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DNase Production by *Clostridium septicum*

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Sixty-two *Clostridium septicum* isolates were assayed for extracellular DNase activity. All of the *C. septicum* isolates tested produced greater DNase activity than did the other DNase-producing clostridial isolates tested. The molecular weight of the DNase of *C. septicum* was determined to be approximately 45,000. DNase is a major extracellular protein produced by this organism.

Clostridium septicum is a gram-positive, rod-shaped, anaerobic bacterium which is an etiologic agent of traumatic and nontraumatic gas gangrene and septicemia (9). Human infection with this organism follows a fulminant course and has a 50 to 70% mortality rate (6). Like that of many other *Clostridium* species, the virulent nature of *C. septicum* is due, in part, to the production of toxins and other biologically active agents (9). Four toxins, alpha, beta, gamma, and delta, are produced by *C. septicum*. The alpha- and delta-toxins are oxygen-stable and oxygen-labile hemolysins, respectively (2), the beta-toxin is DNase, and the gamma-toxin is hyaluronidase (8). The activity of the DNase produced by *C. septicum* relative to that of other *Clostridium* species has not been investigated. In this study, the DNase activity in the culture supernatants of 62 clinical isolates of *C. septicum* and several other *Clostridium* species was compared.

The clostridial isolates tested were obtained from the culture collection of the Anaerobe Laboratory of Indiana University Hospital. Of the 62 *C. septicum* isolates, 17 were from the Anaerobe Laboratory of the Centers for Disease Control, Atlanta, Ga.; the remainder were isolated in our laboratory from clinical specimens or had been referred to us from other clinical laboratories for confirmation. The isolates were identified by using the conventional methods of Holdeman et al. (5) or of Dowell and Hawkins (3), as described by Allen (1). The isolates were subcultured into thioglycolate broth and then inoculated into brain heart infusion (BHI) broth (Difco Laboratories) supplemented per liter with 5.0 g of yeast extract, 0.5 g of cysteine hydrochloride, 5.0 mg of hemin, and 1.0 mg of vitamin K. All cultures and subcultures were incubated for 48 h at 35°C in an atmosphere of 5% CO_2, 10% H_2, and 85% N_2.

The supernatants of the BHI broth cultures were tested for DNase activity by using the DNA-methyl green assay as modified by Miyakawa et al. (7). For the DNase assay, 0.5 ml of the BHI culture supernatant filtrate was mixed with 2.5 ml of the DNA-methyl green substrate solution and then incubated at 37°C in a water bath. The substrate solution consisted of 0.05% calf thymus DNA suspended in 50 mM Tris hydrochloride (pH 7.4)–2 mM $CaCl_2$–1 mM $MgSO_4$–0.02% NaN_3. Five microliters of 1.0% methyl green stock solution, prepared in 0.02 M acetate buffer (pH 4.2) and the impurities extracted with chloroform, was added to the DNA solution. A tube containing 0.5 ml of sterile BHI broth and 2.5 ml of substrate solution served as the control. The DNase activity was detected by measuring the A_{640} at hourly intervals and calculating the percent decrease in the A_{640} relative to the control tube. The percent decrease was calculated by using the equation $D = (1 - X_t/C_t) \times 100$ where X_t is the A_{640} of the test sample at time t and C_t is the A_{640} of the control sample at time t (7).

Sixty-two clinical isolates of *C. septicum* were tested for DNase activity by using the DNA-methyl green assay. The assay tubes were incubated at 37°C, and the A_{640} of the solution was measured hourly for the first 12 h and at 20 h. All of the *C. septicum* isolates tested exhibited DNase activity, and after 20 h of incubation, the average percent decrease in the A_{640} was 94% (standard deviation [SD], 1.25%).

