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SHORT COMMUNICATION

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A novel *Streptomyces* gene, *samR*, with different effects on differentiation of *Streptomyces ansochromogenes* and *Streptomyces coelicolor*

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Abstract A 1.4-kb DNA fragment from *Streptomyces ansochromogenes* accelerated mycelium formation of *S. ansochromogenes* when present on a multicopy plasmid. The DNA fragment contains one complete open reading frame, designated *samR*, encoding a protein with 213 amino acids that contains a likely DNA-binding helix-turn-helix motif close to its N-terminus. The deduced SamR protein resembles the product of the *hppR* gene, which is involved in the regulation of catabolism of 3-(3-hydroxyphenyl) propionate in *Rhodococcus globerulus*. A *samR* disruption mutant was constructed that presented a bald phenotype and failed to form aerial hyphae and spores. We suggest that *samR* plays an important role in the emergence of aerial hyphae from substrate mycelium. An almost identical gene of *Streptomyces coelicolor* was also subjected to gene disruption. Surprisingly, the mutant was able to develop an aerial mycelium, but it remained white and deficient in sporulation instead of forming gray spores.

Keywords *Streptomyces* differentiation · *samR* gene · Gene disruption

Introduction

Streptomycetes are aerobic gram-positive soil bacteria that undergo morphological and physiological differentiation in response to environmental factors (Chater 1989). *Streptomyces ansochromogenes* was isolated from soil in the northeast of China. The main antibiotics produced by this strain are nikkomycins X and Z. Nikkomycin, a nucleoside peptide antibiotic and competitive inhibitor of chitin synthetase, inhibits the biosynthesis of chitin in cell walls due to its structural resemblance to UDP-*N*-acetylglucosamine (Brillinger 1979; Mueller et al. 1981). The antibiotic inhibits the growth of fungi, insects and acarids, is non-toxic to mammals, plants and bees, and is easily degraded in nature (Hector et al. 1990). *S. ansochromogenes* has a typical developmental process from substrate mycelium to spores, though the aerial hyphae of this strain differ from those of *Streptomyces coelicolor* in their absence of tight coiling. Some of the genes involved in aerial growth may therefore differ between the two species. For this reason, it is quite interesting to study the interplay of morphological differentiation and antibiotic biosynthesis in *S. ansochromogenes*, which involves a complex genetic network of regulations rather than a simple linear cascade (Chater 1989).

We previously described a *proX*-like gene of *S. coelicolor* that is controlled by the sporulation-specific sigma factor σ^{whiG} (Tan et al. 1998), which is encoded by the *whiG* gene and is essential for the initiation of sporulation. Here, we describe gene disruption experiments which show that a gene (*samR*) located downstream of the *proX*-like gene plays important, but somewhat different, roles in the development and differentiation of *S. ansochromogenes* and *S. coelicolor*.

Materials and methods

Bacterial strains, plasmids, phages and media

The following strains were used: *Escherichia coli* JM109 and JM101, *Streptomyces ansochromogenes* 7100 (wild-type strain) and *Streptomyces coelicolor* J1501 (*hisA1 uraA1 strA1* Pgl$^-$ SCP1$^-$ SCP2$^-$ and a wild-type strain for morphological differentiation; Chater et al. 1982). The vector systems used were: pBluescript M13, the helper phage KO7 (Vieira and Messing 1987), and plasmids pIJ702, pSET152 and pKC1139 (temperature- sensitive replicon, *E. coli-Streptomyces* shuttle plasmid, Kieser et al. 2000). LB medium was prepared following Sambrook et al. (1989). The liquid growth medium YEME, the protoplast regeneration medium R2YE and the minimal medium (MM) were prepared according to Hopwood et al. (1985).

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DNA manipulation

Plasmid and chromosomal DNAs were isolated according to Hopwood et al. (1985) and Tan and Chater. (1993); DNA fragments were purified using the method in Tan et al. (1998). Single-stranded DNA was prepared according to Tan and Chater (1993); DNA sequencing and analysis were done according to Sanger et al. (1977) and Bibb et al. (1984). The Southern blotting procedure was described by Hopwood et al. (1985). The procedure used to transform *E. coli* was described by Sambrook et al. (1989) and that for *Streptomyces* by Hopwood et al. (1985).

