The Occurrence of Enterotoxigenic Isolates of *B. cereus* in Foodstuffs

Josef BRYCHTA[1], Jiří SMOLA[2], Petr PIPEK[3], Jaroslav ONDRÁČEK[2], Vladimír BEDNÁŘ[2], Alois ČÍŽEK[3] and Tomáš BRYCHTA[3]

[1]State Veterinary Institute, Jihlava, Czech Republic; [2]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic; [3]Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

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Enterotoxigenic *Bacillus cereus* was detected in a variety of meat stuffs (36), ready-to-cook products (5), and swabs (7). The bacterial colonies isolated from PEMBA agar were identified as *B. cereus*. The 85 isolates were examined for the enterotoxin production using both TECRA-VIA and BCET-RPLA kits (ELISA – 47, RPLA – 38). Thirty two isolates (66%) were positive for enterotoxin using the ELISA test while only 15 isolates (39%) gave positive results in the RPLA test system. In total, 178 (91.8%) and 164 (84%) of the strains isolated in our laboratory (from various foods) were enterotoxigenic as determined using TECRA-VIA and BCET-RPLA, respectively. Parallel enterotoxin positive results obtained using both tests were demonstrated in only 9 isolates from 19 assessed (47.4%). Coincidental negative results from both kits were established for 3 isolates (15.8%) only. The isolates of *B.cereus* from meat were resistant to cephalothin (57%), clindamycin (14%), oxytetracycline (14%), and erythromycin (7%). The isolates from swabs were resistant to cephalothin (83%), erythromycin (16%), clindamycin (16%) and enrofloxacin (16%).

Keywords: *Bacillus cereus*; enterotoxin; bacterial resistance; foodstuffs; ELISA; RPLA

B. cereus is considered to be ubiquitous microorganism occurring in soil, dust, water etc. It is logical that *Bacillus cereus* can also be found in foodstuffs of vegetable and animal origins. *B. cereus* can have a negative influence on human health (Notermans & Batt 1998; Granum *et al*. 1993, 1995; Granum 1994), it is an etiologic agent of the diarrhoeic and emetic syndromes in humans (Batt 1998).

B. cereus is highly resistant and can survive extremely severe environmental conditions. This ability to survive is based on its well known spore forming capability. The spores of *B. cereus* are highly hydrophobic and readily adhere to stainless steel, as well as to glass and rubber surfaces (Flint *et al*. 1997, 2001). The so called short clearing programmes are not able to eliminate the spores present on such surfaces. The spores are activated by heat. They can germinate quickly, adhering to surfaces and creating biofilms. The subsequent removal and destruction of *B. cereus* are extremely complicated and difficult procedures (Svensson

et al. 2000). The hydrophobic capability of *B. cereus* is a very important factor related to this adhesion. Adherence has been suggested as a significant virulence factor of *B. cereus* (Marshal 1984, 1992). Hence, the meat processors try to take advantage of a number of auxiliary steps in order to reduce the microbial contamination of meat (Pipek *et al*. 1999, 2005).

B. cereus causes food poisoning characterised by a number of different syndromes (emetic and diarrhoeal) with variable intensities (Christianson *et al*. 1989). *B. cereus* produces a number of toxic products that are important virulence factors and participate in the course of foodborne human gastrointestinal diseases (Thompson *et al*. 1984; Beecher & Wong 1997; Ryan *et al*. 1997). Generally, diagnostic methods aim at the detection of these enterotoxigenic strains, using methods for the direction enterotoxin complexes or PCR methods for the determination of genes responsible for the production of individual enterotoxins (Phelps & Mc Killip 2002; Ehling-Schulz *et al*. 2005). Of the large number of the toxins produced, this contribution presents the results concerning the detection of the major enterotoxins haemolysin BL (HBL) and nonhaemolytic enterotoxin (NHE).

