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Modeling the behavior of *Listeria monocytogenes* during enrichment in half Fraser broth; impact of pooling and the duration of enrichment on the detection of *L. monocytogenes* in food

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ABSTRACT

A stochastic model describing the growth of *Listeria monocytogenes* during enrichment in half Fraser was developed for the purpose of estimating the effects of modifications to the first enrichment step of the EN ISO 11290-1 detection method. Information pertaining to the variability of growth rates, physiological state of the cell, and the behavior of individual cells contaminating the food were obtained from previously published studies. We used this model to investigate the impact of pooling enrichment broths (wet pooling) on the performance of the standard method. For validation of the model, the numbers of *L. monocytogenes* occurring in 88 naturally contaminated foods following pre-enrichment were compared to model-simulated microbial counts. The model was then used to perform simulations representative of the natural contamination observed for smoked salmon in the European baseline survey of 2010–2011. The model-estimated *L. monocytogenes* levels following individual enrichment or following the pooling of five broths where only one would be contaminated were compared. The model indicated a 10% loss of method sensitivity resulting from wet pooling. The model also predicted a 5% decrease in the sensitivity of the method when the duration of the enrichment was reduced from 24 to 22 h.

1. Introduction

Listeria monocytogenes is a Gram-positive bacterium responsible for listeriosis, a severe foodborne illness which can result in gastroenteritis, meningitis, septicemia, spontaneous abortion, and perinatal infections (Kathariou, 2002). Despite its low incidence, listeriosis is associated with a high rate of mortality, particularly in the elderly and immuno-compromised individuals (Kathariou, 2002). Worldwide estimates for 2010 indicate that this pathogen was responsible for 23150 illnesses, 5463 deaths, and the loss of 172 823 disability-adjusted life-years (de Noordhout et al., 2014). Not only does this pathogen have a significant impact on the costs of public health, but its detection in a food product has economic consequences for the food manufacturer including those arising from the recall and withdrawal of contaminated products, lost sales for incriminated food products, and, in some jurisdictions, costs arising from litigation and out of court settlements (Buzby et al., 2001).

L. monocytogenes is readily controlled using standard processing procedures such as pasteurization. Problems with this organism are predominantly associated with ready-to-eat (RTE) foods where pH may be permissive for growth and where there may be insufficient levels of salt or preservatives to restrict its growth (Gandhi and Chikindas, 2007). *Listeriae* possess the ability to grow over a wide pH range (5.0–9.6), but can also survive in foods with pH levels outside of this range particularly if they are first acid adapted (Gahan et al., 1996; O'Driscoll et al., 1996). Outbreaks of listeriosis have been associated with processed meat, seafood, dairy products and produce (Rocourt et al., 2000; Lundén et al., 2004; Anonymous, 2009; Stephan et al., 2015). Post processing contamination of food remains a critical concern, as *L. monocytogenes* is able to grow at

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refrigeration temperatures. Various jurisdictions have imposed regulatory frameworks with the purpose to control and monitor *L. monocytogenes* contamination in high risk RTE foods that are able to support the growth of *L. monocytogenes*. Both the European Union and Canada require regular testing of the processing environment and product, and high risk foods able to support the growth of *L. monocytogenes* must comply with the regulation requiring the absence of *L. monocytogenes* in food samples (Anonymous, 2005; www.inspection.gc.ca). Manufacturers are faced with two problems in complying with these regulations. Firstly, since foods contaminated with *L. monocytogenes* typically contain low levels of cells and contamination can be sporadic during the production run, it necessitates the testing of multiple samples from single batches. Secondly, comprehensive environmental and product testing for *L. monocytogenes* is expensive, and represents a significant additional cost particularly for small enterprises.

One approach to reduce the economic burden associated with comprehensive testing for *L. monocytogenes* is pooling multiple samples following the pre-enrichment step. Pooling, also known as "wet pooling", is the combining of multiple pre-enriched samples into a single sample which is then used in a rapid detection method or for the completion of the standard method. The advantage of wet pooling is that it can significantly reduce the costs per sample in a sampling program. In a study on artificially contaminated smoked salmon, this approach was found to be effective in pools containing six pre-enriched samples where only one sample was contaminated (Vitas et al., 2014).

