

Toxin production of *Bacillus cereus* isolates from a milk powder plant

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1 Introduction

Bacillus (B.) cereus known to cause spoilage in a variety of foods is of high significance in food hygiene by causing food-poisoning mostly in connection with rice, but also with milk and milk products.

Milk powder as a basic material for the production of sensitive food like babyfood and desserts is of main interest in this context.

Types of food-borne illness caused by *B. cereus* toxins are diarrhoea or emesis or a combination of both (6). Toxins identified are two enterotoxin complexes and two single toxins.

Two enterotoxin complexes

They show highest toxicity if all three components of each complex are present.

- One is hemolysin BL with three single components (B, L₁, L₂) and molecular weights (MW) of about 37.5, 38.2 and 43.5 kDa respectively (1). The L₂ component is recognised in the Oxoid RPLA[®] kit (2). The three components together possess hemolytic, cytotoxic, dermonecrotic, and vascular permeability activities. They induce rapid fluid accumulation in ligated rabbit ileal loops (1).
- The other is a non-hemolytic complex with MW's of 39, 45 and 105 kDa. Neither of the fractions is as cytotoxic alone as when the fractions are combined. The 45 kDa protein is the main antigen detected in the Tecra[®] ELISA ("diarrhoeal toxin"). The 39 kDa protein showed some similarity to the L₁ protein of hemolysin BL (2).

Two single toxins

No routinely applicable tests for the detection are available.

- The ring structure of the emetic toxin is known, the MW is 1.2 kDa. The so called cereulide is a dodecapeptide, it structurally resembles the antibiotic valinomycin and also causes swelling of the mitochondria of HEP-2 cells. AGATA et al. suggest that cereulide causes emesis through the 5-HT₃ receptor and stimulation of the vagus afferent. By heating for 30 min. at 121°C the activities of both vacuole-formation and emesis effect were never lost (3).
- The enterotoxin T was discovered in 1995. It causes VERO-cell cytotoxicity, fluid accumulation in ligated mouse ileal loop, was positive in vascular permeability assay, and lethal to mice upon injection. The protein has a calculated MW of 41039 Da (4).

Within the scope of this paper the toxin production of *B. cereus* strains isolated from milk powder and the environment of the production unit is examined.

2 Materials and methods

2.1 *B. cereus* strains

106 *B. cereus* strains isolated from 1057 samples of milk powder } same
139 *B. cereus* strains isolated from 171 samples from the environment } dairy

4 reference strains (Dr. A. Christiansson, Swedish Dairies' Association), used as controls

2.2 Tests for the detection of toxins

- Detection of cytotoxicity (VERO-cell test):
 - A VERO-cell (African green monkey kidney cells) culture was overlaid with sterile filtered culture supernatant of each *B. cereus* strain. After 48h the degeneration of the VERO-cells was microscopically evaluated.
 - The release of lactate dehydrogenase (LDH) of the VERO-cells was measured photometrically (LDH-L testkit, Sigma). Damage of cellmembranes leads to significant increase of LDH (5).
- Detection of the diarrhoeal enterotoxin:
 - The Tecra[®] ELISA (Bioenterprises, Australia) was used according to the manufacturers' instructions. It detects the 45 kDa component of the non-hemolytic enterotoxin complex (2).

3 Results

3.1 Cytotoxicity (n = 245 *B. cereus* strains)

3.1.1 Microscopic evaluation

- Judgement:
 - Different criteria of cytotoxicity are roundness of the polygonal cells, retraction of the runners, vacuolisation of the cytoplasm, and death of the VERO-cells.
- ⇒ Cell damage was caused by 117 of the tested *B. cereus* strains as demonstrable by microscopy.

3.1.2 LDH-release

- Judgement:
 - Measurement of the positive-control is taken as 100%, the single reading of each strain is set in relationship to the positive-control.
 - A statistically significant increase of LDH-release >20% was defined as positive (T-test) (Figure 1).
- ⇒ 142 *B. cereus* strains were LDH-positive.

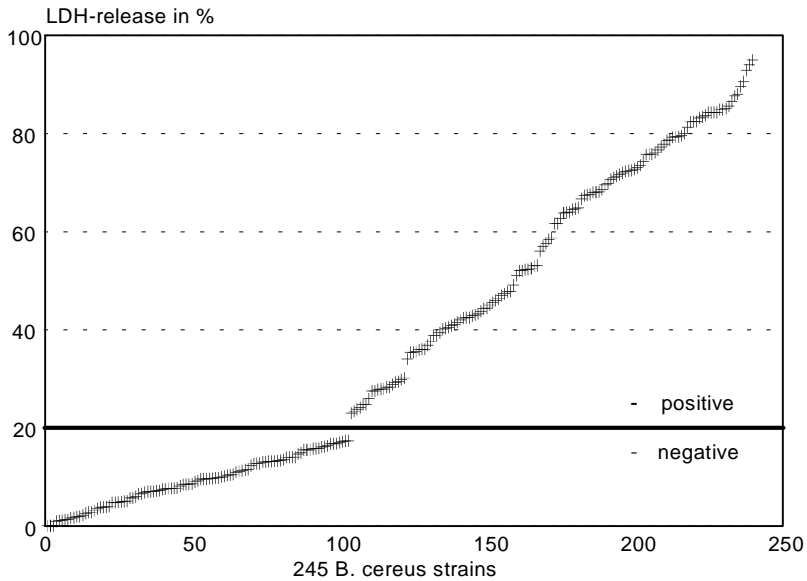


Figure 1: Release of lactate dehydrogenase (LDH) from VERO-cells, ascending order of the results of the *B. cereus* strains (n = 245)

