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Bacillus cereus: an important foodborne pathogen

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Bacillus cereus: ένα σημαντικό τροφιμογενές παθογόνο

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ABSTRACT. *B. cereus* is a spore-forming bacterium, frequently found in the environment. Most of the strains can grow at a temperature range of 10° - 42° C. *B. cereus* grows under aerobic conditions, but anaerobic growth is, also, feasible. D₁₂₁ values of the spores of *B. cereus* strains are usually in the range of 0.03 to 2.35 min. The pathogen produces at least five different enterotoxins (HBL, Nhe, CytK, BceT and FM) and one emetic toxin. The enterotoxins HBL, Nhe and CytK are the etiological agents of the *B. cereus* diarrhoeal disease. The enterotoxins are heat sensitive and can be inactivated by heating at 56° C for 5 min. They are, also, sensitive to low pH and proteolytic activity of enzymes and, subsequently, are inactivated in the acid environment of the stomach. *B. cereus* emetic toxin has been kept stable even in a heat treatment at 121° C for 2 h in *in vitro* tests. The emetic toxin is highly resistant to low pH (as low as 2) and to proteolysis. Thus, the emetic toxin cannot be inactivated in the acidic environment of the stomach and the enzyme proteolytic activity in the intestinal tract. *B. cereus* causes either a diarrhoeal or an emetic type of foodborne disease. The diarrhoeal disease is caused by the *B. cereus* enterotoxins, which are formed in the intestinal tract after the spores' germination and the subsequent growth of the vegetative cells. The symptoms are watery diarrhoea, abdominal cramps and pain. The emetic disease is caused by the ingestion of the preformed toxins in the foods. The symptoms are nausea and vomiting, occasionally followed by abdominal pain or diarrhoea. Foodborne outbreaks caused by *B. cereus* have been associated with various foods. The emetic disease has often been associated with the consumption of rice, pasta and other starchy foods, while the diarrheal disease is often linked to the consumption of dairy products, vegetables and meat. The most common food sources for *B. cereus* infections in humans are milk and dairy products. Among the reported foodborne outbreak cases in North America, Europe and Japan attributed to *B. cereus* represent a percentage of 1% to 22%. Most *B. cereus* foodborne cases were associated with the consumption of cooked foods that were cooled slowly and stored under improper refrigeration conditions. Foodborne diseases caused by *B. cereus* constitute a major problem in restaurants and catering services. Application of control measures, such as Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points system (HACCP), in food processing lines can prevent contamination of the foods with pathogens like *B. cereus*.

Keywords: *Bacillus cereus*, foodborne pathogen bacteria, toxins, spore-forming bacteria

ΠΕΡΙΛΗΨΗ. Ο *Bacillus cereus* είναι ένα σπορογόνο βακτήριο ευρύτατα διαδεδομένο στο περιβάλλον. Τα περισσότερα στελέχη του αναπτύσσονται σε θερμοκρασίες μεταξύ 10° και 42° C, κυρίως σε αερόβιες συνθήκες, αλλά μπορεί να αναπτυχθούν και σε αναερόβιες συνθήκες. Η τιμή D₁₂₁ για τους σπόρους του *B. cereus* είναι συνήθως μεταξύ 0.03 min και 2.35 min. Το βακτήριο παράγει τουλάχιστον πέντε διαφορετικές εντεροτοξίνες (HBL, Nhe, CytK, BceT και FM) και μια εμετική τοξίνη. Οι εντεροτοξίνες HBL, Nhe και CytK είναι οι αιτιολογικοί παράγοντες στην πρόκληση της διαρροϊκής νόσου. Οι εντεροτοξίνες

