Resistance of Vegetative Cells and Microcysts of *Myxococcus xanthus*

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Received for publication 23 December 1968

The resistance of vegetative cells and of microcysts of *Myxococcus xanthus* to several destructive agents was compared. Fruiting-body microcysts were 300 times more resistant to 60°C, 5.4 times more resistant to ultraviolet light, and 19.3 times more resistant to sonic vibration than were vegetative cells. Whereas resistance to sonic vibration developed during the conversion of rods to refractile spheres, resistance to heat did not appear until after the conversion was complete. Both vegetative cells and microcysts of the yellow variant of this strain were more resistant to ultraviolet irradiation than was the tan variant.

A number of reports have demonstrated that microcysts, the resting cells produced by most myxobacteria, are more resistant than the corresponding vegetative cells. Baur (1) showed that microcysts of *Myxococcus ruber* withstood desiccation and wet and dry heat better than did vegetative cells. In 1936, Imshenetsky and Solnetzewa (6) reported comparable findings for *Cytophaga hutchinsonii* and *C. ellipsospora* [reclassified by Stanier (7) as *Sporocytophaga myxococoides* and *S. ellipsospora*, respectively]. Leadbetter (Bacteriol. Proc. p. 42, 1963) found that, whereas microcysts of *S. myxococoides* were markedly more resistant to desiccation, osmotic gradients, and sonic vibration, they were not much more resistant to heat than were vegetative cells. In none of these cases were the kinetics or rates of killing presented.

The purpose of this investigation was to compare quantitatively the resistance of vegetative cells and microcysts of *M. xanthus* to heat, sonic vibration, ultraviolet (UV) irradiation, and desiccation.

**MATERIALS AND METHODS**

**Organisms.** A normal culture of *M. xanthus* FB dissociates into yellow and tan variants, maintaining a ratio of about 1:10. Stable variants FB<sub>y</sub> (yellow) and FB<sub>t</sub> (tan) were isolated and described by Burchard and Dworkin (2). All three strains form microcysts in fruiting bodies on solid media or in liquid media by the glycerol induction technique (4).

**Cultivation methods.** Vegetative cells were grown in liquid CT (3) at 30°C for 16 to 18 hr to a concentration of about 6 × 10<sup>8</sup> cells/ml, at which point the cells were still growing exponentially. Microcysts formed on solid medium were cultured by the method described by Dworkin and Niederpruem (5). Microcysts in liquid medium were induced by the glycerol technique (4). Viable counts were determined in duplicate on CT agar by the spread plate technique. The plates were dried for 1 hr at 30°C, inverted, and incubated for 6 days.

**Experimental conditions.** For the sonic vibration experiments on vegetative cells and glycerol microcysts, cultures were harvested, washed in a buffer containing 0.01 M K<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> (pH 7.6) and 8 × 10<sup>-4</sup> M MgSO<sub>4</sub> (Mg-P), resuspended to 2.5 × 10<sup>7</sup> cells/ml in CT-1 (CT medium with the concentration of Casitone reduced to 1%), and divided into 5.0-ml portions. Each sample was treated in an MSE ultrasonic power unit (type MR20; Measuring & Scientific Equipment, Ltd., London, England) for an interval varying from 10 to 30 sec (with the sample cooled in an ice bath) and immediately plated.

Sonic vibration experiments on cells during microcyst formation were performed in the following manner. Vegetative cells were harvested, washed in Mg-P, resuspended in CT-1 at a concentration of 2.5 × 10<sup>8</sup> cells/ml, and induced to make microcysts by the glycerol technique. At 15 min after the addition of glycerol and every 20 min thereafter, a sample was ground in a tissue homogenizer to break up clumps which tended to form during microcyst formation. The dispersed suspension was allowed to stand at room temperature for 5 min, during which time most of the remaining clumps settled to the bottom. A 5-ml amount of a 1:10 dilution of the supernatant suspension was exposed to sonic vibration for 30 sec, and an additional 5.0 ml was saved as a control. Both portions were counted in a Petroff-Hausser counting chamber and plated quantitatively.

