fbfB, a Gene Encoding a Putative Galactose Oxidase, Is Involved in Stigmatella aurantiaca Fruiting Body Formation

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Received 5 September 1997/Accepted 16 December 1997

Stigmatella aurantiaca is a gram-negative bacterium which forms, under conditions of starvation in a multicellular process, characteristic three-dimensional structures: the fruiting bodies. For studying this complex process, mutants impaired in fruiting body formation have been induced by transposon insertion with a Tn5-derived transposon. The gene affected (fbfB) in one of the mutants (AP182) was studied further. Inactivation of fbfB results in mutants which form only clumps during starvation instead of wild-type fruiting bodies. This mutant phenotype can be partially rescued, if cells of mutants impaired in fbfB function are mixed with those of some independent mutants defective in fruiting before starvation. The fbfB gene is expressed about 14 h after induction of fruiting body formation as determined by measuring β-galactosidase activity in a merodiploid strain harboring the wild-type gene and an fbfB-△trp-lacZ fusion gene or by Northern (RNA) analysis with the Rhodobacter capsulatus pufB4 fragment fused to fbfB as an indicator. The predicted polypeptide FbfB has a molecular mass of 57.8 kDa and shows a significant homology to the galactose oxidase (GaoA) of the fungus Dactyltium dendroides. Galactose oxidase catalyzes the oxidation of galactose and primary alcohols to the corresponding aldehydes.

Stigmatella aurantiaca is a member of the order Myxobacterales. Myxobacteria are gram-negative, rod-shaped soil bacteria which are distinguished from most other bacteria mainly by two properties. First, they are able to move by gliding, a property which they share with a few other prokaryotes. Second, under conditions of starvation they form fruiting bodies, a property which is unique to the myxobacteria. The life cycle of the myxobacteria is bipartite. It is composed of the vegetative phase and the developmental cycle, into which cells enter under conditions of starvation. At the end of this cycle, cells form simple structures (vegetative cells) and the developmental cycle, into which cells enter under conditions of starvation. The myxobacterial fruiting body is a complex process, mutants impaired in fruiting body formation have been induced by transposon insertion with a Tn5-derived transposon. The gene affected (fbfB) in one of the mutants (AP182) was studied further. Inactivation of fbfB results in mutants which form only clumps during starvation instead of wild-type fruiting bodies. This mutant phenotype can be partially rescued, if cells of mutants impaired in fbfB function are mixed with those of some independent mutants defective in fruiting before starvation. The fbfB gene is expressed about 14 h after induction of fruiting body formation as determined by measuring β-galactosidase activity in a merodiploid strain harboring the wild-type gene and an fbfB-△trp-lacZ fusion gene or by Northern (RNA) analysis with the Rhodobacter capsulatus pufB4 fragment fused to fbfB as an indicator. The predicted polypeptide FbfB has a molecular mass of 57.8 kDa and shows a significant homology to the galactose oxidase (GaoA) of the fungus Dactyltium dendroides. Galactose oxidase catalyzes the oxidation of galactose and primary alcohols to the corresponding aldehydes.

The development of the fruiting body is strictly coupled to a time- and compartment-specific synthesis of regulatory factors, which stimulate the expression of several genes or gene families (18, 23, 24). Inactivation of the genes involved in the synthesis of these regulatory factors would lead to a defect in fruiting. The myxobacteria's capacity to glide permits a tight cell-cell contact and an efficient intercellular communication via diffusible signal molecules. These features allow the transmission of positional information about the single cell which is needed for the coordination of the metabolism and of the movement of the cells during fruiting.

