Food Control 69 (2016) 20–29

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

Bacillus cereus hazard and control in industrial dairy processing environment

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A R T I C L E I N F O

Article history:
Received 1 October 2015
Received in revised form
1 March 2016
Accepted 10 April 2016
Available online 14 April 2016

Keywords:
Bacillus cereus
Enterotoxin
Biofilm
Hazard
Cleaning-in-place (CIP)

A B S T R A C T

Bacillus cereus is one of the most important spoilage microorganisms in dairy environment and its growth may result in various dairy defects. Moreover, it is a great safety concern for dairy industry as it is associated with incidences of food poisoning by producing enterotoxin. Because of its outstanding ability to adhere to stainless steel surfaces of dairy plant and form biofilm, *B. cereus* can lead to serious hygiene problems and economic loss due to spoilage of dairy products and equipment impairment. Biofilms are more resistant to antimicrobials and cleaning regimes compared to planktonic cells, and this makes their elimination from dairy industry a big challenge. *B. cereus* biofilms may develop particularly in storage and piping systems that are partly filled during operation or where residual liquid has remained after a production cycle. These biofilms in pasteurizer and storage tanks can be a source of post-pasteurization recurrent contamination. Cleaning-in-place (CIP) regimes, commonly used in dairy industry, showed a varied effectiveness in eliminating *B. cereus* biofilms. Optimization of alkali-based CIP significantly increases *B. cereus* biofilm cell removal, as compared to the reference CIP usually used in dairy industry. Thus, optimization of the existing cleaning processes and development of novel and effective strategies as well are of utmost importance to the dairy industry, as these may lead to quality improvement of products and processes. This review discusses the characteristics, and spoilage and toxigenic potential of *B. cereus* in dairy industry, with an emphasis on biofilm development and emerging control strategies.

Contents

1. Introduction 20
2. Taxonomy 21
3. Functional morphology of cells 21
4. Ecology 22
5. Toxigenicity 22
6. Contamination and risk in the dairy production chain 23
7. Role in toxigenicity and spoilage of dairy products 24
8. Biofilm and its hazard in the dairy environment 25
9. Biofilm control strategies 26
10. Outlook 27
References 27

1. Introduction

Bacillus cereus comprises the largest group of endospore-forming bacteria and is now attracting a great interest among researchers as the members are not only associated with foodborne

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http://dx.doi.org/10.1016/j.foodcont.2016.04.012

outbreaks but also responsible for spoilage of food products. The *B. cereus* group, also known as *B. cereus sensu lato* (*s.l.*), consists of seven closely related species, namely *B. cereus sensu stricto* (*s.s.*), *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus anthracis*, *Bacillus weihenstephanensis* and *Bacillus cytotoxicus*. Since a close genetic relationship exists among all the seven species of the *B. cereus* group (Fig. 1), it was suggested that the entire group should represent a single species (Guinebretière et al., 2013).

Although *B. cereus s.s.*, or *B. cereus* as it is usually called, is mainly associated with gastrointestinal disorders, it is an opportunistic human pathogen associated with a multitude of other infections, such as severe eye infections, periodontitis, necrotizing fasciitis, endocarditis, nosocomial acquired bacteraemia, osteomyelitis, sepsis, liver abscess, pneumonia and meningitis, particularly in postsurgical patients, immunosuppressed individuals, intravenous drug abusers and neonates. In the idiophase, it produces several compounds, like degradation enzymes, cytotoxic factors and cell-surface proteins, which might contribute to virulence. However, there is still little recognition and appreciation of the role of *B. cereus* in these serious, and frequently fatal, clinical infections in humans (Ramarao & Sanchis, 2013).

In dairy environment, *B. cereus* can negatively affect product quality. It produces various extracellular enzymes which can be responsible for a decrease in the organoleptic quality of milk and dairy products.

B. cereus can be introduced into the dairy environment from various sources during production, handling and processing, mainly from improperly cleaned and sanitized equipments. The hydrophobic properties of endospores and their resistance towards heat, desiccation and disinfectants allow them to attach to processing equipment and survive cleaning procedures (Ryu & Beuchat, 2005; Simmonds, Mossel, Intaraphan, & Deeth, 2003). Adherence to stainless steel surfaces of dairy plants can result in biofilm formation which can be an important reservoir for recurrent contamination of dairy products (Kumari & Sarkar, 2014a; Shaheen, Svensson, Andersson, Christiansson, & Salkinoja-Salonen, 2010). An effective control of these bacteria in dairy products and processing environment is still a challenging task. As spore-forming bacteria are ubiquitous in nature, contamination has been shown to occur along the whole processing line; pasteurizer, filling machine, packaging boards and blanks can be a source of contamination (Eneroth, Svensson, Molin, & Christiansson, 2001).

