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Review

A review on growth/no growth *Salmonella* models

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ABSTRACT

"Hurdle technology" is a worldwide technique of food preservation based on the application of a combination of generally mild treatments which act as "obstacles" that microflora must overcome to start to grow. Then, bacteria invest their energy in trying to maintain their homeostatic equilibrium instead of multiplying. While the action mechanisms underlying these treatments are not fully understood, it is very useful to know their effect on bacteria cells as well as the extension of such effects. Growth/no growth models have been developed to offer a response to this need. A review on growth/no growth microbial modeling is presented in this paper, addressing the most important factors and approaches employed. Five growth/no growth models of *Salmonella* published in the period 2001–2011 are reviewed, and their boundary regions were represented (Temperature *vs* pH) at two water activity values (0.983 and 0.990) and two cut-off probabilities (0.1 and 0.5). With this illustration, a picture of the relative grade of conservatism of the five models is provided. Additionally, the most important predictive tools in food microbiology (or tertiary models), including a software for growth/no growth modeling (Microbial Responses Viewer), are commented. Finally, some caveats in growth/no growth *Salmonella* modeling are addressed for future research.

Contents

1. Introduction

Since ages, professionals and scientists in the field of food quality and safety have aimed at preventing the growth of pathogens and spoilage microorganisms in foods. The application of hurdle technology, consisting of the application of more than one intervention treatment, has a long history. For example, from ancient times, is it well known the traditional manufacture of Spanish cured ham, which makes use of salt and chill during ripening to prevent the growth of microorganisms. The different intervention treatments (salt and chill in our example), when applied altogether, often produce a global inhibitory effect on microbial growth greater than the "sum" of the effects of the different treatments applied separately. This successful interaction between treatments is called "synergy". Due to this synergy, the individual treatments or hurdles may be set at lower intensities than would be required if only a single hurdle were used as the preservation technique (Leistner & Gorris, 1995). The mode of action of the hurdles resides in the disruption of several

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or just one of the homeostatic mechanisms of microorganisms, and as a result, the microorganisms will not multiply but instead remain inactive or even die (Gould, 1988). When homeostasis of bacteria is altered, the bacterial cells react spending their energy in maintaining their physiological status rather than in multiplying. Hurdles included in a food system should affect microbial cells at different levels, for example, the cell membrane, DNA, enzymes, pH, Eh or water activity (aw); this multi-targeted approach is the essence of hurdle technology. While the physiological basis of this synergy remains incompletely understood, the ability to define the limits to growth under combined environmental factors has enormous practical application in maintaining the microbial safety and quality of foods (Ross & Dalgaard, 2004).

In general terms, quantification of causes and effects is a cumbersome and time-consuming interdisciplinary exercise which, in turn, produces very important and interesting information. Cause-effect quantification has been extensively studied in the field of chemicals. In this sense, Tallarida (2000) proposed different methodologies for studying drug synergism. Recently, López-Expósito, Pellegrini, Amigo, and Recio (2008), when studying the synergistic effect between different mild-derived peptides and proteins against *Salmonella choleraesuis* among other microorganisms, described the so-called "synergy index" to evaluate possible interaction, either synergistic or additive, of various substances applied together.

A further step in the quantification of the relation cause-effect or, even better, quantification of the interaction of causes, e.g. antimicrobial substances, to produce a certain effect, e.g. inhibition, would be the quantification of the inhibition as a function of the level of these substances. Mathematical models have been developed to give response to this. Already in 1921, Bigelow (1921) developed a mathematical model to describe the logarithm nature of thermal death. Nevertheless it was not until the 1980s when mathematical models predicting the behavior of microorganisms experienced a great development. A new field emerged in the food microbiology area: predictive microbiology. McKellar and Lu (2004) presented a detailed review of predictive models published so far. Among them, secondary models aim at predicting a feature of a microorganism as a function of environmental factors. Secondary models for assessing the growth of microorganisms have been extensively studied. These models, together with others further developed for *Salmonella* spp., are reviewed by Muñoz-Cuevas, Metris, and Baranyi (2012). However, growth is not always the feature of interest for producers and scientists; in different occasions, it is the possibility of growth the most important issue of concern for stakeholders. Despite hygiene conditions in food industries have greatly improved in the last decades, resistance of *Salmonella* to stress conditions (for example, thermal or osmotic stress), is still possible (Spector & Kenyon, 2012). Also, some food operations like slicing of meats, may allow for bacterial cross-contamination events, especially at retail points and households, which could result in the presence of *Salmonella* cells in initially non-contaminated foods (Carrasco, Morales-Rueda, & García-Gimeno, 2012). From a food safety perspective, food business operators and stakeholders should avoid the growth of undesirable microflora potentially present in foods, in other words, they should minimize the probability of growth of pathogens and spoilage flora. For the evaluation of the probability of growth, mathematical models have been developed as a function of different factors.

