Catalase Activity of Virulent and Avirulent Strains of Pseudomonas solanacearum

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Few investigators have attempted to illustrate the difference(s) between virulent and avirulent strains of *Pseudomonas solanacearum* E. F. Smith in terms of oxidative-reductive potentialities. Kelman (5) found that strains differed in their ability to reduce triphenyl tetrazolium chloride (TTC) in the culture medium. When grown on this medium, colonies of the virulent strain which were unable to reduce TTC reagent appeared as irregular fluidal white, and those of the avirulent strain which were able to reduce TTC reagent, appeared as round, butyrous, and deep red with narrow bluish borders. Abo-El-Dahab (1) found that the avirulent tobacco and tomato isolates had a higher rate of oxygen uptake than did the virulent strains of the same isolates, and that the avirulent strains reduced TTC more rapidly under aerobic conditions. The virulent strains on the other hand reduced TTC more rapidly under anaerobic conditions. Digat (3) has recently reported that the avirulent mutants of *P. solanacearum* are lacking in functional catalase, and suggested that catalase activity could play a role in virulence.

Preliminary qualitative tests for catalase activity of virulent strains and avirulent mutants of five potato isolates of *P. solanacearum* showed that only one isolate (No. 1) of the virulent strains had higher catalase activity than the avirulent mutants which appeared in culture (4). On the other hand, no difference in the catalase activity was noticed between the virulent strains and the avirulent mutants of each of the other four isolates (No. 2-5). Since results concerning the catalase activities of virulent and avirulent strains of *P. solanacearum* are contradictory (3, 4), the activity of various cellular preparations of the virulent and avirulent strains of each tested isolate of *P. solanacearum* was quantitatively assayed.

MATERIALS AND METHODS.—Resting cell suspensions (6) and acetone-dried cells (6) of potato isolates of *P. solanacearum* (4), were prepared from 48-hr-old cultures showing the characteristic colony type of each strain on tetrazolium glycerol nutrient agar medium (5 g peptone, 3 g beef extract, 20 ml glycerol, 20 g agar, 1,000 ml H_2O, 0.005% triphenyl tetrazolium chloride). The nitrogen content of the resting cell suspensions or the acetone-dried cells was estimated, using the micro-Kjeldahl method. Cultural filtrates of 3-day-old cultures grown on glycerol broth

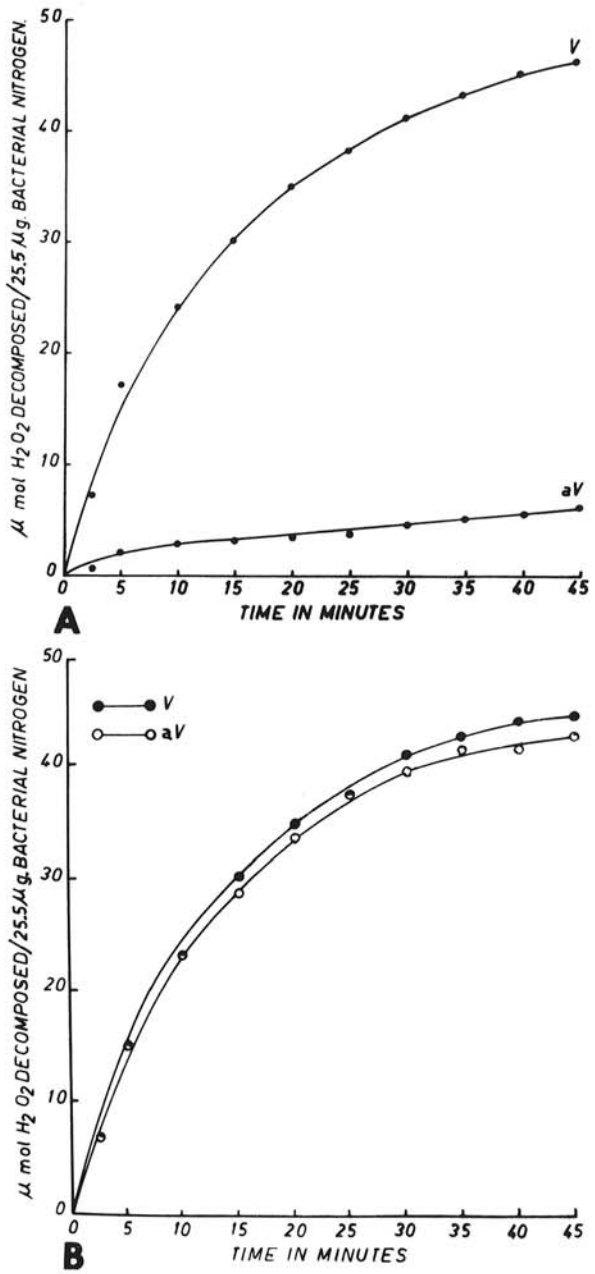

Fig. 1. Catalase activity of resting cell suspensions of virulent (v) and avirulent (av) strains of *Pseudomonas solanacearum*. **A)** Isolate 1. **B)** Isolate 3.

medium (5 g peptone, 3 g beef extract, 20 ml glycerol, 1,000 ml H_2O) or grown on synthetic broth medium (7 g K_2HPO_4, 3 g KH_2PO_4, 0.5 g Na citrate, 0.1 g $MgSO_4 \cdot 7H_2O$, 5 g ammonium sulfate, 20 ml glycerol, 1,000 ml H_2O) were prepared by filtration through a Seitz filter. Cell-free extracts of heavy cell suspensions of the isolate to be tested were obtained by grinding the cells with powdered alumina in a sterile porcelain mortar (6).

Catalase activity (2) of the intact washed cells, cellular preparations, or cultural filtrates were determined by the addition of 1 ml of the cell suspension (255 μg N/ml), or 1 ml of the acetone-dried cell preparation suspended in phosphate buffer pH 7.0 (294 μg N/ml), or 5 ml of the culture filtrate of the strain to be tested of *P. solanacearum* to 100 ml of 0.01 N H_2O_2 solution. The mixture was incubated at 25 C for 45 min. The decomposition of H_2O_2 was measured by titrating the remaining substrate with 0.0052 N potassium permanganate solution after stopping the enzymatic reaction with 10 ml 2% (v/v) sulfuric acid. A sample of 10 ml was taken from each assay mixture at 5-min intervals, and the remaining H_2O_2 was titrated. Four replicates were used in each treatment.

RESULTS AND DISCUSSION.—From the data presented in Fig. 1, it was concluded that the resting cell suspension of the virulent strain (isolate 1) of *P. solanacearum* (Fig. 1-A) had higher catalase activity than did the avirulent mutant. On the other hand, no difference in the catalase activity was noticed between the virulent strain and the avirulent mutants of each of the other four isolates (No. 2, 3 [Fig. 1-B], 4, 5).

None of the avirulent mutants of the five tested isolates was lacking in functional catalase. The catalase activity of acetone-dried cell preparations and cell-free extracts obtained by grinding cells with alumina powder gave results very similar to those of the resting cell suspensions. Culture filtrates of virulent strains and the avirulent mutants of the five isolates had no catalase activity, indicating that this enzyme does not diffuse into the medium. This fact was further supported by Digat (3), who reported that while the catalase activity of *P. solanacearum* is extracellular, it is located in the bacterial slime; i.e., the capsular layer. Nevertheless, the results obtained in the present work contradict the conclusions reached by Digat (3) in that the avirulent mutants of *P. solanacearum* are lacking in functional catalase, and that the bacterial catalase could bear any significance as far as virulence of this bacterium is concerned.

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