Growth, inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii* — development and evaluation of predictive models

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ABSTRACT

Mathematical models for growth, heat inactivation and histamine formation by *Morganella psychrotolerans* and *Morganella morganii* were studied to evaluate the importance of these bacteria in seafood. Curves for growth and histamine formation by *M. psychrotolerans* in broth and seafood were generated at constant and changing storage temperatures ($n = 12$). Observed and predicted times to formation of 100, 500 and 2000 ppm histamine were used for evaluation of an existing *M. psychrotolerans* histamine formation model [Emborg, J., Dalgaard, P., 2008-this issue-this issue. Modelling and predicting the growth and histamine formation by *Morganella psychrotolerans*. International Journal of Food Microbiology. doi:10.1016/j.ijfoodmicro.2008.08.016] Growth rates for *M. psychrotolerans* and *M. morganii* were determined at different constant temperatures from 0 °C to 42.5 °C whereas heat inactivation was studied between 37.5 °C and 60 °C. A *M. morganii* growth and histamine formation model was developed by combining these new data (growth rate model) and data from the existing literature (maximum population density and yield factor for histamine formation). The developed *M. morganii* model was evaluated by comparison of predicted growth and histamine formation with data from the existing literature.

Observed and predicted growth rates for *M. psychrotolerans*, at constant temperatures, were similar with bias- and accuracy factor values of 1.15 and 1.45, respectively ($n = 11$). On average times to formation of critical concentrations of histamine by *M. psychrotolerans* were acceptably predicted but the model was not highly accurate. Nevertheless, predictions seemed useful to support decisions concerning safe shelf-life in relation to formulation, storage and distribution of chilled seafood. Parameters for the effect of temperature on growth and inactivation of *M. psychrotolerans* and *M. morganii* differed markedly with T_{min} of −8.3 to −5.9 °C vs. 0.3 to 2.8 °C, T_{opt} of 26.0 to 27.0 °C vs. 35.9 to 37.2 °C and T_{max} 32.0 to 33.3 °C vs. 44.0 to 47.4 °C, $D_{50\ °C}$ of 5.3 min vs. 13.1 min and z-values of 6.8 °C and 7.2 °C. At temperatures above ~15 °C *M. morganii* grew faster than *M. psychrotolerans*. Bias- and accuracy factor-values of 1.41 and 2.44 ($n = 93$) showed the predicted growth of *M. morganii* to be faster than previously observed in fresh fish and broth. In agreement with this, predicted times to formation of critical histamine concentrations by *M. morganii* were on average shorter than observed in fresh fish. A combined model was suggested to predict histamine formation by both psychrotolerant and mesophilic *Morganella* during storage of fresh fish between 0 °C and 37 °C.

1. Introduction

Histamine fish poisoning (HFP) is a common seafood-borne disease with more than 16 000 reported cases world-wide and an actual occurrence likely to be much higher (Dalgaard et al., 2008). Previously, it was expected that to cause HFP seafood had to be exposed to elevated temperatures, above 7–10 °C, allowing mesophilic histamine producing bacteria such as *Morganella morganii*, *Raoultella planticola* and *Hafnia alvei* to grow to high levels and form histamine in toxic concentrations above 500–1000 ppm (Lehane and Olley, 2000; Taylor, 1986). However, recent studies of HFP in Japan and Denmark showed the psychrotolerant histamine producing bacteria *Morganella psychro-*

tolerans and *Photobacterium phosphoreum* to cause more incidents of HFP than the well known mesophile histamine producing bacteria (Dalgaard et al., 2008). Histamine formation by *M. psychrotolerans* and *P. phosphoreum* cannot be prevented by chilling of seafood to 0–5 °C alone. Thus, to manage histamine formation the time of storage and distribution (the safe shelf-life) must be limited depending on storage conditions and product characteristics. It is therefore interesting that an extensive mathematical model has been developed to predict growth and histamine formation by *M. psychrotolerans* depending on both storage conditions and product characteristics (Emborg and Dalgaard, 2008-this issue). This model includes the effect of temperature (0–20 °C), atmosphere (0–100% CO_2), NaCl (0.0–6.0%) and pH (5.4–6.5) and may be important for management of histamine formation in chilled seafood provided of course that the model can be successfully validated for these products.

