Regulation of Extracellular Protease Production in *Bacillus cereus* T: Characterization of Mutants Producing Altered Amounts of Protease

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Twenty-nine mutants of *Bacillus cereus* T were selected on casein agar for their inability to produce large amounts of extracellular protease. They all formed spores, and 27 were also auxotrophs for purines or pyrimidines. Upon reversion to prototrophy, a large fraction regained the capacity to produce protease. Conversely, reversion to normal protease production resulted in loss of the purine or pyrimidine requirement in a large fraction of the revertants. One spontaneous low-protease-producing pyrimidine auxotroph studied in detail grew as well as the wild type and produced spores which were identical to those produced by the wild type on the basis of heat resistance, dipicolinic acid content, density, and appearance in the electron microscope. The rate of protein turnover in the mutant was the same as the wild type. The mutant did grow poorly, however, when casein was the principal carbon source. A mutant excreting 5 to 10 times as much protease as the wild type was isolated as a secondary mutation from the hypoproducer discussed above. Loss of the pyrimidine requirement in this case did not alter the regulation of protease production. Although the secondary mutant grew somewhat faster in most media than the wild type, the final cell yield was lower. The spores of this mutant appeared to have excess coat on the basis of both electron microscopic and chemical studies. There appear to be closely related but distinct catabolic controls for both extracellular protease and spore formation. These controls can be dissociated as for the hypoproducers but can also appear integrated as for the hyperprotease producer.

A variety of bacilli produce extracellular proteases (for recent review, see 19). In most cases the enzyme is produced during the post-exponential growth phase, although some species do produce the enzyme during exponential growth when grown under appropriate conditions (3, 4, 13). Although the specific regulatory mechanisms are not known, the production in most species can be inhibited by high concentrations of glucose or amino acids, or both (9, 19, 23). In the case of *Bacillus megaterium* KM, specific amino acids have been implicated (3, 4), although a wide spectrum is required for maximal inhibition (3, 13).

We had previously found that *B. cereus* T mutants selected for their ability to sporulate in the presence of a high concentration of amino acids or glucose produced excessive amounts of extracellular protease (9). Many of the former class turned out to be purine auxotrophs. This relation apparently was not coincidental, since revertants to prototrophy no longer overproduced the enzyme. Because a wide spectrum of catabolic and biosynthetic mutants appeared to affect protease production, a general catabolic regulatory mechanism was postulated.

Another aspect of this problem is the relation of extracellular protease production to spore formation. Although there is a correlation in time between the initiation of formation of the spore and the protease, mutants capable of sporulating but unable to produce the protease (13) or producing very low levels (N. Angelo and A. Aronson, Bacteriol. Proc., p. 29, 1967) have been isolated. Conversely, nonsporulating mutants capable of producing the enzyme have been found (20). The isolation of such mutants implies that the two physiological properties are not necessarily coupled.

In the present paper, *B. cereus* T mutants se-
ected for low protease production and a spontaneous hyperprotease producer are characterized in terms of ability to sporulate and the possible physiological basis for regulation of protease formation. The results reported here and our previous findings are consistent with a model of general catabolic control of enzyme production closely related to but distinct from the control of spore formation (9).

**MATERIALS AND METHODS**

Cultures and growth. *B. cereus* T was grown from spore inocula (10^7/ml final concentration) at 30 C in Erlenmeyer flasks with 10 to 25% liquid volume in either a New Brunswick gyroratory water-bath shaker (0.5-inch stroke, 150 to 200 rev/min) or for volumes larger than 20 ml, in a New Brunswick incubator shaker (1-inch stroke, 300 rev/min). M (medium) (5) was modified by the addition of 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.6. A synthetic medium, CDGS, was used as previously described (16) or supplemented as indicated in specific experiments. Modified G Tris-casein consisted of G Tris medium without glucose supplemented with 0.2% vitamin-free sodium caseinate (Fisher).

Low protease producers were selected by plating cells on modified G Tris-casein. Small white colonies with no detectable halo were picked for further screening. Some of the mutants were spontaneous, whereas others were picked from cultures treated with N-methyl-N'-nitro-N-nitrosoguanidine (9). Twenty-nine such mutants were selected (all capable of sporulating) and found to produce 3 to 7% of the wild-type level of extracellular protease in G Tris medium. Twenty-seven were found to be auxotrophs for either purines or pyrimidines. Since they all seemed to be phenotypically very similar, only one (designated C), a spontaneous mutant, is discussed here in detail.

