Toxin genes and cytotoxicity levels detected in Bacillus cereus isolates collected from cooked food products delivered by Swiss Army catering facilities

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Abstract: Heated food is known to be often contaminated with B. cereus, leading to cases of diarrhoeal or emetic diseases. Battalion kitchens or army catering facilities present a food safety risk, as temperature abuse and long storage time can result in serious public health problems affecting a high number of served people. In contrast to civil catering facilities, no microbiological monitoring systems are currently implemented in Swiss military kitchens. In this study toxin gene profiles and cytotoxicity levels of 21 isolates of B. cereus originating from six different food categories were determined. Nearly all isolates (95%) harbored the nhe gene, whereas no hbl could be detected. Seven isolates displayed the cytK2 gene and one cereulide-producer was isolated out of vegetables. While most isolates displayed low cytotoxicity, highly cytotoxic strains were detected, with three isolates even exceeding the cytotoxicity level of the reference strain for high-level toxin production, underpinning that cytotoxicity cannot be deduced only from presence or absence of toxin genes. These findings further underline the importance of rapid cooling of foods or maintenance over 65℃ before serving. This is especially important in mass catering facilities, such as military kitchens, in which food is often prepared a long time in advance.

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Characterization of *Bacillus cereus* group isolates from powdered food products

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ABSTRACT

Mashed potato powder as well as powdered infant formula (PIF) are frequently contaminated with *Bacillus cereus sensu lato (B. cereus s.l.)*, mainly with its spores. These products have also been implicated in foodborne illnesses. Here, we characterized *B. cereus s.l.* isolates originating from powdered products based on sporulation assays, toxin gene profiling, and *panC* typing combined with a SplitsTree analysis. Furthermore, cytotoxicity assays with *B. cytotoxicus* isolates were performed. 78% of PIF tested positive for *B. cereus s.l.*, whereas 92% of all mashed potato powders were positive. In total, 43 isolates were further characterized. The *nhe* and *cytK2* genes were most frequently detected. Moreover, a cereulide-producer was detected from PIF. Most isolates were assigned to *panC* group III, but members of group II, IV, V, and VII could also be found. Nine *B. cytotoxicus* were isolated out of nine mashed potato powders. All *panC* group VII isolates were positive for *cytK1*. Cytotoxicity assays of these nine isolates revealed one highly cytotoxic strain, while all other isolates exhibited no detectable cytotoxicity, underpinning that cytotoxicity of a certain *B. cereus* group strain cannot be deduced from the sole presence or absence of toxin genes.

Keywords: *Bacillus cytotoxicus; Bacillus cereus* group; Vero cell assay; mashed potato; powdered infant formula

1. Introduction

Bacillus cereus sensu lato (B. cereus s.l.), a group of Gram-positive spore-forming bacteria, is ubiquitous in nature and can therefore widely be found as part of the microflora of agricultural products (Stenfors Arnesen et al., 2008). The group comprises several genetically closely related species, with *B. cereus sensu stricto* as well as *B. anthracis, B. mycoides, B. pseudomycoides, B. thuringiensis, B. weihenstephanensis, B. cytotoxicus,* and *B. toyonensis* as the most prominent members.

B. cereus is known as an important foodborne pathogen that can cause two distinct forms of illness (Stenfors Arnesen et al., 2008). Firstly, the diarrheal syndrome that is linked to three enterotoxins - Hbl, Nhe and CytK - and secondly, the emetic syndrome caused by cereulide toxin preformed in food. *B. thuringiensis* forms characteristic parasporal crystals with insecticidal activity, enabling the use of *B. thuringiensis*-based insecticides in agriculture (Chattopadhyay et al., 2004). *B. thuringiensis* is known as a common contaminant of milk (Bartoszewicz et al., 2008). However, its relevance as a causative agent of foodborne disease has been controversially discussed (EFSA, 2016, Jackson et al., 1995, McIntyre et al., 2008). The thermotolerant species *Bacillus cytotoxicus*, which has been described 2013, characteristically harbors the *cytK1* variant of the cytotoxin K gene (Guinebretière et al., 2013). The description of this novel *B. cereus* group member was based on five strains, four of which were linked to food poisoning, including an outbreak caused by strain NVH 391-98^T that led to three fatalities of diarrheal disease in France in 1998 (Guinebretière et al., 2013, Lund et al., 2000).

