Genetic and physiological characterization of rpoB mutations that activate antibiotic production in Streptomyces lividans

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Antibiotic production in Streptomyces lividans can be activated by introducing certain mutations (rif) into the rpoB gene that confer resistance to rifampicin. Working with the most typical (rif-17) mutant strain, KO-417, the rif-17 mutation was characterized. The rif-17 mutation was shown to be responsible for activating antibiotic production and for reducing the growth rate of strain KO-417, as demonstrated by gene-replacement experiments. Gene-expression analysis revealed that introduction of rif into S. lividans elevates expression of the pathway-specific regulatory gene actII-ORF4 to nearly the same level seen in Streptomyces coelicolor. The rif effect on antibiotic production was still evident in the genetic background of relC, indicating that the rif mutation can provoke its effect without depending on ppGpp. Accompanying the restoration of antibiotic production, rif mutants also exhibited a lower rate of RNA synthesis compared to the parental strain when grown in a nutritionally rich medium, suggesting that the mutant RNA polymerases may behave like ‘stringent’ RNA polymerases. These results indicate that the rif mutation can alter the gene-expression pattern independent of ppGpp. The impaired growth of strain KO-417 (rif-17) was largely restored by introducing the second rif mutation (rif-18) just adjacent to the rif-17 position. Proteome analysis using two-dimensional PAGE revealed that the rif mutant strain KO-418 (rif-17 rif-18) displayed a temporal burst of expression especially of two enzymes, glutamine synthetase (type II) and oxidoreductase, during the late growth phase.

Keywords: RNA polymerase, stringent response

INTRODUCTION

Antibiotic production in Streptomyces spp. is believed to occur in a growth-phase-dependent manner (Martin & Liras, 1989; Demain & Fang, 1995; Bibb, 1996). The initiation of antibiotic biosynthesis usually starts at the transition between vegetative growth and morphological development (Chater, 1993). While the physiological signals and underlying regulation mechanisms that regulate antibiotic production remain to be fully elucidated, most of the published data are consistent with the intrinsic role of growth rate (the cessation of growth) on the onset of secondary metabolism (Champness & Chater, 1994). Streptomyces coelicolor A3(2), the most frequently studied species of Streptomyces, takes advantage of four kinds of antibiotics that it produces, including the pigmented antibiotics actinorhodin (Act) and undecylprodigiosin (Red). Streptomyces lividans is a close relative of S. coelicolor A3(2) and its merits include fast growth, good sporulation and almost no restriction of foreign DNA (Kieser et al., 2000). A large proportion of the S. lividans genome is identical to that of S. coelicolor A3(2), and the established genetic and physical maps of these two species are well-aligned to each other (Leblond et al., 1993). However, the Act and Red genes are normally poorly expressed in S. lividans. Why S. lividans cannot produce Act, even though it carries the entire biosynthetic gene clusters required for...
production of this antibiotic, is a point of interest. How to activate this silent antibiotic biosynthetic gene cluster can also give some clues about the regulation system for antibiotic production. We have previously reported that can also give some clues about the regulation system for to activate this silent antibiotic biosynthetic gene cluster production of this antibiotic, is a point of interest. How

**METHODS**

**Bacterial strains, plasmids and culture conditions.** The *S. lividans* strains used in this study are listed in Table 1. Spontaneous rif mutants from *S. lividans* were obtained from colonies that grew within 7 days of spores being spread onto

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**Table 1. *S. lividans* strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1326</td>
<td>Prototrophic wild-type</td>
<td>D. A. Hopwood*; Kieser <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>KO-417</td>
<td>rif-17</td>
<td>Rif-resistant isolate from 1326; Hu <em>et al.</em> (2002)</td>
</tr>
<tr>
<td>KO-418</td>
<td>rif-17 rif-18</td>
<td>Double Rif-resistant isolate from KO-417; this study</td>
</tr>
<tr>
<td>KO-419</td>
<td>rif-17 rif-12</td>
<td>Double Rif-resistant isolate from KO-417; this study</td>
</tr>
<tr>
<td>KO-420</td>
<td>rif-positive</td>
<td>Spontaneous revertant isolate from KO-417; this study</td>
</tr>
<tr>
<td>KO-421</td>
<td>relC</td>
<td><em>rplK</em> mutant of 1326; this study</td>
</tr>
<tr>
<td>KO-422</td>
<td>relC rif-1</td>
<td>Rif-resistant isolate from KO-421; this study</td>
</tr>
<tr>
<td>KO-423</td>
<td>relC rif-2</td>
<td>Rif-resistant isolate from KO-421; this study</td>
</tr>
</tbody>
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**Fig. 1.** Growth and Act production in the *S. lividans* wild-type and mutant strains. Spores were spread on R4 agar and then incubated at 30 °C for 3 days. Blue colour represents the antibiotic Act.
tide chain termination method using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer).