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Microbial formation of histamine in naturally contaminated seafood is a dynamic process where initial microbial contamination, storage conditions and product characteristics select the specific microflora that grow to high levels and then may produce histamine in toxic concentrations (Dalgaard, 2006; Emborg et al., 2005; Frank et al., 1983; Okuzumi et al., 1984a,b). Use of a predictive microbiology model for evaluation of histamine formation by *M. psychrotolerans* in seafood therefore requires that: i) the initial concentration of the micro-organism, storage conditions and product characteristics are known, ii) the model predicts histamine formation in agreement with challenge studies and storage trials i.e. that the model has been successfully validated and iii) the models range of applicability is known for example with respect to the temperatures where histamine formation by *M. psychrotolerans* is faster than observed for the mesophilic bacteria *M. morganii*.

Successfully validated predictive microbiology models can be most valuable for evaluation of food safety and quality (McMeekin et al., 2006). At the same time prediction of growth or time to toxin formation that differs markedly from responses with naturally contaminated food may lead to inappropriate risk management decisions (Dalgaard and Jørgensen, 1998; Ross et al., 2000). For evaluation of predictive microbiology growth models the bias- and accuracy factors are well established indices of model performance. Models that on average predict growth rates to be from 25% slower to 42% faster than observed in food have been considered acceptable (Dalgaard, 2002; Ross, 1996; Ross et al., 2000). If a similar approach is used for histamine formation then a mathematical model would be considered acceptable when the predicted time to a critical histamine concentration is from 42% shorter to 25% longer than observed in product studies. However, to our knowledge such validation studies have not previously been performed for histamine formation models.

The objectives of the present study were to predict growth of both *M. psychrotolerans* and *M. morganii* in seafood and to evaluate their relative importance for histamine formation. Firstly, an existing mathematical model for growth and histamine formation by *M. psychrotolerans* was evaluated using new data from the present study together with literature data for seafood and broth. Secondly, the effect of temperature (0 °C–60 °C) on growth and inactivation kinetics of *M. psychrotolerans* and *M. morganii* were quantified to evaluate their growth and inactivation during seafood processing or storage. Finally, mathematical models were developed for the effect of temperature (the entire biokinetic range) on histamine formation by both *M. morganii* and *M. psychrotolerans*. This *M. morganii* histamine formation model was evaluated using available data from the literature.

2. Materials and methods

2.1. Validation of M. psychrotolerans growth and histamine formation model

Growth and histamine formation data for model evaluation were generated using canned tuna meat, thawed garfish meat, tuna juice and broth.

Canned tuna meat with or without added NaCl were studied ($n=3$). Drained tuna meat from yellowfin tuna (*Thunnus albacares*) was obtained form a local retailer (Amanda Seafood Ltd., Frederikshavn, Denmark) and prepared as previously described (Emborg and Dalgaard, 2008-this issue). NaCl was added to the tuna meat as an aqueous solution (33% w/v) to a final concentration of 3% water phase salt (WPS). The meat was left overnight at 0 °C prior to inoculation and packaging. A mixture of four strains of *M. psychrotolerans* (Mix-Mp) was studied. The strains (LMG 23374[T], U2/5, JB-T11 and JB-T12) were previously isolated from fresh and cold-smoked tuna (Emborg and Dalgaard, 2006; Emborg et al., 2005). The tuna meat was inoculated with 1% (v/w) of Mix-Mp to an initial concentration of ca. 4 log(CFU/g) as previously described (Emborg and Dalgaard, 2008-this issue). Salted

tuna meat was packed in (i) 100% N_2 ($n=1$) or in (ii) 55%N_2/45% CO_2 ($n=1$) as previously described (Emborg and Dalgaard, 2008-this issue) and stored at 10 °C. In addition tuna meat without added NaCl was packed in 100% N_2 ($n=1$) and kept at changing temperatures (96 h at 0.7 °C followed by 29.5 h at 10.1 °C and 252 h at 4.3 °C) to allow the growth and histamine formation model to be evaluated for storage at dynamic temperatures. Non-inoculated control samples were packed in 100% N_2 ($n=1$) and stored at 10 °C. During storage duplicate samples were taken at regular time intervals from each experiment and analysed for microbial growth and histamine formation. These analyses and determination of product characteristics were performed as described in Section 2.4.