Plate microcysts to be tested for heat resistance were harvested in deionized water, and the large number of unconverted vegetative cells were removed by osmotic shock. (The culture was suspended in 1 M KCl and centrifuged; the pellet was resuspended in...
distilled water. The vegetative cells lysed immediately whereas the microcysts were unaffected. Clumps of microcysts were routinely dispersed by 30-sec sonic vibration, a treatment previously proved not to alter viability significantly.

Occasionally, vegetative cells clumped when suspended in deionized water. This could be prevented by suspending the cells in Mg-P. Accordingly, Mg-P was the suspending medium routinely used in experiments to test heat resistance. In each experiment, a 14.5-cm test tube containing 5.0 ml of Mg-P was heated in a water bath to the desired temperature. A 0.2-ml amount of a heavy cell suspension was added, and 30 sec was allowed for equilibrium to be established. At intervals, a tube was removed and plunged into an ice bath. The samples were immediately plated quantitatively.

Glycerol microcysts to be exposed to heat were removed from the glycerol-CT medium at 3 and 9 hr, washed once in Mg-P, and treated in the same manner as plate microcysts, i.e., subjected to osmotic shock and sonic vibration.

Vegetative cells to be exposed to UV light were washed and resuspended in cold Mg-P to 10⁷/ml. Amounts of 5 ml of this suspension were placed in 9.0-cm glass petri dishes, and the uncovered plates were gently rotated under a 15-w GE germicidal lamp at 56.0 cm. After exposure, the cells were cooled, shielded from direct light, and plated. The same procedures were followed in experiments with microcysts.

For experiments to test resistance to desiccation, 2-week-old plate microcysts were harvested in distilled water, washed once, resuspended, sonically treated for 30 sec, and diluted to 10⁸ cells/ml in ice-cold distilled water. Samples (1 ml) were evenly distributed on membrane filters (diameter, 5 cm; pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) which had been autoclaved in 1 liter of distilled water. Suction was applied until no more water left the filter. Each filter was placed in a sterile glass petri dish and stored in a desiccator under partial vacuum. The filters appeared dry within 1 hr. At intervals, filters were removed, placed on plates of CT, and incubated for 6 days. Colonies developed on the filters and were counted. This method (and several modifications of it) was used in experiments with vegetative cells.

RESULTS

Sonic vibration. Figure 1a shows the decrease in viable vegetative cells as a function of the dosage of sonic vibration. After treatment for 30 sec, less than 0.5% of the population was viable. In the same type of experiment with 6-hr microcysts (in glycerol for 6 hr), a linear rate of death was also observed (Fig. 1a). After 30 sec, more than 80% of the microcysts were viable. A com-
parison of the sensitivity of the two cell types can be made by calculating the ratio of the slopes, \( K \), from the equation describing logarithmic death: \( \ln b - \ln a = K t \). Here \( a \) is the initial number of viable cells and \( b \) is the number of viable cells at time \( t \). According to these curves, 6-hr microcysts were about 19 times more resistant to sonic oscillation than vegetative cells.

Figure 1b represents the appearance of resistance to sonic vibration during microcyst formation. Although resistance coincided with the appearance of optical refractility, not all refractile cells were viable.

**Heat.** Figure 2 shows the rates at which vegetative cells and fruiting-body microcysts were killed at 45, 50, and 60 C. Vegetative cells were killed at 45 C and at increasing rates at 50 and 60 C. In contrast, microcysts were stable to 50 C for at least 20 min and were killed only slowly at 60 C.

**UV irradiation.** The viability of vegetative cells and of microcysts exposed to UV light is shown in Fig. 3. Experiments have shown that the UV resistance of 8-hr-old glycerol microcysts was identical to that of fruiting-body microcysts. In addition, a progressive enrichment of surviving FBc cells was observed, both with vegetative FB and with FB microcysts (Fig. 4).

**Desiccation.** In spite of repeated and varied attempts to measure resistance to desiccation, vegetative cells immediately lost viability upon being dried on the Millipore filters. On the other hand, approximately 50% of the fruiting body microcysts retained viability after 6 days of desiccation.

Table 1 summarizes the differential resistance of microcysts and of vegetative cells to 60 C, to UV light, and to sonic vibration.

**DISCUSSION**

Fruiting-body microcysts are clearly more resistant to the agents tested than are vegetative cells. Glycerol-induced microcysts display intermediate degrees of resistance, becoming progressively more resistant the longer they are incubated in the glycerol medium.

