DETERMINING DIFFERENCES IN CHARACTERISTICS OF *BACILLUS CEREUS* ISOLATED FROM VARIOUS FOODS

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Abstract. The aim of this study was to determine the differences in characteristics of *Bacillus cereus* prevalent in different food products. Presumptive *B. cereus* was isolated from food samples on MYP agar. Assignment of isolates to *B. cereus* was confirmed by principal confirmatory tests and using chromogenic medium. 93.2% of cultures from ready-to-eat products, 100% and 84.6% of cultures isolated respectively from dried milk products and dry products of non-milk origin were growing on BACARA medium and confirmed as *B. cereus*.

The main characteristics of *B. cereus* cultures isolated from the products of different type did not differ but the type of the food had an influence on the possibility of cultures to grow at the refrigeration temperature and on the susceptibility to antibiotics ($p \leq 0.05$). Mesophiles were dominating cultures in all products. The most adapted to growing at low temperatures were *B. cereus* isolated from ready-to-eat products.

Keywords: *Bacillus cereus*, products, chromogenic medium, characteristic.

IŠ ĮVAIRIŲ MAISTO PRODUKTŲ IŠSKIRTŲ *BACILLUS CEREUS* SAVYBIŲ SKIRTUMŲ ĮVERTINIMAS

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Santrauka. Darbo tikslas buvo nustatyti skirtinguose maisto produktuose paplitusių *Bacillus cereus* savybių skirtumus. Numanomos *B. cereus* išskirtos naudojant MYP agarą. Išskirtos kultūros *B. cereus* rūšiai priskirtos naudojant pagrindinius patvirtinimo testus ir chromogeninę terpę. 93,2 proc. kultūrų, išskirtų iš paruoštų tiesiogiai vartoti produktų, 100 proc. ir 84,6 proc. kultūrų, išskirtų atitinkamai iš sausų pieno ir ne pieno kilmės produktų, augo ant BACARA terpės ir buvo patvirtintos kaip *B. cereus*.

Iš skirtingų tipų produktų išskirtų *B. cereus* pagrindinės savybės nesiskyrė, bet maisto rūšis, iš kurios buvo išskirtos kultūros, turėjo įtakos kultūrų augimui šaldymo sąlygomis ir jautrumui antibiotikams ($p \leq 0,05$). Visuose produktuose vyravo mezofilinės padermės. Labiausiai prisitaikiusios augti žemose temperatūrose buvo *B. cereus*, išskirtos iš paruoštų tiesiogiai vartoti produktų.

Raktažodžiai: *Bacillus cereus*, produktai, chromogeninė terpė, savybės, jautrumas antibiotikams.

Introduction. *B. cereus* spores and vegetative cells are spread in the environment and can be found in raw, dried or processed food (Giffel et al., 1996). *B. cereus* are found in more than 50% of various types of rice, milk, meat and fish products. These bacteria can also be found on vegetables and in sauces, soups, steamed dishes and cakes (Bennet & Belay, 2001).

EU official documents do not contain any provisions on control of *B. cereus* or other *Bacillus* species in foods, although some member states specify criteria of *B. cereus* for some foods in their national documents. Therefore, in 2005 the EC requested EFSA to indicate food categories, food production and preparation processes in which *B. cereus* or other *Bacillus* species could pose a risk to human health. Experts recommended introducing the HACCP system, temperature control, correct washing of hands, disinfection of equipment, and rapid chilling of products for reducing the number of spores in products (EFSA, 2005). The Commission Regulation (EC) No. 2073/2005[1] does not provide *B. cereus* criteria for any food product though Commission Regulation (EC) No. 1441/2007[2] includes the criterion of presumptive *B. cereus* for dried baby foods. Lithuanian Hygiene Norm HN 26:2006[3] provides for the control of *B. cereus* in bakery confectionery with filling (10^3-10^4 CFU g^{-1}), in meals and ready-to-eat culinary products (10^3-10^4 cfu g^{-1}).

B. cereus may produce toxins (Schoeni & Wong, 2005) which cause food poisoning usually when insufficiently chilled food is kept for several hours before consumption and contains > 10^6 cfu *B. cereus* g^{-1} (Rhodehamel & Harmon, 2001; FDA, 2007). Poisoning may also be caused by grains and starchy foods consumption. Boiled rice is usually referred to the *B. cereus* related diseases (Forsythe, 2000). *B. cereus* is commonly found in dairy products. As a result of Weerkamp & Stadhouders (1993) study of 293 dairy products purchased at the local market, *B. cereus* was found in 17% of fermented milk products, in 52% of ice-cream and 29% of milk powder samples. The average number of *B. cereus* in milk products was 15-280 cfu ml^{-1}

