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Sigma factor WhiG and its regulation constitute a target of a mutational phenomenon occurring during aerial mycelium growth in *Streptomyces ambofaciens* ATCC23877

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Abstract

The genetic instability of *Streptomyces ambofaciens* affects the pigmentation of colonies and generates a variety of mutants the majority of which display large genome rearrangements. Among them, the Pig-pap mutants, which probably result from a mutational event occurring during aerial mycelium growth, display specific features, since they are unable to sporulate and do not harbor any large detectable genome rearrangements. To identify the mutational event causing their phenotype, three Pig-pap mutants originating from three independent mutational events were characterized. These mutants exhibited a *whiG*-like phenotype which was suppressed by the introduction of one copy of *Streptomyces coelicolor whiG*. Their own *whiG* gene was devoid of mutations and appeared to be transcribed at a level similar to that of the WT. However, *whiH*, the expression of which depends on σ^{WhiG}, was not transcribed in any of the three Pig-pap mutants, suggesting that the σ^{WhiG} was absent or inactive. This suggests that in these Pig-pap mutants, the regulation of σ^{WhiG} might be affected. Finally, the introduction of *S. coelicolor whiG* in one of these Pig-pap mutants restored not only pigmentation and sporulation, but also the ability to once again form white papillae. Analyses of transgene *whiG* in these papillae revealed that it constitutes a mutational target during aerial mycelium formation when integrated into the genome of this Pig-pap mutant.

Keywords: *Streptomyces*; Differentiation; *whi* genes; Sigma factor; Genetic instability

1. Introduction

Streptomyces are Gram$^+$ mycelial soil bacteria which undergo a complex cycle of morphological differentiation. On solid medium, spore germination gives rise to the vegetative mycelium with interconnected hyphae from which the aerial mycelium expends and differentiates into coiled chains of mature spores [8]. In *Streptomyces coelicolor*, many genes involved in the differentiation process have been characterized and split into two classes of genes grouped on the basis of the differentiation step with which they interact. The first class, including the *bld* genes (for bald), is essential for aerial mycelium erection while the second, among which are the *whi* genes (for white), is implicated in the formation of mature pigmented spores [8]. The *whi* genes have been qualified as "early" or "late" genes [9]. At least six "early" *whi* genes (*whiA*, *whiB*, *whiG*, *whiH*, *whiI* and *whiJ*), required for achieving full septation of the aerial hyphae, have been identified in *S. coelicolor* [1,2,7,15,29,36]. To our knowledge, six "late" *whi* genes (*sigF*, *whiD*, *whiE*, *whiL*, *whiM* and *whiO*), involved in the maturation of spores and the synthesis of spore pigment, have been identified in the same species [10,17,18,29,37]. Finally, as their role during aerial differentiation remains unknown, the *whiK* and *whiN* (renamed *bldN*) genes are qualified as "indeterminate" [4,29]. In *S. coelicolor*, the RNA polymerase sigma factor WhiG is absolutely required for the shift from aerial growth to sporulation [7,17,28]. Consequently, a *whiG* mutant presents

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straight aerial hyphae [14]. *whiG* is similar to the genes encoding the sigma factors σ^D of *Bacillus subtilis* and σ^F of coliform bacteria, both controlling expression of motility and chemotaxis genes [7,30]. Homologues of WhiG have been identified in other *Streptomyces* species such as *S. avermitilis* [31], *S. griseocarnum* [39] and *S. aureofaciens* [20]. All of these sigma factors belong to the σ^{70} family and are characterized by four conserved regions, among which 2.4 and 4.2 (highly conserved) are involved in −10 and −35 promoter sequence recognition for the genes regulated by these sigma factors [24]. σ^{WhiG} activates the promoter of the two "early" *whiH* and *whiI* genes and of the *orfTH4* gene [1,37,40]. In *S. aureofaciens*, *rpoZ*-encoded sigma factor also regulates the transcription of *whiH* [21] and that of the *ren71* gene [22]. The regulation of *whiG* seems to be complex and remains unclear [9]. In *S. coelicolor*, while *whiG* is transcribed during vegetative mycelium development, σ^{WhiG} seems to be active only during the beginning of the sporulation process [17] and the cause of such activation is not known. Despite the close similarities of σ^{WhiG} to σ^{FliA} of *Salmonella typhimurium*, no *flgM* homologue, which encodes the anti-σ^{FliA} factor, has been found in the *S. coelicolor* genome sequence [9]. This suggests that, if *whiG* post-transcriptional regulation implies an anti-σ factor, the latter may be quite different from FlgM.

