Role of Spore Coat Proteins in the Resistance of \textit{Bacillus subtilis} Spores to \textit{Caenorhabditis elegans} Predation\textsuperscript{\textdagger}

Maria-Halima Laaberki and Jonathan Dworkin*

Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received 5 May 2008/Accepted 23 June 2008

Bacterial spores are resistant to a wide range of chemical and physical insults that are normally lethal for the vegetative form of the bacterium. While the integrity of the protein coat of the spore is crucial for spore survival in vitro, far less is known about how the coat provides protection in vivo against predation by ecologically relevant hosts. In particular, assays had characterized the in vitro resistance of spores to peptidoglycan-hydrolyzing enzymes like lysozyme that are also important effectors of innate immunity in a wide variety of hosts. Here, we use the bacteriovorous nematode \textit{Caenorhabditis elegans}, a likely predator of \textit{Bacillus} spores in the wild, to characterize the role of the spore coat in an ecologically relevant spore-host interaction.

We found that ingested wild-type \textit{Bacillus subtilis} spores were resistant to worm digestion, whereas vegetative forms of the bacterium were efficiently digested by the nematode. Using \textit{B. subtilis} strains carrying mutations in spore coat genes, we observed a correlation between the degree of alteration of the spore coat assembly and the susceptibility to the worm degradation. Surprisingly, we found that the spores that were resistant to lysozyme in vitro can be sensitive to \textit{C. elegans} digestion depending on the extent of the spore coat structure modifications.

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Bacilli are soil bacteria that exist in their habitat either as vegetative cells or, more abundantly, as spores (23, 30). The spore is a dormant state that confers in vitro resistance to a broad range of insults that are bactericidal for the vegetative form of bacteria, including extreme temperatures, mechanical stresses, or exposure to chemicals (5). The ability of spores to resist these treatments is at least partially attributed to the spore coat, a proteinaceous layer composed of at least 70 protein species in \textit{Bacillus subtilis} (11). The coat is organized in two distinct layers, an outer coat and an inner coat. The proper assembly of the spore coat requires the expression of at least five morphogenetic proteins (11), including CoTE that controls the recruitment and the binding of a large subset of proteins to the coat (36). A \textit{\Delta}cotE mutation results in spores that lack the outer coat and frequently display an aberrant inner coat that appears to be disconnected from the outer surface of the forespore (6, 36). Further, the precise timing of CoTE expression is critical for the proper recruitment of proteins to the coat during assembly, and delaying CoTE expression during sporulation results in spores lacking the outer spore structures (3).

The modifications in the coat structure observed in the \textit{\Delta}cotE mutant spores are assumed to be responsible for their sensitivity to peptidoglycan-degrading enzymes, such as hen egg white lysozyme (36). Lysozymes are widely distributed among organisms and are considered effectors of innate immunity in metazoan or as digestive enzymes in unicellular and multicellular eukaryotes (27). In soil, \textit{Bacillus} species encounter different types of predator organisms, including protozoa and bacteriophagous nematodes. Recently, the relevance of in vivo studies in understanding the role of the spore coat structures (15) was demonstrated using the protozoan \textit{Tetrahymena thermophila}. The bacteriovorous nematode \textit{Caenorhabditis elegans} appears to interact with soil bacteria like the endospore-forming \textit{Bacillus cereus} in the wild (9). \textit{C. elegans} expresses and presumably secretes into its intestinal lumen bacteriolytic proteins, including lysozyme-like proteins (19, 20) encoded by numerous genes (>10) in its genome that show homology to either c-type, i-type, or Ch-type lysozymes (M.-H. Laaberki and J. Dworkin, unpublished data). In the present study, we investigated the fate of \textit{Bacillus subtilis} cells during \textit{C. elegans} predation. We tested the ability of \textit{C. elegans} to ingest and digest vegetative \textit{Bacillus subtilis} cells or spores. In addition, we characterized the role of coat structures in the resistance to \textit{C. elegans} ingestion.

