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A New Putative Sigma Factor of *Myxococcus xanthus*

DAVID APELIAN AND SUMIKO INOUYE*

Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, New Jersey 08854-5635

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A third putative sigma factor gene, *sigC*, has been isolated from *Myxococcus xanthus* by using the *sigA* gene (formerly *rpoD* of *M. xanthus*) as a probe. The nucleotide sequence of *sigC* has been determined, and an open reading frame of 295 residues (M_r = 33,430) has been identified. The deduced amino acid sequence of *sigC* exhibits the features which are characteristic of other bacterial sigma factors. The characterization of a *sigC-lacZ* strain has demonstrated that *sigC* expression is induced immediately after cells enter into the developmental cycle and is dramatically reduced at the onset of sporulation. A deletion mutant of *sigC* grows normally in vegetative culture and is able to develop normally. However, in contrast to the wild-type cells, the *sigC* deletion mutant cells became capable of forming fruiting bodies and myxospores on semirich agar plates. This suggests that *sigC* may play a role in expression of genes involved in negatively regulating the initiation of fruiting body formation.

Myxococcus xanthus is a gram-negative gliding bacterium. *M. xanthus* and other myxobacteria live in the soil and feed on microorganisms and organic debris (15, 27). Upon nutrient depletion, *M. xanthus* cells migrate to aggregation centers where they form fruiting bodies. During development, a small percentage of the cells in the fruiting bodies differentiate into spherical myxospores. Myxospores are metabolically dormant and withstand prolonged periods of nutrient deprivation. When nutrient replenishment occurs, the myxospores germinate into the vegetative rod-shaped cells. The elaborate developmental program of *M. xanthus* ensures that the spores are highly localized and ready to resume their social feeding behaviors when germination occurs.

M. xanthus utilizes the appropriately timed expression of development-specific genes during fruiting body formation and sporulation. Dramatic changes have been shown to occur in the pattern of protein production during the developmental cycle of *M. xanthus* (11). A similar result was observed at the transcriptional level by random insertion of the *lacZ* gene into the chromosome of *M. xanthus* (16). RNA polymerase sigma factors are required for the initiation of transcription at particular promoter sequences (7), and stage-specific sigma factors in *Bacillus subtilis* have been shown to temporally and spatially regulate gene expression in a cascade-like fashion during endospore formation (31).

M. xanthus sigA has been cloned and sequenced previously (12). On the basis of extensive sequence homology of *M. xanthus sigA* to the primary sigma factor genes of *Escherichia coli* and *B. subtilis*, *sigA* has been proposed to encode the major sigma factor of *M. xanthus*. The *M. xanthus sigB* gene has also been cloned and sequenced, and it also shows significant sequence homology to known sigma factor genes (1). Characterization of *M. xanthus sigB* by *lacZ* fusion analysis revealed that its expression occurs at a late developmental stage within myxospores. A deletion mutant of *sigB* exhibited greater than 10-fold reduction in spore yield during late development. Additionally, the viability of myxospores harvested during late development of Δ*sigB* decreased more than 1,000-fold. These data suggest that *sigB* is involved in the proper maturation of myxospores. Here we describe a new *M. xanthus* gene, *sigC*. The *sigC* gene exhibits significant sequence similarity to sigma factor genes. The *sigC* expression is induced upon initiation of development. A *sigC* deletion mutant strain was found to exhibit abnormal fruiting body formation. The present data, along with previous results (1, 12), indicate that a family of sigma factor genes plays an important role in regulating the gene expression of the *M. xanthus* life cycle.

MATERIALS AND METHODS

Materials. Restriction enzymes were purchased from either Bethesda Research Laboratories or New England Biolabs. T4 DNA ligase was obtained from Bethesda Research Laboratories, and DNA polymerase I Klenow fragment was obtained from Boehringer Mannheim. Sequenase (version 2.0) was obtained from United States Biochemical Corp. [α-^{32}P]dCTP and [α-^{35}S]dATP were obtained from Amersham. Nitrocellulose was purchased from Schleicher & Schuell.

Cells and growth conditions. *M. xanthus* FB (DZF1) was used in this study and grown in CYE medium (3). *E. coli* strains SB221 (22) and JM83 (34) were used for propagating plasmids. Both *E. coli* strains were grown in Luria broth. CF agar (clone fruiting agar) (6) and 1/2CTT agar (semirich medium) (9) were prepared as described previously.

Plasmids and phages. pBR322X (in which A and C at bases 338 and 343 in pBR322 [23] were changed to C and G, respectively, to create an *Xho*I site in the tetracycline resistance gene) was used to clone an 8.4-kb *Xho*I genomic fragment (pX8.4) from DZF1. pX8.4 was identified from screening plasmids by Southern hybridization with the 324-bp *Pst*I-*Xho*I fragment of *sigA* as a probe. pUC9 (33) and pBluescript (Stratagene Cloning Systems) were used for subsequent subcloning and sequencing experiments. P1*clr100*Cm (25) was used for transduction of plasmids into *M. xanthus* as described previously (28).

DNA manipulations and sequencing. Chromosomal DNA was prepared as described previously (35). Plasmid preparation was done by the alkaline extraction procedure as originally described by Birnboim and Doly (2). Blot hybridization was performed according to the method of Southern (30) with 40% formamide–5× SSPE (1× SSPE is 180 mM NaCl–10 mM NaH_2PO_4 [pH 7.4]–1 mM EDTA [pH 7.4]–0.5% sodium dodecyl sulfate–5× Denhardt's solution–5 ×

* Corresponding author.