HBL, also known as haemolytic enterotoxin, consists of three protein components characterised as B, L1, and L2 (Ryan *et al*. 1997). Component B binds the HBL toxin to the targeted intestinal cells, the exact receptors remaining unknown. This is followed by the binding of the lytically active L1 and L2 components. In such a way, colloidal osmotic lysis occurs (Beecher & Wong 1997; Beecher *et al*. 1995), resulting in the destruction of the enterocytes cell membranes. Component L2 has a molecular mass of 43 kDa and appears to be a key factor in pathogenesis as it has been shown to increase the activity of other HBL enterotoxic components (Granum & Lund 1997; Prüss *et al*. 1999). The toxin component L2 can be detected *in vitro* by the RPLA test produced by OXOID Ltd. (Brno, Czech Republic). As this component needs not be produced by all *B. cereus* strains, the RPLA test (Anonymous 2003b) was selected for use throughout this work.

NHE is a three component toxin which comprises proteins with molecular weights of 39, 45, and 105 kDa (Lund & Granum 1996; Phelps & Mc Killip 2002). In this study, a commercially produced ELISA based test (Anonymous 2003a) was chosen for the detection of this complex of the test toxins. The test confirmed the reported sensitivity of less than 1ng/ml of the sample prepared.

MATERIALS AND METHODS

Material. The isolates of *B. cereus* under study originated from the samples of foodstuffs (27 samples), ready-to-cook products (7 samples), and swabs (6 samples) received in our laboratory by routine microbiological testing. The selection of samples was made randomly. There was no indication or prior knowledge of the samples, producers, or risks to the consumers.

Methods. Cultivation procedures – The laboratory tests were performed in compliance with the accredited method for *B. cereus* count determination based on CSN ISO 7932 (Anonymous 1995). This method comprises colony count techniques following the cultivation at 30°C on the recommended growth media MYP agar, PEMBA agar, Chromogenic *B. cereus* agar with subsequent confirmation.

Evidence of enterotoxins. Two commercial tests for *B. cereus* determination were used:
BCET-RPLA test from OXOID code TD950
ELISA-(BDE VIA 48) test from Tecra International Pty, Ltd

The isolates for enterotoxin testing were prepared following the manufacturers' instructions. It was noted that neither test was able to detect and/or quantify the complete spectrum of *B. cereus* toxicity. The RPLA method reacting with L2 component does not detect the strains producing only NHE, while the ELISA kit specifies 45 kDa and 105 kDa proteins from the NHE complex but misses the HBL complex (Lund & Granum 1996). However, a positive reaction detected with one or both commercial kits used in parallel was considered as evidence for the strain being enterotoxin-positive. This was in accordance with Granum & Lund (1997) who stated that, if the culture supernatants gave cytotoxic results, they could be deemed enterotoxin-positive.

Methodology of quantitative determination of *B. cereus* sensitivity to antimicrobial substances was used for monitoring the resistance of Gram-positive bacteria (producer National Veterinary Institute, Uppsala, Sweden). The antimicrobial substances for the testing of sensitivity and resistance, respectively, were obtained through commercial composition of the set for Gram-positive bacteria with the following substances:

Table 1. Evidence for enterotoxin production by *B. cereus* isolates coming from meat products and spices

Description of samples	Enterotoxins proof test		
	ELISA (NHE)		RPLA (HBL) HBL (ng)
	absorbance (A)	interpretation	
Instant goulash soup	0.963	+	0
Instant vegetable soup with pasta	0.557	+	0
	0.212*	–	ND
	0.723	+	0
Instant soup	0.327	+	32
	1.060	+	0
Instant vegetable soup with pasta	0.881	+	0
Instant goulash soup	1.166	+	0
Vegetable bouillon	0.792	+	0
	0.917*	+	ND
Instant vegetable soup with pasta	0.278	–	0
	0.864	+	0
Instant soup	0.327	+	32
Coctail salad	1.021	+	0
	0.810*	+	ND
Parisien style salad	0.619	+	8
Beef steak spread	2.458	+	0
	2.182*	+	ND
Beef steak spread	0.564	+	0
Dried meat product	0.996	+	0
	0.386*	+	ND
Brawn	0.670	+	0
	0.514*	+	ND
Dried salami	0.118	-	0
Fermented meat product	0.375	+	0
	0.614*	+	ND
Chicken thighs	0.459	+	64
Chicken wings	0.150	–	16
Game	0.712	+	0
Steak tartare	0.424	+	64
Game seasoning mixture	0.548	+	0
	0.439*	+	ND
Ground meat seasoning	0.724	+	0
Steak seasoning mixture	0.816	+	0
	0.748*	+	ND
Seasoning mixture for grilling	0.555	+	0
Positive (in sum)	32		6
Negative (in sum)	4		21