Enzymes, antibiotics and chemical reagents

The restriction enzymes and T4 DNA ligase were purchased from Sino-American Biotechnology; exonuclease III and nuclease were from Promega. The T7 sequencing and Taq sequencing kits were from Amersham Pharmacia and Promega, respectively. The working concentration of ampicillin was 50 μg/ml. Apramycin, a gift from Dr. Brenda Leskiw (University of Alberta, Canada), was dissolved in H_2O to give the working concentrations 30 μg/ml for R2YE, and 5 μg/ml for MM and for YEME. Polyethyleneglycol 1000 (Merk-Schuchard) was used for *Streptomyces* protoplast transformation. Both 5-bromo-4-chloro-3-indolyl-β-D-galactose and isopropyl-β-D-thiogalactopyranoside were used for transformant selection in *E. coli* at working concentrations of 40 μg/ml in LB medium. α-^{32}P-dCTP (Yahui Biological and Medical Technology, Beijing) was used for DNA sequencing. The non-radioactive digoxigenin-11-dUTP kit (Boehringer Mannheim) was used for probing DNA.

Nucleotide sequence accession numbers

The nucleotide sequences of *samR* and *samR*-like genes have been submitted to GenBank under accession numbers AF 369921 and AF 170560, respectively.

Results

Cloning of a DNA fragment accelerating differentiation

A DNA library of *S. ansochromogenes* was constructed in *E. coli*. A 400-bp DNA fragment located downstream of the *proX*-like gene of *S. coelicolor* A3(2) was used as a probe (Tan et al. 1998); a 4.6-kb DNA fragment was then cloned and analyzed by restriction enzyme digestions. A 1.4-kb *Pvu*II fragment of the cloned DNA was inserted into pIJ702 (using the *Pst*I site rendered blunt by treatment with Klenow enzyme). The resulting recombinant plasmid was then introduced into *S. ansochromogenes* 7100. The transformants gave rise to abundant gray spores after incubation for 3 days, whereas the parent *S. ansochromogenes* strain gave rise to white aerial hyphae without gray spores at this time (at least 5 days were needed for spores to become detectable). The 1.4-kb DNA fragment inserted into a high-copy-number plasmid (pIJ702) can thus accelerate *S. ansochromogenes* differentiation.

DNA sequencing and analysis

The nucleotide sequence of the 1.4-kb *Pvu*II DNA fragment was determined. The 1479-bp DNA fragment contains one complete ORF with a potential start codon at position 485 (GTG). The G+C content of the whole sequence is 74 mol%, and a typical ribosome-binding site (GGAG) is located at 9–12 base positions from the start codon. The deduced protein consists of 213 amino acid residues.

Fig. 1A, B Comparison of SamR, SamR-like and HppR proteins. SamR is encoded by *samR* from *Streptomyces ansochromogenes*; SamR-like by the *samR*-like gene from *Streptomyces coelicolor* J1501 and HppR by the *hppR* gene from *Rhodococcus globerulus*. **A** Alignment of SamR with HppR; identical residues are listed in the *middle*, similar residues are indicated by +. A likely DNA binding helix-turn-helix is close to the N-terminus. **B** Alignment of SamR with SamR-like; only the residues differing between the two proteins are listed in the *middle*, similar residues are indicated by + and non-conserved residues by *X*

A

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                                   Helix — turn — helix
SamR: 6    SQTLDRGLRVLKLLADTDHGLTVTELSTKLGVNRTVVYRLLATLEQHALVRRDLGGRARV 65
           SQTL RGL+ L+L+A T  G+T+ E++  LGV+RT+  RLL T+    L++R   G+ R
HppR: 30   SQTLARGLQALELVATTPDGMTIQEVADALGVHRTIASRLLTTVADFRLIKRGADGKYRA 89

