I. *Stigmatella aurantiaca*, a Prokaryotic Organism for Studying Intercellular Signalling and Morphogenesis

The organisms

*Stigmatella aurantiaca* belongs to the myxobacteria that are Gram-negative soil bacteria. Myxobacteria show both, features of unicellular and multicellular organisms. As the eukaryotic organism *Dictyostelium discoideum* they are thought to lie on the boundary between unicellular and multicellular organisms. Myxobacteria grow and divide as separate cells. But they may be regarded as a multicellular organisms whose cells feed in swarms and which under conditions of starvation assemble to well defined regular three dimensional structures called fruiting bodies which enclose about $10^5$ dormant cells, the myxospores. The shape of the fruiting bodies is species specific and is genetically determined. The fruiting body of *S. aurantiaca* consists of a stalk bearing several sporangioles on branches at its top. Myxobacteria secrete hydrolytic enzymes together with slime with which they degrade particulate organic matter of the soil. It has been shown that the growth rate increases with cell density if myxobacteria were grown on a macromolecular substrate as sole nutrient, such as casein. This suggests that cells feed cooperatively and the association in a swarm allows them to feed more efficiently. The advantage of cooperative feeding may have driven the evolution of fruiting body formation. When nutrients are again available after a period of starvation, myxospores germinate and form vegetative cells. The multicellular nature of the fruiting body ensures that a swarm of cells is formed for a new growth cycle.

Myxobacteria move by gliding on solid surfaces. This facilitates the stabilisation of a swarm and of fruiting body formation. Gliding permits tight cell-cell contact and efficient signal exchange between the cells by diffusible molecules. Both features are a prerequisite for the transmission of positional information of the single cell necessary for the coordination of the metabolism and movement of the cell in the course of fruiting body formation. One of the developmental signals – Stigmolone – that is involved in early cell aggregation has been recently isolated and characterised by Wulf Plaga et al. Apart from their ability to form fruiting bodies, myxobacteria form a broad range of secondary metabolites. All these unique features are reflected in the size of the genome and its organisation. The size of the myxobacterial genome has been shown to be about 9.5 Mbp.

A gene cluster involved in *S. aurantiaca* fruiting body formation

Susanne Müller, Barbara Silakowski and Diana Hofmann

To investigate the genes involved in *S. aurantiaca* fruiting body formation and the co-ordination of their expression, Tn5 transposon insertion mutagenesis was performed. Three different mutant types impaired in fruiting body formation were detected by screening the insertional mutants. They include mutants that form neither fruiting bodies nor aggregates, mutants that undergo only part of the differentiation process. One of the mutants (AP182) that formed clumps during starvation was analysed further. Mixing of the
cells of this strain with those of a mutant (AP191), which was unable to form aggregates prior to starvation, lead to a partial phenotypic complementation. Instead of clumps, a mushroom-like structure, similar to a chlamydom, was obtained. Sequencing of the mutant gene of strain AP192 and of the adjacent genomic segments resulted in four open reading frames that were involved in fruiting body formation. One of the other three genes, fbfA, fbfC, and fbfD are arranged in a divergent orientation. FbFD shows significant homology to the secreted copper enzyme galactose oxidase from the fungus Dactylium dendroides. FbfA encodes a polypeptide that is homologous to chitin synthases. The start codon of fbfC overlaps with the stop codon of fbfA. FbfC has no significant homology to any of the known proteins. FbD has similarities to the human phosphoprotein synapsin I. Insertional mutagenesis did not impair fruiting body formation; in such experiments fruiting bodies are formed by the combined action of two wild-type strains. Thus the mutant strain cells can be easily distinguished from wild-type cells using a fluorescent labelling with the dye mbH. Further investigations of the gene cluster are performed at the GBF in Braunschweig.

CsgA, a prominent cold-shock(-like) protein
I. Stamm and W. Plaga

Several related proteins of about 7 kDa constitute a prominent fraction of the S. aurantiaca cell extract. One of the genes encoding such a protein was cloned and named csgA. The delineated protein sequence of 68 amino acid residues displays a high sequence identity with bacterial cold-shock(-like) proteins. A RNA chaperon-function was proposed for these proteins in E. coli. Using a csgA::trp-lacZ fusion gene that was introduced into Stigmatella by electroporation the transcription was analyzed during development and at lowered temperature. These experiments indicated csgA to be constitutively transcribed at a high level. The csgA promoter was used to express the gene for the green fluorescent protein (GFP). GFP fluorescence was found to be detectable in whole fruiting bodies as well as in single cells. The GFP-labelled cells are easily distinguished from wild type cells using a fluorescence-activated cell sorter (FACS). Fruiting body formation is not impaired in csgA expressing wild type strains. Thus the csgA gene under the control of the csgA promoter seems to be suitable to label Stigmatella. With this kind of labelling it should be possible to analyze the fate of mutant strain cells in phenotypic complementation experiments during fruiting body formation; in such experiments fruiting bodies are formed by the combined action of two mutant strains which are unable to develop properly on their own.

one of the strains can be supplemented by the other mutant and vice versa. For the analysis of the time dependence of the expression of the genes fbfA, fbfB, fbfC, and fbfD during fruiting body formation or indole induced sporulation merodiploid strains were constructed. They harboured the wild type genes and in addition a 3′ truncated fbf gene with 5′ regions of variable length. The truncated genes were fused to the promotorless hybrid indicator gene Δap-lacZ and the neo gene for selection. β-Galactosidase activity increased 8 or 14 hours after the beginning of starvation in the merodiploid strains but not during indole induced sporulation. This unequivocally proves the four fbf genes to be involved in the morphogenic process of fruiting. RT-PCR analyses of fbf gene transcription revealed these genes to be induced during starvation. Low levels of fbf gene transcript are found in vegetative cell and in the case of fbfC or fbfD during induced sporulation. Analysis of the protein patterns of the wild-type and the mutant strains by 2D electrophoresis is in progress.