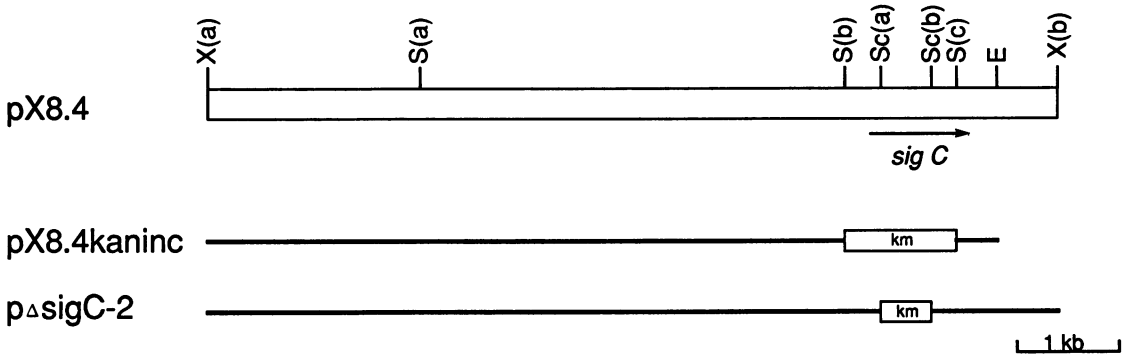

Fig. 1. Schematic diagram for the 8.4-kb DNA fragment encompassing the *M. xanthus sigC* gene and the regions used to construct *sigC* deletion mutants. The arrow represents the *sigC* ORF and its orientation. X, *Xho*I; S, *Sal*I; E, *Eco*RI; Sc, *Sac*II. Only the *Sac*II sites in 1.1-kb *Sal*I fragment are shown. The thick lines indicate upstream and downstream regions used to construct *sigC* deletion mutants. Boxes labeled km represent the kanamycin resistance gene and are not drawn in actual sizes.

10^5 cpm of ^{32}P-labeled probe per ml at 37°C. Posthybridization washes with 2× SSPE were done at room temperature.

A 1.1-kb *Sal*I fragment from pX8.4, which was identified by hybridization with the 324-bp *Pst*I-*Xho*I fragment of *M. xanthus sigA* (12), was cloned into pBluescript (Stratagene Cloning Systems) to form pS1.1. Nested deletions were created by using the *Exo*III-mung bean method (United States Biochemical Corp.). Four fragments (123, 132, 268, and 540 bp) were also obtained from the 1.1-kb *Sal*I fragment by digestion with *Rsa*I. These fragments were cloned into pUC9.

The DNA sequence of *sigC* was determined on both strands by the chain termination method (26) with double-stranded plasmids. Customized synthetic oligonucleotides were used as sequencing primers to clarify ambiguities. Oligonucleotides were also used to sequence the open reading frame (ORF) region of *sigC*, which extended beyond *Sal*I(c) (Fig. 1 and 2). dITP (United States Biochemical Corp.) was used to minimize compressions which can result from the high GC content of myxobacterial DNA.

Construction of Δ*sigC* strain and *sigC-lacZ* fusion gene. The 1.1-kb *Sal*I fragment of pX8.4 between sites *Sal*I(b) and *Sal*I(c) in Fig. 1 was replaced with a 1.3-kb *Sal*I fragment, containing the kanamycin resistance gene of Tn*5*, from pUC7Kan5 to form pX8.4Kan. pUC7Kan5 was created by cloning a Klenow-treated 1.3-kb *Sma*I-*Hin*dIII fragment containing the kanamycin resistance gene of Tn*5* into pUC7 that was cut with *Pst*I and Klenow treated. pX8.4Kan was digested with *Eco*RI and ligated with the 5.4-kb *Eco*RI P1inc fragment from pP1incE, a derivative of the pP1EK (10) having an *Eco*RI linker added at the *Sma*I site. This last step formed pX8.4KanInc (Fig. 1 and 2). pX8.4KanInc was introduced into the *M. xanthus* chromosome by P1 transduction (28). Double-crossover recombination of this construct into the chromosome at each of the regions flanking the kanamycin resistance gene resulted in the deletion of the first 197 codons of the *sigC* gene, including 196 bases of upstream DNA. Deletion of the *sigC* gene was verified by Southern hybridization (30) of *Pst*I chromosomal digests of the deletion strain, with the radioactively labeled 8.4-kb *Xho*I fragment from pX8.4 as a probe. Since the kanamycin resistance gene used to construct the deletion contained a *Pst*I site, the deletion mutant was identified by the shift of the band in the *Pst*I digest compared with the pattern for the wild type (data not shown). *Xho*I chromosomal digests of the deletion mutants were also probed with the 1.1-kb *Sal*I fragment and shown not to hybridize at the position of the 8.4-kb fragment (data not shown).