SamR: 66   GLGVLRLGRQVHPLVREAGLPALASLAEDIGATAHLTLVDGSEALAVAVVEPTWTDYHVA 125
           G G+  L R ++  +R+   P L  LA  +GA+  L + +GSEA+AVAVVEP    Y V+
HppR: 90   GGGLAALARDLYAGLRDEATPLLRRLANSLGASVALFVAEGSEAVAVAVVEPKNARYWVS 149

SamR: 126  YRAGFRHPLDRGAAGRAILA---------ARQSPSADPGYTLTHGELEAGATG 169
           +R G RHP+DRGAAG A+LA         +R + +   GY +++GE+E G  G
HppR: 150  FREGGRHPIDRGAAGYALLAGQPPVPGESSRVTDARRDGYVVSYGEVEPGYWG 202
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B

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                                   Helix — turn — helix
SamR:       1   MTAETSQTLDRGLRVLKLLADTDHGLTVTELSTKLGVNRTVVYRLLATLEQHALVRRDLG 60

SamR-like:  1   MTAETSQTLDRGLRVLKLLADTDHGLTVTELSTKLGVNRTVVYRLLATLEQHALVRRDLG 60

SamR:       61  GRARVGLGVLRLGRQVHPLVREAGLPALASLAEDIGATAHLTLVDGSEALAVAVVEPTWT 120
                                          X+                +
SamR-like:  61  GRARVGLGVLRLGRQVHPLVREAGLPALRALAEDIGATAHLTLVDGAEALAVAVVEPTWT 120

SamR:       121 DYHVAYRAGFRHPLDRGAAGRAILAARQSPSA--------DPGYTLTHGELEAGATGAAA 172
                                          X  XXXXXXXXX
SamR-like:  121 DYHVAYRAGFRHPLDRGAAGKAILAARQ-PSQPTGGEAVDDPGYTLTHGELEAGACGAAA 179

SamR:       173 PLVGVTGVEGSVGVVMLADAVPERVGERVVEAAREVAEALR 213
                  +                X         XX
SamR-like:  180 PLLGVTGVEGSVGVVMLADVVPERVGERVKHAAREVAEALR 220
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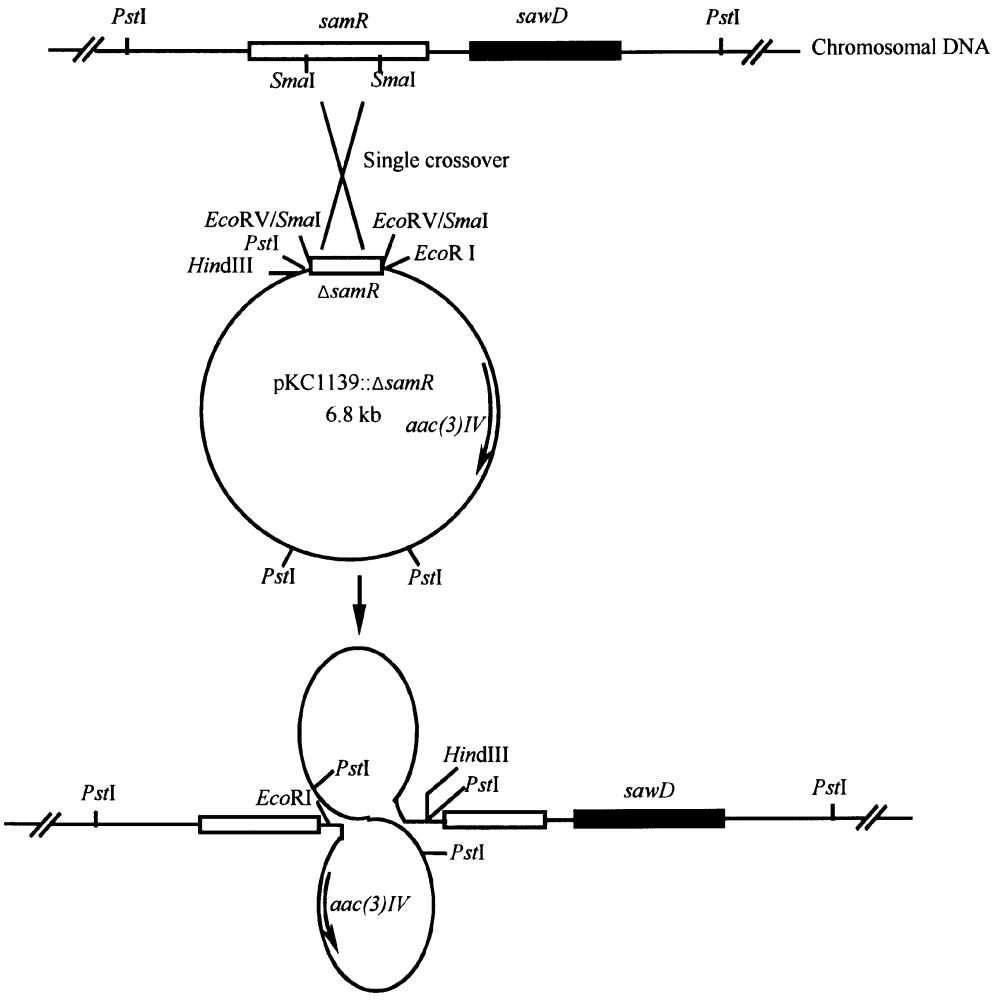

