PEB2.29 **Waking up in acid: germination and outgrowth of *Bacillus cereus* spores**

Masja Nierop Groot (1,2), C van Melis (1,3), M Tempelaars (1,3), R Moezelaars (1,2), T Abee (1,3)
(1) TI food and Nutrition, the Netherlands
(2) Wageningen UR Food and Biobased Research, The Netherlands
(3) Wageningen UR, Food Microbiology Laboratory, The Netherlands

Bacterial spores are a prime concern in food preservation and current practice to control them involves intensive thermal treatments which often compromise the organoleptic and physical quality of products. Lower intensity treatments are desired but should be developed in combination with preservatives to control outgrowth of surviving spores. Although the effectiveness of weak organic acids on spore outgrowth has been established, critical targets and molecular mechanisms of inactivation are largely unknown. An additional complexity in the design of safe processes is that germination heterogeneity can be induced by applying stresses, such as heating or the use of acids.

Spores of *Bacillus cereus* were used for phenotype and transcriptome profiling during germination and outgrowth in the presence of weak organic acids (acetic, lactic and sorbic acid) or hydrogen chloride at pH 5.5. Comparative analyses revealed distinct gene expression profiles that mark various stages in spore germination. Germination was relatively efficient under most acid stress conditions and genes expressed at this stage largely overlap suggesting a tightly controlled spore germination program, however, both generic and weak acid-specific responses could be distinguished in the outgrowth phase and its relevance will be discussed. Depending on the type and concentration of acid used, different germination and outgrowth phenotypes were observed. In mildly stressed spores, germination was triggered albeit with reduced outgrowth capacity. For more severe stress, spores either germinated but failed to grow out or germination was prevented and spores remained dormant. An interesting observation was that although mildly stressed *B. cereus* spores germinated homogeneously, at more severe stress different subpopulations of dormant and germinated spores could be distinguished. We show that heterogeneity and delay in germination under sorbic acid stress could be clearly visualized by flow cytometry analysis, and subsequent assessment of outgrowth capacity of sorted single, germinated spores. Understanding of molecular mechanisms for inactivation of spores by individual organic acids and their critical targets could be used for the design of improved preservation strategies by maximising synergistic or complementary effects of acids and minimizing heterogeneity in germination.