Since the *Sal*I(b) site is located 202 bases upstream of a putative initiation codon of *sigC* and the deletion of a 1.1-kb DNA fragment between *Sal*I(b) and *Sal*I(c) may disrupt an upstream gene, another deletion mutant was constructed by replacing the 483-bp *Sac*II fragment in the *sigC* gene (see Fig. 1 and 2) with the 1.3-kb *Hin*cII fragment containing the kanamycin resistance gene. pUC-S1.1, containing the 1.1-kb *Sal*I fragment in pUC9, was digested with *Sac*II, subjected to Klenow treatment, and ligated with the 1.3-kb *Hin*cII fragment of the kanamycin gene. The 1.9-kb *Sal*I fragment was isolated and ligated with the 11.7-kb *Sal*I fragment of pX8.4 obtained by partial digestion. In this fragment, the 1.1-kb *Sal*I(b)-*Sal*I(c) fragment was eliminated as a result of the partial digestion. The resulting plasmid is designated pX8.4Δ*Sac*II. pΔ*sigC*-2, containing the P1inc fragment, was constructed by ligating the following three fragments: the 4.0-kb *Eco*RI-*Bam*HI fragment of pBR322, the 9.2-kb *Bam*HI-*Hin*dIII fragment from pX8.4Δ*Sac*II, and the 5.4-kb partial *Hin*dIII-*Eco*RI fragment of pP1EK (10). Subsequently, pΔ*sigC*-2 was transduced into DZF1 as described above, and the deletion mutation (Δ*sigC*-2) was verified by the same method used for Δ*sigC*-1.

A *sigC-lacZ* fusion plasmid was constructed. The 763-bp *sigC* portion of the *sigC-lacZ* fusion gene was prepared by digestion the pS1.1 subclone with *Nco*I (indicated by an arrow in Fig. 2), filling in with Klenow enzyme, and digestion with *Kpn*I. Then, a 6.0-kb fragment containing the *lacZ* gene of *E. coli* was isolated from pKM005 (18) by digestion with *Bam*HI, filling in with Klenow enzyme, and digestion with *Sal*I. This created a fragment which consisted of the *lacZ* gene fused with *sigC* in the same reading frame. The 0.76-kb *sigC* fragment, the 6.0-kb *lacZ* fragment, and a *Sal*I-*Kpn*I-digested pBluescript vector were ligated together to form pB-C/Z. Then, the 6.75-kb *Hin*dIII-*Kpn*I fragment from pB-C/Z containing the *sigC-lacZ* fusion gene, a 5.4-kb *Kpn*I-*Eco*RI fragment from pPIEK containing the P1inc region, a 2.5-kb *Eco*RI-*Sal*I fragment from pUCKan5 containing the kanamycin resistance gene of Tn*5*, and a *Sal*I-*Hin*dIII-digested pBR322 vector were ligated together to yield pC/ZKanInc. pC/ZKanInc was transduced into the strain DZF1 by the P1 phage as described above. Insertion of the plasmid into the chromosome was verified by Southern blot analysis as described above (data not shown).

Developmental conditions. Glycerol sporulation was per-

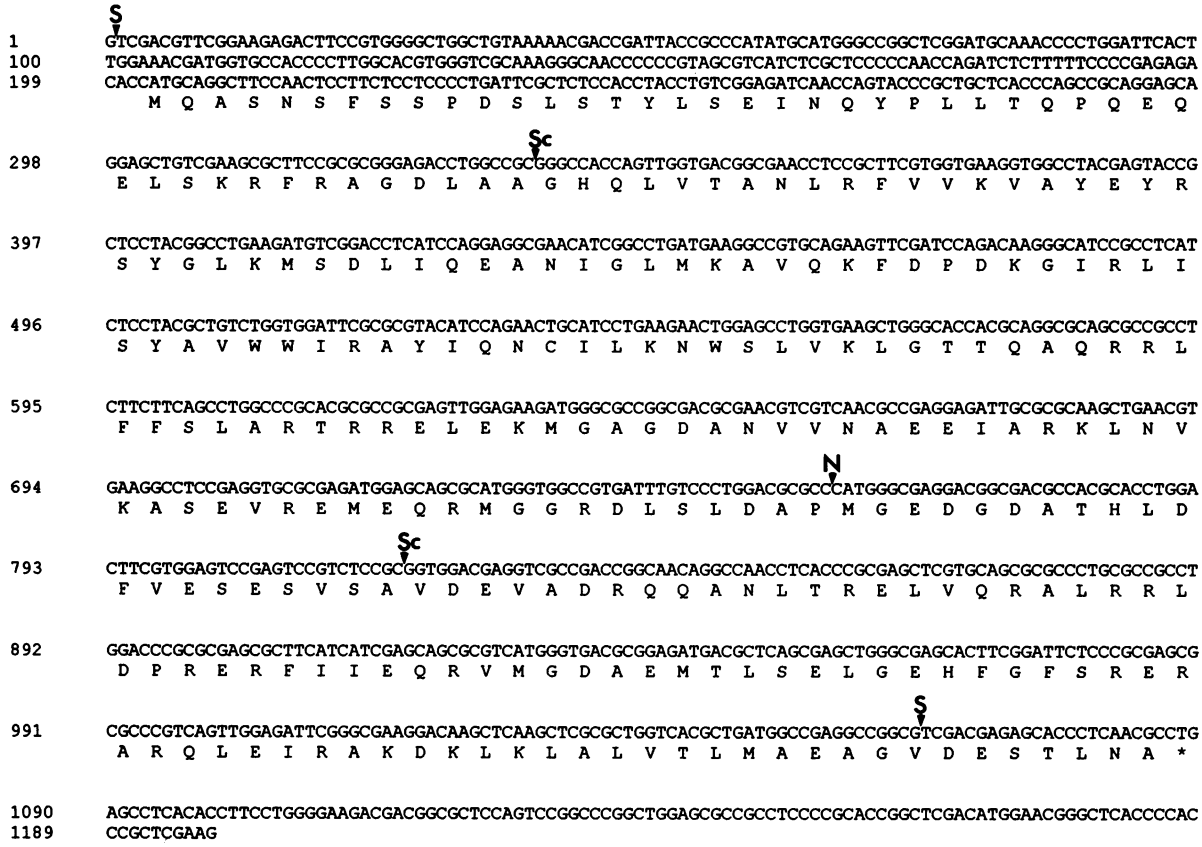

FIG. 2. Nucleotide sequence of the *sigC* region and its deduced amino acid sequence. Numbers on the right enumerate the nucleotide bases. The *Sal*I(b) and *Sal*I(c) sites are located at bases 1 and 1064, respectively. Arrows with letters indicate locations of the restriction sites, S, *Sal*I; Sc, *Sac*II; N, *Nco*I. The GenBank accession number is L12992.

