

Incidence and spread of *Bacillus cereus* in a milk powder plant as determined by phenotypic and molecular typing of isolated strains

6. SUMMARY

Bacillus (B.) cereus is as well a pathogenic as a spoilage microorganism. This is due to its toxinogenic properties and its highly active metabolism characterized by a formation of many extracellular enzymes. Accordingly the occurrence of *B. cereus* in milk powder and subsequently produced baby food is to be judged with respect to the protection of consumers and, in addition, with respect to quality assurance.

The aim of the work presented was to collect data on phenotypical and molecular characteristics of the organism to perform a risk consideration with regard to consumers protection and to implement a hygiene management system in a milk powder plant.

Between 1994 and 1998, 1365 milk powder samples and 215 environmental samples were examined for the presence of *B. cereus*. From milk powder 146 (11 % of the examined samples contained *B. cereus*) and from the environment of the production unit 183 (85 % of the examined samples contained *B. cereus*) strains were isolated.

Phenotypical characterization was performed using conventional bacteriological methods. Toxin formation was examined with a commercial test kit (Tecra[®]-ELISA) and a cytotoxicity assay using VERO-cells. For molecular characterization RAPD-PCR (randomly amplified polymorphic DNA) was applied. Results of RAPD-PCR were used to distinguish between strains from milk powder and from the environment and to clarify whether these strains belong to an inhouse flora or enter the plant via the raw milk. A cluster analysis of both groups of strains did not show similarities between the strains indicating that there is no inhouse flora of *B. cereus* present. The input of the organism with raw milk is estimated to be less than 1 spore/ml.

According to the result of the Tecra[®]-ELISA, a 45 k Da-component of the non-hemolytic toxin was produced by 97.9 % of all strains. Cytotoxicity was measured by release of lactate dehydrogenase (LDH). 58.4 % of the strains were positive in the cytotoxicity assay. All strains positive with this assay were positive with the ELISA as well.

Using the data collected, a risk consideration for the occurrence of *B. cereus* in milk powder leads to a safety limit of 10 spores/g powder. This will not be exceeded in an optimized production process, however, due to the input via raw milk it will not be reduced as well. In addition, the application of this limit takes into consideration a possible multiplication of the organism during subsequent processing and handling by the consumer. The education of consumers should aim on a proper risk communication about aerobic spore formers and also make clear that risks due to this organisms cannot be completely avoided in milk powder.