JOURNAL OF BACTERIOLOGY, Nov. 1988, p. 5279–5288
0021-9193/88/115279-10$02.00/0

Vol. 170, No. 11

Genetic Identification and Cloning of a Gene Required for Developmental Cell Interactions in *Myxococcus xanthus*

RONALD E. GILL,* MILLARD G. CULL, AND SUSAN FLY

Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262

Received 28 January 1988/Accepted 9 August 1988

Developmental mutants of *Myxococcus xanthus* have been previously described which appear to be defective in required cell-cell interactions. These mutants fall into four phenotypic classes, Asg, Bsg, Csg, and Dsg, each of which is unable to differentiate into spores but can be rescued by extracellular complementation by wild-type cells or by mutants of a different class. We report the identification of one of the loci in which mutations result in a Bsg phenotype. The cloned locus was contained on a 12-kilobase *Eco*RI fragment and then localized by subcloning and a combination of in vitro and transposon mutagenesis. All mutations in this locus behave as a single complementation group, which we designate *bsgA* (formerly *ssbA*). Each of the *bsgA* mutations results in a nonsporulating phenotype, which can be rescued by extracellular complementation. Furthermore, we report that the *bsgA* mutants have a distinctive interaction with wild-type cells when vegetatively growing, swarming colonies converge.

Myxococcus xanthus is a gliding, gram-negative soil bacterium which undergoes a distinctive multicellular developmental cycle (17, 19). When cells are plated at high density on agar medium with limiting nutrient, streams of cells move to aggregation centers, which give rise to compact three-dimensional aggregates called fruiting bodies. Within the fruiting body, some of the cells undergo differentiation into dormant, environmentally resistant myxospores.

It is the multicellular aspects of *M. xanthus* development which make it particularly unusual among the procaryotes. Implied by its multicellular nature are requisite interactions between cells that control and coordinate the actions of individual cells. Indeed, Hagen et al. (7) isolated a collection of developmental mutants that behave as if defective in required cell-cell developmental interactions. These mutants apparently grow normally during vegetative growth but fail to complete development and produce myxospores when plated on starvation medium. However, these same mutants do produce myxospores when placed on developmental medium in a mixture with wild-type cells. This behavior has been termed extracellular complementation. There is no evidence that such cocultivation results in genetic changes in the mutant cells. Instead, the mutants behave as if they have lost the ability to produce a required extracellular signal but remain able to respond to such a signal produced by the wild-type cells. These mutants were further subdivided into four groups based on pairwise mixing of the individual mutants. Pairs of mutants which result in production of spores are defined as belonging to different groups (given the phenotypic designations Asg, Bsg, Csg, and Dsg) and are thought to be deficient in the production of different developmental signals.

Recent studies have begun to shed some light on the nature of these developmental defects and the molecular consequences of extracellular complementation. Studies by Gill and Cull (6), Kroos and Kaiser (10), and Kuspa et al. (13) have shown that representatives of the Asg, Bsg, and Csg mutants are blocked in their ability to transcribe characteristic sets of developmental genes. Furthermore, these studies showed that the ability of the mutant cells to transcribe these developmental genes is restored by extracellular complementation.

The purpose of this paper is to report the genetic identification and cloning of one of the loci in which mutations result in a Bsg$^-$ phenotype. All mutations that map to this locus belong to a single complementation group, to be designated *bsgA* (formerly *ssbA* [6]). In addition to the very early developmental defect caused by these mutations (6, 10), we report here the identification of a novel aspect of the mutant phenotype which is manifest during vegetative growth of the cells.

MATERIALS AND METHODS

Bacterial strains, phages, and transposons. The strains of *M. xanthus* used in this study are listed in Table 1. The transposon Tn*5* and its use as a mobile genetic marker have been reviewed (3, 11). Tn*5*-132 is a variant of Tn*5* in which the kanamycin resistance determinant has been replaced by tetracycline resistance but which retains most of the IS*50* elements that comprise the long terminal inverted repeats of Tn*5* (4). The in situ replacement of Tn*5* insertions in *M. xanthus* by Tn*5*-132 has been described (1). The coliphage P1 *clr-100 cam* is a Cm^r, temperature-sensitive, inducible variant of P1 (18). The myxophage Mx8*cp2* and its use for generalized transduction between *M. xanthus* strains has been described (15). The transposon Tn*10*mini-*kan* (25) is a derivative of Tn*10* which consists of the distal 78 base pairs of each of the Tn*10* inverted repeats flanking a kanamycin resistance determinant derived from Tn*903*.

Bacterial growth and determination of developmental phenotype. *Escherichia coli* was grown in LB medium (5) supplemented, when appropriate, with kanamycin (50 μg/ml), ampicillin (200 μg/ml), or chloramphenicol (12.5 μg/ml). *M. xanthus* was grown vegetatively in CTT medium (8) supplemented, when appropriate, with kanamycin (50 μg/ml) or oxytetracycline (15 μg/ml) (1). Solid media contained 1.5% Bacto-Agar (Difco Laboratories). The developmental phenotype of individual *M. xanthus* colonies was determined by transfer to CF agar (8) with a sterile toothpick. After 5 days, plates were examined microscopically for refractile

* Corresponding author.

TABLE 1. Bacterial strains[a]

Strain	Description	Derivation	Source or reference
DK435	*bsgA435*	UV on DK101	7
DK454	*bsgA454*	UV on DK101	7
DK460	*bsgA460*	UV on DK101	7
DK468	*bsgA468*	UV on DK101	7
DK751	*bsgA751*	ICR-191 on DK101	7
DK2466	Spo$^+$ Tn*5*ΩDK2466	Mx8(pool Tn*5* in DK101) × DK454 → Km^r [Spo$^+$]	Kuner (Ph.D. thesis)
M101	Spo$^+$ *sglA1*[b]	Single colony isolate of DK101	D. Kaiser
M102	Spo$^+$, fully motile	Single colony isolate of DK1622	D. Morandi and D. Kaiser
M174	*bsgA262*	P1(pREG1262) × M102, gene replacement	This work
M201	Spo$^+$ Tn*5*-132ΩDK2466 (Tc^r)	P1::Tn*5*-132 × DK2466 → Tc^r [Km^s Spo$^+$]	This work
M203	*bsgA460* Tn*5*ΩDK2466	Mx8(DK2466) × DK460 → Km^r [Spo$^-$]	This work
M206	*bsgA435* Tn*5*ΩDK2466	Mx8(DK2466) × DK435 → Km^r [Spo$^-$]	This work
M207	*bsgA454* Tn*5*ΩDK2466	Mx8(DK2466) × DK454 → Km^r [Spo$^-$]	This work
M226	M102::Tn*5*ΩDK2466 Spo$^+$	Mx8(DK2466) × M102 → Km^r [Spo$^+$]	This work
M230	*bsgA454* Tn*5*-132ΩDK2466	Mx8(M207) × M102 → Km^r [Spo$^-$] = M227; P1::Tn*5*-132 × M227 → Tc^r [Km^s Spo$^-$]	This work
M234	*bsgA435* Tn*5*-132ΩDK2466	Mx8(M206) × M102 → Km^r [Spo$^-$] = M231; P1::Tn*5*-132 × M231 → Tc^r [Km^s Spo$^-$]	This work
M237	*bsgA460* Tn*5*-132ΩDK2466	Mx8(M203) × M102 → Km^r [Spo$^-$] = M236; P1::Tn*5*-132 × M236 → Tc^r [Km^s Spo$^-$]	This work
M250	*bsgA299*	P1(pREG1299) × M102, gene replacement	This work
M251	*bsgA300*	P1(pREG1300) × M102, gene replacement	This work
M252	*bsgA301*	P1(pREG1301) × M102, gene replacement	This work (6)
M485	*bsgA355*	P1(pREG1355) × M102, gene replacement	This work

[a] ΩDK2466 designates a particular site in *M. xanthus*, site number 2466 from the laboratory of Dale Kaiser, at which Tn*5* has inserted. Mx8(DK2466) × DK460 → Km^r [Spo$^-$] indicates that Mx8 grown on DK2466 was used as a donor in transduction with DK460. Transductants were selected on kanamycin and then screened for the Spo$^-$ phenotype. Similarly, P1(pREG1262) × M102 indicates that phage P1 grown on *E. coli* carrying the pREG1262 plasmid was used to transduce strain M102.