The *B. cereus* group species do not show a clear phylogenetic separation and generally form three major clades, in which species are intermingled. SpoAB typing allows the assignment of a strain to a certain clade (Ehling-Schulz et al., 2005; Fricker et al., 2011). For gaining a deeper insight into the population structure of the *B. cereus* group, an AFLP system has been established by Guinebretiere et al. (2008), which allows for assignment of *B. cereus*

group strains to 7 phylogenetic subtypes. *panC* has been found to be a suitable housekeeping gene to assign new strains to these subtypes (Guinebretière et al., 2010). The ability of strains to cause food poisoning was suggested to vary depending on phylogenetic affiliation with *panC* groups I to VII rather than species affiliation (Guinebretière et al., 2010). To date, strains causing emetic illness have exclusively been associated with *panC* group III (Guinebretière et al., 2010).

According to EFSA, *B. cereus* holds fourth place as a cause of foodborne outbreaks in the European Union (EFSA, 2015). It has been stated by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) that *B. cereus s.l.* is an organism of concern in PIF with regard to the strength of evidence of a causal association between the presence of the microorganism in PIF and illness in infants (FAO/WHO, 2006). *B. cereus s.l.* is a frequent contaminant of dried milk products (Becker et al., 1994; Di Pinto et al., 2013; Reyes et al., 2007). Powdered infant formula (PIF) could also represent a source for isolates of the *B. cereus* group, which could have severe consequences as neonates are highly susceptible for infections. *B. cytotoxicus* has been detected in infant foods in China, showing that the possibility of food poisoning outbreaks due to *B. cytotoxicus* is a risk in this particularly vulnerable consumer group (Zhang et al., 2017).

Although the production of powdered products involves heating and drying processes, which pose harsh living conditions for most bacteria, isolates of the *B. cereus* group and in particular *B. cytotoxicus* have mainly been isolated from dehydrated potato products (Contzen et al., 2014) (Kim and Goepfert, 1971; King et al., 2007; Turner et al., 2006). *B. cereus* group isolates are capable of producing spores, which are able to survive stress conditions encountered in the production of powdered products. Foodborne illnesses caused by isolates of the *B. cereus* group in association with potato products have been reported (Doan and Davidson, 2000; Lindqvist et al., 2000). Especially the newly described species *B. cytotoxicus*

that was discovered during an outbreak in France with three fatalities (Lund et al., 2000) has gained attention in recent times. First studies attributed its high cytotoxicity to the possession of *cytK1* (Fagerlund et al., 2007; Lund et al., 2000) and provided phylogenetic data (Guinebretière et al., 2013; Sorokin et al., 2006). Though the number of characterized *B. cytotoxicus* strains is low to date, many of them originated from mashed potatoes and have been linked to food poisoning cases (Guinebretière et al., 2013). A recent study by Contzen *et al.* has shown that *B. cytotoxicus* can frequently be detected in different dehydrated potato products and occurs far more wide-spread than previously suggested (Contzen et al., 2014). Although *B. cytotoxicus* is generally assumed to be highly cytotoxic (Fagerlund et al., 2004; Guinebretière et al., 2010; Hardy et al., 2001), Fagerlund *et al.* suggested that presence of the *cytK1* gene does not correlate with cytotoxic activity (Fagerlund et al., 2007). As cytotoxicity data has so far only been published for three *B. cytotoxicus* isolates (Fagerlund et al., 2007), further cytotoxicity testing is crucial to assess the food poisoning risk related to this new *B. cereus* group species.

Therefore, the objective of the present study was to isolate and characterize *B. cereus* species out of powdered food products including PIF, mashed potato powder, and fruit powder. In addition, we aimed to determine the cytotoxic potential of all isolated *B. cytotoxicus* strains.

