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Toward a next generation of predictive models: A systems biology primer

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ABSTRACT

In the area of predictive microbiology, most models focus on simplicity and general applicability, and can be classified as black box models with the main emphasis on the description of the macroscopic (population level) microbial behavior as a response to the environment. Their validity to describe pure cultures in simple, liquid media under moderate environmental conditions is widely illustrated and accepted. However, experiments have shown that extrapolation of these models outside the range of experimental validation is not allowed as such. In general, the applicability and robustness of existing models under a wider range of conditions and in more realistic situations can definitely be improved by unraveling the underlying mechanisms and incorporating intracellular (microscopic) information. Following a systems biology approach, the link between the intracellular fluxes and the extracellular measurements is established by techniques of metabolic flux analysis. The modeling approach presented in this paper will lead to more accurate predictive models for more complex systems, such as co-cultures and structured environments, based on a top–down systems biology approach. A theoretical case study in predictive microbiology is presented in which the potentials of metabolic network-based models are illustrated. This tutorial paper is directed toward food scientists, who want to get familiar with the mathematical framework used in metabolic flux analysis and adopt these tools in predictive microbiology; the paper is also oriented toward researchers in systems biology, who want to explore the potential and limitations of systems biology tools when applied to challenging (non-steady state) conditions as encountered with bacterial populations in food products.

1. Introduction

In predictive microbiology, an important area of food microbiology, focus is on the mathematical description and prediction of the evolution (growth, survival and inactivation) of pathogenic and spoilage microorganisms in food products. Since the 1980s, there is a remarkable increase of interest in predictive microbiology (McMeekin, Olley, Ratkowsky, & Ross, 2002). Implementation of these predictive models contributes to the improved control of food safety and spoilage, e.g., by quantifying the effect of storage and distribution on microbial proliferation via the HACCP system. Recently, predictive microbiology has been accepted as a tool to define safety of (certain) food products in Europe. Predictive models are also being applied in software packages (e.g., ComBase [UK–US], Sym'Previus [FR] and the Pathogen Modeling Program (PMP) [US]), useful in both academic and industrial environments. In addition, predictive models can be an essential tool for risk control in the optimization of food engineering processes.

First publications in the domain of predictive microbiology mainly focused on the growth and inactivation dynamics of species exposed to (a constant value of) a single environmental condition. This behavior is described by a primary predictive model, which quantifies microbial cell count as a function of time. In the classic approach, bacterial growth is modeled autonomously by a logistic type equation. These models do not include mechanistic knowledge to describe the transitions between the different growth phases. The stationary phase, for example, is described by including the asymptotic value, i.e., the maximum cell number, as a parameter. Primary models developed in the 90s are still widely used but mainly empirical (see, e.g., Baranyi and Roberts (1994), Buchanan, Whiting, and Damert (1997), Zwietering, Jongenburger, Rombouts, and van 't Riet (1990) and Geeraerd, Herremans, and Van Impe (2000)).

In a second step, secondary models are developed which describe the influence of changing environmental conditions on

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primary models, i.e., on its parameters. Most currently used secondary models can be subdivided in four classes: (*i*) square root models (e.g., Ratkowsky, Olley, McMeekin, and Ball (1982), Ratkowsky, Lowry, McMeekin, Stokes, and Chandler (1983) and Ross, Ratkowsky, Mellefont, and McMeekin (2003)), (*ii*) cardinal parameter models (e.g., Rosso, Lobry, and Flandrois (1993), Rosso, Lobry, Bajard, and Flandrois (1995) and Sautour, Dantigny, Divies, and Bensoussan (2001)), (*iii*) neural networks (e.g., Geeraerd, Herremans, Cenens, and Van Impe (1998) and Panagou, Tassou, Saravanos, and Nychas (2007)), and (*iv*) response surface models (e.g., Baranyi, Ross, McMeekin, and Roberts (1996) and Geeraerd et al. (2004)). According to Geeraerd et al. (2004), secondary models fall into two groups. A first group consists of models which include (some) biologically or graphically interpretable parameters and can be extended toward more environmental factors via a multiplicative approach. Furthermore, they are parsimonious and have a high fitting quality. The second group of secondary models, i.e., neural networks and response surface models, do not presume a priori knowledge of the underlying relationship. These models are characterized by a high flexibility.

Most existing primary and secondary models enable an accurate description of microbial dynamics under (non-stressing) dynamic conditions for liquid systems. However, the last decennium, it has been widely recognized that these models fail when applied to real food products and under more realistic, more stressing conditions. The above models consider rather simple liquid systems, mainly controlled by temperature, pH, water activity, acids and preservatives. However, more complex elements, like background flora, microbial competition, stress and stress adaptation, and physicochemical properties of the food structure are rarely taken into account. This is described as the *completeness error*, and is considered as (one of) the largest source(s) of error in predictive microbiology (McMeekin & Ross, 2002).