Models to predict the likelihood, as a function of intrinsic and extrinsic environmental factors, that growth of a microorganism of concern could occur in a food were first explored in the 1970s. These models were concerned with prediction of the probability of formation of staphylococcal enterotoxin or botulinum toxin within a specified period of time under defined conditions of storage and product composition (Genigeorgis, 1981; Gibson, Bratchell, & Roberts, 1987). Phenomena that have been modeled using this approach include germination of spores, population growth, survival, and toxin formation. These types of models became known as "probability" models. In the late 1990s, it seemed that the only way to manage the risk to consumers from certain pathogens was to ensure that the organism was never present in foods, or to ensure that it was not able to grow in foods that could become contaminated. The latter imperative led to the re-development of "growth/no-growth boundary", or "interface" modeling (Ross & Dalgaard, 2004), more general terms to describe combinations of environmental conditions that just prevent growth. Some authors have discussed the potential of growth boundary models for the design of safe foods (Masana & Baranyi, 2000; McMeekin et al., 2000; Ratkowsky & Ross, 1995; Schaffner & Labuza, 1997).

In this review, we will present the methodology of development of a probability or growth/no-growth (G/NG) model and we will describe and compare the probability models developed for *Salmonella* so far. Also, we will present the computational tools available to assess the boundary growth region of *Salmonella*. Finally, prospective in the field of (G/NG) modeling and application is envisaged.

2. Growth/no growth microbial modeling

2.1. Modeling approaches

During the last 20 years, different approaches to model the interface between "growth" and "no growth" have been proposed.

Deterministic modeling attempts to predict only one position (e.g. $P_{\mathrm{GROWTH}} = 0.1$) for the boundary. One of the first attempts to G/NG modeling, was that by Pitt (1992), who related in an empirical equation the temperature and aw limits for *Aspergillus* spp. growth. In the same line, Masana and Baranyi (2000) built a parabolic model describing the pH/aw interface for *Brochothrix thermosphacta*. In other cases, some researchers (Ratkowsky & Ross, 1995) observed that the form and parameters of Cardinal Parameters Models imply absolute limits to microbial growth, so they suggested approaches to define the G/NG interface based on estimates of cardinal parameters. Augustin and Carlier (2000) and Le Marc et al. (2002) presented approaches based on assumed interactions between factors and are not fitted to G/NG data. Other authors (Battey, Duffy, & Schaffner, 2001; Jones & Walker, 1993; Jones, Walker, Sutherland, Peck, & Little, 1994) have combined growth and death models in which the rate of growth and rate of death under specified conditions are estimated simultaneously; the G/NG interface can be inferred from those combinations of conditions where growth rate and death rate are equal. Masana and Baranyi (2000) discussed the need for approaches that model the transition between conditions leading to high probability of growth and those leading to low probabilities of growth; this transition, as recognized by many workers (Masana & Baranyi, 2000; McKellar & Lu, 2001; Presser, Ross, & Ratkowsky, 1998; Salter, Ratkowsky, Ross, & McMeekin, 2000; Tienungoon, Ratkowsky, McMeekin, & Ross, 2000) is usually abrupt, and often at or beyond the limits of resolution of instruments commonly used to measure such differences. Also, although consistency is observed between replicates in the same experiment, some differences have been detected in microbial responses between experiments, suggesting differences in the physiology of the inoculum and/or its concentration. This observation leads to the idea that "absolute" boundary may not exist, but a growth boundary in dependence of the physiological state of cells and the size of the inoculum (Koutsoumanis, 2008).

The logistic regression approach has been widely adopted for probability and G/NG modeling. In general, probability models are devoted to data which can be measured as "positive" or "negative". For example, if we consider the variable "detection of toxin", only two responses are possible: "detectable" or "not detectable". The same can be said for the variable "detection of growth". In these cases, responses can be coded as 1 (positive response) or 0 (negative