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Application of polynomial models to predict growth of mixed cultures of *Pseudomonas* spp. and *Listeria* in meat

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Abstract

Three models for one rapid and one slow growing strain of *Pseudomonas fragi* and one slow growing strain of *P. fluorescens* were developed in a meat broth; they were designed to take account of variations in growth and to provide a growth response interval. These models, and another for *Listeria monocytogenes* (*Lm*14 model), were used to predict the growth of spoilage *Pseudomonas* spp. and pathogenic *Listeria* in meat products. The *Pseudomonas* and *Listeria* models provided satisfactory predictions concerning inoculated strains grown in decontaminated beef meat. It was also possible to use the *Pseudomonas* models to predict the growth of the natural flora (mainly *Pseudomonas* spp.) of refrigerated meat stored under aerobic conditions. In experiments with mixed populations, three situations were observed: (1) in decontaminated meat, *L. monocytogenes* inoculated alone grew well at 6°C, and this result was correctly predicted by the model; (2) in decontaminated meat inoculated with *Listeria* and *Pseudomonas* strains, *L. innocua* grew well and was not affected by the presence of *Pseudomonas*, and the growth of both organisms was correctly predicted by the models; (3) in naturally contaminated meat inoculated with *Listeria*, the strain did not grow until *Pseudomonas* had reached the stationary phase. The models satisfactorily predicted the growth of *Pseudomonas* spp. but not that of *Listeria*. In conclusion, the *Lm*14 model cannot be used for refrigerated meat stored aerobically as the results suggest a 'fail-safe' level which may be too high: meat had already reached a spoilage state even though no increase in the level of *Listeria* was observed. The *Pseudomonas* models accurately predicted the growth of naturally occurring *Pseudomonas* spp.

Keywords: *Listeria* models; Meat products; Mixed populations; Polynomial models; Predictive microbiology; *Pseudomonas* models

1. Introduction

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 shelf-life of these products and can be responsible for considerable financial losses. The main flora responsible for such spoilage during aerobic storage are the pseudomonads (Coates et al., 1995; Widders et al., 1995). Gill and Newton (1977) showed that *Pseudomonas* spp. grew faster than other strains present in meat at temperatures between

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2 and 15°C. Several pathogenic bacteria also grow within this temperature range and may cause food-borne outbreaks: *Listeria monocytogenes* has been shown to be responsible for outbreaks of food poisoning in France and in many countries (Rocourt and Bille, 1997; Jacquet et al., 1999). A recent outbreak in 1999 in France was due to the consumption of rillettes (De Valk et al., 2000).

Models which can predict the growth of *Listeria* (Hudson, 1994; McClure et al., 1994; Lebert et al., 1998a) or *Pseudomonas* (Fu et al., 1991; Muermans et al., 1993; Neumeyer et al., 1997; Giannuzzi et al., 1998) are available, but these models were developed independently, using different methodologies. The aim of this study was: (1) to develop predictive models for *P. fluorescens* and *P. fragi* since these species are frequently detected in meat products (Pooni and Mead, 1984; Molin and Ternström, 1986; Drosinos and Board, 1995); (2) to predict the growth of both spoilage *Pseudomonas* spp. and pathogenic *Listeria* in meat products. Since models for *Listeria* (Lebert et al., 1998a) were already available, *Pseudomonas* models were developed using the same methodology (media, materials, protocols). The models were tested for both genera in decontaminated or naturally contaminated meats.

2. Materials and Methods

2.1. Bacterial strains

Three strains of *Pseudomonas* were selected from 59 different types of *Pseudomonas*, previously clustered into two groups (Lebert et al., 1998b). The first group comprised *P. fragi* strains characterised by shorter lag times than the second group which was made up of *P. fluorescens* strains. Two *P. fragi* were selected from group 1: *P. fragi* 162 (*Pfr*162) isolated from beefsteak which had shorter lag times than *P. fragi* K1 (*Pfr*K1) which was isolated from minced beef. *P. fluorescens* 58 (*Pfl*58) was selected from group 2 for its long lag phase. It had been isolated from poultry after a 7-day storage period. *L. monocytogenes* 14 (*Lm*14) was isolated from an industrial site, *L. monocytogenes* LMI (*Lm*LMI) from minced meat and *L. innocua* CLIP 20595 (*Li*CLIP) from meat.

The strains were stored at 4°C on Tryptic Soy Agar (TSA; Difco, OSI, Maurepas, France) slants and transferred monthly. *Listeria* strains were first cultured on TSA slants at 37°C for 8 h, while *Pseudomonas* strains were cultured at 24°C for the same duration. Subcultures of both strains were then performed in a meat medium (MM) (Lebert et al., 1998b), which was incubated in a horizontally rotating water bath (Aquatron, Infors SARL, Massy, France) at 150 rpm for 17 h at 30°C for *Listeria* or 24°C for *Pseudomonas*. It was used to inoculate broth media and beef tissues.

2.2. Bacterial growth in broth

Pseudomonas strains were grown in a Tryptic Meat Broth (TMB) buffered at different pH values using a NaOH solution (0.5 N, Prolabo, France) or a HCl solution (35%, Prolabo). Sodium Chloride (Merck, Nogent sur Marne, France) was added as required to the TMB which was sterilised by passage over a 0.2-μm nitrocellulose filter (Whatmann, Poly Labo, Strasbourg, France).

