BASELINE

SELECTION AND IMPROVING OF FIT-FOR-PURPOSE SAMPLING PROCEDURES FOR SPECIFIC FOODS AND RISKS

COOPERATION

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PROJECT DELIVERABLE

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Workpackage Collaborators

3 CNTA:
Carlos J.: González: cgnavarro@cnta.es
María José Sáiz: mjsaiz@cnta.es

4DKFZ:
Lutz Edler: edler@dkfz-heidelberg.de
Maria Muench: m.muench@dkfz-heidelberg.de
Simone Braun: simone.braun@dkfz-heidelberg.de

5UBO:
Alain Claude Roudot: alain-claude.roudot@univ.brest.fr
alain-claude.roudot@orange.fr
Queffelec Nathalie: nathalie.queffelec@univ-brest.fr
Dominique Parent-Massin: dominique.parent-massin@univ-brest.fr
François-Gilles Carpentier: francois-gilles.carpentier@univ-brest.fr

6HFSO
Árpád Ambrus: arpad.ambrus@mebih.gov.hu
Lazlo Meszaros meszaros.laszlo@mebih.gov.hu

11 UCO
Gonzalo Zurera Cosano: bt1zucog@uco.es
Rosa María Carcía-Gimeno: bt1gagir@uco.es
Antonio Valero-Diaz: bt2vadia@uco.es
Fernando Pérez-Rodríguez: b42oerif@uco.es
Elena Carrasco-Jiménez: bt2cajie@uco.es

14 AFSSA
Marianne Chemaly: m.chemaly@ploufragan.afssa.fr

15 TEAGASC
Geraldine Duffy: Geraldine.Duffy@teagasc.ie
Lucy Rivas: lucy.rivas@teagasc.ie

Writing Team:

Dr. Lutz Edler edler@dkfz-heidelberg.de
Maria Muench m.muench@dkfz-heidelberg.de
Dr. Simone Braun simone.braun@dkfz-heidelberg.de
DKFZ
German Cancer Research Centre
Department of Biostatistics (CO60)
Im Neuenheimer Feld 280
69120 Heidelberg

Project Coordinator:

Prof. Gerardo Manfreda
Alma Mater Studiorum – Università di Bologna
Tel: +39 051 20 9 7854
E-mail: gerardo.manfreda@unibo.it

Project website address: http://www.baselineeurope.eu/

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List of Abbreviations

CFU	Concentration of microorganisms
MOs	Microorganisms
MRA	Microbiological Risk Assessment
QnMRA	Quantitative Microbiological Risk Assessment

Executive Summary

BASELINE Delivery 6.1 is the first report on the achievements made in Work package 6 on Model Development. D6.1 aims to give an overview of the project status after the first 12 months on this topic. It includes the progress of the work towards the objectives, a description of the work performed from month 1 to month 12 of the project, the main results achieved and the next steps.

From month 1 to month 12 the partners CNTA, DKFZ, UCO, AFSSA, and TEAGASC of subtask 6.1 in WP 6 focused their attention on the review of models and methods on the development of microbial populations in food as available in scientific literature and as implemented in three major data storage and analysis systems.
This overview was performed as planned in the project to function as a starting platform for the modelling and statistical evaluation of data recruited during the course of BASELINE, in particular in the WP1-5. It also serves as basis for the development of new fit-for-purpose models in BASELINE WP 6.
Predictive mathematical models of the microbial evolution in culture media and/or food matrices were described comprising
Empirical/curve fitting models, in particular logistic type models;
Mechanistic models, making use of biological characteristics of the growth curve such as the

- Verhulst model,
- Gompertz model,
- Baranyi-Roberts model;

Self-limiting models determined by an exhaustion of nutrients of the micro-organisms (MOs) and an accumulation of toxic growth inhibiting products comprising

- physiological states,
- inhibition products,
- substrate,
- lag-phase followed by exponential growth and stationary (survival) phase before entering death phase.

Besides the Gompertz model, which is still used as a classical model for sigmoidal shaped growth data, we conclude that the Baranyi-Roberts model as well as the simple Three Phase Model may be used as a first choice before one tries out more complicated models to available data sets. For studying intrinsic factors of microbial growth, e.g., temperature T, water activity or the ph value, the secondary model approach is the approach of choice, both, for quantitative outcomes where several models have been developed differing in the way how the parameters are combined mathematically, and for qualitative outcomes. In the latter case, secondary quantal models, i.e., models where the quantitative endpoint is replaced by a qualitative endpoint (e.g., growth/no growth) can play a larger role than it has been anticipated in the past, in particular, when microbial growth is restricted to finite limits of factors. The provide means to model growth (quasi-) thresholded by the level of one or more than one factor. Secondary quantal predictive models are applicable when the probability of growth is used for risk management and the presence of microbes to be controlled. The practical applicability of this approach has been demonstrated by BASELINE partners (see Valero et al., 2009).

Microbial cell survival models aid to understand and model the survival of pathogens in foods, including survival during the processing of food. Such, they constitute one of the earliest forms of

predictive microbiology for prevention of food pathogen caused disease or death. A descriptive index is the D-value (decimal reduction time) describing the time required for a reduction of the population size by one order of magnitude, i.e., a reduction on the log scale by one unit. Directly related to the D-value is the z-value. Even though D- and z-values have been used widely their values is limited in practice since not such many survival curves observed empirically obey the assumed underlying first-order kinetics. Therefore non-linear survival models need to be applied, e.g., when the curve exhibits a so-called “shoulder”, a lag time or when the curve is truly of bi-phasic nature. No agreed indices exits for that case and the paradigm of on “indexed-fits-all” must be questioned, if not abandoned.