To detect genes involved in fruiting body formation of S. aurantiaca, Tn5 insertional mutagenesis was performed with the transposon Tn5lacZ (29). The defect in one of the mutants obtained, AP182, in which the fbfB (frUITI) gene was inactivated, can be rescued partially by mixing the cells of this mutant strain with cells of the nonaggregating mutant AP191. Recently, we described the inactivation of an independent gene involved in fruiting body formation, fbfA, which is located near fbfB and encodes a putative chitin synthase (37). The mutant phenotype is partially rescued by mixing the cells of this mutant with those of AP191 before inducing fruiting body formation. In this communication, the characterization of the fbfB gene encoding a polypeptide with sequence homologies to the galactose oxidase (GaoA) of Dactyltium dendroides is reported.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains and plasmids are described in Table 1. S. aurantiaca DW4/3-1 and its derivatives (30) were grown at 32°C in incandescent light. For cultivation, Casitone medium (1% Casitone [Difco], 0.15% MgSO4 • 7H2O, pH 7.0) or tryptone medium (1% tryptone [Difco], 0.2% MgSO4 • 7H2O, pH 7.2) was used and 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 after conjugation, Trypticase peptone agar was used (0.025% Trypticase peptone [Becton Dickinson], 0.05% MgSO4 • 7H2O, 0.05% CaCl2 • 2H2O, pH 7.2). For fruiting body formation assay, starvation agar was used (containing only 0.1% CaCl2 • 2H2O) (29). Escherichia coli strains were grown in Luria broth at 37°C, supplemented when necessary with chloramphenicol (34 µg/ml), kanamycin sulfate (50 µg/ml), and tetracycline base (10 µg/ml). If necessary, media were solidified with 1.5% agar or, in the case of soft agar, with 0.75% agar (Difco).

Transfer of conjugable plasmids from E. coli to S. aurantiaca (10). A total of 5 x 105 exponentially growing S. aurantiaca cells were mixed with a total of 5 x 106 exponentially growing cells of E. coli and filtered onto a membrane filter (0.45-µm pore size; 25-mm diameter; Schleicher & Schuell, Dassel, Germany).
The cells were washed twice with 5 ml of Casitone medium. The filter was placed onto a Casitone plate and incubated overnight at 32°C. Cells were scrapped 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.

Fruiting body formation assay (29) and germination assay (32). Exponentially growing S. aurantiaca cells were sedimented, washed in HEPES buffer (100 mM HEPES, 10 mM CaCl₂, pH 7.2), sedimented again, and resuspended in HEPES buffer to a concentration of 4 × 10⁶ cells per ml. Aliquots of 5 μl were spotted onto starvation agar and incubated at 32°C for 24 h in incubator light. For the phenotypic complementation assay, an equal number of cells of two different mutants were mixed for fruiting body formation.

For the germination assay, the 5-ml cell suspensions were spotted onto filter paper (Schleicher & Schuell) about 1 cm² in size. The filter papers were shifted onto starvation agar plates and incubated at 32°C for 10 days in incubator light. For the germination assay, the 5-ml cell suspensions were spotted onto filter paper (Schleicher & Schuell) about 1 cm² in size. The filter papers were shifted onto starvation agar and incubated at 32°C for 24 h in incubator light. For the phenotypic complementation assay, an equal number of cells of two different mutants were mixed for fruiting body formation.

Induction of spore formation by indole. Spore formation was induced by addition of indole (Sigma) to a final concentration of 0.5 mM to late-log-phase culture (2 × 10¹⁰ to 3 × 10¹⁰ cells per ml in shake flask) in tryptone medium at 32°C. After 2 h after the addition of indole, the cells formed shorter rods, and they started to become refractile after about 4 h. A total of 60 to 80% of the initial cells converted into sonication-resistant cells (9, 12).

β-Galactosidase assay (37). Fruiting bodies which had been scraped off starvation agar plates or vegetative cells were suspended in a buffer containing 50 mM 3-N-morpholinopropansulfonic acid (MOPS) at pH 7.5, 10 mM MgCl₂, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride and sonicated (Branson sonifier; cell disruptor 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). To remove cell debris, the samples were centrifuged at 15,000 × g at 4°C for 15 min. The supernatant was assayed for β-galactosidase activity with the substrate 4-methylumbelliferyl-β-D-galactopyranoside (4-MUG) (Sigma). A total of 0.1 ml of the supernatant containing 10 μg of protein was mixed with 0.3 ml of 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl, 1 mM MgCl₂, 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 wavelengths of 360 nm for excitation and 450 nm for emission.

