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Microbial challenge tests and predictive modelling software for evaluating and improving food safety – A case study with *Listeria monocytogenes* and ready-to-eat foods

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Introduction

The pathogenic bacterium *Listeria monocytogenes* has been identified as a major risk associated with consumption of ready-to-eat (RTE) foods. *L. monocytogenes* has frequently been isolated from different types of RTE foods due to its ubiquitous nature and ability to persist and proliferate in the food processing environment. In a recent European Union (EU) baseline survey, the prevalence of *L. monocytogenes* in seafood and meat products was established as 10.4% and 2.1%, respectively (EFSA, 2013). Furthermore, *L. monocytogenes* is highly tolerant towards salt (> 10%) and is able to grow at chill temperatures, typically being used for storage of RTE foods. According to the EU regulation on RTE foods (EC, 2005), it is the responsibility of the food processors to document that the critical limit of 100 *L. monocytogenes*/g is not exceeded throughout the shelf-life of their products, either by eliminating contamination or limiting/preventing the potential growth of the pathogen. In regulation EC 2073/2005, challenge tests and predictive modelling are specified as two of the main approaches for demonstrating compliance with the legal requirements for *L. monocytogenes*.

Challenge tests

To assess the growth potential of *L. monocytogenes* in RTE foods, challenge tests are often performed. The EU Community Reference Laboratory (CRL) has developed a technical guidance document that describes the procedure for establishing the growth potential of *L. monocytogenes* in artificially contaminated (i.e. inoculated) samples of RTE foods stored under foreseeable conditions (EU CRL, 2008, Beaufort, 2011). The growth potential is estimated as the difference between the concentration of *L. monocytogenes* ($\log_{10}$, cfu/g) at the beginning and at the end of the challenge test. The EU CRL procedure includes the following points as a minimum to be considered: product characteristics, shelf-life of the product, number of batches, choice of the strains, preparation of the inoculum, preparation and inoculation of the test unit, storage conditions and microbiological analysis. RTE foods subjected to challenge testing should be characterized carefully with respect to for example pH, salt, preservatives, packaging conditions and the background microbiota (e.g. lactic acid bacteria) to make sure that the samples are representative of the variability typically associated with the examined type of product. For the same reason, at least three batches of the same product should be examined to account for product variability. Vermeulen et al. (2011) addressed this point as particularly important for challenge testing in order not to underestimate the growth potential of *L. monocytogenes*. To include the importance of strain variability, at least three strains of *L. monocytogenes* should be used for the inoculum including one reference strain. *L. monocytogenes* should be subcultured at a temperature close to the storage temperature of the product in order to adapt the strains to the experimental conditions.

Predictive modelling software

Today, several predictive modelling software packages are available and they include a wide range of models for both spoilage and pathogenic microorganisms. Incorporation of predictive microbiology models in software packages is important in order to facilitate their use by the food industry, regulatory authorities and other interested parties. At DTU Food, we have developed the Seafood Spoilage and Safety Predictor (SSSP) software which was released for the first time in 1999. A new version is scheduled for 2014 and on that occasion the software will be renamed the Food Spoilage and Safety Predictor (FSSP). At the moment, more than 6000 persons from 113 countries use our free software (http://sssp.dtuaqua.dk/), which is available in 18 different languages (e.g. Italian) and includes (i) models to predict the growth of specific spoilage bacteria in fresh fish, (ii) models to predict histamine formation in marine fin-fish and (iii) models to predict growth and the growth boundary of *L. monocytogenes* and lactic acid bacteria in seafood and meat products. Our predictive model for *L. monocytogenes* includes the effect of 12 environmental parameters (temperature, pH, salt, CO_2, smoke components, nitrite, acetic acid, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid) as well as their interactive effects (Mejlholm and Dalgaard, 2009). This model has been successfully validated on data from more than 1000 experiments with seafood and meat products, both with respect to the growth rate as well as the growth boundary of *L. monocytogenes* (Mejlholm et al., 2010). Recently, the SSSP software was approved by the Danish Veterinary and Food Administration as a means to predict growth of *L. monocytogenes* and to document compliance of RTE foods with regulation EC 2073/2005. Similarly to our model for *L. monocytogenes* we have also developed an extensive model for lactic acid bacteria to be able to model the importance of microbial interaction (i.e. the Jameson effect) between the two types of microorganisms. Without the Jameson effect, the maximum cell concentration of *L. monocytogenes* in e.g. cold-smoked salmon has been shown to be overestimated by as much as 5-6 $\log_{10}$ units (i.e. 100.000-1.000.000 times too high). This new combined model for *L. monocytogenes* and lactic acid bacteria will be included in the FSSP software to be released in 2014. To be able to use predictive microbiology models in an appropriate way, knowledge about the product characteristics affecting growth of e.g. *L. monocytogenes* is a prerequisite. Without a careful product characterization, predictions are likely to be misleading rather than indicative.

Discussion

Development and validation of predictive microbiology models is a time demanding and labour intensive job. As an example, our model for *L. monocytogenes* was developed and validated continuously over a period of more than 5 years by consecutively adding the effect of new environmental parameters and by expanding the range of products for which the model is valid. However, when developed and successfully validated, predictive microbiology models possess a range of advantages as compared to challenge tests. Challenge tests are important in order to generate data for model development and validation, but, for evaluation of the growth potential of microorganisms as well as for product development the use of predictive microbiology models seem more promising for several reasons. Firstly, the use of predictive microbiology models is both faster and cheaper than challenge tests. Predictions can be obtained within days (including time to carefully determine product characteristics) and it is relatively easy to change one or more of the

environmental parameters in order to obtain combinations of product characteristics and storage conditions that prevent or limit the growth of *L. monocytogenes* to an acceptable level. In contrast, the requirements for challenge tests to be carried out at foreseeable storage conditions of RTE foods, means that this type of experiments typically will take 4 to 6 weeks and sometimes even longer. Secondly, challenge tests are only valid for the examined product, so each time the product formulation or the process is changed new experiments has to be carried out. In this context, predictive microbiology models have a great advantage as the impact of substituting e.g. one set of preserving parameters with another can be predicted relatively fast. If challenge tests for some reasons are a requirement, predictive microbiology models can be used constructively to reduce the number of experiments to an absolute minimum by providing suggestions for suitable product formulations. Finally, challenge tests provide only information about the growth potential of e.g. *L. monocytogenes*. For example, if no growth of *L. monocytogenes* is observed, no additional information is given on how much (or how little) the product characteristics and/or storage conditions can be changed without resulting in growth. Our predictive model for *L. monocytogenes* provides a quantitative measure on the distance between the product (i.e. combination of product characteristics and storage conditions) and the predicted growth boundary of the pathogen. Thus, in addition to prediction of no growth, a quantitative measure is also given reflecting the safety of the product and *vice versa* if growth is predicted.

Conclusion

Successfully validated predictive microbiology models are powerful tools for evaluating and improving food safety, particularly when models are included in user-friendly application software. Today the best predictive microbiology models can, in many situations, replace the use of challenge testing. Furthermore, these models provide information on the distance to the growth boundary. This information is important for food safety management and cannot conveniently be obtained by challenge testing.

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