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Stigmatella aurantiaca Fruiting Body Formation Is Dependent on the *fbfA* Gene Encoding a Polypeptide Homologous to Chitin Synthases

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***Stigmatella aurantiaca* is a prokaryotic organism that undergoes a multicellular cycle of development resulting in the formation of a fruiting body. For analyzing this process, mutants defective in fruiting body formation have been induced by transposon mutagenesis using a Tn*5*-derived transposon. About 800 bp upstream of the transposon insertion of mutant AP182 which inactivates a gene (*fbfB*) involved in fruiting, a further gene (*fbfA*) needed for fruiting body formation was detected. Inactivation of *fbfA* leads to mutants which form only non-structured clumps instead of the wild-type fruiting body. The mutant phenotype of *fbfA* mutants can be partially suppressed by mixing the mutant cells with cells of some independent mutants defective in fruiting body formation. The *fbfA* gene is transcribed after 8 h of development as determined by measuring the induction of β-galactosidase activity of a *fbfA-Δtrp-lacZ* fusion gene and by Northern (RNA) analysis using an insertion encoding a stable mRNA. The predicted polypeptide FbfA shows a homology of about 30% to NodC of rhizobia, an *N*-acetylglucosamine-transferase which is involved in the synthesis of the sugar backbone of lipo-oligosaccharides. These induce the formation of the root nodules in the *Papilionaceae*. Besides the predicted molecular mass of 45.5 kDa, the hydropathy profile reveals a structural relationship to the NodC polypeptide.**

Multicellular morphogenesis is a feature of many eukaryotes and of a group of social prokaryotes, the myxobacteria. They are gram-negative soil bacteria growing on insoluble organic substrates such as decaying wood or leaves. Myxobacteria interact with each other, forming swarms. The cells move by gliding and secrete slime containing lytic enzymes that degrade biopolymers. Upon starvation, cells glide into aggregation centers from which arise the fruiting bodies, structures with a defined species-specific morphology and diverse complexity, harboring spores (10, 44). As in eukaryotic multicellular morphogenesis, direct cell-cell interaction and communication as well as positional signalling are predicted to play an important role in fruiting body formation of myxobacteria (8, 21, 23). *Stigmatella aurantiaca* belongs to the order of the myxobacteria. The fruiting body of *S. aurantiaca* is differentiated into a stalk and several delicate pedicels bearing a sporangiole at the top which contain between 10^4 and 10^5 myxospores. The morphological changes during development occur in a defined, temporal sequence, and in *S. aurantiaca*, the whole process takes about 24 h. Thus *S. aurantiaca* provides a simple and well-suited model system to study the development of morphological structures. Sporulation of vegetative cells may be induced independently from fruiting body formation by using indole and some of its derivatives (12).

To detect genes involved in the morphogenesis of the *S. aurantiaca* fruiting body, transposon mutants defective in fruiting body formation have been induced with the promotor probe transposon Tn*5lacZ* (36). The *fbfA* gene which has been shown to be essential for fruiting body formation is the topic of this report. It is localized about 150 bp upstream of the 5′ end of the *fbfB* gene which was also detected by transposon insertion and was shown to be involved in fruiting (44a).

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MATERIALS AND METHODS

Bacterial strains, phage, and growth conditions. Bacterial strains, phage, and plasmids are described in Table 1. *S. aurantiaca* DW4/3-1 and its derivatives (38) were grown at 32°C in Casitone medium (1% Casitone [Difco], 0.15% $MgSO_4$ $7H_2O$, pH 7.0) supplemented with streptomycin sulfate (120 μg/ml) and when necessary with kanamycin sulfate (50 μg/ml). To obtain colonies from single cells of *S. aurantiaca*, Trypticase peptone medium was used (0.025% Trypticase peptone [Becton Dickinson], 0.05% $MgSO_4$ $7H_2O$, 0.05% $CaCl_2$ $2H_2O$, pH 7.2). To initiate development, cells were plated onto starvation agar (containing only 0.1% $CaCl_2$) (36). *Escherichia coli* strains were grown in Luria broth at 37°C supplemented when necessary with ampicillin (50 μg/ml), chloramphenicol (34 μg/ml), nalidixic acid (50 μg/ml), kanamycin sulfate (50 μg/ml), and tetracycline base (10 μg/ml). All media were solidified with 1.5%, or in the case of soft agar with 0.75%, agar (Difco).

Transfer of conjugable plasmids from *E. coli* to *S. aurantiaca* (13). Exponentially growing cells of *S. aurantiaca* (5×10^8 cells) were mixed with 5×10^8 exponentially growing cells of *E. coli* and collected on a membrane filter (0.45-μm pore size, 25-mm diameter; Schleicher & Schuell, Dassel, Germany). After washing of the cells twice with 5 ml of Casitone medium, the filter was placed on a Casitone plate and incubated overnight at 32°C. Cells were scraped off the filter and suspended in 5 ml of Casitone medium. Portions were plated in soft agar onto Trypticase peptone plates containing antibiotics for selection and incubated for 7 days at 32°C. Resistant clones were transferred into 3 ml of Casitone medium and further incubated to obtain the required cell density.

Transfer of conjugable plasmids from *E. coli* to *E. coli* (29). A total of 5×10^8 exponentially growing donor and recipient cells were mixed and collected on a membrane filter (0.45-μm pore size, 25-mm diameter; Schleicher & Schuell). The filter was placed on a Luria broth plate and incubated for 3 h at 37°C. Cells were scraped off the filter and suspended in 3 ml of Luria broth. Portions were plated on Luria broth plates containing the antibiotics for selection and incubated overnight at 37°C.

