JOURNAL OF BACTERIOLOGY, Dec. 2006, p. 8299–8302 Vol. 188, No. 23
0021-9193/06/$08.00+0 doi:10.1128/JB.00578-06

NOTES

CbgA, a Protein Involved in Cortex Formation and Stress Resistance in *Myxococcus xanthus* Spores[▽]

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Received 24 April 2006/Accepted 15 September 2006

CbgA plays a role in cortex formation and the acquisition of a subset of stress resistance properties in *Myxococcus xanthus* spores. The *cbgA* mutant produces spores with thin or no cortex layers, and these spores are more sensitive to heat and sodium dodecyl sulfate than their wild-type counterparts.

In nature, biofilms formed by the soil bacterium *Myxococcus xanthus* feed on prey bacteria to obtain amino acids, which are used as sources of carbon, nitrogen, and energy (3, 5). When starved for amino acids, large groups of *M. xanthus* cells migrate to aggregation centers and build multicellular fruiting bodies. Rod-shaped cells inside these structures differentiate into spherical spores that are more resistant to sonication, heat, UV irradiation, and toxic substances than vegetatively growing cells (13, 20).

Expression studies suggest that over 500 proteins may be involved in endospore formation in *Bacillus subtilis* (6, 19, 24). Although it is likely that *M. xanthus* also uses a large number of proteins to construct a spore, relatively few proteins known to play roles specific to spore development within its fruiting bodies have been identified (8, 10, 11, 15, 16, 18).

CbgA was first tagged as a potential *M. xanthus* sporulation protein based upon its sequence similarity (35% identity and 57% similarity) to SpoVR, a *B. subtilis* protein that plays an important role in the formation of the endospore cortex (2). To examine CbgA's role in sporulation, an insertion in the chromosomal copy of the *cbgA* gene in wild-type strain DK1622 was created as previously described (4). (Table 1 shows bacterial strains, plasmids, and primers used.) MXAN 5829 is the gene immediately downstream of *cbgA* (Fig. 1), and DNA sequencing data suggest that *cbgA* and MXAN 5829 are located in different operons. To confirm that the insertion in *cbgA* did not have a polar effect on the transcription of MXAN 5829, we used a real-time quantitative reverse transcription-PCR (qRT-PCR) protocol similar to that of Lancero et al. (14). We found that the expression levels of MXAN 5829 during growth in CTTYE (Casitone, Tris-HCl, yeast extract, KH_2PO_4, $MgSO_4$) broth and development on TPM (Tris-HCl, KH_2PO_4, $MgSO_4$) agar plates were similar for wild-type cells and the *cbgA* insertion mutant (data not shown), indicating that the insertion in *cbgA* did not affect MXAN 5829 expression.

When *cbgA* and wild-type cells were spotted onto TPM starvation agar and their development was monitored using phase-contrast microscopy (4), we found that *cbgA* cells were capable of aggregating and forming fruiting bodies with about the same timing as wild-type cells (data not shown). However, scanning electron microscopy (SEM) analysis (22) revealed clear differences in the morphologies of wild-type and *cbgA* fruiting bodies (Fig. 2). For the most part, wild-type cells yielded the characteristic *M. xanthus* dome-shaped fruiting bodies (Fig. 2A and D). In contrast, the *cbgA* mutant formed towers of cells or elongated cell clusters that had snake-like appearances (Fig. 2B, C, E, and F). Thus, it appears that the *cbgA* mutant forms fruiting bodies with abnormal shapes.

SEM (22) and transmission electron microscopy (25) analyses were used to compare the morphology of *cbgA* mutant spores to that of wild-type spores (Fig. 2 and 3). Surface-exposed spores in *cbgA* mutant fruiting bodies (Fig. 2G) were the same size and spherical shape as those found in

TABLE 1. Bacterial strains, plasmids and primers

Strain, plasmid, or primer	Relevant characteristic(s) or sequence	Source or reference
Strains		
AG840	pAG331::*cbgA* (within *cbgA*)	This study
DK1622	Wild-type motility and development	(12)
Plasmids		
pCR2.1-TOPO	Kan^r	Invitrogen
pAG331	621-bp fragment extending from bp 525 to bp 1146 of the *cbgA* gene	This study
Primers		
OAG106 up	5′-CCGAAGAAGGCCGAGGAC GAG-3′ (amplicon size, 621 bp)	Invitrogen
OAG107 down	5′-GAACTCGGGCGTCAGGAAC GTG-3′	

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▽ Published ahead of print on 22 September 2006.

