Risk profile of *Bacillus cereus* and public health implications

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Introduction

Bacillus cereus belongs to a group of closely related aerobic sporeforming species, which is being referred to as the *B. cereus* group. Although *B. cereus* is a well-known cause of food-borne illness it is not commonly reported because of its usually mild symptoms. It can cause two types of food poisoning, known as the emetic and the diarrhoeal types. The emetic type is frequently associated with the consumption of food rich in carbohydrates such as rice and pasta (Dierick et al., 2005), whereas the diarrhoeal type is often associated with cooked meat and meat products (Arnesen et al., 2007; Granum and Lund, 1997). It is generally admitted that contamination levels above 10^5 CFU g^{-1} are required to provoke illness. *B. cereus* is often present as an intrinsic contaminating microorganism in Refrigerated Processed Foods of Extended Durability (REPFED), pasteurized milk, rice dishes and pastas. During shelf life it may become a major part of the microflora. Because of its resistant spores, significant numbers of *B. cereus* have also been found in herbs and spices, vegetables and dehydrated foods. The presence of both vegetative cells and spores in food commodities has been reported and their role in food safety and food spoilage elaborated.

Risk potential of *B. cereus* in the food chain

FAO/WHO defines risk as a function of the probability of an adverse effect and the magnitude of that effect. In other words the risk is a statistical or probabilistic concept, which is directly linked to a hazard, being essentially a mathematical function of two components: probability (P) of occurrence of an undesired event (adverse effect) and the effect (E), also called severity, the latter considered as the magnitude of the consequence of its occurrence. Existing data on P and E outlines the following as main conclusions of risk portrayal:

- *B. cereus* as a sporeforming organism is a ubiquitous microorganism in the (production) environment and the heterogeneity of the food categories involved in food poisoning, together with the versatility of *B. cereus* itself represents a challenge for its control in the food chain.
- It is apparently the combination of the prevalence of a particular type of *B. cereus* (*B. cereus* constitutes very heterogeneous species group), and its toxin producing capacity, with the type of food and environmental factors (temperature abuse), which determine the risk profile. At present, and unlike other foodborne pathogens such as Salmonella spp., there is no indication that a particular type is or becomes dominating in foodborne outbreaks.
- Overall, at-risk foods are either foods which have been subjected to a mild heat treatment (pasteurized dairy foods or cooked chill foods) with an extended shelf life under refrigeration or dehydrated foods (e.g. milk powder, powder for dairy desserts, dehydrated soups, spices etc). The risk originates in inactivation of the competitive vegetative microflora by heat treatment or by reduced water activity. Surviving *B. cereus* spores are able to support germination, growth and toxin production of *B. cereus* during storage or after reconstitution (if the food forwards appropriate conditions). Particular at-risk products, which have been recognized in foodborne outbreaks, are pasteurized milk and dairy products, REPFED's, potato puree, rice or pasta containing dishes or salads and milk powder.

- No clear evidence is obtained that *B. cereus* concentration less than 10^5 CFU ml^{-1} will cause food poisoning. Compared to the high counts (> 10^6 CFU ml^{-1}) of *B. cereus* regularly observed in milk, cases of food poisoning are relatively rare. High numbers of *B. cereus* may be obtained, even if storage conditions encompassed respect of the cold chain (<7°C) due to the multiplication of psychrotolerant strains. There is insufficient information available to define the exact number of elevated levels of *B. cereus* that may cause food poisoning.
- Numbers of *B. cereus* are only part of the predisposing factor of *B. cereus* food poisoning: whether symptoms (response to exposure to defined levels) will occur due to ingestion of contaminated food is a function of three variables: the pathogen (*B. cereus* strain), the food and the host ingesting the contaminated food.
- With regard to the emetic strains it is well acknowledged that there is variety in the amount of cereulide produced (high producing versus low producing) by various established emetic toxin producing *B. cereus* strains. However, also the exact food composition has been shown to influence the toxin production for the same *B. cereus* strain.
- Overall there are many variables that determine the pathogenicity of a biological hazard which are in the case of *B. cereus* largely unknown and need to be clarified. The ability for and the extent of toxin production by a *B. cereus* strain is influenced by its complex and diverse genetic composition (which provides the arsenal of toxin genes and its regulatory systems) and its immediate environment (food or gastro-intestinal tract) which does not only provide the substrates and conditions for growth but as well the environment conditions (temperature, redox potential, substrates, etc) that will dictate whether and which of these genetic determinants will be switched on.
- *B. cereus* comprises both mesophilic and psychrotolerant strains. Although psychrotolerant strains are most important as food contaminants, mesophilic strains are most important for the onset of disease. Whereas both types can produce diarrhoeal toxins, emetic toxin seemed to be restricted to mesophilic strains of *B. cereus*. However, one recent report indicated possible emetic toxin production by psychrotolerant bacilli at low incubation temperatures. Public health significance of this new finding needs further investigation. Capacity of psychrotolerant *B. cereus* to produce diarrhoeal toxins in human intestines is suggested to be weaker than that of mesophilic strains. This hypothesis also requires verification under experimental condition mimicking condition of the human GIT.
- In recent years, considerable knowledge has been gained regarding *B. cereus* emetic toxin, mainly as a result of optimized detection methods (computerized boar semen bio-assay and HPLC-MS). Lack of quantitative, robust and reproducible detection methods for diarrhoeal toxins (HBL, NHE; entT and cytK) hindered real progress in the research of these toxins. It is therefore an imperative of future research to provide effective detection tools that will help answering some of the crucial questions, such as i) what are the amounts of diarrhoeal toxins produced in intestines, ii) what is intoxicative dose of different diarrhoeal toxins, and iii) how can one model the relationship between *B. cereus* counts and toxin production.
-Detection and isolation of presumptive *B. cereus* seems to be straightforward using the ISO method, but there are taxonomic problems or issues in the whole *B. cereus* group to be taken into account. There is a need to a more exact characterization of the pathogenic target organism(s) within the *B. cereus* group and hence a reliable detection method for it for identification to species level and for definition of the genotype, the pathotype and the phenotype (psychrotolerant character and functional toxic activity by biological assays).
- Whenever outbreaks occur, it is important to pick multiple and if feasible all isolates (also consider atypical non or weak haemolytic isolates) and apply all the appropriate phenotypic and molecular methodologies: i.e. classical isolation and biochemical confirmation, PCR detection for confirmation of identity to species level, detection of toxin genes (pathotype); verification of toxin production capacity of the isolates (phenotype) and the psychrotolerant character of the isolated strains. More specific isolation media should be developed which allow a more direct evaluation of important phenotypes.
- *B. cereus* can produce multiple toxins and one strain can produce more than one toxin. It is currently believed that HBL and emetic toxin production are mutually exclusive. It is not clear whether this statement can be understood as an absolute rule. Further characterization of