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είναι ευαίσθητες στη θερμική επεξεργασία και αδρανοποιούνται με θέρμανση στους 56° C για 5 min. Επίσης, είναι ευαίσθητες σε χαμηλές τιμές pH, στη δράση των πρωτεολυτικών ενζύμων και αδρανοποιούνται στο όξινο περιβάλλον του στομάχου. Έχει διαπιστωθεί σε *in vitro* μελέτες ότι η εμετική τοξίνη του *B. cereus* παραμένει σταθερή ακόμη και μετά από θέρμανση στους 121° C για 2 h, ενώ είναι ιδιαίτερα ανθεκτική σε χαμηλές τιμές pH (μέχρι 2) και στην πρωτεόλυση. Συνεπώς, μπορεί να παραμείνει δραστική στο όξινο περιβάλλον του στομάχου και στην επίδραση της πρωτεολυτικής δράσης των ενζύμων του εντερικού σωλήνα. Ο *B. cereus* προκαλεί μια διαρροϊκή και μια εμετική μορφή τροφιογενούς νόσου. Η πρώτη μορφή προκαλείται από τις εντεροτοξίνες του *B. cereus* που παράγονται στον εντερικό σωλήνα μετά τη βλάστηση των σπόρων και την ανάπτυξη των βλαστικών μορφών του βακτηρίου, με κύρια συμπτώματα τα υδαρή κόπρανα και το κοιλιακό άλγος. Η δεύτερη μορφή προκαλείται από την πρόσληψη της προσχηματισμένης στα τρόφιμα τοξίνης. Τα συμπτώματα είναι ναυτία και έμετος που περιστασιακά συνοδεύονται από κοιλιακό άλγος ή διάρροια. Οι τροφιογενείς λοιμώξεις που προκλήθηκαν από τον *B. cereus* έχουν συσχετιστεί με διάφορα είδη τροφίμων. Η εμετική μορφή συχνότερα έχει συσχετιστεί με την κατανάλωση ρυζιού, ζυμαρικών και άλλων αμυλούχων τροφίμων, ενώ η διαρροϊκή με την κατανάλωση γαλακτοκομικών προϊόντων, λαχανικών και κρέατος. Τα τρόφιμα που συχνότερα εμπλέκονται στην πρόκληση νόσου από το *B. cereus* είναι το γάλα και τα γαλακτοκομικά προϊόντα. Μεταξύ των τροφιογενών λοιμώξεων που έχουν αναφερθεί στη Βόρεια Αμερική, στην Ευρώπη και την Ιαπωνία, οι περιπτώσεις που αποδόθηκαν στον *B. cereus* αποτελούσαν το 1% έως 22%. Όμως, τα περισσότερα κρούσματα τροφιογενούς νόσου από τον *B. cereus* έχουν συσχετιστεί με την κατανάλωση μαγειρεμένων τροφίμων που ψύχθηκαν αργά και διατηρήθηκαν σε ακατάλληλες συνθήκες ψύξης. Οι τροφιογενείς νόσοι από τον *B. cereus* αποτελούν σημαντικό πρόβλημα σε εστιατόρια και επιχειρήσεις τροφοδοσίας. Η εφαρμογή μέτρων, όπως η τήρηση των αρχών της Ορθής Βιομηχανικής Πρακτικής (Good Manufacturing Practice, GMP) και του Συστήματος Ανάλυσης των Κρίσιμων Σημείων Ελέγχου (Hazard Analysis Critical Control Points System, HACCP), στην παρασκευή των τροφίμων μπορούν να αποτρέψουν τη μόλυνση των τροφίμων με παθογόνα βακτήρια, όπως είναι ο *B. cereus*.

Λέξεις ευρετηρίασης: *Bacillus cereus*, τροφιογενή παθογόνα βακτήρια, τοξίνες, σπορογόνα βακτήρια

Introduction

B. cereus has been recognized as an important foodborne pathogen. The European Commission has classified it as a hazard group 2 microorganism, due to its ability to cause infections in humans (European Commission Council Directive 93/88/EEC 1993).

B. cereus can cause two foodborne diseases, an emetic (vomiting) intoxication due to the ingestion of a toxin preformed in the food (Kramer and Gilbert 1989) and a diarrhoeal infection due to the ingestion of bacterial cells/spores, which produce enterotoxins in the small intestine (Granum and Lund 1997, Granum 1994).

