IMMUNOLOGICAL SPECIFICITIES OF SPORE AND VEGETATIVE CELL CATALASES OF *BACILLUS CEREUS*

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Baillie and Norris (J. Appl. Bacteriol. **26**:102, 1963) demonstrated two molecular forms of catalase in ultrasonic extracts of sporulating cells of *Bacillus cereus* by means of starch gel electrophoresis. One of the catalases was thermolabile (inactivated at 60 C in 5 min) and was the only form present in young vegetative cells; the other was relatively heat-resistant (resisting 80 C for 30 min) and appeared during spore-formation, persisting to become the sole catalase of the mature spore.

There are two possible explanations of the marked difference in heat resistance of these catalases: the two molecules may be entirely different, the spore catalase having a higher intrinsic stability; or the vegetative-cell catalase may become bound during sporulation to other cell components, thus acquiring a higher degree of structural rigidity. The retention of heat resistance by the spore catalase in aqueous extracts suggests that dehydration is not a major factor in heat stability. To study the relationship between these two molecular forms of catalase, antisera prepared against extracts of *B. cereus* cells of various ages were used to determine the immunological specificities of the enzymes.

Ultrasonic extracts of cells at various stages in spore formation were examined by immunoelectrophoresis in agar with antisera prepared in rabbits by injection of similar extracts (Baillie and Norris, J. Bacteriol., **87**:1221, 1964). The gels prepared on lantern slides were allowed to develop for 48 hr at 30 C and then were washed for an additional 48 hr in several changes of distilled water. The wells and trenches were then filled with molten 1% agar in distilled water, and the gels were allowed to dry out in an incubator at 30 C.

Catalase-anticatalase zones were detected by flooding the dried gels with 10% hydrogen peroxide when zones having catalase activity were sharply defined by the formation of small gas bubbles in the agar film (Uriel, Ann. N.Y. Acad. Sci. **103**:956, 1963).

Sporulating-cell extracts exhibited two catalases which gave reaction arcs when tested against a sporulating-cell antiserum. The component which migrated most rapidly towards the anode was the heat-sensitive catalase of the young

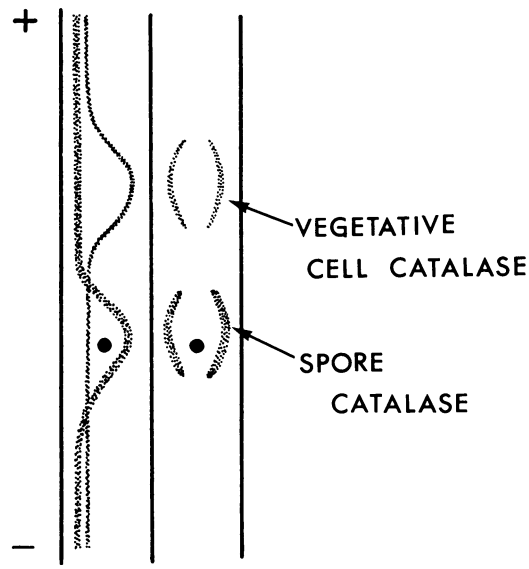

FIG. 1. *Diagrammatic representation of an immunoelectrophoretic analysis of an ultrasonic extract of sporulating cells of Bacillus cereus (in the antigen wells and the left-hand trench) against antiserum prepared by injecting a similar extract into a rabbit (in the center and right-hand trenches). The catalase-anticatalase lines were revealed by the production of minute gas bubbles when the washed and dried gel was flooded with 10% hydrogen peroxide. (For full details of the preparation of antigen extracts and antisera, and for a description of the electrophoretic technique, see Baillie and Norris, J. Bacteriol.* **87**:*1221, 1964.)*

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vegetative cell, and the more slowly moving component proved to be the heat-resistant spore catalase. The two catalase-anticatalase arcs crossed each other, showing that the enzymes were antigenically distinct. Figure 1 shows a diagrammatic representation of the results of an analysis of sporulating-cell extract by use of the antigen trench method of Osserman (J. Immunol. **84**:93, 1960).

An earlier attempt to study the immunological specificity of *B. cereus* spore catalase (Sadoff, p. 180. *In* H. O. Halvorson [ed.] *Spores* II, Burgess Publishing Co., Minneapolis, 1961) by immunodiffusion failed to demonstrate catalase activity in the antigen-antibody complex. Sadoff did, however, demonstrate the formation of heat-resistant catalase in the early stages of spore-formation in *B. cereus*, and presented evidence indicating that the heat-resistant and heat-sensitive catalases are quite distinct enzymes. To this finding, we would now add their lack of antigenic relationship as evidence for the existence of two structurally different molecular forms of catalase in sporulating cells of *B. cereus*.

CYSTINE-INDEPENDENT THERMORESISTANT MUTANTS OF POLIOVIRUS

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The genetic basis of the resistance of poliovirus to inactivation at 50 C has been shown by several groups of workers (Youngner, J. Immunol. **78**:282, 1957; Papaevangelou and Youngner, Proc. Soc. Exptl. Biol. Med. **108**:505, 1961; Medearis et al., Proc. Soc. Exptl. Biol. Med. **104**:419, 1960). In addition, Pohjanpelto (Virology **6**:472, 1958) described a phenotypic resistance which could be obtained by stabilizing viruses sensitive to 50 C with L-cystine. However, Wallis and Melnick (J. Bacteriol. **86**:499, 1963) recently concluded that resistance to 50 C of polioviruses is solely a phenotypic rather than a genotypic character. Their conclusion is based on evidence that thermoresistance can be imposed by extracellular stabilization of sensitive virus by L-cystine in the medium in which the virus is yielded from infected cells. In particular, they reported that a mutant previously described in our laboratory as genotypically resistant to inactivation at 50 C was, in their hands, merely a phenotypic variant stabilized by cystine.

The present report describes experiments done to test the conclusions of Wallis and Melnick regarding the genetic basis of resistance to 50 C of poliovirus. The results below show that, in the case of previously described mutants resistant to 50 C, the resistance is independent of cystine stabilization and represents a genotypic, not a phenotypic, character.

To test the claim of Wallis and Melnick that our thermoresistant (R) mutant could be converted to a highly thermosensitive state by one passage in cystine-free medium, we passed the resistant (R) and sensitive (S) mutants in medium containing different amounts of cystine. In addition, virus was harvested at 6 and 48 hr to test their further claim that "the time of harvest of the culture determines the phenotypic characteristic of heat-susceptibility," since they stated that intracellular virus is always heat-sensitive. The results of our experiments (Table 1) clearly indicate that the R mutant, grown in the absence of cystine, retained its resistance to 50 C when tested as either intracellular (6 hr) or extracellular (48 hr) harvests. On the other hand, the presence or absence of cystine markedly affected the thermostability of the S mutant. Clearly, 50 to 100 μg/ml of L-cystine in the medium produced a marked stabilization of extracellular (48 hr) S virus. Intracellular virus retained its S character even in the presence of high concentrations of cystine in the medium. With cystine absent from the medium, both intra- and extracellular harvests of the S mutant were thermosensitive.

To further illustrate the genotypic nature of the resistance of the R mutant, in contrast to the phenotypic nature of the resistance of cystine-stabilized S virus, treatment of the