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Biochemical and toxic diversity of *Bacillus cereus* in a pasta and meat dish associated with a food-poisoning case

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Abstract

A dish of pasta and minced meat caused severe food-poisoning involving both emesis and diarrhoea in two adult persons. Emetic toxin producing strains of *Bacillus cereus* formed the majority (68% of 122) of strains identified in this food. Haemolytic diarrhoeal toxin was produced by 26% of the strains studied and 6% of the strains produced neither emetic nor haemolytic diarrhoeal toxin. The *B. cereus* strains isolated from this dish could be divided into four biochemically distinct groups and two different colony morphologies. All emetic toxin producing strains ($n = 83$) were negative for both haemolytic enterotoxin and starch hydrolysis in contrast to haemolytic enterotoxin producers ($n = 32$). Colonies of emetic toxin producing strains were poorly haemolytic, $\leqslant 2$ mm zones, in contrast to the diarrhoeal colonies, 4–5 mm zones. This disparity persisted after extended incubation using blood agar supplemented with lithium chloride. Despite the wide diversity of *B. cereus* biotypes in this single food all emetic toxin producers exhibited narrow haemolysis with negative starch hydrolysis. The findings emphasize that colonies with different properties should be isolated when food-poisoning cases are studied.

Keywords: *Bacillus cereus*; Emetic toxin; Diarrhoeal toxin

1. Introduction

Bacillus cereus is a common food-poisoning organism. Its capacity to produce both emetic and diarrhoeal disease is well known (Kramer and Gilbert, 1989; Granum and Lund, 1997; Gilbert and Humphrey, 1998; Beattie and Williams, 2000). It was earlier suspected that one strain might cause both diseases, since both types of symptoms often occur simultaneously. Agata et al. (1996) showed that a specific class of *B. cereus* causes the emetic symptoms.

B. cereus has been reported as the causative agent in 1–22% of foodborne outbreaks in Europe, Japan and North America over the period 1960–1992. In Norway and The Netherlands, with their effective surveillance, it is the most frequently isolated bacterial foodborne pathogen (Beattie and Williams, 2000). Emetic toxin producers may also occur in some foods, e.g. certain beans (Mikami et al., 1994). In food-poisoning cases only one or a few colonies are commonly analysed for toxin production. In the absence of any commercial method for detecting the emetic toxin, colonies are analysed only for the diarrhoeal toxin in routine laboratories. This is likely to lead to underestimation of *B. cereus* as a causative agent of foodborne diseases.

In this study a large number ($n = 122$) of isolates from a single food involved in food-poisoning by *B. cereus* was analysed to reveal the biochemical and toxic diversity of *B. cereus* that may exist in a food commodity. The emetic toxin was detected in vitro by the boar spermatozoan motility test and identified as cereulide by LC-MS analysis (Andersson et al., 1998).

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2. Materials and methods

2.1. Sample

A frozen sample of a home-made pasta dish, which had caused food-poisoning in two adults, was received by the National Veterinary and Food Research Institute (EELA) in Helsinki from the Municipal Food Control Laboratory of Seinäjoki, Finland. The ingredients of the dish were pasta, minced meat, milk, eggs and salt; the mixture was baked in oven and left to cool at room temperature. Two persons ate the dish about 4 h after baking and repeatedly 2 days later. On the first day one of the persons got mild symptoms with nausea soon after eating. After the latter meal both got more severe symptoms with vomiting and nausea about 1 h after eating and diarrhoea after some hours. The symptoms were typical to those described for emetic *B. cereus* food-poisoning cases (Gilbert and Humphrey, 1998). The dish was stored in refrigerator between the two meals. The diners felt a bitter taste in the dish already at the first meal.

B. cereus was subsequently enumerated from the sample on bovine blood agar (BA) according to the method of NMKL No. 67 (1997) at the Seinäjoki laboratory.