Growth was followed by measuring the optical density at 655 nm in a Coleman 8 colorimeter. The percentage of refractile spores was determined by counting at least 500 cells in a Petroff-Hauser chamber employing a Zeiss phase microscope. Counts were reproducible to ±10%.

Chemical and enzyme measurements. Protease was assayed with azocasein (California Biochemical Corp.) suspended to 20 mg/ml in 0.05 M Tris-hydrochloride, pH 7.8. Samples (1 ml) were removed from the cultures and centrifuged in an RC-2B Sorvall centrifuge at 10,000 rev/min for 10 min, and various amounts of supernatant fluid or other enzyme preparations were added. The volume was adjusted to 2 ml, and the tubes were incubated at 37 C for 10 min. The reaction was stopped by filtration through Whatman no. 1 paper, and the absorbancy at 580 nm (A580) of the filtrate was determined. Despite the use of an insoluble substrate, assays were highly reproducible (±3%) and proportional to enzyme concentration up to an A580 of 0.4. With an insoluble substrate, the rate increased with increasing substrate concentration much as would be expected for an absorption isotherm (17). Many of the assays were also checked with a soluble substrate, azocasein (California Biochemical Corp.; reference 23), at a saturating concentration of 10 mg/ml with completely analogous results.

Results are reported as A580/0.5 ml of supernatant fluid, corrected to 200 μg of protein in the cell pellet, unless otherwise indicated.

Orotidine-5-phosphate decarboxylase (EC 4.1.1.23) was assayed by the procedure of Lieberman et al. (10). Crude extracts were prepared from 500 ml of cells growing exponentially in G Tris medium. The cells were collected by centrifugation, washed three times with 15 ml each of 0.02 M Tris-0.002 M MgCl2 (pH 8.0) suspended in 2 ml of this buffer and broken by sonic treatment (Branson model S75, setting no. 4 for 90 sec). Intact cells and debris were removed by centrifugation at 10,000 rev/min for 10 min. Initial slopes were used to calculate units, as per Lieberman et al. (10).

Protease was purified from culture supernatant fluid (14- to 15-hr cultures; see Fig. 1) and characterized on gels as previously described (9). Labeled protease was prepared from the supernatant fluid of cultures used for the turnover experiments. After electrophoresis, gels were stained with Amido Schwarz and scanned in a Joyce-Loebl densitometer. The gels were then frozen on dry ice and sliced with a Yeda macrotome. Two 1-mm slices were placed in scintillation vials, 0.5 ml of Nuclear-Chicago solubilizer was added, and the vials were gently shaken on a reciprocal platform shaker for 12 to 16 hr at 25 C to permit the gel slices to swell. A 10-ml amount of Omnifluor (New England Nuclear Corp.) was then added, and the vials were counted in a Packard 3000 series scintillation counter (45% efficiency for 14C).

The rate of turnover of cellular protein was determined by adding l-leucine-UL-C14 (0.02 μCi/ml, 200 mCi/mM; New England Nuclear Corp.) to cultures at 2 hr after the end of exponential growth. After 60 min, 1 mg of unlabeled L-leucine per ml was added, and 2-ml samples were removed at 1-hr intervals. All samples were precipitated with an equal volume of 20% trichloroacetic acid and collected, after standing on ice for 20 min, by centrifugation at 10,000 rev/min for 10 min. The pellets were dissolved in 0.2 N NaOH, precipitated with 10% trichloroacetic acid, and dissolved in 1 to 2 ml of 0.2 N NaOH. A sample was removed for protein determination (11); another sample was precipitated with an equal volume of 20% trichloroacetic acid, collected on glass fiber filters (Reeve Angel, 9340AH), dried, and counted in Omnifluor scintillation mix (New England Nuclear Corp.) in a Packard 3000 series scintillation counter.