2. Materials and methods

2.1 Sampling material and enrichment procedure

A total of 13 powdered mashed potato products and nine PIF from different brands were bought in supermarkets in Switzerland. Furthermore, 11 *B. cereus* group isolates originating from self-control of a powdered infant formula producer were included in the study. In addition, four strains of *B. cereus s.l.* were included in this study that had been isolated out of fruit powders. Two different approaches of enrichment were used for the purchased products. First, 10 g of powder was mixed with 90 ml buffered peptone water (Oxoid, Basel, CH) in a

123 stomacher bag using the Stomacher® 400 Circulator (Seward, Worthing, UK) for 30 s. The
124 samples were subsequently incubated at 37°C overnight. After overnight incubation, one loop
125 of the overnight culture was streaked onto Mossel (Mossel et al., 1967) and sheep blood agar
126 plates (BD Difco™ Columbia Blood Agar Base) that were incubated at 37°C overnight.
127 Second, an approach was used that has already been described by Contzen *et al*. in order to
128 detect *B. cytotoxicus* (Contzen et al., 2014). This included enrichment of the powder in 90 ml
129 CGY medium (Beecher and Wong, 1994) followed by incubation at 50°C overnight. The next
130 day, a loopful of the enriched culture was streaked onto Mossel and blood agar plates that
131 were subsequently incubated at two different temperatures, 37°C (Mossel) and 50°C (blood
132 agar), respectively. In the present study, the minor modification was made that the culture and
133 the blood plates were incubated at 46°C instead of 50°C. Mossel plates were checked for
134 colonies showing an egg-yolk lecithinase-positive and mannitol-negative phenotype
135 characteristic for isolates of the *B. cereus* group.
136
137 *2.2 DNA extraction and toxin gene profiling*
138 DNA was extracted from all isolates using the GenElute Bacterial Genomic DNA Kit
139 according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO). Toxin gene
140 profiles were determined using a PCR approach as previously described by Ehling-Schulz *et*
141 *al*. (Ehling-Schulz et al., 2006) with minor modifications: The GoTaq PCR system (Promega
142 AG, Dübendorf, Switzerland) was used at (i) 2 min at 95°C, (ii) 30x [45 s at 95°C, 45 s at
143 51°C, 2 min at 72°C]; (iii) 5 min at 72°C. The respective forward primer used for detection of
144 the *nhe* complex is located in *nheA* while the reverse primer is located in *nheB*, thus enabling
145 detection of the first and second gene of the *nhe* operon. The respective primers for *hbl* are
146 located in *hblD* and *hblA*, thus allowing for detection of the second and third gene of the *hbl*
147 operon. Moreover, a duplex PCR was carried out to distinguish between *cytK1* and *cytK2* as
148 previously described (Guinebretière et al., 2006).

149

150 *2.3 Genotyping using panC*

151 A PCR-based genotyping approach targeting *panC* was performed (Guinebretière et al.,
152 2008). In cases in which previously published *panC* primers did not result in an amplicon,
153 additional primers designed in this study were used (see Table 1). The following cycling
154 conditions were used: (i) 2 min at 95°C, (ii) 30x [45 s at 95°C, 45 s at 60°C, 50 s at 72°C];
155 (iii) 5min at 72°C. The PCR products were purified with the GenElute™ PCR Clean-Up Kit
156 according to the manufacturer's instructions. Subsequently, the sample's concentration and
157 purity were measured using a NanoDrop™ Fluorospectrometer (Witec AG, Luzern, CH).
158 Sequencing was outsourced (Microsynth™, Balgach, CH). Sequences of *panC* were assigned
159 to seven (I-VII) phylogenetic groups as previously described
160 (https://tools.symprevius.org/Bcereus/english.php) (Guinebretière et al., 2008, 2010).
161 Cluster analysis of *panC* sequences was performed with the SplitsTree™ software
162 (http://www.splitstree.org). Several reference strains were included in the SplitsTree analysis
163 (*panC* type I: DSM 12442; *panC* type II: WSBC10311; *panC* type III: Ames; *panC* type IV:
164 ATCC 14579; *panC* type V: BCT-7112; *panC* type VI: WSBC 10204; *panC* type VII:
165 NVH391-98).

166

167 *2.4 Detection of B. thuringiensis parasporal crystal*

168 A sporulation assay was performed to identify *B. thuringiensis* isolates. To this end, all
169 isolates were streaked onto T3 plates (Travers et al., 1987), which were incubated for three
170 days at 30°C to promote sporulation. A tiny amount of colony material was mixed with
171 double distilled water on a microscope slide until a homogenous suspension resulted. All
172 strains were checked for the presence of parasporal crystals with diamond, bipyramidal, or
173 spherical shape using a phase contrast microscope (1000 x, oil immersion) (EFSA, 2016).