Growth conditions were adapted on the basis of the results obtained by Lebert et al. (1998b). Since the amount of oxygen had been shown to be a limiting factor for *Pseudomonas* growth, cultures were thus carried out in 1-l Erlenmeyer flasks containing 250 ml of broth: an optimised air/liquid exchange surface and rapid agitation made it possible to obtain a sigmoïdal growth curve and to calculate growth parameters. The TMB was inoculated with the second subculture to give an initial concentration of approximately 10^7 CFU/ml. The number of viable bacteria was verified on TSA plates inoculated with this culture and incubated at 24°C for 24 h. Meanwhile, the Erlenmeyer flasks were incubated in a horizontally rotating water bath at 150 rpm at the appropriate temperature. One millilitre of the culture was removed at regular intervals and the optical density (OD) read on a spectrophotometer (UV-160A, Shimadzu, Japan) at 600 nm. Samples with an OD of over 0.5 were diluted in TMB until the OD fell below 0.5. No more than 10% of the working volume was removed during growth. Each growth curve was plotted using a minimum of 30 measure-

ments. *Pseudomonas* models were constructed from experiments in TMB (see Section 2.4).

2.3. Bacterial growth on beef meat tissues

Two types of experiment were performed: (1) growth in decontaminated *Biceps femoris* muscle (the meat was decontaminated, cut and inoculated with *Listeria* or *Pseudomonas* strains as described by Lebert et al. (1998a)); (2) growth in naturally contaminated *Biceps femoris* muscle or minced meat, the dominant background microflora being grown alone or in the presence of inoculated *Listeria*.

Minced meats were purchased from a local supermarket, and experiments planned to begin 2 days before the sell-by date. In the supermarket, the meats were available in either oxygen-permeable bags (used for experiments B and D) or in vacuum packs (experiments A and C) (Table 6).

Meat samples were incubated in Petri dishes in which relative humidity (RH) close to 100% was sustained throughout the experimental period. The Petri dish comprised two glass dishes: one (12 cm diameter $\times$ 2 cm high) was placed in another larger one (14 cm diameter $\times$ 3 cm high) which was then covered. Meat cubes were placed in the inner dish, the outer dish being filled with sterile water.

At each sampling time, three meat cubes or three samples of minced meat (10 g) were removed and each sample was plated in duplicate using a spiral plater (DS, Interscience, Saint Nom la Bretèche, France). Bacterial counts were performed (temperature of incubation): on TSA (24°C) for non-selective counts to control any contamination in decontaminated meat and the level of background microflora; on Palcam Agar (30°C) (Merck) for *Listeria*, and on *Pseudomonas* Agar Base (24°C) (Oxoïd, Unipath, Basingstoke, UK) supplemented with *Pseudomonas* CFC Supplement (Oxoïd), for *Pseudomonas*. The pH was determined at alternate sampling time points using a pH-meter probe (Ingold, Bioblock Scientific, Illkirch, France).

2.4. Growth conditions

Growth models were built using data from 16 experiments carried out in Erlenmeyer flasks with a central composite design (Benoist et al., 1994) with three factors (NaCl %, temperature, pH) and five levels. pH values ranged from 5.4 to 7.0, the NaCl content from 0 to 6.5%, and the temperature from 2 to 14°C. Tests at the central point of the design (8°C, NaCl 3.5% and pH 6.2) were performed in duplicate. The models were tested in meat broth and on the meat surface. Growth conditions and the corresponding strains are listed in Tables 3–6.

2.5. Fitting of the curve

Growth curves were obtained from OD data in broth experiments ($\mathrm{Log}_{10}(\mathrm{OD})$) and from viable bacterial counts on selective Palcam Agar or *Pseudomonas* Agar for growth in meat ($\mathrm{Log}_{10}(\mathrm{CFU/g})$). Growth curves were fitted using a modified Gompertz equation (Zwietering et al., 1990). Growth parameters (A, logarithmic increase in the bacterial population, L, lag time, GT, generation time) were calculated using non-linear regression with STAT-ITCF statistical software (Gouet J.P. and Philippeau G., Institut Technique des Céréales et des Fourrages, Paris, France).

2.6. Statistical analysis

Polynomial models were generated for the three strains. As the results of only 16 experiments were available, multiple linear regressions on log10(Y) (where $Y = \mathrm{GT}$ or L) were performed using STAT-ITCF software. Correlations were made using the following terms: Constant, T, pH, Salt, T^2, pH^2, Salt^2, $T \times \mathrm{pH}$, $T \times \mathrm{Salt}$, $\mathrm{pH} \times \mathrm{Salt}$, where T is temperature (°C), Salt is NaCl %. The significant terms were given by the software.

2.7. Comparisons of observed and predicted data

In order to evaluate the goodness of fit of the *Lm*14 model (Lebert et al., 1998a) and the three *Pseudomonas* models (present study), the bias and accuracy factors proposed by Ross (1996) were calculated from literature data and data from this study. Taking the definition of Neumeyer et al. (1997), the factors were defined as:

$$\text{bias factor} = 10^{\dfrac{\sum \log(\mathrm{GT}_{\text{predicted}}/\mathrm{GT}_{\text{observed}})}{n}}$$

$$\text{accuracy factor} = 10^{\frac{\sum \log|GT_{predicted}/GT_{observed}|}{n}}$$

where $GT_{observed}$ is the generation time experimentally observed; $GT_{predicted}$ is the generation time predicted by the model; n is the number of observations.

A value greater than 1 for the bias factor indicates that the predicted generation time is, on average, greater than the observed generation time and is thus fail dangerous. Conversely, a value below 1 indicates that the model is fail-safe. The accuracy factor measures the average difference between observed and predicted values. The larger the value of the accuracy factor the less accurate the average estimate, while a value of 1 indicates that there is perfect consistency between all predicted and observed values.

