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Variation of cardinal growth parameters and growth limits according to phylogenetic affiliation in the *Bacillus cereus* Group. Consequences for risk assessment

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ABSTRACT

The growth rates of strains covering the seven major phylogenetic groups of *Bacillus cereus sensu lato* (as defined by Guinebretiere et al., 2008) at a range of temperature (7 °C–55 °C), pH (4.6–7.5) and a_w (0.929–0.996, with 0.5%–10% NaCl as humectant) were determined. Growth rates were fitted by non-linear regression to determine the cardinal parameters T_{min}, T_{opt}, T_{max}, pH_{min}, pH_{opt}, a_{wmin} and μ_{opt}. We showed that cardinal parameters reflected the differences in the temperature adaptation observed between *B. cereus* phylogenetic groups I to VII. The ability of growing at low pH (up to 4.3) or low a_w (from a_w 0.929 and up to 10% NaCl) varied among strains. The strains of groups III and VII, the most tolerant to heat, were also the most adapted to high NaCl (all strains growing at 8% NaCl) and the ones of groups I and VI the least adapted (no growth at 7% NaCl). All strains of groups II and VII were able to grow at pH 4.6, and only a few strains of group VI. Phenotypic differences between the two psychrotrophic groups II and VI were revealed by contrasted acid and salt tolerance. The cardinal values determined in this work were validated by comparing with cardinal parameters of a panel of strains published elsewhere and with predictions of growth in a range of foods.

1. Introduction

Growth limits for temperature, pH or water activity are major characteristics of foodborne pathogenic bacteria and important determinants of food safety hazards. The pH value of 4.5 for instance, which is the pH growth limit of proteolytic *Clostridium botulinum*, is a recognized international standard. Acid foods at pH lower than 4.5 do not represent a botulism hazard, which has major consequences for the food industry (EFSA, 2005b; ICMSF, 1996). Although they have a major biological and practical significance, those growth/no growth limits of bacterial pathogens may not be appropriate to predictions of the increases in populations in foods. Risk characterization is partly based on the assessment of the concentrations reached in a food before consumption. Risk management options will favour conditions keeping bacterial concentrations below a critical level. This is of particular relevance for the foodborne pathogenic bacterium *Bacillus cereus* that causes two types of foodborne poisonings, an emetic syndrome and a diarrheic syndrome, representing a significant part of outbreaks of foodborne poisonings in many countries, and widely distributed in food materials (EFSA, 2005a; Granum, 2007). Both syndromes are associated with multiplication and relatively high concentrations of *B. cereus* cells in the foods ($>10^5$ cfu/g) and consequently with concentration changes during food storage.

Several theoretical approaches have been proposed to predict microbial growth in foods. Among those Cardinal Parameter Models (CPM) offer the advantage to use parameters having a biological significance. These cardinal parameters (CPs) T_{min}, T_{opt}, T_{max}, pH_{min}, pH_{opt}, a_{wmin}, and a_{wopt} allow to predict the maximum specific growth rate μ_{max} as a function of the

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incubation temperature, and pH and a_w of the growth medium. The CPs X_{min} and X_{max} represent the value of X_i below and above which no growth occurs (and μ_{max} is equal to 0), and X_{opt} the value at which μ_{max} is equal to μ_{opt} and reaches a maximum (Ross and Dalgaard, 2004).

Genetic variability must be accounted and is included in *Listeria monocytogenes* and *Escherichia coli* CP values for instance (Membre et al., 2005). The *B. cereus* Group (or *B. cereus sensu lato*) phylogenetic structure was recently resolved in seven major phylogenetic groups (I–VII), showing clear differences in their ability to grow at low or high temperatures and to cause food poisoning (Guinebretiere et al., 2008, 2010). However whether it is similar for growth at low pH or low a_w – high NaCl concentration is not known. The aim of this work is to determine whether strain from different phylogenetic affiliations exhibit different cardinal growth parameters (temperature, pH and a_w) and whether this is related to growth limits establish in growth/no growth tests. The relevance of the determined CPs for growth predictions will be discussed.

