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Influence of temperature, pH and NaCl concentration on the maximal growth rate of *Brochothrix thermosphacta* and a bioprotective bacteria *Lactococcus piscium* CNCM I-4031

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Abstract:

The maximum specific growth rate (μ_{max}) of *Brochothrix thermosphacta*, a spoilage bacteria of cooked peeled shrimp, and *Lactococcus piscium* CNCM I-4031, a bioprotective strain, was investigated under different conditions of temperature, NaCl concentrations and pH. The basic modelling approach used was the Gamma concept (γ-concept) and the model developed was then adapted to shrimp. Cardinal growth parameters were quite similar for the two strains, except for NaCl. No NaCl was required for growth and the $NaCl_{max}$ was three-times higher for *B. thermosphacta* than for *L. piscium* (62 and 23 g l^{-1} respectively). However, tolerance to NaCl was higher in seafood than in liquid broth, possibly due to presence of osmoltically active molecules. *L. piscium* and *B. thermosphacta* were psychrotolerant, with T_{min} = −4.8 and −3.4 °C, T_{opt} = 23.4 and 27.0 °C and T_{max} = 27.2 and 30.8 °C respectively. The optimal pH was neutral and growth possible till pH = 4.8 for the two strains, assuming possible applications of the bioprotective strain in lightly marinated seafood. The μ_{max} of *B. thermosphacta* in shrimp was a little higher than in *L. piscium* whatever the environmental conditions. Validation of the model showed that the γ-concept was suitable for predicting μ_{max} of *B. thermosphacta* in shrimp. Data generated in this study can be used to adapt the model to other foods with few additional experiments and the effect of different parameters may be added in the future. The model was less accurate for the bioprotective strain and the effect of NaCl must be studied in more detail directly in the matrix.

Highlights

► Effect of pH, temperature and NaCl on *Lactococcus piscium* has been modelled. ► Same work was performed on *Brochothrix thermosphacta* and data validated in shrimp. ► Cardinal values were closed except $NaCl_{max}$ (3 times higher for *B. thermosphacta*). ► *B. thermosphacta* grew faster in shrimp than *L. piscium*. ► Bioprotective effect of *L. piscium* is not due to a better adaptation to shrimp matrix.

Keywords: Modelling**;** Biopreservation; Shrimp; Lactic acid bacteria; *Brochothrix thermosphacta*; Spoilage bacteria; Environmental conditions

1. Introduction

The purpose of predictive microbiology is to perform quantitative estimations of microbial kinetics in food or liquid media by using suitable mathematical modelling. The use of predictive microbiology allows anticipating the behaviour of bacteria in any environmental condition (Brul et al., 2007). Pathogenic and spoiling microorganisms may contaminate food but often at low levels. However, under some storage conditions, these microorganisms may reach critical levels. So knowledge of bacterial behaviour with respect to environmental parameters is necessary to optimise the process and estimate shelf-life. The principle of biopreservation is to combat undesirable microorganisms by applying bioprotective bacteria. This additional step cannot replace good manufacturing and hygienic practises, but it helps to reduce the extent of technological treatments (NaCl, pH, preservatives etc.) and/or prevent the development of undesirable microorganisms at abuse temperatures (Calo-Mata et al., 2008; Pilet and Leroi, 2011). Recently, a strain of lactic acid bacteria, *Lactococcus piscium* CNCM I-4031 isolated from fresh salmon steak, was observed to delay sensory shelf-life of tropical cooked peeled shrimp packed under modified atmosphere. The inoculation of *L. piscium* at a level of 10^5 Colony Forming Unit (CFU) g^{-1} significantly increased the shelf-life of this product (Matamoros et al., 2009a) and this was attributed to the inhibition of *Brochothrix thermosphacta* (Fall et al., 2010) recently identified as a major spoiling organism in cooked peeled shrimp (Laursen et al., 2006; Jaffrès et al., 2011).

B. thermosphacta has been identified as a specific spoilage organism of meat products for years (Gardner, 1981; Dainty and Mackey, 1992) and occurs in air or vacuum packed pork, beef, lamb and cured meat (Kakouri and Nychas, 1994; Sheridan et al., 1997; Nychas et al., 2008; Vasilopoulos et al., 2010; Pennacchia et al., 2011). Some authors have studied the effect of temperature and to a lesser extent pH, NaCl and modified atmosphere composition on the growth and metabolism of *B. thermosphacta* (Blickstad and Molin, 1984; Baranyi et al., 1996; Masana and Baranyi, 2000; Pin et al., 2002; Cayré et al., 2005; Koutsoumanis et al., 2006). Different models have been proposed in the literature taking into account the simultaneous effect of temperature, pH and NaCl or water activity (Mc Clure et al., 1993; Braun and Sutherland, 2004), using a polynomial approach. A model is also freely available in the ComBase Predictor software (www.combase.cc) using approximately the same structure. Different modelling approaches exist in predictive microbiology (Brul, et al., 2007). The γ-concept described by Zwietering et al. (1996) assumes independent functions γ of each environmental condition, with values generally restricted between 0 and 1, identified in a specific medium (generally liquid) and connected by multiplication (equation 1). Another parameter, $\mu_{opt\ matrix}$, takes into account the specificity of the food matrix. This concept was successfully validated in some foods (Pinon et al., 2004; Leporq et al., 2005; Membré et al., 2005) and used for the development of software (www.symprevius.net) designed to predict the response of a range of pathogens to key factors. Some spoilage microorganisms are included but not *B. thermosphacta* nor bioprotective strains such as *L. piscium*. *L. piscium* is a recently described species (Williams et al., 1990) for which few data are available on growth parameters. A cardinal parameter model for lactic acid bacteria including the effect of temperature, pH, NaCl and a number of other environmental parameters is freely available as part of the Seafood Spoilage and Safety Predictor software (http://sssp.dtuaqua.dk) but does not differentiate the lactic acid bacteria species, and particularly *L. piscium*.