MATERIALS AND METHODS

Care of bacterial strains and \textit{C. elegans} strains. \textit{Bacillus subtilis} strains used in this study (Table 1) were all derivatives of \textit{B. subtilis} PY79 (35). Genetic manipulations of \textit{B. subtilis} were performed by standard methods (4). The \textit{gerD-cwD} (JDB1486) mutant was obtained by transforming \textit{B. subtilis} PY79 with genomic DNA from strain TB1 (kindly provided by Simon Cutting, University of London, United Kingdom) and by selecting for resistance to neomycin (2.5 \mu g/mL). The germination deficiency in comparison to the wild-type strain on rich medium of the \textit{gerD-cwD} mutant was as low (<0.0001%) as described previously (34). The strains ectopically expressing \textit{cotE} at \textit{amyE} under the control of different promoters in a \textit{\Delta}cotE\textit{cotE} background were obtained after transformation of the laboratory \textit{\Delta}cotE (JDB1323) mutant with the genomic DNA from strains AH2914, AH2915, AH2921, AH2920, and AH2942 to give the strains JDB2122, JDB2124, JDB2127, JDB2125, and JDB2129, respectively. \textit{AH} strains were kindly provided by A. Henriques (Universidade Nova de Lisboa, Portugal) and are in a different \textit{B. subtilis} background (3). The sensitivity of the \textit{AH} strains in \textit{C. elegans} was the same as for the PY79 derivative strains (data not shown). The bacterium was maintained in Luria broth liquid media or agar media. \textit{B. subtilis} strains were sporulated in liquid Difco sporulation medium, and spores were purified as described previously (10). Spores were purified using a 33% diatrizoate meglumine–8% diatrizoate sodium solution and washed with cold water until 100% of phase-bright spores were obtained. The spores were stored at 4°C in

* Corresponding author. Mailing address: Department of Microbiology, College of Physicians and Surgeons, Columbia University, 701 W. 168th St., Room 1218, New York, NY 10032. Phone: (212) 342-3731. Fax: (212) 305-1468. E-mail: jonathan.dworkin@columbia.edu.

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TABLE 1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference(s) or source</th>
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<tbody>
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<td>JDB3</td>
<td>Wild-type PY79 strain, prototrophic</td>
<td>35</td>
</tr>
<tr>
<td>JDE304</td>
<td>Escherichia coli OP50</td>
<td>I. Greenwald</td>
</tr>
<tr>
<td>JDB1931</td>
<td>Listeria innocua</td>
<td>D. Portnoy</td>
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<td>JDB1323</td>
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<td>JDB1308</td>
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<tr>
<td>JDB1346</td>
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</tr>
<tr>
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<td>cotO::tet</td>
<td>14</td>
</tr>
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<td>JDB1381</td>
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<tr>
<td>JDB2129</td>
<td>cotE::cat amyE::P::cotG::catE neo</td>
<td>This study and reference 3</td>
</tr>
</tbody>
</table>

**RESULTS**

Sensitivity of *B. subtilis* to digestion by *C. elegans*. We tested the resistance of vegetative *B. subtilis* cells to *C. elegans* digestion by feeding a population of young adult *C. elegans* N2 worms (about 600 for each sample) with vegetative cells of wild-type *B. subtilis* (JDB3). We determined the number of surviving bacteria per worm and recovered only 16 ± 1 CFU per worm (mean ± standard deviation), which indicates that few viable bacteria were recovered from the nematode intestines (Fig. 1). We compared this recovery with that obtained following incubation of the worms with cultures of *Listeria innocua*.}