Fig. 2 The scheme of gene disruption and the relevant restriction sites. An internal *Sma*I DNA fragment of *samR* gene was inserted into the *Eco*RV site of pKC1139, resulting in recombinant plasmid pKC1139::Δ*samR*, which was used for gene disruption. The *Pst*I sites were employed to check the pattern of the disruption mutants after gene crossover. *aac(3)IV* is an apramycin-resistance gene

In database searches (Altschul et al. 1997), the deduced protein was homologous with that encoded by the *hppR* gene of *Rhodococcus globerulus* (Fig. 1A) (42% amino acid sequence identity and 61% amino acid sequence similarity). The product of *hppR* is a protein that regulates the degradation of 3-(3-hydroxphenyl)propionate (Barnes et al. 1997); a likely helix-turn-helix DNA-binding motif was found in the N-terminal region (Fig. 1). Therefore, the product of this gene may be also a regulatory protein. The gene was designated *samR* (*Streptomyces* aerial mycelium regulator).

Gene disruption of *samR*

A gene-disruption experiment was carried out in order to clarify the function of *samR*. The 271-bp internal *Sma*I fragment of *samR* was inserted into the *Eco*RV site of pKC1139, resulting in a recombinant plasmid pKC1139::Δ*samR* (Fig. 2), which was introduced into *S. ansochromogenes* 7100. The transformants were further examined by plasmid isolation and restriction enzyme digestions. The transformants were then grown on MM at 28 °C to prepare spore suspensions. A 0.1-ml aliquot of each spore suspension (10^8/ml) was spread on MM agar containing apramycin, and about 100 putative disruption mutants were obtained after incubation at 39 °C.

DNA from both the wild-type strain and the disruption mutants was digested with *Pst*I (Fig. 2), and transferred to nylon membrane after agarose gel electrophoresis. The results of Southern hybridization showed that the DNA from the wild-type strain displayed a 4.6-kb positive signal, but the DNA from the disruption mutants had the predicted 3.6-kb and 5.3-kb signals (data not shown).

The biological function of *samR* and *samR*-like genes

The *samR*-disruption mutants grown on MM agar supplemented with either glucose or mannitol as carbon source failed to form aerial hyphae (Fig. 3C, D), whereas abundant spores were formed by the wild-type strain 7100 after incubation at 28 °C for 6 days (Fig. 3A, B). The morphological features of the mutants following incubation at 28 °C for 6 days were also observed by phase-contrast microscopy using impression preparations (Chater 1972) (data not shown). The mutants showed no sign of aerial hyphae or spore formation, suggesting that *samR* plays an