3. Results

3.1. Development of the three Pseudomonas growth models

As previously reported by Lebert et al. (1998b), the lag times (L) for *Pfl*58 were longer than those for *P. fragi* strains. In general, however, the L for *Pfr*162 was longer than that observed for *Pfr*K1, except under particularly favourable conditions (high temperature and pH, or low NaCl %). *Pfr*162 had a shorter GT than *Pfr*K1, while *Pfl*58 had a far longer GT than the two *P. fragi* strains. So as to take growth variations into account, and calculate a growth response interval, three predictive models were developed using *Pfr*162 (*Pfr*162 model, fast strain), *Pfr*K1 (*Pfr*K1 model, slow strain) and *Pfl*58 (*Pfl*58 model, slow strain) (Tables 1 and 2). These models were tested in broth experiments (Table 3) under conditions which differed from those of the experimental design. Two conditions fell within the experimental range (NaCl 3.5%), while four were outside the range of study (NaCl 0 and 6.5%). In five experiments, good predictions were observed, regardless of the model or conditions. At 4°C and NaCl 6.5%, only a slight increase in population was observed for the two *P. fragi* strains and no growth was observed after 30 days for *Pfl*58. The models predicted very low growth, but prediction was calculated at the boundaries of the model.

Table 1
Parameters of the generation time (h) models developed for three *Pseudomonas* strains[a]

Parameter	*Pfr*162		*Pfr*K1		*Pfl*58	
	Value	*P*	Value	*P*	Value	*P*
Constant	4.6878	*	4.3663		8.0793	*
T	−0.1766	**	−0.2161	**	−0.1875	*
pH	−1.1050		−0.8747		−2.0859	
Salt	0.2706	*	0.1649		0.1437	
T^2	0.0026	*	0.0023		0.0049	*
pH^2	0.0855		0.0603		0.1641	
$Salt^2$	0.0164	***	0.0207	**	0.0154	*
$T \times pH$	0.0129		0.0195		0.0074	
$T \times Salt$	−0.0013		−0.0035		−0.0010	
$pH \times Salt$	−0.0440	**	−0.0301		−0.0235	*
R^2	0.9937		0.9901		0.9856	

[a] *Pfr*, *P. fragi*; *Pfl*, *P. fluorescens*; Salt, % NaCl; R^2, coefficient of determination.
*1%<P<5%; **0.1%<P<1%; ***P<0.1%.

Table 2
Parameters for the lag time (h) models developed for three *Pseudomonas* strains[a]

Parameter	*Pfr*162		*Pfr*K1		*Pfl*58	
	Value	*P*	Value	*P*	Value	*P*
Constant	4.3606		1.5554		0.8679	
T	−0.1093	*	−0.0353	*	−0.2723	*
pH	−0.9334		−0.3312		0.4593	
Salt	0.2069		0.4389		0.3567	
T^2	0.0015	*	0.0002		−0.0029	*
pH^2	0.0737		0.0379		−0.0677	
$Salt^2$	0.0061	*	−0.0089	*	−0.0011	
$T \times pH$	0.0001		−0.0055		0.0445	*
$T \times Salt$	0.0012	**	−0.0013		−0.0103	**
$pH \times Salt$	−0.0155		−0.0311		−0.0107	
R^2	0.9477		0.8981		0.9789	

[a] *Pfr*, *P. fragi*; *Pfl*, *P. fluorescens*; Salt, % NaCl; R^2, coefficient of determination.
*1%<P<5%; **0.1%<P<1%.

3.2. Observed and predicted growth in decontaminated meat inoculated by Pseudomonas strains

The study conditions and results are presented in Table 4. Experiments with the two *P. fragi* strains were repeated three times at 4°C. Reproducibility was satisfactory for two experiments for each strain:

Table 3
Observed (obs.) and predicted (pred.) lag times *L* (h) and generation times GT (h) during additional experiments performed using broth at pH 5.8[a]

