Expression in Bacillus subtilis of the 51- and 42-Kilodalton Mosquitocidal Toxin Genes of Bacillus sphaericus

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A 3,080-base-pair KpnI-HindIII DNA fragment from Bacillus sphaericus 2362 coding for 51- and 42-kilodalton mosquitocidal proteins was cloned into Bacillus subtilis DB104 by using the vector pUB18. In B. subtilis these proteins were not detected during vegetative growth but were expressed during sporulation at levels comparable to those found in B. sphaericus.

In the course of sporulation Bacillus sphaericus 2362 produces a parasporal crystal which contains several proteins that are toxic to mosquito larvae (1, 3, 4). Recently, we cloned into Escherichia coli and sequenced a 3.5-kilobase (kb) DNA fragment which codes for toxin proteins of 51 and 42 kilodaltons (kDa) (1, 2). In B. sphaericus these genes are expressed only during sporulation at a final level corresponding to about 5% of the dry weight of the cells (4, 7), while in E. coli they are expressed at much lower levels throughout the growth cycle (2). Since both Bacillus subtilis and B. sphaericus form endospores, it is plausible that the B. sphaericus sporulation-associated expression of these proteins would also occur in B. subtilis. Recently, de Marsac et al. (5) have presented evidence for the expression of toxin genes from B. sphaericus 1593M in B. subtilis.

B. subtilis DB104 is a strain that is deficient in extracellular alkaline and neutral proteases (6). Plasmid pUB18 is a pUB110 derivative containing a M13mp18 multiple cloning site (8); T. H. Zaghloul, Ph.D. thesis, University of California, Davis, 1986. A 3.1-kb KpnI-HindIII fragment (Fig. 1) from pGA-5 (2) was ligated into pUB18, and the preparation was used to transform B. subtilis DB104. The procedures used for the transformation of cells and plasmid miniscreening have been described previously (8). The resulting plasmid was designated pC7a. B. subtilis DB104 containing pC7a was grown at 37°C in medium containing a mineral base (4); 0.1% (wt/vol) D-glucose; and the following, per liter: 10 g of tryptone, 5 g of yeast extract, and 5 mg of kanamycin. Periodically, samples were removed, centrifuged, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were electroblotted onto nitrocellulose paper and detected by immunoblot assays by using antisera to all the crystal proteins or antisera which discriminated between the 42- and 51-kDa proteins. These procedures have been described previously in detail (3, 4).

A growth curve of B. subtilis DB104 containing pC7a is presented in Fig. 2. Exponential growth ceased about 2 h and 45 min after inoculation. After 8 h no significant decrease in turbidity was noted (tested up to 48 h). Figure 3 presents the kinetics of synthesis of the 51- and 42-kDa proteins. Neither protein was detected during exponential growth (lane a). The 42-kDa protein was first detected about 5 h after the cessation of exponential growth (lane d), while the 51-kDa protein was detected at 7 h after the cessation of exponential growth (lane e). Since the final level of the 42-kDa protein was greater than that of the 51-kDa protein, the difference in the time of their detection was probably a function of the difference in their amounts. The maximal level of the proteins was reached at or before 14 h after the inoculation of the culture (lane g) and remained approximately constant (lane h) up to 48 h (last time point sampled; data not shown) (lane i represents a sample taken at 36 h and contains twice the amount of cells present in lanes a to h). Degradation products of about 27 and 24 kDa were present in most of the samples containing the 42- and 51-kDa proteins. The concentration necessary to kill 50% of the second and third instar larvae of Culex pipiens (LC₅₀) was determined as described previously (3) by using the culture harvested at 36 h after the initiation of growth (lane i). The LC₅₀ was found to be 27 ng (dry weight) of cells per ml, as compared with 18 ng (dry weight) per ml found with a culture of B. sphaericus 2362 (3).

In B. sphaericus the amount of the 51- and 42-kDa proteins is approximately equivalently consistent with the suggestion that these two genes are in a single transcriptional operon (1). In B. subtilis the amount of the 51-kDa protein appeared to be considerably less than that of the 42-kDa protein. In order to test whether the 51-kDa protein is degraded to the 24- and 27-kDa peptides detected in Fig. 3, immunoblots of sample (lane g) were assayed by using antisera (3) which discriminated between the 42- and 51-kDa proteins. No major degradation products of the 51-kDa protein were detected (Fig. 4, lane b). The 24- and 27-kDa peptides had antigenic determinants of the 42-kDa protein (Fig. 4, lane c) and, therefore, appeared to be degradation products of this

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FIG. 1. Restriction map of the DNA fragments from B. sphaericus 2362 which were cloned into B. subtilis DB104. Abbreviations: E. EcoRI; H. HindIII; K. KpnI. Thick lines delineate the DNA coding for the toxin proteins.
protein. We also tested the possibility that the 176-base-pair DNA fragment between the 51- and 42-kDa proteins (Fig. 1) contained a promoter which functioned in B. subtilis and accounted for the greater amount of the 42-kDa protein. pC7a (Fig. 1) was cut with EcoRI (there was an EcoRI site in the mp18 multiple cloning site adjacent to the KpnI site) and religated. The resulting plasmid contained a 1.9-kb EcoRI-HindIII DNA fragment, with the intact gene coding for the 42-kDa protein and the upstream region which included part of the gene coding for the 51-kDa protein (Fig. 1). In an experiment for which the results were analogous to those presented in Fig. 2 and 3, no cross-reacting material was detected in the samples (1 mg [dry weight] of cells) taken during exponential growth or at 24 and 36 h after inoculation of the culture (data not shown).

The results of this study indicate that in B. subtilis (Fig. 2 and 3), as in B. sphaericus (4), the 51- and 42-kDa proteins are expressed only in the course of sporulation and that the 96-base-pair DNA fragment between the KpnI site and the initiation codon for the 51-kDa protein (Fig. 1) (1) contain all or most of the promoter region. This sporulation promoter appears to function in B. subtilis with an efficiency comparable to that of B. sphaericus, since the LC50% of the two cell preparations for larvae of C. pipiens (an approximation of the amount of toxin) were similar. Unlike the case of B. sphaericus, in which the amount of the 42- and 51-kDa proteins was about the same (4), the amount of detectable 42-kDa protein in B. subtilis was greater than that of the 51-kDa protein (Fig. 3). We have no explanation for this difference, which cannot, however, be readily accounted for by the degradation of the 51-kDa protein (Fig. 4) or the promoter activity of the DNA fragment between the genes coding for the two proteins.

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LITERATURE CITED