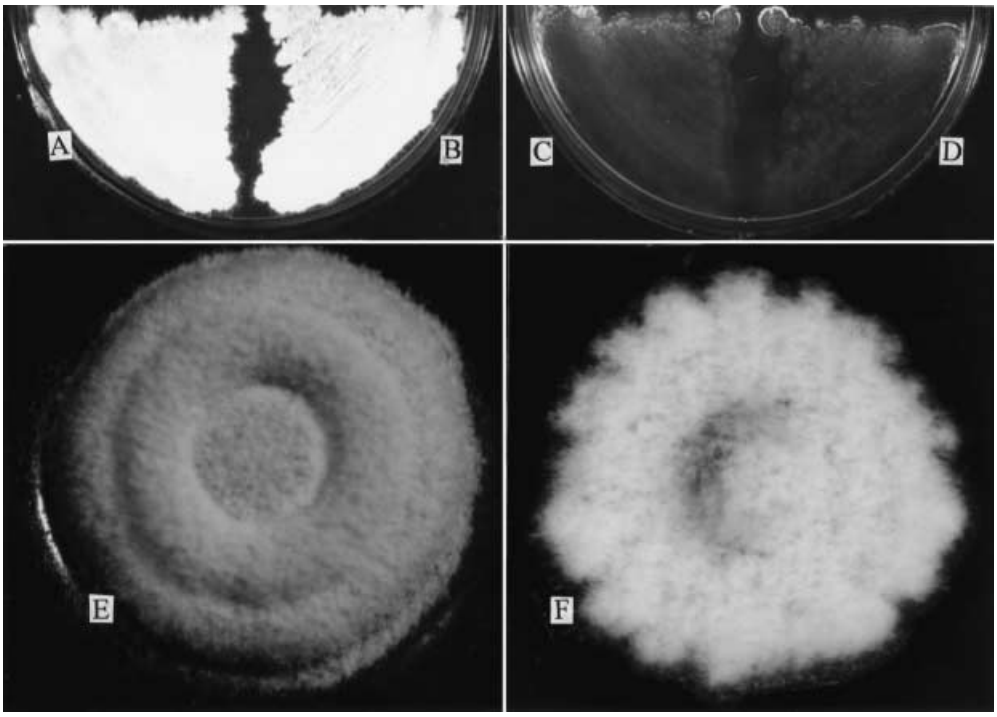

Fig. 3A–F The effect of *samR* and *samR*-like gene disruption on morphological differentiation. **A** *S. ansochromogenes* 7100 (wild-type strain), **B** *S. ansochromogenes* 7100 (pKC1139), **C, D** *samR* disruption mutants, **E** *S. coelicolor* J1501, **F** *samR*-like gene disruption mutant. All plates are shown after incubation at 28 °C for 6 days, on minimal medium (MM) agar supplemented with 1% mannitol as carbon source

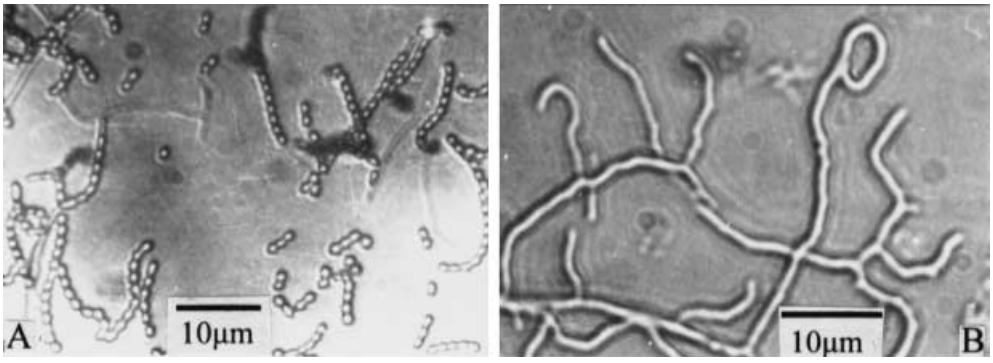

Fig. 4A, B Morphological feature of *samR*-like disruption mutant and wild-type strain. **A** Spores and spore chains of *S. coelicolor* J1501 (wild-type), **B** aerial hyphae of *samR*-like disruption mutant. Both strains were grown on minimal medium supplemented with 1% mannitol at 28 °C for 6 days and observed by phase-contrast microscope. *Bars* (10 μm) indicate the size of spore chains and aerial hyphae