The aim of the present work is to review the characteristics, the toxins and types of the foodborne diseases, as well as the food sources and reported outbreaks of *B. cereus*.

Characteristics of *Bacillus cereus*

B. cereus is an aerobic spore-forming bacterium, frequently found in the environment and usually isolated in soil, dust, air, water and decaying plants. Its growth temperature varies between the strains of the pathogen. Mesophilic strains of *B. cereus* can grow at a temperature range of 10° - 42° C (optimum 30° - 37° C). It has, also, been reported that some thermophilic strains can even grow at temperatures as high as 55° C (Kramer and

Gilbert 1989). However, psychrotrophic strains of *B. cereus* are able to grow at 4° - 10° C (EFSA 2005).

B. cereus is not a particularly acid tolerant bacterium and many strains can grow over a pH range of 4.75 to 9.3 (EFSA 2005). The minimum water activity (a_w) value, allowing vegetative growth for many strains of the pathogen, is 0.92 (Kramer and Gilbert 1989).

Although there are inconsistent results on the effect of NaCl on *B. cereus* growth, it is generally recognized that the majority of *B. cereus* strains can grow in laboratory media with a NaCl content of less than 7% (Claus and Berkeley 1986, Raevuori and Genigeorgis 1975).

B. cereus grows under aerobic conditions, but anaerobic growth is, also, feasible. Toxin production is lower under anaerobic conditions than in aerobic ones (Kramer and Gilbert 1989).

Vegetative cells of *B. cereus* are sensitive to heat, but, in contrast, spores have a broad range of heat resistance (Goepfert et al. 1972). Most of the spores of *B. cereus* strains are moderately heat resistant with a D_{121} value of 0.03 min, but certain spores are extremely heat resistant with a D_{121} value of 2.35 min (Kramer and Gilbert 1989). *B. cereus* isolates from foodborne outbreaks show D_{100} values ranging from 6 to 27 min (Rajkowski and Mikolajcik 1987).

A heat process at 121.1° C for 3 min is generally considered safe for the elimination of *Clostridium botulinum* in canned foods. This heat process is, also, sufficient to eliminate the spores of *B. cereus* (Rajkowski and Mikolajcik 1987). Foods subjected to less severe heat treatments may occasionally carry spores of *B. cereus* (EFSA 2005).

Toxins of *B. cereus*

B. cereus produces at least five different enterotoxins, Haemolysin BL (HBL), Nonhemolytic enterotoxin (Nhe), Cytotoxin K (CytK), Enterotoxin T (BceT), Enterotoxin FM (EntFM) and one emetic toxin. The enterotoxins HBL, Nhe and CytK are the etiological agents of the *B. cereus* diarrhoeal disease, but enterotoxins BceT and EntFM are less toxic and are usually not involved. They are polypeptides or protein complexes, while the emetic toxin is a polypeptide. The enterotoxins HBL and Nhe are multicomponent and chemically related proteins, whereas the enterotoxin CytK is a single protein. The enterotoxin HBL is a haemolysin and consists of three proteinic components (L1, L2 and B), which are all required for the enterotoxin activity. It shows haemolytic, dermonecrotic and vascular permeability activities and it is, also, considered as the primary virulence factor in *B. cereus* diarrhoea (Granum 2007). The non-haemolytic enterotoxin (Nhe) consists of three proteinic components (NheA, NheB and NheC), which are all required for the enterotoxin activity. Cytotoxin (CytK) has been discovered recently and it is, also, involved in *B. cereus* diarrhoeal disease. This enterotoxin is a protein with a molecular weight of 34 kDa and it is similar in structure to β -toxin of *Clostridium perfringens* (Kramer and Gilbert 1989). The enterotoxins (HBL, Nhe and CytK) are cytotoxic and act on the cell membrane by making holes or channels (Beecher et al. 1995).