In order to include more complex type of information, one needs the statistical distribution of the variables resulting in stochastic models that can incorporate both variability and uncertainty. Stochastic modelling eliminates the need to make kinetic assumptions that are usually hard and sometimes impossible to confirm. However, stochastic models come also with higher complexity, need more data and may not be feasible in all practical situations. Nevertheless, their value is in developing a conceptual understanding of processes behind the growth and survival of microbial populations.

Predictive models on growth, survival and death available in ComBase, USDA Pathogen Modelling Program 7.0, and Seafood Spoilage and Safety Predictor (SSSP) are described with a short introduction to their usage.

The models presented in D6.1 allow the characterisation of survival and growth of human pathogens in process lines to be investigated in BASELINE WP 1-5 at predetermined checkpoints for the examination of presence or absence as well as for the quantitative amount (e.g., count per food unit) or concentration. Available predictive modelling software on growth, survival and death is useful for BASELINE, but needs fit-for-data adaptation and guidance as well as training.

All partners achieved their results for the period without major problems or delays.

Introduction

The objectives of WP 6 consisting of the

- Review of existing and definition of new mathematical models in predictive microbiology;
- Investigation of correlations between food risk factors and traceable environmental parameters and contamination indicators;
- Consolidation of existing and new models for microbial growth as a function of intrinsic environmental factors and extrinsic parameters,

aim at supporting in the general objective of BASELINE to develop predictive mathematical models for biological risks and investigate and model sources and pathways of chemical contaminants to improve sampling schemes.
Model development in the framework of Microbiological risk assessment (MRA) occurs at various levels: the level of risk assessment and risk management, the level of exposure assessment and sampling as it will be developed in the course of the next milestones of WP 6, and the level of the population dynamics of the agents as it will be described in the following four chapters of delivery 6.1.

D 6.1 provides basic mathematical and statistical tools to investigate the behaviour and development of the microbiological agents (the microbes) at a restricted period of time of their prevalence in the food chain,e.g., at some risk assessment checkpoints where one of several sampling schemes within one food chain- chosen for the specific product-risk combinations identified for BASELINE- will be implemented. D 6.1 is therefore a prerequisite of the development of sampling schemes covering growth and death/survival of microbial populations, which may be initiated by one single microbe in a food batch or food lot form which samples are taken. Note, that this modelling applies solely for the microbial biohazardous contamination studied in BASELINE. For chemical and toxins investigated under the framework of chemical risks in BASELINE no dynamic is assumed and their concentration or amount in a food batch or food lot is assumed to be unknown, but fixed over the respective food chain chosen for the specific product-risk combinations as identified for BASELINE.

Predictive mathematical models of the microbial evolution in culture media and/or food matrices are used for predicting

N(t) = number / microbial concentration at time t in a food unit (e.g. 1g or 25g).

That comprises

- Traditional purely empirical/curve fitting models, in particular logistic type models
- Mechanistic models, which make use of biological characteristics of the growth curve, which are the
  - Verhulst model,
  - Gompertz model,
  - Baranyi-Roberts model;

The generic class of self-limiting models determined by an exhaustion of nutrients of the microorganisms (MOs) and an accumulation of toxic growth inhibiting products comprising

- physiological states,
- inhibition products and substrates,
- lag-phase followed by exponential growth and stationary (survival) phase before entering death phase.

In predictive microbiology, modelling is organized in the three stages of

- Primary models for the evolution and the variation of microbial populations versus time under particular environmental and cultural conditions;
- Secondary models for the influence of environmental factors (temperature, pH) on the parameters of the primary models, where we apply in a first step the Square Root Model and the Gamma Model;
- Tertiary models for the prediction of microbial growth from user defined scenarios.

Microbiological risk assessment can be defined as the structured, systematic approach to integrate and evaluate information from many diverse sources along the food chain for assessing and characterising the risk posed by a hazard of microbiological origin in a food within a certain population. This is achieved by estimating (i) the magnitude of a potential adverse outcome and (ii) the probability that the adverse outcome will occur (see e.g., ILSI 2009, USING MICROBIOLOGICAL RISK ASSESSMENT (MRA) IN FOOD SAFETY MANAGEMENT). Envisaging MRA as a science-based process, it consists therefore of the generic four risk assessment steps of hazard identification, hazard characterisation, exposure assessment and risk

characterisation where mathematical and statistical modelling is a constituent part focussing on hazard characterization and exposure assessment.
In MRA, modelling has been embedded in a Quantitative MRA (QnMRA; to distinguish from qualitative MRA) approach aiming at the provision information that can (i) assist targeting potential control and risk reduction measures, (ii) form a basis for cost-benefit analysis of the relative merits of competing risk mitigation strategies and (iii) help to focus the direction of research programmes. Specifically, modelling in the framework of QnMRA uses mathematical and statistical techniques to infer the probability of adverse outcomes by appropriately combining a formal representation of the risk-generating system with the rules of inferring probability, that risk generating system has two basic elements (a) the food production system and the food chain (from farm–to-fork) and the dynamic of the development of the microorganism along that system/chain.
Note, model development in the framework of Microbiological risk assessment (MRA) occurs at various levels. Three major levels may be distinguished: the level of risk assessment and risk management as described above, at the level of exposure assessment and sampling as it will be developed in the course of the next milestones of WP 6, and the level of the population dynamics of the agents as it will be described in the following four chapters of delivery 6.1.