**FIG. 1.** Survival of *B. subtilis* spores in the *C. elegans* intestine. Adult *C. elegans* worms were fed with either *B. subtilis* PY79 vegetative cells or spores or *Listeria innocua* or *Escherichia coli* OP50 cells. After mechanical disruption of the nematodes (~600 worms per sample), the surviving bacteria were determined after growth on LB agar. The values represent the mean CFU/worm ± standard deviation (error bars) for three experiments.
**FIG. 2.** Role of the coat morphogenetic proteins in the resistance of spores to *C. elegans* digestion. (A) Measurement of the survival of *B. subtilis* wild-type and coat mutant spores in *C. elegans*. L4-young adults were fed with wild-type spores (wt) or cotE, safA, spoVID, cotA, cotG, cotO, yutH, and yxsE mutant spores. Survival of mutant spores is represented relative to the survival of wild-type spores. The experiment was performed three times in duplicate, and error bars indicate the standard deviations. (B) Survival of *B. subtilis* spores within the *C. elegans* intestine. Merged Nomarski and red fluorescence and Nomarski photomicrographs of the upper intestine of *C. elegans* L3 larvae are shown. The worms were maintained without food (“no food”) or were fed with spores carrying the P_{optig}mCherry reporter in a wild-type background; ΔcotE P_{optig}mCherry spores, and ΔspoVID P_{optig}mCherry spores. Note the autofluorescence of the intestinal granules on the merged Nomarski and red fluorescence images. The white arrowheads indicate the borders of the intestinal lumen. RFP, red fluorescent protein. Bar = 0.025 mm.

*inocua* (JDB1931), another nonpathogenic gram-positive bacterium, or a gram-negative bacterium, *Escherichia coli* OP50 (JDE304). In both cases, the number of recovered bacteria was at least 10-fold greater than for *B. subtilis* vegetative cells with 311 ± 48 CFU/worm obtained for *E. coli* and 199 ± 15 CFU obtained for *L. innocua* (Fig. 1). Vegetative cells of *B. subtilis* were detected at less than one CFU per worm in worms at earlier stages (larval stages 1 to 3) of development (data not shown). Although we cannot rule out the possibility that vegetative cells of *B. subtilis* are less taken up by the nematode, this result suggests that vegetative *B. subtilis* cells were highly sensitive to digestion by the nematode.

In contrast with vegetative *B. subtilis* cells, large numbers of bacteria were easily recovered from the worms when *C. elegans* were fed wild-type *B. subtilis* spores (6,869 ± 575 CFU/worm [Fig. 1]). We confirmed that the bacteria recovered were spores and not vegetative cells by demonstrating their ability to survive high heat treatment (80°C, 20 min). Although there are numerous differences between spores and vegetative cells that could account for the increased survival of spores following ingestion by *C. elegans*, we focused on the possible protective role of the spore coat. We examined three strains carrying mutations in coat morphogenetic proteins (cotE, safA, and spoVID) necessary for lysozyme resistance (15). In addition, we examined the correlation between the in vitro chemical sensitivity of spores and their survival following ingestion by *C. elegans* by testing strains carrying mutations in the cotG, cotH, cotO, yutH, and yxsE genes that exhibit increased sensitivity to various reagents. We also tested the spores of the cot4 mutant that do not exhibit any sensitivity in vitro using standard assays (28). CotH, CotO, and CotG are required for the recruitment of a small subset of proteins to the coat and are considered minor morphogenetic proteins (21, 29, 37). While cotO spores, unlike cotG mutant spores, have been reported to be sensitive in vitro to lysozyme (21), we found using the same lysozyme assay (24) that cotO mutant spores were as resistant as wild-type spores were (data not shown). The cotH, yutH, and yxsE mutant spores are slightly sensitive to hypochlorite, and the yutH and yxsE spores were also partially digested by *T. thermophila* (15).

We compared the survival of these mutant spores with wild-type spores by determining the number of recovered bacteria per worm after 16 h of incubation at 20°C with L4-young adult *C. elegans*. In this assay, there was no significant difference between the values obtained for the wild-type spores and for the spores of the cotA (JDB1343), cotG (JDB1345), cotO (JDB1348), cotH (JDB1346), yutH (JDB1381), and yxsE (JDB1382) strains (98% ± 25%, 125% ± 9%, 91% ± 27%, 139% ± 25%, 96% ± 5%, and 81% ± 16% as a percentage of the wild-type spores, respectively [Fig. 2A]). In contrast, between 10- and 20-fold-less cotE (JDB1323), spoVID (JDB1371), and safA (JDB1380) spores were recovered from the worm intestine (5% ± 2%, 9% ± 3%, and 12% ± 4% as a percentage of wild-type spores, respectively [Fig. 2A]). There was no significant difference observed between the recovery of cotE spores and either spoVID or safA spores.

