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Bacillus cereus in powdered foods

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 391-98T 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 widespread 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
stomacher bag using the Stomacher® 400 Circulator (Seward, Worthing, UK) for 30 s. The samples were subsequently incubated at 37°C overnight. After overnight incubation, one loop of the overnight culture was streaked onto Mossel (Mossel et al., 1967) and sheep blood agar plates (BD Difco™ Columbia Blood Agar Base) that were incubated at 37°C overnight. Second, an approach was used that has already been described by Contzen et al. in order to detect B. cytotoxicus (Contzen et al., 2014). This included enrichment of the powder in 90 ml CGY medium (Beecher and Wong, 1994) followed by incubation at 50°C overnight. The next day, a loopful of the enriched culture was streaked onto Mossel and blood agar plates that were subsequently incubated at two different temperatures, 37°C (Mossel) and 50°C (blood agar), respectively. In the present study, the minor modification was made that the culture and the blood plates were incubated at 46°C instead of 50°C. Mossel plates were checked for colonies showing an egg-yolk lecithinase-positive and mannitol-negative phenotype characteristic for isolates of the B. cereus group.

2.2 DNA extraction and toxin gene profiling

DNA was extracted from all isolates using the GenElute Bacterial Genomic DNA Kit according to the manufacturer’s instructions (Sigma-Aldrich, St. Louis, MO). Toxin gene profiles were determined using a PCR approach as previously described by Ehling-Schulz et al. (Ehling-Schulz et al., 2006) with minor modifications: The GoTaq PCR system (Promega AG, Dübendorf, Switzerland) was used at (i) 2 min at 95°C, (ii) 30x [45 s at 95°C, 45 s at 51°C, 2 min at 72°C]; (iii) 5 min at 72°C. The respective forward primer used for detection of the nhe complex is located in nheA while the reverse primer is located in nheB, thus enabling detection of the first and second gene of the nhe operon. The respective primers for hbl are located in hblD and hblA, thus allowing for detection of the second and third gene of the hbl operon. Moreover, a duplex PCR was carried out to distinguish between cytK1 and cytK2 as previously described (Guinebretière et al., 2006).
2.3 Genotyping using panC

A PCR-based genotyping approach targeting panC was performed (Guinebretière et al., 2008). In cases in which previously published panC primers did not result in an amplicon, additional primers designed in this study were used (see Table 1). The following cycling 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]; (iii) 5 min at 72°C. The PCR products were purified with the GenElute™ PCR Clean-Up Kit according to the manufacturer’s instructions. Subsequently, the sample’s concentration and purity were measured using a NanoDrop™ Fluorospectrometer (Witec AG, Luzern, CH). Sequencing was outsourced (Microsynth™, Balgach, CH). Sequences of panC were assigned to seven (I-VII) phylogenetic groups as previously described (https://tools.symprevius.org/Bcereus/english.php) (Guinebretière et al., 2008, 2010).

Cluster analysis of panC sequences was performed with the SplitsTree™ software (http://www.splitstree.org). Several reference strains were included in the SplitsTree analysis (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).

2.4 Detection of B. thuringiensis parasporal crystal

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

A Vero cell assay was used to determine cytotoxicity of all isolated *B. cytotoxicus*. Assays were performed using WST-1 bioassay as described elsewhere (Moravek et al., 2006). Reference strains for low (RIVM Be90) and high-level toxin production (NVH 0075-95) were included in every run. In order to obtain cell-free culture supernatants, strains were grown in 30 ml CGY broth in an Erlenmeyer flask and were adjusted to an OD$_{600}$ of 0.05 using an overnight culture of the isolate. The day cultures were incubated at 30°C (120 rpm shaking) until an OD of 7 was reached. After centrifugation at 11000 rpm for 10 min and filtration through 0.2 µm sterile filters, aliquots of 1 ml supernatants were supplemented with 10 µL 0.1 M Na$_2$ EDTA and stored at -80°C.

3. Results

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

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

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

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

3.3 Cytotoxicity testing of B. cytotoxicus isolates

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

4. Discussion

The present study revealed a high prevalence of B. cereus group species in mashed potato powder and PIF products. B. cereus group species were detected in 92% of tested mashed potato powders. Based on varying sample sizes, prevalence rates for B. cereus in dehydrated potato products of 74% (Turner et al., 2006) and 10 to 40% (King et al., 2007) have been previously reported. The prevalence in PIF in the current study (78%) is similar to 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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### 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_fwd     | CGATATCCTCGTGATATTGATAGA                        | Sorokin et al. (2006)         |
|             | panC_rev     | TCCGCTTTAATCTACAGTGGCTTTTC                     | Sorokin et al. (2006)         |
| nhe         | NA2F         | AAGCIGCTCTCGIATTC                               | Ehling-Schulz et al. (2006)   |
|             | NB1R         | ITIGTGAATAAGCTGTGG                              | Ehling-Schulz et al. (2006)   |
| hbl         | HD2F         | GTAAATTAIGATGAICAATTTTC                         | Ehling-Schulz et al. (2006)   |
|             | HA4R         | AGAATAGGCATTTCATAGATT                          | Ehling-Schulz et al. (2006)   |
| ces         | CesF1        | GGTGACACATTATCATATAAAGGTG                      | Ehling-Schulz et al. (2006)   |
|             | CesR2        | GTAAGCGAACCTGTCTGTAACAAACA                     | Ehling-Schulz et al. (2006)   |
| cytK1       | CK1F         | CAATTCCAGGGGCAAGTGC                            | Guinebretiere et al. (2006)   |
|             | CK1R         | CCTCGTTGCATCTGTTTCATGAG                        | Guinebretiere et al. (2006)   |
| cytK2       | CK2F         | CAATCCCTGGCCGTAGTGC                            | Guinebretiere et al. (2006)   |
|             | CK2R         | GTGIAGCCTGGACGAAGTTGG                          | Guinebretiere et al. (2006)   |
Table 2: Toxin genes detected by PCR in a total of 43 *B. cereus s.l.* isolates collected from powdered infant formula (PIF), mashed potato powder, and fruit powder.

|                        | nhe | hbl | cytK1 | cytK2 | ces |
|------------------------|-----|-----|-------|-------|-----|
| PIF<sup>P</sup> isolates (n = 11) | 11  | 4   | 0     | 8     | 0   |
| PIF<sup>R</sup> 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   |

PIF<sup>P</sup> Samples obtained at the level of production

PIF<sup>R</sup> Samples obtained at retail level

Table 3: Assignment of 43 *B. cereus s.l.* isolates originating from different food sources to panC groups

| panC group | PIF<sup>P</sup> isolates (n = 11) | PIF<sup>R</sup> 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                             |

NS = no assignment to any of the panC groups I-VII.

PIF<sup>P</sup> Samples obtained at the level of production

PIF<sup>R</sup> 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.
(b) panC III
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.