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Microbial variability in growth and heat resistance of a pathogen and a spoiler: All variabilities are equal but some are more equal than others

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ABSTRACT

Quantitative microbiology is used in risk assessment studies, microbial shelf life studies, product development, and experimental design. Realistic prediction is, however, complicated by different sources of variability. The final concentration of microorganisms at the moment of consumption is affected by different sources of variability: variability in the storage times and temperatures, variability in product characteristics, variability in process characteristics, variability in the initial contamination of the raw materials, and last but not least, microbiological variability.

This article compares different sources of microbiological variability in growth and inactivation kinetics of a pathogen and a spoiler, namely experimental variability, reproduction variability (within strain variability), strain variability (between strain variability) and variability between individual cells within a population (population heterogeneity). Comparison of the different sources of microbiological variability also allows to prioritize their importance. In addition, the microbiological variability is compared to other variability factors encountered in a model food chain to evaluate the impact of different variability factors on the variability in microbial levels encountered in the final product.

1. Introduction

About one third of agricultural production is lost or wasted annually (FAO, 2011). These losses are caused among others by poor post-harvest technologies and conditions that cause spoilage and damage of food products, food safety issues, inadequate market systems, and the appearance of high quality standards. To reduce the loss caused by food safety and quality issues, the implementation of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), followed by the adoption of Good Hygienic Practices (GHP) are important strategies. The Hazard Analysis Critical Control Point (HACCP) system is a food safety management system that is mandatory in the European Union for food producers to systematically control food production processes (EC, 1993), and is supplementary to the good practices. Implementation of the good practices and HACCP is beneficial not only for food safety, but also for food waste reduction. When the growth and/or reduction of pathogens and spoilage microorganisms in food is not sufficiently controlled it can lead to undesired food losses and/or foodborne outbreaks and burden the sustainability of the food supply. Combinations of various mild, though growth limiting, factors can be applied to control the growth of pathogen and spoilage organisms in food, and therefore extending the shelf life of food products. Since zero food safety risk or food waste does not exist, minimizing is the ultimate goal to reduce the disease burden and food waste caused by microorganisms. Quantitative microbiology is used to evaluate the effectiveness of certain processes and product designs in controlling the growth and reduction of microorganisms. This quantitative modelling approach assists formulation of HACCP plans by identifying hazards and critical control points and in specifying limits and corrective actions (McMeekin et al., 2002). The concept of predictive microbiology, according to McMeekin et al. (2002), is that a detailed knowledge of microbial responses to intrinsic food properties and environmental conditions enables objective evaluation of the effect of processing, distribution and storage operations on the microbiological safety and quality of foods. Therefore, predictive modelling is applicable for many activities along food production chains, not only in the area of HACCP, but also in risk assessment studies, microbial shelf life studies, product research and development, and experimental design (McMeekin et al., 2007). Realistic prediction is, however, complicated by different sources of variability (Zwietering, 2015). The final concentration of microorganisms at the moment of consumption depends on the variability in the storage times and temperatures, variability in product characteristics, variability in process characteristics and variability in the initial contamination of the raw materials. Also the variability introduced by microorganisms is of relevance. Fitness and/or robustness of strains depend on the physiological state, cell history, the genetic and phenotypic variability within a population (e.g. population heterogeneity), and

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diversity between strains belonging to the same species (e.g. strain variability) (see e.g. Den Besten et al., 2010; Ryall et al., 2012; Van Boeijen et al., 2010, and references therein). All these variability factors have influence on the microbial kinetics along the food productions chain and challenge the precise prediction of the true behaviour of the microorganisms. Quantitative information on the variability factors is required to prioritize and rank their importance and to evaluate where and whether these can be controlled. Over the years, various studies have investigated growth and inactivation kinetics resulting in a wealth of information on growth rate and inactivation parameters (e.g. *D*- and *z*-values), and databases like Combase (www.combase.cc) give easy access to numerous studies. Comparison of these data in a structured way can be done in a meta-analysis where the findings of many individual studies are quantitatively integrated to provide global estimates of kinetic parameters and to quantify their variabilities (Den Besten and Zwietering, 2012). This structured approach allows to identify dominant influencing factors on parameters (Van Asselt and Zwietering, 2006). Recent work in our laboratory aimed to quantify different sources of microbiological variability in growth and inactivation kinetics of a pathogen (Aryani et al., 2015a, 2015b; Metselaar et al., submitted for publication) and a spoiler Aryani et al., submitted for publication), namely experimental variability, reproduction variability (within strain variability), strain variability (between strain variability) and variability between individual cells within a population (population heterogeneity). This allows for comparison of these different sources of variability and to prioritize their importance. Therefore in this study we will compare different sources of microbiological variability in growth and inactivation kinetics of *Listeria monocytogenes* as a model pathogen and for *Lactobacillus plantarum* as a model spoilage organism. In addition, the microbiological variability will be compared to other variability factors encountered in a model food chain, namely, variability in storage and heating temperature and variability in storage and heating time, to evaluate the impact of different variability factors in the variability encountered in the microbial levels in the final product.

