J Ind Microbiol Biotechnol (2010) 37:673–679
DOI 10.1007/s10295-010-0710-0

ORIGINAL PAPER

Overexpression of ribosome recycling factor causes increased production of avermectin in *Streptomyces avermitilis* strains

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Received: 25 January 2010 / Accepted: 5 March 2010 / Published online: 30 March 2010

Abstract Ribosome recycling factor (RRF), encoded by *frr* gene, is involved in the release of ribosomes from the translational post-termination complex for a new round of initiation. In this study, the *frr* gene with either its own promoter or with *ermE*p* was cloned into a multi-copy vector, pKC1139, and a single-site integrative vector, pSET152, respectively. The resulting plasmids were transformed into *Streptomyces avermitilis* wild-type strain ATCC31267, avermectin high-producing mutant strain 76-02-e, and the engineered strain GB-165 that produces only avermectin B. The results showed that overexpression of *frr* increased avermectin yield (by 3- to 3.7-fold in the wild-type strain) and revealed an *frr* gene "copy number effect"; i.e., multiple copies of *frr* had a greater promoting effect on avermectin production than a single copy in each of the three transformed *S. avermitilis* strains. Comparison of the growth and expression of the *ave* genes in an *frr*-overexpressing strain and wild-type ATCC31267 indicated that *frr* overexpression promoted cell growth as well as the expression of *ave* genes (including pathway-specific positive regulatory gene *aveR* for avermectin biosynthesis and *ave* structural genes), leading in turn to avermectin overproduction. These findings provide an effective approach for the improvement of antibiotic production in *Streptomyces*.

Keywords Avermectin · *frr* Gene · Ribosome recycling factor (RRF) · *Streptomyces avermitilis*

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Abbreviations

EF-G	Elongation factor-G
HPLC	High-performance liquid chromatography
PMSF	Phenylmethanesulfonyl fluoride
PVDF	Polyvinylidene fluoride
RRF	Ribosome recycling factor
RT-PCR	Reverse transcription PCR

Introduction

Avermectins, a series of eight structurally related secondary metabolites (A1a, A1b, A2a, A2b, B1a, B1b, B2a, B2b) produced by *Streptomyces avermitilis*, have highly potent anthelmintic and insecticidal activity and are used commercially for broad-spectrum parasite control in the medical, veterinary, and agricultural fields [2, 5]. Since the avermectin biosynthetic pathway is well elucidated [5, 7], and the complete *S. avermitilis* genome sequence is known [8, 14], it is possible to engineer this industrially important bacterial strain for the overproduction of avermectins.

Protein biosynthesis, which occurs on the ribosome, consists of four steps: initiation, elongation, termination, and ribosome recycling. The subsequent translation cycle requires breakdown of the ribosome–mRNA–tRNA post-termination complex and recycling of the ribosomal subunits. The ribosome recycling factor (RRF), together with the elongation factor-G (EF-G), is involved in catalyzing the disassembly of the post-termination complex and recycling the ribosome [9]. RRF is widely distributed in prokaryotes and eukaryotic organelles, and all prokaryotes examined to date have *frr* genes encoding RRF. RRF has been shown to strongly stimulate in vitro protein synthetic

activity in *Escherichia coli*, suggesting that the efficiency of protein synthesis is increased by recycling components of the post-termination complex [15]. An increased expression of RRF in *Streptomyces coelicolor* was recently shown to cause enhanced protein synthesis during the late growth phase and subsequent overproduction of the antibiotic [4]. However, whether RRF activates antibiotic biosynthesis in other *Streptomyces* species remains to be determined. In the study reported here, we investigated the positive role of RRF in avermectin production through overexpression of the *frr* gene in *S. avermitilis*.