formed by adding glycerol (to a final concentration of 0.5 M) to exponentially growing cells in CYE (4). Liquid starvation was carried out by pelleting exponentially growing cells from CYE and resuspending them in an equal volume of MCM buffer (10 mM morpholinepropanesulfonic acid [MOPS], 2 mM $CaCl_2$, 4 mM $MgSO_4$ [pH 7.2]) as described previously (24). Fruiting body formation on CF agar was carried out by concentrating exponentially growing cells from CYE to 4,000 Klett units in TM buffer (10 mM Tris [pH 7.6], 8 mM $MgSO_4$) and plating them on CF agar as previously described (11). Cells were plated on 1/2CTT by the same method used for fruiting body formation on CF agar. Cells were harvested from CF plates by using a straight-edged spatula to scrape the agar surface. Harvests were suspended in TM buffer. Cells were disrupted by sonication at 40 W for 2 min in an ice bath by using a heat system sonicator with a cup horn (Ultrasonics Inc.). The sonicated cells were then centrifuged to separate the sonication-sensitive fraction and the spore fraction. The remaining spore fraction was washed twice in TM buffer and disrupted by sonication in the presence of 100-μm-diameter glass beads (Sigma Chemical Co.) at 30 W for 5 min as described previously (32). These fractions were used in the β-galactosidase assay as described previously (16). The β-galactosidase specific activity (nanomoles of *o*-nitrophenyl-β-D-galactopyranoside [ONPG] per milligram of protein per minute) was normalized by estimating the total amount of protein (in milligrams) in each sample by the Bradford method (Bio-Rad Laboratories).

Nucleotide sequence accession numbers. The nucleotide sequence of *sigC* has been assigned GenBank no. L12992; the revised nucleotide sequence of *sigB* has been assigned accession no. X55500.

RESULTS

Cloning and sequencing of *sigC*. The major vegetative sigma gene of *M. xanthus*, *sigA* (formerly *rpoD*), has been cloned and sequenced previously (12). The deduced amino acid sequence of *sigA* displays extensive similarity to those of the major vegetative sigma factors of *E. coli* and *B. subtilis*. When the 324-bp *Pst*I-*Xho*I fragment, which encodes the highly conserved carboxy-terminal coding region of the *M. xanthus sigA* gene, was used as a probe, four additional fragments from *Xho*I-digested *M. xanthus* chromosomal DNA (1) (Fig. 3) were found to hybridize to the probe. An 8.4-kb *Xho*I fragment, which hybridized to the *sigA* probe, was identified and cloned into pBR322X, yielding pX8.4. A 1.1-kb *Sal*I fragment was identified from pX8.4 by using the same 324-bp *Pst*I-*Xho*I fragment as a probe and subcloned into Bluescript KS+ (creating pS1.1). The nucleotide sequence of the 1.1-kb *Sal*I fragment and a region downstream of the *Sal*I(c) site was determined, and an ORF was deduced from the nucleotide sequence as shown in Fig. 2.

The ORF of *sigC* was established on the basis of a comparison of conserved amino acid residues found in the

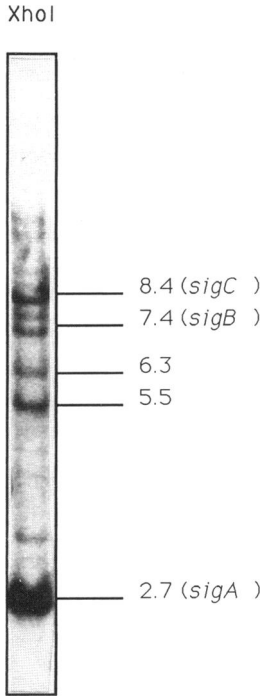

FIG. 3. Southern blot analysis of the chromosomal DNA from *M. xanthus* DZF1 digested with *Xho*I. A 324-bp *Pst*I-*Xho*I-digested fragment from *sigA* was used as a probe. Numbers on the right indicate molecular sizes in kilobases.

deduced SigA, SigB, and SigC sequences (see Fig. 7). The designation of the initiation codon at bases 203 to 205 is supported by the codon usage upstream of the putative initiation site. G and C bases constitute only 60.3% of the third positions of codons within the 202 bases upstream of the putative initiation codon. In contrast, G and C bases constitute 94.2% of the third positions of codons within the bases of the predicted ORF. *M. xanthus* DNA has a GC content of 68% (14), and as a result, all the genes characterized thus far have a very high GC content at the third positions of codons within their ORFs (13). The *sigA* and *sigB* genes contain 94.0 and 94.6% GC in the third positions of their codons, respectively. The stop codon of *sigC* is located in frame at bases 1088 to 1090. The *sigC* gene encodes a protein sequence which consists of 295 amino acid residues, which is the same size as the *sigB* product (for the revised sequence, see Discussion). The *sigC* gene encodes a highly charged protein (14.6% acidic residues and 14.9% basic residues) with an estimated pI of 6.0. The similarity of the deduced SigC sequence to those of other sigma factors will be described in the Discussion.