NHE = nonhaemolytic enterotoxin; HBL = haemolytic enterotoxin; ND = not performed; + = positive assessment; – = negative assessment; * = repeated tests (after 2 years)

Penicillin (Pc), Oxytetracycline (Tc), Cephalotin (Cl), Fusidic acid (Fu), Oxacyline + 2% NaCl (Ox), Gentamycin (Ge), Erythromycin (Em), Neomycin (Nm), Chloramphenicol (Cm), Enrofloxacin (Ef), Clindamycin (Cl), andTrimethoprim (Trim).

Statistics. The results obtained were assessed by means of Mc Namara test (Hend 2006).

RESULTS

Cultivation

The results of the tests of raw materials and samples from processing units and retail shops confirmed that the frequency of *B. cereus* isolation from the samples was high, but that the above-limit recovery of *B. cereus* was very sporadic. During 2002–2004, *B. cereus* was detected in the following samples of foodstuffs and at the levels (CFU/g): cheese cream (4×10^5 CFU/g), egg melange (1.6×10^3 CFU/g), cheese spread (7×10^3 CFU/g), condensed sweetened milk (3×10^2 CFU/g), and processed cheese (4×10^3 CFU/g and 1.5×10^4 CFU/g). None of these samples demonstrated the occurrence of *B. cereus* enterotoxins. During 2002–2004, the proportion of unsatisfactory samples of foodstuffs because of *B. cereus* CFU (in accordance with the Czech Republic legislation in force) was 0.4%. (In 2002, 2 samples; from 482 were unsatisfactory in 2003 it was 1 from 236 samples, in 2004 were 3 from 514 samples.) This level of contamination corresponded to the recent and historic data from other countries (Champagne *et al.* 1994; Ahmed *et al.* 1995; Te Gieffel *et al.* 1996; Pirttijärvi *et al.* 2000; Lake *et al.* 2004).

Production of enterotoxins

Meat products isolates. Thirty two isolates (89%) out of 36 tested by ELISA test were found to be enterotoxin producers, while 11% of isolates did not produce NHE enterotoxin. The use of the RPLA test showed the production of enterotoxin by 6 isolates of 27 tested (22%). The ELISA test revealed a higher percentage of the enterotoxin production (89%) in comparison with the RPLA test (22.2%). Positive results with both tests were found in 5 of 8 parallel evaluated isolates (62%). The conformity in negative results of both methods was demonstrated in the case of two isolates (25%). Statistical evaluation of both methods (ELISA and RPLA) by means of Mc Nemara test indicated a highly significant difference in performance ($P = 5.7 \times 10^{-5}$). The results showed that the simultaneous production of NHE enterotoxin with HBL was observed most frequently with the isolates of *B. cereus* originating primarily from spreads, instant products, and various spices. The difference between the numbers of isolates tested with RPLA and ELISA was given by the former Czech

Table 2. Evidence for enterotoxin production by *B. cereus* isolates coming from foodstuffs samples and ready-to-cook products

Description of samples	Enterotoxins proof test		
	ELISA (NHE)		RPLA (HBL) HBL (ng)
	absorbance (A)	interpretation	
Yeast dumpling	0.282	–	32
	0.282	–	32
Pasta	0.746	+	0
Rice pudding	0.263	–	64
Potato salad	0.320	+	32
Positive (in sum)	2		4
Negative (in sum)	3		1
Positive (ELISA+ RPLA)	1		0
Negative (ELISA+ RPLA)	0		0

NHE = nonhaemolytic enterotoxin; HBL = haemolytic enterotoxin; + = positive assessment; – = negative assessment

Table 3. Evidence for enterotoxin production by *B. cereus* isolates coming from swab samples taken from processing area

Description of samples	Enterotoxins proof test		
	ELISA (NHE)		RPLA (HBL) HBL (ng)
	absorbance (A)	interpretation	
Swab – dairy plant	0.922	+	64
	0.672	+	16
	0.466*	+	ND
	0.231	–	32
Swab – meat production	0.251	–	8
	0.605	+	128
Swab – cooked products	0.085	–	0
Positive (in sum)	4		5
Negative (in sum)	3		1
Positive (ELISA+ RPLA)	3		0
Negative (ELISA+ RPLA)	1		0

NHE = nonhaemolytic enterotoxin; HBL = haemolytic enterotoxin; ND = not performed; + = positive assessment; – = negative assessment; * = repeated tests (after 2 years)

legislation which preferred RPLA test. The ELISA test was applied later while the previously tested isolates were accidentally destroyed by a laboratory worker (Table 1).