The purpose of the present study is to model μ_{max} of *B. thermosphacta* and *L. piscium* separately as a function of temperature, pH and NaCl concentrations with the multiplicative approach. The model is then developed for cooked peeled shrimp.

The results also provide information on the adaptation of *L. piscium* and *B. thermosphacta* to this matrix. They allow determining the range of environmental conditions for which a bioprotective effect may be expected.

$$\mu_{\max} = \mu_{optmatrix} . \gamma(T) . \gamma(pH) . \gamma(NaCl) \quad (1)$$

2. Materials and methods

2.1. Bacterial strains and subcultures conditions

L. piscium CNCM I-4031 (formerly EU2241) and *L. piscium* EU2229 were isolated from salmon steak by Matamoros et al. (2009b). The strains of *L. piscium* CIP 104371^T, *L. lactis* subsp. *lactis* CIP 102975, *L. raffinolactis* CIP 102300^T, *L. plantarum* CIP 102506^T, *L. garviae* CIP 102507^T and *B. thermosphacta* CIP 103251^T were obtained from the Pasteur collection (Paris, France). *B. thermosphacta* CD340 was isolated from cooked, peeled, brined and drained tropical shrimp stored under modified atmosphere (Jaffrès et al., 2009). Eight *B. thermosphacta* strains were isolated from cold-smoked salmon and various tropical and artic shrimp products (Table 1). These *B. thermosphacta* strains belong to the Ifremer collection (Nantes, France). The strains were stored at –80°C in their growth medium with 10% (v/v) of sterile glycerol. *L. piscium* and *B. thermosphacta* were subcultured twice successively (24 h at 26°C) in Elliker broth (Biokar Diagnostics, Beauvais, France) and Brain Heart Infusion (BHI, Biokar), respectively, before inoculation in growth medium for μ_{max} determination in a liquid medium and for challenge tests in cooked peeled shrimp.

2.2. Growth medium

A modified Elliker broth (MEB) containing: tryptone 20 g l^{-1}, yeast extract 5 g l^{-1}, gelatin 2.5 g l^{-1}, glucose 7 g l^{-1}, sodium acetate 1.5 g l^{-1}, sodium chloride 4 g l^{-1} and ascorbic acid 0.5 g l^{-1} was used to monitor the growth of *L. piscium* and *B. thermosphacta*. After sterilisation, pH was adjusted to the desired values with NaOH (1N) or HCl (1N) and the media were filtered sterilised. For NaCl concentration, high NaCl concentrated MEB was prepared (100 g 1^{-1}) and the target values were obtained by appropriate dilution with the same non salted medium. The pH was then adjusted to 7.0 before filter sterilisation.

2.3. Experimental conditions

The parameters were studied in the following order: pH, NaCl and temperature. The effect of pH was studied in a range from 4.6 to 7.4 with a 0.2 step in MEB at 26°C. This temperature had previously been estimated to be closed to the optimum for *L. piscium* (Matamoros, et al., 2009b) and *B. thermosphacta* (Baranyi et al., 1996). The effect of NaCl concentrations from 0 to 80 g l^{-1}, with a 2 - 5 g l^{-1} step, was studied in MEB, pH =7.0, and was close to the optimum observed in the first set of experiments. The effect of temperature was studied from 0°C to 35°C with a 2 - 5°C step in MEB (pH and NaCl close to the optimum). For each value of the parameters studied, 200 µl of the non-inoculated medium were placed in ten wells of honeycomb sterile microplates (Thermo Electron Corporation, Vantaa, Finland). The second sub-

subculture of each strain was diluted a hundred-fold to reach approximately 10^6 CFU ml^{-1}. The dilution medium was MEB with the same pH and NaCl concentration as those of the conditions studied. Two hundred µl of the diluted culture were inoculated in the first well, and then eight successive half-dilutions were performed from the first to the ninth well. The tenth well was used for sterility control. At 0°C and 5°C, growth was performed in flasks by inoculating 1% (v/v) of a diluted subculture to obtain an initial concentration of 10^3 CFU ml^{-1}.

2.4. Growth monitoring

Microplates were placed in a Bioscreen C (Labsystem, Helsinki, Finland) and incubated at 26°C for pH and NaCl studies and at a target value for the temperature study. Growth was monitored by measuring the optical density (OD) at 600 nm every 20 min. Before each measurement, microplates were shaken for 15 s. For each parameter value studied, 9 optical density curves were generated. The detection time (DT) was determined as the time to reach an OD fixed at the middle of the exponential growth phase. DT were plotted versus the Naperian logarithm of the initial inoculation level and the μ_{max} was estimated as being the opposite of the slope of the linear regression (Augustin et al., 1999; Baranyi and Pin, 1999). This calculation was performed by an automated program developed on Matlab version 6.5.0 (MathWorks Inc., Natick, Massachusetts, US), by the authors. Bacteria cultivated in flasks were enumerated on Elliker and BHI plate count agar at 26°C and μ_{max} estimated with the modified Gompertz model (Zwietering et al., 1990).