Expression of *sigC* during the developmental cycle. To determine the expression of *sigC* under various conditions, we constructed a *sigC* and *lacZ* fusion. The *sigC-lacZ* fusion construction was inserted into the *M. xanthus* chromosome by P1 transduction (28). Integration of this construct into the *M. xanthus* chromosome fuses the *lacZ* gene with the 187-residue *sigC* N-terminal region under the control of the *sigC* promoter and putative upstream regulatory regions. Insertion by this method results in the tandem arrangement of the fusion construct followed by the wild-type *sigC* gene. Reconstitution of the wild-type copy of the *sigC* is supported by the wild-type phenotype in the fusion strain.

The fusion strain, designated C/Z, was plated on CF agar (6) containing 5-bromo-4-chloro-3-indolyl-β-D-Galactopyranoside (X-Gal) and incubated at 30°C. Fruiting body formation and sporulation proceeded normally in the C/Z strain. However, blue color induction was observed in C/Z after 2 h of incubation, and the color induction intensified for more than 1 day. No color induction was observed in the wild-type control under these conditions.

To measure the β-galactosidase activity during development on CF agar more closely, developing cells, which consisted of rod-shaped cells and spores, were harvested at each time point shown in Fig. 4 and disrupted by sonication as described in Materials and Methods. The β-galactosidase activity was assayed in both the sonication-sensitive fraction and the spore fraction, with ONPG as a chromogenic substrate, and the results are shown in Fig. 4.

Expression of *sigC-lacZ* was detected exclusively in the sonication-sensitive fraction during fruiting body formation on CF agar. The specific activity increased from the time of plating until the time of sporulation (approximately 30 h), at which time the specific activity was dramatically reduced, as shown in Fig. 4A. When expression of *sigC-lacZ* was assayed during sporulation in MCM starvation buffer (24), the specific activity peaked just prior to the time of sporulation and decreased dramatically during the period of sporulation (Fig. 4B). Again, expression was detected only in the nonspore fraction of the C/Z strain. When expression of *sigC-lacZ* was assayed during glycerol induction of sporulation (4), we observed an increase of β-galactosidase activity just prior to sporulation (Fig. 4C). Expression of *sigC-lacZ* was also detected in the spore fraction during glycerol induction at a level equal to that of the nonspore fraction (Fig. 4C). The wild-type strain exhibited no β-galactosidase activity under these experimental conditions.

It is interesting that C/Z colonies, after several days of growth on CYE (rich medium) plates with X-Gal, showed blue color induction at the colony center (data not shown). The blue color intensity increased gradually for several days but remained confined to the colony center. Analysis of harvests from C/Z growing in liquid CYE from exponential growth through the stationary phase revealed low β-galactosidase specific activity (approximately 2 to 3 U) during the stationary phase.

Effects of *sigC* deletion on development. To examine the function of *sigC*, we constructed *sigC* deletion mutant strains, designated Δ*sigC*-1 and Δ*sigC*-2. In the case of Δ*sigC*-1, a 1.1-kb fragment between 2 *Sal*I sites [S(b) and S(c) in Fig. 2] of pX8.4 was replaced by the kanamycin resistance gene of Tn*5* (see Materials and Methods). A 196-bp region upstream of the predicted ORF was deleted in Δ*sigC*-1. Since the possibility exists that the expression of an upstream ORF of *sigC* was disrupted by this construct, we also constructed another deletion mutant (Δ*sigC*-2) in which only an internal coding region (amino acid residues 45 to 206) was deleted and replaced with the kanamycin resistance gene (see Materials and Methods). Growth curves of Δ*sigC*-1 and Δ*sigC*-2 in liquid CYE and 1/2CTT liquid media appeared normal through the stationary phase compared with that of the wild-type strain. Growth of Δ*sigC*-1 and Δ*sigC*-2 also appeared normal on CYE agar. Both deletion mutant strains were capable of sporulation by glycerol induction and by liquid starvation in MCM buffer (24). However, they exhibited aberrant fruiting bodies when plated on CF agar. Figure 5 shows the fruiting body pattern of Δ*sigC*-1. In contrast to the oval and evenly spaced fruiting bodies of the wild-type strain, both deletion mutant strains displayed