Isolates from ready-to-cook foodstuffs. By means of the ELISA test, it was possible to confirm 2 of the 5 isolates tested (40%) as the enterotoxin producers. Using the RPLA test, the production of enterotoxins was found in the case of 4 isolates (80%). The conformity of the positive results of both methods was 20% while the conformity of negativity was not proven (Table 2). It is apparent from these results that the isolates of *B. cereus* with a high production of NHE enterotoxin come largely from the pasta products. However, the HBL enterotoxin production was found in rice, where the NHE enterotoxin was not found. The low number of isolates tested made the performance of the statistical evaluation by Mc Nemara test impossible.

Swab isolates from processing environment. By means of ELISA test, it was possible to indicate 4 of the 7 isolates tested (57.1%) as enterotoxin producers. None of the other isolates produced toxins. Using the RPLA test, the enterotoxin production was found in 4 of 5 isolates tested (80%) (Table 3). The conformity of the enterotoxin production determined by both tests simultaneously reached 50%

Table 4. Summary of NHE and HBL enterotoxins detection from *B. cereus* isolates – samples coming from foodstuffs

Sample	ELISA					RPLA					Confirmity (ELISA+RPLA)		
	n	positive		negative		*n*	positive		negative		positive *n*/%	negative *n*/%	altogether
		n	(%)	*n*	(%)		*n*	(%)	*n*	(%)			
Meat products	36	33	91.7	3	8.3	27	6	22.2	21	33.3	5	2	8
Ready-to-cook products	5	2	40.0	3	60.0	5	4	80.0	1	20.0	1	0	5
Swabs from processing	7	4	57.1	3	42.9	6	5	83.3	1	16.7	3	1	6
Total	48	39	81.2	9	18.7	38	15	39.4	23	60.5	9/47.4	3/15.8	19

while the conformity of negative results was 16.6%. It is apparent from these results that *B. cereus* isolates from the processing environment represent a significant majority of isolates producing both enterotoxins. The isolates originated largely from swabs taken in milk and meat processing plants. For the reason of a low number of the samples, tested it was not possible to perform the statistical evaluation by Mc Nemara test.

Resistance to selected antimicrobial substances

The testing of the resistance to antimicrobials was not the main purpose of this experiment, being rather a sort of bonus. In our opinion, the number of isolates was not large enough to guarantee statistical significance.

Isolates from meat products. The following resistance was found of isolates (14 *B. cereus* isolates from meat products) to: penicillin (100%), cephalotin (57%), clindamycin (14%), oxytetracycline (14%) and erythromycin (7%) – Table 5.

Isolates from swabs and ready-to-cook products (6 isolates). The following resistance was found of isolates to penicillin (100%), cephalotin (83%), erythromycin (16%), clindamycin (16%) and enrofloxacin (16%) was found (Table 6).

DISCUSION

Unfortunately, we did not find more studies testing both ELISA and RPLA simultaneously. Using the ELISA test the production of enterotoxins was demonstrated in 39 isolates (81.2%) while the RPLA test revealed the enterotoxin production in 15 isolates only (39.4%). As shown in Table 4, parallel enterotoxin positive results were found on only 47.4% occasions (9 of 19 tested isolates) and coincidental negative results were found in the tests with three isolates only (15.8%). Similar results were achieved by Rusul and Yaacob (1995), who proved the presence of enterotoxins in food in 91.8% samples with ELISA test and in 84% samples with RPLA test.