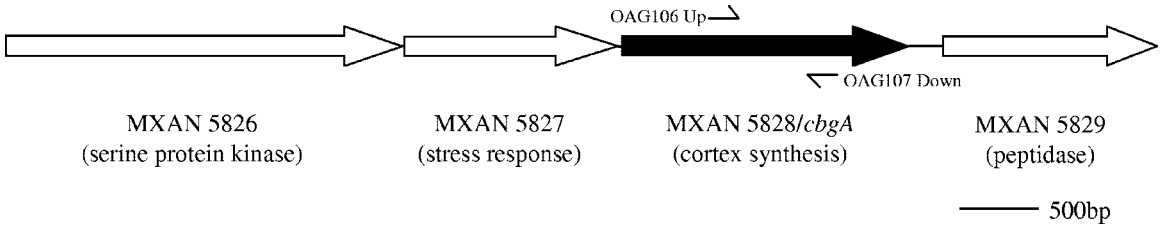

FIG. 1. Organization of the *cbgA* locus. Arrows show the locations of the indicated genes and the predicted directions of gene transcription. The potential functions of the proteins encoded by these genes are shown in parentheses. OAG106 up and OAG106 down are the primers used to generate the 621-bp *cbgA* fragment in plasmid pAG331.

wild-type fruiting bodies (Fig. 2D). Furthermore, phase-contrast microscopy showed that wild-type and *cbgA* fruiting bodies had similar numbers of spherical cells (data not shown), indicating that cells in the *cbgA* fruiting bodies were not undergoing lysis.

Transmission electron micrographs of wild-type DK1622 spores revealed all of the structural features previously described for *M. xanthus* spores (10, 23). These structures include a spore core, an inner membrane and an outer membrane, a cortex, and an exterior spore coat (Fig. 3A). The *cbgA* mutant produced spores that lacked cortexes (Fig. 3B) or spores that had relatively thin cortex layers (Fig. 3C). The outer protective coat that normally surrounds the cortex, however, was indistinguishable in *cbgA* spores and wild-type spores.

The *B. subtilis* spore coat provides a protective barrier against bactericidal enzymes such as lysozyme (9), and mutations in *cotE*, *spoVID*, or *yrbA*, which affect coat assembly, render spores lysozyme sensitive (1, 21, 26). Presumably, the compromised spore coat in the mutant spores allows lysozyme to gain access to the peptidoglycan in the cortex. To confirm that the coats of the *cbgA* spores were intact, we exposed *cbgA* and wild-type spores to 250 μg/ml of lysozyme for 12 h as previously described (17, 21). The yield of viable, lysozyme-resistant *cbgA* mutant spores was similar to that of wild-type spores (data not shown). In addition, using the procedure of Inouye et al. (10), we were able to extract the major *M. xanthus* coat protein, protein S, from the surfaces of *cbgA* spores (data not shown). Taken together, our findings indicate that the coats of *cbgA* mutant spores are likely to be intact. These data also suggest that an intact cortex is not absolutely required for *M. xanthus* spores to maintain their characteristic shape, nor is it essential for synthesis of the spore coat.