To compare the DNase activity of *C. septicum* to that of other clostridial isolates, the supernatants of several clinical clostridial isolates were tested for DNase activity. Fifteen isolates of each species were tested in the assay, which was conducted as described above for 20 h. *C. septicum* consistently exhibited the highest DNase activity throughout the assay, and at 20 h, the average percent decrease in the A_{640} of the *C. septicum* assay tubes was 91% (SD, 1.5%). An intermediate level of DNase activity was present in all of the supernatants obtained from *C. clostridiiforme* (51%; SD, 14.3%), *C. cadaveris* (46%; SD, 1.2%), *C. perfringens* (42%; SD, 8.2%), and *C. sporogenes* (40%; SD, 1.8%). *C. butyricum*, *C. difficile*, *C. glycolicum*, *C. paraputrificum*, *C. ramosum*, and *C. tertium* exhibited no extracellular DNase

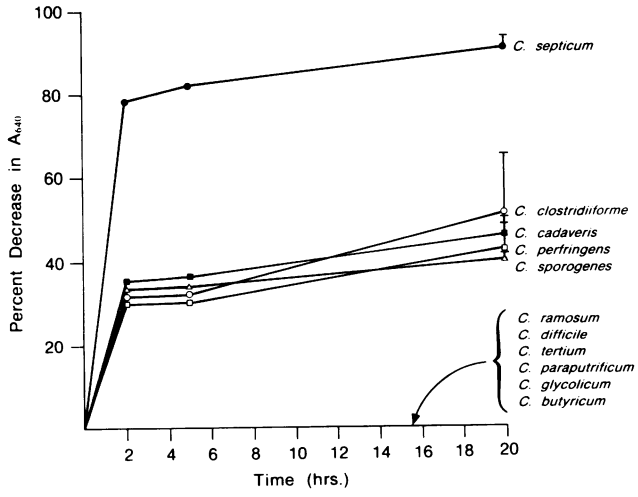

FIG. 1. Comparison of average DNase activity present in culture supernatants of 15 strains of each of 11 *Clostridium* species examined.

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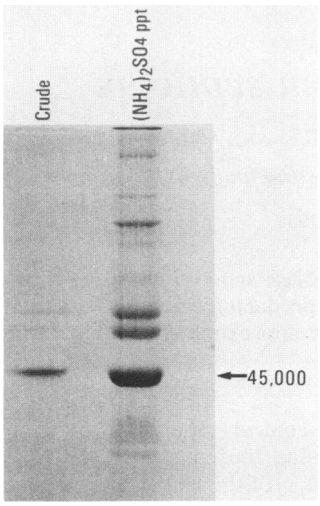

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of crude *C. septicum* culture supernatant and $(NH_4)_2SO_4$ precipitate (ppt) of *C. septicum* culture supernatant. The 45,000-dalton protein band corresponds to the extracellular DNase.

activity in this assay (Fig. 1). When the protein concentrations of the supernatants were determined by using the Bio-Rad protein assay, all of the supernatants exhibited similar protein concentrations.

The approximate molecular weight of the DNase was determined by polyacrylamide gel electrophoresis. The supernatant proteins were precipitated from 1 liter of *C. septicum* broth culture by the addition of $(NH_4)_2SO_4$ to 80% saturation. The precipitate was suspended in 10 ml of 50 mM Tris hydrochloride (pH 8.0) and dialyzed against the same buffer overnight. The dialyzed solution was chromatographed through a Sephadex G-100 column by using 50 mM Tris hydrochloride (pH 8.0) buffer, and the DNase-containing fractions were subjected to polyacrylamide gel electrophoresis. After electrophoresis at 120 V for 4 h, the proteins were eluted from the gel by the method of Hager and Burgess (4) and tested for DNase activity by using the DNA-methyl green assay. A protein with an approximate molecular weight of 45,000 exhibited DNase activity and was the major extracellular protein present in the crude culture supernatant (Fig. 2).

This study revealed that all *C. septicum* isolates tested produce DNase, *C. septicum* culture filtrates degrade DNA more rapidly than do filtrates of other clostridia tested, and the DNase is a major extracellular protein of *C. septicum*. These results suggest that the production of DNase is an important feature of *C. septicum*. Whether this property is associated with the virulence of *C. septicum* remains to be investigated. However, rapid DNase activity in the culture filtrate may be used as a key phenotypic characteristic for the identification of *C. septicum*.

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