The isolates with the highest levels of enterotoxin production were obtained from instant soups, spreads, and deli products. These products were previously heat treated and were intended for direct consumption without subsequent treatment such as cooking, etc. Thus they represented a high risk to consumers' health. It is, however, suggested that the highest risk for consumers is foodstuffs with long expiration periods, such as cured meats for grilling, spreads and specialities, etc. Formerly published data (Gaillard *et al.* 1998) confirm our thesis that pH and cold temperature cannot be considered as quite secure factors for the restriction of the growth and activity of *B. cereus*.

Table 5. Frequency of selected MIC values of antimicrobial substances found by 14 *B. cereus* isolates testing – meat products

MIC (mg/l)	% R	≤ 0.015	≤ 0.03	≤ 0.06	≤ 0.12	≤ 0.25	≤ 0.5	≤ 1	2	4	≥ 8	≥ 16	≥ 32
Penicillin	100.0	0	0	0	0	0	0	0	7.2	21.4	71.4	0	0
Cephalotin	57.1	0	0	0	0	0	0	0	7.1	7.1	28.6	57.1	0
Erythromycin	7.1	0	0	0	7.1	0	57.1	14.3	14.3	0	0	0	7.1
Chloram-phenicol	0.0	0	0	0	0	0	0	0	42.8	42.8	14.3	0	0
Clindamycin	14.3	0	0	0	0	0	7.1	14.3	64.3	14.3	0	0	0
Oxytetra-cycline	14.3	0	0	0	0	57.1	0	14.3	14.3	14.3	0	0	14.3
Fusidic acid	0.0	0	7.1	0	50.0	35.7	7.1	0	0	0	0	0	0
Gentamicin	0.0	0	0	0	0	14.3	0	42.8	35.7	0	7.1	0	0
Neomycin	0.0	0	0	0	0	0	71.4	0	7.1	14.3	0	7.1	0
Enrofloxacin	0.0	0	0	35.7	0	57.1	0	7.1	0	0	0	0	0

MIC = minimal inhibitory concentration; % R – percentage of resistant isolates; Sensitive-extend of concentration; Intermediary sensitive - extend of concentration; Resistant-extend of concentration

Table 6. Frequency of selected MIC values of antimicrobial substances found by 6 *B. cereus* isolates testing – ready-to-cook products and swabs

MIC (mg/l)	% R	≤ 0.015	≤ 0.03	≤ 0.06	≤ 0.12	≤ 0.25	≤ 0.5	≤ 1	2	4	≥ 8	≥ 16	≥ 32	≥ 64
Penicillin	100.0	0	0	0	0	0	0	0	0	0	100.0	0	0	0
Cephalotin	83.3	0	0	0	0	0	0	0	0	16.7	0	83.3	0	0
Erythromycin	16.7	0	0	0	16.7	0	33.3	16.7	16.7	0	16.7	0	0	0
Chloram-phenicol	0.0	0	0	0	0	0	0	0	66.7	16.7	0	16.7	0	0
Clindamycin	16.7	0	0	0	0	0	16.7	33.3	33.3	16.7	0	0	0	0
Oxytetra-cycline	0.0	0	0	0	0	66.7	0	0	33.3	0	0	0	0	0
Fusidic acid	0.0	0	16.7	0	66.7	16.7	0	0	0	0	0	0	0	0
Gentamicin	0.0			0	0	16.7	0	16.7	66.7	0	0	0	0	0
Neomycin	0.0	0	0	0	0	0	66.7	0	33.3	0	0	0	0	0
Enrofloxacin	16.7	0	0	33.3	16.7	33.3	16.7	0	16.7	0	0	0	0	0

MIC = minimal inhibitory concentration; %R – percentage of resistant isolates; Sensitive-extend of concentration; Intermediary sensitive-extend of concentration; Resistant-extend of concentration

The isolates from ready-to-cook products and from swabs were characterised by lower quantities of enterotoxin production in comparison with the group of isolates coming from meat products. The presence of *B. cereus* in the processing area was considered a significant source of the secondary contamination of products and was also seen as a sign of an inadequate plant management, demonstrating that cleaning and sanitation procedures had not been performed carefully on the production equipment.

Statistically highly significant difference ($P = 5.7 \times 10^{-5}$) between both methods of the enterotoxin production on determination was evident only in the case of the group of isolates from meat products. However, it was concluded that the assessments of the production and presence of both enterotoxin complexes (NHE and HBL) in *B. cereus* coming from foodstuffs must be considered in parallel and not carried out by one test only. Similar bacterial resistance was found by (Turnbull 2004).

