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The impact of spore aggregation on viable and total counts of *Bacillus subtilis*

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Introduction: Decontamination of surfaces is a vitally important process in industrial settings, however in order to assess the efficacy of an antimicrobial, it is imperative that an accurate bacterial enumeration method is in place to avoid over or under-estimating the remaining bacterial count. Aggregation can be common in biofilm forming organisms such as *B. cereus*, therefore *Bacillus subtilis* spores are a good safe alternative to model pathogenic organism. In these communication spores of *B. subtilis* are exposed to different conditions and their total counts as well as viable counts are assessed by plating and flow cytometry (FCM).

Aims: To assess the perceived viable counts of spores across a range of different pH conditions and compare these with the total counts via FCM and to compare these results with spore counts with a non-ionic surfactant present to gain an insight into 'true' counts.

Methods: Spores were examined in LB broth and PBS (100 mM) at pH 1, 3 5 and 7. Flow cytometry (FCM) was implemented to measure viability, physiology and total counts of spores. Viability was also analysed by plating. Particle size distribution (PSD) was also carried out on spores with and without tween 20 and the levels of aggregation compared.

Results: Tween yielded significantly higher total counts in most cases. At pH-7 in LB, tween increased counts by 55%. This highlights the level of error in count one could expect from standard enumeration techniques. The PSD data clearly showed an increase in aggregation as the pH lowered. The presence of tween 20 broke apart these aggregates, leaving a much more homogenous single population.

Conclusions: The impact of spore aggregation on viable counts is an overlooked aspect possibly due to limitations in methodological analyses. As such, tools which provide total counts such as flow cytometry are extremely valuable in this line of research.

Biography

Nikos E Mavroudis is a Senior Lecturer and Programme Leader in Food Science and leads the laboratory of Food Engineering & Separation of Actives (FoESA) in the Dept. of Applied Sciences in Northumbria University at Newcastle. Previously he was a Research Scientist and Project Leader for Unilever R&D for 9 years and has been responsible for developing the separation expertise within Unilever R&D Vlaardingen, the Netherlands. His research interests are focused on Food Security & Food Safety, particularly relevant is his interest on bacterial flow cytometry and surface decontamination. He has published 10 research articles in peer review journals and 12 patents/ patent filings and his work has attracted ca 325 citations excluding self-citations.

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