Experiments with thawed garfish were performed as described by Dalgaard et al. (2006). Frozen garfish were thawed during 12 h at 10 °C, then gutted, filleted using a dedicated garfish-filleting device and cut into pieces of approx. 6 cm. Garfish meat was inoculated with a mixture of three isolates of *M. psychrotolerans* (JB-T11, JB-T12 and JB-T16) as described for tuna meat above. The strains were all previously isolated from fresh tuna (Emborg et al., 2005). Approx. 100 g of inoculated garfish fillet with 4 log(CFU/g) was packed in MAP with 40% CO_2/60% N_2 as described for canned tuna meat above and then stored at 5 °C. Samples (triplicate) were removed at regular intervals during storage for microbial and chemical analyses (see Section 2.4).

Tuna juice was prepared as described by Dalgaard (1995) but with a lower buffer concentration of 1.5 g/L of H_2KPO_4 and 1.5 g/L of HK_2PO_4 and without added trimethyleamineoxide, cystein and methionin. pH was adjusted using HCl/NaOH. Two experiments using a mixture of three isolates of *M. psychrotolerans* (JB-T11, JB-T12 and JB-T16) were carried out at 5 °C. The tuna juice was inoculated to an initial concentration of ca. 2 log(CFU/mL). Inoculated tuna juice in test tubes were incubated in anaerobic jars with 100% N_2 or 25% CO_2/75% N_2. During storage duplicate samples were removed for microbial analysis and determination of biogenic amines as described in Section 2.4.

Finally, growth and histamine formation ($n=3$) were studied at 1.7 °C using broth (LB-AA) with pH 5.9 and containing varying concentrations of buffer (1.5, 5.0 and 7.0 g/L of both H_2KPO_4 and HK_2PO_4). The LB-AA broth was prepared as previously described (Emborg and Dalgaard, 2008-this issue). Broth in test tubes was inoculated with the type strain of *M. psychrotolerans* (LMG 23374[T]) and stored in 100% N_2. During storage duplicate samples were removed for determination of growth and histamine formation (Section 2.4).

Lag time (t_{lag}, h) and maximum specific growth rate (μ_{max}, h^{-1}) were estimated from the *M. psychrotolerans* growth curves obtained at constant temperatures as described above ($n=11$). t_{lag} and μ_{max} were estimated from log-transformed cell concentrations by fitting of the expanded Logistic model (Eq. (1))(Emborg and Dalgaard, 2008-this issue). To evaluate the existing *M. psychrotolerans* growth and histamine formation model the lag times determined here (observed values) were compared to t_{lag}-values predicted at corresponding environmental conditions by the model of Emborg and Dalgaard (2008-this issue). Bias and accuracy factor values were calculated to compare the observed and predicted μ_{max}-values. A bias-factor of for example 1.10 shows predicted growth rates to be on average a factor 0.10 (10%) faster than observed whereas an accuracy factor of for example 1.25 indicate an average total deviation of 25% between observed and predicted μ_{max}-values (Dalgaard and Jørgensen, 1998; Ross, 1996). Observed and predicted times to formation of 100, 500 and 2000 ppm of histamine were compared. Times to the three specific observed concentrations of histamine were determined by interpolation if required. Data used for this model evaluation originated from the experiments described above ($n=8$) as well as from studies of fresh tuna (Emborg et al., 2005) and cold-smoked tuna (Emborg and Dalgaard, 2006). To predict growth and histamine formation the model of Emborg and Dalgaard (2008-this issue) was used. In brief, the specific storage conditions (temperature and CO_2