Fruiting body formation assay (36). In order to test fruiting body formation, exponentially growing *S. aurantiaca* cells were sedimented, washed in HEPES buffer (100 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 10 mM $CaCl_2$, pH 7.2), sedimented again, and resuspended in HEPES buffer to a concentration of 4×10^{10} cells per ml. Portions of 5 μl were spotted onto starvation agar and incubated at 32°C for 24 h. For the phenotypic complemen-

TABLE 1. Bacterial strains, phage, and plasmids

Strain, phage, or plasmid	Description	Reference or source
E. coli strains		
C600 Nal^r	e14$^-$ (McrA$^-$) *supE44 thi-1 thr-1 leuB6 lacY1 tonA21* Nal^r	Stratagene
JM101	*E. coli* K-12 *supE thi-1* Δ(*lac-proAB*) (F′ *traD36 proAB lacI^{q}ZΔM15*)	53
S17-1	*E. coli* K-12 *thi pro hsdR* mutant *hsdM$^+$ recA* (RP4 Sm^r Tp^r)	46
S. aurantiaca strains		
AP182	DW4/3-1, *fbfB*::Tn*5lacZ* (Km^r Sm^r)	36
AP191	DW4/3-1, *fbf*::Tn*5lacZ* (Km^r Sm^r)	36
BS14	DW4/3-1, *fbfA*::*neo* (Km^r Sm^r)	This work
BS20	DW4/3-1, *fbfA*::(*neo pufBA*) (Km^r Sm^r)	This work
BS23	DW4/3-1, *fbfA*::(*fbfA*::{Δ*trp-lacZ-neo*}) (merodiploid for *fbfA*) (Km^r Sm^r)	This work
DW4/3-1	Wild type (Sm^r)	37
Phage		
λ EMBL3		Stratagene
Plasmids		
pBS SK(−)	Amp^r	Stratagene
pUC18	Amp^r	53
pUC4KIXX	Amp^r Km^r	Pharmacia
pSUP102	Cm^r Tet^r	45
pSZ182	pSUP102 containing a 20-kbp *Sal*I fragment from AP182 harboring Tn*5lacZ* (Cm^r Km^r)	This work
pSK24	3.6-kbp *Eco*RI-*Sma*I fragment containing *fbfA* in pBS SK(−) (Amp^r)	This work
pSK3	5.2-kbp *Eco*RI-*Sal*I fragment containing *fbfA* in pBS SK(−) (Amp^r)	This work
pBS1	12-kbp *Sal*I fragment containing *fbfA* in pSUP102 (Cm^r)	This work
pBS2	*fbfA*::*neo* in pBS SK(−) (Amp^r Km^r)	This work
pBS3	pSUP102 harboring a 13.5-kbp *Sal*I fragment containing *fbfA*::*neo* (Cm^r Km^r)	This work
pBS7	*fbfA*::(*pufBA neo*) in pBS SK(−) (Amp^r Km^r)	This work
pBS8	pSUP102 harboring a 14-kbp *Sal*I fragment containing *fbfA*::(*pufBA neo*) (Cm^r Km^r)	This work
pBS9	*fbfA*::(Δ*trp-lacZ-neo*) in pBS SK(−) (Amp^r Km^r)	This work
pBS10	pSUP102 harboring a 17-kbp *Sal*I fragment containing *fbfA*::(Δ*trp-lacZ-neo*) (Cm^r Km^r)	This work
Mini-Tn*5lacZ1*	Amp^r Km^r	7

tation assay, an equal number of cells of two different mutants were mixed for fruiting body formation.

β-Galactosidase assay. Fruiting bodies scraped off agar plates or vegetative cells were suspended in a buffer containing 50 mM 3-*N*-morpholino-propanesulfonic acid (MOPS) at pH 7.5, 10 mM $MgCl_2$, 10 mM dithiothreitol, and 1 mM phenylmethanesulfonyl fluoride and sonicated (Branson Sonifier, Cell Disrupter B15) with glass beads (diameter, 0.1 mm) at 4°C for 1 min in an Eppendorf tube with a cup horn (Branson EDP 101-151-003). Samples were centrifuged at 15,000 × *g* at 4°C for 10 min to remove cell debris. The supernatant was assayed for β-galactosidase activity with the substrate 4-methylumbelliferyl-β-D-galactopyranoside (4-MUG) (Sigma) (41). A total of 0.1 ml of the supernatant was mixed with 0.3 ml of 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% bovine serum albumin, and 10 μg of 4-MUG and incubated for 30 min at 37°C. The reaction was stopped with 3 ml of 0.1 M glycine buffer (pH 10.3). The fluorescence intensity was measured with a Shimadzu RF-5000 fluorescence spectrophotometer with a wavelength of 360 nm for excitation and 450 nm for emission.

DNA manipulations and sequencing. Plaque lifts, colony hybridization, restriction analysis, and plasmid subcloning were performed according to standard protocols (42). Chromosomal DNA from *S. aurantiaca* was prepared as described elsewhere (34). The sequence for both strands of the 3.6-kbp genomic *Sma*I-*Eco*RI fragment containing *fbfA* was determined as follows. Exonuclease III-generated directed deletions (17) were constructed from pSK24. Single-stranded DNA of subclones was sequenced at 70°C with *Taq* polymerase (TaqTrack; Promega). The sequence of the second strand was obtained by sequencing double-stranded DNA of pSK3 with Sequenase version 2.0 (U.S. Biochemical) by using synthetic oligonucleotide primers derived from the first DNA strand.

PCR. PCR was carried out with the Vent DNA polymerase (New England Biolabs). Amplification was performed at a final concentration of 0.01 pM template, 100 pM each primer, 150 μM each deoxynucleoside triphosphate, and 2 U of Vent DNA polymerase in a total volume of 100 μl. The reaction mixture was overlaid with 100 μl of mineral oil. The conditions for the amplification with the Trio-Thermoblock (Biometra) were as follows: the initial denaturation step was at 94°C for 3 min, annealing was at 65°C for 1 min, extension was at 72°C for 2 min, subsequent denaturation was at 94°C for 1 min, and there were 30 cycles. The PCR products were purified with the Geneclean Kit (Bio 101).