[b] *sglA1* is a motility mutation which reduces the swarming of colonies growing on solid media.

spores and for heat-resistant spores by the method of LaRossa et al. (14).

Extracellular complementation of sporulation. Exponentially growing cells were harvested, washed, and suspended at a concentration of 5×10^9 cells per ml in TPM (10 mM Tris hydrochloride [pH 7.6], 1 mM potassium phosphate, 8 mM $MgSO_4$). Equal volumes of pairs of strains to be tested were mixed, and 10-μl samples were spotted onto TPMP (6) plates. After 4 days at 30°C, spores derived from the Km^r parent were detected as previously described (14). The Km^s tester strains used were M101 and M102 (both Spo$^+$), DK480 (Asg), DK731 (Csg), and DK439 (Dsg).

Plasmid manipulations. The general methods used for the formation, isolation, and characterization of recombinant plasmid DNA are those described by Maniatis et al. (14a). All enzymes used in plasmid constructions were used according to the manufacturers' specifications. Restriction enzymes and T4 ligase were from Boehringer Mannheim Biochemicals; T4 DNA polymerase was from Pharmacia Fine Chemicals. Restriction enzyme fragments used in ligations were routinely purified by agarose gel electrophoresis, electroelution of the desired fragment into dialysis tubing, and purification by NACS chromatography (Nucleic Acid Chromatography System; Bethesda Research Laboratories, Inc.) according to the manufacturer's specifications.

Construction of pREG429. The plasmid pREG429 (Fig. 1) is an integrating vector designed to facilitate the cloning and subsequent genetic analysis of DNA adjacent to Tn*5* insertions in *M. xanthus*. It is capable of replication in *E. coli* but not in *M. xanthus*. In addition to ampicillin resistance and plasmid replication functions derived from pBR322, this plasmid contains (i) a fragment of bacteriophage P1 which confers P1 incompatibility (23), making it possible to efficiently transfer the plasmid by P1 specialized transduction, and (ii) a portion of Tn*5* which encodes kanamycin resistance, a strong selectable marker in both *M. xanthus* and *E. coli*, and also contains most of the left IS*50* element, which will provide the needed homology to integrate the plasmid at chromosomal Tn*5*-132 insertions in the *M. xanthus* chromosome.

The plasmid pREG429 was constructed as shown in Fig. 1. The 6.6-kb *Eco*RI-5 fragment of bacteriophage P1 was purified from a λ clone supplied by N. Sternberg and inserted into the *Eco*RI site of pBR322 to give pREG411. To inactivate the *Eco*RI site adjacent to the pBR322 *bla* gene, pREG411 was first partially digested with *Eco*RI and then linear molecules were gel purified. The linearized plasmids were then treated with Klenow enzyme to produce blunt ends, recircularized with T4 DNA ligase, and transformed into *E. coli* C600. Double digestions with *Eco*RI and *Bam*HI were used to identify a plasmid, pREG422, in which the proper *Eco*RI site had been inactivated. A fragment of Tn*5* encoding kanamycin resistance and containing most of the IS*50* element (Fig. 1A) was added to pREG422 as follows. ColE1::Tn*5* (obtained from D. Berg) was cut with *Hpa*I, and *Bam*HI linkers (Collaborative Research, Inc.) were ligated onto the *Hpa*I blunt ends. This was followed by digestion with *Sal*I. The resulting 2.5-kilobase (kb) *Bam*HI-to-*Sal*I fragment encoding kanamycin resistance was purified and inserted into *Bam*HI-*Sal*I-cut pREG422, with selection for resistance to kanamycin and ampicillin.

Formation of P1-pREG429 specialized transducing phage. Bacteriophage P1 has been used to transfer DNA from *E. coli* to *M. xanthus* (12, 16, 20, 22). It will inject its DNA into *M. xanthus* cells but does not multiply or lysogenize in this bacterium (12). A novel property of pREG429 and its derivatives is the formation of P1-pREG429 cointegrates in *E. coli*, which makes it possible to package the plasmid DNA into P1 particles with high efficiency. Bacteriophage P1 normally lysogenizes *E. coli* as a self-replicating plasmid.

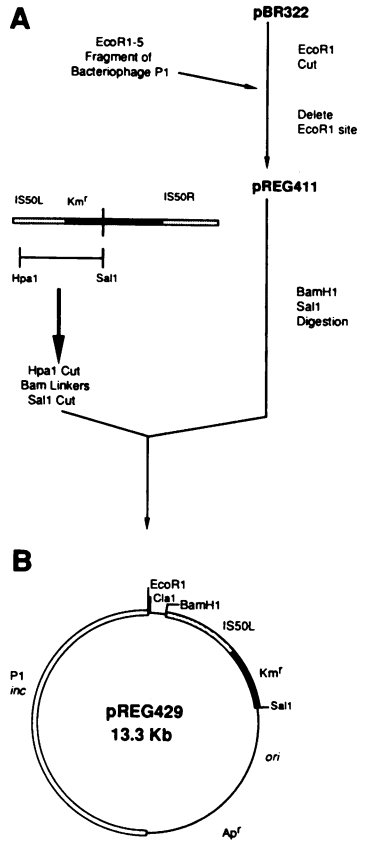

FIG. 1. Construction of pREG429. (A) Flow chart showing the modification of pBR322 by the addition of the 6.6-kb *Eco*RI-5 fragment of bacteriophage P1, which encodes P1 incompatibility (*inc*), and deletion of one of the *Eco*RI sites to give pREG411. pREG411 was then modified by the addition of a 2.5-kb Tn*5*-derived *Hpa*I-*Sal*Ifragment encoding kanamycin resistance (depicted by the solid bold line) and containing most of the left IS*50* ( ). The resulting plasmid, pREG429 is shown in panel B.