In the course of the experiment, we observed that the densities of the spores on the agar plates for the cotE, spoVID, and safA strains were lower in comparison to the plates containing the wild-type spores, consistent with the digestion of the cotE, spoVID, and safA spores by the nematode (data not shown). We confirmed that the number of spores of cotE, safA, and spoVID mutants were indeed reduced in the worm intestine by monitoring the fate of spores containing red fluorescent pro-
tein from the wild-type, cotE, spoVID, and safA strains in the gut (Fig. 2B, cotE and spoVID) (safA strain not shown). These observations indicated that the overall structure of wild-type spores is maintained in the worm intestine and that wild-type spores accumulated in the worm intestine (Fig. 2B). In contrast, fluorescence associated with the cotE, safA, and spoVID spores was considerably reduced in the worm intestine (Fig. 2B and data not shown). Differential interference contrast microscopy of nematodes fed with spores of the cotA, cotO, cotG, yuhH, and yxE mutants confirmed that the spores of these strains accumulate in the intestine of worms as in the case of wild-type spores (data not shown). Thus, only the major alterations in coat formation that are seen in mutants of the major morphogenetic proteins like CotE, SafA, and SpoVID render spores sensitive to digestion by the worm.

Analysis of the ingestion and digestion of cotE mutant spores. We examined the possibility that the lower recovery of ΔcotE spores was due to decreased ingestion by the nematodes by measuring the kinetics of spore ingestion and excretion. We fed nematodes with spectinomycin-resistant (Spec+) spores of B. subtilis strain JDB1334 that has a spectinomycin resistance cassette inserted in a locus (amyE) that does not affect sporulation. We transferred these worms to agar plates containing either no bacteria or a lawn of either E. coli OP50, a lawn of ΔcotE spores, a lawn of wild-type spores (wt), or without any source of food (without food). The number of spectinomycin-resistant CFU per worm was determined before transfer and at 20 min, 40 min, and 3 h following the transfer to each plate. The results are represented as a percentage of the number of CFU per worm before the transfer. The data shown are from one experiment but are representative of two independent experiments.

\[ \text{No. of spectinomycin-resistant CFU/worm} = \frac{\text{CFU after transfer}}{\text{CFU before transfer}} \times 100 \]

Table 2: Ingestion of cotE spores by C. elegans

<table>
<thead>
<tr>
<th>Time after transfer to plate</th>
<th>No. of spectinomycin-resistant CFU/worm (%) of CFU/worm before transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OP50</td>
</tr>
<tr>
<td>20 min</td>
<td>12</td>
</tr>
<tr>
<td>40 min</td>
<td>7</td>
</tr>
<tr>
<td>3 h</td>
<td>0</td>
</tr>
</tbody>
</table>

* L4 larvae were fed spectinomycin-resistant B. subtilis spores and transferred to agar plates containing either a lawn of E. coli OP50, a lawn of ΔcotE spores, a lawn of wild-type spores (wt), or without any source of food (without food).

Given that ΔcotE spores were more sensitive to worm digestion, we hypothesized that they would be more easily used as a food source by C. elegans than wild-type spores. We examined this possibility by comparing the development of L1 larvae at 20°C on either metabolically active bacteria (E. coli OP50 and vegetative B. subtilis cells) or on dormant wild-type and ΔcotE spores. The worms reached adulthood on either E. coli strain OP50 or B. subtilis vegetative cells in less than 3 days (Table 3), which was similar to the results reported previously (2) and confirming both the sensitivity of vegetative B. subtilis to worm ingestion and that these bacteria are indeed taken up by the nematode. Consistent with our hypothesis, worms developed faster on ΔcotE spores than on wild-type spores (5.3 and 4.3 days, respectively [Table 3]; \( P < 0.05 \)). Surprisingly, however, the worms developed on wild-type spores that are presumably resistant to C. elegans digestion, albeit more slowly than on vegetative cells (5.3 and 2.6 days, respectively [Table 3]). In fact, nematodes grown on wild-type spores manifested the hallmarks of development under caloric restriction described for C. elegans mutants that have an eating defect (1). Specifically, we observed that C. elegans fed on spores were smaller and thinner than the nematode fed on vegetative cells of B. subtilis or E. coli OP50 and that adult hermaphrodites generally contained fewer eggs (data not shown). This observation indicated that growth on spores compared to metabolically active bacteria led to limited nutrient availability and suggested that spores become sensitive to the worm’s digestion.