174

175 *2.5 Vero cell cytotoxicity assay*

176 A Vero cell assay was used to determine cytotoxicity of all isolated *B. cytotoxicus*. Assays
177 were performed using WST-1 bioassay as described elsewhere (Moravek et al., 2006).
178 Reference strains for low (RIVM Bc90) and high-level toxin production (NVH 0075-95) were
179 included in every run. In order to obtain cell-free culture supernatants, strains were grown in
180 30 ml CGY broth in an Erlenmeyer flask and were adjusted to an OD_{600} of 0.05 using an
181 overnight culture of the isolate. The day cultures were incubated at 30°C (120 rpm shaking)
182 until an OD of 7 was reached. After centrifugation at 11000 rpm for 10 min and filtration
183 through 0.2 µm sterile filtres, aliquots of 1 ml supernatants were supplemented with 10 uL 0.1
184 M Na2 EDTA and stored at -80°C.

185

186 **3. Results**

187 *3.1 Identification of B. cereus group species and toxin profiling*

188 We detected *B. cereus s.l.* in 78% of purchased PIF and 92% of mashed potato
189 powders. In total, 28 strains were isolated out of the purchased PIF and mashed potato
190 samples. Six products harbored *B. cereus s.l.* of two or more different colony morphologies
191 on blood agar. Including the 11 strains provided by a PIF producer and the four strains that
192 originated from fruit powder, a total of 43 strains have been characterized.

193 Parasporal crystals were detected in one of the 43 isolates (P21), which exhibited small,
194 round-shaped crystals and originated from powdered infant formula. Nine isolates were
195 classified as *B. cytotoxicus* based on presence of *cytK1* and their affiliation to *panC* group VII.
196 These isolates originated from mashed potato powder from nine different brands. An
197 overview of all other toxin genes detected by PCR is provided in Table 2. All isolates
198 displayed one or more enterotoxin genes, and seven strains carried all three enterotoxin genes
199 (*nheA/B, hblD/A*, and *cytK*). One cereulide-producer was isolated out of a PIF product
200 collected on retail level.

201

202 *3.2 Affiliation of isolates to panC groups and visualization of genetic relatedness in SplitsTree*

203 The 43 isolates represented five different *panC* groups (Table 3). No representatives of
204 group I and VI could be found. All *panC* group VII isolates were positive for *cytK1*. The *B.*
205 *thuringiensis* isolate belonged to *panC* group III. Most of the strains were affiliated with
206 group III, including the strain positive for *ces* (strain P22). In addition to *panC* typing was
207 performed. The similarity of *panC* nucleotide sequences of the isolates was depicted by a
208 SplitsTree (Figure 1). The isolates formed clusters consistent with the results of *panC* typing.
209 Apart from *B. cytotoxicus* isolates, all isolates from mashed potato powder formed a highly
210 homogeneous group and belonged to the cluster exclusively comprising *panC* group III
211 isolates, while strains that originated from PIF and fruit powder showed a higher degree of
212 heterogeneity.

213 *3.3 Cytotoxicity testing of B. cytotoxicus isolates*

214 Cytotoxicity in a Vero cell assay was determined for all *B. cytotoxicus* isolates. Seven
215 out of nine isolates exhibited no detectable cytotoxic effect. One isolate showed very low
216 cytotoxicity and another isolate exhibited cytotoxicity 4.5 times as toxic as the highly toxic
217 reference strain (Figure 2).

218

219 **4. Discussion**

220 The present study revealed a high prevalence of *B. cereus* group species in mashed
221 potato powder and PIF products. *B. cereus* group species were detected in 92% of tested
222 mashed potato powders. Based on varying sample sizes, prevalence rates for *B. cereus* in
223 dehydrated potato products of 74% (Turner et al., 2006) and 10 to 40% (King et al., 2007)
224 have been previously reported. The prevalence in PIF in the current study (78%) is similar to
225 a large study from Becker *et al*. who stated that 70% of the powdered infant formula in

Germany were positive for *B. cereus s.l.* (Becker et al., 1994). Results obtained by *panC* typing were consistent with clusters formed by SplitsTree based on *panC* sequences. A correlation of toxin patterns and *panC* types could however not be seen, except for *panC* group IV, which exclusively comprised isolates positive for *nhe, hbl*, and *cytK2*. Toxin gene profiling of all isolates investigated in frame of this study revealed that all *B. cereus s.s.* harbor *nheA/B*, consistent with previous publications reporting that *nhe* is present in almost all *B. cereus s.s.* (Ehling-Schulz et al., 2011).