Materials and methods

Bacterial strains, plasmids, and growth conditions

Bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were grown at 37°C in Luria–Bertani (LB) medium, and *S. avermitilis* strains were grown at 28°C on solid YMS medium [6] for sporulation or in liquid YEME medium [11] containing 25% sucrose for the growth of mycelia for use in DNA extraction and protoplast preparation. For avermectin production, seed medium (3% soluble starch, 0.2% malt extract, 0.2% soya peptone, 0.0005% $CoCl_2{\cdot}6H_2O$) was used for all *S. avermitilis* strains. We used fermentation medium A (5% cornstarch, 1.2% yeast meal, 0.05% $K_2HPO_4{\cdot}3H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$, 0.4% KCl, 0.2% $CaCO_3$, 0.0005% $CoCl_2{\cdot}6H_2O$) for the wild-type strain and its transformants, fermentation medium G [9.5% cornstarch, 2.5% peanut protein powder, 0.7% cottonseed protein, 0.3% maltose, 0.03% $MgSO_4{\cdot}7H_2O$, 0.00075% $CoCl_2{\cdot}6H_2O$, 0.01% $(NH_4)_2SO_4$, 0.03% $K_2HPO_4{\cdot}3H_2O$, 0.1% $CaCO_3$] for the engineered strain GB-165 and its transformants, and fermentation medium E [12% cornstarch, 0.2% soybean meal, 2.6% peanut meal, 0.8% yeast meal, 0.025% $(NH_4)_2SO_4$, 0.0025% $CoCl_2{\cdot}6H_2O$, 0.003% $MnSO_4{\cdot}4H_2O$] for the avermectin high-producing mutant strain 76-02-e and its transformants. Liquid fermentation medium II (5% soluble starch, 1.2% yeast extract, 0.05% $K_2HPO_4{\cdot}3H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$, 0.4% KCl, 0.0005% $CoCl_2{\cdot}6H_2O$) was used to cultivate mycelia for biomass and RNA isolation. Medium RM14 [13] was used for the regeneration of protoplasts and for the selection of transformants. When necessary, media were supplemented with antibiotics (apramycin was added at 10 μg/ml to YMS, 5 μg/ml to YEME, 20 μg/ml to RM14, or 100 μg/ml to LB; ampicillin was added at 100 μg/ml to LB).

Construction of plasmids for *frr* expression in *S. avermitilis*

A 976-bp DNA fragment carrying the promoter and coding region of *frr* was amplified by PCR from genomic DNA of *S. avermitilis* ATCC31267 with primers F1 (CCGA CATGACCGCGATCAC) and R1 (CGGAATTCGTCATG CGCATCGTACGTGG; the *Eco*RI site is underlined) and cloned into TA cloning vector pMD18-T to produce pFRR. The sequence of *frr* in pMD18-T was verified by nucleotide sequencing. The 1-kb *Eco*RI/*Bam*HI fragment of *frr* was excised from pFRR and inserted into the corresponding sites of multi-copy vector pKC1139 and integrative vector pSET152 to give the *frr* gene expression vector pFRR-1139 and pFRR-152, respectively.

The 700-bp *frr* open reading frame (ORF) was amplified with primers F4 (GAAGATCTCCTACTCAAGACACG CAGGAG; the *Bgl*II site is underlined) and R4 (CCC AAGCTTGTCATGCGCATCGTACGTGG; the *Hin*dIII site is underlined) and then cloned into *Hin*dIII/*Bam*HI-digested pJL117 to produce pFRRerm-117. The 1-kb *Bgl*II fragment containing the *ermE*p* and *frr* ORF was cut from pFRRerm-117 and cloned into the *Bam*HI site of pKC1139 and pSET152 to give *frr* expression vectors pFRRerm-1139 and pFRRerm-152, respectively. *frr* was controlled by the *Streptomyces* strong constitutive promoter *ermE*p* in these vectors.

The resulting plasmids, pFRR-1139, pFRR-152, pFRRerm-1139, and pFRRerm-152, together with pSET152 and pKC1139 as control vectors, were introduced separately into *E. coli* ET12567 to propagate non-methylated DNA prior to their transformation into protoplasts of *S. avermitilis* strains. *S. avermitilis* protoplast preparation, transformation, and regeneration were performed as described previously [13]. Putative transformants were regenerated on RM14 medium containing apramycin for 7–10 days to confirm resistance. After transformation, the pKC1139-based vector remained multi-copy in the cell, while the pSET152 derivative was integrated into the chromosome, thereby introducing one extra copy of *frr* into *S. avermitilis*. Transformants were confirmed by colony PCR analysis with primers Apr-1 (AGCTCATCGGTCAGCTTCTC) and Apr-2 (GGCAT CGCATTCTTCGCATC), which are specific for the apramycin resistance gene *aac(3)IV*, resulting in the generation of a 0.73-kb band. No such PCR product was detected from the analysis of control parental strains. Since *S. avermitilis* does not sporulate on RM14 medium, transformants regenerated on RM14 plates were transferred to solid YMS medium for sporulation.