With regard to the demonstrable antibiotic resistance profiles of the isolates under study, it was considered suitable to perform antimicrobial resistance testing, in the case of antibiotical therapy of the extraintestinal disease related to *B. cereus*.

It was suggested that the introduction of novel probiotics into the foodstuffs intended for human consumption or for animal feeds may be advantageous, however, that it would be also desirable to repeat and verify the enterotoxin producing status of the isolated *B. cereus* using a full suite of methods. The verification of resistance with regard to the antimicrobial substances is considered to be of equal importance.

CONCLUSIONS

RTE can be a significant source of strong toxinogenic isolates of *B. cereus*. The toxinogenity of isolates should be certified by both tests available (ELISA and RPLA).

The ability of *B.cereus* to form biofilms (and their resistance to antibacterial agents and disinfectants) should be considered while cleaning and disinfecting the production area.

The isolates may be very resistant to drugs and can jeopardise human health.

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References

Ahmed R., Sankar-Mistry P., Jackson S., Ackermann H.W. Kasatiya S.S. (1995): *Bacillus cereus* phage typing as an epidemiological tool in outbreaks of food poisoning. Journal of Clinical Microbiology. **33**: 636–640.

Anonymous (1995): AOAC. Differentiation of members of *Bacillus cereus* group: Microbiological method. Sec. 17.8.02, Method 983.26. In: Cunniff P.A. (ed.): Official Methods of Analysis of AOAC International. 16th Ed. AOAC International, Gaithersburg: 54–55.

Anonymous (2003a): *Bacillus cereus* RPLA Toxin Detection Kit (BCET-RPLA TD950).

Anonymous (2003b): *Bacillus cereus*. OXOID Manual, No. 4: 21–38.

Batt C.A. (1998): A risk assessment approach for foodborne *Bacillus cereus* and its toxins. Journal of Applied Microbiology (Symposium Supplement), **84**: 51–61.

Beecher D.J., Wong A.C.L. (1997): Tripartite hemolysin BL from *Bacillus cereus*. Journal of Biological Chemistry, **272**: 233–239.

Beecher D.J., Pulido J.S., Barney N.P., Wong A.C.L. (1995): Extracellular virulence factors in *Bacillus cereus* endophthalmitis: Methods and implication of involvement of hemolysin BL. Infection and Immunity, **63**: 632–639.

Braun P., Fehlhaber K., Klug C., Kopp K. (1999): Investigations into the activity of enzymes produced by spoilage-causing bacteria: a possible basis for improved shelf-life estimation. Food Microbiology, **16**: 531–540.

Champagne C.P., Laing R.R., Roy D., Mafu A A., Griffiths M. (1994): Psychrotrops in dairy products: their effects and their control. Critical Reviews in Food Science and Nutrition, **34**: 1–30.

Christiansson A., Naidu A.S., Nilsson I., Wadstrom T., Pettersson H.E. (1989): Toxin production by *Bacillus cereus* dairy isolates in milk at low temperatures. Applied and Environmental Microbiology, **55**: 2595–2600.

Ehling-Schulz M., Svensson B., Guinebretiere M.-H., Lindbäck T., Andersson M., Schulz A., Fricker M., Christiansson A., Granum P.E., Märtlbauer E., Nguyen-The Ch., Salkinoja-Salonen M., Scherer S. (2005): Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology. **151**: 183–197.

Flint S., Bremer P.J., Brooks J.D. (1997): Biofilms in dairy manufacturing plant – description, current concerns and methods of control. Biofouling, **11**: 81–97.

Flint S., Palmer K., Bloemen J., Brooks R. (2001): The growth of *Bacillus stearothermophilus* on stainless steel. Journal of Applied Microbiology, **90**: 151–157.

Gaillard S., Leguerinel I., Mafart P. (1998): Model for combined effects of temperature, pH, and water activity on thermal inactivation of *Bacillus cereus* spores. Journal of Food Science, **63**: 887–889.