[1] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

[2] Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs

[3] Lietuvos higienos norma HN 26:2006 "Maisto produktų mikrobiologiniai kriterijai" (Lithuanian Hygiene Norm HN 26:2006)

(g^{-1}), and the limits varied from 0 to 800 cfu ml^{-1}(g^{-1}). It was found that 25% of pasteurized milk shelf-life problems were associated with *Bacillus* spp. proliferation (Weerkamp & Stadhouders, 1993; Juan et al., 2007). Mesophilic aerobic bacteria count and *B. cereus* count increased by increasing the pasteurized milk storage time and temperature. Psychrotrophic *B. cereus* was found in 25% of raw milk samples (Larsen & Jorgensen, 1997). Sometimes even modern milk-processing equipment does not protect from the opportunity of *B. cereus* growth in some points of the production line. *B. cereus* was detected from 0.3 cfu g^{-1} to 600 cfu g^{-1} in 54% samples of baby food (261 units) selected from 17 countries. *B. cereus* can cause poisoning when dried milk is used as a component of any food or when bacteria multiply in the reconstituted milk or infant formulas. In the first case, it is difficult to determine the nutritional source of poisoning (Rhodehamel & Harmon, 2001).

It is impossible to eliminate *B. cereus* from food products. The results of research have shown that *B. cereus* cells can survive the heat-treatment (mesophilic *B. cereus*) and grow in food under refrigeration conditions (0-6°C) (psychrotrophic *B. cereus*), so the initial number of these bacteria in food must be very low (Dufrenne et al., 1995; Choma et al., 2000, Valero et al., 2000; Valero et al., 2003).

Research was carried out to explore the possibility of *B. cereus* isolated from foodstuffs to transmit the antimicrobial resistance to other bacteria. It was found that 31% of milk and 28% of meat products were contaminated by *B. cereus*. Almost all the isolated *B. cereus* cultures had low susceptibility to ampicillin, cephalothin and oxacillin. Except for the resistance to streptomycin, resistance to other antimicrobial agents (clindamycin, erythromycin, tetracycline, and neomycin) has been sporadic. When foodstuffs are contaminated with microorganisms resistant to antimicrobial substances they may be vectors of spreading resistance. It was not confirmed that *B. cereus* isolated from food products transmitted the antimicrobial resistance to other bacteria (Schlegelova et al., 2003).

B. cereus is widely analyzed by many scientists, though literary sources comparing of properties of *B. cereus* isolated from various foodstuffs are lacking. In Lithuania, investigations concerning the prevalence of *B. cereus* and their characteristics were initiated several years ago by the authors of this article.

The aim of this study was to determine the differences in main characteristics (biochemical reactions, growth temperature ranges, and susceptibility to antibiotics) of *Bacillus cereus* widespread in different food products.

Materials and methods. The subjects of the experiments included 106 cultures of *B. cereus* isolated from 200 samples of ready-to-eat products, 58 samples of dried milk products and 55 samples of dry products of non-milk origin.

Isolation and typing of strains. Food products were obtained in big supermarkets while dried milk samples were received from Lithuanian milk processing plants in 2008–2010. Samples of products were taken and prepared for microbiological testing according to standard procedures.

The number of presumptive *B. cereus* in food products was determined according to ISO 7932:2004[4]. Five or more colonies (if there were less than five, all colonies) were picked from the MYP agar plates and transferred to nutrient agar slants for further confirmation as *B. cereus*. Principal confirmatory tests – hemolytic activity, resistance to lysozyme, catalase, motility, anaerobic growth, acid production from carbohydrates, anaerobic utilization of glucose, starch hydrolysis, liquefying of gelatin – were determined according Rhodehamel & Harmon, 2001.

Growth on BACARA medium. Presumptive *B. cereus* which were identical to differential characteristics of *B. cereus* species or had 1–4 atypical characteristics were checked for the growth onto chromogenic BACARA medium (*AES chemunex*). BACARA medium was inoculated by gently touching the surface of agar with a loop of culture. The inoculum was allowed to be fully absorbed before incubating at 30°C for 24 h. Orange colonies surrounded by a zone of precipitation were assigned as *B. cereus*.

Determination of growth temperature of isolated *B. cereus* strains. Growth of *B. cereus* strains isolated from different foodstuffs was investigated at 3±2ºC and 42ºC. The strains were ascribed to mesophilic strains when they were unable to grow at 3±2ºC but grew at 42ºC; psychrophilic strains when they were able to grow at 3±2ºC and at 42ºC and psychrotrophic strains when they were able to grow at 3±2ºC and did not grow at 42ºC. All *B. cereus* cultures were inoculated with a loop into separate tubes with brain heart infusion. The tubes were incubated at 30ºC for 24 h and then the culture suspensions were inoculated on the petri dishes with Plate Count Agar (PCA) (CM 325, *Oxoid*) divided into four sectors. The plates were incubated in an inverted position at 3±2ºC and at 42ºC. After incubation the plates were observed for the presence or absence of colony growth (Wijnands et al., 2002).