Table 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Reference or source
Streptomyces avermitilis strains		
ATCC31267	Wild-type strain, avermectin producer	ATCC
GB-165	Δ*aveD*, an engineered strain constructed from green spore-producing mutant, producing only avermectin B	Our laboratory
76-02-e	Avermectin high-producer, derived from ATCC31267 by continuous mutagenesis	Our laboratory
31267 (pKC1139)	ATCC31267 with multi-copy vector pKC1139	This study
31267 (pSET152)	ATCC31267 with integrative vector pSET152	This study
31267 (pFRR-1139)	ATCC31267 with multi-copy vector pFRR-1139	This study
31267 (pFRR-152)	ATCC31267 with integrative vector pFRR-152	This study
31267 (pFRRerm-1139)	ATCC31267 with multi-copy vector pFRRerm-1139	This study
31267 (pFRRerm-152)	ATCC31267 with integrative vector pFRRerm-152	This study
GB-165 (pKC1139)	GB-165 with multi-copy vector pKC1139	This study
GB-165 (pSET152)	GB-165 with integrative vector pSET152	This study
GB-165 (pFRR-1139)	GB-165 with multi-copy vector pFRR-1139	This study
GB-165 (pFRR-152)	GB-165 with integrative vector pFRR-152	This study
GB-165 (pFRRerm-1139)	GB-165 with multi-copy vector pFRRerm-1139	This study
GB-165 (pFRRerm-152)	GB-165 with integrative vector pFRRerm-152	This study
76-02-e (pKC1139)	76-02-e with multi-copy vector pKC1139	This study
76-02-e (pSET152)	76-02-e with integrative vector pSET152	This study
76-02-e (pFRR-1139)	76-02-e with multi-copy vector pFRR-1139	This study
76-02-e (pFRR-152)	76-02-e with integrative vector pFRR-152	This study
76-02-e (pFRRerm-1139)	76-02-e with multi-copy vector pFRRerm-1139	This study
76-02-e (pFRRerm-152)	76-02-e with integrative vector pFRRerm-152	This study
Escherichia coli strains		
DH5α	General cloning host, *supE*44 Δ*lacU*169 (φ80 *lacZ*ΔM15) *hsdR*17 *recA endA*1 *gyrA*96 *thi-1 relA*1	Sambrook et al. [16]
ET12567	DNA methylation-deficient host, *dam*$^-$ *dcm*$^-$ *hsdM*$^-$ *hsdR*$^-$ *zjj*-201	MacNeil et al. [13]
Plasmids[a]		
pMD18-T	TA-cloning vector, Amp^R	TaKaRa, Japan
pIJ2925	Cloning vector, *lacZα*, Amp^R	Janssen et al. [10]
pJL117	pIJ2925 derivative carrying the *Streptomyces* strong constitutive promoter *ermE*p* from the 0.3-kb *Bgl*II fragment of pIJ4090, Amp^R	Our laboratory
pKC1139	*E. coli-Streptomyces* shuttle vector, *lacZα*, Apra^R, *oriT*, *rep*^{ts}	Bierman et al. [1]
pSET152	*E. coli-Streptomyces* shuttle vector, *attB*, *lacZα*, Apra^R, *oriT*	Bierman et al. [1]
pFRR	pMD18-T containing the 976-bp *frr* gene with its native promoter	This study
pFRR-1139	pKC1139 containing the 976-bp *frr* gene with its native promoter	This study
pFRR-152	pSET152 containing the 976-bp *frr* gene with its native promoter	This study
pFRR2	pMD18-T containing the 0.7-kb *frr* ORF	This study
pFRRerm-117	pJL117 with the 0.7-kb *frr* ORF inserted immediately downstream of *ermE*p*	This study
pFRRerm-1139	pKC1139 containing *ermE*p* and the *frr* ORF excised from pFRRerm-117	This study
pFRRerm-152	pSET152 containing *ermE*p* and the *frr* ORF from pFRRerm-117	This study