Southern hybridization. Southern blot analysis was performed according to a standard protocol (42). Prehybridization was carried out for 2 h at 60°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), and 50 μg of denatured herring sperm DNA per ml. Hybridization was performed overnight at 60°C after addition of the ^{32}P-labelled DNA probe. DNA probes were labelled by using random oligonucleotide primers (Boehringer, Mannheim, Germany). Filters were washed twice for 30 min at 60°C in 0.1× SSC–0.1% SDS.

RNA isolation and Northern (RNA) hybridization. *S. aurantiaca* RNA from vegetative cells and developing cells was isolated as described previously (5). RNA was subjected to electrophoresis on 1% agarose-formaldehyde gels. Gels were prepared as follows: a suspension of 1 g of agarose in 72 ml of diethylpyrocarbonate-treated H_2O was melted. After cooling to about 65°C, 10 ml of 10× MOPS running buffer (200 mM MOPS, 10 mM EDTA, 50 mM NaAc, 100 mM NaOH) and 18 ml of formaldehyde (37%) were added. The RNA to be electrophoresed was dried and suspended in 9 μl of sample buffer (10% 10× MOPS running buffer, 50% formamide, 18% formaldehyde). After heating to 65°C for 10 min, 1 μl of stop buffer (50% glycerol, 6 mM EDTA, 0.05% bromophenol blue) was added. Electrophoresis was performed at 70 V for 4 h. The RNA was transferred to a nylon membrane (Biodyne B; Pall) with a vacuum blotter (Appligene). After UV cross-linking using the Stratalinker (Stratagene), prehybridization of the filters was at 42°C for 5 h in 50% formamide–4× Denhardt's solution–0.1% glycine–5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–0.1% SDS–0.25 mg of denatured herring sperm DNA per ml. Hybridization was carried out overnight at 42°C in 55% formamide–1.1% Denhardt's solution–5.5× SSPE–0.1% SDS–10% dextran sulfate–4 mM sodium diphosphate–0.1 mg of denatured herring sperm DNA per ml containing the ^{32}P-labelled DNA probe. Filters were washed at room temperature twice for 15 min in 2× SSPE–0.1% SDS and at 37°C twice for 15 min in 0.1× SSPE–0.1% SDS.

Construction of *S. aurantiaca* insertional mutants. (i) Mutant BS14: introduction of a kanamycin resistance cassette into the *fbfA* gene. For introducing an *Eco*RI site into the *fbfA* gene, the gene was amplified in two parts by PCR: the oligonucleotides B1 (GGCCGGATCCATGGAGCTATTTCCCATTCA) and PCRBS1 (GGCCGAATTCCGCTGTCAGACACCCCGACA) were used for amplification of the first 612 bp, generating the gene fragment *fbfA1*. The oligonucleotides PCRBS2 (GGCCGAATTCTACCGCCGCCACGTGCTGGA) and HE1 (GGCCCTTAAGAAGCTTTCATGTGGAGAGGCCCTCCG) were used for the amplification of the remaining 632 bp, generating fragment *fbfA2. fbfA1* was digested with *Bam*HI and *Eco*RI and inserted into the *Bam*HI-*Eco*RI sites of pBS SK$^-$, resulting in plasmid pBS/*fbfA1*. Fragment *fbfA2* was digested with *Eco*RI and *Hin*dIII and inserted into the corresponding restriction sites of pBS/*fbfA1*. The kanamycin resistance gene, *neo*, of pUC4KIXX (2) was inserted into the *Eco*RI site of the resulting recombinant *fbfA* gene of the above plasmid, generating plasmid pBS2. The *recA$^+$ E. coli* strain JM101(pBS1), which harbored

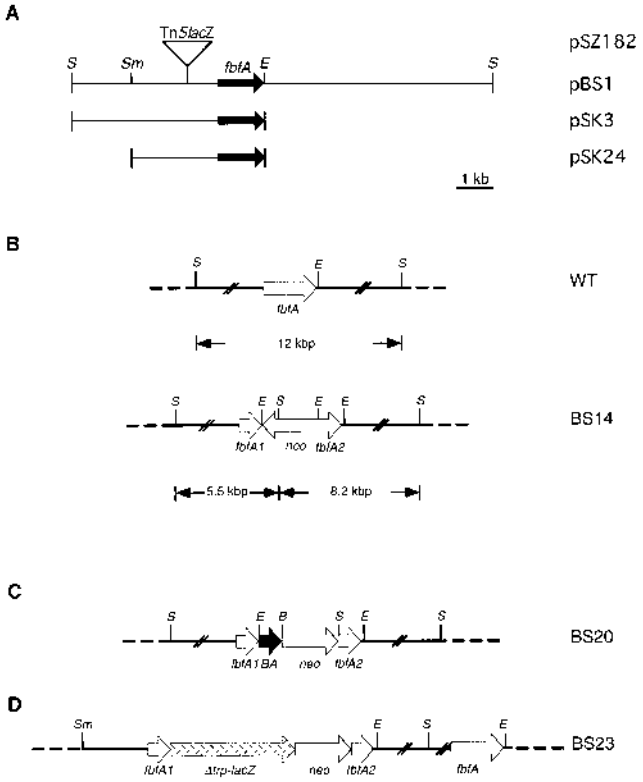

FIG. 1. (A) Localization of the transposon Tn*5lacZ* and of the *fbfA* gene on a *Sal*I restriction fragment derived from strain AP182 and of *fbfA* on a *Sal*I-*Eco*RI and *Sma*I-*Eco*RI restriction fragment derived from the wild-type strain DW4/3-1. (B) Depiction of the structure of the mutant *fbfA* gene of strain BS14 which is disrupted by the Tn*5*-derived *neo* gene. (C) Schematic depiction of the *fbfA* gene of strain BS20, into which a cassette composed of the *Rhodobacter pufBA* gene and the *neo* gene of Tn*5* was inserted. (D) Schematic depiction of the *fbfA* gene region of the merodiploid strain BS23. A cassette composed of the *trp-lacZ* cassette isolated from a mini-Tn*5lacZ* (7) and the *neo* gene of Tn*5* was inserted into one of the *fbfA* genes. Restriction endonuclease sites are as follows: B, *Bam*HI; E, *Eco*RI; S, *Sal*I; Sm, *Sma*I.