strains is necessary to elucidate what is the relation between genotype or origin of a strain and its ability to produce toxins or the compatibility of various toxins especially for the diarrhoeal strains. Furthermore there is only a limited knowledge on the prevalence of toxin genes among other strains and species of the *B. cereus* group. This needs to be further investigated, including the potential impact, in term of public health, of their presence (or not) in the food chain. This implies need for quicker and specific methodologies.

- In order to restrict the presence and numbers of *B. cereus* at-risk food stuffs, principles of Good Manufacturing Practices (GMP) and HACCP in the production process are to be strictly respected. These encompass at least hygienic design of equipment, adequate cleaning and disinfection, development of high care zones for aseptic filling, rapid chilling after heat treatment and control of the cold chain during storage and transport. If the food business operator (FBO) does not control these requirements that are necessary for the production and processing of safe foods a continuous post contamination with *B. cereus* may occur.
- *B. cereus* as a hazard and the management of the risk should be part of the concerned FBO's obligatory food safety management system (FSMS). In order to verify the effectiveness of the above mentioned control measures in the frame of its FSMS, it is recommended that the FBO active in the production of at-risk foods establishes a microbial assessment scheme to monitor *B. cereus* in raw materials, in processing line or processing environment and in intermediate or end products on a regular basis.
- As mentioned by the BACILLUS CEREUS EU project within the scope of hazard characterization and exposure assessment, and supported by the outbreak analysis, at present the most efficient management option for control of *B. cereus* in the food chain seems to be cold-chain maintenance (< 7°C) throughout the whole food chain (processing/storage/ transport) being a responsibility of the food processor, retailer, caterer and consumer.
- Especially for emetic strains, it is of importance that heated products are not kept outside the fridge or that no serious temperature abuse (> 10°C) is encountered during storage for a short period. Portioned chilling to rapidly lower the temperatures of precooked food is recommended to prevent growth of emetic strains.
- *B. cereus* growth may also be inhibited by producing food products with a non-neutral pH or develop product formulation according to hurdle technology to restrict growth of *B. cereus* throughout the shelf life. Appropriate product formulation and shelf life conditions are a management option, which is available to the food business operator to control the potential risk of *B. cereus* in the food chain.
- Infant foods should be considered as a high risk food class due to growth capabilities of *B. cereus* in these products and to the particularities of this group of consumers. Recently legislation was passed with regard to a process hygiene criterion at the end of the production process for presumptive *B. cereus* in dried infant formulae and dried dietary foods for medical purposes intended for infants below six months of age (EC 1441/2007 amending EC 2073/2005 on microbial criteria for foodstuffs): m = 50 cfu/g to M = 500 cfu/g (n=5, c=1).
- In contrast to the use of probiotics, amongst which sporeforming organisms such as *B. cereus*, in animal feed which are evaluated scientifically by EFSA and comprehensively regulated by the European Commission, up to now no strict regulations for their use in human food are applied. Initial steps of guidelines for a probiotic product to be used for humans, which are taken on worldwide level by the Food and Agricultural Organisation of the United States (FAO) and the World Health Organisation (WHO) and on the European level by EFSA (Qualified Presumption of Safety approach) should be further elaborated and implemented.

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