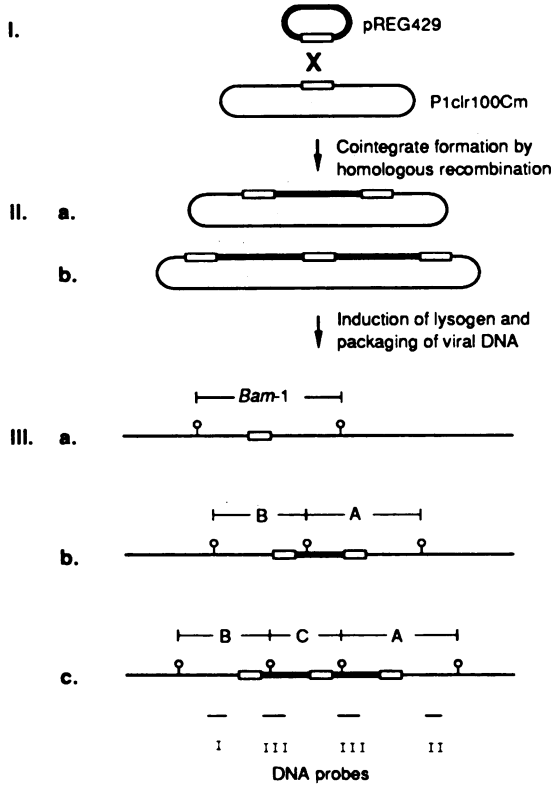

FIG. 2. Diagramatic representation of cointegrate formation and the expected structure of the resulting specialized transducing phage. (I) Recombination between the P1 *inc* segments (□) of pREG429 (▬) and P1 (——), resulting in cointegrate formation. Cointegrates would have the structure shown in IIa, or that shown in IIb if more than one copy of pREG429 were integrated into the P1 molecule. The DNA molecules which are expected to be packaged after induction of a lysogen containing a P1-pREG429 cointegrate are shown: (IIIa) a wild-type P1 genome in which no pREG429 has integrated, (IIIb) DNA packaged from a cointegrate molecule containing a single integrated pREG429 molecule, (IIIc) DNA packaged from a cointegrate molecule containing two copies of the integrated pREG429 plasmid. Relevant *Bam*HI sites (♆) are shown. The *Bam*-1 fragment contains the P1 *inc* region through which recombination takes place to form the cointegrates. *Bam*HI fragments A, B, and C are characteristic of specialized transducing phage which contain one or more integrated copies of the pREG429 plasmid. Also shown in IIIc is the location of the three hybridization probes used in the Southern hybridization shown in Fig. 3. Probes I and II are *Eco*RI fragments (designated *Eco*RI-5 and *Eco*RI-15 [23]) that are contained in the *Bam*-1 fragment and are located to the right and left, respectively, of the P1 *inc* region. Probe I is specific for fragment B; probe II is specific for fragment A. Probe III is an *Eco*RI-*Xho*I fragment of pREG429 which spans the *Bam*HI site and contains pBR322 and IS*50* sequences but has no homology to P1. This probe will hybridize to fragments A, B, and C.

When an infecting P1 attempts to lysogenize an *E. coli* strain carrying pREG429, the incoming P1 and the resident plasmid are unable to coexist as independent replicons due to the action of the P1 incompatibility region carried by the pREG429 plasmid. However, the incoming P1 can lysogenize and be maintained as a cointegrate between P1 and the resident pREG429 plasmid, formed by recombination between their homologous P1 incompatibility sequences (Fig. 2). The resulting cointegrate plasmids consist of an entire P1 genome into which one or often multiple tandem copies of pREG429 are integrated (data not shown).

When P1 is induced to lytic growth, P1 DNA packaging normally begins at a unique site and continues in a processive manner for three or four P1 headfuls (2). Therefore, packaging of the P1-pREG429 cointegrate molecules would be expected to result in the formation of specialized transducing phage in which the packaged DNA consists of the entire pREG429 plasmid flanked by P1 DNA. A diagramatic representation of such phage DNA and the location of unique *Bam*HI restriction fragments which can be used to identify these DNAs are shown in Fig. 2. The P1 *inc* fragment used in the construction of pREG429 is contained within the *Bam*-1 fragment of P1 *clr-100 cam* (Fig. 2, IIIa), which would therefore be altered by this recombination event. In its place, two new *Bam*HI fragments would be expected, designated A and B (IIIb), or fragments A, B, and C if multiple copies of pREG429 were present (IIIc).

The data shown in Fig. 3 demonstrate the existence of these specialized transducing particles. DNA was purified from phage produced from the induction of a P1 *clr-100 cam* lysogen and a P1 *clr-100 cam*-pREG429 cointegrate strain. The DNA was digested with *Bam*HI, and the fragments were separated on a 0.45% agarose gel. The ethidium bromide-stained gel (Fig. 3, lanes A and B) shows the nearly complete absence of fragment *Bam*-1 in the DNA from phage pro-

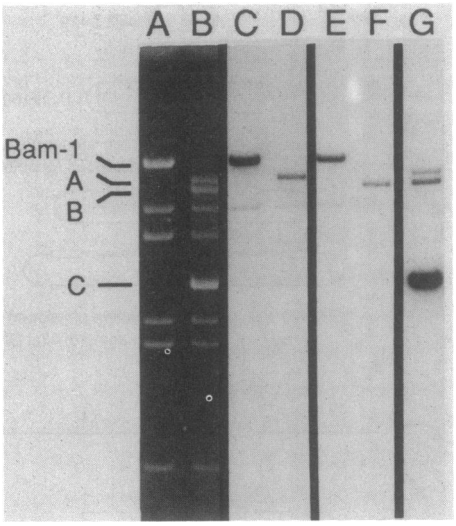

FIG. 3. Comparison of *Bam*HI-digested DNA purified from an induced P1 *clr-100 cam* lysogen and induction of a lysogen containing a P1-pREG429 cointegrate. Lanes: A and B, ethidium bromide-stained gel of P1 *clr-100 cam* (A) and cointegrate phage DNA (B); C and D, Southern blot of P1 *clr-100 cam* phage DNA (C) and cointegrate phage DNA (D) hybridized to probe II; E and F, Southern blot of P1 *clr-100 cam* phage DNA (E) and cointegrate phage DNA (F) hybridized to probe I; G, Southern blot of cointegrate phage DNA hybridized with probe III. Location of the hybridization probes is shown in panel IIIc of Fig. 2, as is the origin of the *Bam*HI fragments *Bam*-1, A, B, and C.

duced by the cointegrate strain. This would be expected if the majority of the phage contained an integrated pREG429 molecule. The appearance of three new *Bam*HI fragments in cointegrate phage DNA (Fig. 3, lane B, fragments designated A, B, and C; correlating with the respective fragments in Fig. 2) is also consistent with the proposed structure of the transducing phage. These fragments hybridized to probes II and I, respectively, which are specific for the right (probe II) and left (probe I) ends of the P1 fragment *Bam*HI-1 (Fig. 3, lanes D and F). Furthermore, both of these fragments hybridized with the pREG429-specific probe (probe III, lane G). These data confirm that fragments A and B are the right and left pREG429-*Bam*-1 junction fragments, respectively.

Fragment C comigrates with authentic linear pREG429 (data not shown) and hybridized only to the pREG429-specific probe (probe III, compare lane G with lanes D and F). Such a fragment would result from integration of a tandem dimer (or higher form) into the P1 genome (Fig. 2). The existence of phage DNA containing multiple integrated copies of the pREG429 plasmid was confirmed by digestion of cointegrate phage DNA with enzymes which cut in P1 but not in the pREG429 sequences (data not shown).

Transfer of plasmid DNA to *M. xanthus* by specialized transduction. As shown above, induction of a P1 *clr-100 cam* lysogen of a pREG429-containing strain resulted in the production of specialized transducing phage with the structure shown in Fig. 2. When these phage were used to infect *M. xanthus*, homologous recombination between the flanking P1 incompatibility sequences could regenerate the original circular pREG429 plasmid. The circular plasmid may, in turn, recombine with homologous sequences on the chromosome to integrate the plasmid. The required homology can be provided by either IS*50*, if the host bacterium contains a Tn*5* element, or cloned *M. xanthus* DNA contained in the plasmid. Although the plasmid is unable to replicate in *M. xanthus*, it is stably maintained in this integrated form.

In practice, *E. coli* C600 carrying the pREG429 plasmid or its derivatives containing additional cloned DNA were infected with P1 *clr-100 cam* phage at a multiplicity of approximately 1. After preadsorption at room temperature for 20 min, the cells were diluted 20-fold into fresh LB and shaken at 32°C for 1 h. Chloramphenicol and kanamycin were then added to a final concentration of 12.5 and 50 μg/ml, respectively, and cells were grown overnight at 32°C with shaking. Phage lysates were prepared by thermal induction of the lysogenic cultures as previously described (24), except that 0.2% glucose was added at the time of thermal induction. The plasmid was transferred to *M. xanthus* by mixing 10^7 exponentially growing cells with 10 to 100 μl of the P1 lysate. After 20 min at room temperature, the cells were mixed with CTT soft agar, overlaid onto CTT agar containing 50 μg of kanamycin per ml, and incubated for 5 days at 33°C.

