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Comparison of Growth and Toxin Production in Two Vaccine Strains of *Bacillus anthracis*

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Two vaccine strains of *Bacillus anthracis* were monitored in a 10-liter fermentor to compare growth patterns and toxin production. Under identical conditions, the Sterne strain produced all three components of anthrax toxin, whereas strain V770 produced only the protective antigen.

Although its exact role in pathogenesis is still unknown, the toxin produced by *Bacillus anthracis* is probably one of several factors contributing to the high levels of mortality for this disease. The toxin has been studied by various groups (1, 3-5).

Three components have been identified. The protective antigen (PA) component of the toxin complex appears to be most important to immunity (6). Another component, edema factor (EF), acts in combination with PA to produce local edema in test guinea pigs (7). The third component, lethal factor (LF), working in combination with PA, kills test rats (1). Neither EF nor LF is biologically active alone; each must be assayed in the presence of PA. Likewise, PA has no biological activity, but can be measured serologically. The molecular arrangement of these individual components is unclear; the synergistic effects of LF and EF with PA suggest bonding, aggregation, or interaction of some sort (3).

The Sterne strain of *B. anthracis* is widely used as a veterinary vaccine. It also has been used by several laboratories to produce crude anthrax toxin (1, 3, 4, 7). These preparations have been examined for PA, LF, and EF. The V-770-NP1-R strain of *B. anthracis* was used to produce PA for human vaccine use (5), but was not examined for LF or EF activity. Therefore, we began a comparison of these two vaccine strains.

A synthetic medium (5) containing glucose as the primary carbon source was employed in these fermentation studies. Ten liters of medium was used in a 14-liter-capacity vessel (Fermentation Design, Edison, N.J.). Cultures were grown at 37°C and agitated at a rate of 150 rpm. A foam stopper in the top of the vessel allowed minimal exchange of gases with the surrounding atmosphere; no extra gases were added. The inoculum was prepared by propagating 2 ml of frozen stock culture in 50 ml of medium; serial transfer of 10 ml after 24 h of growth provided a total of 500 ml of culture (5% of the total volume in the fermentor vessel). Cultures were sampled hourly to determine growth rate and toxin production.

Growth was estimated by measuring the increase in absorbance at 540 nm. Similar values were obtained from three separate fermentation runs for each strain studied. As shown in Fig. 1 (data from representative experiments), both strains showed similar patterns. Initial cell concentration for strain V770 was 2.1×10^5 colony-forming units per ml. The maximum value (9 h) was 2.4×10^8 colony-forming units per ml, and the final value was 2.3×10^7 colony-forming units per ml. Utilization of glucose also provided a measure of growth. Glucose remaining in the medium was measured by the method of Dubois et al. (2). Measurements of pH were made simultaneously and are shown in Fig. 2. Here also there was a marked similarity between the two strains.

Each sample from the fermentor was centrifuged to remove bacteria and then assayed for the presence of PA, EF, and LF. PA was measured by double diffusion in agar as described by Thorne and Belton (6). Polyvalent antiserum was prepared by injecting a horse with spores of the Sterne strain of *B. anthracis* (serum was graciously provided by J. A. Carman, Medical Research Establishment, Salisbury, England). PA was first detected in culture supernatants at 16 h postinoculation for both the V770 and Sterne strains. No increase in PA titer was seen between 16 and 20 h. The presence of LF in culture supernatants was determined by intravenous injection of 1 ml of the test material into F344 rats as described by Beall et al. (1). Positive samples resulted in deaths 60 to 90 min after injection. EF was determined by injecting 0.2 ml of culture supernatant intradermally into Hartley guinea pigs as previously described (4).

Induration at the injection site was noted after 24 h for positive samples. For both the EF and LF assays, uninoculated medium provided a negative control. These results are summarized in Table 1. Attempts to improve toxin levels by increased aeration, change of agitation rate, or prolonged time of incubation in the fermentor vessel were all unsuccessful.

Under the conditions described, the Sterne strain produced all three components of anthrax toxin. The V770 strain, however, produced only PA when cultured by this method. Culture supernatants were also tested at 10-fold concentration to detect low levels of toxin. No EF or LF activity was seen in any of the V770 material.