**Gene-replacement analysis of rpoB.** The procedure used for gene-replacement analysis is illustrated in Fig. 4. Total chromosomal DNA was prepared from the two rif mutants KO-417 (rif-17 Act-positive) and KO-418 (rif-17 rif-18 Act-positive). A 4354 bp SacI–SacI fragment from KO-417 and KO-418 was cloned into the SacI site of the multiple-cloning sites in a pBluescriptSK(+)-vector to generate pLC1 and pLC2, respectively. The insert includes the rpoB coding region but lacks 15 aa residues at the carboxyl terminus. A 1.1 kb EcoR1–EcoR1 fragment containing the apramycin-resistance gene (aac(3)IV; accession no. X99319) was cloned into both pLC1 and pLC2 in a step-wise manner, generating pLC3 and pLC4, respectively. Plasmids pLC3 and pLC4 were passaged through the methylation-deficient E. coli strain DM1 (dam dem) and introduced into S. lividans strains as described previously (Kieser *et al.*, 2000). The R2YE plates were flooded with 1 ml of an apramycin (Sigma) solution, to give a final concentration of 50 µg ml⁻¹. Integration and looping-out of the plasmids by homologous recombination were confirmed by Southern hybridization.

**Western blotting and RT-PCR analysis.** Western analysis was carried out as described previously (Hu *et al.*, 2002). RT-PCR was carried out by using the Thermoscript RT-PCR System Kit (Invitrogen).

**Assay for ppGpp and determination of RNA synthesis.** The intracellular ppGpp content was assayed as described by Ochi (1987) using HPLC analysis. RNA synthesis after Casamino acid deprivation or during growth in a Casamino acid medium was determined by measuring [2-¹⁴C]uracil incorporation into acid-precipitable material, as described previously (Ochi, 1990a).

**Two-dimensional gel electrophoresis.** Cells were collected from a GYM plate covered with a cellophane sheet. They were then disrupted by sonication three times for 30 s on ice, and centrifuged at 14000 g for 20 min. The supernatants were used as protein extracts and 150 µg total protein from each sample was applied to an Immobiline Dry Strip (pH 4–7, 18 cm; Amersham Pharmacia) for isoelectric focusing using the Multiphor II Electrophoresis Unit (Amersham Pharmacia). An ExcelGel (XL SDS 12–14%; Amersham Pharmacia) was applied to an Immobiline Dry Strip (pH 4–7, 18 cm; Amersham Pharmacia) for isoelectric focusing using the Multiphor II Electrophoresis Unit (Amersham Pharmacia). An ExcelGel (XL SDS 12–14%; Amersham Pharmacia) was used for the second dimension SDS-PAGE.

**Peptide-mass-fingerprinting analysis and N-terminal-sequencing analysis.** The gels were stained with Coomassie blue. Spots of interest were cut out and subjected to peptide-mass-fingerprinting analysis and N-terminal-sequencing analysis. Gel pieces were washed and dried under vacuum, before being digested with trypsin (Promega). After trypsin treatment, peptides were extracted with 25 µl of 50% acetonitrile.

### Table 2. Position of mutations in rpoB of S. lividans

<table>
<thead>
<tr>
<th>Strain</th>
<th>Position of mutation in rpoB*</th>
<th>Amino-acid position</th>
<th>Amino-acid exchange</th>
<th>Resistance to Rif (µg ml⁻¹)†</th>
<th>Act production‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1326</td>
<td>-§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO-417</td>
<td>1318 (C→T)</td>
<td>440</td>
<td>Arg→Cys</td>
<td>&gt;250</td>
<td>++ +</td>
</tr>
<tr>
<td>KO-418</td>
<td>1318 (C→T)</td>
<td>440</td>
<td>Arg→Cys</td>
<td>&gt;250</td>
<td>++</td>
</tr>
<tr>
<td>KO-419</td>
<td>1280 (A→C)</td>
<td>427</td>
<td>Asp→Ala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO-420</td>
<td>Reversion of 1318 (T→C)</td>
<td>427</td>
<td>Asp→Asn</td>
<td>&gt;250</td>
<td>++</td>
</tr>
<tr>
<td>KO-421</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO-422</td>
<td>1309 (C→T)</td>
<td>437</td>
<td>His→Tyr</td>
<td>&gt;250</td>
<td>++</td>
</tr>
<tr>
<td>KO-423</td>
<td>1310 (A→G)</td>
<td>437</td>
<td>His→Arg</td>
<td>&gt;250</td>
<td>++</td>
</tr>
</tbody>
</table>