Cloning strategy. The strategy used to clone *M. xanthus* DNA adjacent to a Tn*5* insertion by using pREG429 is illustrated in Fig. 4. Plasmid pREG429 does not autonomously replicate in *M. xanthus*. It can only be stably maintained in these bacteria by integration into the host chromosome by homologous recombination between the IS*50* sequences contained on the plasmid and the IS*50* element of an endogenous Tn*5* element. The consequence of integrating the cloning vector at the Tn*5* element is that it is now physically joined to the DNA to be cloned. Digestion of DNA from these cells with either *Cla*I, *Bam*HI, or *Eco*RI produced a fragment which contained all vector sequences required for selection and maintenance in *E. coli* plus a segment of chromosome DNA extending outward from Tn*5*. After ligation at low DNA concentration to promote circularization of the restriction fragments (a unimolecular event), the recombinant plasmids were recovered by transformation into *E. coli*.

To clone the DNA adjacent to the Tn*5*ΩDK2466 insertion site, 5 μg of chromosomal DNA from each strain was digested separately with *Cla*I, *Eco*RI, and *Bam*HI. After phenol extraction and ethanol precipitation, DNA (1 μg/ml) was ligated at 14°C for 16 h and transformed into *E. coli* C600. Transformants were selected for resistance to kanamycin and ampicillin.

Isolation of mutations in cloned DNA. Mutations at restriction sites were created in vitro by cutting with the appropriate restriction enzyme, creating flush-ended molecules with T-4 DNA polymerase, and ligating to reform a circular molecule. The properties of phage λ1105, which contains the Tn*10*mini-*kan* transposon, and its use in isolating transposon insertion mutations in plasmids in *E. coli* have been described previously (25). The location and orientation of transposon insertions within the 12-kb *M. xanthus* fragment of pREG1394 were determined by restriction enzyme mapping with *Bam*HI, which cuts symmetrically near the ends of the transposon and five times within the 12-kb fragment, and double digestions with *Eco*RI, which cuts at the ends of the fragment, and *Hin*dIII, which cuts only once asymmetrically within the transposon.

Cloning *bsg* alleles from strains without Tn*5*ΩDK2466. The scheme used to clone the mutant *bsg* alleles is shown in Fig. 5. To provide a means of integrating the vector near the *bsg* locus without requiring Tn*5* homology, a *Bam*HI-derived clone, pREG1153, containing the 7.5 kb of *M. xanthus* DNA indicated by the lower bar in Fig. 5A was obtained from Spo^+ cells by using the scheme shown in Fig. 4. The pREG1153 plasmid was transduced into the strains from

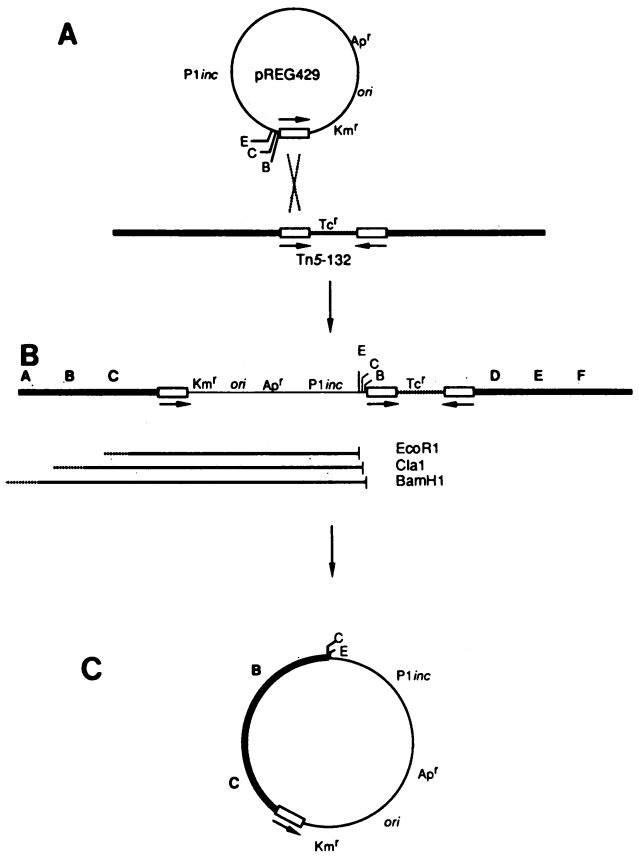

FIG. 4. General cloning scheme using the integrating vector pREG429. (A) Transduction of pREG429 into an *M. xanthus* strain which contains Tn*5*-132 may result in homologous recombination between the IS*50* sequences of the plasmid and the IS*50* sequences of the chromosomal Tn*5*-132. IS*50* sequences are designated by open boxes with arrows denoting their relative orientation. The location of the unique *Eco*RI (E), *Cla*I (C), and *Bam*HI (B) sites on the plasmid are indicated. Arbitrary chromosomal markers ABC and DEF are shown in boldface type. Recombination between pREG429 and the left or right IS*50* element of Tn*5*-132 occurs at approximately equal frequency; only one of the two alternatives is depicted here. The result of this recombination is integration of the plasmid into the chromosome as shown in panel B. Cleavage of DNA prepared from such a transductant with *Eco*RI, *Cla*I, or *Bam*HI generates the fragments shown. Each fragment contains all of the essential portions of the integrated vector plus flanking chromosomal DNA, up to the first recognition site for the respective enzyme. DNA was recovered by ligation of the restriction fragments at low concentration to promote circularization and transformation into *E. coli*. The plasmid shown in panel C depicts the possible outcome from *Cla*I digested DNA, if a *Cla*1 site were located between chromosomal markers A and B.

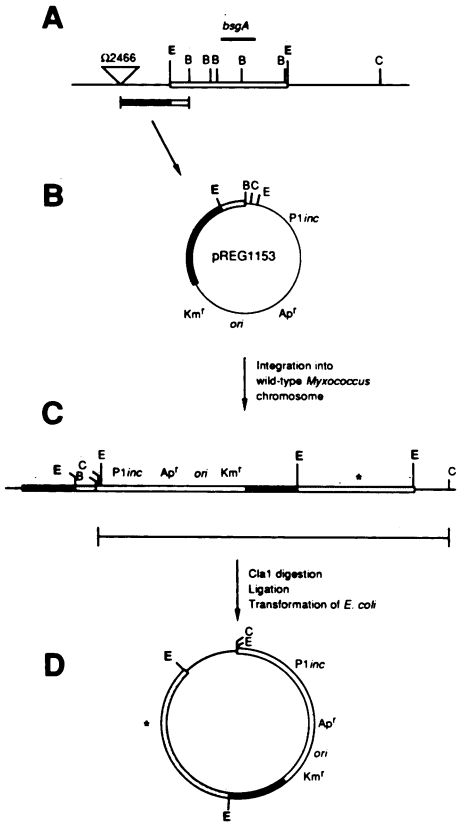

FIG. 5. Cloning mutant *bsg* alleles from strains without Tn*5* ΩDK2466. (A) Partial restriction map of the DNA adjacent to ΩDK2466. The 12-kb *Eco*RI (E) fragment containing the *bsg* locus is indicated (▭). The location of the *Bam*H1 sites (B) within this fragment are shown for reference, as is the *Cla*I site (C) at the end of the pREG1149 clone. The 7.5-kb segment of *M. xanthus* DNA contained in pREG1153 (panel B), extending from the Ω2466 site to the indicated *Bam*HI site, is indicated by the lower bar in panel A. To clone *bsg* mutations (indicated by asterisks in panel C), pREG 1153 was transduced into the *bsg* mutant strain. Integration of the plasmid into the chromosome occurred by recombination between the cloned *M. xanthus* DNA and its chromosomal homologue, resulting in the sequence arrangement shown in panel C. Purified DNA from this strain was cut with *Cla*I, which generated the fragment indicated by the lower line in panel C, ligated to circularize the fragments, and transformed into *E. coli*. The resulting plasmid, which contained the *bsg* mutation, is shown in panel D.

which the *bsg* alleles were to be cloned. Integration of pREG1153 into the chromosome occurs by recombination through homology provided by the 7.5-kb cloned *M. xanthus* DNA. Because the 7.5-kb *M. xanthus* DNA in pREG1153 does not contain the *bsgA* locus, integration of the plasmid does not alter the *bsgA* genotype of the strain and so can be used as a straightforward way to clone *bsgA* mutations (Fig. 5C). To recover the *bsgA* locus from the *M. xanthus* transductants containing pREG1153, chromosomal DNA was purified, cut with *Cla*I, ligated to circularize the fragments, and transformed into *E. coli*. The resulting plasmid, which contains the *bsgA* locus, is shown in Fig. 5D. For use in genetic complementation studies, the *bsg* locus was subcloned on the 12-kb *Eco*RI fragment into the unique *Eco*RI site of pLJS46.