the conjugable plasmid pBS1, was transformed with pBS2. For the construction of pBS1, a 12-kbp *Sal*I fragment containing the *fbfA* gene was isolated from a λ EMBL3 gene library of *Sal*I-digested chromosomal DNA of the *S. aurantiaca* wild-type strain DW4/3-1 and inserted into the *Sal*I site of pSUP102. For the generation of a recombinant plasmid in which the wild-type *fbfA* gene was replaced by the modified *fbfA* gene of pBS2, the transfected cells were grown overnight in liquid culture containing kanamycin sulfate and chloramphenicol. In order to isolate the recombinant plasmid, which should confer resistance to kanamycin and chloramphenicol, plasmids isolated from the cells of the above overnight culture were used to transfect *E. coli* S17-1. The S17-1 transformants which were resistant to chloramphenicol and kanamycin were crossed by conjugation with *E. coli* C600 Nal^r. After selection for the resistance to chloramphenicol, kanamycin, and nalidixic acid, a C600 derivative harboring the pSUP102 construct pBS3 containing the inactivated *fbfA* gene and conferring resistance to both chloramphenicol and kanamycin was obtained. Strain S17-1 was then transfected with pBS3, and kanamycin-resistant *S. aurantiaca* transconjugants were obtained with a frequency of 2.5×10^{-5}. Six percent of the kanamycin-resistant clones were generated by replacing the wild-type gene with the *fbfA* gene inactivated by the insertion of the Tn*5*-derived *neo* gene, whereas the remaining clones were merodiploid strains containing the inactivated gene in addition to the wild-type *fbfA* gene.

(ii) Mutant BS20: insertion of the *Rhodobacter capsulatus* fragment *pufBA* into the *fbfA* gene. Adapter BS5/6 with the restriction sites *Eco*RI, *Sal*I, *Mlu*I, and *Hin*dIII was cloned into the *Eco*RI-*Hin*dIII sites of pUC18. A *Bss*HII-*Hin*dIII fragment (590 bp) encoding *fbfA2* was inserted into the *Mlu*I and *Hin*dIII sites of this modified plasmid. From this plasmid, the *fbfA2* fragment was isolated after digestion with *Eco*RI and *Hin*dIII and inserted into the *Eco*RI and *Hin*dIII sites of plasmid pBS*fbfA1* from which the *Sal*I site had been removed previously. Next, an *Eco*RI-*Sal*I restriction fragment encoding the *R. capsulatus pufBA* gene which had been fused to the *neo* gene upstream of the promoter was inserted into the *Eco*RI and *Sal*I sites of the plasmid constructed above, thus generating plasmid pBS7. For the replacement of the wild-type *fbfA* localized on pBS1 with the modified *fbfA* gene, pBS7 was introduced into JM101(pBS1). Transformation of S17-1 with the plasmids isolated from the strain obtained by electroporation and transfer of the plasmid(s) from S17-1 into C600 Nal^r with subsequent selection for the resistance to nalidixic acid, chloramphenicol, and kanamycin resulted in a derivative of C600 Nal^r harboring plasmid pBS8, in which wild-type *fbfA* was replaced with the modified gene. Transformation of S17-1 with pBS8 and transfer of the plasmid into the *S. aurantiaca* wild-type strain resulted in kanamycin-resistant transconjugants with a frequency of 4×10^{-5}. In BS20, the wild-type *fbfA* gene was replaced with the modified *fbfA* by a double crossover.

(iii) The merodiploid strain mutant BS23: construction of the Δ*trp-lacZ-fbfA* fusion gene. An *Eco*RI-*Hin*dIII 3.3-kbp promoterless Δ*trp-lacZ* fusion gene isolated from the minitransposon Tn*5lacZI* (7) was cloned into the *Eco*RI-*Hin*dIII sites of pBS*fbfA1* (see above). Next, a 2.1-kbp fragment from pBS7 containing a fusion of the *neo* gene from pUC4KIXX and the 590 bp of *fbfA2* was cloned into the *Hin*dIII site of this plasmid, giving plasmid pBS9. This plasmid was then transformed into *E. coli* JM101(pBS1) harboring plasmid pBS1. For the isolation of the recombinant plasmid pBS10, in which the wild-type *fbfA* gene of pBS1 was replaced with the fusion gene *fbfA1*-Δ*trp-lacZ-neo-fbfA2*, the plasmid(s) was first isolated from the derivative of JM101(pBS1) and transferred to S17-1. The transformed S17-1 derivative was crossed by conjugation with strain C600 Nal^r. pBS10 was isolated from the resulting transconjugants which were selected for resistance to kanamycin, chloramphenicol, and nalidixic acid and transferred to S17-1. The strain harboring pBS10 was crossed with the *S. aurantiaca* wild type. Kanamycin-resistant transconjugants were obtained with a frequency of 5×10^{-5}. One of the transconjugants, BS23, was used in this study. It is a merodiploid strain which contains the wild-type *fbfA* and the *fbfA* fusion gene.

Nucleotide sequence accession number. The nucleotide sequence of *fbfA* is available in the GenBank database under accession number Z11601.