Only one *B. thuringiensis* strain was detected by screening for parasporal crystals (data not shown). However, this method may not be fully reliable, as tiny or irregular crystals can be missed (EFSA, 2016). The strain detected in our study was isolated from PIF and assigned to *panC* group III, consistent with previous assignments of *B. thuringiensis* to this *panC* group (Guinebretière et al., 2008). While there were no reports of *B. thuringiensis* in PIF, they are known to be a common contaminant of milk (Bartoszewicz et al., 2008). *B. thuringiensis*-based insecticides are used worldwide in agriculture and are highly effective against different groups of insects (Chattopadhyay et al., 2004) including the Colorado potato beetle - the most destructive insect pest of potato - that is also widespread in Switzerland (Wang et al., 2017). Still, no *B. thuringiensis* strains were detected in mashed potato powder samples investigated in this study.

The cluster analysis and *panC* typing revealed that most of the isolated strains belonged to group III, which has previously been suggested to harbor cytotoxic strains (Guinebretière et al., 2010). To date, outbreaks of emetic illness due to *B. cereus s.l.* have exclusively been associated with this *panC* type (Guinebretière et al., 2010). Notable, apart from *B. cytotoxicus* isolates, this cluster included all isolates obtained from mashed potato powders, while isolates originating from PIF showed much higher phylogenetic heterogenicity.

Mashed potatoes are often served in child-care institutions or hospitals, where they are likely to be held at temperatures promoting growth of germinated bacteria (Turner et al., 2006), before being served to particularly vulnerable groups of humans. To prevent becoming ill with diarrheal or emetic syndrome when eating mashed potatoes, it is essential to keep the food above 60°C or to dispose of it within 2 h as Turner *et al*. have shown (Turner et al., 2006).

FAO and WHO classified *B. cereus s.l.* as an organism of concern in PIF with regard to the strength of evidence of a causal association between the presence of the microorganism in PIF and illness in infants (FAO/WHO, 2006). Indeed, several studies reported high contamination levels of *B. cereus s.l.* in PIF (Rowan et al., 1997; Zhang et al., 2017). Due to the increasing numbers of *B. cereus* infections in infants (Gaur et al., 2001; Hilliard et al., 2003; Wang et al., 2009), EFSA suggests the numbers of *B. cereus s.l.* spores in PIF should be as low as possible (EFSA, 2005). Lequin *et al*. reported three preterm infants with fatal hemorrhagic meningoencephalitis due to *B. cereus* infections (Lequin et al., 2005).

Although *ces*-positive strains have been rarely reported from food samples, their occurrence often resulted in fatalities (Dierick et al., 2005; Naranjo et al., 2011; Takabe and Oya, 1976). In the present study, one cereulide-producer was isolated out of a PIF product which is consistent with other studies (Andersson et al., 2004; Zhang et al., 2017). The presence of a cereulide-producing strain in PIF raises concern, given the fact that this toxin can be preformed in the reconstituted PIF. It was shown by Shaheen *et al.* that PIF containing cereal as well as dairy ingredients are especially conducive for cereulide production (Shaheen et al., 2006).

In contrast to mashed potato powders, no *B. cytotoxicus* could be detected in PIF. Nine isolates were found in mashed potato powder that harbored the *cytK1* variant, which is known to have necrotic and hemolytic activity and whose toxic potential is stated to be higher compared to *cytK2* (Fagerlund et al., 2004). Up to now, only few strains of *B. cytotoxicus*

have been further characterized (Guinebretière et al., 2013). This low number could be due to the fact that isolated *B. cereus s.l.* strains are normally summarized under the term of "presumptive *B. cereus*" comprising all different group members (Ehling-Schulz and Messelhäusser, 2013). The present study revealed a high prevalence of *B. cytotoxicus* in mashed potato powders. This is in accordance with the study of Contzen *et al.* who found a prevalence of 88% in mashed potato powder, flakes and granules (Contzen et al., 2014). All nine *B. cytotoxicus* isolated in the present study could be assigned to *panC* group VII, which is known to exclusively comprise *B. cytotoxicus* (Guinebretière et al., 2008, 2013). Depicting the isolates in a SplitsTree has shown that *B. cytotoxicus* isolates (M12-M20) represent a very remote cluster within the *B. cereus* group, consistent with other phylogenetic analyses using MLST (Fagerlund et al., 2007; Sorokin et al., 2006). It stays unclear why *B. cytotoxicus* has been mostly associated with mashed potato powders (Contzen et al., 2014) or potato purée (Guinebretière et al., 2013), considering that also PIF contain a high level of carbohydrates like starch, sucrose or lactose. Contzen et al. hypothesized that soil may be the source of contamination for mashed potato powders, as they had found *B. cytotoxicus* on a raw potato (Contzen et al., 2014).