2.5. Determination of $\mu_{opt\ shrimp}$ in cooked peeled shrimp

Frozen tropical peeled and beheaded shrimp (*Penaeus vannamei*) farmed in Colombia (51/60 size i.e. 51 to 60 shrimps per pound) were obtained from a local production site, Miti industry (Nantes, France). Frozen shrimp were cooked at ONIRIS (Nantes, France) in industrial equipment by immersion in boiling salted water (15 g l^{-1} NaCl). When water temperature went back to 100°C, shrimp were cooked for 2.5 min, then cooled in melting ice with 15 g l^{-1} NaCl, vacuum-packed in plastic bags (1 kg/ bag) and stored at –80°C for 9 days. The frozen cooked peeled shrimp were then sterilized by ionization at 5.12 ± 0.29 kGy (IONISOS, Pouzauges, France). NaCl was measured with a Chloride Analyser 926 (Corning, Halstead, UK), on a 2 g homogenate of shrimp. After processing, the concentration of NaCl in shrimp was 0.96 % in water phase (WP) and pH = 6.82. Shrimp were inoculated with *L. piscium* CNCM I-4031 and *B. thermosphacta* CD340 by spraying 10% v/w of a diluted subculture onto the surface of the shrimp to reach a final level of approximately 3.5 log CFU g^{-1} and 2 log CFU g^{-1} respectively. After inoculation, the samples were mingled to ensure good microbial distribution. The shrimp were then packed in 125 g portions under modified atmosphere (50% N_2 - 50% CO_2) (Multivac T252, Wolfertschwenden, Germany) with a shrimp/gas ratio of approximately 2/1. The shrimp were stored at 12°C for 28 days and removed at regular time intervals for microbial analysis, as described by Fall et al. (2010).

2.6. Curve fitting and statistical analysis

The experimental data obtained in a liquid medium were fitted with the cardinal models proposed by Rosso et al. (1993) (Equation 2) to determine γ (X) for each factor.

$$\gamma(X)=\frac{\mu^{*}_{opt}(X-X_{\max})(X-X_{\min})^{n}}{(X_{opt}-X_{\min})^{n-1}\left[(X_{opt}-X_{\min})(X-X_{opt})-(X_{opt}-X_{\max})((n-1)X_{opt}+X_{\min}-nX)\right]} \quad (2)$$

for $X_{\min}\leq X\leq X_{\max}$ and $\gamma(X)=0$ for $X<X_{\min}$ or $X>X_{\max}$

with X_{min} and X_{max} being the minimum and maximum values of X where the bacteria can no longer grow, X_{opt} the value of X where the highest μ_{max} is observed, μ^{*}_{opt} the optimal growth rate in liquid medium and n a curvature parameter.
In food products, some of the influencing factors are not taken into consideration (composition, nutrient availability, packaging conditions such as modified atmosphere etc.) and the growth rate is (purposely) lowered compared to growth in culture broth. A single experiment with shrimp at fixed temperature, pH and NaCl (in water phase) allowed determining μ_{max} in those conditions and thus calculating the last parameter of the model, $\mu_{opt\ shrimp}$ specific to shrimp (equation 1).
Matlab software was used for curve fitting (regression by the non linear least squares method and the Trust-region algorithm) and the quality of the model was estimated with statistical indices such as R-squared (R^2) and Root Mean Square Error (RMSE).

2.7. Validation of the model

To validate the model, other experiments were performed in cooked peeled shrimp as described above, with various final NaCl concentrations, pH and incubation temperatures. These data include those of *B. thermosphacta* CD340 growth in cooked peeled shrimp in pure culture obtained by Fall et al. (2010) and Fall et al. (2012).
An accuracy factor and a bias factor were determined to provide an objective indication of model performance (Ross, 1996).

3. Results

For *B. thermosphacta* and *L. piscium*, the best fit for equation 2 was always obtained with the curvature parameter n = 2. For NaCl and pH factors, the best RMSE values were obtained with only two cardinal parameters and the assumption of a symmetric curve around the X_{opt} parameter. Therefore equations 3, 4 and 5 were used for temperature, NaCl and pH functions respectively.

$$\gamma(T)=\frac{(T-T_{\max})(T-T_{\min})^{2}}{(T_{opt}-T_{\min})\left[(T_{opt}-T_{\min})(T-T_{opt})-(T_{opt}-T_{\max})(T_{opt}+T_{\min}-2T)\right]} \quad (3)$$

for $T_{\min}\leq T\leq T_{\max}$ and $\gamma(T)=0$ for $T<T_{\min}$ or $T>T_{\max}$

$$\gamma(NaCl)=\frac{1}{(NaCl_{\max}-NaCl_{opt})^{2}}(NaCl-2NaCl_{opt}+NaCl_{\max})(NaCl_{\max}-NaCl) \quad (4)$$

for $2NaCl_{opt}-NaCl_{\max}\leq NaCl\leq NaCl_{\max}$ and $\gamma(NaCl)=0$ for $NaCl<2NaCl_{opt}-NaCl_{\max}$

or $NaCl>NaCl_{\max}$

$$\gamma(pH)=\frac{1}{(pH_{opt}-pH_{\min})^2}(pH-pH_{\min})(2pH_{opt}-pH_{\min}-pH) \quad (5)$$

for $pH_{\min}\leq pH\leq 2pH_{opt}-pH_{\min}$ and $\gamma(pH)=0$ for $pH < pH_{\min}$ or $pH > 2\ pH_{opt}-pH_{\min}$

3.1. Growth of B. thermosphacta

Figures 1, 2 and 3 show the evolution of μ_{max} as a function of NaCl, temperature and pH respectively. Table 2 summarised the cardinal values estimated by the models in MEB. The high R^2 and low RMSE values confirmed the quality of the model. *B. thermosphacta* CD340 grew very well without NaCl. The minimum NaCl concentration that stopped the development of *B. thermosphacta* was around 60 - 65 $g.l^{-1}$ (Fig. 1). The optimum temperature was around 25-27°C and then μ_{max} decreased quickly as the temperature increased. No growth was observed at a temperature higher than 31°C. *B. thermosphacta* was psychrotolerant and grew at refrigerated temperature. Growth was very weak but nonetheless continued at 0°C (Fig. 2). Neutral pH was optimal for *B. thermosphacta* but development was still possible at pH 4.8 (Fig. 3). The μ_{opt} in Elliker medium was estimated at 1.08 h^{-1} (generation time = 38 min).