The spore coat protein was extracted from spores washed as previously described (2). Samples were removed to determine total counts in a Petroff-Hauser chamber. Coat protein was solubilized (2) by treatment of 5 x 10^10 to 5 x 10^13 spores with 2 ml of 1% sodium lauryl sulfate - 0.05 m diethioethyl (pH 10.5) at 37 C for 90 min. The extraction was repeated once, and the sodium lauryl sulfate was removed from the pooled supernatant fluids by addition of 0.05 ml of saturated KCl, chilling to 0 C for 40 min, and centrifugation at 10,000 rev/min for 10 min. Cold 50% trichloroacetic acid was then added to a final concentration of 15%. After standing on ice for 20 min, the precipitated protein was collected by centrifugation and the pellets were dissolved in 0.2 N NaOH for protein determina-
Dipicolinic acid was extracted and measured by the method of Janssen et al. (8).

Electron Microscopy. For thin sectioning, spores were fixed directly in 1% OsO₄ as previously described (7). For freeze etching, all samples were centrifuged, and the pellets were frozen in Freon 22 and stored in liquid N₂ until used. Samples were fractured as described by Moor and Mühlethaler (14) and by Holt and Leadbetter (6).

RESULTS

General properties of mutant and wild type.

The growth and extracellular protease production of the wild type and mutant C₄ are shown in Fig. 1. In G Tris medium, the rate and amount of growth were about the same (see Table 5). The time of appearance of protease activity in the supernatant fluid was the same, but the level remained low in C₄ for a prolonged period (until the commencement of cell lysis). In addition, the time of appearance of refractile spores was the same. The spores produced by C₄ were as heat resistant (80°C for 10 min) as those produced by the wild type (Table 5) and had the same density in Renografin gradients (22). Whereas the amount of dipicolinic acid per spore was the same in the two strains, the kinetics of synthesis were somewhat different (Fig. 2). The initial rate of accumulation was more rapid in the mutant and reached a maximum level somewhat earlier than the wild type.

Physiological basis for low enzyme level. Several possible explanations for the lower enzyme level excreted by C₄ were tested. (i) A possible altered release mechanism was examined by simply measuring enzyme contents of crude cell extracts (Table 1). Very low levels of activity were found in C₄ extracts. Reconstitution experiments, i.e., mixing known amounts of the wild and C₄ cultures before centrifugation and breakage revealed no loss of activity. The presence of an "inhibitor" in C₄ cells, therefore, seems unlikely.

(ii) The possibility of an altered enzyme was tested by purifying C₄ and wild-type enzymes by acetone fractionation (9) and comparing them by gel electrophoresis (Fig. 3). Both preparations contained one principal protein band (containing greater than 95% of the recovered enzyme activity) with the same mobilities. In addition, the heat sensitivities of the two purified enzyme preparations were the same and approximately similar to the heat inactivation curves previously published (9). In the present case, the data were...

**Fig. 1.** Growth and extracellular protease production by wild-type Bacillus cereus T (a) and mutant C₄ (b). A 20-ml amount of G Tris medium in 250-ml side-arm flasks was inoculated with spores to 10⁴/ml. The flasks were incubated at 30°C in a New Brunswick gyratory water-bath shaker. Samples (1 ml) were removed at the times indicated to determine protease activity. Extensive clumping occurred between 11 and 12 hr. Phase white structures first appeared at 15 hr in both cultures. Symbols: ○, A₆₆₅ nm (growth); ○, A₆₆₅ nm/0.5 ml (protease); □, per cent refractile spores.

**Fig. 2.** Synthesis of dipicolinic acid in sporulating cultures of Bacillus cereus T wild (●) and mutant C₄ (○) grown in G Tris medium at 30°C as in Fig. 1. Samples (1 ml) were removed at times indicated for dipicolinic acid (DPA) determinations. Final spore counts (Petroff-Hauser): wild, 7.8 × 10⁸/ml; C₄, 7.5 × 10⁸/ml.
Table 1. Distribution of proteolytic activity in supernatant fractions and cell extracts

<table>
<thead>
<tr>
<th>Culturea</th>
<th>Time (hr)</th>
<th>Total unitsb</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Supernatant</td>
</tr>
<tr>
<td>Wild</td>
<td>10</td>
<td>670</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1,200</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1,840</td>
</tr>
<tr>
<td>C4</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>73</td>
</tr>
<tr>
<td>Wild + C4</td>
<td>14</td>
<td>1,750</td>
</tr>
</tbody>
</table>

* Cells were grown in 500 ml of G Tris medium. Samples (100 ml) were removed at indicated times. The cells were washed three times with 30 ml of 0.05 M Tris-hydrochloride (pH 7.8), suspended in 2 ml of 0.05 Tris, and broken by passage through a French press at 9,000 psi. Intact cells and large fragments were removed by centrifugation at 12,000 rev/min for 10 min. Little activity was associated with this pellet (9). The volume of the supernatant was adjusted to 2 ml.