important role in developmental changes from substrate mycelium to aerial hyphae in *S. ansochromogenes*. The mutants could be complemented by the complete *samR* gene present on pIJ702 and displayed abundant gray spores. They could also be complemented by the complete *samR* gene cloned on a single-copy-number vector (pSET152) which resulted in a phenotype similar to that of wild-type strain 7100.

The *samR*-like gene from *S. coelicolor* J1501, which is located in the cosmid E51 and in the *Ase*I E fragment of the chromosomal physical map, encodes 220 amino acids, and was also cloned and sequenced. The deduced proteins from *samR* and from the *samR*-like gene have about 91% amino acid identity, the main difference being near the C-terminal region (Fig. 1B). The function of the *samR*-like gene was also studied by gene disruption. To do this, an internal fragment (nucleotides 107–502 of the coding sequence) was amplified by PCR and inserted into pKC1139 (*Eco*RV site). Subsequent steps were as described for *S. ansochromogenes*. Surprisingly, the *samR*-like gene disruption mutants had a white phenotype in comparison with the gray phenotype of the wild-type strain (*S. coelicolor* J1501) or a *S. coelicolor* J1501(pKC1139) control under the same conditions (Fig. 3E, F). Phase-contrast microscopy showed that the *samR*-like disruption mutant displayed the morphological feature of aerial hyphae which were straight with a little bit of coiling and non-sporulating (Fig. 4B), whereas the wild-type strain formed spore chains and free spores after incubation at 28 °C for 6 days (Fig. 4A). The *samR*-like disruption mutant could be complemented by the complete *samR*-like gene when present on a single copy-number plasmid (pSET152) and showed the phenotype of gray spores as in the wild-type strain J1501. The *samR*-like gene, when present on a multicopy plasmid (pIJ702), could also accelerate spore formation of *S. coelicolor* J1501, which manifested abundant gray spores.

Discussion

The regulation of carbon utilization influences the expression of genes involved in morphological development and in antibiotic production by *Streptomyces* species. Differentiation in *Streptomyces* is a complicated process, which includes a regulatory network consisting of many genes. In *Streptomyces coelicolor*, all characterized *bld* mutants fail to form aerial hyphae on MM agar supplemented with glucose as sole carbon source, whereas in most of them spore formation is partially restored on MM agar supplemented with mannitol (Pope et al. 1996). In our experiments, the bald phenotype of *samR* mutants of *S. ansochromogenes* was not affected by carbon sources such as glucose and mannitol.

The *samR* gene shares a high similarity with the regulatory gene *hppR* of *R. globerulus* (Barnes et al. 1997), but high similarity with regulatory genes other than *hppR* was not found in database searches. The *hppR* gene is a member of the *iclR* family, which plays an important role in the catabolic pathway of aromatic compounds, suggesting that *samR* may be an essential gene for the environmental response in *Streptomyces* development and differentiation. This may be important for future studies of the molecular mechanisms of these processes.

Further studies (unpublished) showed that another gene, *sawD*, is located downstream of *samR* and encodes a serine protease. Disruption of this gene resulted in the white phenotype of disruption mutants in contrast to the gray phenotype of the wild-type strain (unpublished result), and it may be interesting to look for possible interactions between *samR* and *sawD* in *S. ansochromogenes* differentiation.

samR-like gene disruption mutants in *S. coelicolor* J1501 produced aerial hyphae, but failed to form spores. This result was unexpectedly different from that with the *samR* disruption mutant of *S. ansochromogenes* under the same conditions. It suggests that the *samR* and *samR*-like genes are controlled by different regulatory mechanisms in the development and differentiation of different *Streptomyces* species.

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