The ability of worms to develop on spores, albeit more slowly than on vegetative cells, may indicate that ingested spores had germinated and were therefore sensitive to digestion. We therefore investigated the role of spore germination on nematode growth rate by incubating L1 nematodes with spores of B. subtilis that are defective for germination due to an insertion in the gerD-cwlD genes (strain JDB1486) (34). The gerD-cwlD spores are indeed highly defective in germination, with less than 0.0002\% of the spores germinating on rich medium in comparison to wild-type spores (not shown) (34). The growth of C. elegans was slower in the presence of gerD-cwlD spores (8.5 days [Table 3]) than on either wild-type or ΔcotE spores (5.3 and 4.3 days, respectively [Table 3]), suggesting that the germination of B. subtilis spores and their subsequent sensitivity to digestion permitted the growth of C. elegans. We excluded the possibility that the nematode could develop on

Table 3: Growth rate of C. elegans on B. subtilis spores

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean no. of days to adulthood (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td></td>
</tr>
<tr>
<td>Vegetative cells</td>
<td>2.6 (±0.17)</td>
</tr>
<tr>
<td>Spores</td>
<td>5.3 (±0.29)</td>
</tr>
<tr>
<td>ΔcotE mutant spores</td>
<td>6.3 AC (±0.43)</td>
</tr>
<tr>
<td>gerD-cwlD mutant spores</td>
<td>8.5 B (±0.71)</td>
</tr>
</tbody>
</table>

* C. elegans L1 larvae were fed with either E. coli OP50 strain, B. subtilis PY79 wild-type strain, PY79 spores, ΔcotE mutant spores, gerD-cwlD mutant spores, or ΔcotE gerD-cwlD mutant spores.

* The number of days for C. elegans to reach adulthood at 20°C is indicated. The values are the means from three independent experiments. Values with the same letter (A, B, or C) are significantly different (\( P < 0.05 \)).
NMM agar by demonstrating that L1 larvae incubated on a plate without any source of food (spores or metabolically active bacteria) did not grow in the course of the experiment (data not shown). Finally, ΔcotE gerD-cwlD spores (JDB1494) supported a growth rate (6.3 days [Table 3]) intermediate to those of the ΔcotE and gerD-cwlD spores (4.3 and 8.5 days, respectively [Table 3]). This result indicates that part of the growth rate on ΔcotE spores is due to germination of the spores during the incubation, similarly to the wild-type spores, but also that the ΔcotE gerD-cwlD spores are a better source of nutrient than the gerD-cwlD spores, indicating that both ΔcotE mutant spores are digested due to their increased sensitivity to digestion.

Role of the spore coat structures in sensitivity to C. elegans digestion. The sensitivity of ΔcotE spores to C. elegans digestion prompted us to study how coat protein composition afforded protection during the digestive process. This composition can be affected by modulating the timing of CotE protein expressed ectopically in a ΔcotE mutant background (3). Early expression of CotE from the amyE locus is observed when it is under the control of its own promoters (P_{cotEP1}, P_{cotEP2} or P_{cotEP1P2}), whereas late expression is observed when it is under the control of the gerE and cotG promoters (P_{gerE} and P_{cotG}) (3). Expression of cotE under P_{gerE} or P_{cotG} and, to a lesser extent under the P_{cotEP2} promoter, led to a similar, but not identical, defect in overall protein composition and the structure of the coat to that observed in a ΔcotE null mutant strain (3). However, coat composition and structure were more similar to those in the wild-type spores in strains expressing cotE under P_{cotEP1P2} (3).