DNA manipulations, sequencing, and PCR. Restriction analysis and plasmid subcloning were performed according to standard protocols (35). Chromosomal DNA from S. aurantiaca was prepared as described previously (27). The sequence of pkSk4, containing the fbfB gene, was determined with exonuclease III-generated directed deletions (13) and synthetic oligonucleotides. PCR was carried out with Vent DNA polymerase (New England Biolabs). Amplification was performed at final concentrations of 0.1 mM template, 1 μM each primer, 300 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 Tris-Thermoblock (Bo- metrica) were as follows: the initial denaturation step was at 94°C for 4 min and the subsequent denaturation was at 94°C for 4 min, and there were 3 cycles. The PCR products were purified with the Qiaquick PCR purification kit (Qiagen).

Southern hybridization. Southern blot analysis was performed according to standard protocols (35). Prehybridization was carried out for 2 h at 60°C in 5× SSC (0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt’s solution–0.5% sodium dodecyl sulfate (SDS)–50 μg of denatured herring sperm DNA per ml. Hybridization was performed overnight at 60°C after addition of the DNA probes, which were 3²P-labelled with a nick translation kit (Boehringer, Mannheim, Mannheim, Germany). The filters were washed in 0.1× SSC–0.1% SDS twice for 30 min at 60°C.

RNA isolation and Northern hybridization. S. aurantiaca RNA from vegetative cells and developing cells was isolated as described previously (4). RNA electrophoresis was performed as follows. A suspension of 1 g of agarose in 72 ml of diethylpyrocarbonate-treated H₂O was melted. After the suspension was heated to 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 was to be electrophoresed was dried and suspended in 9 μl of sample buffer (10% 1× MOPS running buffer, 50% formamide, 18% formaldehyde). After the mixture was heated to 65°C for 10 min, 1 μl of stop buffer (50% glycerol, 6 mM EDTA, 0.05% bromphenol 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 (Appiogene). After UV cross-linking of the nylon (Stratagene), the prehybridization of the filters was carried out at 42°C in 50% formamide–4× SSC (0.15 M NaCl, 0.1 M NaH₂PO₄, and 1 mM EDTA)–pH 7.0 (7.0)–0.1% SDS–0.25 mg of denatured herring sperm DNA per ml. Hybridization was carried out overnight at 42°C in 55% formamide–2× SSC (0.15 M NaCl, 0.1 M NaH₂PO₄, and 1 mM EDTA)–pH 7.0 (7.0)–0.1% SDS–0.25 mg of denatured herring sperm DNA per ml. The filters were washed at room temperature twice for 15 min in 2× SSC–0.1% SDS and at 37°C twice for 15 min in 0.1× SSC–0.1% SDS.
RESULTS AND DISCUSSION

For the identification of genes involved in S. aurantiaca fruiting body formation, transposon mutagenesis with Tn5lacZ was performed. Three mutant types affected in fruiting body formation have been obtained. These include mutants which form neither fruiting bodies nor aggregates, mutants which are able to aggregate into nonstructured clumps, and mutants which undergo only a part of the differentiation process (29). One of the mutants (AP182) which forms only clumps during starvation (Fig. 1B) was selected for further analysis. Mixing the AP182 cells with those of strain AP191 prior to starvation leads to a partial phenotypic complementation. Mutant AP191 forms neither fruiting bodies nor aggregates (Fig. 1D) but shows a normal gliding behavior. Instead of the clumps, a mushroom-like structure, similar to a champignon (cf. Fig. 1F), has been obtained.

In a λ EMBL3 library of Sall-restricted chromosomal DNA of the mutant strain AP182 (29, 37), a 20-kbp Sall restriction fragment harboring the Tn5lacZ insertion was detected with the neo gene derived from Tn5 (3) as a probe. After subcloning of this fragment into the Sall site of pSUP102 (resulting in plasmid pSZ182), the position and orientation of the transposon were determined by restriction analyses and sequencing starting with the insertional sequence elements (Fig. 2). With this 20-kbp Sall fragment as probe, the corresponding 12-kbp Sall fragment was detected in a λ EMBL3 library of Sall-restricted chromosomal DNA of the wild-type strain DW4/3-1. This 12-kbp fragment was cloned into the Sall site of the conjugable plasmid pSUP102, resulting in plasmid pBS1 (Fig. 2).