The majority of *B. cereus* strains are capable to synthesize enterotoxins at a temperature range of 6 to 21° C. Toxin production is, also, favoured at a pH range between 6.0–8.5 (Griffiths 1990), they are heat sensitive and can be inactivated at 56° C for 5 min. They are, also, sensitive at low pH and to enzymes' proteolytic activity and, subsequently, they are inactivated in the acid environment of the stomach. Therefore, while the enterotoxins production is feasible in food, it is presumably less important to assess the risk of diarrhoeal infection (van Netten et al. 1990).

The emetic toxin, also named as cereulide, is a peptide with a molecular weight of 1.2 kDa. It is produced in higher amounts between 20° - 25° C, while it is not usually feasible at temperatures below 8° C (Haggblom et al. 2002). It is highly resistant at a low pH, as low as 2, and to proteolysis. Thus, the emetic toxin can be stable in the acidic environment of the stomach and the enzyme proteolytic activity in the intestinal tract (Granum 2007).

In addition, the emetic toxin has been kept stable at 121° C for 2 h in *in vitro* tests (Rajkovic et al. 2008). This observation is important because it may remain active upon sterilization (121° C for 3 min), which is generally recognized as a safe heat process, as mentioned above. The cases of emetic disease with a low number of *B. cereus* cells (10^2 cfu/g) present in heat processed foods may be attributed to the presence of the emetic toxin (EFSA 2005). Moreover, due to its hydrophobic properties, toxic residuals of emetic toxin cannot be completely washed away from contaminated grain foods (e.g. rice) during the washing procedure (Rajkovic et al. 2008).

B. cereus foodborne diseases

B. cereus causes either a diarrhoeal or an emetic type of foodborne disease.

1. Diarrhoeal disease

The diarrhoeal disease is caused by the *B. cereus* enterotoxins, which are formed in the intestinal tract after the germination of the spores and the subsequent growth of the vegetative cells. An infectious dose of 10^5 to 10^7 viable cells or spores per g of the contaminated food is usually enough for the onset of the symptoms of the diarrhoeal disease (Granum 2007). However, some strains may cause the disease with an infectious dose as low as 10^3 - 10^4 cfu/g (Granum and Lund 1997). The disease is characterized by a fairly long incubation time, usually 8 - 16 h. The delayed onset of symptoms is due to the transit time of the pathogen through the stomach and the small intestine, which may be almost 6 h (Takumi 2000), and the time required for the growth of *B. cereus* cells to hazardous levels in the intestine. When spores of *B. cereus* are consumed with contaminated foods, the incubation time is even longer due to the germination of the spores. The symptoms are watery diarrhoea, abdominal cramps and pain. Nausea may accompany diarrhoea, but vomiting

Table 1. *B. cereus* foodborne outbreaks and implicated foods.

Type of food	Region/Country	No. outbreaks (persons affected)	Years	References
Rice	England & Wales	28	1992-2008	HPA 2010
Rice	Verginia (USA)	1 (14)	1993	Khodr et al. 1994
Spaghetti	Switzerland	1	1996	Mahler et al. 1997
Poultry	England & Wales	14	1992-2008	HPA 2010
Chicken	USA & European Union	10	1988-2007	Greig and Ravel 2009
Chicken	UK	2 (300 & 30)	1998	Ripabelli et al. 2000
Red meat	England & Wales	9	1992-2008	HPA 2010
Beef	USA, Australia, New Zealand	5	1988-2007	Greig and Ravel 2009
Pork	USA & European Union	2	1988-2007	Greig and Ravel 2009
Eggs	Australia	1	1988-2007	Greig and Ravel 2009
Seafood	USA & Asia	3	1988-2007	Greig and Ravel 2009
Seafood	England & Wales	3	1992-2008	HPA 2010
Cod fish	Spain	1 (4)	1986-1989	van Netten et al. 1990
Salad/ Fruit	England & Wales	5	1992-2008	HPA 2010
Salad/ Fruit	Australia & Canada	6	1988-2007	Greig and Ravel 2009
Dairy products	USA & European Union	3	1988-2007	Greig and Ravel 2009
Pasteurized milk	Netherlands	1 (42)	1988	Christiansson 1992
Pasteurized milk	Netherlands	1 (280)	1986-1989	van Netten et al. 1990
UHT milk	Japan	1(201)	1991	Christiansson 1992
Desserts	England & Wales	2	1992-2008	HPA 2010
Cakes	Italy	2 (95 & 78)	2000	Ghelardi et al. 2000