Determination of antibiotic susceptibility. The susceptibility of *B. cereus* to antibiotics was determined by the agar diffusion method (Кощеев, 1987). This method allows the detection of the bacteriostatic and bactericidal impact of antibiotics on the investigated bacteria. The tested cultures were pre-cultivated on PCA slants for 24 h at 30°C. The cultures from the slants were transferred into 10 ml of buffered peptone solution with a loop and suspended to obtain the inoculum density equivalent to a 0.5 McFarland standard. 1 ml of adjusted suspension was transferred to 100 ml of PCA dissolved and cooled to 45°C. The mixture of medium and culture suspension was poured into 90 mm petri dishes. The medium was allowed to solidify. Then hollows of 7.5 mm diameter were cut in agar using a special tool. Aliquots of

[4] ISO 7932:2004 Microbiology of food and animal feeding stuffs – Horizontal method of the enumeration of presumptive *Bacillus cereus* – Colony-count technique at 30°C

prepared antibiotic solutions (0.05 ml) were dropped into the hollows made in the agar. The plates were incubated in a straight position at 30±0,5°C for 24 h. The diameters of the complete inhibition zones were measured, including the diameter of the hollow. The width of the inhibition zone (i.e. the zone free from bacteria) was calculated using the following formula[5]: H = D – d / 2; where: H – the inhibition zone in mm; D – the total diameter of hollow and inhibition zone in mm; d – the diameter of hollow in mm. There is some correlation between the width of inhibition zone and the susceptibility of bacteria to antibiotics. When the inhibition zone width in petri dish is >10 mm, from 10 to 5 mm, from 5 to 4 mm or <4 mm, the degree of bacterial susceptibility to antibiotics is respectively strong bactericidal, weak bactericidal, weak bacteriostatic and bacteria are resistant to evaluated antibiotic.

To determine the susceptibility of *B. cereus* to antibiotics the following antibiotics were used: penicillin G (*Fluka BioChemika*); neomycin (*Fluka BioChemika)*; erythromycin (*Fluka BioChemika*); streptomycine (*Sigma*); oxytetracycline (*Fluka BioChemika*); chlortetracycline (*Fluka BioChemika*). The antibiotic solutions were prepared in the following concentrations: penicillin, 250 and 1000 μg ml^{-1}; erythromycin, 0.5, 4, 50, 100, 250 and 500 μg ml^{-1}; oxytetracycline, 2, 8, 32, 100, 250 and 500 μg m^{-1}; chlortetracycline, 1, 10, 50, 100 and 250 μg ml^{-1}; neomycin, 0.5, 4, 50, 100, 500, 800 and 1000 μg ml^{-1}; streptomycine, 32, 128, 500 and 950 μg ml^{-1}. Antibiotic solutions were prepared in sterile water, with an exception of primary dissolving of oxytetracycline – in phosphate buffer (pH 4.5) and erythromycin – in 20 % ethanol.

Analysis of data. The test was repeated 3 times, All data were analyzed with the statistica v.7.0 statistical software. In all cases, the differences were considered significant at a confidence interval of 95% ($p \leq 0.05$).

Results. The samples of 200 ready-to-eat products, 58 samples of dried milk products and 55 samples of dry products of non-milk origin were investigated for isolation of *B. cereus* cultures. Presumptive *B. cereus* were found in 29.5% of investigated ready-to-eat products, in 58.6% of dried milk products and in 23.6% of dried products of non-milk origin. The presumptive *B. cereus* growing in the selective *B. cereus* BACARA medium were assigned to *B. cereus*. It was determined that 4 cultures (6.8%) and 2 cultures (15.4%) isolated on MYP agar respectively from ready-to-eat products and dry products of non-milk origin did not grow on chromogenic medium, while all cultures isolated from dry milk products were growing on the selective BACARA medium. Contamination of investigated foodstuffs by *B. cereus* grown in chromogenic medium is presented in Table 1. The highest numbers of *B. cereus* were determined in sweet dishes and whole milk powder, where the numbers reached the levels up to $2.0 \cdot 10^4$ and $2.1 \cdot 10^4$ cfu g^{-1}, respectively. Lower numbers of *B. cereus* were determined in fish dishes, dried whey and dry mixes for preparing meals.