The aim of this review was to give an overview of hazards caused by *B. cereus* in dairy environment and its control strategies.

2. Taxonomy

B. cereus group consists of seven closely related species, as mentioned earlier. Ecological populations of the *B. cereus* group are so closely related that they cannot be distinguished by 16S rRNA gene sequencing. Based on molecular data from fluorescent amplified fragment length polymorphism patterns, ribosomal gene sequences, partial *panC* (pantothenate synthetase) gene sequence, 'psychrotolerant' DNA sequence signatures and growth temperature, the *B. cereus* group has been divided into seven phylogenetic groups (Table 1). While all the mesophilic rhizoidal colony-forming strains of *B. mycoides* and *B. pseudomycoides* are clustered in group I, all the psychrotolerant strains of *B. mycoides*, *B. cereus s.s.* and *B. thuringinesis* along with *B. weihenstephanensis* are placed in group VI. *B. cereus s.s.* and *B. thuringinesis* are highly polyphyletic being spread over group II, III, IV, V and VI. The strains of *B. anthracis* are clustered in group III. The moderate thermotolerant *B. cytotoxicus* belongs to group VII (Guinebretière et al., 2008, 2013). Hence, the '*B. cereus* group' is an informal, however, widely used term describing a genetically highly homogeneous subdivision of the genus *Bacillus* and caters a dilemma from the taxonomic viewpoint (Stenfors Arnesen, Fagerlung, & Granum, 2008).

3. Functional morphology of cells

B. cereus is a large (1.0–1.2 μm by 3.0–5.0 μm), rod-shaped, motile, endospore-forming, aerobe-to-facultative, gram-positive bacterium which grows on common agar media, such as nutrient agar and plate count agar, to large colonies (3–8 mm dia) with a flat, greyish and 'ground-glass' appearance, often with irregular borders. On blood agar, the colonies are surrounded by zones of β-haemolysis. In selective culture media, such as mannitol-egg yolk-polymyxin (MYP) agar, polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA) and Bacara medium, *B. cereus* colonies are pink, peacock blue and orange-pink, respectively, surrounded by a halo or precipitation zone (Tallent, Kotewicz, Strain, & Bennett, 2012). Its spores are ellipsoidal, centrally or paracentrally placed and have net negative charge. The hydrophobic nature of *B. cereus* spores and presence of appendages (1–30 in number of 0.45–3.8 μm × 13.6 nm) on the surface result in firm adhesion to food processing surfaces, like stainless steel (Ankolekar & Lebbé, 2010; Tauveron, Slomianny, Henry, & Faille, 2006).

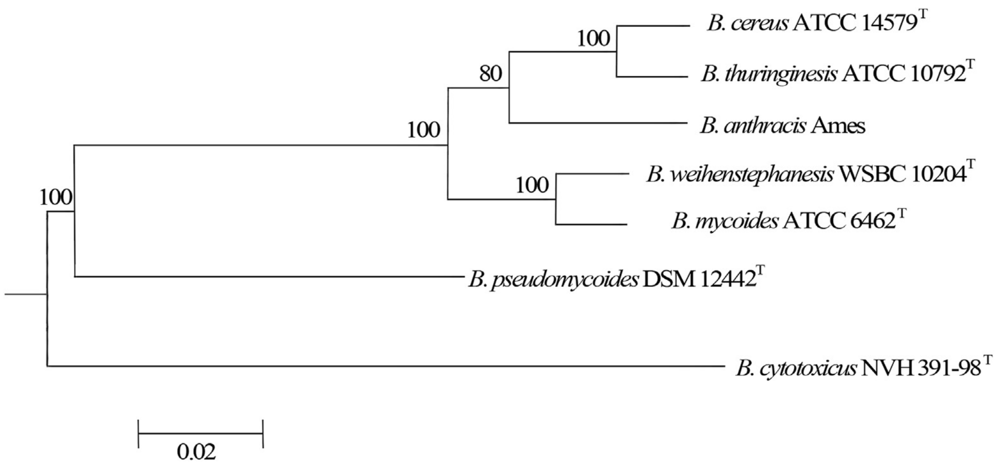

Fig. 1. The phylogenetic position of *Bacillus cereus* group species, based on concatenated sequences from genes included in MLST scheme of Tourasse, Helgason, Økstad, Hegna, and Kolstø (2006). Bootstrap values above 75% are given at each branch point. Bar, 0.02 substitutions per site (based on Guinebretière et al., 2013).