ORF, Open reading frame;

[a] Apra^R, Apramycin resistance; Amp^R, ampicillin resistance

Fermentation and high-performance liquid chromatography analysis of avermectins

For avermectin production, spores from various *S. avermitilis* strains cultivated on YMS plates for 7–10 days were inoculated into 50 ml seed medium in 250-ml flasks. After incubation for 24 h at 28°C on a rotary shaker (180 rpm), the cultures were trans-inoculated (5% inoculation) into three parallel 250-ml flasks containing 50 ml fermentation medium each and grown for 10 days at 28°C on a rotary shaker (220 rpm). For the identification of avermectins in fermentation culture by high-performance liquid chromatography (HPLC) analysis, 0.5 ml fermentation broth was extracted with 4.5 ml methanol for 30 min. The extract was centrifuged at 4000 *g* for 10 min, and the supernatant was directly applied to an HPLC system (model 600; Waters, Milford, CT) with a C18 column (10 μm; internal diameter 4.6 × 150 mm) developed with methanol–water (85:15) at flow rate 1.0 ml/min. Avermectins were detected by UV absorption at 246 nm, with authentic samples of avermectin B1 used as internal standards.

RNA isolation and reverse transcription–PCR analysis

Mycelia of *S. avermitilis* grown in fermentation medium II were collected on days 4 and 7, flash-frozen in liquid nitrogen, and ground into a fine powder. RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and the sample was treated with DNase I (TaKaRa, Shiga, Japan) to remove chromosomal DNA contamination. This treated RNA sample was then used as a template for PCR amplification in order to confirm that the amplified products were not derived from chromosomal DNA.

Reverse transcription (RT)–PCR was conducted using the TaKaRa RNA PCR kit (AMV) ver.3.0 according to the manufacturer's protocol. Primer pairs, listed in Table 2, were used to analyze *aveA1*, *aveA2*, *aveA3*, *aveA4*, and *aveR* transcript levels in the various strains of *S. avermitilis*. For semi-quantitative RT–PCR analysis, which was carried out to ensure that the produced DNA had not reached plateau value, each set of PCR reactions for each tested transcript was repeated using different cycle numbers (see Table 2). The *hrdB* gene, which encodes the major sigma factor in *Streptomyces*, was used as the positive internal control in the RT–PCR assays. Samples of the PCR amplification products (7 μl) were loaded onto a 2.0% agarose gel and stained with ethidium bromide after electrophoresis.

Table 2 Primers used in reverse transcription–PCR analysis

Primer	DNA sequence (5′ to 3′)	Gene	Cycle number
hrdB-S	TACTGCGCAGCCTCAACCAG	*hrdB*	25
hrdB-AS	GCCGATCTGCTTGAGGTAGTC		
aveA1-S	ACGCTTCCGACGTCTTCCG	*aveA1*	25
aveA1-AS	TTGTCCTCGGTCCACGGAG		
aveA2-S	CCTGTACGAGGTGGTCGAG	*aveA2*	30
aveA2-AS	TTGAGGGCTGATGTGTGTATC		
aveA3-S	AGCTCGTCGACGCGCTTAG	*aveA3*	30
aveA3-AS	TCGAACGCCTCCCACGAC		
aveA4-S	CGCGTTACTGCCGATCTC	*aveA4*	35
aveA4-AS	AGTCCTGGGACATGAGGC		
aveR-S	CCGCGACTTCCTCACCG	*aveR*	30
aveR-AS	GGACTCGCTCAGCAG		

Western blotting analysis

Ground *S. avermitilis* mycelial paste was prepared as described in the preceding section, suspended in ice-cold extraction buffer containing protease inhibitors (50 mM HEPES, pH 7.4, 137 mM NaCl, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A), mixed by gentle inversion, placed on ice for 5 min, and mixed again. Cell debris was removed by centrifugation, and the supernatant was used as a total protein extract. Samples from each protein analysis (100 μg total protein) were separated by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and the proteins were transferred onto a polyvinylidene fluoride membrane. Western blots were developed with polyclonal antibodies using an ECL detection system (Amersham, Arlington Heights, IL) as described by the manufacturer. Polyclonal antibody against AveR was prepared in rabbits as described previously [3] and used as the primary antibody at a dilution of 1:1500.