A 3.6-kbp Smal-EcoRI fragment containing the site of transposon insertion of strain AP182 was cloned into pBS SK(−), resulting in pSK24 (Fig. 2). After sequencing, two putative open reading frames (ORFs) named fbfA (37) and fbfB, which are arranged in a divergent orientation on the fragment, have been detected (Fig. 2). Both genes have been localized on an 862.5-kbp SpeI and a 676.8-kbp XbaI restriction fragment of the S. aurantiaca genome (28). The distance between the start codons of fbfA and fbfB is 153 bp. The size of the fbfB gene is 1,581 bp (Fig. 3). The site of the transposon insertion in mutant AP182 is 550 bp downstream of the ATG start codon of the fbfB gene. A putative Shine-Dalgarno sequence (GGAGA) is detected 8 bp upstream of the start codon (Fig. 3). fbfB, which we suggest to be involved in fruiting body formation, encodes a putative polypeptide (FfbB) composed of 526 amino acid residues with a molecular mass of 57.8 kDa. Protein database searches for the deduced polypeptide with BLASTP 2.0.3 (1) revealed a significant homology between FfbB and the secreted copper enzyme galactose oxidase (GaoA) from the deutero-mycete fungus D. dendroides (Fig. 4) (25). In addition, some similarity to the copper enzyme glyoxal oxidase (Glx) from the lignin-degrading Basidiomycete Phanerochaete chrysosporium was found (19, 44). GaoA catalyzes the oxidation of primary alcohols and of the C-6 hydroxyl group of galactose to aldehydes. During this reaction, molecular oxygen is reduced to hydrogen peroxide by a radical mechanism (42). The four amino acid residues Tyr-313, Tyr-536, His-537, and His-622 of GaoA, which form the copper binding site, and Cys-269, which forms the thioether cysteinylthrosine with Tyr-313 (17), are conserved in FfbB (Fig. 4). As FfbB may act on the outside of the bacterial cell during development, the N-terminal domain of the putative polypeptide was screened for a signal sequence. The N-terminal sequence (40 amino acids) of FfbB (MAG LPRGVVSVLL AMPWPLGRVGREAS LRLRPWHLR ES) was analyzed with the program SignalSeq of the Heidelberg Unix Sequence Analysis Resources (43). Two hypothetical cleavage sites, indicated by * in the above sequence, have been proposed, of which, if any, the site for the longer signal sequence seems more probable.

To rule out the possibility that the phenotype of mutant AP182 is due to a second-site mutation, fbfB had to be inactivated. For this purpose, the conjugable plasmid pBS21 was constructed. It harbors an insertion of a 5′- and 3′-truncated fbfB gene fused to the Rhodobacter pufBA fragment and the Tn5 neo gene (see Materials and Methods). pBS21 was transferred into E. coli S17-1 and subsequently conjugated into wild-type S. aurantiaca to obtain strains in which the wild-type gene is replaced by two genes truncated at the 5′ or 3′ end, respectively. One of the kanamycin-resistant transconjugants, BS34, was used for further analyses. As expected, Southern blot analysis showed that BS34 is a merodiploid strain containing two truncated fbfB genes (Fig. 5 and 6). Wild-type cells form well-defined fruiting bodies during starvation (Fig. 1A), whereas mutant BS34 cells generate only nonstructured aggregates (Fig. 1C). Fruiting body formation of mutant BS34 is partially restored by mixing the mutant cells with cells of mutant AP191, which though competent for gliding are not able to form aggregates during starvation (Fig. 1D). Mixing of the mutant cells before starvation leads to the formation of a structure composed of a stem and a cap which looks like a champignon (Fig. 1F). A fruiting body structure resembling that of a morel (Fig. 1G) is obtained if cells of BS34 are mixed with those of the fbfA mutant BS14, defective in fruiting (Fig. 1E). Mutants BS14 and BS34 generate spores during the development of their fruiting bodies. These spores have the capability of germinating in our germination assay (see Materials and Methods).

We have not been able to detect the transcript of the fbfB gene generated during fruiting body formation by using an fbfB-derived probe for Northern analysis. To prove fbfB transcription, strain BS34 was constructed. BS34 contains a 5′-truncated fbfB gene fused to the pufBA fragment from R. capsulatus and a 5′-truncated fbfB fragment (Fig. 5). pufBA encodes an mRNA that has a half-life of about 30 min (20, 21). It has been shown recently that the time at which the transcription of a pufBA gene fusion starts can be easily determined by Northern analysis with the pufBA gene as a probe (37). The pufBA transcript was detected in mutant BS34 about 14 h after the beginning of starvation (Fig. 7) but not in vegetative cells. No significant amount of fbfB gene transcript or part of it was detected. This suggests that the expression of fbfB is low and/or that its transcript is very unstable.