A mutation in the *spoVR* gene of *B. subtilis*, which affects production of the endospore cortex, results in a 3- to 10-fold decrease in heat resistance and resistance to toxic chemicals such as chloroform (2). To examine whether the stress resis-

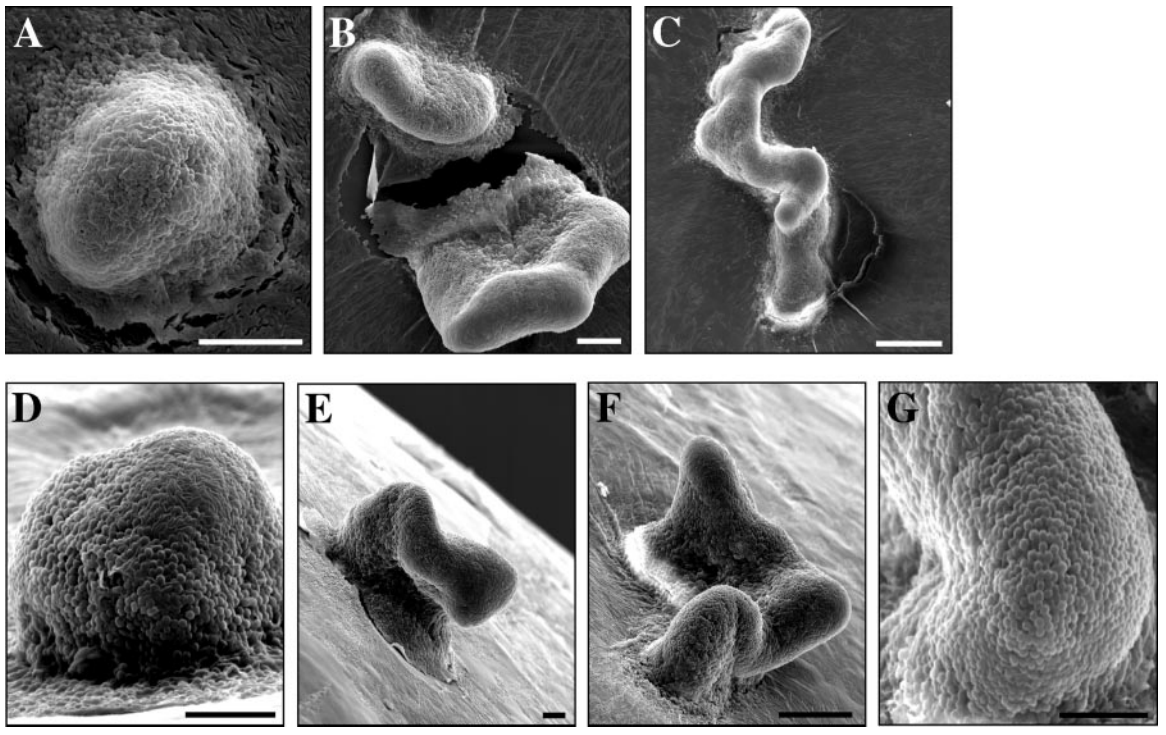

FIG. 2. High-resolution images of wild-type and *cbgA* fruiting bodies. SEM analysis was performed on wild-type (DK1622) fruiting bodies (A and D) and on *cbgA* mutant (AG840) fruiting bodies (B, C, E, F, and G) formed after 5 days of development on TPM agar. Black bars = 15 μm. White bars = 30 μm.