symptoms are not usually observed (Gilbert 1979). The duration of disease is generally 12-24 h, and occasionally several days. The symptoms of the *B. cereus* diarrhoeal disease are, also, similar to those caused by *Clostridium perfringens* infections (Granum 2007).

2. Emetic disease

The emetic disease is caused by the ingestion of the preformed toxins in foods. Generally, a large population of *B. cereus* cells (10^5 - 10^8 /g of food) is required to produce a sufficient toxin amount in the food for the onset of the emetic disease (Granum 2007). According to Agata et al. (2002), the amount of emetic toxin implicated in cases of *B. cereus* emetic disease is usually in the range of 0.01 to 1.28 µg/g of food. The emetic type occurs in 1-6 h after the consumption of the contaminated food (Mortimer and McCann 1974). The symptoms are nausea and vomiting, occasionally followed by abdominal pain or diarrhoea. The duration of disease is generally 6-24 h.

The disease is similar to that of *Staphylococcus aureus* food poisoning in both its symptoms and incubation period. The rapid onset indicates preformed toxin. The emetic disease is self-limiting and recovery is, also, complete (Granum 2007).

B. cereus food sources and foodborne outbreaks

Since *B. cereus* is a common soil saprophyte, it is easily spread to foods of plant origin. However, the pathogen is usually found in food of animal origin like meat, eggs and dairy products (Kramer and Gilbert 1989). Hauge (1955) provided the first and most comprehensive description of *B. cereus* diarrhoeal disease in the 1950's (after investigating four outbreaks), associated with the consumption of vanilla sauce in a Norwegian hospital. Foodborne outbreaks caused by *B. cereus* have been associated with various types of food (Table 1). The emetic disease has been quite often associated with the consumption of rice, pasta and other starchy foods, while the diarrheal

disease has been often linked to the consumption of dairy products, vegetables and meat (Jay et al. 2005).

The case number of *B. cereus* infections are usually underestimated because the pathogen causes a mild disease with a short duration (often < 24 h) and sporadic cases of foodborne *B. cereus* outbreaks are not officially reported. In addition, the food source of the outbreak is rarely confirmed and epidemiological investigation is usually not conducted in many countries. Among the reported foodborne outbreaks in North America, Europe and Japan, cases attributed to *B. cereus* outbreaks represented a percentage ranging between 1% to 22% (Griffiths and Schraft 2002). In a survey conducted in Europe in the years 1993–1998, 278 outbreaks were caused by *B. cereus*, representing a percentage of 1.3% of the total number of reported foodborne outbreaks (WHO 2000).

In many countries, the case number of foodborne diseases caused by *B. cereus* is not exactly known. However, the dominant type of reported *B. cereus* disease varies between different countries. For example, in Japan, the case number of emetic diseases is ten times higher than the diarrhoeal type, whereas in Europe and North America, the diarrhoeal disease is often reported as the most common type. This variation is presumably due to the different types of consumed foods or the different cooking methods among the countries (Granum 2007).

