

## Sporeformers in Food; Implication of Natural Diversity on Food Safety and Food Quality

Bacterial spores are extremely resistant and therefore widespread in food ingredients and foods. Efficacy of control of sporeformers by the food processing industry is challenging due to diversity between strains and species with respect to their spore robustness and ability to rapidly germinate. Food processing and food characteristics can impose significant selective pressure on sporeformers, whereby most robust sporeformers and/or the best growers predominate. This symposium will focus on diversity of relevant food sporeformers (both pathogens and spoilage organisms) and the implication for controlling food safety and quality. We will address these issues with leading experts in the spore research field, discussing quantification of natural diversity between strains and between species, genetic biomarkers for high spore heat resistance and diversity in growth performance of germinated spores, thereby highlighting the relevance for these aspects in the actual control of sporeformers in food.

### Variability in Heat Resistance of Sporeformers; How Diverse is Diversity?

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Realistic prediction of microbial inactivation in food requires quantitative information on variability introduced by the microorganisms. *Bacillus* species form heat-resistant spores and variability in spore heat resistance varies among species. Therefore, the spore heat resistance of the spoilage organisms *Bacillus subtilis* and *Geobacillus stearothermophilis*, and the foodborne pathogen *Bacillus cereus* were characterized in detail using twenty strains per species. This allowed comparison of variability in spore heat resistance between and within *Bacillus* species. In addition, reproduction variability was determined using two biologically independent spore crops for each strain which were heat treated on different days. Reproduction variability was significantly lower than strain variability for all three species. A meta-analysis on spore heat resistance of *Bacillus* species demonstrated that strain variability explained at least 50% of all variability in heat resistance of sporeformers. This indicates that integration of microbiological variability in prediction makes predictions not more accurate, yet more realistic.

## Genetic Biomarkers for High Heat Resistance of *Bacillus* Spores: Relevance for Optimal Design of Heat Treatment

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Spores of various *Bacillus* species can display very high levels of heat resistance, allowing for survival of even sterilization processes that are commonly applied in the food industry. This includes spores of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* and various other species. However, high level heat resistance of spores is seen only for certain strains of these species. This makes it hard to predict if their presence will cause problems in final heat-treated products. So far, it was not possible to establish whether spores of *Bacillus* isolates could survive high heat treatments (i.e., at least 30 min 100°C) unless their survival was assessed in an experimental setting. We recently identified a mobile genetic element that is responsible for high level heat resistance of *Bacillus subtilis* spores. It encodes proteins that are specifically expressed during spore formation. The presence of this element in the genomes of wide array of *Bacillus* species was investigated and could be linked directly with high-level heat resistance of spores of certain *B. subtilis* strains. Moreover, this was also demonstrated for high-level heat resistance of spores of certain *B. amyloliquefaciens* and *B. licheniformis* strains. In addition, we established that the copy number of the element correlated positively with further increased heat resistance of spores. The presence or absence of this element can now be established by PCR, and based on the established heat inactivation kinetics of the spores of a large range of individual strains, optimal heat treatments can be designed to inactivate such spores in foods.

## Combined Approaches to Differentiate the Common Mister *B. licheniformis* and the Super Spoiler

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Sporeforming bacteria are ubiquitous in the environment and exhibit a wide range of diversity leading to their natural prevalence in foodstuff. In most cases, *Bacillus* and related genus enter the industrial plants via spore contaminations of raw material or dehydrated ingredients and may persist in the environment. While spore is the resistant but dormant state of these bacteria, food spoilage and food poisoning is only due to the vegetative cells multiplication of specific strains. Better knowledge on biodiversity, in particular regarding spore resistance, germination, growth and spoilage abilities may explain why specific strains, called here super spoiler, are able to survive process and develop in food during shelf life. Based on combined screening approaches, either common *Bacillus* contaminant or super spoiler may be identified. Deep characterization of these contaminants provides an added value when conducting challenge tests in food