T (°C)	NaCl (%)	Strain	Log N_0	*A*	*L* (h)		GT (h)	
					Obs.	Pred.	Obs.	Pred.
4	6.5	*Pfr*162	7.1	0.4	NG	116 *63–212*	NG	35 *28–45*
		*Pfr*K1	7.3	0.2	NG	83 *17–396*	NG	46 *33–65*
		*Pfl*58	7.0	NG	NG	564 *260–1226*	NG	47 *32–70*
4	0.0	*Pfr*162	7.3	2.4	8.7 *7.2–10.1*	10.3 *5.5–19.3*	7.1 *6.6–7.5*	6.2 *4.8–7.9*
		*Pfr*K1	7.3	2.2	5.3 *3.1–7.4*	4.4 *0.9–22.3*	9.2 *8.5–9.8*	8.8 *6.3–12.4*
		*Pfl*58	7.1	2.2	14.6 *13.1–16.0*	14.2 *6.4–31.7*	8.2 *7.8–8.7*	10.1 *6.7–15.1*
7	3.5	*Pfr*162	7.0	2.3	18.0 *17.3–18.7*	17.8 *11.4–27.8*	5.8 *5.6–6.0*	6.2 *5.2–7.4*
		*Pfr*K1	7.3	1.9	16.4 *15.3–17.5*	16.4 *5.2–52.1*	6.9 *6.5–7.3*	7.0 *5.5–8.9*
		*Pfl*58	7.0	2.2	47.2 *46.0–48.4*	60.0 *34.0–106.2*	9.0 *8.6–9.3*	8.3 *6.2–11.2*
7	0.0	*Pfr*162	7.2	2.3	5.4 *4.4–6.4*	5.4 *3.2–9.3*	4.1 *3.8–4.3*	3.7 *3.0–4.6*
		*Pfr*K1	7.4	2.0	4.9 *3.7–6.1*	2.8 *0.7–11.1*	5.2 *4.8–5.6*	5.1 *3.8–6.9*
		*Pfl*58	7.2	1.6	9.5 *8.6–10.3*	10.3 *5.3–20.4*	4.6 *4.3–4.9*	5.4 *3.8–7.6*
10	3.5	*Pfr*162	7.0	2.3	10.9 *10.2–11.6*	10.3 *6.6–16.0*	4.0 *3.8–4.2*	4.0 *3.4–4.8*
		*Pfr*K1	7.3	2.0	10.2 *9.4–11.0*	10.3 *3.3–32.4*	4.6 *4.3–4.8*	4.1 *3.2–5.2*
		*Pfl*58	7.1	1.9	23.3 *21.9–24.7*	30.1 *17.1–53.1*	5.8 *5.3–6.3*	5.3 *4.0–7.1*
10	0.0	*Pfr*162	7.2	2.1	3.2 *2.6–3.8*	3.1 *1.8–5.3*	2.7 *2.5–2.9*	2.5 *2.0–3.1*
		*Pfr*K1	7.4	1.8	3.4 *2.8–3.9*	1.8 *0.4–7.5*	3.3 *3.1–3.5*	3.3 *2.4–4.4*
		*Pfl*58	7.0	1.6	5.5 *4.6–6.3*	6.7 *3.3–13.4*	3.5 *3.1–3.8*	3.5 *2.5–5.0*

[a] An observed value for a strain was compared with the predicted value of the corresponding model of the strain. *Pfr, P. fragi*; *Pfl, P. fluorescens*; Log No., Log10 of population at time zero; *A*, increase in population; italic ranges, lower and upper confidence intervals at 95%; NG, no growth observed after 10 days for *Pfr*162 and *Pfr*K1, and after 30 days for *Pfl*58.

in the third cases (experiments 1 and 5 in Table 4), variations were greater.

Experimental *L* values were always longer than predicted *L* values and predicted GT values were generally a little higher than observed GT values. In terms of GT values, the predictions of the models were considered satisfactory.

3.3. Observed and predicted growth of Pseudomonas spp. in naturally contaminated meat

The three models were tested to predict the growth of *Pseudomonas* spp. in naturally contaminated meat. Since the TSA and CFC counts rapidly began converging at the beginning of the exponential

Table 4
Observed (obs.) and predicted (pred.) growth parameters, L (h) and GT (h) during experiments on meat[a]

Strain	T (°C)	pH	log N_0	A	L obs.	L pred.	GT obs.	GT pred.
*Pfr*162	4.0	5.5	4.8	6.1	19.2	11.1	3.8	6.5
					14.9–23.6	*5.2–23.6*	*3.4–4.3*	*4.8–8.9*
*Pfr*162	4.0	5.7	4.6	6.5	15.0	10.5	4.8	6.3
					14.9–23.6	*5.4–20.4*	*4.4–5.2*	*4.8–8.2*
*Pfr*162	4.0	5.7	4.0	6.2	25.7	10.5	5.2	6.3
					20.2–31.2	*5.4–20.4*	*4.5–5.8*	*4.8–8.2*
*Pfr*K1	4.0	5.6	5.3	6.0	36.6	4.2	6.3	9.3
					29.5–43.7	*0.7–26.3*	*5.5–7.3*	*6.3–13.6*
*Pfr*K1	4.0	5.8	4.1	7.0	8.4	4.4	5.3	8.8
					3.1–13.7	*0.9–22.3*	*4.8–5.8*	*6.3–12.4*
*Pfr*K1	4.0	5.9	4.5	6.6	14.5	4.5	6.3	8.6
					8.8–20.2	*0.9–21.3*	*5.7–6.9*	*6.2–12.0*
*Pfl*58	4.0	5.7	4.4	5.8	62.6	14.7	7.7	10.5
					47.6–77.6	*6.3–34.2*	*6.0–9.3*	*6.8–16.1*
*Pfr*162	7.0	6.0	3.5	6.6	11.2	5.3	3.8	3.7
					6.2–16.2	*3.2–8.7*	*3.3–4.3*	*3.0–4.5*
*Pfr*K1	7.0	6.1	3.7	6.6	14.4	3.0	3.8	5.1
					9.4–19.4	*0.8–10.7*	*3.3–4.3*	*3.9–6.6*
*Pfr*162	10.0	5.6	4.3	6.8	7.4	3.2	2.1	2.5
					5.9–8.8	*1.7–5.9*	*1.9–2.2*	*1.9–3.2*
*Pfr*K1	10.0	5.4	3.7	7.7	3.2	1.8	2.4	3.3
					1.1–5.4	*0.3–11.4*	*2.2–2.5*	*2.2–4.9*

[a] Meat was decontaminated, inoculated with one strain of *Pseudomonas* and incubated in air with a relative humidity close to 100%. The observed value for a strain was compared with the predicted value of the corresponding model of the strain. Italic ranges, lower and upper confidence intervals at 95%. *Pfr, P. fragi*; *Pfl, P. fluorescens*.

phase, *Pseudomonas* spp. were considered to be the dominant microflora. Curves obtained from CFC counts alone were used to calculate growth parameters, because the total flora counts were sometimes higher than *Pseudomonas* counts at time zero. Fig. 1a,b shows that, at 6°C, the experimental points were within the curves generated by the three models. In Fig. 1c, growth was similar to that predicted using the *Pfr*162 model.