Genetic instability, which occurs in *Streptomyces ambofaciens*, affects pigmentation of colonies. Four main phenotypes are currently observed in the progeny of a pigmented colony (WT): pigmented colonies, pigment-defective colonies and pigmented colonies with pigment-defective sector and/or white papillae. Mutants deriving either from pigment-defective colonies or from pigment-defective sectors frequently display large genome rearrangements such as deletions and/or amplifications, and most of them are able to sporulate [23,26,27]. Conversely, mutants deriving from white papillae, which probably result from genetic instability events occurring during aerial mycelium formation, are devoid of any large genome rearrangement. Further, among the Pig-pap mutants which were characterized, all but one exhibit the same phenotype consisting of an inability to sporulate [27]. These genotypic and phenotypic features prompted us to try to identify the type of mutational event which generated Pig-pap mutants at high frequency during the development of *S. ambofaciens*. For this, three mutants deriving from three independent mutational events were studied, and their characterization is reported here.

2. Materials and methods

2.1. Bacterial strains, growth conditions and phenotypic characterization

Streptomyces strains studied in this work derived from *S. ambofaciens* ATCC23877, whose genetic instability has been detailed in previous works [26,27]. Pig-pap mutants (pap1, pap2 and pap3) were derived from white papillae isolated from WT subclones WT8, WT1 and WT12 of strain ATCC23877, respectively, as follows [26]. Each papilla was carefully picked up using a toothpick from WT8, WT1 and WT12 respectively, and subcultured 2 or 3 times, each time choosing a white colony, until the progeny of each Pig-pap mutant was homogeneous. For transcriptional experiments, WT subclone WT8 was used as reference. Strains pap1(*whiG*), pap2(*whiG*) and pap3(*whiG*) were derived from one exconjugant of pap1, pap2 or pap3 containing the plasmid pSET152(*whiG*). The absence of apramycin in the medium enabled comparison, under the same conditions, of the phenotypes of the different *S. ambofaciens* strains. Strains pap3Gp1 to pap3Gp10 were derived from ten papillae isolated from strain pap3(*whiG*) and subcloned twice on HT solid medium without selection to obtain a homogeneous progeny. To ensure that, under these conditions, pSET152 and pSET152(*whiG*) were not highly unstable, we estimated the frequency of loss of apramycin resistance, which is conferred by these plasmids. For this, two exconjugants, WT8(pSET152) and WT8(pSET152(*whiG*)), were grown on HT solid medium without apramycin for 4 days. The colonies were then replicated on HT solid medium supplemented with apramycin. For each plasmid, the loss of apramycin resistance was estimated below 10^{-5}. *S. coelicolor* WT strain M145 and *whiG* mutant C71 were used for microscopic observations. *Escherichia coli* MRF'307 (resistant to tetracycline and kanamycin) [6] was used as host for cloning and conjugation experiments.

Streptomyces strains were grown at 30 °C in Hickey–Tresner (HT) liquid or solid medium [32] and in YEME liquid medium for pulsed-field gel electrophoresis [16]. *E. coli* strains were grown at 37 °C in Luria–Bertani (LB) liquid or solid medium [38]. Conjugation between *E. coli* and *S. ambofaciens* was done on AS-1 medium [3]. Antibiotics were used at the following final concentrations: ampicillin at 50 μg/ml; apramycin, tetracycline and kanamycin at 30 μg/ml (Sigma).

The capacity of a strain to sporulate was determined either by observing colony fragments by light microscopy after Gram staining or in scanning electron microscopy (SEM) [26]. In this case, fragments were fixed with osmic acid (2%) in a desiccator for 48 h. After drying out, samples were pasted onto their support, metallized with palladium gold (SC7640 Sputter Coater Polaron) and then observed in SEM (Cambridge Stereoscan S240).

2.2. Plasmids and conjugation procedures

DNA of plasmids pIJ6204, pIJ2157, pIJ6301, pIJ6201 and pIJ6205 (kindly provided by Professor K. Chater) containing, respectively, *S. coelicolor* genes *whiA*, *whiB*, *whiG*, *whiH* and *whiJ* was digested by *Xba*I to release a fragment containing the corresponding *whi* gene which