2. Material and methods

2.1. Strain selection and media

Strains used in this work are listed in Table 1. Growth/no growth limits at a range of pH and at a_w near growth limits were determined for all 41 strains. Cardinal temperatures, pHs and a_ws were determined for two strains per each group (II to VII). Filament growth of group I strains (*Bacillus pseudomycoides*) was not suitable for A_{600} growth curves and CPs of those strains were consequently not determined. The assignment to groups I to VII was mainly obtained from previous works (Guinebretiere et al., 2008, 2010, 2012), otherwise by analysis of the sequence of the *panC* gene as previously described Guinebretiere et al. (2010).

2.2. Estimation of μ_{max} as a function of temperature, pH and a_w

The methods used in this work derive from those described in a previous work (Membré et al., 2002). Strains taken from stock

Table 1
Origin of *Bacillus cereus sensu lato* strains.

Designation of strains used in this work	Phylogenetic group[a]	Reference[b] for assignation to phylogenetic groups	Equivalent designation	Origin
[a]CIP 52-19	I	1		Unknown
WSBC 10275	I	1		Environment
INRA C36	I	This study		Food
DSMZ 12442	I	1		Type strain
CIP 105701	I	1		Unknown
CIP 105702	I	1		Soil
RIVM BC120	**II**	**1**	**WSBC 10819**	Diarrhoeal outbreak
NVH 0861-00	**II**	**1**		Diarrhoeal outbreak
INRA Bc05-F1	II	1	INRA KBAA5	Environment
RIVM BC938	II	1		Food
INRA 15	II	1		Food
RIVM BC126	II	1		Patient feces
F4810/72	**III**	**1**	**DSMZ4312**	Emetic outbreak
DSMZ 4222	**III**	**1**	**F837/76**	Clinical infection
NVH 0500-00	III	1		Diarrhoeal outbreak
INRA C26	III	2	INRA TL811	Food
F2085/98	III	1		Diarrhoeal outbreak
DSMZ 2301	III	1	FH3502/72	Diarrhoeal outbreak
NVH 200	III	1		Diarrhoeal outbreak
ATCC 10987	III	1		Food
F4430/73	**IV**	**1**	**DSM 4384, B4ac**	Diarrhoeal outbreak
ATCC 14579	**IV**	**1**		Type strain *B. cereus*
NVH 1230-88	IV	1		Diarrhoeal outbreak
ADRIA I16	IV	1		Food
F4815/94	IV	1		Diarrhoeal outbreak
AFSSA 98HMPL63	IV	1		Diarrhoeal outbreak
F2769/77	**V**	**1**		Diarrhoeal outbreak
NVH 141/1-01	**V**	**1**		Diarrhoeal outbreak
INRA C33	V	1		Food
ADRIA I11	V	2		Food
UH TSP9	V	1		Environment
INRA KBAB4	**VI**	**This study**		Environment
ADRIA I21	**VI**	**1**		Food
INRA 5	VI	1		Food
WSBC 10204T	VI	1		Type strain *B. weihenstephanensis*
ADRIA I3	VI	1		Food
INRA C1	VI	1	INRA TZ415	Food
NVH 391-98	**VII**	**1**		Diarrhoeal outbreak
NVH 883-00	**VII**	**1**		Food
INRA AF2	VII	1		Diarrhoeal outbreak
AFSSA 08CEB44BAC	VII	3		Diarrhoeal outbreak

Bold characters: cardinal temperature, pH and a_w were determined for those strains.

[a] CIP Collection de l'Institut Pasteur; WSBC: Weihenstephan *Bacillus cereus* collection, Weihenstephan, Germany; INRA, Institut National de la Recherche Agronomique, UMR408, Avignon; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellculturen, Braunschweig, Germany; RIVM, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands; NVH, The Norwegian School of Veterinary Science, Oslo; F strains were obtained from the Public Health Laboratory Service (PHLS), London, UK; ATCC, American Type Culture Collection, Manassas, VA; ADRIA Normandie, Villers Bocage, France; UHDAM, University of Helsinki, Department of Applied Chemistry and Microbiology, Helsinki, Finland; AFSAA (ANSES since 2010), Agence Française de Sécurité sanitaire des Aliments, Maisons-Alfort, France.

[b] 1, Guinebretière et al. (2008); 2, Guinebretière et al. (2010); 3, Guinebretière et al. (2012).