* Numbering originated from the start codon of the ORF.
† Determined after 3 days incubation on GYM agar.
‡ Determined after 5 days incubation on R4 agar. —, No production; ++, considerable production; ++++, abundant production.
§ Wild-type rpoB gene.
acetonitrile/5% trifluoroacetic acid (TFA). The extracts were dried by using a Speed-Vac and reconstituted by adding 6 μl of 50% acetonitrile/0.1% TFA. Resulting samples were spotted onto a MALDI-TOF/MS sample target with a–cyano-4-hydroxy-cinnamic acid (Fluka). Angiotensin II (human; Sigma) and insulin chain B (bovine; Sigma) were used for external calibration. Samples were analysed using a REFLEX II MALDI mass spectrometer (Bruker). Mascot (Matrix Science) was used to identify the protein from the mass data. For N-terminal-sequencing analysis, gel spots from the two-dimensional polyacrylamide gels were blotted onto a PVDF membrane. After staining, the spots were cut out and the membrane was subjected directly to N-terminal-sequencing analysis using a G1000A Protein Sequencer (Hewlett Packard).

RESULTS

rif mutant KO-417 has a growth defect
The rif mutant KO-417 (previously designated RC-1) has a mutation (rif-17) that alters Arg140 to Cys in the RNA polymerase β-subunit; phenotypically, this mutant strain demonstrates the most potent ability to activate production of both the blue-coloured antibiotic Act and the red-coloured antibiotic Red (Hu et al., 2002). We first confirmed this fact using R4 medium (Fig. 1). We also found that KO-417 has significantly impaired growth, as determined by growth of the strain in GYM liquid medium (Fig. 2). After serial transplantation of KO-417 onto GYM plates without Rif, growth-compensatory mutants were generated at a high frequency. Two types of mutants were generated. One had the ability to produce antibiotics, and mutants belonging to this type were found to have an additional rpoB mutation adjacent to the original rif mutation (as represented by KO-418 and KO-419); the other lost the ability to produce antibiotics, and mutants belonging to this type were found to simply revert to the wild-type (as represented by KO-420) (Table 2). The latter type no longer showed resistance to Rif. The restoration of growth was confirmed using liquid GYM medium to grow strain KO-418 (rif-17 rif-18) (Fig. 2). It should be noted that the rif double mutant KO-418 (and KO-419) still retained the ability to produce Act, although the amounts of Act produced by this strain were less than those produced by the original rif mutant, KO-417 (Fig. 1). KO-418 (and KO-419) also displayed restoration of sporulation – sporulation was severely impaired in the original strain, KO-417 (Fig. 1). The mutant strains revealed a normal ability to accumulate ppGpp, as determined by using KO-417 and KO-418 in a nutritional-shift-down assay (Fig. 3a).

rif-17 and rif-18 are responsible for the altered phenotypes
We next conducted gene-replacement analysis of the rif mutation. The mutant rpoB alleles from KO-417 (rif-17 Act-positive) and KO-418 (rif-17 rif-18 Act-positive) were cloned into a pBluescriptSK(+) vector to generate pLC1 and pLC2, respectively. Plasmids for gene replacements were constructed by inserting an apramycin-resistance cassette into pLC1 and pLC2. The resulting plasmids, pLC3 (containing rif-17) and pLC4 (containing rif-17 rif-18), were introduced into S. lividans 1326 by protoplast transformation. Apramycin-resistant transformants were selected and passed through three rounds of non-selective cultivation on GYM agar to facilitate the second crossover. As illustrated in Fig. 4, among the apramycin-sensitive double-crossed recombinants that were derived from a single-crossed type-B recombination (aprampcin-resistant, Rif-sensitive, Act-negative), we could identify two types of recombinants – type I, in which the mutant rpoB gene sequence was present, and type II, which contained the wild-type rpoB sequence. The ratio of type I (aprampcin-sensitive, Rif-resistant) to type II (aprampcin-sensitive, Rif-sensitive) recombinants was 40% for pLC4, which is in agreement with the original rif mutation.
RNA polymerase mutations in *S. lividans*