Characteristics of *B. cereus* isolated from different foodstuffs and growing in chromogenic media are listed in Table 2. All the 100 cultures grown on the chromogenic medium were hemolytic, their microscopic view were single rods or chains of rods. All cultures of *B. cereus* isolated from ready-to-eat products showed a strong hemolysis when compared with control strain *B. cereus* ATCC 11178, while 18 cultures isolated from dried milk showed a weak hemolysis. Starch hydrolysis was typical of 54.5%, 47.1% and 63.6% of the cultures isolated respectively from ready-to-eat products, dried milk samples, and dry products of non-milk origin. All cultures liquefied gelatin. Nine cultures isolated from ready-to-eat products had one non-typical characteristic for *B. cereus*: one culture did not utilize glucose in anaerobic conditions; 7 cultures produced acid from xylose; and 1 culture produced acid from mannitol. Two cultures isolated from dried milk products had one non-typical characteristic for *B. cereus*: one culture did not produce acid from mannitol, while the other did not grow in anaerobic conditions. One culture isolated from a dry product of non-milk origin was not motile (unlike all other cultures); other did not grow in anaerobic conditions and did not utilize glucose. It seems this culture could be an obligate aerobe.

Results on the determination of *B. cereus* growth at different incubation temperatures showed that not a single *B. cereus* strain isolated from dry products of non-milk origin could grow at 3±2°C, while 12.7% of *B. cereus* isolated from ready-to-eat products were subdivided into psychrotrophic strains, and 9.1% into psychrophilic strains. Four cultures (11.8%) isolated from dried milk products were assigned to psychrophilic strains. 78.2%, 100% and 88.2% of *B. cereus* isolates respectively from ready-to-eat products, dry products of non-milk origin and dried milk products were mesophiles which could not grow below 10°C. 21.8% of cultures isolated from ready-to-eat products could grow at 3±2°C. The growth of *B. cereus* isolated from ready-to-eat products appeared in two days at 3±2°C, while the growth of cultures isolated from dried milk products started in 7 days (2.9% of cultures) and 10 days (8.8% of cultures). The ncubation time of 10 days at 6.5°C is specified in the method for determining psychrotrophic microorganisms (ISO 6730:2005)[6].

The susceptibility to antibiotics of 100 cultures isolated from different foodstuffs was determined. Results are shown in Figs. 1–3.

The results show that the susceptibility to antibiotics of different cultures isolated from dried milk products and dry products of non-milk origin was very similar ($p>0.05$). The cultures isolated from ready-to-eat products were more susceptible to concentrations of erythromycin ≥ 4 μg ml^{-1}, oxytetracycline ≥ 8 μg ml^{-1}, neomycin ≥ 500 μg ml^{-1} and chlortetracycline ≥ 10 μg ml^{-1} ($p \leq 0.05$).

[5] ISO 20645:2004 Textile fabrics – Determination of antibacterial activity – Agar diffusion plate test (ISO 20645:2004)

[6] LST ISO 6730:2005/P:2007 Pienas. Psichrotrofinių mikroorganizmų kolonijas sudarančių vienetų skaičiavimas. Kolonijų skaičiavimo 6,5°C temperatūroje metodas (tapatus ISO 6730:2005)

Table 1. **The number of *B. cereus* in different foodstuffs**

Foodstuff	Total number of samples	Number of samples contaminated by *B. cereus*, percent	The range of *B. cereus* count, cfu*/g
Ready-to-eat products			
Salads of raw vegetables	36	19.4	$1.0 \cdot 10^2 – 1.9 \cdot 10^3$
Salads of treated components	82	29.3	$1.0 \cdot 10^1 – 7.0 \cdot 10^2$
Potato dishes	14	35.7	$2.0 \cdot 10^1 – 2.0 \cdot 10^2$
Floury dishes	12	50.0	$1.0 \cdot 10^2 – 6.0 \cdot 10^2$
Meat dishes	14	28.6	$1.0 \cdot 10^1 – 5.0 \cdot 10^2$
Fish dishes	8	25.0	$1.0 \cdot 10^2 – 1.3 \cdot 10^2$
Garnish dishes	14	35.7	$5.0 \cdot 10^1 – 6.0 \cdot 10^2$
Sweet dishes	20	50.0	$1.0 \cdot 10^2 – 2.0 \cdot 10^4$
Dried milk products			
Whole milk powder	15	73.3	$3.0 \cdot 10^1 – 2.1 \cdot 10^4$
Skimmed milk powder	15	100.0	$6.0 \cdot 10^1 – 4.0 \cdot 10^2$
Dry whey	27	29.6	$1.0 \cdot 10^1 – 1.0 \cdot 10^2$
Dry products of non-milk origin			
Dry cream (of plant origin)	8	12.5	$8.0 \cdot 10^1$
Deserts	15	26.7	$1.0 \cdot 10^1 – 1.0 \cdot 10^2$
Chips	8	12.5	$3.5 \cdot 10^1$
Dry mixes for garnish (mashed potatoes, rice)	15	46.7	$2.0 \cdot 10^1 – 1.8 \cdot 10^3$
Dry mixes for dishes (for pancakes, soups)	9	0	$<1.0 \cdot 10^1$

