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Food Microbiology 24 (2007) 1–6

FOOD MICROBIOLOGY

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Prevalence of *Bacillus cereus* in dried milk products used by Chilean School Feeding Program

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Received 18 February 2006; received in revised form 12 April 2006; accepted 13 April 2006
Available online 9 May 2006

Abstract

The prevalence of *Bacillus cereus*, in a total of 381 samples of dried milk products (milk with rice, milk substitute, milk powder, milk-cereal-rice, pudding milk, flan, and mousse) used by the Chilean School Feeding Program, was investigated. The potential of 94 selected isolates of *B. cereus* to produce diarrhoeal enterotoxin (by the BCET-RPLA test) in BHI culture, as well as the ability of enterotoxigenic-strains to grow at psychrotrophic temperatures were also verified. *B. cereus* was found in 175 of 381 of the samples analysed (45.9%), reaching levels from 3.0 to 10^4 spores g^{-1}. As expected, the higher prevalence and counts were observed in those products that contained whole rice, cereals and pulses extruded, and food additives. Of the 94 isolates of *B. cereus* tested for diarrhoeal enterotoxin production, 28 (29.8%) were positive, and none of these was able to grow at $\leqslant 7\,^{\circ}C$. The prevalence of *B. cereus* in dried milk products analysed was fairly high, although it was present in low number. However, as they were composed to a large extent of enterotoxigenic mesophilic strains, the potential risk for the safety of reconstituted products held at improper temperature should not be neglected.

Keywords: Bacterial contamination; *Bacillus cereus*; Dried milk products

1. Introduction

Bacillus cereus is a Gram-positive, spore-forming bacterium that is widely distributed in nature (Claus and Berkeley, 1986). It is a common contaminant in a wide variety of foods, including milk and dairy products, cereals (especially rice), and food additives (Kramer and Gilbert, 1989; Becker et al., 1994). The bacterium is responsible for two different forms of foodborne disease—the emetic syndrome, caused by ingestion of a preformed toxin in the food, and the diarrhoeal syndrome, caused by a different toxin that can be formed in the food but also in the small intestine (Carlin and Nguyen-the, 1998).

B. cereus has been reported as the causative agent in 7% of school foodborne disease outbreaks in North America over the period of 1998–2000 (Daniels et al., 2002). Consequently, because children are most vulnerable to foodborne illness, the safety of food served in schools is a matter of concern.

In Chile, more than 1.3 million meals are served daily to school children by the School Feeding Program (Kain et al., 2002). An important fraction of these meals include dried milk products, such as milk powder, milk substitute, and dairy desserts (e.g. flan, mousse, milk pudding, and rice with milk), which are reconstituted in the school kitchens and frequently held at abusively high temperature for long periods before being consumed.

Dried milk products are known to be frequently contaminated with *B. cereus*, principally with its spores (Kramer and Gilbert, 1989; Becker et al., 1994). Although these do not germinate in these food types, when they are reconstituted and held at room temperature, viable spores may germinate and the vegetative cells can proliferate and produce toxin; which could potentially even occur at refrigeration temperatures (Meer et al., 1991; Jaquette and Beuchat, 1998), given that psychrotrophic variants of *B. cereus* have the capacity to grow and generate toxin at storage temperatures above 6 °C (Granum et al., 1993).

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0740-0020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2006.04.004

Therefore, if these foods are contaminated with *B. cereus*, especially enterotoxigenic strains, it might represent a potential risk for the safety, and consequently for the health, of school children.

The prevalence of *B. cereus* in dried milk products has been reported by various investigators, also it has been found in a large number of ingredients utilized in dried milk formulations. In particular, the prevalence of *B. cereus* reported in dried milk products ranged from 10% to 100%, reaching levels from 0.3 to 10^3 cells or spores g^{-1} (Ahmed et al., 1983; Becker et al., 1994; Wong et al., 1988). In spite of the high incidence in these products, only a few outbreaks have been reported in which milk or milk-related products containing *B. cereus* were suggested to be the cause of the disease (Becker et al., 1994; Notermans et al., 1997), principally of diarrhoeal syndromes.