RESULTS AND DISCUSSION

For the isolation of nonselectable developmental mutants of *S. aurantiaca*, transposon insertion mutagenesis using Tn*5lacZ*

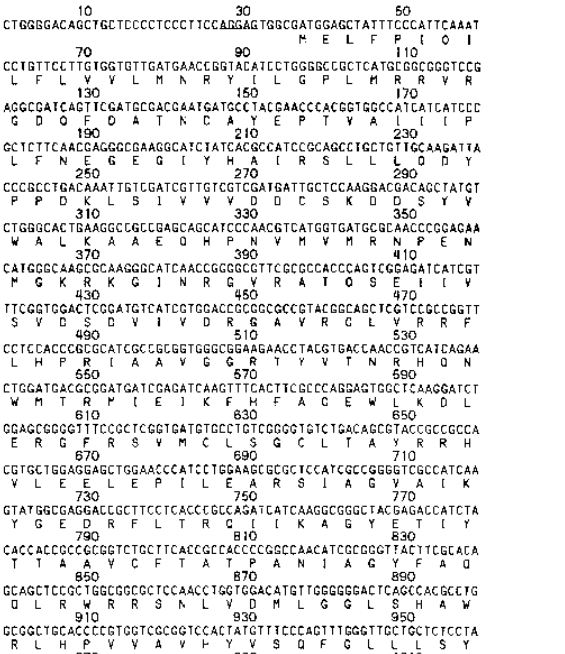

FIG. 2. Nucleotide sequence of *fbfA*. The derived amino acid sequence for FbfA is shown in single-letter code. The putative ribosome-binding site is underlined.

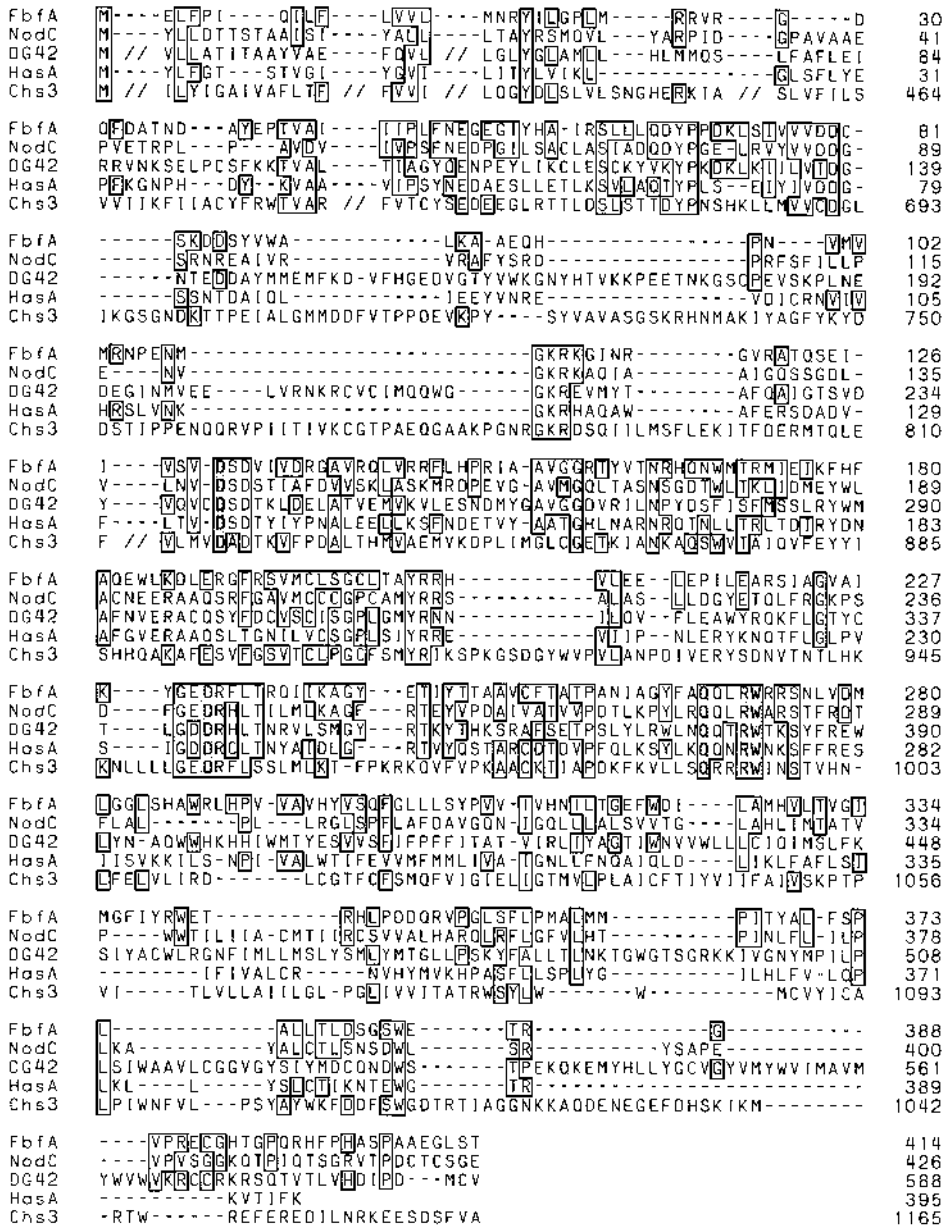

FIG. 3. Alignment of the putative FbfA polypeptide from *S. aurantiaca* with NodC from *R. meliloti*, DG42 from *X. laevis*, HasA from *S. pyogenes*, and Chs3 from *S. cerevisiae*. The amino acids in boldface correspond to Asp-441, Asp-562, Gln-601, Arg-604, and Trp-605 of *S. cerevisiae* Chs2p (33). It is suggested that these amino acids are located in the active site and form the catalytic center of the enzyme. Amino acid residues are numbered as indicated. Identical amino acid residues are boxed. Parts of the polypeptide sequences which have been omitted are indicated by double slashes.

was performed (36). The insertion in one of the mutants defective in fruiting body formation (AP182) was localized on a 862.5-kbp *Spe*I and a 676.8-kbp *Ase*I fragment of the *S. aurantiaca* chromosome (35). The region of insertion was isolated on a 20-kbp *Sal*I fragment of a phage clone, which was detected in a λ EMBL3 library of *Sal*I-restricted chromosomal DNA of the mutant by using the kanamycin resistance gene of pUC4KIXX (3) (derived from Tn*5*) as a probe. The fragment was cloned into the conjugable plasmid pSUP102 (45), resulting in plasmid pSZ182.