*cfu – colony forming units

Table 2. **Determining the characteristics of the *B. cereus* cultures**

No of the culture of *B. cereus*	Hemolysis	Anaerobic growth	Resistance to lysozyme	Motile	Catalase	Aerobic glucose fermentation	Anaerobic glucose fermentation	Xylose fermentation	Mannitol fermentation	Growth on the chromogenic medium	Liquefying of gelatin	Starch hydrolysis
ATCC 11778 (control)	+	+	+	+	+	+	+	–	–	+	+	+
Ready-to-eat food products												
1-22	+	+	+	+	+	+	+	–	–	+	+	+
23-46	+	+	+	+	+	+	+	–	–	+	+	–
47	+	+	+	+	+	+	–	–	–	+	+	+
48-51	+	+	+	+	+	+	+	+	–	+	+	+
52-54	+	+	+	+	+	+	+	+	–	+	+	–
55	+	+	+	+	+	+	+	–	+	+	+	+
Dried milk products												
1-16	+	+	+	+	+	+	+	–	–	+	+	+
17-32	+	+	+	+	+	+	+	–	–	+	+	–
33	+	+	+	+	+	+	+	–	+	+	+	–
34	+	–	+	+	+	+	+	–	–	+	+	–
Dry products of non milk origin												
1-6	+	+	+	+	+	+	+	–	–	+	+	+
7-9	+	+	+	+	+	+	+	–	–	+	+	–
10	+	–	+	+	+	+	–	–	–	+	+	–
11	+	+	+	–	+	+	+	–	–	+	+	+

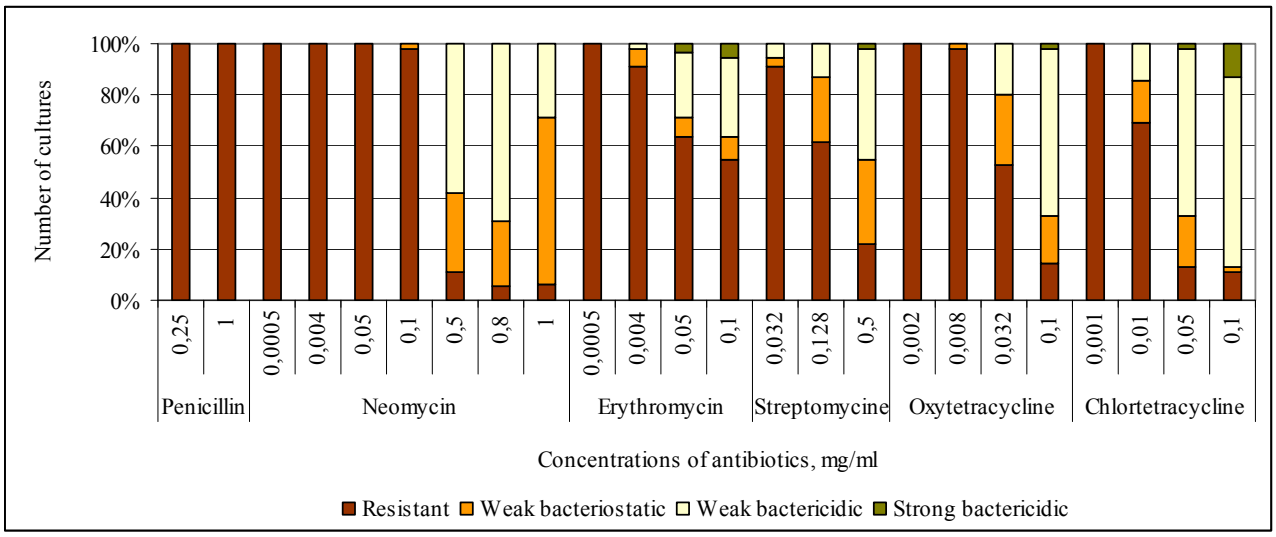

Fig. 1. **The susceptibility of *B. cereus* isolated from ready-to eat products to antibiotics**