Because the prevalence of *B. cereus* in dried milk products is a theme of public health concern, the aim of this study was to determine the prevalence and contamination levels of *B. cereus* spores in a range of dried milk products used by the Chilean School Feeding Program. In addition, 94 selected isolates of *B. cereus* recovered from the products analysed were screened for the potential to produce diarrhoeal enterotoxin (by the BCET-RPLA test), and positive enterotoxigenic strains were also tested for the ability to grow at psychrotrophic temperatures (4 and 7 °C) in BHI culture.

2. Materials and methods

2.1. Prevalence of Bacillus cereus in dried milk products

2.1.1. Sample collection

A total of 381 samples of dried milk products (Table 1) were collected directly from central warehouses of four concessionary companies that provide food services to the School Feeding Program of the Chilean government, which geographically are located in the VII and VIII regions of the country. These were visited during a period of six months, and each warehouse was visited two times during this period. The taking of samples was carried out utilizing a plan of simple sampling and normal inspection according to the Norm Chilean Official NCh 44 of 78 (INN, 1978). All samples, which were packaged into polyethylene bags in 500 and 1000 g units, were transported in boxes of tightly sealed cartons, and were kept at environmental temperature until analysed within 2–4 h.

2.1.2. Enumeration of B. cereus spores and aerobic spore-forming bacteria

To enumerate the aerobic spore-forming bacteria and *B. cereus* spores, samples of 25 g were reconstituted with 225 ml of PSS [peptone saline solution: 8.5 g l^{-1} NaCl and 1 g l^{-1} of neutralized bacteriological peptone (Oxoid, Hampshire, England)], and homogenized for 1 min at 230 rpm in a Stomacher 400 (Seward, London, UK). A volume of 10 ml of the homogenate (dilute 1:10) was transferred into sterile tubes, and placed in a circulating water bath at 80 °C for 10 min, and then cooled in melting ice prior to further serial dilutions in PSS, until 1:1000. This heat treatment was made to destroy vegetative bacteria and fungi and to make easier the quantification and isolation of *B. cereus* from its spores that are thermoresistant.

In order to detect and enumerate low levels of *B. cereus* spores, the three-tubes MPN procedure was performed as specified in Harmon et al. (1992). In total, 1-ml volumes of each dilution was used to inoculate 3 series of 3 tubes containing tryptone soya broth (TSB; Oxoid) supplemented with 100 mg l^{-1} of polymyxin B sulphate (Sigma–Aldrich Co. Ltd., Irvine, UK). After incubation at 30 °C for 24 h, the tubes were examined visually for turbidity, and a loopful of culture from positive MPN tubes (turbid) was streaked onto mannitol–egg yolk–polymyxin agar (MYP; Difco, Detroit, Michigan, USA) plates. The plates were incubated at 30 °C for 24–48 h. Typical colonies (pink colonies surrounded by a zone of precipitation) were purified on tryptone soya agar (TSA; Oxoid), and further confirmed by establishing the following morphological and biochemical properties as described in Harmon et al. (1992): Gram-positive rods with spores and positive for glucose fermentation, Voges–Proskauer reaction, gelatine hydrolysis, nitrate reduction, motility, and tyrosine degradation. The value counts (MPN g^{-1}) were obtained consulting the MPN table (Peeler et al., 1992). Additionally, in order to detect and enumerate high levels of spores

Table 1
Number and formulations of dried milk products analysed

Products	No. of samples	Formulation
Milk substitute	94	Whole milk powder, milking serum, greasy matter, cereals and pulses extruded, flours, minerals, antioxidants, emulsifiers, flavouring, edulcorates and colorants
Rice with milk	56	Rice, whole milk powder, emulsifiers, thickeners, sugar, minerals and permitted flavours
Milk pudding	54	Whole milk powder, sugar, starch, minerals, flavourings, edulcorates, and permitted colours
Flan	55	Whole milk powder, sugar, starch, greasy matter, gelificants, minerals, edulcorates, colorants, and permitted flavours
Semolina with milk	49	Whole milk powder, semolina, sugar, cereal starch, edulcorates, permitted flavours and colours
Whole milk powder	29	Whole powder milk
Milk–rice–cereal	27	Whole milk powder, sugar, cereal extruded (rice), edulcorates, permitted flavours and colours
Mousse	17	Whole milk powder, sugar, modified starch, foaming agent, thickeners, flavours y colorants