3.4. Observed and predicted growth of a mixed population of Pseudomonas and Listeria

Interactions between the two genera were studied in a decontaminated meat, inoculated with *Pseudomonas* and *Listeria* strains successively (Table 5). The growth of *Listeria* was predicted using the *Lm*14 model (Lebert et al., 1998a). Generation times for *Listeria* were similar when grown alone or with *P. fragi,* and were predicted satisfactorily by the models. The differences were greater for the lag time, but values remained within the confidence intervals which were large at this temperature. 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 the two organisms was neither inhibited nor stimulated. *L. monocytogenes* did not affect the growth of *Pseudomonas*. No interactions were observed between the strains, and their growth parameters were satisfactorily predicted by their respective models.

Interactions between *Pseudomonas* and *Listeria* were then carried out in naturally contaminated meat (cubes of meat or minced beef) inoculated with *Listeria*. *Listeria* was always inoculated at a level below that of *Pseudomonas*. *Pseudomonas* spp. rapidly became the dominant bacteria in samples. No *Listeria* growth was observed until *Pseudomonas* reached the stationary phase. In experiment A, *Listeria* counts remained constant at 4 log(CFU) until the stationary phase of *Pseudomonas,* and then increased up to 6 log(CFU) after incubation for 26 days. Slight increases (1.5 log(CFU)) were observed

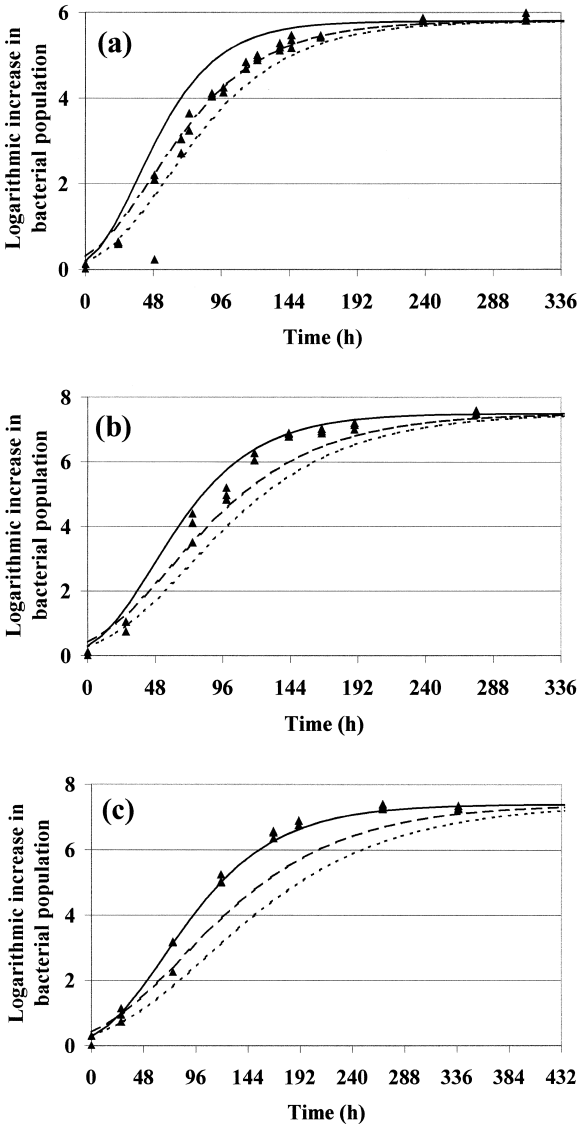

Fig. 1. Comparison of the growth of *Pseudomonas* spp. in naturally contaminated meat with *Pseudomonas* models. (a,b) Replicate experiments at 6°C, relative humidity of air≈100%, pH 5.8; (c) 4°C, relative humidity of air≈100%, pH 5.8. Experimental data (▲); *P. fragi* 162 model (———); *P. fragi* K1 model (– · –); *P. fluorescens* model (· · ·).

in experiments B and C after *Pseudomonas* had reached the stationary phase. In experiment D, the population remained constant for 12 days.

Differences in lag times between experiments can be explained in terms of the storage conditions. When meat was stored in oxygen-permeable film, the microflora present in the meat was already aerobic and probably composed mainly of *Pseudomonas* spp.: the *L* for *Pseudomonas* spp. was close to zero. However, with vacuum-packaged meat, the *L* differed significantly from zero, and it took longer for the *Pseudomonas* to outgrow the anaerobic flora present in the meat. No differences were observed between GT values.

Predicted values are presented in Table 6. For meat stored in an oxygen-permeable film, calculated *L* values were longer than observed *L* values. They were shorter than those seen when meat had previously been vacuum-packaged. In the four experiments, the GT values observed fell within those calculated using the *Pfr*162 and *Pfr*K1 models.

3.5. Bias and accuracy factors for Pseudomonas and Listeria models

Tables 7 and 8 present the bias and accuracy factors for the three *Pseudomonas* models and the Neumeyer model (Neumeyer et al., 1997) calculated from the data produced in this study and from the data in the literature. In both cases, the bias factors of the *Pfr*162 and Neumeyer models were similar, both values being below but close to 1; the accuracy factors were also similar and greater than one, so the *Pfr*162 and Neumeyer models underestimated the values of the growth parameters. Conversely, the bias factors of the *Pfr*K1 and *Pfl*58 models were greater than one and the accuracy factors close to 1.4 on average: they overestimated the values of the growth parameters.