The position and orientation of the transposon on the 20-kbp *Sal*I fragment were determined by restriction analysis and sequencing starting with the insertion sequence (IS) elements of the transposon (Fig. 1A). The corresponding 12-kbp *Sal*I wild-type restriction fragment was isolated from a λ EMBL3 gene library of *Sal*I-digested chromosomal DNA of the wild-type strain DW4-3/1 and cloned into the *Sal*I site of the mobilizable plasmid pSUP102, resulting in plasmid pBS1.

For sequence analysis, two subclones of the original 12-kbp *Sal*I genomic fragment were constructed. One of them, pSK24, contains a 3.6-kbp *Eco*RI-*Sma*I restriction fragment inserted into pBS SK(−) (Fig. 1A). The 3.6-kbp *Eco*RI-*Sma*I fragment, containing the insertion site of the transposon, was sequenced. A total of 804 bp downstream of the transposon integration site, a gene encoding an open reading frame of 1,242 nucleotides was found (Fig. 1A). This gene, named *fbfA*, encodes a putative polypeptide with a molecular mass of 45.5 kDa (Fig. 2) which we suggest is involved in fruiting body formation. The predicted polypeptide has strong homology to the NodC polypeptide of *Rhizobium meliloti* (48), the hyaluronan synthase of *Streptococcus pyogenes* (6), the developmental protein from *Xenopus laevis* DG42 (39), and the chitin synthase 3 of the yeast *Saccharomyces cerevisiae* (50) (Fig. 3). In the case of the rhizobial NodC polypeptide, the homology is about 30% for perfect matches and spans the whole protein. If similar amino acids are included, the homology reaches about 60%. The distribution of hydrophobicity along the peptide backbone is also strongly conserved between FbfA and NodC (data not shown). NodC is an integral outer membrane protein (18, 20) with a receptor-like domain structure (19). Expression of *nodC* is induced by root exudates (14, 30, 51), and a mutation within the *nodC* gene abolishes both the chemotactic responses to luteolin (1) and formation of root nodules during nodulation (4, 40). Nodulation is a symbiotic bacterium-plant interaction resulting in the formation of nodules on the roots of the plant. Nodules are specialized organs whose function is nitrogen fixation carried out by the endosymbiotic rhizobia. It has been shown recently that NodC is an *N*-acetylglucosaminyltransferase which catalyzes the synthesis of the tetra- or pentasaccharide backbone of the nodulation factors (11, 22, 47). Nodulation and the formation of fruiting bodies have a number of common features. Both are complex multicellular processes requiring coordinated developmental events such as cell-cell communication and exchanging of signals as well as direct cell-cell contact and result in morphological changes (10).

To prove that *fbfA* is involved in fruiting body formation, the gene had to be inactivated. For this purpose, the derivative pBS3 of the conjugable plasmid pBS1 was constructed by replacing the wild-type *fbfA* gene with an in vitro-modified gene which had been disrupted by the insertion of a kanamycin resistance cassette (see Materials and Methods). pBS3 was transferred into *E. coli* S17-1 and subsequently conjugated into wild-type *S. aurantiaca*, giving a strain in which the *fbfA* gene was replaced with the modified gene. Southern blot analysis showed that the strain (BS14) carried only the modified *fbfA* gene (Fig. 1B and 4). Whereas wild-type cells form well-defined fruiting bodies during starvation (Fig. 5a), mutant BS14 cells form only nonstructured aggregates (Fig. 5b). As no open reading frame is detected up to 1,000 bp downstream of *fbfA* (data not shown), we suggest that the loss of FbfA function, and not polar effects of the insertion on genes downstream of *fbfA*, leads to the mutant phenotype. Fruiting body formation of mutant BS14 can be partially restored by mixing the mutant cells with cells of certain other mutant strains such as AP191, which are unable to form aggregates during starvation (Fig. 5c). Mixing of the cells of both mutants and subsequent starvation lead to the formation of a structure composed of a stem and a head (Fig. 5d) which encloses the myxospores (data not shown). This structure is also detected as an intermediate form during fruiting body formation of the wild-type *S. aurantiaca* (see Fig. 4c in the work of White [52]).

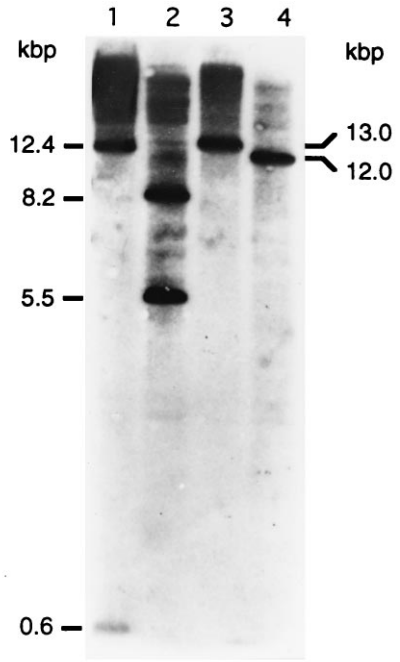

FIG. 4. Southern analysis of chromosomal DNA of BS14 and *S. aurantiaca* wild-type DW4/3-1, using the *fbfA* gene as a probe. Lane 1, BS14 chromosomal DNA restricted by *Eco*RI. A 12.4- and a 0.6-kbp fragment were detected. Lane 2, BS14 chromosomal DNA restricted by *Sal*I. Insertion of the gene for the neomycinphosphotransferase leads to an additional *Sal*I site. Lane 3, DW4/3-1 chromosomal DNA restricted by *Eco*RI. A 13-kbp fragment was labelled. Lane 4, DW4/3-1 chromosomal DNA restricted by *Sal*I. A 12-kbp fragment was labelled. The size of the fragments was estimated by using *Hin*dIII-restricted λ DNA as a reference.

