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Organelle Nature of a Cell Granule from a Thermophilic Bacterium.* (20131)

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Recent interest in mitochondria of animal and plant cells has quickened the search for specific granules in bacteria(1). In a former paper from this laboratory(2), we presented evidence to show that a fraction isolated from a thermophilic bacterium(3), *Bacillus stearothermophilus* (No. 2184), was, indeed, a specific cell granule. The data rested upon photomicrographs and upon certain enzyme data. Some doubt could still be raised at the organelle nature of the granules on the basis that the particles isolated are not the particles as seen in the intact cells. The percentage of granules in whole cells is roughly 60% of the total protein, a figure which is entirely out of proportion by comparison to figures for organelles of animal cells. Further, staining technics on whole cells could lead to false interpretations due to diffusion artifacts, a fact well recognized by Koelle(4) and Novikoff(5).

Recently, we have obtained data by the use of triphenyltetrazolium chloride (TPTZ) that leaves little doubt as to the existence in the intact cells of the granules obtained after treatment with lysozyme.

Cells of the thermophilic bacterium No. 2184 when treated with TPTZ and substrate reveal zones in the areas where oxidation is carried on due to the deposition of insoluble formazan which can be seen with the phase microscope as highly refractile and remarkably clear areas. Bielig, Kausche, and Haardick(6) have already pointed out the usefulness of TPTZ with the conventional microscope in locating areas of oxidation in bacteria. The granules isolated after lysozyme treatment show the same properties toward TPTZ as do the original cells. In each case where formazan is seen within the cell, the same deposition can be seen in the granule. When no formazan is deposited in the cell, no formazan is deposited in the granules.

The most striking illustration of such parallel behavior is seen with the pyruvic oxidase system. This system oxidizes pyruvate in a solution containing fresh cells or fresh granules (10 mg), diphosphothiamine (50 μg), pyruvate (0.05 M), TPTZ (0.05%), phosphate buffer of pH 6.5 (1.0 ml), magnesium sulfate (4×10^{-2} M) in a total volume of 3.0 ml. At 60°C the reaction time to show pronounced color development is rapid with granules (15 min.) and somewhat slower with whole cells (30 min.). When viewed under the phase microscope after the reaction, the cells show definite refractile areas (Fig. 1). These areas appear to be in the granules themselves, although distinct discernment of the granule is not quite possible without counterstaining, a practice that destroys the for-

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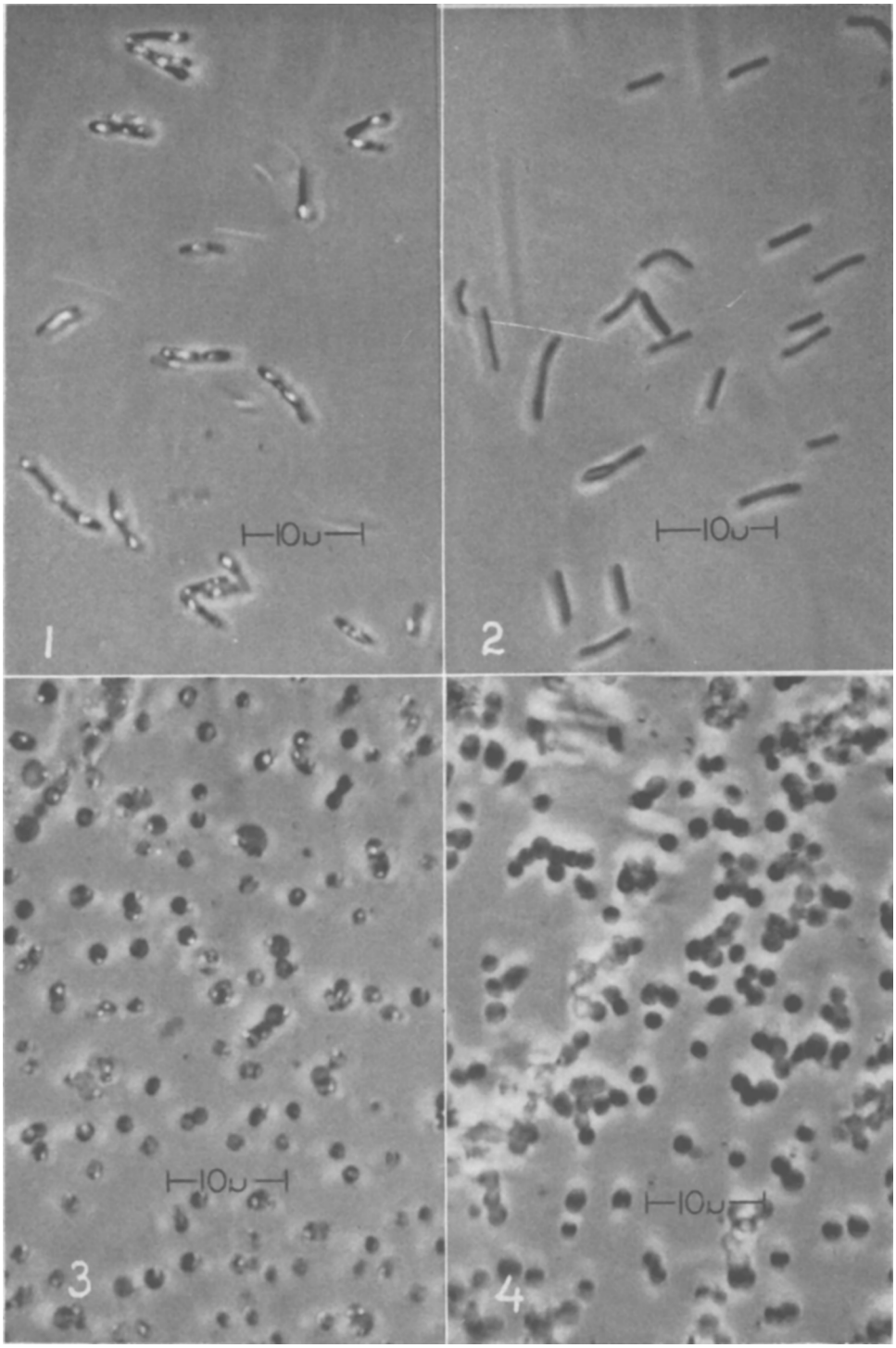