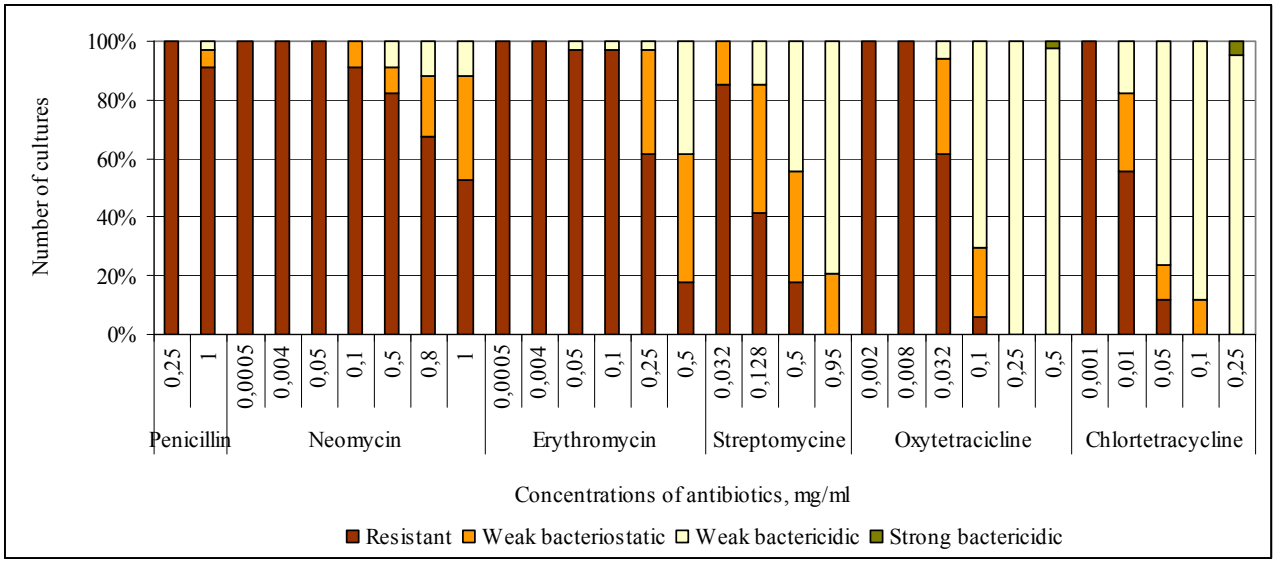

Fig. 2. **The susceptibility of *B. cereus* isolated from dried milk products to antibiotics**

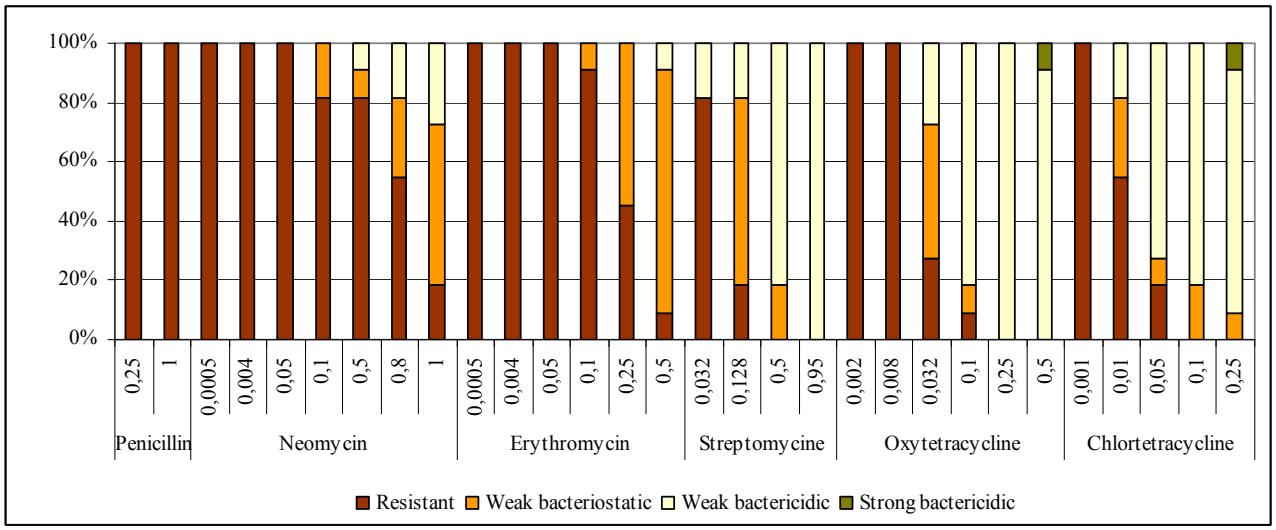

Fig. 3. **The susceptibility of *B. cereus* isolated from dry products of non-milk origin to antibiotics**