Table 9 summarises the bias and accuracy factors for the *Lm*14 model calculated from the data in the literature. On average, the values were 0.96 and 1.27, respectively, for the bias and accuracy factors.

4. Discussion

Since large variations were observed between bacterial growth profiles (Lebert et al., 1998b), it was decided to build three models for one rapid and one slow growing strain of *P. fragi* and for one slow growing strain of *P. fluorescens*. It was assumed that any growth would be within a zone delimited by the three predictive growth curves. This method differed from those employed by Neumeyer et al. (1997),

Table 5
Observed and predicted lag times (h) and generation times (h) for a single strain or mixed cultures grown on the surface of decontaminated meat at 4°C, relative humidity of air≈100%[a]

Type of experiment	Strain	pH	log N_0	*A*	*L* obs.	*L* pred.		GT obs.	GT pred.	
						*Pfr*162	*Lm*14		*Pfr*162	*Lm*14
Listeria	*Lm*14	6.3	4.1	4.8	35.9		56	32.7		28.4
					10.4–61.5		*10–301*	*29.0–36.4*		*13.0–38.9*
Listeria + *Pseudomonas*	*Pfr*162	5.9	4.0	6.4	8.2	10.1		5.2	6.1	
					4.1–12.4	*6.4–16.1*		*4.8–5.6*	*5.0–7.3*	
	*Li*CLIP	5.9	3.1	4.1	69.2		67	27.7		26.4
					41.1–97.3		*10–446*	*22.6–32.9*		*14.3–48.9*

[a] Italic ranges, lower and upper confidence intervals at 95%; *Pfr*, *P. fragi*; *Lm*, *L. monocytogenes*; *Li*, *L. innocua*.

Table 6
Observed and predicted growth parameters (h) for microflora present in naturally contaminated meat incubated at 6°C with relative humidity of air≈100%; *Listeria* was inoculated into meat[a]

		pH	Strains	log N_0	A	*L* obs.	*L* pred.			GT obs.	GT pred.		
							*Pfr*162	*Pfr*K1	*Pfl*58		*Pfr*162	*Pfr*K1	*Pfl*58
A	Meat surface	5.6	Total flora	7.1	2.9								
			Ps. sp.	4.0	6.1	19.0	7.0	3.2	11.9	5.7	4.4	6.3	7.0
						5.3–32.7	*3.8–12.9*	*0.6–15.5*	*5.4–26.1*	*5.2–6.2*	*3.5–5.7*	*4.5–8.8*	*4.7–10.5*
			*Li*CLIP	3.7	1.9	NG after 12 days							
B	Minced meat	5.8	Total flora	4.6	5.6								
			Ps. sp.	4.4	6.0	0.0	6.7	3.3	11.7	4.6	4.3	6.1	6.5
						−5.7–5.7	*3.9–11.6*	*0.8–13.5*	*5.8–23.5*	*3.9–5.2*	*3.5–5.4*	*4.5–8.2*	*4.5–9.3*
			*Lm*LMI	<2.0	0.9	NG after 9 days							
C	Minced meat	5.9	Total flora	6.3	3.9								
			Ps. sp.	3.1	6.7	17.2	6.6	3.3	11.5	5.0	4.3	6.0	6.3
						11.4–22.9	*3.9–11.2*	*0.8–13.1*	*5.9–22.6*	*4.6–5.5*	*3.5–5.3*	*4.5–8.0*	*4.5–8.9*
			*Lm*LMI	2.3	1.8	NG after 17 days							
D	Minced meat	5.9	Total flora	5.8	4.3								
			Ps. sp.	5.2	4.9	0.0	6.6	3.3	11.5	5.3	4.3	6.0	6.3
						−10.3–10.3	*3.9–11.2*	*0.8–13.1*	*5.9–22.6*	*3.8–6.7*	*3.5–5.3*	*4.5–8.0*	*4.5–8.9*
			*Li*CLIP	3.8	0.3	NG after 12 days							

[a] Italic ranges, lower and upper confidence intervals at 95%; *Ps.*, *Pseudomonas*; *Lm*, *L. monocytogenes*; *Li*, *L. innocua*; NG, no growth observed during the indicated period.

who proposed a model developed using the fastest strain available, or by McClure et al. (1994) who used a cocktail of strains.

Our results confirmed the findings of Shelley et al. (1986), Cox and Mac Rae (1988) and Lebert et al. (1998b), who had reported that *P. fragi* grew faster than *P. fluorescens*. In meat broth, the differences between the growth parameters of *P. fragi* strains decreased when the temperature rose above 8°C. Lebert et al. (1998a) had obtained similar results with *Listeria*, where the differences decreased as temperatures rose above 14°C.