While these experiments indicated that early expression of cotE from either P_{cotEP1}, P_{cotEP2} or P_{cotEP1P2} is required for the proper assembly of the outer coat, spores derived from these strains were as resistant to lysozyme as the wild-type spores were (3). Thus, these strains uncoupled, for the first time, lysozyme resistance observed in vitro and outer coat assembly (3) and allowed us to test the distinct role of each of these phenotypes in sensitivity to digestion by C. elegans. Surprisingly, the spores of all the strains tested except for the ΔcotE P_{cotEP1P2}-cotE strain (JDB2129) were susceptible to ingestion by worms (Fig. 3). Using the measurements of spores per worm and further supported by data obtained in growth rate experiments and microscopy observations, we can distinguish three classes of mutants. The first class comprises ΔcotE, P_{gerE}-cotE (JDB2122) recovered at a level comparable to that obtained for the ΔcotE spores (12% ± 1%, and 8% ±
0.3% as a percentage of wild-type spores, respectively [Fig. 3A]). These spores supported the growth of worms as well as \( \Delta \text{cotE} \) spores did (data not shown) and are not distinguishable from \( \Delta \text{cotE} \) spores in the intestine (Fig. 3B). The second class comprises the \( \Delta \text{cotE} \; \text{P}_{\text{cotE}}\text{-cotE} \) (JDB2124) and \( \Delta \text{cotE} \; \text{P}_{\text{cotEP7}}\text{-cotE} \) (JDB2125) spores which were recovered at fourfold- and threefold-higher levels, respectively, than the level of \( \Delta \text{cotE} \) spores (37% ± 4% and 31% ± 4%, respectively [Fig. 3A]). These spores supported nematode growth at a rate similar to the \( \Delta \text{cotE} \) spores (data not shown) and are clearly seen in the worm intestine (Fig. 3B). The third class of mutants, including the \( \Delta \text{cotE} \; \text{P}_{\text{cotEP1}}\text{-cotE} \) (JDB2127) and \( \Delta \text{cotE} \; \text{P}_{\text{cotEP1MP}}\text{-cotE} \) (JDB2129) spores, was recovered at 6- and 10-fold-higher levels than the level of \( \Delta \text{cotE} \) spores (51% ± 7%, 80% ± 7%, and 8% ± 0.3%, respectively [Fig. 3A]) and supported the growth of the worms as well as wild-type spores did (data not shown) and accumulated in the intestine (Fig. 3B). Thus, the viability of these three classes of mutant spores following ingestion by the worm correlates with their respective defect in coat protein composition and structural modification of the coat.

**DISCUSSION**

*Bacillus* species are abundant soil bacteria (~10^6 organisms/gram [18]) and likely serve as a source of nutrients for the bacterivorous nematode *C. elegans* (9). Indeed, *B. subtilis* vegetative cells are efficiently digested by the worm (Fig. 1) (7), consistent with the longer life span observed for *C. elegans* grown on vegetative *Bacillus subtilis* compared to the life span for *C. elegans* grown on *E. coli* OP50 (8). In contrast, we observed that wild-type *B. subtilis* spores were highly resistant to worm digestion (Fig. 1) as would be predicted from their resistance to phagocytosis by the protozoan *T. thermophila* and by their survival in the mammalian gastrointestinal tract (11, 15, 34). In a previous study, we found that other *Bacillus* species (*Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*) are also highly sensitive to the worm ingestion and that *B. anthracis* spores are fully resistant to the worm digestive process (16). In *B. subtilis*, the proper recruitment and assembly of the spore coat are crucial for the spore resistance properties, and in particular, mutations in coat morphogenetic proteins (*CotE, SpoVID*, and *SafA*) result in lysozyme sensitivity (25, 26, 36). Our finding that spores carrying mutations in the genes encoding these proteins were sensitive to digestion (Fig. 2A) is consistent with the role of the overall structure and composition of the coat in providing resistance to nematode predation. In contrast, spores carrying mutations in minor morphogenetic proteins, (*CotG, CotO, and CotH*) exhibited little or no effect on coat structure (21, 29) and were as resistant as the wild-type spores were (Fig. 2A).