At EELA, the frozen food was thawed and analysed for *B. cereus* on BA (BBL, BD, Cockeysville, Maryland, USA; 5% citrated blood according to NMKL 67 (1997), and on mannitol–yeast extract–polymyxin–agar (MYP) (LabM, Lancashire, UK) according to ISO 7932 (1993) using replicate plates.

2.2. Analysis of the isolates

The diarrhoeal toxin was analysed by immunolatex assay using the BCET-RPLA (Oxoid Ltd, Hampshire, UK), which measures the haemolytic component of *B. cereus* diarrhoeal enterotoxin. Starch hydrolysis was detected from starch agar plates (Nutrient agar, Oxoid, with 1% of starch, Merck, Darmstadt, Germany) incubated at 30°C for 3–5 d as described by Pirttijärvi et al. (1999). The ability to produce lecithinase was read from MYP plates grown for 30/48 h at 30°C (ISO, 7932). Emetic toxin was detected by the rapid sperm micro-assay (Andersson et al., 2004) and by chemical analysis with LC-iontrap-MS (Häggblom et al., 2002). The width of the haemolytic zone was measured on BA (5% of citrated bovine blood) after 24 h at 30°C. Zones under 4 mm were considered narrow.

In addition to the analyses of the toxins and starch and lecithin hydrolysis, 12 strains isolated from the suspected food along with strains isolated from vanilla sauce ($n = 2$), green tea ($n = 2$) and rice ($n = 1$) and connected to food-poisoning cases were tested for haemolysis on BA supplemented with polymyxin B sulphate (8.0 mg l^{-1}; Sigma P-1004, Sigma Chemical, St. Louis, Missouri, USA) and BA supplemented with polymyxin B sulphate (8.0 mg l^{-1}) and lithium chloride (5.0 g l^{-1}; Sigma L-0505).

3. Results

The minced meat and pasta dish had caused severe food-poisoning, involving both emesis and diarrhoea, in two adult persons. The following day the remains of the food were plated on BA in the local laboratory and found to contain 7.3×10^7 cfu g^{-1} of *B. cereus*. The single isolate sent to EELA by the local laboratory for analysis of toxin production was found to produce haemolytic diarrhoeal toxin. A food sample, stored frozen, was also sent to EELA and was repeatedly enumerated for *B. cereus* after thawing. The *B. cereus* count was 1.3 and 1.8×10^5 cfu g^{-1} on MYP and BA, respectively. A total of 127 colonies were isolated from the replicate plates of BA and MYP (Table 1) and 122 (96%) were identified as *B. cereus*.

All colonies identified as *B. cereus* were tested for haemolysis, lecithinase, starch hydrolysis, and production of the emetic toxin (cereulide) and the haemolytic diarrhoeal toxin (Table 2). Thirty-seven colonies (30%) gave a wide (> 4 mm) and 85 colonies (70%) a narrow (1–2 mm) zone of haemolysis on BA. All colonies giving narrow haemolysis were negative for starch hydrolysis and positive for lecithinase production. Eighty-three (98%) of the 85 starch negative isolates produced toxin that behaved as cereulide in the boar sperm micro-assay. None of these isolates produced the haemolytic diarrhoeal toxin.

All the colonies that showed a wide zone of haemolysis ($n = 37$) on BA hydrolysed both starch and lecithin. None of these isolates produced a toxin behaving similarly to cereulide in the sperm micro-

Table 1
The origins of 127 colonies from *B. cereus* isolation plates of a minced meat pasta dish implicated in food-poisoning

Agar and dilution	*B. cereus*	Other *Bacillus* species
MYP, 10^{-4}, all colonies	26	1
BA, 10^{-4}, all colonies	35 (5[a])	
MYP, 10^{-3}, all colonies in a single sector	37	4
BA, 10^{-3}, detached colonies	24 (20[a])	
Total	122	5

MYP = mannitol yeast extract polymyxin agar, BA = bovine blood agar.
[a] Colony morphology more rough than typical for *B. cereus*.