RESULTS

Identification of Bsg mutations genetically linked to Tn*5*ΩDK2466. An insertion of the transposon Tn*5* (designated ΩDK2466) has been isolated near the mutation responsible for the Bsg phenotype in the strain DK454 (J. Kuner, Ph.D. thesis, Stanford University, Stanford, Calif., 1980). However, the relationship between this mutation and other mutations which also result in a Bsg phenotype was not resolved. We have begun a genetic analysis of this phenotypic group by screening additional Bsg mutants from the Hagen et al. collection (7) to identify other strains with mutations linked to Tn*5*ΩDK2466. The results of our transduction analysis demonstrating genetic linkage between

TABLE 2. Identification of *bsg* mutations linked to Tn5ΩDK2466

Recipient strain	% fraction of transductants[a]	% (fraction) cotransduction of *bsg* mutations and Tn5ΩDK2466 with donors[b]				
		M206 (*bsg-435*)	M207 (*bsg-454*)	M203 (*bsg-460*)	M155 (*bsg-468*)	M156 (*bsg-751*)
DK435	26 (39/150)					
DK454	22 (49/225)					
DK460	29 (146/500)					
M101 (Spo^+)	100 (50/50)	26 (26/100)	29 (29/100)	23 (23/100)	<0.05 (0/200)	<0.05 (0/200)
M102 (Spo^+)	100 (50/50)	23 (17/75)	27 (41/150)	25 (19/75)	<0.05 (0/200)	<0.05 (0/200)

[a] Spo^+ transductants obtained with a DK2466 (Spo^+) donor. Mx8 grown on the donor strain DK2466 (Spo^+ Tn5ΩDK2466) was used to transduce kanamycin resistance to the recipient strain. Data are expressed as fractions of total kanamycin-resistant transductants that were also Spo^+. Other Bsg strains (7), DK433, DK440, DK442, DK468, DK473, DK478, DK631, DK751, DK770, DK772, and DK773, were also used as recipients in crosses with DK2466 donor but failed to show cotransduction of Spo^+ with Tn5ΩDK2466 (0 of 200 for each strain).

[b] Mx8 stocks grown on each donor strain carrying Tn5DK2466 and the indicated *bsg* mutation were used to transduce kanamycin resistance to the recipient strain. Data are expressed as fractions of total kanamycin-resistant transductants that were also Spo^-.

Tn5ΩDK2466 and a sporulation defect in three of the mutants having a Bsg phenotype is shown in Table 2. When DK2466, a Spo^+ strain containing Tn5ΩDK2466, was crossed to each of the Bsg^- mutants listed in Table 2, three strains (DK435, DK454, and DK460) gave rise to kanamycin-resistant transductants that were also Spo^+. There was a similar cotransduction frequency between Tn5ΩDK2466 and the sporulation defect in these strains (26, 22, and 29%, respectively), suggesting the possibility that the mutations are clustered. In contrast to these three strains, 16 of the Bsg mutants from the Hagen collection could not be transduced to a kanamycin-resistant, Spo^+ phenotype with a Tn5ΩDK 2466 donor (Table 2).

Introduction of Tn5DK2466-linked Bsg mutations into wild-type strains and characterization of the mutant phenotype. The sporulation defects from DK435, DK454, and DK460 were transduced into Spo^+ strains to determine whether the Bsg phenotype is the consequence of a single mutation or multiple mutations. A Km^r Spo^- transductant of each of these strains (M206, M207, and M203, respectively), derived in the previous experiments, was used as a donor strain to transduce kanamycin resistance into the Spo^+ strains M101 and M102. Individual transductants were screened on CF agar for their developmental phenotype. The sporulation defects in these strains were cotransduced with Tn5ΩDK2466 at a frequency comparable to that of their respective wild-type alleles (Table 2). Representative Spo^- transductants from this experiment were further characterized by mixing experiments and found to retain the essential components of the Bsg phenotype. First, the Spo^- transductants did sporulate when allowed to develop in mixtures with their respective wild-type parent, M101 or M102. Second, sporulation by the Spo^- transductants of M101 was rescued in mixtures with Asg, Csg, or Dsg tester strains but not by any of the Bsg parental strains. Therefore, it appears that both the sporulation defect and the specificity of extracellular complementation may result from a single mutation.

A close examination of the *bsg* transductants of M102 has revealed that these mutations have an additional, previously undescribed, effect on cells growing in the vegetative state. The M102 parent is a Spo^+ fully motile (A^+ S^+) strain that produces swarming colonies on CTT agar. As swarming mutant and wild-type colonies approached one another, the leading edge of the mutant colony stopped advancing and a ridge of cells was formed at the colony edge (Fig. 6). This behavior was the specific consequence of the interaction of a mutant and wild-type colony; it was not observed when either two wild-type colonies or two mutant colonies converged.

The distinctive interaction of a Bsg^- mutant colony with a wild-type colony was a characteristic common to each of the three *bsg* mutations transduced into M102 from the Hagen strains. It was also a characteristic of each of the in vitro-constructed mutants described below. Furthermore, the motility aspects of the phenotype were apparently genetically inseparable from the sporulation defect. All of the Spo^- transductants reported in the last row of Table 2 had this peculiar phenotype, whereas the Km^r Spo^+ transduc-

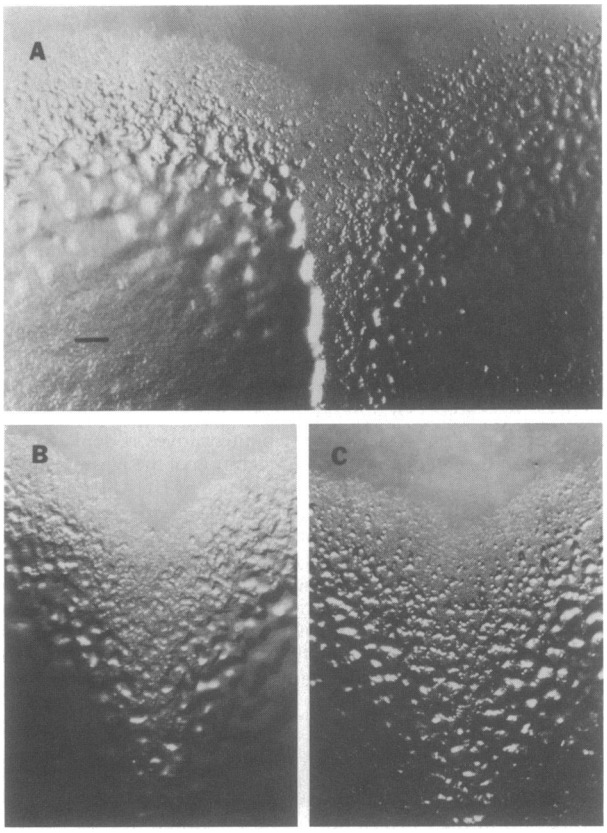

FIG. 6. Characteristics of converging colonies of mutant and wild-type cells. Spots containing 2×10^6 cells of each type to be tested were placed approximately 2 cm apart on the surface of CTT agar plates and incubated at 33°C. Photographs were taken after 2 or 3 days, when the swarming cells from the two spots had converged. (A) Characteristic pattern at the convergence of mutant (left) and wild-type (right) cells. At the leading edge the swarming mutant cells appear to form an abrupt ridge where the two cell types meet. This pattern is not seen when two mutant swarms (B) or two wild-type swarms (C) converge. Bar, 1 mm.

tants all had normal motility (data not shown). In reciprocal crosses in which the Tc^r Spo$^-$ recipient strains (M234, M230, and M237) were transduced with phage grown on M226 (Km^r Spo$^+$, normal motility), the motility and sporulation phenotypes were again inseparable; 22% (17 of 75) of the transductants were Spo$^+$ and had normal motility, and the remaining 78% were all of the parental mutant phenotype. These data argue that a single mutation results in both the developmental and the vegetative phenotypes.

