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Study of analysis methods to monitor sporulation during fermentation of Bacillus subtilis to produce endospores

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Bacterial endospores, mainly of *Bacillus subtilis, B. atrophaeus* or *Geobacillus stearothermophilus,* are used as biological indicator for the validation of sterilization processes in various industrial processes and applications. Commonly used spores for sterilization studies are spores of *B. subtilis*, due to their high degree of resistance to chemical and physical treatments, reproducible inactivation response, and ease of use. To prevent falsified results in the validation of sterilization processes, theses spores must have high and comparable D-values as well as a homogeneous distribution of the resistance within every charge. The main influence on the resistance properties is the fermentation process, which lead to the necessity of well-defined methods and analytics of the spore formation for the production process.

To produce suspensions containing homogeneously equipped spores, a reproducible fed batch fermentation strategy with optimized concentrations of media components has been developed (VLB; data not yet published). To increase spore resistance, different influence factors such as fermentation time, temperature and composition of sporulation media were investigated.

A systematic screening of fermentation capabilities towards optimal spore formation, production and efficiency was performed. A collection of commonly used sterilization-recommended *B. subtilis* strains was evaluated by applying a set of different molecular biological techniques and classical microbiological assays. Quantitative real time PCR was used to study transcriptomic changes during the sporulation; here major sporulation events (e.g., sigma factors; from Stuelke, Joerg, SubtiWiki) were studied. MALDI-TOF-TOF analyses were conducted to follow changes in the protein spectra (Momo et al, 2013) from during initiation of sporulation until maturation of the dormant spore. The EloTrace® System (Junne et al, 2010) allowed us to characterize the morphological and physiological of the transition of the sporulating cultures using an electro-optical approach. Established and reproducible methods for measuring spore resistance were used to determine batch-specific resistance properties to selected sterilization methods (hydrogen peroxide or UV-C radiation treatments; Raguse et al, 2016).