The results of the performed cytotoxicity assays in this study suggest that there are few strains which are highly cytotoxic, and which could lead to food poisoning outbreaks, while most *B. cytotoxicus* seem to be non-toxic. However, up to now, cytotoxicity assays have – with one exception - only been performed with strains related to food poisoning cases, thus leading to an overestimation of the cytotoxicity of *B. cytotoxicus* (Fagerlund et al., 2007). The results of the present study support the assumption of Fagerlund *et al.* that harboring the *cytK1* gene is not a sufficient criterion for highly cytotoxic strains (Fagerlund et al., 2007). Fagerlund *et al.* have also shown that the different levels of expression of *cytK1* could not be due to differences in the PlcR-PapR quorum sensing system, which acts as key transcriptional regulator for extracellular virulence factors in *B. cereus* group strains (Fagerlund et al., 2007).

Furthermore, YvrGH and YvfTU two-component systems have also been studied and neither seem to be responsible for the differences in the expression of *cytK1*.

In conclusion, this study shows that *B. cereus s.l.* in mashed potato powders as well as PIF pose a potential food safety risk. Further research is needed to extend the hitherto very limited knowledge on the ecological niches of *B. cytotoxicus* and mechanism of its cytotoxicity. Due to the ubiquity, resistance, and persistence of *B. cereus s.l.* and colonization of processing facilities with spores (Carlin, 2011), contamination of food products is almost impossible to avoid. It is therefore essential that producers uphold highest quality control standards, while consumers should assure good practices such as proper holding times and storage temperatures to protect especially vulnerable consumer groups such as infants or hospital inpatients.

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Competing interests

The authors declare that they have no competing interests.

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455 **TABLES AND FIGURES**

456 **Table 1:** Primers used in this study.

Target gene	Primer	Primer sequence (5' → 3')	Reference
panC	panC_Cyto_for	CGTTATCCAAGGGATATAAAGCGA	This study
	panC_Cyto_rev	TCTACATAATCAACTATACCGTTTG	This study
panC	panC_fwd	CGATATCCTCGTGATATTGATAGA	Sorokin *et al.* (2006)
	panC_rev	TCCGCATAATCTACAGTGGCTTTC	Sorokin *et al.* (2006)
nhe	NA2F	AAGCIGCTCTTCGIATTC	Ehling-Schulz *et al.* (2006)
	NB1R	ITIGTTGAAATAAGCTGTGG	Ehling-Schulz *et al.* (2006)
hbl	HD2F	GTAAATTAIGATGAICAATTTC	Ehling-Schulz *et al.* (2006)
	HA4R	AGAATAGGCATTCATAGATT	Ehling-Schulz *et al.* (2006)
ces	CesF1	GGTGACACATTATCATATAAGGTG	Ehling-Schulz *et al.* (2006)
	CesR2	GTAAGCGAACCTGTCTGTAACAACA	Ehling-Schulz *et al.* (2006)
cytK1	CK1F	CAATTCCAGGGGCAAGTGTC	Guinebretiere *et al.* (2006)
	CK1R	CCTCGTGCATCTGTTTCATGAG	Guinebretiere *et al.* (2006)
cytK2	CK2F	CAATCCCTGGCGCTAGTGCA	Guinebretiere *et al.* (2006)
	CK2R	GTGIAGCCTGGACGAAGTTGG	Guinebretiere *et al.* (2006)

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463 **Table 2:** Toxin genes detected by PCR in a total of 43 *B. cereus s.l.* isolates collected from
464 powdered infant formula (PIF), mashed potato powder, and fruit powder.