The altered motility associated with these *bsgA* mutations was not observed in the Spo$^-$ transductants of M101. The S-system motility mutation (*sglA1*) in M101 drastically reduces the swarming colonial morphology, which may simply make it impossible to observe the mutant phenotype. Alternatively, the mutant behavior may result from a modification or interaction with the S system of motility itself.

Cloning of the Bsg locus linked to Tn*5*ΩDK2466. The integrating vector pREG429 was used to facilitate the cloning and subsequent genetic analysis of DNA adjacent to the Tn*5*ΩDK2466 insertion site (Fig. 4). The construction and properties of this plasmid are described in Materials and Methods (20, 22). First, plasmid pREG429 was introduced into *M. xanthus* M201. This strain is Spo$^+$ and contains the transposon insertion Tn*5*-132ΩDK2466, which provides homology for the integration of the plasmid into the *M. xanthus* chromosome. Southern analysis of *Eco*RI-*Hin*dIII-cut DNA from individual transductants confirmed that they resulted from the integration of the transduced plasmid into one or the other of the IS*50* elements of the endogenous Tn*5*-132. Of 12 strains that were analyzed, 7 had the plasmid integrated into one of the IS*50* elements, whereas 5 had the plasmid in the other.

DNA prepared from one of the strains having pREG429 integrated in each orientation was used for subsequent cloning experiments. A total of six cloned DNA fragments were obtained, containing *M. xanthus* DNA extending outward in one or the other direction from the Tn*5*ΩDK2466 to an endpoint at a *Cla*I, *Bam*HI, or *Eco*RI site. One of these plasmids, pREG1149, was able to transduce DK435, DK454, and DK460 to a Spo$^+$ phenotype. Subcloning experiments localized the Spo$^+$ allele of pREG1149 to the *M. xanthus*-derived 12.6-kb *Eco*RI fragment (pREG1255) and 1.8-kb *Bam*HI restriction fragment (pREG1258). A map of the *M. xanthus* DNA in pREG1149 and the location of the subcloned *Eco*RI and *Bam*HI fragments are shown in Fig. 7A. These data suggest that these mutations are indeed clustered and on the same side of the Tn*5*ΩDK2466 insertion site.

Although pREG1255 transduced each of the mutants to Spo$^+$ at a high frequency, Km^r Spo$^-$ transductants did arise (Table 3). Similar results were also noted when pREG1255 was transduced into any of the in vitro-constructed *bsg* mutants described below (Table 3). We took advantage of the restriction site heterogeneity between the wild-type *bsg* locus and the *bsg* mutations created by removal of an *Sst*II site (M252, *bsgA301*) or an *Xho*I site (M250, *bsgA299*) to determine the genotype of the Spo$^+$ and Spo$^-$ transductants. Southern blots were prepared from DNA isolated from 25 Spo$^+$ and 25 Spo$^-$ transductants of pREG1255 into M252 and M250 and cut with the appropriate enzymes (*Sst*II and *Xho*I, respectively). Blots were probed with the ^{32}P-labeled 1.8-kb *Bam*HI fragment. All of the Spo$^+$ strains showed heterozygous pattern, whereas the Spo$^-$ strains had only bands corresponding to the mutant parent (data not shown). This indicates that these Spo$^-$ partial diploids had undergone an apparent gene conversion event, resulting in two copies of the mutant allele and loss of the incoming wild-type allele.

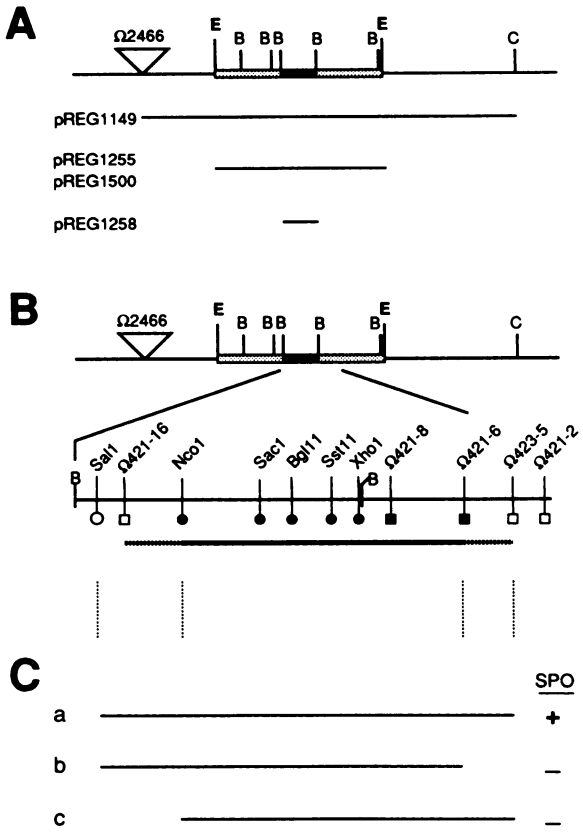

FIG. 7. Mutations which define the limits of the *bsgA* locus. (A) The upper line is a map of the DNA adjacent to Ω2466 site. The plasmid pREG1149 contains the sequences from the Ω2466 site to the *Cla*I site (C). The stippled box indicates the 12-kb *Eco*RI fragment from pREG1255 and pREG1500. The positions of *Bam*HI (B) and *Eco*RI (E) sites are shown only for this fragment. The 1.8-kb *Bam*HI fragment from pREG1258 is indicated by the solid bold line. (B) Expanded representation of the region in which *bsg* mutations were isolated. The location of restriction site mutations (○, ●) and transposon mutations (□, ■) are indicated: (●, ■) Spo- mutations, (○, □) mutations which did not abolish sporulation. The deduced limit of the *bsg* locus is shown. (C) Lines indicate the portion of DNA from the lower portion of panel B which was present in the plasmids: (a) pREG1445, (b) pREG1446, and (c) pREG1447. The ability of these plasmids, when integrated at the Mx8 *att* site, to complement known *bsgA* mutations is indicated on the right.

Whereas pREG1255 transduced each of the mutants to Spo$^+$ at a comparable frequency, the mutants were transduced at substantially different frequencies by the smaller pREG1258 plasmid (Table 3; 0.01, 72, and 47%, respectively). These data suggest that the 1.8-kb fragment of pREG1258 contains only a portion of the wild-type gene and that recombination between the mutation site on the chromosome and the truncated end of the cloned gene is required to produce a wild-type gene after integration. This would lead to a situation in which the position of the mutation within a gene would determine the size of this interval and therefore the frequency of obtaining the recombination event required to result in a Spo$^+$ phenotype. This interpretation is supported by data discussed below, which further define the limits of the *bsg* locus.