The $\mu_{opt\ shrimp}$ in shrimp was determined with one experiment performed in cooked peeled shrimp with 0.96% NaCl (WP), pH = 6.82 and stored at 12°C under modified atmosphere packaging. μ_{max} observed was 0.196 h^{-1} , so, according to equation (1), $\mu_{opt\ shrimp}$ in shrimp was 0.64 h^{-1} (generation time = 65 min) at optimal temperature, pH and NaCl conditions (Table 2). Figure 4 shows the $\mu_{max\ shrimp}$ observed and the $\mu_{max\ shrimp}$ predicted by the model for four experiments performed in shrimp. The bias factor and accuracy factor were 0.97 and 1.20 respectively. μ_{max} predicted by the model of Mc Clure et al. (1993) and with ComBase predictor are also indicated.

The variability of μ_{max} versus temperature between nine strains of *B. thermosphacta* isolated from various seafood products (shrimp and smoked salmon) and the strain isolated from pork sausage has been studied in our laboratory in a range from 10 to 35°C with the absorbance detection time technique previously described. The μ_{opt} varied from 0.76 to 1.16 h^{-1} (Table 1) but the overall behaviour was similar for all the strains. T_{opt} was close to 25°C, while very low or no growth was recorded at 30°C and growth was never observed at 35°C.

3.2. Growth of L. piscium CNCM I-4031

Cardinal values are presented in Table 2 and the model of μ_{max} fitted as a function of NaCl, temperature and pH in Figs. 1, 2 and 3 respectively. The μ_{opt} in Elliker broth was 0.75 h^{-1} (generation time = 55 min). NaCl was not required for the growth of *L. piscium* and NaCl concentration over 23 g l^{-1} stopped development. The pH_{opt} was close to that of *B. thermosphacta* (around 7.0 - 7.4) and growth was possible till pH = 4.8. At refrigerated temperature (between 0 to 15°C) *L. piscium* grew at approximately the same rate as *B. thermosphacta* (Fig. 2). The increase from 15 to 25°C had less effect on the μ_{max} of *L. piscium*. Optimum growth was observed for a temperature around 23-25°C, then μ_{max} decreased drastically with the increase of temperature, since the ratio between the growth rate at optimal temperature (23.4°C) and the growth rate at 27°C was around 6.7. No growth was observed at 29°C and over. The μ_{max} of other *Lactococcus* sp. was determined with the absorbance detection time method between 10 and 35°C. The *L. piscium* type strain grew at 30°C (μ_{max} = 0.24 h^{-1}) but not at 35°C, whereas another *L. piscium* EU2229 isolated from

raw salmon did not grow at 30°C. The reference strains *L. lactis, L. garviae, L. plantarum* and *L. rafinolactis* continued to grow at 35°C (μ_{max} respectively 1.1, 1.79, 0.46 and 0.38 h^{-1}).
As for *B. thermosphacta*, the $\mu_{opt\ shrimp}$ of *L. piscium* CNCM I-4031 in shrimp was estimated on the basis of one experiment performed with cooked peeled shrimp (NaCl = 0.96% WP, pH = 6.82 and T = 12°C). The μ_{max} observed was 0.18 h^{-1} and the $\mu_{opt\ shrimp}$ calculated 0.46 h^{-1} (generation time 90 min) at optimal temperature, pH and NaCl conditions (Table 2). The model predicting the growth of *L. piscium* was tested with the four environmental conditions presented in Fig. 4. The bias and the accuracy factor were 0.69 and 1.45 respectively.

4. Discussion

B. thermosphacta has been identified as a specific spoilage organism of meat products for years, explaining why most of the studies aimed at predicting growth or metabolism as function of environmental parameters have been performed with strains isolated from meat. In the present study strains isolated from various lightly preserved seafood products were tested. Among them, *B. thermosphacta* CD340 was studied in more detail as it was the target strain used to characterise the bioprotective effect of *L. piscium* CNCM I-4031 (Fall et al., 2010; Fall et al., 2012). Although isolated from marine salted products, *B. thermosphacta* CD340 can easily grow in the absence of NaCl. Up to 10 - 15 g l^{-1}, μ_{max} was not significantly lowered (Fig. 1). Mc Clure et al. (1993) observed similar values with a strain isolated from fresh bacon cultivated in Tryptone Soya Broth (TSB) ($NaCl_{opt}$ ≤ 7 g l^{-1}). The maximum NaCl concentration tolerated was 62 g l^{-1}. This value was a little lower than that of Mc Clure et al. (1993) who observed weak growth in the presence of 80 g l^{-1}. Similar results were found by Blickstad (1984), both aerobically and anaerobically and are in agreement with the growth/no growth boundary of Masana et al. (2000). The pH_{opt} and pH_{min} of *B. thermosphacta* CD340 were 7.1 and 4.8 respectively. Our model developed in the present study makes it ease to compare prediction with observation from various studies and for a wide range of environmental conditions. Mc Clure et al. (1993) estimated 6.8 ≤ pH_{opt} ≤ 6.9, like ComeBase Predictor, but the range of predictions is limited between 5.5 and 7.0 in the two cases. Pin and Baranyi (1998) did not observe large difference of μ_{max} between pH = 5.2 and 6.4 at 11°C in TSB with a cocktail of three strains from different meats . The same observation was made for naturally contaminated fresh ground meat at 15°C (Koutsoumanis et al., 2006). On the other hand, in our study near optimal NaCl and temperature, the inhibitory effect of pH was higher than Pin and Baranyi (1998) (μ_{max} ratio between pH = 6.4 and 5.2 was 2.8) and smaller than Mc Clure et al, (1993) and ComBase (the same ratio with ComBase was obtained for a substitution value of pH of 5.5 for 5.2). Masana et al. (2000) have observed pH_{min} for growth very closed of our pH_{min} parameter. Although the same difficulties were encountered when comparing the results regarding the effect of temperature, most studies have reported growth at 0°C - 2°C (Mc Clure et al., 1993; Baranyi et al., 1995; Pin and Baranyi, 1998; Koutsoumanis et al., 2000; Braun and Sutherland, 2004; Cayré et al., 2005; Koutsoumanis et al., 2006; Zhou et al., 2009). T_{opt} = 27.0°C estimated in this study is in agreement with the results of Mc Clure et al. (1993) (23 ≤ T_{opt}≤ 24°C) and Baranyi et al. (1995, 1996) (25 ≤ T_{opt} ≤ 30°C). T_{max} (30.8°C) is also close to values reported by Gardner (1981) (T_{max} > 30°C) and Baranyi et al. (1996) (31.2°C for *B. thermosphacta* NCFB 2891). In the present study, the 10 strains tested had the same T_{opt} and T_{max} range. All these data tend to prove that the cardinal growth values of *B. thermosphacta* are similar whatever the origin of the strains. The μ_{opt} from the literature are difficult to compare, as the growth media and strains tested are often