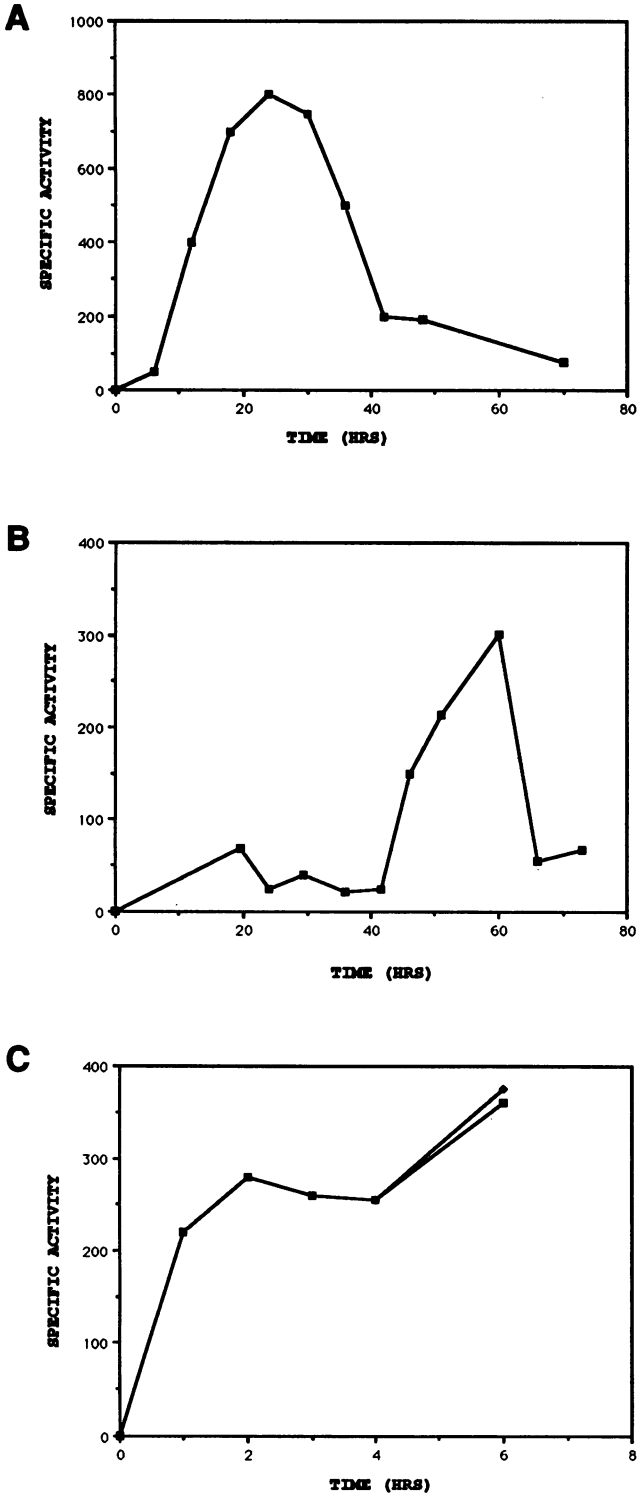

FIG. 4. β-galactosidase activity of the C/Z strain during development and glycerol induction. β-Galactosidase specific activity (nanomoles of ONPG per milligram per minute) in the sonication-sensitive fraction of the *sigC-lacZ* strain during development on CF agar (A) and during MCM induction of sporulation (B); (C) β-galactosidase specific activity in the sonication-sensitive (■) and spore (◆) fraction during glycerol induction of the *sigC-lacZ* strain.

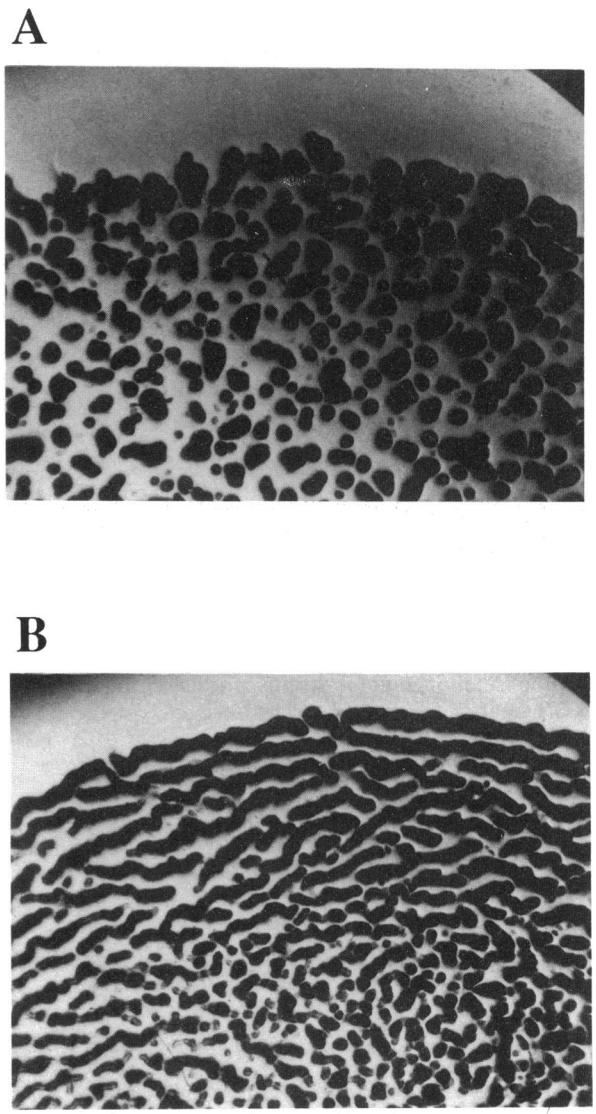

FIG. 5. Morphology of developing *M. xanthus* Wild-type (A) and Δ*sigC* (B) fruiting bodies after 96 h of development on CF agar. Photographs were taken by using an incandescent light source and a dissecting microscope.

elongated and concentric fruiting ridges which were concentrated toward the colony edge. Toward the center of the colony there appeared to be the normally shaped fruiting bodies that were somewhat smaller in size than those of the wild type. Spore counts and viability of Δ*sigC*-1 were at levels comparable to those of the wild type throughout development (data not shown).

