Modeling microbial competition in foods. Application to the behaviour of Listeria monocytogenes and lactic acid flora in diced bacon
M. Cornu¹, E. Billoir², H. Bergis¹, A. Beaufort¹, V. Zuliani³

¹Lerqap, Afssa, 23 av. du Gal de Gaulle, F-94706 Maisons-Alfort, France (m.simon-cornu@afssa.fr)
²INRA, UR341 Mathématiques et informatique appliquées, F-78350 Jouy en Josas, France
³IFIP, institut de la filière porcine, Pôle Viandes Fraîches et Produits Transformés, 7 av. du Gal de Gaulle, F-94704 Maisons Alfort, France (veronique.zuliani@ifip.asso.fr)

Abstract
The current models developed in predictive microbiology to describe interactions between microflora in foods are reviewed, with a special focus on the Jameson-effect and Lotka-Volterra approaches. One case-study is further explored: modelling the sparse growth of Listeria monocytogenes in diced bacon along the shelf-life.

Keywords
Challenge testing, Maximal Population Density, competitive growth, pork meat product.

Introduction
One of the major advances of predictive microbiology since the end of the 1990s has been the increasing interest in the fate of the hazards (e.g. Salmonella, L. monocytogenes, Escherichia coli, Staphylococcus aureus...) in situ, i.e. in the food itself, instead of in culture media. This has been emphasized by the creation of databases, e.g. ComBase and Sym'Previus.

In this context, different approaches have been proposed to model microbial interactions between one pathogenic micro-organism of interest and a specific microflora, or a group, i.e. lactic acid bacteria (LAB), or even mesophilic aerobic flora. They are reviewed in this paper, with a special interest for their eligibility to be integrated into simple and robust predictive models. The discussion of these different approaches is applied to the case-study of L. monocytogenes and LAB in diced bacon.

Jameson effect models
In the late 1990’s and early 2000’s, there were numerous observations that (i) many microbial interactions in foods are limited only to a reduction in the maximum population density, without any significant effect on the lag time and the growth rate [5; 6]), (ii) the minority population decelerates when the majority - or the total - population count reaches its maximum [14; 20; 33]. On this basis, Cornu [9] proposed a model relying on the hypothesis that decelerations of both populations would be simultaneous and would result from the competition for a common limiting resource. Ross et al. [37] proposed to call this phenomenon the Jameson effect, after Jameson [22] who had studied growth of Salmonella in an enrichment broth. To quote the fine comparison of Mellefont et al.[30], the Jameson effect “can be described as a race between species to use the resources of the environment to maximise their growth and population numbers. When those resources are depleted, the race is over, and the growth of each species in the population stops”.

Since then, numerous papers have referred to the Jameson effect, concerning growth of:
- Listeria spp. in fishery products [2; 12; 15; 17; 29], in meat products [7; 25; 31; 35; 36], in vegetables [11; 16; 39], on surfaces [21],
- shiga-toxin producing E. Coli in enrichment broth [41], and in meat products [7],
- Salmonella in meat and poultry products [7], in broth [23] and in alfalfa sprouts[27].

This simultaneous deceleration is a simplistic principle which is of course not applicable to every interaction [9; 24; 30] but presents the major advantage to enable simple and parsimonious modelling. Thus, most modelling approaches of the Jameson effect are achieved through a modification of the standard primary growth model and can be conceptualized through the following generic system of equations, in which $N_i$ and $N_B$ stand respectively for
two populations, and all parameters are standard and easily obtainable in “pure cultures” (which means in absence of the other population):

\[
\begin{align*}
\frac{1}{N_A} \frac{dN_A}{dt} &= \mu_A(t) = \mu_{maxA} \cdot \alpha_A(t) \cdot f(t) \\
\frac{1}{N_B} \frac{dN_B}{dt} &= \mu_B(t) = \mu_{maxB} \cdot \beta_B(t) \cdot f(t)
\end{align*}
\]  

where \(\alpha(t)\) is an acceleration function (e.g. that of the Baranyi model), and \(f(t)\) is a logistic deceleration function based on the assumption that both populations inhibit each other to the same extent that they inhibit their own growth, e.g. \(f(t) = \left(1 - \frac{N_A(t)+N_B(t)}{N_{maxtot}}\right)\) with proposals to derive \(N_{maxtot}\) from \(N_{maxA}\) and/or \(N_{maxB}\) [9; 10], or \(f(t) = \left(1 - \frac{N_A(t)}{N_{maxA}}\right) \cdot \left(1 - \frac{N_B(t)}{N_{maxB}}\right)\) [17; 29]. All these proposed deceleration functions are equivalent when \(N_A(t) \ll N_B(t)\) or \(N_A(t) \gg N_B(t)\). Notice that no analytical solution can be provided for the system of equations with the above proposals, which does not help fitting.