FIG. 1. Whole cells of *Bacillus stearothermophilus* (No. 2184) treated with triphenyltetrazolium chloride (TPTZ) and pyruvate, showing refractile areas under the phase microscope. Cells were immobilized in 1.0% methyl cellulose.

FIG. 2. Control whole cells showing the absence of refractile areas when substrate is omitted.

FIG. 3. Cell granules, isolated from thermophile No. 2184, showing refractile areas with TPTZ and pyruvate.

FIG. 4. Control cell granules.

mazan localization. A control on whole cells without pyruvate does not show refractile areas (Fig. 2) except on prolonged standing in which case the substrates already present within the cells cause deposition of formazan. The isolated cell granules, also, show clear refractile areas with TPTZ and pyruvate (Fig. 3), whereas the control without substrate (Fig. 4) remains negative for days.

The refractile areas in the bacteria cannot be confused as spores which, it could be argued, might arise due to the unusual conditions. A sporulating bacterium can be seen in Fig. 2. The spore, under the phase microscope, does not have the brilliant appearance of the formazan areas.

Cells that have been dried show no activity with pyruvate and TPTZ, nor do the granules isolated therefrom. No refractile areas can be seen under the phase microscope with these preparations. Drying readily inactivates the pyruvic oxidase system, as do freezing and surface inactivation.

Results with other substrates bear out fully those obtained with pyruvate. The granules and the cells show good enzyme activity and refractile bodies with TPTZ and malate, succinate, lactate, glucose, isocitrate, oxalacetate, and fumarate. The supernatant from the separation of the granules shows good enzyme activity only with malate, slight activity at the end of an hour with glucose and lactate, but no activity with pyruvate, succinate, and isocitrate.

In addition to the enzymes mentioned above, the granules show aconitase and alpha ketoglutaric oxidase activity (shown by means other than the TPTZ technic) thus rounding out the group of enzymes making up the Krebs tricarboxylic acid cycle. Table I gives the activity of several of these enzymes. The results with TPTZ on the substrates of the Krebs cycle have been amply verified by studies on oxygen consumption with the Warburg apparatus with the exception of aconitase, which was determined colorimetrically by a method given by Dickman and Cloutier (7).

TABLE I. Activity at 45°C of Some Enzymes Located in the Cell Particle.

Enzyme	Activity/10 mg protein/hr (O_2)
Succinoxidase	36 μl
Cytochrome b	50 "
Alpha ketoglutaric oxidase	47 "
Pyruvic oxidase	254 "
Aconitase	176 μM citrate formed
Isocitric dehydrogenase	59 μl
Malic dehydrogenase	175 "

Conclusions. Photomicrographs and enzyme data obtained with triphenyltetrazolium chloride and various substrates support earlier conclusions that a fraction isolated from a thermophilic bacterium consists of cell granules. Formazan, deposited at local sites of oxidation, appears under the phase microscope as brilliant refractile particles. The isocitric acid cycle appears in the cell granule.

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