Discussion. *B. cereus* were found and isolated from all investigated foods. The highest numbers of *B. cereus* were determined in sweet dishes and whole milk powder, where numbers reached levels up to $2.0 \cdot 10^4$ and $2.1 \cdot 10^4$ cfu g^{-1}, respectively. Lower numbers of *B. cereus* were determined in fish dishes, dried whey and dry mixes for preparing meals. The number of *B. cereus* in various foodstuffs has been reported by various investigators and the contamination of dried milk products by *B. cereus* ranged from 10% to 100%, reaching levels from 0.3 to 10^3 cfu g^{-1} (Becker et al., 1994; Reyes et al., 2007), 98.7% of ready-to-eat products were contaminated below $1.0 \cdot 10^3$ cfu g^{-1}, for 0.7% the ranges were $1.0 \cdot 10^3 – 1.0 \cdot 10^4$ cfu g^{-1}, and the contamination 0.5% of the samples was above $1.0 \cdot 10^4$ cfu g^{-1} (Rosenquist et al., 2005). The obtained results, except for dried milk samples, were very close to the literary data. In dried milk samples a higher number of *B. cereus* ($2.0 \cdot 10^4$ cfu g^{-1}) was determined. The number of *B. cereus* found in the analyzed food products did not exceed $1.0 \cdot 10^5$ cfu g^{-1} which according to the literary sources may cause product defects of microbiological origin and toxin production (Weerkamp & Stadhouders, 1993).

Cultures that did not grow on chromogenic medium had 3 or 4 non-typical characteristics of *B. cereus* species. That confirms that presumptive *B. cereus* isolated from MYP agar according to the typical morphological characteristics and results of hemolysis reaction on blood agar does not necessarily grow on chromogenic media and could belong to the another species of *Bacillus* spp. BACARA medium appeared as more selective for isolation of *B. cereus* as MYP. Using a new chromogenic medium enables rapid isolation and identification of *Bacillus* species according to colony morphology and color (Reissbrodt et al., 2004). BACARA medium ensures excellent recovery of *B. cereus* and colonies do not require confirmation (Frisker et al., 2008), because chromogenic agents within the chromogenic medium detect the enzymatic activity of *B. cereus* allowing their colonies to be clearly differentiated on the petri dish after 24 h (Bourgeois et al., 1995). This new technology improves *B. cereus* recovery and isolation by shortening the duration of analysis.

Determination of the temperature-tolerance of *B. cereus* strains enables the differentiation of the strains. The growth of psychrophilic cultures isolated from dried products was slower than psychrophilic and psychrotrophic cultures isolated from ready-to-eat products. The fact that the all products (especially ready-to-eat products) analyzed contained more meshophilic as psychrotrophic *B. cereus* strains, it is clear that these products held at room temperature may cause a risk. In addition, psychrotrophic bacteria can multiply in some foods stored in the refrigerator so the initial food contamination by these bacteria should be very low.

Susceptibility of *B. cereus* to antibiotics was determined to detect a wider range of antibiotic effects to *B. cereus* because *B. cereus* is a potential pathogenic bacterium with a wide reservoir of antibiotic resistance which can be transferred to other pathogenic microorganisms. Most authors agree that the presence of such a high number of multiple-antibiotic resistant strains of *B. cereus* in food and human samples is a matter of concern (Teuber, 1999; Jorgensen, 2004). It is the reason to pay more attention to this problem. Our results showed that all 55 and 11 *B. cereus* cultures isolated respectively from ready-to-eat products and dry products of non-milk origin were resistant to concentrations of penicillin at 250 – 1000 μg ml^{-1} and neomycin at 0.5 – 50 μg ml^{-1}. Three cultures isolated from dried milk products were susceptible to penicillin in concentration of 1000 μg ml^{-1}. This concentration had a weak bactericidal impact on one culture and weak bacteriostatic impact to the other 2 cultures. The implication is that these cultures possibly do not produce β-lactamase, which could break the β-lactam ring of penicillin, so the cultures are not resistant to this antibiotic. This phenotypic resistance of *B. cereus* strains to β-lactam antibiotics is also mentioned in studies of other authors (Jensen et al., 2001; Schlegelova et al., 2003). 31 culture (91.2%) isolated from dried milk products were resistant to penicillin at concentrations of 250 – 1000 μg/ml. All cultures isolated from dried milk samples were resistant to 0.5–50 μg ml^{-1} neomycin. The impact of the investigated concentrations of other antibiotics on isolated cultures was different and varied depending on their concentrations. The growth inhibition of cultures isolated from dried milk products by streptomycine was stronger when compared with the cultures isolated from other investigated products. The concentration of streptomycin 32 μg ml^{-1} had a weak bacteriostatic impact on all cultures isolated from dried milk products while 5.5% and 18.2% of cultures isolated respectively from ready-to-eat and dried non-milk origin products were resistant to this concentration. Streptomycine concentration of 950 μg ml^{-1} had no strong bactericidal impact on the cultures isolated from dried products but streptomycine concentration of 500 μg ml^{-1} had a strong bactericidical impact on one culture. The impact of investigated concentrations of erythromycin on all cultures isolated from dried milk products was not strongly bactericidal. This indicates that these cultures were more resistant to the impact of this antibiotic, because the impact of 50 μg ml^{-1} and 100 μg ml^{-1} erythromycine was strongly bactericidal on, respectively, 3.6% and 5.5% of cultures isolated from ready-to-eat products. *B. cereus* cultures isolated from dried milk products were mostly resistant to oxytetracycline. The concentration of this antibiotic at 500 μg ml^{-1} had a strong bactericidal impact on 2.9% of the cultures isolated from ready-to-eat products; while a lower concentration (100 μg ml^{-1}) of oxytetracycline had a strong bactericidal impact on 1.8% cultures isolated from ready-to-eat products. *B. cereus* cultures isolated from dried products were less susceptible to chlortetracycline than cultures isolated from ready-to-eat products. Concentrations of 250 μg ml^{-1} had a stronger impact on the cultures isolated from dried products, while 50 μg ml^{-1} and 100 μg ml^{-1} concentrations of chlortetracycline had a strong bactericidal impact on 1.8% and 12.7% cultures isolated from ready-to-eat products. The obtained results show