Results

Overexpression of *frr* leads to increased avermectin production

To determine the efficacy of RRF in avermectin biosynthesis, the *frr* gene with its own promoter or with *ermE*p* was cloned into the multi-copy vector pKC1139 and the integrative vector pSET152, respectively, to generate *frr*

expression vectors. The resulting recombinant plasmid was transformed into the strains ATCC31267, GB-165, and 76-02-e, respectively. The resulting transformants and their host strains, as well as vector control strains, were cultured separately in the appropriate fermentation medium for 10 days. HPLC analysis of the fermentation products revealed that the control strains, which contained only pSET152, produced nearly the same amount of avermectin as the host strains, while the introduction of pKC1139 caused a small reduction in avermectin yield. In contrast, introduction of the plasmid containing the *frr* gene led to enhanced avermectin production in all three *S. avermitilis* strains (Fig. 1), indicating that the increased production was due to overexpression of *frr* gene. The avermectin stimulation effect by *frr* overexpression was different in the three host strains and exhibited an *frr* copy number effect. In comparison to the wild-type strain, avermectin yield was increased significantly by 3- to 3.7-fold in transformants 31267(pFRR-1139) and 31267(pFRRerm-1139), both of which contained multiple *frr* copies, but was increased by only 71–86% in transformants 31267(pFRR-152) and 31267(pFRRerm-152), both of which contained *frr* on the integrative vector (Fig. 1a). Likewise, multiple *frr* copies had a greater promoting effect on avermectin yield than did the one extra *frr* copy in strain GB-165 and 76-02-e, whereas both the *frr* copy number effect and avermectin stimulation effect were not so sharply marked as in wild-type strain (Fig. 1b, c). There was no obvious difference in avermectin yield between the transformant containing recombinant plasmid pFRR-1139 (or pFRR-152) and its cognate transformant containing recombinant plasmid pFRRerm-1139 (or pFRRerm-152), suggesting that *ermE*p* is not superior to the *frr* native promoter in driving the expression of *frr* in *S. avermitilis*.

Overexpression of *frr* enhances *S. avermitilis* growth

To investigate whether avermectin overproduction in *frr*-overexpressing strains was due to improved cell growth, we analyzed the growth and avermectin production of *frr*-overexpressing strain 31267(pFRR-1139), its parent ATCC31267, and vector control strain 31267(pKC1139) in liquid fermentation medium II. As shown in Fig. 2, the transformation of pKC1139 had no significant effect on cell growth or avermectin production, whereas the overexpression of *frr* had a clear enhancing effect on both of these processes. The highest biomass of strain 31267(pFRR-1139) was 22.4 mg/ml on day 6, which represents a 27 and 32% increase relative to that of the parent strain ATCC31267 (17.6 mg/ml) and vector control strain 31267(pKC1139) (17 mg/ml), respectively, on day 7. These findings indicate that increased avermectin production in *frr*-overexpressing strains was due, at least in part, to an enhancement of cell growth.