Phenotypic complementation is also possible for various *Rhizobia* mutants. *nod* mutants as well as *exo* mutants, which are defective in the production of exopolysaccharides, do not induce nodulation. However, nodulation is restored when a *nod*/*exo*$^+$ strain is mixed with a *nod*$^+$/*exo* strain (15). We do not know whether the *S. aurantiaca* mutants used in the complementation experiments are defective in the production of exopolysaccharides; however, fruiting bodies do contain large amounts of polysaccharides, although the function of these molecules is unknown (9).

In order to determine the timing of *fbfA* transcription during development, we performed a Northern analysis; however, no hybridizing transcript could be detected with labelled *fbfA* DNA or the corresponding antisense RNA (data not shown). This might be due to a very low transcription rate and/or the

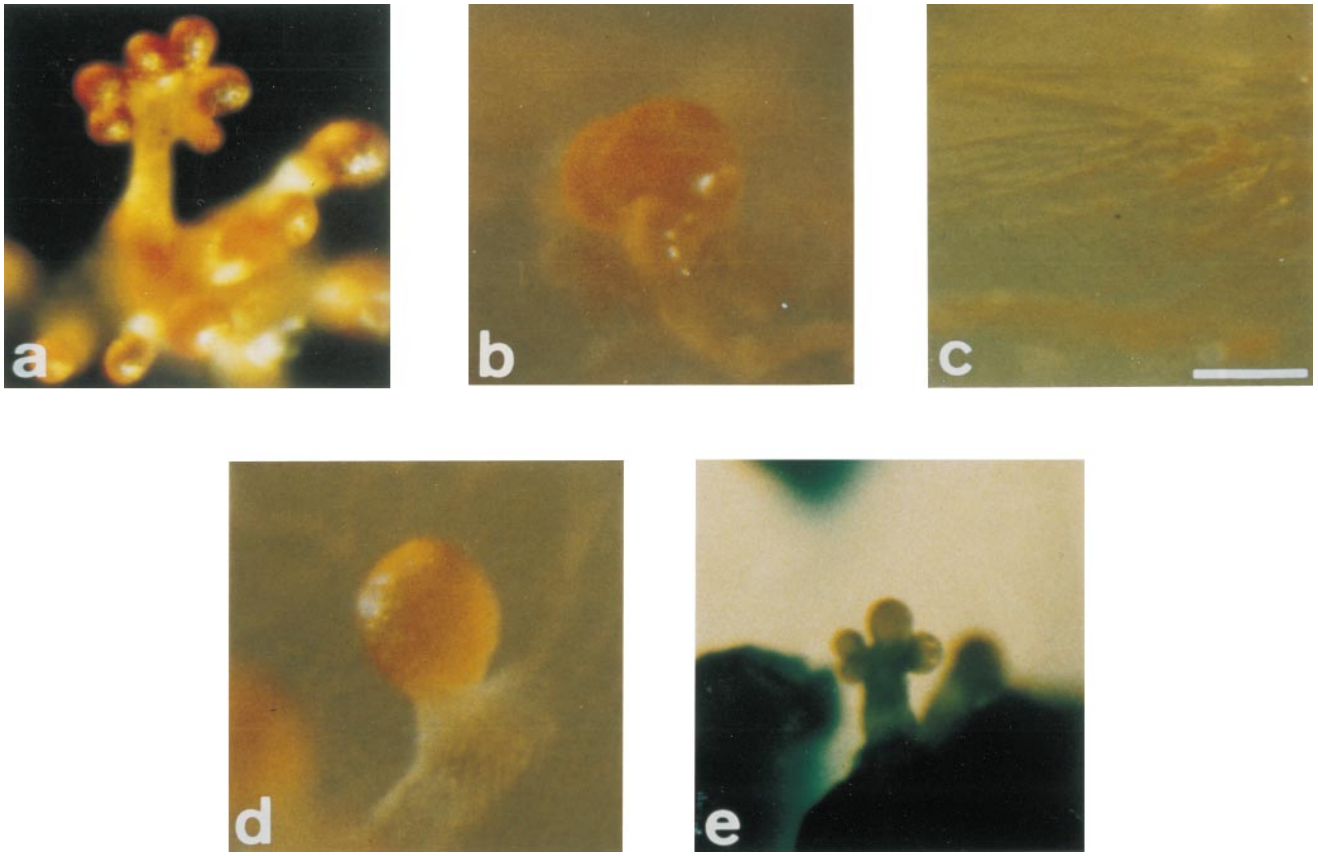

FIG. 5. Fruiting body morphology of various derivatives of *S. aurantiaca*. (a) Fruiting body of the *S. aurantiaca* wild-type DW4/3-1 is differentiated into a stalk, branches, and sporangioles. (b) Mutant BS14, whose *fbfA* gene is disrupted by the *neo* gene, forms only clumps. (c) The Tn*5lacZ* insertional mutant AP191 shows no cell aggregation. (d) Mixing of the cells of the mutant strains BS14 and AP191 leads to a structure composed of a stem and a head. (e) Starvation of cells of the merodiploid strain BS23, harboring a functional *fbfA* gene and a *fbfA*-Δ*trp-lacZ* hybrid gene, on water agar containing X-Gal resulted in fruiting bodies which are stained blue after about 70 h. Bar, about 40 μm for panels a, b, c, and d and about 80 μm for panel e.