Cross-contamination of processed foods with *B. cereus* spores has, also, been observed (Kramer and Gilbert 1989). Due to the fact that spores of *B. cereus* have strong adhesive properties, they might form biofilms and persist on the surface of the food processing equipments (Andersson et al. 1995). Furthermore, foods could, also, be contaminated with *B. cereus* spores by the addition of contaminated ingredients, such as texturing agents (Guinebretiere and Nguyen 2003), liquid eggs, herbs and spices (ICMSF 2005). Spores of *B. cereus* were, also, found in paper mill industries and in packaging materials (Pirttijarvi et al. 2000), which could represent an additional source of contamination of the packaged foods. Dry foods, such as milk powder, infant formulae, spices and herbs, are often contaminated with *B. cereus* spores (EFSA 2005).

The most common food sources for human infections by *B. cereus* are the milk and the dairy

products (Granum et al. 1993, Gilbert 1979). Raw milk is often contaminated with *B. cereus* spores during an improper or not hygienic milking. In addition, the raw milk may be further contaminated with its spores during the transportation or processing. For instance, raw milk can be contaminated with *B. cereus* strains that persist in the farm bulk tanks and the collection milk tanks of the dairy plants (Svensson et al. 2004). Contamination of pasteurized and powdered milk with *B. cereus* strains, persisting in pasteurizing and drying process, respectively, has been reported (Eneroth et al. 2001, Svensson et al. 1999).

Certain strains of *B. cereus* spores can survive milk pasteurization and germinate during subsequent refrigerated storage. After spores' germination, psychrotrophic cells can easily grow because of the less competition from the low number of other bacterial cells survived in the pasteurization process of the milk. Notermans et al. (1997) found that psychrotrophic strains of *B. cereus* grew during refrigerated storage of milk at 4–7° C. However, other studies revealed that emetic *B. cereus* strains were presumably unable to grow and produce the emetic toxin below 10° C (EFSA 2005). Thus, proper refrigeration storage of milk at 4° C should prevent development of *B. cereus* emetic toxin (EFSA 2005).

Most *B. cereus* foodborne cases were associated with the consumption of cooked foods that were cooled slowly and stored under improper refrigeration conditions. In an inappropriate heating process, the spores of the pathogen could survive and could, also, germinate into vegetative cells. Further growth of vegetative cells of *B. cereus* could, also, be enhanced due to the less antagonistic activity of the low populations of other bacteria that survived the heating process. Particularly important are thermophilic *B. cereus* strains, which are able to grow at temperatures as high as 55° C, as they might multiply rapidly during an elongated cooling process (EFSA 2005).

Starch may promote the growth of *B. cereus* and the production of emetic toxin (Griffiths and Schraft 2002). In particular, cooked rice, kept at unrefrigerated storage for several hours before re-heating, led to several emetic intoxication outbreaks (Jay et al. 2005). The emetic toxin was produced during the inappropriate refrigerated storage of the cooked rice and was not destroyed during the re-heating process (Kramer and Gilbert 1989).

Foodborne diseases caused by *B. cereus* are a major problem in restaurants and catering services (Guinebretiere et al. 2006). Failure in refrigeration or extended delays in preparation and service of cooked products were factors that often led to *B. cereus* emetic disease (EFSA 2005).

Since the cause of foodborne outbreaks is not often identified, cases of *B. subtilis*, *B. licheniformis* and *B. pumilus* infections, which show similar symptoms to those of *B. cereus* infections, have occasionally been reported as *B. cereus* foodborne illnesses in humans (EFSA 2005).

Control measures

Since *B. cereus* spores are widespread in the environment, application of control measures to

prevent the contamination of the foods with the pathogen spores and toxins is important for the consumers' safety. Application of control measures, such as GMP and HACCP, in food processing lines can prevent food contamination with *B. cereus*. The dairy industry should apply control measures to avoid post-pasteurization contamination of milk with this pathogen (EFSA 2005).

Cooked foods should be consumed soon after preparation and in case of a later consumption, they should be kept hot (>63° C) or rapidly cooled. Refrigeration below 4° C is necessary to prevent growth of all types of *B. cereus*, including psychrotrophic strains. The European Union should, also, set limits for the population levels of *B. cereus* and its toxins in various foodstuffs (EFSA 2005).

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