Δ*sigC*-1 and Δ*sigC*-2 were grown in CYE and plated on 1/2CTT agar (9) to observe motility at the colony edge. However, fruiting bodies began to form after approximately 15 h of incubation at 30°C (Fig. 6). Fruiting bodies were confined to the region of the colony where cells were initially administered. The wild-type strain also formed a small number of fruiting bodies (less than 3% of Δ*sigC*-1) after approximately 50 h of incubation at 30°C on 1/2CTT agar plates, suggesting that the *sigC* deletion mutation made the cells more sensitive to the nutrient starvation signal for development. The cells at the colony edge of Δ*sigC*-1

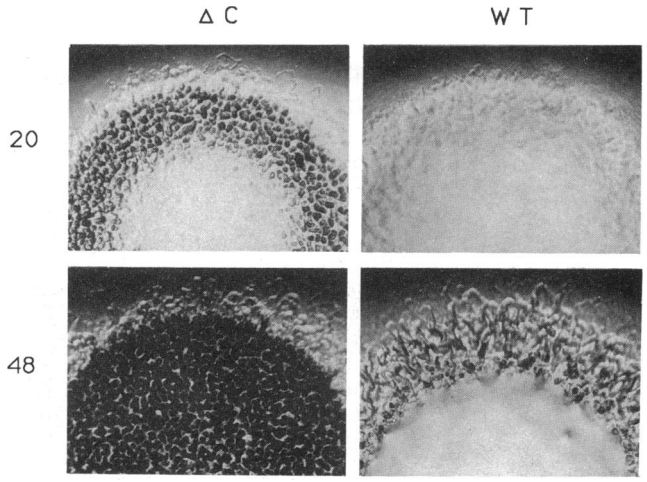

FIG. 6. Morphology comparison of Δ*sigC*-1 (ΔC) and wild-type (WT) *M. xanthus* plated on 1/2CTT agar. The number of hours of incubation at 30°C are indicated on the left.

showed wild-type levels of motility and did not form fruiting bodies after prolonged incubation at 30°C. Fruiting bodies from the deletion mutant strains produced myxospores on 1/2CTT agar.

DISCUSSION

Structural and functional features of prokaryotic sigma factors are well conserved and have been designated as regions 1 through 4 (7, 17). The amino acid sequence deduced from the DNA sequence of *sigC* was compared with those of *sigA* and *sigB* of *M. xanthus* (1, 12), and their alignments are shown in Fig. 7. Region 1 has been shown to consist of subregions 1.1 and 1.2. Region 1.1 is found only in the primary sigma factors, while region 1.2 is reasonably well conserved among known sigma factors (17). The absence of region 1.1 in SigC and SigB suggests that SigB and SigC are secondary sigma factors. Similarities between the sigma factors of *M. xanthus* start in region 1.2 as shown in Fig. 7. In region 1.2, there are 10 identical residues of 26 residues between SigA and SigC and 11 between SigA and SigB. In contrast, there are 16 identical residues between SigC and SigB.

Region 2 is divided into 4 subregions (2.1, 2.2, 2.3, and 2.4) and is known to be the most conserved region in all known sigma factors (7, 17). As shown in Fig. 7, SigC shares 93% identity in region 2 (77 identical residues of 83) with SigB. In contrast, the identities in region 2 between SigA and SigC and between SigA and SigB are 46 and 47%, respectively. Three sigma factors share the highest identity in region 2.2, known to be the most conserved domain among sigma factors. In region 2.4, which is known to be involved in the recognition of the −10 region of the promoter, only two amino acid residues of 21 are different between SigC and SigB. On the other hand, SigA shows that 7 amino acid residues are different from those of SigB or SigC in this region.

Region 3 is divided into two subregions, of which region 3.1 is more conserved among the primary sigma factors (17). The similarity between SigA and SigB or SigC is poor, while SigC and SigB show 71% identity in this region.

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Mx SigA   DPVRLYLRKMGSVSLLTREGEVEIAKRIEDGEKEVL  232
Mx SigB   GSLAMYLSEINHYSLLKVEEEQELARRFLKGD     43
Mx SigC   DSLSTYLSEINQYPLLTQPQEQELSKRFRAGD     42
                    1.2

Mx SigA   RAERAKSELVEANLRLVVSIAKKYTNRGLQFLDLIQEGNIGLMKAVDKFEYKRGYKFSTYATWWIRQAITRAIADQARTIRIPVHM  551
Mx SigB   LAAGHRMVTANLRFVVKVAYEYRSYGIKMSDLIQEGNIGLMKAVQKFDPDKGIRLISYAVWWIRAYIQNYILKSWSLVKLGTT  126
Mx SigC   LAAGHQLVTANLRFVVKVAYEYRSYGLKMSDLIQEANIGLMKAVQKFDPDKGIRLISYAVWWIRAYIQNCILKNWSLVKLGTT  125
          2.1                 2.2                 2.3                 2.4

Mx SigA   IETINKLIRTSRYLVQE---IGREPT----PEEIAEKMELPLDKVRKV-LKIAKEPISLETPIGEEE------DSHLGDFIEDKSLVSPADAV  630
Mx SigB   QAHGKLFFSLARTRRELEKFGSGDAAV-NVDDIARRLHVKPGEVREMEQRMGGRDLSLDAPMGEDGGNSHVDFVVSAAAPQDDEFADKEEA  216
Mx SigC   QAQRRLFFSLARTRRELEKMGAGDANVVNAEEIARKLNVKASEVREMEQRMGGRDLSLDAPMGEDGDATHLDFVESESVSAVDEVADRQQA  216
                                    3.1                                                 3.2

