

Development and validation of a reference material for food microbiology using *Bacillus cereus* spores

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Food microbiology laboratories have an increasing need for tools to evaluate their analytical performances. Reference Materials (RM) are appropriate means to assess the precision and fidelity of their analytical results. A food microbiology RM is a material that contains a precise, homogenous and stable number of microorganisms. Currently commercially available RM have important drawbacks: short shelf-life (6 months), low assigned value, high uncertainty, heterogeneity problems, or storage and transport at frozen temperatures.

REQUASUD is a Walloon organization that includes a network of six food microbiology laboratories, which take part to internal and external proficiency testing. An Internal Reference Material (IRM) was developed by REQUASUD for the enumeration by its member laboratories of total flora (ISO 4833), and for the setup of control charts. The IRM was validated following the ISO Guidelines ISO 13528, ISO 5725 (Statistical analysis and preparation of standards), and ISO 19036 (Uncertainty in food microbiology).

The main innovation of this research was the use of *B. cereus* spores as RM because of their natural resistance and stability at room temperature. The development of this IRM was made in three steps:

1. Sporulation of *B. cereus* was optimized (different strains, nutrients, incubation times and temperatures) in order to yield high amounts of viable spores;
2. "Easy-to-handle" units of RM containing appropriate amounts of spores (representative of contamination level of food matrices) were produced;
3. Batches of IRM were then submitted to validation for homogeneity, stability and assigned value according to ISO standards.

A sporulation protocol was developed which yielded, in seven days, a culture containing more than 95% viable spores of *B. cereus* ATCC 13061. Several solid supports were inoculated with 10^4 *B. cereus* spores, dried and stored at room temperature. Two supports (calcium carbonate and lactose pellets), turned out to bring the most interesting results. Two batches of 500 inoculated pellets were manufactured and tested for validation.

The validation of a RM requires that the variance between IRM units does not contribute for more than 10 % to the global variance of the proficiency testing (PT): $s_{\text{IRM}}^2 < 0,1 \cdot s_{\text{PT}}^2$

where s_{PT} is the robust mean of the standard deviation of REQUASUD's last 20 proficiency testing. The homogeneity of both IRM batches was proven and their assigned values were calibrated using a certified RM. Their stability was also evaluated using control charts: only the calcium carbonate IRM showed a very good stability for at least 6 months, at room temperature. The lactose IRM was stable for 14 weeks and then a rapid drop was observed.

This work revealed new paths for the development and validation of IRM. Spores of *B. cereus* displayed an excellent stability during at least six months at room temperature, which is a clear advantage compared to the existing reference materials. A manufacturing and validation process was also compiled in a procedure that will be useful to laboratories willing to develop and validate new reference materials.