Alternative sigma factors
Barbara Stilakowski, Susanne Müller and Chi-Hyuk Chung

The genes of two alternative sigma factors, sigB and sigC have been cloned. These sigma factors harbour two domains that were shown for σ32 of E. coli to be necessary for DNA binding and thus for its proteolytic cleavage. Merodiploid strains containing the wild type gene and the corresponding 3′ truncated gene to an indicator gene were analysed for the expression of the sigma factor genes during development or heat shock. sigB was shown to be expressed early during indole-induced sporulation and fruiting body formation as well during heat shock.

results agree with those of the RT-PCR analysis of sigB transcription. Inactivation of either sigB or sigC by insertional mutagenesis did not impair fruiting body formation, indole-induced sporulation or the heat shock response. No changes in either the spores’ ultrastructure (H. Linsdorf, GBF, Braunschweig) or in spore germination have been detected.

A gene cluster of S. aurantiaca DW4/3-1 for Myxothiazol biosynthesis
H. Ehret and B. Stilakowski in collaboration with H. Reichenbach, GBF, Braunschweig

Sequence analyses downstream of the developmental fbfB gene resulted in the detection of the mtaA (myxothiazol) gene cluster. The first ORF, mtaA, (formerly designated hesA), encodes a phosphopantetheinyl transferase. P-pant transferases activate polyketide synthases (PKS) by the transfer of the P-pant moiety from coenzyme A to a conserved serine residue of the PKS. Downstream of mtaA a second ORF, mtaB, (formerly pksA) was found, of which 7 kbp were sequenced. It encodes a PKS. Inactivation of mtaA by insertional mutagenesis or deletion of part of the gene or insertional inactivation of mtaB gene impairs myxothiazol synthesis. In addition mutations defective in mtaB fail to form a sofar unknown metabolite. This suggests MtaA to be a functional wildtype and a 3′ truncated mtaB gene to which an indicator gene was fused. Measurement of indicator gene expression showed, mtaB to be expressed under all conditions tested, such as vegetative growth, fruiting, induced sporulation and heat shock. The project was stopped at this stage at the ZMBH.
Fluorescence-based analysis of gene expression: Identification of pheromone target genes

The pheromone stigmolone (2,5,8-trimethyl-8-hydroxy-nonan-4-one) is instrumental in early steps of fruiting body formation. To identify stigmolone-responsive genes a promoter trap vector (pYTAPI) was constructed which allows the creation of random promoter fusions to gfp in S. aurantiaca. With the aid of a flow cytometer a selection strategy exploiting the differential fluorescence induction (DFI) of these promoter fusions by stigmolone is feasible. First analyses of the random insertion mutants by flow cytometry have shown, that about 2.5% of the mutants express the gfp during vegetative growth. Screening for genes affected by the addition of stigmolone is in progress.

HspA (SP21): Transcriptional regulation and biological function

HspA (formerly SP21) of S. aurantiaca gene were detected after heat shock and only one after indole treatment. A His-tagged fusion protein of Hspa (HspA element to exist just upstream of the -35, -10 regions of the transcription by more than half. Deleting a sequence ranging from bp -86 to bp -140 upstream of the hspA promoter abolishes hspA transcription. The second domain ranges from bp -141 to bp -223 upstream of the hspA promoter and carries putative regulator-binding sites. Heat shock and phosphorylation enhance binding of the regulator(s) to the hspA promoter. Deletion of this region completely blocks hspA transcription. This polypeptide tends to assemble into a large complex that consists of 26 subunits. A His-tagged fusion protein of Hpa (HspA) was produced in E. coli. This polypeptide tends to assembling into a large complex that consists of 26 subunits with a molecular mass of 500 kDa as judged by size exclusion chromatography. This oligomer of HspA interacts with unfolded cytrate synthetase (CS) and prevents the enzyme’s precipitation. The unfolded B-chain of insulin is not protected from precipitation. A stable complex is formed between HspA and unfolded CS because the unfolded enzyme does not dissociate from the complex. Though thermostoler-
Characterisation of genes involved in \textit{B. bassiana} virulence

For the identification of virulence factors of a \textit{B. bassiana} strain that is highly adapted to the Colorado potato beetle, two complementary strategies are employed. Firstly, in a REMI (“restriction enzyme mediated integration”) transformation approach mutants are generated. Mutants that result from a monolocal recombination event are tested for their ability to grow on their host-insect. In the case of a significant reduction of their virulence the disrupted genomic region will be cloned and sequenced. Secondly, a mRNA differential display analysis is performed to identify fungal genes that are transcriptionally up regulated by the interaction with the host using cultures grown in the absence or presence of host structures These genes are cloned from a wild-type genome DNA library. The significance of the thus found potential virulence factor genes for pathogenesis are tested by site-specific inactivation of the single cloned genes or of groups of functionally related genes and the determination of the pathogenicity phenotype of the corresponding null mutants.

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PUBLICATIONS


**THESES**

Dissertation


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