either inhibited or stimulated. No interactions were observed between the strains, and their growth parameters were satisfactorily predicted by their respective models.

In naturally-contaminated meat inoculated with *Listeria,* Figure 1 shows that the experimental points for *Pseudomonas* were within the curves generated by the three models. In the four cases, observed GT were well predicted by the models. In experiments A and C, growth was inside the curves predicted by *Pfr*K1 and *Pfl*58 models (slow models). In experiments B and D, growth was near the curves predicted by *Pfr*162 models (fast model). A and D experiments (meat stored in vacuum packs) were characterised by longer lag times than those observed in experiments B and D (meat stored in oxygen-permeable bags): these differences can be due to the presence of different bacterial flora on the meat because of the way of storage. In meat stored in oxygen-permeable film, the microflora was already aerobic and mainly composed of *Pseudomonas* spp., that explains a small L. With vacuum-packaged meat, it took longer for the *Pseudomonas* to outgrow the anaerobic flora present in the meat. No *Listeria* growth was observed until *Pseudomonas* reached the stationary phase, slight increases (1 to 2 log(CFU)) were then observed. Predictions of *Listeria* growth were calculated for an A of 4.1 as found in Table 2 and *Lm*14 model failed to predict the slow growth of *Listeria.*

Conclusions

In experiments with mixed populations, three situations were observed: (1) in decontaminated meat, *L. monocytogenes* inoculated alone grew well at 6°C and the growth was correctly predicted by the model; (2) in decontaminated meat that was inoculated with *Listeria* and *Pseudomonas* strains, *L. monocytogenes* grew well and was not affected by the presence of the *Pseudomonas*, growth of both organisms were correctly predicted by the models; (3) in naturally contaminated meat inoculated with *L. innocua*, the strain did not grow until the *Pseudomonas* had reached the stationary phase. The models satisfactorily predicted the growth of *Pseudomonas* spp. but not that of *Listeria.*
In consequence, the *Lm*14 model can not be used for refrigerated meat stored aerobically as it indicated a too 'fail-safe' prediction: meat had already reached a spoilage state even though no increase of *Listeria* was observed. The *Pseudomonas* models accurately predicted the growth of naturally occurring *Pseudomonas* spp.

Pertinent literature

Lebert, I., Bégot, C. and Lebert, A. (1998) Development of two *Listeria monocytogenes* growth models in a meat broth and their application to beef meat. Food Microbiol. 15, 499-509.

Robles Olvera, V. (1999) Comparaison de la croissance de *Pseudomonas species* et *Listeria species* en milieu liquide et en viande de boeuf. Thèse de Docteur ès science, Université Blaise Pascal, Clermont-Ferrand II.

Rocourt, J. and Bille, J. (1997) Foodborne Listeriosis. World Health statistics quartely 50, 67-73.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M. and Van't Riet, K. (1990) Modeling the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875-1881.

Table 1: Observed and predicted values (lag time (L) and generation time (GT)) of *Pseudomonas* spp. grown on the surface of naturally contaminated beef muscle at a relative humidity of air ≈ 100% and pH 5.8. In small prints: lower and upper confidence intervals at 95%.

T (°C)	$logN_0$	A	L obs. (h)	L pred. (h) *Pfr*162 model	L pred. (h) *Pfr*K1 model	L pred. (h) *Pfl*58 model	GT obs. (h)	GT pred. (h) *Pfr*162 model	GT pred. (h) *Pfr*K1 model	GT pred. (h) *Pfl*58 model
6	4.4	5.8	19 (13–26)	6.7 (3.9–11.6)	3.3 (0.8–13.5)	12 (5.8–24)	5.1 (4.5–5.6)	4.3 (3.5–5.4)	6.1 (4.5–8.2)	6.5 (4.5–9.3)
6	3.0	7.5	17 (12–22)	6.7 (3.9–11.6)	3.3 (0.8–13.5)	12 (5.8–24)	4.4 (4.1–4.8)	4.3 (3.5–5.4)	6.1 (4.5–8.2)	6.5 (4.5–9.3)
4	3.0	7.4	18 (12–24)	10.3 (5.5–19.3)	4.4 (0.9–22.3)	14 (6.4–32)	5.7 (5.2–6.2)	6.2 (4.8–7.9)	8.8 (6.3–12.4)	10 (6.7–15)

Table 2 : Observed and predicted lag times and generation times (h) for single strain or mixed cultures grown on the surface of decontaminated meat at 4°C, relative humidity ≈ 100%. In small prints: lower and upper confidence intervals at 95%.
*Pfr*162: *P. fragi* 162; *Lm*14: *L. monocytogenes* 14; *Li*CLIP: *L. innocua* CLIP 20595