Defining the limits of the *bsg* locus. The limits of the *bsg* locus were determined by transposon mutagenesis of the

TABLE 3. Complementation of *bsg* mutants[a]

Recipient strain	Fraction (%) of Km^r transductants which are Spo$^+$ with donor plasmid		
	pREG1255	pREG1258	pREG1500
M102 (Spo$^+$)	50/50 (100)	50/50 (100)	72/72 (100)
M234 (*bsg-435*)	107/124 (86)	2/187 (0.01)	72/72 (100)
M230 (*bsg-454*)	125/158 (79)	60/84 (72)	72/72 (100)
M237 (*bsg-460*)	62/75 (83)	57/121 (47)	72/72 (100)
M485 (*bsg-355*)	66/72 (91)		72/72 (100)
M174 (*bsg-262*)	54/72 (75)		
M251 (*bsg-300*)	59/72 (82)		72/72 (100)
M252 (*bsg-301*)	58/72 (80)		
M250 (*bsg-299*)	64/72 (89)		72/72 (100)

[a] Phage P1 was grown on *E. coli* carrying the designated plasmids and lysates used to transduce the recipient to Km^r (carried by vector portion of each of the plasmids). The data reported are the percentage of Km^r transductants that were able to sporulate. Plasmids pREG1255 and pREG1258 integrate by homologous recombination between the cloned DNA and its chromosomal homolog. Plasmid pREG1500 contains the Mx8*att* site (21); integration takes place at the chromosomal attachment site of bacteriophage Mx8. The extent of the cloned *M. xanthus* DNA in each plasmid is shown in Fig. 7A.

12-kb *Eco*RI fragment in *E. coli* and subsequent introduction of the transposon mutations into Spo$^+$ *M. xanthus* cells for determination of the resulting developmental phenotype. The Km^r derivative of Tn*10*, Tn*10*mini-*kan*, was used in these experiments to circumvent the problem of an unexpectedly strong specificity for insertion at a site near one end of the cloned fragment encountered with Tn*5*. In fact, each of more than 50 independent transpositions of Tn*5* was located at or very near the same site in the cloned DNA. Insertion site specificity was not a problem, however, when the transposon Tn*10*mini-*kan* was used.

Transpositions of Tn*10*mini-*kan* into pREG1394 were isolated in *E. coli* as previously described (25). The location and orientation of each transposon insertion were determined by restriction enzyme mapping as described in Materials and Methods. Individual plasmids with Tn*10*mini-*kan* inserted in the cloned *M. xanthus* DNA were transduced into *M. xanthus* wild-type strain M102 by P1 transduction, with selection for Km^r of the transposon. Strains in which the transduced Tn*10*mini-*kan*-containing DNA had replaced its normal chromosomal counterpart were identified by sensitivity to oxytetracycline (encoded on vector sequences), and their developmental phenotype was determined. These gene replacements are in contrast to the alternative in which integration of the entire plasmid takes place, resulting in the formation of a partial diploid strain.

The location, orientation, and resulting developmental phenotype of pertinent Tn*10*mini-*kan* insertions are shown in Fig. 7B. All other insertions tested either had no effect on developmental phenotype or, in some cases, so severely affected vegetative growth that a developmental phenotype could not be reliably determined (data not shown).

In vitro mutations were introduced at five additional positions in this locus by inactivation of the *Nco*I, *Bgl*II, *Sst*II, and *Xho*I restriction sites and by deletion of a small (approximately 50 base pairs) *Sac*I fragment as described in Materials and Methods. Each of these mutations, when recombined into the *M. xanthus* chromosome by gene replacement, also resulted in a Bsg$^-$ phenotype. A similar mutation at the *Sal*I site shown in Fig. 7B did not affect the developmental phenotype of wild-type cells.

This analysis confirms the existence of a locus in and near the 1.8-kb *Bam*HI fragment that is essential for a Spo$^+$ phenotype. This locus is bounded by Tn*10*mini-*kan* insertions, ΩRG421-2 on one side and ΩRG421-16 on the other. Each of the seven mutations that map to this interval results in both a Spo$^-$ phenotype and the peculiar behavior of vegetatively growing cells.

Genetic complementation analysis. Stellwag et al. (21) reported that plasmids that contain the cloned Mx8 attachment site (*att*) are able to integrate at the Mx8 prophage attachment site on the *M. xanthus* chromosome, rather than integrating by recombination between extensive regions of homology. The use of such plasmid vectors to introduce cloned DNA into *M. xanthus* for genetic complementation analysis has been shown to reduce or abolish gene conversion artifacts that arise during integration of recombinant plasmids by recombination between the cloned and chromosomal homologs (22).

To make use of this strategy, the 12-kb *Eco*RI fragment containing the *bsg*$^+$ locus was subcloned into the unique *Eco*RI site of pLJS46, a derivative of pREG429 which contains the bacteriophage Mx8 *att* site. The resulting plasmid, pREG1500, was transduced into each recipient strain listed in Table 3. Integration of the plasmid at the prophage attachment site was confirmed by Southern analysis of 15 transductants of M102 (data not shown). All 72 of the partial diploid strains resulting from transductions of pREG1500 into each of six Spo$^-$ recipients were Spo$^+$ (Table 3). Each of these transductants also showed a wild-type pattern of motility.

The 12-kb *Eco*RI fragment containing the mutant *bsg* locus from each of the eight *bsg* mutant strains listed in Table 3 was also cloned into pLJS46 (Materials and Methods) (Fig. 7). Transductants arising from the reciprocal crosses in which these *bsg* plasmids were transduced into the wild-type strain M102 were also Spo$^+$ and had a normal motility pattern (data not shown). This indicates that the wild-type allele is dominant over each of the mutations that were tested and that both aspects of the mutant phenotype are complemented.

The minimum requirement for complementation of the mutant phenotype is the fragment contained in the plasmid pREG1445 (line a in Fig. 7C), which contains the DNA extending from the *Sal*I site to the *Hin*dIII site within the transposon Tn*10*mini-*kan*Ω423-5. This fragment contains the entire *bsg* locus identified above and very little flanking DNA. Transduction of this plasmid into each of the mutant strains listed in Table 3 and integration of plasmid into the Mx8 attachment site resulted in strains that were Spo$^+$ and had normal motility. In contrast, the plasmids pREG1446 and pREG1447, containing shorter fragments (Fig. 5C), could not complement any of the *bsgA* mutations tested.

A *trans* complementation test was performed to determine the number of complementation groups contained within this *bsg* locus. Each of the *bsg* plasmids was transduced into the *bsg* recipient strains listed in Table 3. The sporulation and motility properties of five transductants for each of the 56 heteroallelic combinations were determined and found to be uniformly of the mutant phenotype (data not shown). Therefore, each of the eight mutations appears to belong to the same complementation group, which is to be designated *bsgA* (formerly *ssbA* [6]).

Evidence for an additional *bsg* locus unlinked to Tn*5*ΩDK2466. Eleven of the Spo$^-$ mutants that have been assigned to the Bsg phenotypic group (7, 14) could not be transduced to a Km^r Spo$^+$ phenotype by phage grown on DK2466 (Table 2). It is not clear by this criterion alone whether there is another unlinked locus in which mutations

confer a Bsg phenotype or whether these additional Bsg mutant strains also contain secondary sporulation defects such that a wild-type strain cannot be formed in a single transduction event.

We have investigated these possibilities further with respect to two of the strains, DK468 and DK751. Two lines of evidence argue that these strains contain a *bsg* mutation at a site other than *bsgA* and which is unlinked to ΩDK2466. First, phage grown on either DK468::Tn*5*ΩDK2466 (Spo^-) or DK751::Tn*5*ΩDK2466 (Spo^-) did not cotransduce the wild-type strain M102 to a Km^r Spo^- phenotype (Table 2). Second, the *bsgA* locus itself was cloned from DK468 and DK751 (Materials and Methods) (Fig. 5), and the 12-kb *Eco*RI fragment containing the *bsgA* locus was subcloned into pLJS46. To determine whether these cloned *bsgA* loci could complement known *bsgA* mutations, the plasmids were transduced into M237 (Tc^r, *bsgA460*) and M252 (*bsgA301*), with selection for kanamycin resistance encoded by the vector. Each of the resulting Km^r transductants (15 from each transduction) was Spo^+ and had normal motility. Therefore, DK468 and DK751 are apparently wild type at the *bsgA* locus and so contain mutations at one or more additional *bsg* loci which are yet to be identified.

