Food Microbiology 31 (2012) 222–228

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

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

Françoise Leroi [a,*], Papa Abdoulaye Fall [a], Marie France Pilet [a,b,c], Frédérique Chevalier [a], Régis Baron [a]

[a] *Ifremer, Laboratoire de Science et Technologie de la Biomasse Marine, Rue de l'île d'Yeu, BP 21105, F-44311 Nantes, France*
[b] *LUNAM Université, Oniris, UMR 1014 Secalim, Nantes F-44307, France*
[c] *INRA, Nantes F-44307, France*

ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form
28 February 2012
Accepted 29 February 2012
Available online 10 March 2012

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

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.

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

* Corresponding author. Tel.: +33240374172; fax: +33240374071.
E-mail address: Francoise.leroi@ifremer.fr (F. Leroi).

0740-0020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2012.02.014

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 (Eq. (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.

$$\mu_{max} = \mu_{opt\ matrix} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(NaCl) \quad (1)$$

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.

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.

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 (1 N) or HCl (1 N) and the media were filtered sterilised. For NaCl concentration, high NaCl concentrated MEB was prepared (100 g l^{-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