METHOD FOR REMOVAL OF VEGETATIVE CELLS FROM BACTERIAL SPORE PREPARATIONS[1]

STERLING K. LONG[2] AND O. B. WILLIAMS

Department of Bacteriology, The University of Texas, Austin, Texas

Received for publication May 14, 1958

One of the more difficult and important problems encountered in analytical work on bacterial spores is the removal of the nonsporulating and unlysed vegetative cells found in all cultures which have attained maximum sporulation. A number of methods reported in the literature which have given satisfactory removal of vegetative cells of a number of species gave no appreciable separation of the spores and cells of *Bacillus stearothermophilus* NCA numbers 1518 and 7900, or *Bacillus cereus*. Reported methods which were here unsuccessful included attempted destruction of the vegetative cells by alteration of the osmotic environment, autolysis in distilled water (Church *et al.*, J. Bacteriol., **68,** 393–399, 1954), slow freezing and thawing (Stewart and Halvorson, J. Bacteriol., **65,** 160–166, 1953), and separation by differential centrifugation in plain water or in high viscosity, aqueous solutions of sucrose.

Satisfactory purification of spore material was accomplished by the procedure given here. An advantage of the method lies in the use of commonly available laboratory equipment.

The *B. stearothermophilus* strains were grown in the tryptose-basamine-dextrose broth of Long and Williams (Bacteriol. Proc., **1957,** 15, 1957). *B. cereus* was grown in a glucose-yeast extract-glutamic acid broth.

After maximum obtainable sporulation had occurred, the spores and remaining vegetative cells were removed from the growth media by centrifugation at 2000 rpm for 1 hr in the cold. The supernatant was carefully removed with sterile pipettes and discarded. The residues were then dispersed by shaking vigorously with glass beads in cold sterile distilled water. The spore and cell materials were again packed by centrifugation as before and the supernatant removed and discarded.

Examination of the packed matter at this stage revealed a pink layer of vegetative cells overlying a gray-white spore layer for *B. stearothermophilus*, and a white layer of cells overlying a gray-white spore layer for *B. cereus*.

The centrifuge tubes were held at an angle and 5 ml of cold sterile distilled water were allowed to flow slowly from a pipette onto the residues. The tubes were rotated gently causing the water to swirl over the surface of the material. During this treatment the pink or white vegetative layers gradually formed into gelatinous globules. As soon as the compact spore layer became visible, swirling was stopped and the tube inverted to remove the vegetative material. The packed spores remained intact during this operation. Microscopic examination of stained smears from the removed material revealed that most of this consisted of vegetative cells and only a few spores.

The spore material was again dispersed by shaking with cold water and centrifugation, and removal of vegetative cell layers was repeated.

Generally, the gelatinous globules of vegetative cells will form through only three washings, probably due to the fact that so few of these cells remain that well-defined layers cannot be formed. At this time some difficulty is also experienced in obtaining packed spores since they have become markedly less adherent. This problem was solved by increasing the period of centrifugation to 1½ to 2 hr. Two additional washings were done to ensure cleanliness of the material.

Successive microscopic examinations of material from spore layers revealed a continuously decreasing vegetative cell content. After the final washing, only an occasional vegetative cell was found per several microscopic fields of stained smears of the spore material.

[1] This work was supported in part by a research grant from Swift and Company, Chicago, Illinois.

[2] Present address: University of Florida Citrus Experiment Station, Lake Alfred, Florida.