Granum P.E. (1994): *Bacillus cereus* and its toxins. Journal of Applied Bacteriology, **76**: 61–66.

Granum P.E., Lund T. (1997): *Bacillus cereus* and its food poisoning toxins. FEMS Microbiology Letters, **157**: 223–228.

Granum P.E., Brynestad S., O'sullivan K., Nissen H. (1993): Enterotoxin from *Bacillus cereus*: Production and biochemical characterization. Bulletin of the IDF, **287**: 38–41.

Granum P.E, Thomas J.M., Alouf J.E. (1995): A survey of bacterial toxins involved in food poisoning: a suggestion for bacterial food poisoning toxin nomenclature. International Journal of Food Microbiology, **28**: 129–144.

Lake R., Hudson A., Cressey P. (2004): Risk profile: *Bacillus* spp. in rice. In: Client Report FW0319,FSANZ, 40.

Lund T., Granum P.E. (1996): Characterization of a nonhaemolytic enterotoxin complex from *Bacillus cereus* isolated after a foodborne outbreak. FEMS Microbiology Letters, **141**: 151–156.

Marshall K.C (1984): Microbial Adhesion and Aggregation. Springer Verlag, Berlin.

Marshall K.C. (1992): Biofilms: an overview of bacterial adhesion, activity, and control at surfaces. American Society for Microbiology, **58:** 202–207.

Notermans S., Batt C.A. (1998): A risk assessment approach for food-borne *Bacillus cereus* and its toxins. Journal of Applied Microbiology (Symposium Supplement), **84**: 51–61.

Phelps R.J., McKillip J.L. (2002): Enterotoxin production in natural isolates of *Bacillaceae* outside the *Bacillus cereus* group. Applied and Environmental Microbiology, **68**: 3147–3151.

Pipek P., Beneš P., Kvasnička F., Wilmink M. (1999): Mechanisch entbeintes Geflügelfleisch und dessen Haltbarkeit. Fleischerei-Technik, **15**: 41–42.

Pipek P., Houška M., Jeleníková J., Kýhos K., Hoke K., Šikulová M. (2005): Microbial decontamination of beef carcasses by combination of steaming and lactic acid spray. Journal of Food Engineering, **67**: 309–315.

Pirttijärvi T.S.M., Andersson M., Salkinoja-Salonen M.S. (2000): Properties of *B. cereus* and other bacilli contaminating biomaterial-based industrial processes. International Journal of Food Microbiology, **60**: 231–239.

Prüss B.M., Dietrich R., Nibler B., Märtlbauer E., Scherer S. (1999): The hemolytic enterotoxin HBL is broadly distributed among species of the *Bacillus cereus* group. Applied and Environmental Microbiology, **65**: 5436–5442.

Rusul G., Yaacob N.H. (1995): Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. International Journal of Food Microbiology, **25**: 131–139.

Ryan P.A., MacMillan J.D., Zilinskas B.A. (1997): Molecular cloning and characterization of the genes encoding the L1 and L2 components of hemolysin BL from *Bacillus cereus*. Journal of Bacteriology, **179**: 2551–2556.

Svensson B., Eneroth A., Brendehaug J., Molin G., Christiansson A. (2000): Involvement of a pasteurizer in the contamination of milk by *Bacillus cereus* in a commercial dairy. Journal of Dairy Research, **67**: 455–460.

Te Gieffel M.C., Beumer R.R., Bonestroo M.H., Rombouts F.M. (1996): Incidence and characterization of *Bacillus cereus* in two dairy processing plants. Netherlands Milk and Dairy Journal, **50**: 479–492.

Thompson N.E., Keterhagen M.J., Bergdoll M.S., Schantz E.J. (1984): Isolation and some properties of an enterotoxin produced by *Bacillus cereus*. Infection and Immunity, **43**: 887–94.

Turnbul P.C.B. (2004): *Bacillus* spp. Availabe at: http://gsbs.utmb.edu/microbook/ch015.htm

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Corresponding author:

Prof. Ing. Petr Pipek, CSc. Vysoká škola chemicko-technologická v Praze. Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie, Technická 5, 160 28 Praha 6, Česká republika
tel.: + 420 220 443 198, fax: + 420 233 337 337, e-mail: petr.pipek@vscht.cz