The precise expression of *cotE* and the subsequent proper assembly of the *cotE*-dependent proteins in the coat appear to be crucial steps in achieving resistance to digestion (Fig. 3). Increased defects in coat structure, particularly the outer coat, led to increased digestion by the worm (Fig. 3). However, even \( \Delta \text{cotE} \; \text{P}_{\text{cotEP7}}\text{-cotE} \) spores that have a coat structure and composition similar to those wild-type spores and in which only a few protein species were found missing by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (3) are still somewhat sensitive to digestion. Thus, either the defect in this small subset of proteins is sufficient to enable the digestion of the spore, or the structure or composition of the coat is more affected in this strain than previously explored. Whatever the ultimate explanation, this result clearly highlights the usefulness of the *C. elegans* model for uncovering subtle coat protein mutant phenotypes.

In contrast to previous experiments using *T. thermophila* (15), we did not observe a correlation between in vitro lysozyme sensitivity and *C. elegans* digestibility. Indeed, the *yutH* and *ysxE* spores that are sensitive both to lysozyme and to digestion by *T. thermophila* (15) were as resistant to *C. elegans* digestion as the wild-type spores were. One explanation could be that these two studies used nonisogenic *B. subtilis* strains. This discrepancy could also be explained by a longer time of residency of the spores in the protozoan digestive vacuole compared to the intestine of a L4 worm (~1 h) (Table 2). Longer persistence in the *T. thermophila* vacuoles would result in longer exposure to digestive enzymes and thus increased degradation of the *yutH* and *ysxE* spores.

\( \Delta \text{cotE} \) mutant spores are degraded during incubation with a high concentration of purified lysozyme (36) and are also readily digested by *C. elegans* (Fig. 2A and B). However, spores produced by cells in which *cotE* expression was delayed were resistant to lysozyme (3) but sensitive to digestion (Fig. 3). Thus, the timing of expression of *cotE* appeared critical for the survival of the spore in the worm gut and may indicate more subtle defects in spore coat assembly in these *cotE* expression mutants.

The *C. elegans* digestive process has been suggested to involve both mechanical disruption mediated by a pharyngeal grinder and enzymatic digestion of the ingested microorganisms in the intestine (19). However, since \( \Delta \text{cotE} \) spores do not exhibit increased survival in mutant worms with a defective grinder (M.-H. Laaberki and J. Dworkin, unpublished data), they, and presumably other defective spores, are likely to be degraded by enzymes secreted into the intestinal lumen. This result is consistent with a previous study showing that wild-type and *cotE* spores exhibit essentially identical resistance to mechanical disruption (12). *C. elegans* expresses and potentially secretes into its digestive system several enzymes that could degrade sensitive spores, including 10 lysozyme genes (*lys-I* to *lys-10*) and 5 insect-lysozyme genes (*lys-I* to *lys-5*) (19, 31), and 5 of the *lys* genes and 2 of the *ilyS* genes are expressed in intestinal cells (13, 17, 20, 32). In addition, numerous peptidases and lipases are expressed and also presumably secreted in the intestine of the nematode (20). A synergistic activity of these enzymes could be responsible not only for the degradation of \( \Delta \text{cotE} \) spores but also for the degradation of the ectopically expressed *cotE* spores that are lysozyme resistant in vitro (3). An understanding of the enzymatic basis for digestion of spore coat mutants as well as vegetative *Bacillus* cells in *C. elegans* will be greatly facilitated by the genetic tools available in this organism. For example, RNA interference could be used to reduce the expression of potential candidate bacteriolytic enzymes (17, 22), and then the effect of these manipulations on bacterial survival could be determined using the assays we have described. Finally, this knowledge will help us refine our understanding of the role of the spore coat structures in resistance to an ecologically relevant predator.
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