2. Materials and methods

2.1. Growth kinetics

Aryani et al. (2015a) quantified the specific growth rate μ_{max} as function of pH, water activity (a_w), temperature and undissociated lactic acid concentration ([*HLa*]). Twenty *L. monocytogenes* strains were used including strains with a long history as lab strain and also strains isolated from various origins that did not have a long laboratory history. The μ_{max} was determined in brain heart infusion (BHI) broth using the two-fold dilution method (Biesta-Peters et al., 2010). All growth experiments were performed in duplicate using the same culture to quantify the experimental variability (degrees of freedom (DF) is 60 for each condition). The growth experiments were performed three times at different days using freshly prepared cultures to quantify the reproduction variability, i.e. within strain variability (DF is 40 for each condition). Strain variability was defined as the difference in μ_{max} of the twenty *L. monocytogenes* strains, i.e. within species variability (DF is 19 for each condition). Secondary growth models were fitted to the μ_{max} data to determine the cardinal growth parameters for each strain and their 95% confidence intervals.

The secondary pH growth model reparameterized by Aryani et al. (2015a) and having solely interpretable parameters, was used to fit the μ_{max} data as function of pH.

$$\mu_{max} = \mu_{opt}\left(1-2^{\frac{(pH-pH_{min})}{(pH_{min}-pH_{1/2})}}\right) \quad (1)$$

where pH_{min} is the pH growth limit, $pH_{½}$ is the pH where μ_{max} is half of the optimal specific growth rate μ_{opt}.

The secondary temperature growth model (Ratkowsky et al., 1982) was used to fit the data as function of temperature.

$$\sqrt{\mu_{max}} = \alpha_T(T-T_{min}) \quad (2)$$

where T_{min} is the temperature growth limit, and α_T is the slope parameter.

The Luong model (Luong, 1985) was used to describe the effect of a_w and [*HLa*].

$$\mu_{max} = \mu_{opt}\left(1-\left(\frac{1-a_w}{1-a_{w_{min}}}\right)^{\alpha_{a_w}}\right) \quad (3)$$

where $a_{w_{min}}$ is the water activity growth limit, and α_{aw} is the shape parameter.

$$\mu_{max} = \mu_{opt}\left(1-\left(\frac{[HLa]}{[HLa]_{max}}\right)^{\alpha_{[HLa]}}\right) \quad (4)$$

where $[HLa]_{max}$ is the growth limit for the concentration of undissociated lactic acid, and $\alpha_{[HLa]}$ is the shape parameter.

Also for *L. plantarum* twenty strains were collected from different origins which were cultured in De Man, Rogosa and Sharpe (MRS) broth buffered with 100 mM sodium phosphate buffer to determine the μ_{max} as function of pH, a_w, temperature and [*HLa*] and to determine the cardinal growth parameters and their 95% confidence intervals of each strain.

Previous work on population heterogeneity of *L. monocytogenes* LO28 revealed the presence of stable resistant variants within the strain population (Metselaar et al., 2013). These variants were genotypically and phenotypically characterized in detail (Metselaar et al., 2015a, 2015b). Eight variants that represented different clusters of variants based on genotypic and phenotypic characteristics were selected, and the μ_{max} of these variants as function of pH, a_w and temperature was determined, as well as the cardinal growth parameters (Metselaar et al., submitted for publication) following a similar approach as Aryani et al. (2015a).

2.2. Inactivation kinetics

The twenty *L. monocytogenes* strains and the twenty *L. plantarum* strains were also heat-treated to determine the *D*-values at different temperatures (Aryani et al., 2015b, Aryani et al., submitted for publication). All inactivation experiments were performed in duplicate

Table 1
Scenarios for the process chain.

Process step	Scenario 1[a]	Scenario 2[a]	Scenario 3[a]	Scenario 4[a]
1. storage at farm	Temp: 0–4 °C Time: 2 h–72 h	Temp: 0–4 °C Time: 2 h–72 h	Temp: 0–4 °C Time: 2 h–72 h	Temp: 0–4 °C Time: 2 h–72 h
2. heating at factory	Temp: 65 °C (0.1 °C) Time: 15 s (0.1 s)	Temp: 70 °C (0.1 °C) Time: 15 s (0.1 s)	Temp: 70 °C (2 °C) Time: 15 s (0.1 s)	Temp: 70 °C (0.1 °C) Time: 15 s (0.1 s)
3. domestic storage	Temp: 6.3 °C (2.7 °C) Time: 0 h–120 h	Temp: 6.3 °C (2.7 °C) Time: 0 h–120 h	Temp: 6.3 °C (2.7 °C) Time: 0 h–120 h	Temp: 8.4 °C (3.0 °C) Time: 0 h–120 h

[a] Standard deviation σ between brackets.