The physical or chemical alteration which confers resistance to sonic vibration has not been identified. Change in cell wall composition, loss of water, capsule formation, and inactivation of autolytic enzymes are among the many possibilities. The parallel incidence of refractility and resistance to sonic vibration may have some significance, perhaps indicating a change in cell wall structure or in the degree of hydration of the cell.

Vegetative cells formed spheroplasts at 45 and 50 C, perhaps as a result of the activation of their autolytic system. At 60 C, however, the cells looked like broken rods, and death could have
These values are considerably more of K fruiting-body microcysts. Based on comparison of K values, however, microcysts were at least 30 times more resistant to 60 C than were vegetative cells.

The resistance of microcysts to UV irradiation is considerably greater than that of vegetative cells, the ratio of the K values being about 5.4. These values were calculated from the portions of the curves after the 10-sec point, since it has not been definitely established that the slopes do indeed change at this point. In several other experiments, only one slope was observed. If one assumes the zero time point on the curves presented to be too high, a straight line could be drawn through the other points.

A definite enrichment for FBp among the survivors was noted with both vegetative cells and microcysts. Preliminary experiments with pure strains of FBp and FBt have indicated that both the vegetative cells and the microcysts of FBp are more resistant to UV than are the corresponding cells of FBt. Since the primary difference between the two strains is the nature of their pigments, it is not unreasonable to suggest that the pigment of FBp is exerting a partial protective effect.

In several experiments, the resistance of glycerol microcysts was compared with that of fruiting-body microcysts. Although at 120 to 150 min the morphological change is just barely complete and resistance to sonic vibration is appearing in the population, 3-hr microcysts showed the same sensitivity to 50 C as did vegetative cells. However, after 9 hr in glycerol, the resistance to heat had increased about five times, but it still did not equal that of fruiting-body microcysts. On the other hand, 8-hr glycerol microcysts were at least as resistant to UV light as were fruiting-body microcysts.

In general, then, it seems that glycerol microcysts are not yet as “mature” as fruiting-body microcysts, but seem to be acquiring resistance comparable to that of fruiting-body microcysts. Resistance to all agents tested does not appear simultaneously. The cells first become resistant to sonic vibration, then to UV irradiation, and finally to heat. These changes probably reflect alterations within the cell which begin at different times and take different periods to be completed. Some of these changes occur after the morphological portion of the conversion is completed.

The level of heat resistance does not approach that of the bacterial endospore, but seems adequate for the conditions a microcyst is likely to encounter. In nature, M. xanthus is usually found in the surface layers of the soil. It is thus subjected to intermittent UV irradiation, elevated temperature, and desiccation. The ability of the microcysts to resist these conditions confers an obvious survival advantage on the organism.

ACKNOWLEDGMENTS
This investigation was supported by Public Health Service grant AI08036 and Public Health Service training grant GM4966 from the National Institute of Allergy and Infectious Diseases.

**TABLE 1. Comparison of death rates for vegetative cells and microcysts exposed to various agents**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell</th>
<th>(-K) (min(^{-1}))</th>
<th>Ratio (K_{MC}/K_{VC})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonic vibration</td>
<td>VC</td>
<td>10.7</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>MC (G)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>60 C</td>
<td>VC</td>
<td>8.15</td>
<td>302</td>
</tr>
<tr>
<td></td>
<td>MC (FB)</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>UV light</td>
<td>VC</td>
<td>4.12</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>MC (FB)</td>
<td>0.762</td>
<td></td>
</tr>
</tbody>
</table>

\* VC = vegetative cells; MC (G) = glycerol-induced microcysts; MC (FB) = fruiting-body microcysts.

resulted from protein denaturation as well as from partial autolysis. There was no change in the appearance of microcysts during heating. The slope of the viability curve for microcysts at 60 C varied significantly with the preparation of fruiting-body microcysts. Based on a comparison of K values, however, microcysts were at least 30 times more resistant to 60 C than were vegetative cells.

The resistance of microcysts to UV irradiation is considerably greater than that of vegetative cells, the ratio of the K values being about 5.4. These values were calculated from the portions of the curves after the 10-sec point, since it has not been definitely established that the slopes do
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