	nhe	*hbl*	*cytK1*	*cytK2*	*ces*
PIF^{P} isolates (n = 11)	11	4	0	8	0
PIF^{R} isolates (n = 8)	8	1	0	5	1
Mashed potato powder isolates (n = 20)	12	2	9	6	0
Fruit powder isolates (n = 4)	4	2	0	4	0

465 PIF^{P} Samples obtained at the level of production
466 PIF^{R} Samples obtained at retail level
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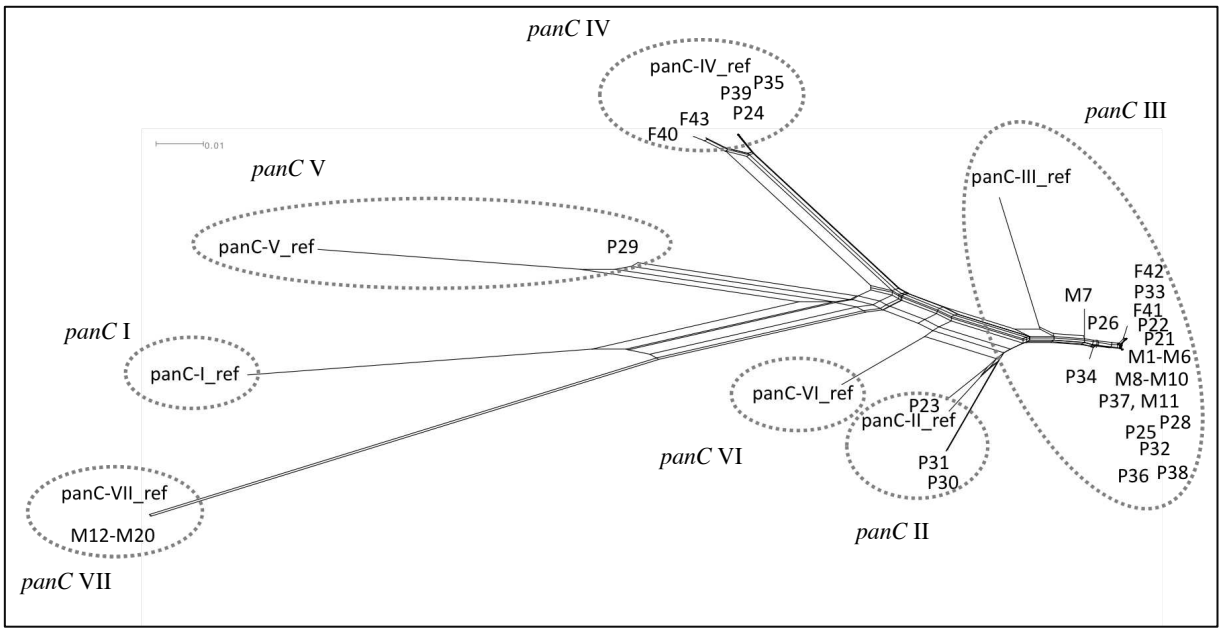
468 **Table 3:** Assignment of 43 *B. cereus s.l.* isolates originating from different food sources to
469 *panC* groups

panC group	PIF^{P} isolates (n = 11)	PIF^{R} isolates (n = 8)	Mashed potato powder isolates (n = 20)	Fruit powder isolates (n = 4)
II	2	1	0	0
III	6	6	10	2
IV	2	1	0	2
V	1	0	0	0
VII	0	0	9	0
NS	0	0	1	0

470 NS = no assignment to any of the *panC* groups I-VII.
471 PIF^{P} Samples obtained at the level of production
472 PIF^{R} Samples obtained at retail level

Figure 1: SplitsTree depicting the degree of similarity of the *panC* sequences. (a) Overview over the full SplitsTree depicting all isolates as well as one reference strain per *panC* type (*panC* type I: DSM 12442; *panC* type II: WSBC10311; *panC* type III: Ames; *panC* type IV: ATCC 14579; *panC* type V: BCT-7112; *panC* type VI: WSBC 10204; *panC* type VII: NVH391-98); (b) Detail zooming in on the region depicting the *panC* type III cluster, while omitting isolates assigned to other *panC* groups. M = isolate originating from mashed potato powder, P = isolate originating from PIF, F = isolate originating from fruit powder.