In general, predictions by the three models were better regarding GT values than *L* values whichever medium was used (broth or meat), as already reported by other authors (Hudson, 1994; Delignette-

Table 7
Bias and accuracy factors calculated for the three *Pseudomonas* models and the model of Neumeyer et al. (1997) using data from the present study

Medium	Model strains	*n*	Neumeyer et al.		*Pfr*162		*Pfr*K1		*Pfl*58	
			Bias	Accuracy	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy
Broth (from Table 3)										
	All	15	0.85	1.19	0.82	1.24	1.02	1.13	1.16	1.19
	*Pfr*162	5	1.00	1.04	0.95	1.08	1.19	1.19	1.36	1.36
	Pfr K1	5	0.81	1.23	0.78	1.28	0.98	1.04	1.11	1.11
	*Pfl*58	5	0.76	1.31	0.73	1.37	0.92	1.18	1.04	1.11
Decontaminated meat (from Tables 4 and 5)										
	All	11	1.16	1.20	1.11	1.16	1.56	1.56	1.76	1.76
	*Pfr*62	5	1.31	1.31	1.26	1.27	1.77	1.77	2.00	2.00
	Pfr K1	5	1.09	1.09	1.03	1.06	1.44	1.44	1.63	1.63
	*Pfl*58	1	0.83	1.20	0.81	1.23	1.18	1.18	1.35	1.35
Naturally contaminated meat (from Fig. 1a–c)										
	Ps sp.	3	1.03	1.09	0.97	1.09	1.37	1.37	1.49	1.49
Naturally contaminated meat + inoculated *Listeria* (From Table 6)										
	*P*s sp.	5	0.96	1.13	0.9	1.18	1.28	1.28	1.37	1.37
Total		34	0.97	1.17	0.93	1.19	1.24	1.3	1.39	1.4

Table 8
Bias and accuracy factors calculated for the three *Pseudomonas* models and the model of Neumeyer et al. (1997) using literature data

Reference	Model medium	Neumeyer et al.			*Pfr*162			*Pfr*K1		*Pfl*58	
		n	Bias	Accuracy	*n*	Bias	Accuracy	Bias	Accuracy	Bias	Accuracy
Li and Torres, 1993	Nutrient broth	7	1.09	1.12	3	0.87	1.17	1.03	1.03	1.31	1.31
Hudson and Mott, 1994	Nutrient broth	6	0.87	1.18	3	0.97	1.16	1.30	1.30	1.52	1.52
Pin and Baranyi, 1998	TSB	12	0.88	1.16	12	0.93	1.15	1.28	1.30	1.52	1.52
Pooni and Mead, 1984	BHI	12	1.08	1.10	4	1.32	1.32	1.45	1.45	2.16	2.16
	Subtotal broth	37	0.98	1.14	22	0.99	1.18	1.27	1.28	1.58	1.58
Cox and Mac Rae, 1988	UHT and raw milk	16	0.86	1.47	16	0.81	1.46	1.05	1.52	1.26	1.61
Fu et al., 1991	Rehydrated milk	7	0.87	1.16	5	0.83	1.20	1.05	1.05	1.21	1.21
Tatini et al., 1991	Milk	9	0.83	1.27	9	0.77	1.32	0.97	1.20	1.07	1.25
Shelley et al., 1986	UHT Milk	24	0.68	1.50	8	0.72	1.41	0.90	1.23	0.98	1.19
Langeveld and Cuperus, 1980	Milk	10	1.04	1.06	8	0.97	1.05	1.21	1.21	1.40	1.40
Marth, 1998	Milk	6	0.84	1.39	5	0.86	1.32	1.09	1.36	1.27	1.49
	Subtotal milk	72	0.81	1.36	51	0.81	1.32	1.03	1.31	1.18	1.39
Muermans et al., 1993	Beef Meat	4	1.26	1.26	3	1.23	1.23	1.72	1.72	1.96	1.96
Lasta et al., 1995	Beef Meat	1	0.95	1.06	1	0.78	1.27	0.92	1.08	1.25	1.25
Delaquis and McCurdy, 1990	Beef Meat	4	0.89	1.13	2	0.91	1.09	1.26	1.26	1.54	1.54
Dainty and Mackey, 1992	Meat	8	0.92	1.13	6	0.99	1.12	1.39	1.39	1.58	1.58
Gill and Newton, 1977	Meat	8	1.00	1.11	6	1.04	1.13	1.39	1.39	1.68	1.68
Scott, 1937	Meat	2	0.68	1.46	2	0.73	1.38	1.00	1.05	1.29	1.29
Marth, 1998	Fish or Meat	6	0.84	1.39	5	0.86	1.32	1.09	1.36	1.27	1.49
	Subtotal meat	33	0.94	1.19	25	0.98	1.18	1.32	1.35	1.57	1.57
	Total	142	0.88	1.26	98	0.89	1.25	1.15	1.31	1.36	1.48

Muller et al., 1995). The bias factors calculated from the data in the literature and produced in this study were on average in the same order: $\text{Bias}_{Pfr62} < 1 < \text{Bias}_{PfrK1} < \text{Bias}_{Pfl58}$. The hypothesis advanced above was correct, inasmuch as the experimental data were within the range delimited by the predictive curves

Table 9
Bias and accuracy factors calculated for the *Listeria monocytogenes* 14 model using literature data