Experiments	Strains	T (°C)	pH	$logN_0$	A	L obs. (h)	L pred. (h) *Pfr*162 model	L pred. (h) *Lm*14 model	GT obs. (h)	GT pred. (h) *Pfr*162 model	GT pred. (h) *Lm*14 model
Listeria +	*Pfr*162	4	5.9	4.0	6.4	8 (4–12)	10 (6–16)		5.2 (4.8-5.6)	6.1 (5.0-7.3)	
Pseudomonas	*Li*CLIP	4	5.9	3.1	4.1	69 (41–97)		67 (10–446)	28 (23-33)		26 (14–49)
Listeria	*Lm*14	4	6.3	4.1	4.8	36 (10–62)		56 (10–301)	33 (29-36)		28 (13-39)
Pseudomonas	*Pfr*162	4	5.7	4.0	6.2	26 (20–31)	11 (5–20)		5.2 (4.5-5.8)	6.3 (4.8-8.2)	

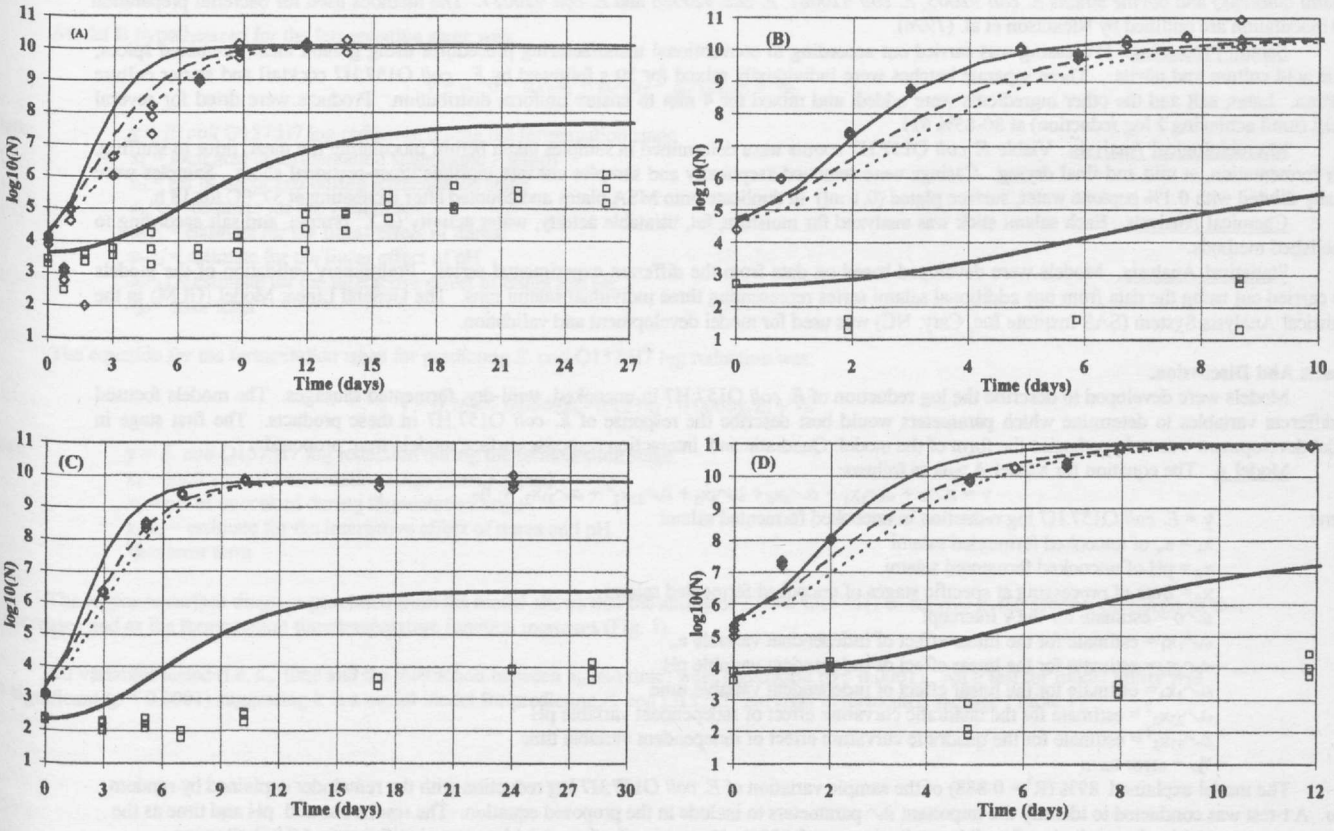

Figure 1: Growth of *Pseudomonas* spp. in naturally-contaminated meat re-inoculated with *Listeria* strains at 6°C, relative humidity of air ≈ 100 %, pH 5.8. The meats were available in oxygen-permeable bags (B and D) or in vacuum packs (A and C).
Experimental data: *Pseudomonas* spp. (✧), *Listeria* (□); Models: *Pfr*162 (——); *Pfr*K1 (— —); *Pfl*58 (·····); *Lm*14 (▬)