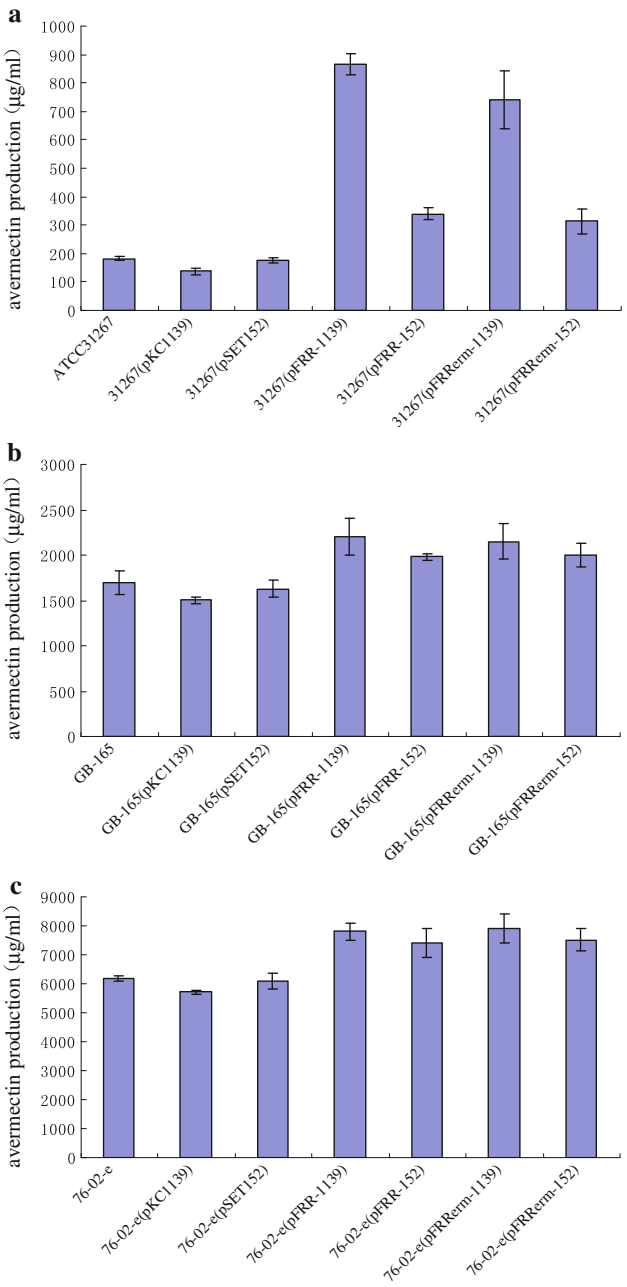

Fig. 1 Effect of enhanced expression of *frr* (gene encoding the ribosome recycling factor) on avermectin production in various *Streptomyces avermitilis* strains. **a** ATCC31267 and its transformants, **b** GB-165 and its transformants, **c** 76-02-e and its transformants

Overexpression of *frr* increases the expression of *aveR* and avermectin polyketide synthase genes

aveR, located at the far left of the avermectin biosynthetic gene cluster, encodes a pathway-specific activator essential for transcription of all avermectin biosynthetic genes [3, 12]. To evaluate the effect of *frr* overexpression on the expression of the *aveR* and *ave* biosynthetic genes, we conducted semi-quantitative RT–PCR analyses to detect

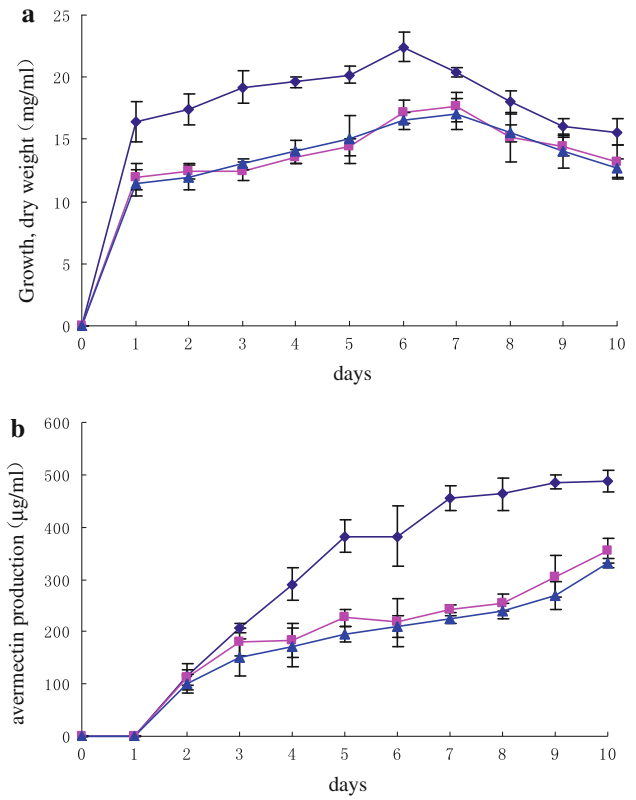

Fig. 2 Comparison of growth (**a**) and avermectin production (**b**) in *frr*-overexpressing strain 31267(pFRR-1139) (*filled diamond*), its wild-type parental strain ATCC31267 (*filled square*), and vector control strain 31267(pKC1139) (*filled triangle*) grown in liquid fermentation medium II