* Enzyme activity was determined as described in Materials and Methods times total volume (100 ml for supernatant fraction; 2 ml for cell extracts).

* Portions (100 ml) of each culture were mixed and processed as described above.

Table 2. Extracellular protease production in various media

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mediumb</th>
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<tr>
<td></td>
<td>CDGS + 0.2% yeast extract</td>
</tr>
<tr>
<td>Wild</td>
<td>6.0</td>
</tr>
<tr>
<td>C4</td>
<td>0.15 - 0.45</td>
</tr>
</tbody>
</table>

* Results are expressed as ΔA450 nm per 10 min per 0.5 ml of supernatant fraction. Values were determined at 1, 3, and 5 hr after the end of exponential growth. The highest values found for a given experiment are reported (see Fig. 1 and reference 9). Single values are reproducible to ±10%; the range reported for some of the lower values are the upper and lower limits from six separate experiments. All values corrected to 200 μg of protein in cell pellets.

required mutant and that most of the other hypoproducers subsequently isolated were auxotrophs for either purines or pyrimidines. In G Tris medium, the maximum amount of enzyme in the supernatant fluid was 3 to 7% of the wild type. This percentage was somewhat higher in CDGS medium plus yeast extract, primarily because of the lower value of the wild type. In a synthetic medium (CDGS) with pyrimidines or pyrimidines plus Casamino Acids, the amount of enzyme released was very low in both the mutant and wild type. The small amount of enzyme excreted by the wild type may be due in part to the slower growth rate and very asynchronous sporulation in the synthetic medium. Although the enzyme content of C4 hardly changed, the identity of the two enzymes and the reversion studies reported below support the hypothesis that C4 is a regulatory mutant.

On the basis of preliminary nutritional studies, it appears that C4 is blocked late in the pyrimidine pathway. The mutant would not grow on minimal medium supplemented with ureidosucinic acid, oroticide, or orotic acid (all at 20 μg/ml). In the case of orotic acid-supplemented cultures, a compound with the chromatographic mobility of orotic acid 5'-phosphate was isolated from the cold trichloroacetic acid-soluble fraction. The results suggest that the mutant is missing the orotic acid 5'-phosphate decarboxylase, and preliminary enzymatic analyses (Table 3) confirm this supposition.

Reversion studies. The fact that most (27/29) hypoproducers, either spontaneous or induced, were auxotrophs suggested a functional relationship between the nutritional requirement and

somewhat erratic, possibly owing to the presence of impurities or enzyme aggregates (Angelo, N., M.S. Thesis, Purdue Univ., 1968).

(iii) The possibility that the mutants may be regulatory was tested by comparing the activity in the supernatant fluids of cultures grown in different media (Table 2). During the course of this study, it was found that C4 was a pyrimidine-

Fig. 3. Densitometer tracing and counts per minute profile (○) of mixtures of partially purified extracellular proteases from Bacillus cereus T wild and mutant C4, electrophoresed in a 7% polyacrylamide gel. A 50-ml amount of the supernatant fluid of a culture (wild) incubated for 60 min in the presence of L-leucine-UL-C4 (see legend to Fig. 4) and 1 liter of the supernatant fluid from a 12-hr C4 culture were used for purification (9). The purified enzymes were mixed (equal activities) and electrophoresed as previously described (9). Staining and scanning were as described in Materials and Methods. Recovery of input counts per minute was 86%.
protease production. Revertants to prototrophy or to normal protease production were selected and scored for the unselected property (Table 4). In both cases, a very high proportion of apparent revertants regained the other property. The reversion frequency to prototrophy was about \( \frac{1}{500} \). The reversion frequency to normal protease production was higher but was not accurately scored. The apparent high frequency was due to hyperprotease producers which grew somewhat more rapidly in G Tris medium. In all cases, however, it appears that the mutants studied were single-point mutations.

