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Short communication

Enterotoxigenic potential of *Bacillus cereus* strains isolated from dairy products at different incubation temperatures

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Abstract

Twenty-three *Bacillus cereus* strains isolated from powdered and pasteurized milk carrying the *hbl*ACD genes were assessed to evaluate their ability to produce the hemolysin BL enterotoxin (HBL) when incubated at 30ºC and 10ºC. All strains showed enterotoxigenic potential at 30ºC and nine strains (39%) produced the toxin at 10ºC. The results indicated that the expression of the *hbl*ACD genes and the subsequent production of the enterotoxin HBL were directly influenced by the incubation temperature. The contamination of dairy products by *B. cereus* with genetic potential to produce enterotoxins represents an important concern for the industry.

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Introduction

Bacillus cereus is a psychrotrophic microorganism, aerobic endospore former pathogen of humans and other animals (Logan, 2012), that causes two foodborne illness syndromes: the diarrhoeal illness, promoted by enterotoxins produced inside the host's small intestine, and the emetic illness, promoted by toxins pre-formed in the food (Oh *et al.*, 2012; Jeßberger *et al.*, 2014). Three types of diarrhoea enterotoxins are produced by *B. cereus*: the haemolytic BL toxin (HBL), non-haemolytic enterotoxin (NHE), and the cytotoxin K (CytK) (Ngamwongsatit *et al.*, 2008; Ankolekar *et al.*, 2009).

According to Lindbäck and Granum (2013), about 40% of *B. cereus* strains harbour the hblACD genes responsible for the HBL codification, for this reason this enterotoxins proteins are considered to be the most important produced by this micro-organism. The presence of *B. cereus* strains that harbour the HBL genes isolated from dairy products has been reported in several studies (Veld *et al.*, 2001; Svensson *et al.*, 2007; Di Pinto *et al.*, 2013; Reis *et al.*, 2013; Fernandes *et al.*, 2014).

Bacillus cereus is a contaminant of raw milk and also frequently isolated from a variety of dairy products (Kumari and Sarkar, 2014). Generally, raw milk and dairy products are contaminated by *B. cereus* from the soil and grass (O'Connell *et al.*, 2013). Some psychrotrophic strains of *B. cereus* are known to grow in dairy at refrigeration temperatures (Montanhini *et al.*, 2014). This growth represents a problem in refrigerated products such as milk and other dairy products (Lee *et al.*, 2011). The present study sought to identify the enterotoxigenic potential of *B. cereus* isolated from dairy products under controlled incubation temperatures.

Material and Methods

Twenty-three strains of *B. cereus sensu stricto* carrying the *hbl*ACD genes confirmed by molecular methods (Reis *et al.*, 2013) were selected for the study, being 14 of pasteurized milk and 9 of powdered milk. These strains were able to grow at 10 ºC for seven days, but not at 7ºC for 10 days, even 64% among them presenting the *cspA* gene signature witch codifies the psychrotrophic behaviour (Montanhini *et al.*, 2014), so they could not be classified as psychrotrophic according to the International Dairy Federation definition (IDF, 2004).

HBL production was evaluated using the BCETRAPLA kit (Oxoid Ltd., Basingtoke, UK) following the manufacturer's instruction. Shortly, the isolates were cultured in brain/heart infusion broth (Merck, Whitehouse Station, USA) at 30°C for 24 hours and 10°C for 7 days. Then 2 mL of each culture were centrifuged at 4°C at 900 G during 20 minutes and applied to the test devices. A sample was considered positive when showed distinct agglutination pattern.

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Results and Discussion

All evaluated strains of *B. cereus* incubated at 30 °C produced the HBL enterotoxin (Table 1). The same result were reported by Dufrenne *et al.* (1995), whose study found 100% of psychrotrophic *B. cereus* strains produced the HBL enterotoxin. Other studies reported the presence of toxigenic psychrotrophic *B. cereus*, particularly in dairy products, so this contamination is recognised to be a hazard to consumers (Christiansson *et al*., 1998; Veld *et al*., 2001; Svensson *et al*., 2007).

Normally, the *B. cereus* presents lower enterotoxigenic potential than those mesophlic strains, regarding to HBL production (Svensson *et al.*, 2007). Wijnands *et al.* (2006) stated that spores of mesophilic strains germinate better and faster in the intestinal condition comparing to psychrotrophic strains.

Although psychrotrophic strains are most important as food contaminant, mesophilic strains appear to be most important for the onset of diseases. However, other factors also are important concerning this onset: the level of contamination, the ability to produce enterotoxins, and the level at witch the enterotoxins are produced.

Moreover, were observed among the evaluated strains, nine (39%) were able to produce the enterotoxin HBL under incubation at 10ºC. Although of best temperature for the enterotoxins production is around 30ºC, *B. cereus* can produce HBL also at marginal refrigeration temperatures, what indicates its adaptive ability to this condition.

The expression of the *hbl*ACD genes and consequently of the enterotoxin HBL production appear to be directly influenced by the incubation temperature. Appling the same methodology used in the present study, Fermanian *et al.* (1997) verified that *B. cereus* strains produced diarrhoea enterotoxins at 32ºC could also produce the toxins at 10ºC. The authors observed that the assessed strains showed different levels of toxicity, what could be explained by the diversity of genetic characteristics and/or their expression by the strains.

The recommended temperature for storage of refrigerated dairy products is 7°C or less. Nevertheless, in tropical regions is not uncommon to find products stored at temperatures above the indicated, what represents a health hazard to the consumer of dairy products stored at these temperatures, concerning enterotoxins produced by toxigenic strains of *B. cereus* able to grow under refrigeration conditions, based on the results obtained in this study.

Table 1. Enterotoxin HBL production by *Bacillus cereus* strains isolated from dairy products incubated at different temperatures

Products	Total of Samples	30ºC, 24 hours	10ºC, 7 days	P-value*
Pasteurized milk	14	14 (100%)	7 (50%)	0.382
Powdered milk	9	9 (100%)	2 (22%)	0.187
Total	23	23 (100%)	9 (39%)	0.090

* The proportions were compared by Fisher Exact Test (P<0,05).

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