Reference	Medium	*n*	Bias	Accuracy
Lebert et al., 1998a	Tryptic meat broth	12	1.04	1.07
Cheroutre-Vialette et al., 1998	Tryptic meat broth	6	0.97	1.18
Buchanan and Phillips, 1990	Tryptose phosphate broth	6	1.41	1.41
Petran and Zottola, 1989	Tryptic soy broth	8	0.90	1.31
MacDonald and Sutherland, 1994	Tryptic soy broth	2	1.06	1.06
Duffy et al., 1994	Listeria selective broth	2	1.00	1.08
	Subtotal broth	36	1.05	1.19
Lebert et al., 1998a	Decontaminated meat	4	0.94	1.33
Robles Olvera et al., 1999	Decontaminated meat	3	0.90	1.12
Grau and Vanderlinde, 1993	Beef fat	15	1.40	1.40
Grau and Vanderlinde, 1993	Beef lean	43	0.98	1.17
McClure et al., 1997	Cooked minced beef	2	1.14	1.14
Farber et al., 1995	Liver paté	4	1.12	1.29
Hudson, 1994	Paté and cooked beef	6	0.64	1.74
Duffy et al., 1994	Minced beef. naturally contaminated	1	1.71	1.71
Manu-Tawiah et al., 1993	Pork chops	1	0.40	2.48
Marth, 1998	Cooked meat	3	0.77	1.29
	Subtotal meat products	82	1.00	1.28
Hudson, 1994	Fish products	4	1.10	1.22
Rosenow and Marth, 1987	Milk products	52	0.84	1.21
Papageorgiou and Marth, 1989	Milk products	8	0.90	1.44
Murphy et al., 1996	Milk products	9	0.90	1.60
Marth, 1998	Milk products	5	0.85	1.18
Buchanan and Phillips, 1990	Whole milk	5	0.84	1.19
	Subtotal dairy products and milk	74	0.85	1.27
Buchanan and Phillips, 1990	Clarified cabbage juice	2	0.58	1.73
Marth, 1998	Lettuce	2	0.50	2.01
Breidt and Fleming, 1998	Cucumber juice	1	1.06	1.06
	Subtotal vegetable products	5	0.61	1.67
Walls and Scott, 1997	Baby food	10	1.50	1.50
	Total	216	0.96	1.27

of the *Pfr*162 and *Pfr*K1 models. These two models were on average at an equal and small distance on either side of the ideal model (bias factor = 1). It was therefore possible to use these models to predict the growth of naturally occurring *Pseudomonas*. The differences between the accuracy factors went in the following order: $\text{Accuracy}_{Pfr62} \approx \text{Accuracy}_{Pfr\text{K}1} < \text{Accuracy}_{Pfl58}$. In comparison with the literature, the values calculated for the bias and accuracy factors were similar to those reported by different authors (Ross, 1996; Neumeyer et al., 1997) or slightly better (Dalgaard and Jorgensen, 1998). The similarity between the Neumeyer model and the *Pfr*162 models can be explained by the fact they were both built from experiments on the fastest strain available.

In experiments with mixed populations, three situations were observed: (1) in decontaminated meat, *L. monocytogenes* inoculated alone grew well at 6°C, and this result was correctly predicted by the model; (2) in decontaminated meat inoculated with *Listeria* and *Pseudomonas* strains, *L. innocua* grew well and was not affected by the presence of *Pseudomonas*, and the growth of both micro-organisms was correctly predicted by the models; (3) in naturally contaminated meat inoculated with *L. innocua*, the strain did not grow until *Pseudomonas* had reached the stationary phase. The models satisfactorily predicted the growth of *Pseudomonas* spp. but not that of *Listeria*.

Numerous authors have studied the growth of *L.*

monocytogenes in naturally contaminated meat products. In processed meat, the rate of growth largely depends on the type of meat: Glass and Doyle (1989) observed rapid growth in poultry products, and slow or inhibited growth in roast beef and summer sausages. In paté (Farber and Daley, 1994) or ground beef (Shelef, 1989), *L. monocytogenes* was capable of surviving at 4°C but incapable of multiplying. Shineman and Harrison (1994) observed that the levels of *L. monocytogenes* remained constant in raw beef and chicken stored at 4°C, but that *Listeria* could grow on the same meat, in which background levels of microflora were lower when cooked. Many authors have assumed that the growth of *L. monocytogenes* is inhibited by lactic acid bacteria or bactericidal agents (Gouet et al., 1978; Champagne et al., 1994; Farber and Daley, 1994) or that background microflora compete with *L. monocytogenes* (Carlin et al., 1996). On the other hand, Marshall and Schmidt (1988) showed that the growth of *L. monocytogenes* was enhanced in milk pre-incubated with *Pseudomonas* spp. Similar results were obtained in decontaminated beef meat by Gouet et al. (1978), who studied the effect of a mixed population on the growth of *L. monocytogenes* at 8°C. When inoculated alone, the *L. monocytogenes* population remained constant; with *Lactobacillus plantarum* it decreased whereas, in the presence of *P. fluorescens* or a more complex flora, the number of *Listeria* fell and then rose again as the *Pseudomonas* reached the stationary phase. An equilibrium may exist between *Pseudomonas* and lactic acid bacteria flora, and may explain such variable results concerning the survival or growth of *L. monocytogenes* in meat products.

Results from the literature have indicated the problems encountered when modelling interactions between bacteria. In our study, it was not possible to predict the growth of *Listeria* in naturally contaminated meat stored at refrigerated temperatures under aerobic conditions. These results were observed for different inoculum levels, types of *Listeria* strain or meat structures. Furthermore, the development of the *Lm*14 model did not take account of the natural microflora in meat. Pin and Baranyi (1998) tried to quantify the interactions between the spoilage bacteria of meat. Four groups of spoilage bacteria were grown alone or together. These authors showed that the group with *Pseudomonas* as the main component rapidly became dominant and was the least inhibited by the others. Dalgaard and Jorgensen (1998) clearly demonstrated that the growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon was slower than that in an inoculated challenge test, and as a consequence it may be said that the models overestimated growth in these products. They suggested including studies on naturally contaminated products when developing and validating models for seafood pathogens. This approach should also be adopted in the case of refrigerated meat products. Nevertheless, Walls and Scott (1997) showed that models could be used to predict the growth of *Listeria* inoculated into sterile baby food. In the case of post-processing contamination where levels of natural flora may be low or absent, models can constitute useful tools for predicting the growth of pathogenic bacteria.

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