**Physiological effects of low protease production.** A possible intracellular function for the protease is an involvement in protein turnover. The time of appearance of protease activity in the medium correlates well with an apparent increase in the rate of protein turnover (15, 21, 24). Protein turnover was determined by incubating cells with \(^{14}\)C-leucine and then measuring the rate of loss of \(^{14}\)C from protein after the addition of a large excess of unlabelled leucine (Fig. 4). With this method of measuring turnover, there is no detectable difference between mutant \( C_4 \) and the control.

A physiological difference can be demonstrated by comparing the growth of \( C_4 \) and wild type in a medium in which casein is the principal carbon source (Fig. 5). The mutant grows poorly, a result which suggests a possible physiological function for the extracellular protease.

**Spontaneous hyperproducers.** It was found that the level of extracellular protease produced by \( C_4 \) increased upon continuous subculturing in G Tris medium. A third subculture was diluted and plated, and 200 colonies were tested for protease production. Two were found to produce very high levels of protease (8 to 10 times the wild type). Both were still pyrimidine auxotrophs, but five of seven prototrophic revertants of one of these hyperproducers still produced very high levels of extracellular protease. Although these revertants had no obvious nutritional requirements, their growth properties were altered (Fig. 6 and Table 5). One studied in detail (9H\( n \)) grew somewhat faster than the wild type in G Tris medium but had a 40 to 50% lower spore yield (Table 5). The slight difference in growth rate is probably significant since the mutant does eventually overgrow the parental strain (\( C_4 \)). Similar differences were found when either glutamate or aspartate (0.2% each) was the principal carbon source in CDGS medium.

![Figure 4](https://jb.asm.org/)

**FIG. 4.** Turnover of protein in Bacillus cereus \( T \) wild (○) and mutant \( C_4 \) (●). Cultures grown in 100 ml of G Tris medium at 30 C in a New Brunswick gyratory water-bath shaker. Phase white structures were visible between 3 and 4 hr. Dipicolinic acid synthesis began between 4 and 5 hr. Labeling and sampling procedures were as described in Materials and Methods.
proteolytic activity grown in casein; spores.

Growth conditions of production in transferrin (9HR) were as indicated in the text. Electron micrographs, especially freeze-etched preparations, seem to confirm this analysis (Fig. 7 and 8). The freeze-etched preparations of 9HR show a thicker plating of the textured coat layers with little if any of the underlying granular layer appearing. The coat layers in section may also be thicker and more densely staining (arrows in Fig. 8C).

**DISCUSSION**

A large number of the mutants selected for low protease production were purine or pyrimidine auxotrophs. This result may in part reflect the medium used for screening, but it also suggests the functional interrelationship between these biosynthetic pathways and the regulation of extracellular protease formation. Three other hypoprotease producers which were also purine or pyrimidine auxotrophs were plated on CDGS medium to permit selection of prototrophic revertants. As in the case of mutant C4, a high frequency of the revertants produced normal levels of protease.

**TABLE 5. Growth rate, spore yield, and heat resistance of wild-type and protease regulatory mutants of Bacillus cereus T**

| Strain | Doubling/hr | Final spore yield | Spore survival (%)
<table>
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<tr>
<td></td>
<td></td>
<td></td>
<td>80°C/10 min</td>
</tr>
<tr>
<td>Wild</td>
<td>0.94</td>
<td>8.0 × 10^8</td>
<td>95</td>
</tr>
<tr>
<td>C4</td>
<td>0.97</td>
<td>7.8 × 10^8</td>
<td>95</td>
</tr>
<tr>
<td>9Hr</td>
<td>1.07</td>
<td>4.9 × 10^8</td>
<td>98</td>
</tr>
</tbody>
</table>

* In 10 ml of G Tris medium in 250-ml side-arm flasks in a New Brunswick shaker bath at 30°C.