For analyzing the progression of fbfB expression during fruiting body formation or indole-induced sporulation (9), the merodiploid strain BS35 was constructed. It contains the wild-type fbfB gene and a 3′-truncated fbfB gene to which a promoterless Sfp-lacZ gene fusion and the neo cassette (2) for transconjugant selection were fused (Fig. 5 and 6). Starvation of BS35 cells on water agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) resulted in fruiting bodies which had the same form as those of the wild type but whose stems were stained blue in the course of 2 days after the beginning of development (Fig. 1H). For the determination of fbfB fusion gene expression during fruiting body formation, cells were scraped off the agar dish and broken by sonication.
Galactosidase activity was determined in the cell extract with the fluorescent substrate 4-MUG (34), as β-galactosidase activity of BS35 cells was low. β-Galactosidase activity starts to increase about 14 h after the beginning of starvation and reaches its maximum level after about 30 h (Fig. 8). No β-galactosidase activity was detected during indole-induced sporulation and in vegetative cells.

Fruiting body formation of the myxobacteria is a multicellular process. Multicellular development requires intercellular signalling for the coordination of the physiology of the single cell as a function of both the location in the swarm and the progress of fruiting. Signalling substances may be diffusible compounds or may be attached to or associated with the cell surface.

McVittie et al. isolated mutants of M. xanthus impaired in fruiting body formation and observed that it was possible to rescue development by mixing certain mutants with others before starvation (26). They demonstrated that the complementation was not genetic and suggested this extracellular complementation to be based on a synergistic interaction, i.e., an exchange of substances involved in developmental interaction. A large number of M. xanthus mutants defective in development and showing synergistic interaction were isolated.

FIG. 1. Fruiting body morphologies of various S. aurantiaca strains. (A) Fruiting body of the wild-type strain DW4/3-1 is differentiated into a stalk, branches, and sporangioles. (B) The Tn5lacZ insertion mutant AP182 can form only unstructured clumps. The transposon is inserted into the fbfB gene. (C) The fbfB mutant BS34 is able only to aggregate into clumps, like AP182. (D) The Tn5lacZ insertion mutant AP191 shows no cell aggregation. (E) The fbfA mutant BS14 aggregates only into clumps. (F) Mixing of cells of BS34 and AP182 following starvation leads to a mushroom-like structure (champignon). (G) Mixing of cells of BS34 and fbfA mutant BS14, which is able only to aggregate into clumps, following starvation leads to a mushroom-like structure (morel). (H) Fruiting body of the merodiploid strain BS35, harboring a functional fbfB gene and a Δorf-lacZorfB/orfB fusion gene, on starvation plates containing 20 μg of X-Gal per ml shows blue staining of the stem. Bars, about 40 μm for panels A, D, and H and about 30 μm for panels B, C, E, F, and G.

FIG. 2. Schematic depiction of the fbfA-fbfB locus and various plasmid constructs. The genes fbfA and fbfB are arranged in a divergent orientation, and the distance between their start codons is 153 bp. The Tn5lacZ insertion in the fbfB gene of the mutant AP182 is 550 bp downstream of the start codon. The insert of pSZ182 is identical to the insert of pBS1 containing Tn5lacZ. E, EcoRI; S, SalI; Sm, SmaI.
They fell into four groups. Mixing a member of one group with one belonging to another group or with the wild type resulted in extracellular complementation of fruiting body formation. The authors concluded that fruiting of *M. xanthus* was governed by at least four developmental signals and that each mutant group lost the ability to produce one of these signals (11). Meanwhile, a fifth factor involved in *M. xanthus* developmental cell-cell signaling has been detected (6, 7).

The fruiting body of *S. aurantiaca* is much more complex than that of *M. xanthus*. Studies of *S. aurantiaca* development will eventually provide the opportunity to learn more about the formation of complex multicellular structures and about the signals which coordinate the physiology of the single cell as a function of the morphogenetic process. During the first hours of development, *S. aurantiaca* forms a diffusible signal, the pheromone, which induces aggregation of the starving cells (40). The structure of the compound has been elucidated recently (16).