(a)



(b)

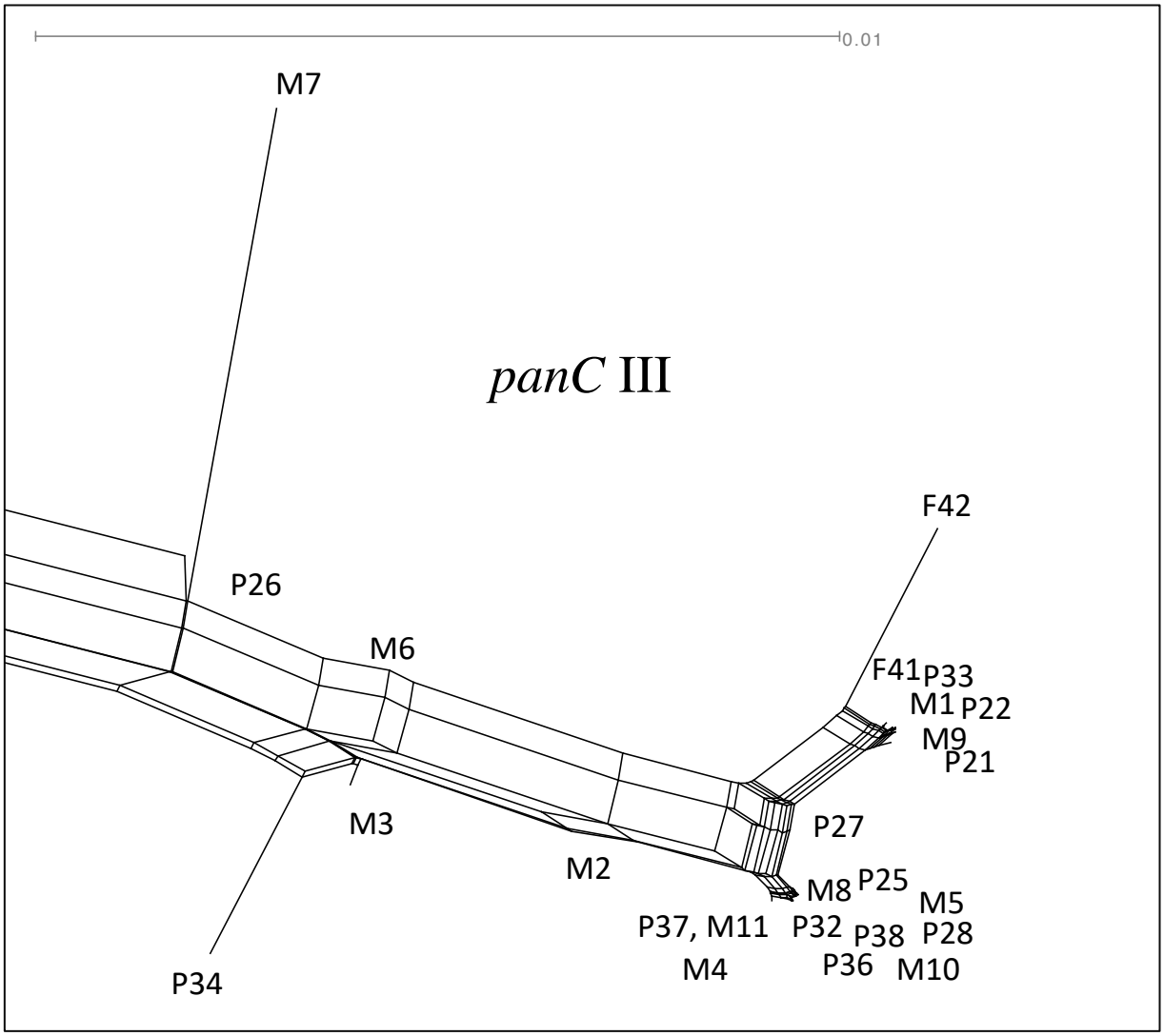

0.01
M7
panC III
F42
P26
M6
F41
P33
M1
P22
M9
P21
M3
P27
M2
M8
P25
M5
P37, M11
P32
P38
P28
M4
P36
M10
P34

Figure 2: Reciprocal cytotoxicity titers of *B. cytotoxicus* isolates M12-M20 and a reference strain for high level toxin production (food poisoning strain *B. cereus* NVH 0075-95). Values indicated are based on supernatants tested in two Vero cell cytotoxicity assays with each dilution of the supernatant tested in duplicate. Error bars represent one standard deviation of the mean.