**TABLE 6. Spore coat protein content of Bacillus cereus T wild type and mutants 9Hr and C4**

<table>
<thead>
<tr>
<th>Source of spore coat</th>
<th>Protein (g/10^11 spores)</th>
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<tbody>
<tr>
<td>Wild</td>
<td>1.60 ± 0.16</td>
</tr>
<tr>
<td>9Hr</td>
<td>2.50 ± 0.25</td>
</tr>
<tr>
<td>C4</td>
<td>1.54 ± 0.15</td>
</tr>
</tbody>
</table>

* Average of three determinations.
FIG. 7. Electron micrographs of frozen-etched Bacillus cereus T spores. The spore coat layers (Co) in wild type (a) and mutant C₄ (b, c) are of approximately similar texture and thickness. The textured spore coat does not completely cover an underlying granular layer (thin arrows). In mutant 9H₆ (d), the textured spore coats are more complete. The thick arrows (a, d) point to a probable additional spore coat layer which has been removed during fracturing. Bar represents 0.5 μm. SB, spore body; E, exosporium.

The implication of biosynthetic pathways in the regulation of extracellular protease synthesis is consistent with our previous studies of hyperproducers (9). It has been assumed that the level of a catabolic intermediate directly or indirectly controls the synthesis of protease. Any change in the catabolic or biosynthetic properties of a cell influence the steady-state level of the hypothetical intermediate and thus affect protease formation. We still find this model the simplest to ac-
FIG. 8. Electron micrographs of thin sections of chemically fixed spores of Bacillus cereus T. In the wild type (a) and mutant C4 (b), the spore coat layers (Co) are of approximately equal thickness. Mutant 9Hm (c) looks similar except for possible indications of a thicker coat (thin arrows). C, cortex; SB, spore body; E, exosporium; EH, exosporial hairs; lead stained. Bar represents 0.25 μm.
count for the wide range of mutations and nutritional conditions which affect protease synthesis.

The appearance of a secondary mutant which overproduced extracellular protease was detected only because of its somewhat greater growth rate in G Tris medium (10 to 15% faster). Revertants to prototrophy (9Hr) still retained the capacity to make excess protease. Although this mutant did have more coat protein per spore, the increase was not comparable to the increased excretion of protease. It is possible, however, that even more coat protein was produced but not deposited on the spore. A similar coupling of the synthesis of spore coat protein and protease has been found in other mutants (1), but the lack of quantitative correlations and the existence of hypoproducers such as C4 indicate that this is not an obligatory coupling.

The occurrence of hyperprotease producers is unlikely to account for all of the enzyme produced by C4 cultures. Even though this mutant excretes a very large amount of protease, it would have to comprise at least 0.2% of the total population to account for all of the enzyme formed. Even with a slightly enhanced growth rate, the mutation frequency would have to be too great to support this interpretation. In addition, C4 cultures have been cloned and they still produce 3 to 7% of the wild-type level of protease in G Tris medium.

Other than an obvious function for the protease as an extracellular scavenger, any intracellular role is still speculative. The rate of protein turnover, as measured by leucine trapping, was the same in C4 as the wild type. Although the small amount of enzyme produced may be sufficient, a B. megaterium mutant with undetectable levels of extracellular protease had normal rates of β-galactosidase turnover (13), whereas a B. subtilis hypoproducer did not turn over protein at an appreciable rate (12). In this latter case, pleiotropic effects of a single mutation may have altered both properties.

The extracellular protease has also been involved in B. cereus antibiotic formation (H. L. Sadoff and E. Celikkol, Bacteriol. Proc., p. 26, 1970) and in the modification of B. cereus aldolase (18). Both functions certainly imply an important intracellular role for the enzyme, especially in post-exponential cells. These modifications should be studied in hypoproducers or protease-negative mutants.

The results reported here and the studies with B. megaterium (13) demonstrate that it is possible to form a spore when extracellular protease production is impaired. At least in the case of C4, the spores appear to be normal by a number of criteria, although more subtle differences dependent on protease function would not have been detected. Perhaps as our criteria for characterizing spores are refined, it will be possible to define functional relationships more adequately.

For the present, our results can be explained by assuming that the production of extracellular protease and spores (or at least spore coat protein) is controlled by related but distinct operons. The two functions can apparently be dissociated by mutations, but alterations in the regulation of extracellular protease production can result in changes in the amount of spore coat produced (i.e., mutant 9 H4 and reference 1). Because of this relationship and the frequent correlation in time between the appearance of extracellular protease and the commencement of spore formation (19), it is likely that metabolically related intermediates (probably in catabolic pathways) either accumulating or depleted at the end of exponential growth are involved in the regulation of both classes of proteins.

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LITERATURE CITED