different. The data in Table 1 (same culture medium) show very slight differences between strains from seafood while the strain isolated from meat had the lowest μ_{opt}. The μ_{opt} in cooked peeled shrimp stored under modified atmosphere was 40% lower compared to the liquid medium. However, growth was still possible ($\mu_{max\ shrimp}$ = 0.044 h^{-1}) in shrimp under usual marketing conditions (1.5 % WP NaCl, pH = 6.8, 4°C, storage under modified atmosphere) and quite rapid ($\mu_{max\ shrimp}$ = 0.115 h^{-1}) at a temperature of 8°C, frequently recorded in consumers' refrigerators. The difference between μ_{max} in liquid medium and shrimp stored under modified atmosphere may partially be explained by presence of CO_2 and N_2, whose inhibitory effect has been demonstrated by Blickstad and Molin (1984). Koutsoumanis et al. (2000) have observed a reduction of μ_{max} of approximately 30% in presence of 50% CO_2 (+ 50% O_2) in naturally contaminated Mediterranean red mullet stored between 0 and 15°C. The bias and accuracy factor of the model were 0.97 and 1.20 respectively which is good according to the interpretation of the bias factor proposed by Ross (1999) for *Listeria monocytogenes* in seafood: a bias factor ranging from: 0.95 - 1.1 good; 1.11 - 1.43 acceptable; ≤ 0.87 and ≥ 1.43 unacceptable. The model from Mc Clure et al. (1993) and the ComBase predictor overestimated μ_{max} for all conditions tested, with bias factor of 2.40 and 2.47 respectively (Fig. 4). The high bias factor value to some extend can be explained by the inhibiting effect of CO_2 in the MAP shrimp. It is noteworthy that the pH values were quite similar in all the validation experiments. In cold-smoked salmon, a higher level of NaCl significantly reduced pH (Leroi et al., 2000) and the same phenomenon was expected in this study. pH may be an important factor that industry can take into account by using organic acids as preservatives. In addition to pH, a *γ*-function for different organic acids could be developed and introduced in the model in the future.

L. piscium is a new species recently described by Williams et al. (1990). The strain was first isolated from diseased Rainbow yearlings. More recently *L. piscium* was found to be part of the dominating microbiota of vacuum-packed beef and pork at refrigerated temperature (Sakala et al., 2002a, 2002b; Jiang et al., 2010), cheese at the beginning of ripening (Carraro et al., 2011), ayu-narezushi (narezushi made using sweetfish) (Matsui et al., 2010) and cabbage and lettuce residue (Yang et al., 2010). Despite the importance of this species in food, very few data are available on its growth characteristics. The cardinal pH values of the bioprotective *L. piscium* CNCM I-4031 are close to those of *B. thermosphacta* (optimum at neutral pH, minimum at pH 4.8). It grew well without NaCl and μ_{max} was quite unchanged until 8 g l^{-1}. However, a strong inhibitory effect was observed and the maximum NaCl concentration tolerated (23 g l^{-1}) was three-times lower than that of *B. thermosphacta.* Sakala et al. (2002b) reported the absence of growth at 40 g l^{-1} of NaCl, but they did not test lower concentrations. This low value could be a serious restriction for use of *L. piscium* CNCM I-4031 as a bioprotective culture in slightly preserved seafood whose NaCl concentration is often higher than 2% (WP). However, Matamoros et al. (2009a) observed good growth of *L. piscium* CNCM I-4031 in cold-smoked salmon containing approximately 5% NaCl (WP). The presence of osmotically active molecules in seafood may be an explanation for this observation. Leblanc et al. (2001) showed that salmon flesh contains choline which can be imported and oxidized by bacteria, leading to the intracellular accumulation of glycine betaine, a molecule with osmoprotective properties. *L. piscium* CNCM I-4031 grew well at low temperature (0°C) and reached maximum growth below 25°C, which is very uncommon among lactic acid bacteria. An overexpression of cold-adaptation proteins during growth at low temperature highlighted by Garnier et al. (2010) may explain the psychrotrophic behaviour of *L. piscium* CNCM I-4031. *L. piscium* is the most psychrotolerant species of the *Lactococcus* genus. An *L. piscium* type strain grew at 30°C but not at 35°C, as found by Williams et al. (1990), whereas the bioprotective strain and another *L. piscium* EU2229 isolated from raw salmon failed to grow at 30°C. Sakala et al. (2002b) observed growth at 5°C but weak and variable