These two strains were also grown in stationary deep cultures (three attempts for each strain) to determine whether agitation, aeration, or both affected the production of toxin components. Sampling was not attempted, since an arbitary portion would not be representative of the culture. After 24 h, growth was evident only on the bottom of the culture vessels; total growth was markedly decreased as compared with cultures grown in the fermentor (representative final cell numbers for the V770 and Sterne strains in stationary culture were 2.1×10^6 and 1.8×10^6 colony-forming units per ml, respectively). Stationary-culture supernatants were assayed after a twofold concentration. Sterne strain material (twofold concentrated) contained only PA. Wright and Puziss (9) also showed that deep cultures produced PA. However, their cultures were flushed with nitrogen to provide an

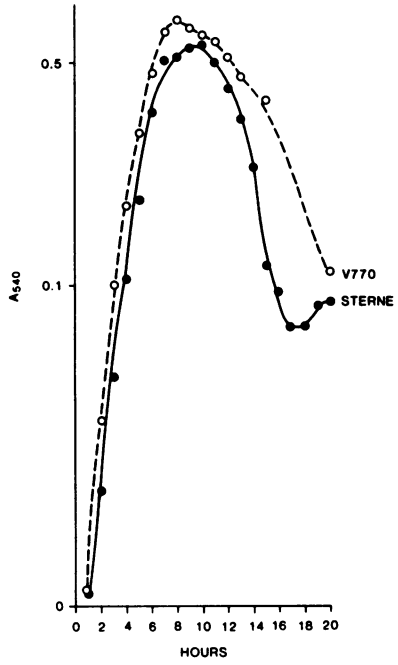

FIG. 1. *Growth patterns of two strains of B. anthracis in a fermentor.* (○) *V770;* (●) *Sterne.*

TABLE 1. *Toxic activity of two B. anthracis strains*

Strain	Level of production[a]		
	PA	LF[b]	EF[c]
Sterne	1:1	1:1	1:1
V-770	1:1	Neg	Neg

[a] Highest dilution of 20-h culture supernatant eliciting a positive response. All samples were negative at a 1:2 dilution.
[b] Measured in rats.
[c] Measured in guinea pigs.

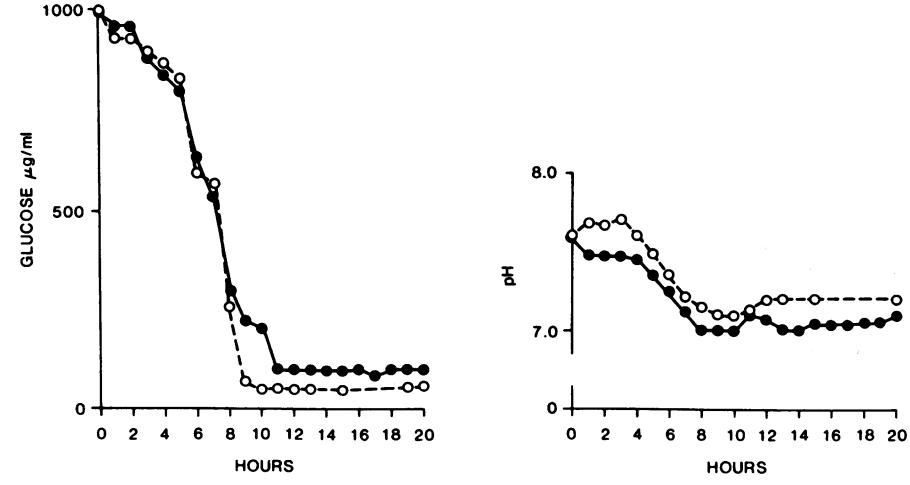

FIG. 2. *Glucose utilization and pH change during growth of two strains of B. anthracis in a fermentor.* (○) *V770;* (●) *Sterne.*

anaerobic atmosphere, whereas ours were not.

Wright and Angelety (8) had reported that satisfactory levels of PA were produced in stationary and stirred cultures, but not in shaken cultures. We have confirmed their observation that stationary cultures produce less growth and less antigen than stirred cultures. We have further demonstrated that fermentor conditions allow production of all components of anthrax toxin (PA, LF, and EF) by the Sterne strain of *B. anthracis*. By establishing optimal conditions for growth and toxin production, we should be able to isolate suitable amounts of each component of the toxin for further biochemical analysis and production of a toxoid.

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