3.2 Diarrhoeal toxin (Tecra[®] ELISA)

- Visual evaluation was done according to the manufacturers' instructions (panel 1-2 negative, 3-5 positive).
- For quantitative evaluation of the ELISA a standard was used (value of record: 1ng toxin/ml) (Table 1, Figure 2)

| Table 1: Qualitative and quantitative evaluation of the Tecra [®] ELISA | | |
|--|--------------------|-----|
| qualitative panel | quantitative ng/ml | n |
| 1 | 0-0.5 | 7 |
| 2 | 1.6 | 1 |
| 3 | 2.5-5.7 | 6 |
| 4 | 4.3-63.9 | 127 |
| 5 | 31.0-303.8 | 111 |

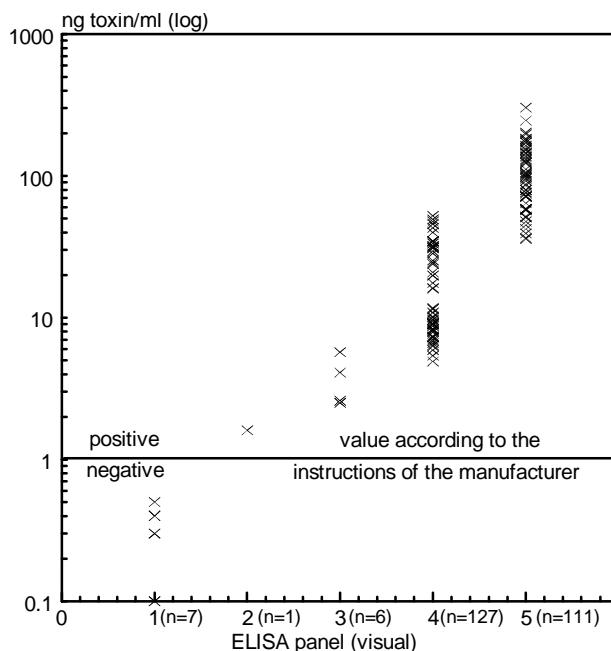


Figure 2: Value of the Tecra[®] ELISA

4 Conclusions

In the VERO-cell test supernatants of 142 *B. cereus* strains (n=245) showed cytotoxicity as evaluated by LDH-release and 117 strains of these also by morphological evaluation. 237 *B. cereus* strains were positive in the Tecra[®] ELISA including all strains positive in the VERO-cell test (Table 2).

| Table 2: Summarized results of toxin detection produced by <i>B. cereus</i> from a milk powder plant | | | |
|---|--------------------------|--------------|--------------|
| Test | | positive (%) | negative (%) |
| VERO-cell test | morphological evaluation | 117 (48) | 128 (52) |
| | LDH-release | 142 (58) | 103 (42) |
| Tecra [®] ELISA | | 237 (97) | 8 (3) |

Maximal cytotoxicity of the non-hemolytic enterotoxin complex depends on the presence of all three components, although binary combinations cause some activity at higher levels (2). The 45 kDa protein of this complex is the main protein reacting with the Tecra[®] ELISA (2), however, it is only one component and not mainly responsible for cytotoxicity by itself. Therefore the VERO-cell test gives a more reliable estimation of cytotoxicity.

Hemolysin BL, enterotoxin T and the emetic toxin which were not determined may also be of influence on the VERO-cell test. Hemolysin BL and enterotoxin T both have cytotoxic activity (1, 4) and the emetic toxin has a HEP-2 cell-vacuolation factor (3).

Nevertheless, to perform a proper risk assessment on the hazard of food poisoning due to a certain *B. cereus* strain an estimation of cytotoxicity by the VERO-cell test is superior to the Tecra[®] ELISA.

5 Summary

Bacillus (B.) cereus is known to cause spoilage and food-poisoning in a variety of foods including milk and milk products.

Typical forms of intoxication with *B. cereus* are diarrhoea and/or vomiting.

An especially concerned product in this context is milk powder because it is used as a basic material in sensitive foods like babyfood and desserts.

Two enterotoxin complexes (hemolysin BL, non-hemolytic toxin) each consisting of three components are described and two single toxins: the emetic toxin and enterotoxin T.

In 96.7% (237 out of 245) of the *B. cereus* strains examined the diarrhoeal enterotoxin was detected qualitatively by the Tecra[®] ELISA. The quantitative record of toxin gave a range from 0 to 303.8ng/ml. 58.0% (142) of the collected strains showed cytotoxicity in the VERO-cell test. All those strains were positive in the Tecra[®] ELISA either.

The Tecra[®] ELISA detects only a single component of the non-hemolytic complex, but the toxicity depends on the presence of all three components. A result of more concern for the health of the consumer can be expected from cytotoxicity testing applying the VERO-cell test. Hemolysin BL, enterotoxin T and probably the emetic toxin are also of influence on this test.

Acknowledgements

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