with the length of the homologous fragment downstream or upstream of the *rif* mutation points, while the ratio of type I to type II recombinants was as low as 8% for pLC3, which was apparently due to the hampered growth of the *rif-17* recombinants. In both cases, the Rif-resistant recombinants exhibited the Act-positive phenotype, while Rif-sensitive recombinants exhibited the Act-negative phenotype. The allele replacements were confirmed by DNA-sequence analysis (data not shown). The Rif-resistant recombinants, which were generated using pLC4 (containing *rif-17 rif-18*), grew as well as the wild-type strain, while the Rif-resistant recombinants, which were generated using pLC3 (containing *rif-17*), all grew slowly. Thus, we conclude that the mutant *rpoB* allele (*rif-17*) is responsible for Rif resistance, the observed growth defect and for activation of Act production in *S. lividans*, while the *rif-18* mutation is responsible for the observed restoration of growth.

**rif effect on relC mutation**

For *S. coelicolor* (and for other *Streptomyces* spp.), the introduction of a certain *relC (= rplK)* mutation into the organism severely restricts its ability to produce Act and Red, due to its failure to synthesize ppGpp (Ochi, 1990a, b; Kawamoto *et al.*, 1997). The *S. lividans* wild-type strain 1326 is able to accumulate ppGpp upon nutritional shift-down (Ochi, 1989). To determine the efficacy of the *rif* mutation in the *relC* background, we screened a *relC*-type mutant from *S. lividans* 1326, as described in Methods. Among the 27 thiopeptin-resistant mutants that developed spontaneously, we found a possible *relC* mutant which had a 6 bp deletion in *rplK*, which resulted in the loss of the dipeptide Val<sup>27</sup>Gly<sup>28</sup> in the ribosomal protein L11. This mutation is of the same type as that detected in the *S. coelicolor* *relC* mutant KO-100 (Ochi *et al.*, 1997). As expected, the presumptive *relC* mutant (designated KO-421) displayed

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Fig. 4. Gene replacement of the *rpoB* locus by integration of pLC3 or pLC4, which contained single- or double-mutated *rpoB* alleles. *rif*<sup>R</sup> indicates the mutated *rpoB* gene. The solid bar in the middle of *rpoB* represents the mutation site.
a significantly reduced ability to accumulate ppGpp as compared to the wild-type strain (Fig. 3a), and showed the relaxed response with respect to RNA synthesis upon nutritional shift-down (Fig. 3b). Thus, the thio-
strepton-resistant mutant KO-421 is a typical relC mutant.

Strikingly, among the rif mutants that were generated spontaneously from the relC strain KO-421, the Act-
positive phenotype was detected at a frequency as high as 80% (81 out of 101). Two representative mutants, KO-422 (relC rif-1) and KO-423 (relC rif-2), can be found in Table 1. Both mutants possess a point mutation within rpoB, as detected by DNA sequencing (Table 2). Act production by the relC rif double mutant KO-422 is shown in Fig. 5, as an example. These results, together with the results from the ppGpp assay, indicate that the rif effect on antibiotic production activation can be provoked even in the relC genetic background.

Expression analysis of actII-ORF4

Stationary-phase production of Act by S. coelicolor A3(2) is transcriptionally regulated (Takano et al., 1992; Gramajo et al., 1993). The ActII-ORF4 protein, which is encoded by the actII-ORF4 gene, has been characterized as a DNA-binding protein that positively regulates the transcription of the Act biosynthesis gene in S. coelicolor A3(2). We analysed the expression pattern of actII-ORF4 by Western blotting and by RT-
PCR, comparing the mutants with the wild-type strain. The expression level of actII-ORF4 in the S. lividans
wild-type strain 1326 was threefold lower than that seen in S. coelicolor A3(2), but increased about fourfold by introducing the rif mutation rif-17 into strain 1326, in agreement with work by Hu et al. (2002). In contrast, expression of actII-ORF4 was found to be severely suppressed in the relC strain KO-421 (data not shown). However, introduction of the rif mutation into the relC strain provoked expression of actII-ORF4 to the level of the rif mutant KO-417, both on the transcription and translation levels (data not shown), thus accounting for the obvious production of Act in the relC rif double mutant.