development at 30°C with 100% of their isolates from fresh vacuum packed beef. Despite the absence of growth at 37°C, it is noteworthy that *L. piscium* has been isolated from human intestine (Kubotaet al., 2010). The bias factor (0.67) showed that the model proposed under-estimated the μ_{max} of *L. piscium* in shrimp. The addition of a classical *γ* interaction function proposed by Augustin et al. (2005) could only increase the inhibition of growth rate when two or more stressing conditions are employed. So as expected in our tested conditions, this kind of model did not significantly change the prediction. On the contrary, the increase of the value of $NaCl_{max}$ parameter improve significantly the quality of the predictions. The assumption that the optimum cardinal values of a microorganism are independent of the matrix does not appear to hold in the case of *L. piscium*, at least for $NaCl_{max}$. Osmotically active solutes may be actively imported from shrimp flesh and intracellular accumulation may trigger restored growth of cultures under inhibitory osmolarities, explaining the under-estimation of the model. Experiments on shrimp with higher levels of NaCl are necessary to confirm this hypothesis.
In this study, only μ_{max} was considered for the comparison of the two strains because no significant time lag was observed in the shrimp matrix and the maximum number of cells in pure culture was always around 10^9 CFU g^{-1}. Dainty and Mackey (1992) raised the question whether the rapid growth of a population might be a sufficient reason for its dominance in certain chilled food products. Pin and Baranyi (1998) concluded that the dominance of *Pseudomonas* spp. in meat versus *Brochothrix* and enterobacteriacea was not due to the difference in their growth rates but to interaction between bacteria in mixed culture. In our study, the μ_{max} of *B. thermosphacta* in shrimp was higher than that of *L. piscium* when grown in isolation whatever the environmental conditions and the reason why *B. thermosphacta* growth is limited in the presence of the bioprotective strains is still unclear.

Conclusion

Most of the models developed in the literature for *B. thermosphacta* cannot be extrapolated to other matrices or extended outside of the domain tested. The present data based on the *γ*-concept allow predicting the growth of *B. thermosphacta* in shrimp and can be adapted to other foods making it possible to reduce the number of fastidious experiments on matrices. Other technological factors such as preservatives and modified atmosphere can also be tested separately in a liquid medium and included in the current model. Although the *γ*-concept seems less accurate for predicting *L. piscium* growth, the data obtained in both the liquid medium and the shrimp matrix demonstrated that the bioprotective strain adapted well at chilled temperatures over a wide range of pH, thereby auguring its possible application in new products such as lightly marinated seafood. Tolerance to NaCl may depend on the presence of osmotically active molecules and must be studied in greater detail directly in the matrix.

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Tables

Table 1: Maximum growth rate (μ_{max} in h^{-1}) of different strains of *B. thermosphacta* as function of temperature.

Strain	Origin	Temperature (°C)					
		10	15	20	25	30	35
CD340	CPDB[a] *Penaeus vannamei*	0.31	0.45	0.78	1.03	0.69	NG
CD290	CPDB *Penaeus vannamei*	0.28	0.49	0.70	0.96	NG	NG
CD251	CPDB *Penaeus vannamei*	0.29	0.41	0.68	0.95	NG	NG
CRE2329	WC[b] *Penaeus vannamei*	0.31	0.48	0.88	1.03	NG	NG
RF199	CP[c] *Pandalus borealis*	0.31	0.48	0.88	1.03	NG	NG
RF200	CP *Pandalus borealis*	0.30	0.47	0.94	1.06	NG	NG
RF202	CP *Pandalus borealis*	0.34	0.51	0.88	1.06	NG	NG
EU2206	cold-smoked salmon	0.29	0.41	0.60	1.16	NG	NG
SF1682	cold-smoked salmon	0.30	0.33	0.69	0.89	1.02	NG
CIP 103251	Pork sausage	0.14	0.33	0.53	0.76	NG	NG

[a] CPDB: cooked, peeled, brined, drained; [b] WC: whole cooked; [c]CP: cooked peeled; NG: no growth for 5 days. Results obtained with one experiment for each strain

Table 2: Growth cardinal values of *B. thermosphacta* CD340 and *L. piscium* CNCM I-4031 in laboratory medium

Cardinal value	*B. thermosphacta*	Statistics	*L. piscium*	Statistics
T_{min}	-3.36°C	R^2=0.9914	-4.80°C	R^2=0.964
T_{opt}	27.01°C	RMSE= 0.02945°C	23.39°C	RMSE= 0.0466°C
T_{max}	30.85°C		27.19°C	
pH_{opt}	7.11	R^2=0.9882	7.36	R^2=0.9966
pH_{min}	4.79	RMSE= 0.0226 pH unit	4.79	RMSE= 0.01266 pH unit
$NaCl_{opt}$	0 g l^{-1}	R^2=0.9941	4.69 g l^{-1}	R^2=0.9738
$NaCl_{max}$	62.24 g l^{-1}	RMSE= 0.06177 g l^{-1}	23.00 g l^{-1}	RMSE = 0.0359 g l^{-1}