Simpler variants of these models (based on the same empirical principle of simultaneous deceleration) consist in an abrupt deceleration function (instead of logistic) [2; 7; 12]. Another empirical variant is to keep a standard primary model for the population of interest only, and to build a secondary model on \(N_{max}\), as a function of the temperature, and then indirectly of the extent of growth of background flora [38; 39] or as a function of \(N_0\) [11].

**Lotka-Volterra models**

To circumvent cases in which the simplistic hypothesis of simultaneous deceleration is not applicable, other models have been proposed:

i. either based on the idea that growth of the minority population is only partly inhibited after the majority population has reached its stationary phase [15]

ii. or on the contrary based on the idea that growth of the minority population stops before the majority population reaches its stationary phase [24]

In these two last models, a new parameter, specific to the mixed culture, is introduced: (i) an inhibition coefficient of the growth rate[15], or (ii) a third \(N_{max}\) specific to the mixed culture [24]. In reference to the hypothesis underlying the Jameson effect, both models could be explained by a differential sensitivity to the unknown reason of growth limitation.

Lotka-Volterra models, historically proposed in ecology, and introduced in predictive microbiology by Dens et al. [13] and Vereecken et al. [40], are another empirical approach of the mixed cultures without referring to the simple simultaneous deceleration hypothesis. The basic scheme of primary model is the system (1) with two, instead of one, deceleration functions:

\[
\begin{align*}
f_A(t) &= \frac{1}{N_{maxA}} \left( N_{maxA} - N_A(t) - \alpha_{AB}N_B(t) \right) \\
f_B(t) &= \frac{1}{N_{maxB}} \left( N_{maxB} - N_B(t) - \alpha_{BA}N_A(t) \right)
\end{align*}
\]

The parameters \(\alpha_{AB}\) and \(\alpha_{BA}\) (coefficients of interaction measuring the effects of one species on the other) have to be estimated in mixed culture.

Such Lotka-Volterra models have been proposed, concerning: growth of *E. Coli* O157:H7 in ground beef [34], growth of *L. monocytogenes* in salami [19], growth of LAB, coliforms, pseudomonads, Brochothrix, Salmonella, and yeasts on sliced pork shoulder [27], growth of *Aeromonas hydrophila* on fish surfaces [18], yeast-yeast and yeast-bacterium interactions during the ripening of smear cheeses [32].

**Mechanistic models**

Again in the late 1990’s, a third class of predictive models were proposed in which the mechanism of the interaction was explicit (decrease of pH, consumption of the limiting substrate, production of an inhibitory by-product...). Such models were far from parsimonious, e.g. with 4 to 5-variable and 20-parameter models [4; 28]. In these approaches, parameters have biological meaning and can be estimated from pure cultures, but at the cost
of an intensive work, which makes them weakly eligible for extensive application in situ. Similar approaches published in the 2000’s are reviewed by Leroy & De Vuyst [26].

**Case-study: Listeria monocytogenes and LAB in diced bacon**

Here, we propose an illustration concerning *L. monocytogenes* and LAB in two related pork meat products: (i) cooked smoked diced bacon (an ingredient used in industrially prepared dishes such as pizzas), (ii) unsmoked uncooked diced bacon (a product usually used in home cooked preparations but occasionally consumed raw by 14% of French people [1]). Both products share close physical-chemical properties.

In the first series, five growth curves were obtained at 8°C under air (i.e. without the modified atmosphere) in one batch of cooked smoked diced bacon: three curves for *L. monocytogenes* in “pure culture” by challenge testing in the cooked smoked product ionized at 5 kGy (Ionisos, Dagneux, France), one for *L. monocytogenes* in “mixed culture” (i.e. challenge testing in the non-ionized product), and one for LAB in “pure culture” by storage trial. Figure 1a presents the fitted primary model to the “pure” LAB growth curve, and various models used to predict the fate of *L. monocytogenes* in “mixed culture”, based on the median growth parameters estimated in “pure culture” and on various interactions hypotheses. The modification of the Jameson effect proposed by Le Marc et al. [24] appears quite promising.

For uncooked unsmoked diced bacon, nine challenge tests (9 batches, 3 from 3 producers) were performed at 8°C under the commercial modified atmosphere. Each challenge test included monitoring of *L. monocytogenes* onto ALOA, of LAB onto MRS, of pH, water activity ($a_w$), organic acids concentrations, and gas composition. In most cases (7/9 batches), no growth of *L. monocytogenes* was observed whereas in the 2 other ones sparse growth was observed (see example Figure1b). The competition with LAB is likely to explain at least partly the quasi-absence of growth even if none of the tested hypotheses appears satisfactory. Stochastic modelling of the lag and stationary phases seems to be required.

**Conclusion**

Further research appears needed to validate the various proposed alternatives to model microbial interactions, and would in particular be useful to model the fate of *L. monocytogenes* in diced bacon along the shelf-life and then to expand the on-going modeling work [3; 8] beyond the process *per se*.

**References**