Mutants impaired in *S. aurantiaca* fruiting body formation were induced by insertional mutagenesis (29). Two of these mutant strains, AP182 and AP191 (Fig. 1B and D), were selected for further analysis because they showed a synergistic interaction. Mixing of the mutant cells before the beginning of starvation resulted in a partial phenotypic complementation of fruiting (Fig. 1F). The gene impaired in AP182, *fbfB*, was modified in vitro and crossed back into the wild-type strain to...
obtain a merodiploid derivative, BS34, harboring two truncated copies of \(fbfB\).

Strain BS34 showed the same phenotype as did mutant AP182. A partial rescue of fruiting body formation was observed after mixing the BS34 cells with those of AP191 before starvation (Fig. 1C and F). The fruiting body obtained after mixing the cells of the \(fbfB\) mutants BS34 and AP191 has a champignon-like shape. This form is also found 15 h after the beginning of starvation of wild-type cells (cf. Fig. 2C in reference 31). Fruiting body formation with the mixture of the mutant cells is obviously blocked at this 15-h stage. With the merodiploid strain BS35, which harbors an indicator gene fused to \(fbfB\), and by Northern analysis with strain BS34, it was shown that \(fbfB\) expression starts about 14 h after the beginning of starvation. Interestingly, fruiting body formation in the mutant BS14, in which another gene involved in fruiting, \(fbfA\), is inactivated, can be partially rescued by mixing the cells with those of mutant AP191 (37). The fruiting body looks like a morel. \(fbfA\) is expressed after 8 h of development, and the shape of the fruiting body obtained in the mixing experiment corresponds to that 12 h after the induction of fruiting body formation of the wild type (cf. Fig. 2B in reference 31). Mixing of cells of an \(fbfA\) and an \(fbfB\) mutant before starvation resulted only in fruiting bodies with a morel-like shape (Fig. 1G).

The partial phenotypic complementation suggests that factors involved in fruiting and which are lacking in one mutant may be obtained from the other. The shapes of the fruiting bodies obtained in the mixing experiments correspond to those observed during development of the wild type (e.g., intracellular macromolecules) which are lacking in one of the strains can be supplemented by the other mutant and vice versa.

Both \(fbfA\) and \(fbfB\) are not expressed during vegetative growth or indole-induced sporulation. Obviously, \(fbfA\) and \(fbfB\) are development-specific genes that are involved in the morphogenetic process of fruiting body, and not of spore, formation. It is tempting to speculate that sporulation and the formation of the structural parts of the fruiting body are partially independent processes which coincide in the late stage. It was shown for \(M. xanthus\) that vegetative cells starved in liquid culture may efficiently convert to spores which seem to be identical with those formed in fruiting bodies (33). This suggests that the formation of the fruiting body structure is not tightly coupled to the formation of starvation-induced spores.

During sequence analyses downstream of the \(fbfB\) region, two ORFs, \(hesA\) and \(pksA\), were detected (36). The disruption of these ORFs by the insertion of the \(neo\) gene has no effect on growth or sporulation of BS34, and the phenotype of BS34 was not altered by the insertions.
fruiting. No coding sequence was detected between fbfB and hesA, which is located about 800 bp downstream of the stop codon of fbfB. These results prove that the inactivation of fbfB and not a polar effect of the mutation on downstream sequences leads to the defect in fruiting.

The putative polypeptide encoded by hesA shows homology with some polypeptides probably involved in the export of antibiotics (14, 15, 22). Most interestingly, the second ORF, pksA (about 2 kbp downstream of the stop codon of fbfB), encodes a putative polyketide synthase which probably is involved in the synthesis of myxothiazol (31a). This compound is an inhibitor of the b-c1 complex of the respiratory chain (41) which possibly protects the fruiting body of S. aurantiaca against fungal attack or vegetative cells against fungal competition for food.

ACKNOWLEDGMENTS

This work was supported by grants Sch a 150/8-1 and Sch a 150/8-2 of the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

We thank Yves Cully for image processing and Berta Reiner for database searches.

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