that individual cultures from various food products differed in their susceptibility to all investigated concentrations of antibiotics. Some authors concluded that the development of populations or subpopulations of pathogens with decreased susceptibility to antibiotics could be led by low pH or high NaCl in food (environmental stress). Increased use of bacteriostatic, rather than bactericidal, food preservation systems may be contributing to the development and dissemination of antibiotic resistance among food-related pathogens (McMahon et al., 2007). Some difficulties may arise in comparing the results of bacteria susceptibility to antibiotics with literary data. Different authors use various methods: agar diffusion, disk diffusion, diffusion and dilution (E-tests), broth or agar dilution (Mishra et al., 2006; Macias et al., 1994; Lang & Garcia, 2004). It is proposed that the agar diffusion method is more sensitive than disk diffusion because the antimicrobial agent directly diffuses into the medium, and thus the culture in the nutrient medium is more exposed to the test material (Bonev et al., 2008).

In Lithuania, the study about the prevalence of *B. cereus* in foods and the resistance of isolates to antimicrobials was initiated in 2009 (Šalomskienė & Žvirdauskienė, 2009), however, only two groups of products (dried milk products and ice cream) were investigated. The study was continued in order to determine the properties of *B. cereus* occurring in high-risk products (ready-to-eat products, dry products of non-milk origin), and to receive a new information about the properties of these bacteria isolated from dry milk products. The isolation and identification of pure *B. cereus* cultures using PCR method is expensive. Only presumptive *B. cereus* can be isolated using the international standard method (LST EN ISO 7932:2005). As the biochemical properties of species from *Bacillus* cereus group are very similar therefore the growth on chromogenic medium was used for confirmation of *B. cereus* in our study.

The determination of some characteristics (e.g. growth temperature) of *B. cereus* cultures isolated from various products provides the opportunities for the adjustment of the finished products storage regimes inhibiting the growth of *B. cereus*. The antimicrobial agents can be transferred from animals to human organism through the food chain. For this reason, results of determining the resistance of isolated *B. cereus* cultures to widely used antimicrobial agents (penicillin, neomycin, oxytetracycline and oth.) contribute to the investigation of this urgent problem around the world. The EU authorities and Lithuanian Ministry of Health as well are engaged into solution of this problem. In 2007, the Lithuanian Ministry of Health has approved the prevention program for antimicrobial-resistant microorganisms for 2008–2014. The research of antimicrobial resistance is important for ensuring the prevention of spreading the resistant microorganisms.

Conclusions

1. *Bacillus cereus* was isolated from all investigated foods: ready-to-eat products, dried milk products and dry products of non-milk origin. The number of *Bacillus cereus* in these products varied respectively $1.0 \cdot 10^1$ – $2.0 \cdot 10^4$ cfu g^{-1}, $1.0 \cdot 10^1$ – $2.1 \cdot 10^4$ cfu g^{-1} and < $1.0 \cdot 10^1$ – $1.8 \cdot 10^3$ cfu g^{-1}.

2. 93.2% cultures from ready-to-eat products, 100% and 84.6% cultures isolated respectively from dried milk products and dry products of non-milk origin were growing on BACARA medium and confirmed as *Bacillus cereus*.

3. *Bacillus cereus* isolates from ready-to-eat products had a higher adaptability to refrigeration temperatures (3±2°C) compared to isolates from dried products. Mesophiles were dominating cultures in all products.

4. The investigation of susceptibility of isolated strains of *Bacillus cereus* to antibiotics showed that they were mostly resistant to penicillin at 250 – 1000 μg ml^{-1}, neomycin at 0.5 – 50 μg ml^{-1}, oxytetracycline at 2 – 8 μg ml^{-1}, erythromycin at 0.5 – 4 μg ml^{-1} and chlortetracycline at 1 μg ml^{-1}. The susceptibility of cultures isolated from ready-to-eat products was higher to investigated antibiotics than that of cultures from dried products.

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