DISCUSSION

In this work we report the identification and cloning of an essential developmental locus in which mutations are responsible for the developmental defect in certain but not all Bsg^- mutants. All of the mutations at this locus belong to a single complementation group and presumably to a single gene, which we designate *bsgA*. The *bsgA* mutants fail to produce fruiting bodies or spores when plated on developmental medium. However, these same mutants transiently regain the ability to sporulate when mixed with wild-type cells or with other mutant cells of the Asg, Csg, or Dsg class. In a previous report (6), we showed that the *bsgA* mutants fail to transcribe normal levels of a number of developmental genes, an observation which has since been corroborated and extended to include additional developmental genes by Kroos and Kaiser (10). Furthermore, we found that under our assay conditions, in spite of a strong inhibitory effect of the mutants on wild-type development, the *bsgA* mutants were rescued for both spore production and expression of developmental genes by extracellular complementation by wild-type cells.

In addition to the developmental aspects of the phenotype conferred by these mutations, our ability to now transduce well-defined *bsgA* mutations into an otherwise wild-type strain has led to the novel observation of an additional component of the mutant phenotype, one which is expressed in vegetatively growing cells. Fully motile strains of *M. xanthus* have a pronounced swarming colonial morphology. We observed that as a swarming *bsgA* mutant and wild-type colony converge, but apparently before the cells are in contact, the leading edge of the mutant colony stops advancing and a prominent ridge of cells is formed at the colony edge. This behavior is the specific consequence of the interaction of a mutant and a wild-type colony; it is not observed when either two wild-type colonies or two mutant colonies converge. Furthermore, to the best of our knowledge, this particular behavior is unique to *bsgA* mutants. We know of no other mutant, including other members of the Bsg extracellular complementation class, which has this phenotype.

We have shown that the fruiting and sporulation defect, the ability to be rescued by wild-type cells, the specificity of that extracellular complementation, and the distinctive motility pattern are all manifestations of single mutations in *bsgA*. It is highly unlikely that this phenotype is due to production of a mutant gene product that either is antagonistic or has a new activity. First, all of the mutations that have been identified in this locus gave rise to the same phenotype. Second, the mutations were recessive to the wild-type allele. In all cases, the cloned wild-type locus corrected all aspects of the mutant phenotype.

The genetic and physiological relationships between *bsgA* mutants and other Bsg mutants have not been totally resolved by the studies reported here. However, with respect to DK468 and DK751, two strains which have often been cited as prototypes of the Bsg mutants (9, 13, 14), it is now clear that they are genetically distinct from the *bsgA* mutants. By transduction and complementation analysis, these strains appear to be $bsgA^+$. The genetic distinction between strains DK468 and DK751 on the one hand and the *bsgA* mutants on the other is also reflected in physiological differences. Whereas the *bsgA* mutants are blocked very early in the developmental program (6, 10), DK465 and DK751 appear, by morphological and biochemical criteria, to be blocked much later (14; Gill, unpublished observations).

ACKNOWLEDGMENT

This investigation was supported by Public Health Service Grant GM-31900 from the National Institute of General Medical Sciences awarded to R.E.G.

LITERATURE CITED

1. **Avery, L., and D. Kaiser.** 1983. In situ transposon replacement and isolation of a spontaneous tandem genetic duplication. Mol. Gen. Genet. **191**:99–109.
2. **Bachi, B., and W. Arber.** 1977. Physical mapping of *Bgl*II, *Bam*H1, *Eco*R1, *Hind*III and *Pst*1 restriction fragments of bacteriophage P1 DNA. Mol. Gen. Genet. **153**:311–324.
3. **Berg, D. E., and C. M. Berg.** 1983. The prokaryotic transposable element Tn*5*. Biotechnology **1**:417–435.
4. **Berg, D. E., C. Egner, B. J. Hirschel, J. Howard, L. Johnsrud, and R. A. Jorgensen.** 1980. Cold Spring Harbor Symp. Quant. Biol. **45**:115–123.
5. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y.
6. **Gill, R., and M. Cull.** 1986. Control of developmental gene expression by cell-to-cell interactions in *Myxococcus xanthus*. J. Bacteriol. **168**:341–347.
7. **Hagen, D. C., A. P. Bretscher, and D. Kaiser.** 1978. Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev. Biol. **64**:284–296.
8. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in non-motile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74**:2938–2942.
9. **Janssen, G., and M. Dworkin.** 1985. Cell-cell interactions in developmental lysis of *Myxcococcus xanthus*. Dev. Biol. **112**: 194–202.
10. **Kroos, L., and D. Kaiser.** 1987. Expression of many developmentally regulated genes in *Myxococcus* depends on a sequence of cell interactions. Genes Dev. **1**:840–854.
11. **Kuner, J., L. Avery, D. E. Berg, and D. Kaiser.** 1981. Uses of tranposon Tn*5* in the genetic analysis of *Myxococcus xanthus*, p. 128–132. *In* E. Schlesinger (ed.), Microbiology—1981. American Society for Microbiology, Washington, D.C.
12. **Kuner, J., and D. Kaiser.** 1981. Introduction of transposon Tn*5* into *Myxococcus* for analysis of developmental and other nonselectable mutants. Proc. Natl. Acad. Sci. USA **78**:425–429.
13. **Kuspa, A., L. Kroos, and D. Kaiser.** 1986. Intercellular signalling is required for developmental gene expression in *Myxococcus xanthus*. Dev. Biol. **117**:267–276.
14. **LaRossa, R., J. Kuner, D. Hagan, C. Manoil, and D. Kaiser.**

1983. Developmental cell interactions of *Myxococcus xanthus*: analysis of mutants. J. Bacteriol. **153**:1394–1404.

14a. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

15. **Martin, S., E. Sodergren, T. Masuda, and D. Kaiser.** 1978. Systematic isolation of transducing phages for *Myxococcus xanthus*. Virology **88**:44–53.

16. **O'Connor, K. A., and D. R. Zusman.** 1983. Coliphage P1 mediated transduction of cloned DNA from *Escherichia coli* to *Myxococcus xanthus*: use for complimentation and recombination analyses. J. Bacteriol. **155**:317–325.

17. **Rosenberg, E.** 1984. Myxobacteria development and cell interactions. Springer-Verlag, New York.

18. **Rosner, J. L.** 1972. Formation induction and curing of bacteriophage P1 lysogens. Virology **48**:679–689.

19. **Shimkets, L. J.** 1987. Control of morphogenesis in myxobacteria. Crit. Rev. Microbiol. **14**:195–227.

20. **Shimkets, L. J., R. E. Gill, and D. Kaiser.** 1983. Developmental cell interactions in *Myxococcus xanthus* and the *spoC* locus. Proc. Natl. Acad. Sci. USA **80**:1406–1410.

21. **Stellwag, E., J. M. Fink, and J. Zissler.** 1985. Physical characterization of the genome of *Myxococcus xanthus* bacteriophage Mx-8. Mol. Gen. Genet. **199**:123–132.

22. **Stephens, K., and D. Kaiser.** 1987. Genetics of gliding motility in *Myxococcus xanthus*: molecular cloning of the *mgl* locus. Mol. Gen. Genet. **207**:256–266.

23. **Sternberg, N., and R. Hoess.** 1983. The molecular genetics of bacteriophage P1. Annu. Rev. Genet. **17**:123–154.

24. **Sternberg, N., D. Tiemeier, and L. Enquist.** 1977. *In vitro* packaging of a λ D*am* vector containing *Eco*R1 DNA fragments of *Escherichia coli* and phage P1. Gene **1**:255–280.

25. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of LacZ operon fusions by transposition. Gene **32**:369–379.