Effect of Casamino acid deprivation on RNA synthesis

The rel mutants, including relA and relC, are charac-
terized by the relaxed response (i.e. failure to inhibit stable RNA synthesis) to amino-acid limitation (Ochi, 1990a, b). We hypothesized that the rif mutations that conferred the Act-positive phenotype may behave like ‘stringent’ RNA polymerases during growth in nutritionally rich media. To assess this possibility, we analysed the rate of RNA synthesis during growth in a nutritionally rich medium (i.e. synthetic medium supplemented with 2% Casamino acids), using the rif mutants KO-417 and KO-418 (Fig. 6a). These strains were grown to mid-exponential phase [100 mg dry cell wt (100 ml culture)−1], and then [2-14C]uracil was added to the culture, followed by a further 60 min incubation. Strikingly, rif mutant KO-417 revealed a fourfold reduction in RNA synthesis, when compared to the wild-type strain. The rif double mutant KO-418 exhibited a less-pronounced reduction in RNA synthesis, but the reduction was still significant (Fig. 6a) despite having no discernible effect on growth (see Fig. 2). Likewise, the relC rif double mutant KO-422 exhibited significantly reduced RNA synthesis compared to the parental relC strain KO-422 (Fig. 6b), although the mutant also showed the relaxed response upon nutritional shift-down (Fig. 3b), reflecting the inability of the mutant to accumulate normal levels of ppGpp (Fig. 3a). Thus, the rif mutants exhibiting the Act-positive phenotype have a RNA polymerase with reduced activity for RNA synthesis during their growth in a nutritionally rich medium.

Total-protein analysis in mutant and wild-type strains

We next searched for proteins that were responsible for the remarkable differences between the rif mutants and the wild-type strain. We employed the rif double mutant
KO-418 (rif-17 rif-18), because this mutant showed almost no impairment of growth. Strains were grown on GYM agar plates to the early (24 h), transition (36 h) or late (60 h) phases of growth. After the extraction of total proteins, the protein samples were analysed by two-dimensional PAGE. Eventually, we found several protein spots whose amounts differed substantially between the mutant and the wild-type strain. The difference between the spot patterns of the strains was especially pronounced in the cells grown to the transition phase (36 h), as shown in Fig. 7 (protein spots are designated by arrows). The two highlighted protein spots (A and B) were abundant in the rif mutant strain, but not in the wild-type strain. Peptide-mass-fingerprinting analysis and N-terminal-sequencing analysis, using a protein sequencer, both revealed that spot A represents glutamine synthetase (GenBank no. CAB43949), encoded by the glnII gene, and spot B represents oxidoreductase (GenBank no. CAC37883), encoded by the SCI1G7.08c gene. The burst of expression of these two proteins in the rif mutant was temporal, since no (or at most faint) spots were detectable in cells in the early or late phase of growth (data not shown).

**DISCUSSION**

We have previously reported that antibiotic production by *S. coelicolor* is dramatically activated by introducing certain mutations into *rpoB* that confer resistance to Rif (Hu & Ochi, 2001; Hu et al., 2002). Our principal findings in this study were that (i) the expression level of actII-ORF4 increases significantly by introducing certain rif mutations into *S. lividans*, irrespective of a relC-positive or relC genetic background, which is accompanied by activation of Act production and remarkable expression of oxidoreductase and glutamine synthetase, and (ii) mutant RNA polymerases behave like ‘stringent’ RNA polymerases. Although the wild-type strain of *S. lividans* does not produce Act, the relC mutation resulted in a significant reduction in actII-ORF4 expression. Therefore, we concluded that the dependence of *S. lividans* on ppGpp to express the key gene actII-ORF4 (and thus for Act production) can apparently be bypassed by a certain mutation in the RNA polymerase. The low level of ActII-ORF4 protein expressed in the wild-type strain of *S. lividans* apparently was not enough to activate Act biosynthesis, as compared with its expression in *S. coelicolor* (see Results).