Mx SigA   INMNLAEQTRKVLATLTPREEKVLRMRFGIGEKSDHTLEEVGQDFEVTRERIRQIEAKALRKLRHPSRSKRLRSFVES        708
Mx SigB   GLINARVRTALMRLDPRERFIIEQRVM-NE-RPMTLKELGEHFGFSRERARQLEIRAKDKLKSELAALMAEVDPEAVAAQQ  295
Mx SigC   NLTRELVQRALRRLDPRERFIIEQRVM-GD-AEMTLSELGEHFGFSRERARQLEIRAKDKLKLALVTLMAEAGVDESTLNA  295
                    4.1                 4.2
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FIG. 7. Amino acid sequence alignment of *M. xanthus* (Mx) SigA, SigB, and SigC. The subregions (in boldface) are denoted by the lines under the aligned sequences. Vertical lines between the sequences indicate identical residues. The lines are drawn between SigA and SigB only when all three proteins share an identical residue. Numbers on the right indicate residue numbers from the N-terminal ends.

Region 4, divided into two subregions, 4.1 and 4.2, is considered to participate in the recognition of the −35 region of the promoter (7). Region 4.2 has the helix-turn-helix DNA-binding motif and is a highly conserved sequence among the primary sigma factors but not among the alternative sigma factors (17). In region 4.2, SigC shows 97% identity to SigB but only 50% identity to SigA. In regions 4.1 and 4.2, only 1 of 17 and 2 of 30 amino acid residues, respectively, are different between SigC and SigB (Fig. 7).

It was found that a single amino acid substitution in regions 2.4 and 4.2 of sigma factors from *E. coli* and *B. subtilis* changes the specificity of promoter utilization by RNA polymerase (5, 29, 37). Between SigB and SigC, there are two amino acid substitutions in region 2.4 and one substitution in region 4.2. In particular, it is interesting that the two amino acid substitutions in region 2.4 are separated by three residues, suggesting that these altered residues are on the same side of the surface of a putative helix in this region. It is possible that this side may be interacting with a specific sequence of a promoter recognized by SigB or SigC.

Note that the originally reported sequence of *sigB* (1) has been revised (revised nucleotide sequence accession no. X55500). This alteration in the nucleotide sequence resulted in an increase in the size of the ORF from 193 residues to 295 residues. The revised sequence has a calculated molecular weight of 33,407 and an estimated pI of 6.7.

The expression of the *sigC-lacZ* fusion was detected only in the sonication-sensitive fraction during development on CF agar plates. The expression also appeared to be temporally regulated, showing a dramatic increase in activity prior to sporulation and a significant decrease in activity through the period of sporulation. We were not able to detect β-galactosidase activity in myxospores, as β-galactosidase activity in the sonication-sensitive fraction decreased. Rapid induction of β-galactosidase after the transfer of cells to CF agar plates might be caused by nutrient downshift. However, this is unlikely, since rapid induction of β-galactosidase was observed when cells were simply transferred from 1/2CTT liquid medium to 1/2CTT agar plates after concentration to 4,000 Klett units. Under this condition, Δ*sigC* cells were able to form fruiting bodies and sporulate, but wild-type cells formed only a few fruiting bodies and were unable to sporulate. The finding that *sigC* is expressed in glycerol-induced spores is consistent with the notion that glycerol sporulation is a bypass of the normal developmental pathway.

On the basis of β-galactosidase activity of the *sigC-lacZ* fusion gene, the *sigC* gene was regulated temporally during fruiting body formation. However, the *sigC* deletion strains (Δ*sigC*-1 and Δ*sigC*-2) were capable of forming fruiting bodies and sporulating during development on CF agar. This result demonstrates that *sigC* is not required for normal development. However, in contrast to the wild-type cells, the ability of Δ*sigC* to form fruiting bodies and to sporulate on 1/2CTT agar plates is unlikely because of impaired growth of the mutant cells in 1/2CTT medium, since the growth rate of the mutant strains in 1/2CTT was almost identical to that of the wild-type strain. These results indicate that *sigC* may play a role in expression of genes that negatively regulate the initiation of development.

Recently, the *rpoS* gene of *E. coli* has been shown to be required for the induction of trehalose synthesis in response to increases in osmolarity and changes the stationary-phase growth conditions (8). *M. xanthus* cells have been shown to accumulate trehalose during osmotic stress and during sporulation (20). To investigate the possible role of *sigC* in the trehalose response of *M. xanthus*, we examined the ability of Δ*sigC* to produce trehalose in response to osmotic stress and during sporulation. The Δ*sigC*-1 strain produced normal levels of trehalose under both of these conditions (data not shown) as determined by the assay previously described (19).

The developmentally expressed sigma factor genes of *M. xanthus* are the first to be characterized in a gram-negative developmental system. The unique life cycle of *M. xanthus* provides an ideal system to explore the gene regulation governing multicellular communication and differentiation. Results obtained thus far suggest that a cascade-like relationship may exist within the sigma gene family of *M. xanthus* as shown in *B. subtilis*. Recently, protein serine/threonine kinases, which have homology to the eukaryotic serine/threonine kinases, have been characterized in *M. xanthus* (21). The protein kinase gene, called *pkn1*, is expressed at the onset of sporulation and is required for normal development. In addition to *pkn1*, many other *pkn* genes have been identified (36). It is tempting to speculate that sigma-directed gene regulation may operate in conjunction with the protein serine/threonine kinase family of *M. xanthus*, which may have a critical role in transmitting signals during the developmental process.

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