Figures

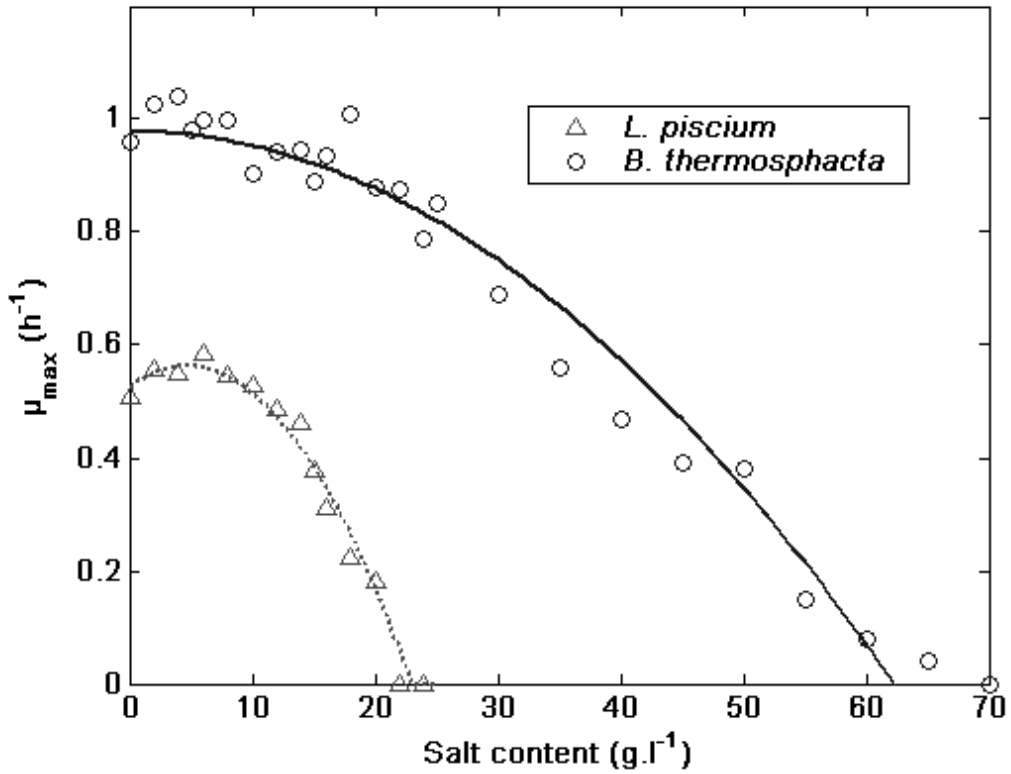

Figure 1: Growth rate of *B. thermosphacta* CD340 (full line) and *L. piscium* CNCM I-4031 (dotted line) in modified Elliker broth versus NaCl: experimental data (symbol) and model (line)

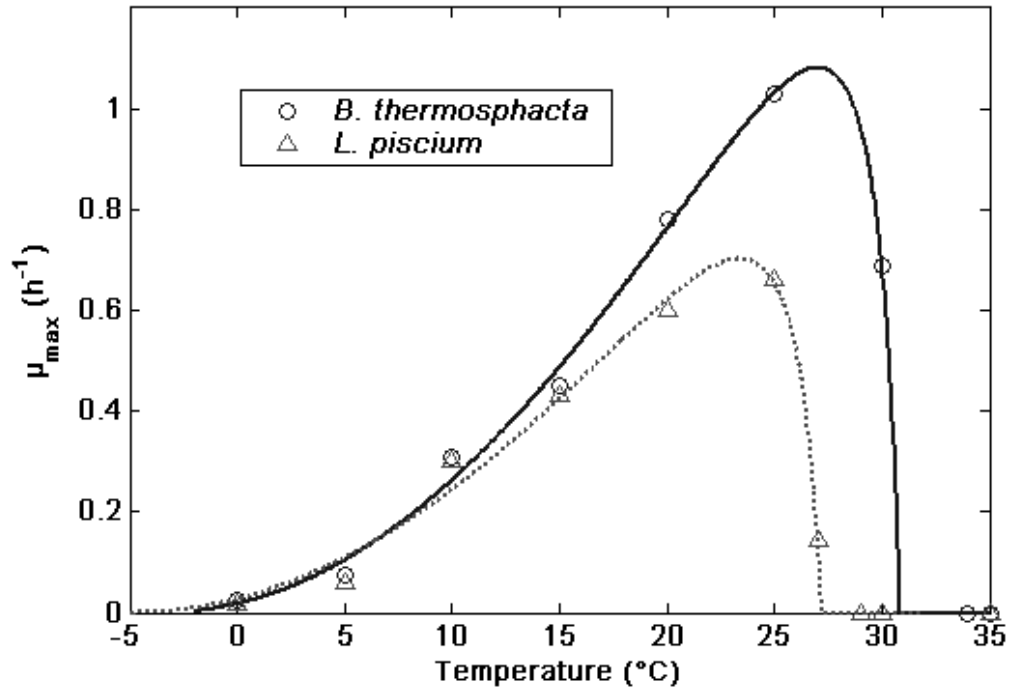

Figure 2: Growth rate of *B. thermosphacta* CD340 (full line) and *L. piscium* CNCM I-4031 (dotted line) in modified Elliker broth versus temperature: experimental data (symbol) and model (line)