The present study provides evidence for the significance of the growth rate of *S. lividans* in antibiotic produc-
tion; the growth rate of the organism was closely linked to the rate of RNA synthesis, as seen in the order wild-type strain > double rif mutant (KO-418) > single rif mutant (KO-417). It is therefore likely that both the timing and the extent of antibiotic production by Streptomyces spp. are crucially decided by the physiological status of the RNA polymerase within the cell. ppGpp, a mediator for the stringent response, has been demonstrated to directly bind to the RNA polymerase β-subunit in E. coli (Chatterji et al., 1998). Reddy et al. (1995) also provided evidence for the location of the ppGpp-binding site on the E. coli RNA polymerase and the proximity relationship with the rif-binding domain. Detailed mapping of the promoter recognition domain on the β-subunit has been done using a collection of various RNA polymerase β-subunit mutants, each with a single amino-acid substitution. Thus, the domains for Rif sensitivity, ppGpp sensitivity, promoter selectivity and σ assembly were found to be lined up along the rpoB gene, which encodes the RNA polymerase β-subunit (Ishihama, 1988). From the result obtained by X-ray analysis of the core RNA polymerase (Zhang et al., 1999), Toulakhonov et al. (2001) proposed that the binding of ppGpp is allosteric and that the binding site is modular. Previous reports, in which various bacteria have been studied, have demonstrated that mutations in rpoB are responsible for the acquisition of resistance to Rif (Aboshkiwa et al., 1995; Jin & Gross, 1988; Singer et al., 1993). Results recently obtained in E. coli show that RNA polymerase mutants selected to confer prototrophy to a rif Δ strain can mimic the effect of ppGpp on the wild-type RNA polymerase (Barker et al., 2001). Therefore, it is reasonable to consider that the RNA polymerase with a rif-type β-subunit may be structurally similar to an RNA polymerase that has been modified by ppGpp, because numerous genetic analyses revealed that rif mutations frequently circumvent the ppGpp phenotype. Indeed, as demonstrated in E. coli, the mutant RNA polymerase may have altered promoter selectivity (Ishihama et al., 1990). In particular, the ppGpp-independent ‘stringent RNA polymerases’ have been described and the model for linking the dual aspects of the stringent response has been proposed (Zhou & Jin, 1998). It is conceivable that the altered conformational status of the RNA polymerase resulting from rif-17 in S. lividans gave rise to different promoter selectivity (or affinity), directly or indirectly leading to the increased actII-ORF4 expression. Although the RNA polymerase with the rif-17 mutation behaved like a stringent RNA polymerase with respect to RNA synthesis (Fig. 6), we can not rule out the possibility that the mutant RNA polymerase generated different promoter selectivity that was capable of activating different pathways for the activation of antibiotic biosynthesis and, hence, did not behave as a stringent RNA polymerase. For instance, the clear difference in certain gene expression (see below) can be originated by a stringent RNA polymerase or simply by a modified promoter selectivity of the mutant RNA polymerase. Although the rif-17 mutation resulted in the abrogation of growth, which was apparently due to severe suppression of RNA synthesis (Fig. 6a), rif-18 (just adjacent to rif-17) could almost completely restore growth (Fig. 2). The effect of rif-18 on the β-subunit can also be explained by the subsequent alteration of the three-dimensional structure of this subunit.

The increase in the production of glutamine synthetase (type II) and oxidoreductase by introducing a rif mutation (Fig. 7) into S. lividans was dramatic. Glutamine synthetase, responsible for the synthesis of glutamine from NH₄⁺ and glutamate, is a key enzyme in NH₄⁺ assimilation and is regulated by nitrogen availability in microorganisms, including Streptomyces strains (Fisher, 1999). At least two types of glutamine synthetase exist in bacteria, GSI (encoded by gltA) and GSII (encoded by gltH). Enteric bacteria and Bacillus subtilis only possess the GSI type, but Streptomyces strains are known to possess the eukaryotic-type glutamine synthetase GSII as well as GSI (Weisschuah et al., 2000). The role of the GSI enzyme in nitrogen metabolism in Streptomyces spp. is unclear. In nitrogen-fixing bacteria, GSII is preferentially expressed during nitrogen-limited growth and nitrogen fixation (Fisher, 1992). There are many kinds of oxidoreductases in S. coelicolor, as assigned by the S. coelicolor genome-sequencing project (http://www.sanger.ac.uk/Projects/S_coelicolor). The oxidoreductase (GenBank no. CAC37883) that was highlighted in this study is encoded by the SC1G7.084 gene, which is located near the type I polyketide synthesis gene cluster. Although our results implicate the intrinsic role of glutamine synthetase and oxidoreductase in secondary metabolism in S. lividans, further investigations are required to establish a causal relationship between these two enzymes and secondary metabolism.

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References


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