instability of the *fbfA* transcript. To further analyze the time at which *fbfA* transcription starts during development, the wild-type gene was replaced with a hybrid *fbfA* gene (strain BS20 [Fig. 1C]) into which the *pufBA* 567-bp fragment of the *puf* operon of *R. capsulatus* (25) and a *neo* cassette were inserted (see Materials and Methods). This *Rhodobacter* DNA fragment encodes an RNA which has no RNase E sites and is highly resistant to 3′ exonucleolytic attack (26), and strain BS20 has the same phenotype as mutant BS14. Northern blot analysis using the 567-bp *Rhodobacter* DNA fragment as a probe revealed that the transcription of the hybrid *fbfA* gene in strain BS20 starts 8 h after the beginning of starvation (Fig. 6). Whereas no traces of this stable mRNA were detected in vegetative cells, the transcript of this *Rhodobacter* fragment was observed even 64 h after the beginning of starvation.

For studying the progression of *fbfA* gene expression during fruiting body formation or indole-induced sporulation (12), the merodiploid strain BS23 was constructed; it contained the wild-type *fbfA* gene and a hybrid *fbfA* gene, into which a Δ*trp-lacZ* fusion (7) and the *neo* cassette (2) for transconjugant selection were inserted (Fig. 1D). Starvation of BS23 cells on water agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) resulted in blue fruiting bodies (Fig. 5e). For the determination of β-galactosidase activity during fruiting body formation, cells were scraped off the agar dish and broken by sonication. As the β-galactosidase activity of mutant BS23 specimens was relatively low, β-galactosidase activity was

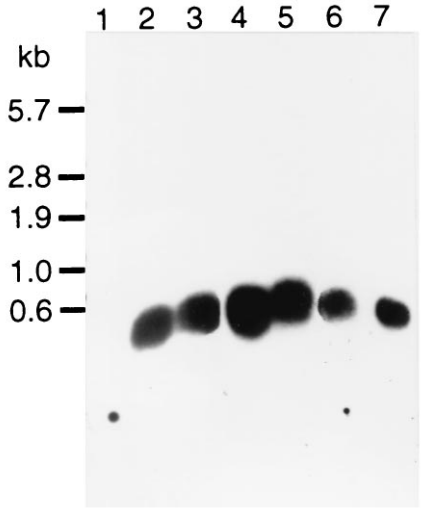

FIG. 6. Northern blot analysis of RNA isolated from strain BS20 at different times after beginning of starvation with the 567-bp radiolabelled *R. capsulatus pufBA* fragment as a probe. Every 30 min after the beginning of starvation, cells were scraped off the agar for isolation of RNA. A signal was detected only after 8 h. Lanes with RNA from starving cells in which no signal had been detected are not shown. Lane 1, RNA isolated from vegetative cells of BS23. Lanes 2 to 7, RNA isolated from cells 8, 9, 15, 20, 44, and 64 h, respectively, after the beginning of starvation. A total of 10 μg of RNA from each sample was used. The size of the fragments was estimated with RNA molecular weight marker I (Boehringer Mannheim).

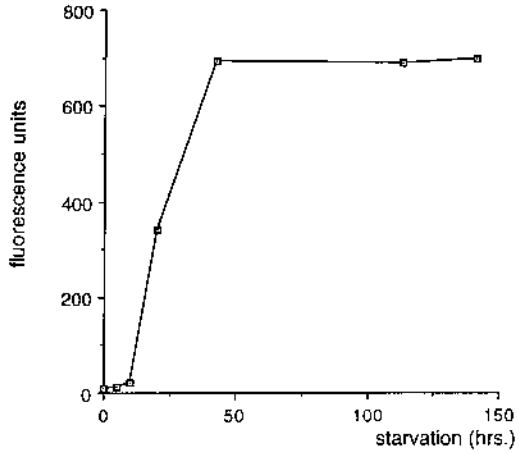

FIG. 7. Determination of the β-galactosidase activity of strain BS23 during fruiting body formation. After 8 h of development, β-galactosidase activity was detectable and reached its maximum after about 40 h of starvation.

determined with the fluorescent substrate 4-MUG. Figure 7 shows that β-galactosidase activity started to increase about 8 h after the beginning of starvation and reached a maximum value after 42 h. No β-galactosidase activity was observed in vegetative cells and during indole-induced sporulation, and no significant β-galactosidase activity was detectable in developing wild-type cells.

In *Myxococcus xanthus*, an organism which is closely related to *S. aurantiaca*, *fbfA* was not detected in a genomic Southern blot analysis of both organisms with the *fbfA* gene as probe under nonstringent conditions (data not shown). The known polypeptides of both organisms have a homology of about 90%.

Developmental genes of *M. xanthus* are sequentially expressed (24, 27, 28). Several serine/threonine kinases involved in the development of *M. xanthus* (24, 27, 28, 31, 32, 49) and a G-protein-related polypeptide in *M. xanthus* (16) have been reported. Protein kinases and G proteins are involved in signalling cascades in eukaryotes. FbfA of *S. aurantiaca* may be part of a signal-transducing pathway which leads to a spatial aggregation of cells, thus stabilizing a morphologic structure, and to a modulation of the cellular physiology. This could be achieved by the direct interaction of the putative FbfA receptors with other cells or indirectly via polysaccharides or other substances of a signalling chain. Alternatively, the FbfA polypeptide may be an *N*-acetylglucosaminyltransferase catalyzing the synthesis of oligomers of *N*-acetylglucosamine (33) which may form the backbone of signalling factors involved in development. Recently, it has been shown unequivocally that NodC from rhizobia is directly involved in the synthesis of the nodulation factors which induce the formation of the root nodules (11, 47). A similar type of signalling factor seems to play a role during the embryonal development of *X. laevis* (43).

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