The international standard method (EN ISO 11290-1) for the detection and enumeration of *Listeria monocytogenes* (Anonymous, 1996, 2004) consists of a sequential double enrichment in half Fraser and Fraser selective broths. The initial incubation in half Fraser is carried out for 24 h at 30 °C, followed by a second enrichment for 48 h in Fraser broth at 37 °C. Over the last decade, a variety of factors have been shown to influence the success of isolation of *L. monocytogenes* using this method (Cornu et al., 2002; Gnanou Besse et al., 2010; Lemaître et al., 2015). In contrast, little information is currently available on the impact of sample preparation and subsequent handling (Vitas et al., 2014). The current standard EN ISO 6887-1 for the preparation of test samples for microbiological analyses (Anonymous, 2016) is under revision and will now include a general approach and experimental design for sample pooling (Annex D verification protocol for pooling samples). Specifically this will allow either pooling of test sample portions or enrichment broths, in order to reduce analytical costs. However, it must first be verified that either practice has no impact on method performance. Unfortunately, this type of study is not only quite laborious but it must also be conducted for each pathogen within each specific food matrix.

Here, we explore the feasibility of using modeling to predict the results of modifications to standard enrichment protocols without the need for full validation testing. We have modeled the impact of wet pooling on the performance of the standard method for the detection of *L. monocytogenes*. Specifically, a model describing the growth of *L. monocytogenes* during pre-enrichment in half Fraser was developed and validated, using data from previous studies conducted with artificially and naturally contaminated foods. A recent revision of the EN ISO 11290-1 method recommended a minimum incubation of 24 h in half Fraser broth, to ensure growth of stressed or low contamination levels of *listeriae* in the samples. Currently, the standard method recommends the incubation time in half Fraser broth should be 24 ± 2 h. As a consequence of this, we have also used this model to examine the impact of enrichment duration on the detection of *L. monocytogenes* in food.

2. Materials and methods

2.1. Naturally contaminated food samples

A collection of 88 foods samples naturally contaminated with *L. mononcyogenes* ($n = 88$) and analyzed between 2004 and 2013 (Gnanou Besse et al., 2005; Gnanou Besse et al., 2016) was used in this study. Samples were collected at both production and distribution levels from multiple food processors and retail stores throughout Europe. The following food categories were represented: dairy ($n = 18$), seafood ($n = 24$), pastries and RTE meals ($n = 9$), vegetable produce ($n = 7$), and a diverse range of meat products ($n = 30$). Pre-enrichment culturing was performed according to the ISO 11290-1 reference method (Anonymous, 1996, 2004). Briefly, samples (25 g) were homogenized in 225 mL of half Fraser broth (AES Laboratories, Combourg, France), then incubated for 24 h at 30 °C. The behavior of the *L. monocytogenes* population over the duration of pre-enrichment was determined with enumerations carried out according to the ISO 11290-2 reference method (Anonymous, 1998) at time 0 and following 24 h incubation. The selective medium utilized for plating was ALOA agar (AES Laboratories). In order to increase the enumeration sensitivity, a total of 1 mL of the initial food suspension or enrichment broth was spread onto three plates. *L. monocytogenes* colonies picks were confirmed according to the ISO 11290-2 method and by the API *Listeria* identification test (bioMérieux, Marcy l'Etoile, France) or using ALOA Confirmation agar (AES Laboratories).

2.2. Stochastic modeling of the behavior of L. monocytogenes during the enrichment step

A stochastic model was used to estimate *L. monocytogenes* levels following pre-enrichment of naturally contaminated food samples. In this model, the fate of each cell present in the half Fraser enrichment broth was evaluated during the 24 h incubation at 30 °C. Each contaminating cell is assumed able to grow with a given probability and when the cell is able to multiply, the change in the bacterial population coming from the cell i is described by the following logistic equation incorporating delayed growth (Rosso et al., 1996; Augustin et al., 1999):

$$x_i(t) = \begin{cases} 1 & , t \le lag_i \\ \dfrac{x_{\max}}{1 + (x_{\max} - 1)\cdot \exp[-\mu_{\max}\cdot(t - lag_i)]} & , t > lag_i \end{cases} \quad (1)$$

where $x_i(t)$ is the bacterial density (CFU) at the time t (h), $x_{\max}$ is the maximum bacterial concentration, lag_i is the lag time of the cell i (h), and $\mu_{\max}$ is the maximum specific growth rate (h^{-1}). The total bacterial population present in the enrichment broth at time t is obtained by summing bacterial populations originating from each cell i.

The *L. monocytogenes* concentration in half Fraser broth at the end of the enrichment step depends on the initial viable cell numbers, the initial physiological state of the cells contaminating the food, the growth properties of the strain and the nutritional properties of the enrichment broth. The following sources of variability were thus included in the model to assess the variability of the fate of *L. monocytogenes* populations naturally contaminating food: initial contamination levels and distribution in food samples, state of the contaminating cells, growth rate, and maximum bacterial population.