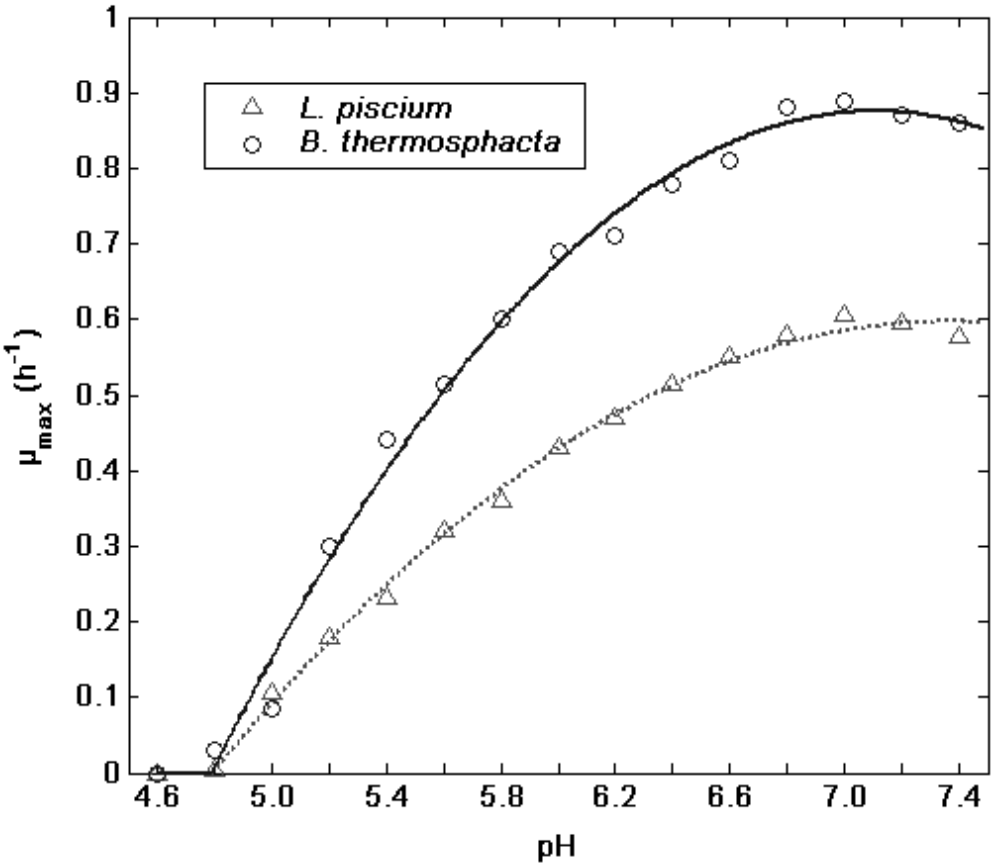

Figure 3: Growth rate of *B. thermosphacta* CD340 (full line) and *L. piscium* CNCM I-4031 (dotted line) in modified Elliker broth versus pH: experimental data (symbol) and model (line)

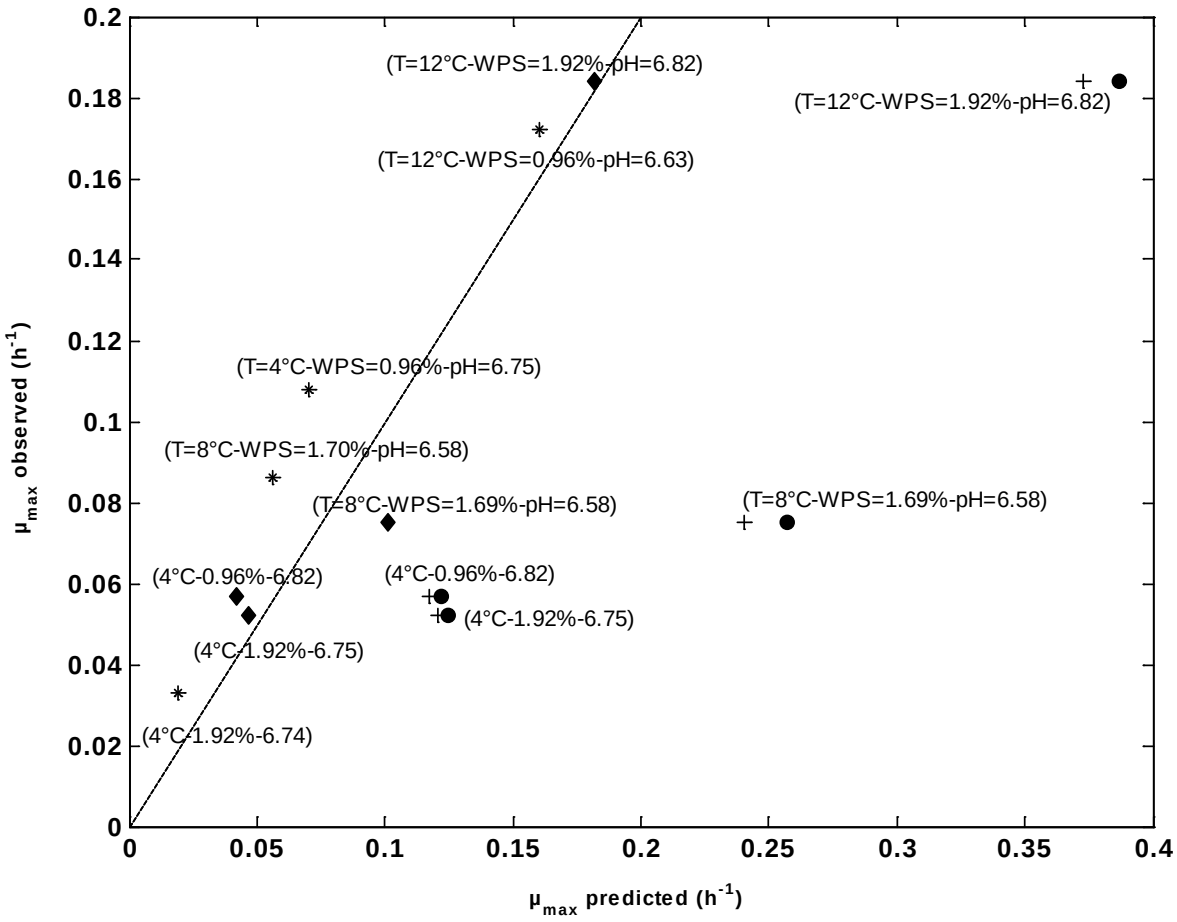

Figure 4: Maximum growth rate of *B. thermosphacta* CD340 observed in cooked peeled shrimp and predicted by ♦ our model (equation 1) (bias factor = 0.97, accuracy factor = 1.20) ; • ComBase predictor (bias factor = accuracy factor = 2.47) ; + model 1 of (Mc Clure, et al., 1993) (bias factor = accuracy factor 2.40) and * μ_{max} of *L. piscium* CNCM I-4031 estimated by our model (bias factor = 0.69, accuracy factor = 1.45)