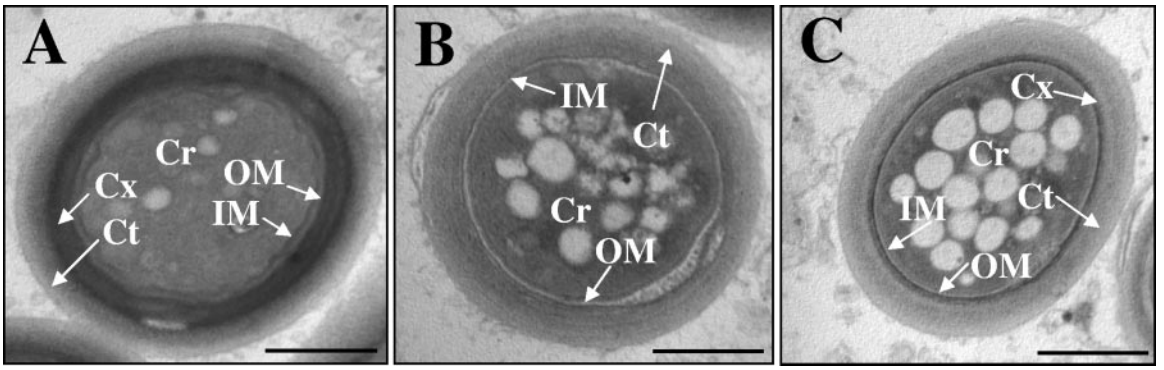

FIG. 3. Ultrastucture of mature spores. Fruiting bodies were isolated after 5 days of development on TPM agar and analyzed using transmission electron microscopy. (A) A wild-type (DK1622) spore is shown with the core (Cr), inner membrane (IM), outer membrane (OM), cortex (Cx), and coat (Ct) labeled. Spores produced by the *cbgA* mutant (AG840) either lack the electron-dense cortex layer (B) or have relatively thin cortex layers (C). Bars = 500 nm.

tance properties of *cbgA* mutant spores were compromised, cells that had developed on TPM agar plates for 5 days were harvested. Assays for resistance to sonication, heat, and UV irradiation were performed as previously described by Sudo and Dworkin (20), except that samples were sonicated before and after treatments (three 10-s bursts with 1-min cooling periods at room temperature between bursts) to disperse the cells. To determine spore resistance to sodium dodecyl sulfate (SDS), harvested cells were exposed to 1.0% SDS and incubated for 1 or 2 h at room temperature on an agitating orbital shaker. Counts of viable spores were determined as previously described (20).

The numbers of *cbgA* mutant spores that were viable after sonication or up to 10 min of exposure to UV irradiation were similar to those of wild-type spores (data not shown). However, *cbgA* mutant spores displayed a greater degree of sensitivity to temperatures of 50°C and 55°C than wild-type spores. As shown in Table 2, the number of *cbgA* spores that survived 50°C treatment was about 12-fold lower than that of wild-type spores, and the number of *cbgA* spores that survived 55°C heat treatment was about 30-fold lower than that of wild-type spores. The *cbgA* spores displayed a higher degree of SDS sensitivity than their wild-type counterparts; the number of *cbgA* spores that survived 1 and 2 h of exposure to SDS was about 50-fold lower than that of wild-type spores.

TABLE 2. Resistance of wild-type and *cbgA* mutant spores to heat and SDS

Strain	Viable spores following[a]:			
	Heat treatment at:		SDS treatment at:	
	50°C, 2 h	55°C, 2 h	1% SDS, 1 h	1% SDS, 2 h
DK1622 (wild type)	100.0 ± 13.2	100.0 ± 16.9	100.0 ± 27.1	100.0 ± 16.6
AG840 (*cbgA*)	8.1 ± 4.1	3.4 ± 1.2	2.4 ± 1.1	2.0 ± 1.3

[a] Medium recipes and procedures for developmental assays are described in the report of Caberoy et al. (4). The indicated spore resistance assays were performed at least three times for each strain. The mean values ± standard deviations for the assays are shown as percentages of the results for DK1622 (wild type). The number of wild-type spores that survived heat treatments ranged from 3×10^6 to 5×10^6, and the number that survived SDS treatments ranged from 6×10^5 to 1×10^6.

This study reports the finding that the *cbgA* gene product is necessary for proper cortex development in *M. xanthus* fruiting body spores. In addition, our results suggest that the *cbgA* gene product and an intact cortex are important for some, but not all, of the resistance properties normally associated with *M. xanthus* spores. The correlation between cortex defects and heat sensitivity was detected in previous studies of *B. subtilis* spore cortex mutants (reviewed in reference 7). Our results also suggest that the *M. xanthus* spore cortex plays an important role in resistance to SDS and perhaps other detergents.

We are thankful to Monsanto Company and TIGR for giving us access to the *M. xanthus* genome sequence prior to submission to GenBank (accession number CP000113).

This research was supported by National Science Foundation award MCB 031674 to J. Dahl and A. Garza.

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