Based on this generally accepted analysis, a quest for more mechanistically-based predictive models has started (Brul, Mensonides, Hellingwerf, & Teixeira de Mattos, 2008; McMeekin et al., 2008). Examples can be found for different aspects of microbial dynamics. (*i*) Van Impe, Poschet, Geeraerd, and Vereecken (2005) introduced a novel class of macroscopic predictive microbial growth models which do take (micro)biological phenomena, governing the microbial growth process, into account. Their work focused on the transition of the exponential growth phase to the stationary phase, which can be induced through toxic product accumulation and/or substrate exhaustion. This modeling approach was also employed to describe the more complex case study of coculture inhibition of *Listeria innocua* mediated by lactic acid production of *Lactococcus lactis* (Poschet, Vereecken, Geeraerd, Nicolaï, & Van Impe, 2005). (*ii*) In order to unravel the initial lag phase dynamics, focus is on the individual cell dynamics and how they relate to the overall population behavior (see, e.g., McKellar (2001), Baranyi (2002) and Baranyi, George, and Kutalik (2009)). Baranyi and Pin (2001) constructed a mathematical relation between the stochastic individual cell dynamics and a deterministic model describing the population. Individual-based modeling techniques have been developed to gain insights into single cell and population lag phase dynamics (Prats, Giro, Ferrer, Lopez, & Vives-Rego, 2008; Standaert et al., 2007). (*iii*) When microbial populations are exposed to (severe) stress (e.g., heat and acid), sigmoid growth curve patterns are often disturbed and typical primary and secondary models and modeling approaches are no longer valid. These unexpected growth curves can be attributed to microbial population heterogeneity, induced by the environmental conditions. Although population heterogeneity is a widely recognized phenomenon of particular interest for, e.g., food safety and quality, only few attempts have been made to include population heterogeneity in the modeling of microbial kinetics (see, e.g., Nikolaou and Tam (2005) and Van Derlinden, Bernaerts, and Van Impe (2009, 2010)). (*iv*) In structured (solid) foods, microbial growth can strongly depend on the position in the food and the general assumption of homogeneity cannot be accepted, i.e., space must be considered as an independent variable. Dens and Van Impe (2001) presented a model that takes into account the variability of microbial growth with respect to space. The presented model describes two phenomena: local evolution of biomass and transfer of biomass through the medium.

(Unexpected) cell dynamics observed in heterogeneous environments and/or under stress conditions due to heterogeneous populations and stress adaptation phenomena cannot be explained using the macroscopic approach generally applied in predictive microbiology. In the future, predictive microbiology must take the modeling one step further by including more micro- and/or mesoscopic information to understand these cell dynamics. The applicability and reliability of existing models under more realistic conditions will definitively be improved by looking inside the black box and unraveling the underlying mechanisms (Brul & Westerhoff, 2007). Incorporating intracellular information in predictive models, following a top–down systems biology approach, will result in more widely applicable mechanistic models.

The intrinsic complexity of biochemical processes, which consist of extensive reaction networks with numerous metabolites, is not reflected by the simple macroscopic level models, classically used in predictive microbiology. However, while more knowledge about the underlying mechanisms of biochemical processes becomes available, new opportunities arise, for instance by using (microscopic level) metabolic network models to build next generation predictive models. These metabolic networks are a blueprint of the reactions that occur inside the micro-organisms during the biochemical process. Metabolic flux analysis (MFA) is an excellent tool to gain in-depth insight (i.e., at the intracellular level) on the impact of environmental conditions on (the fluxes in) the cell metabolism and growth dynamics. Relevant (extracellular) process conditions and key metabolic reactions/pathways can be identified, which is valuable information in the development of predictive models for more complex and realistic situations. Exploitation of MFA as a technique to develop accurate mathematical models in the field of predictive microbiology is a largely unexplored domain which is presented in this paper.

The methodology presented in this manuscript is at the basis of the work published in Vercammen, Van Derlinden, and Van Impe (2011).

2. Methodology

Fundamental microbial research, in general, is conducted at three levels, i.e., the macroscopic, the mesoscopic and the microscopic level. At the *macroscopic level*, the overall population characteristics and behavior are studied. Macroscopic predictive models describe growth and inactivation dynamics of populations. As said in the introduction, macroscopic level models are able to accurately predict population dynamics under non-stressing conditions in liquid food model systems. For process control, monitoring and optimization purposes, macroscopic models are preferred as they have a rather simple structure, i.e., a limited number of model components and parameters. The *mesoscopic level* studies small populations, part of the population like subpopulations or colonies in structured environments. Due to environmental or population heterogeneity, differences in the microbial response are observed and all cells – or their dynamics – can no longer be assumed as identical. Examples of more mesoscopic models can be found in McKellar (1997) and Skandamis, Davies, McClure, Koutsoumanis,