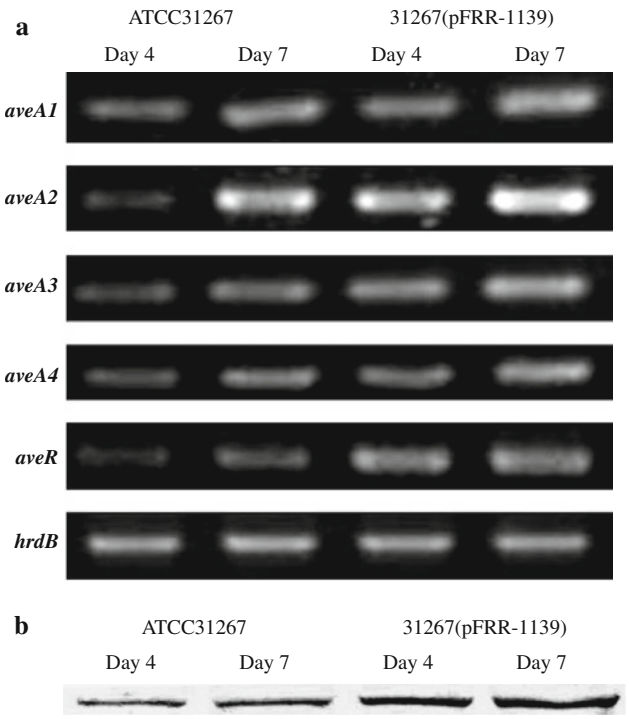

Fig. 3 Effect of *frr* overexpression on the expression of avermectin (*ave*) polyketide synthase (PKS) genes and *aveR*. **a** Semi-quantitative reverse transcription–PCR analysis of *aveA1*, *aveA2*, *aveA3*, *aveA4*, and *aveR* transcript levels in wild-type and *frr*-overexpressing strains of *S. avermitilis*. *hrdB*, which encodes the major RNA polymerase sigma factor in *Streptomyces*, was used as the positive control. **b** Western blotting analysis of AveR protein. Cells were collected from cultures grown on fermentation medium II for 4 and 7 days, respectively

the transcript levels of *aveR* and four avermectin polyketide synthase (PKS) genes (*aveA1*, *aveA2*, *aveA3*, *aveA4*) in *frr*-overexpressing strain 31267(pFRR-1139) and parental strain ATCC31267. Total RNA used for the RT–PCR analysis was prepared from mycelia grown for 4 and 7 days, respectively, in fermentation medium II. Transcript levels of all these *ave* genes were clearly higher at each of the two time points in 31267(pFRR-1139) than in ATCC31267 (Fig. 3a). As a positive control, *frr* overexpression had no obvious effect on the transcription of *hrdB*. Expression of *aveR* was further determined by Western blotting analysis, and strain 31267(pFRR-1139) showed increased expression of AveR protein (Fig. 3b). Thus, overexpression of *frr* stimulates transcriptional and translational expression of the pathway-specific activator AveR, which in turn increases the expression of *ave* structural genes, leading to enhanced avermectin production.

Discussion

Hosaka et al. [4] showed that enhanced expression of the RRF-encoding gene *frr* in *S. coelicolor* led to enhanced protein synthesis during the late growth phase and a remarkably abundant production of actinorhodin (Act). In accordance with these results, we verified the significance of RRF in promoting antibiotic production, since overexpression of *frr* stimulated avermectin production in the various *S. avermitilis* strains tested in our study. The detailed mechanism by which upregulated *frr* enhances antibiotic production remains to be clarified. Hosaka et al. [4] proposed that enhanced protein synthesis during the late growth phase in *frr*-overexpressing *S. coelicolor* would be directly advantageous for the production of proteins from newly transcribed genes, such as those encoding antibiotic biosynthetic enzymes or positive regulatory proteins. Thus, the capacity of a cell to synthesize proteins during the late growth phase is indicative of its ability to accelerate the onset of secondary metabolism. Our finding that overexpression of *frr* in *S. avermitilis* enhances the expression of pathway-specific positive regulatory gene *aveR* and *ave* PKS genes is consistent with this proposal. Although the PKS genes were apparently upregulated through the activation of *aveR*, it is still unclear what factor regulated the expression of *aveR*. One possibility is that the synthesis of regulatory proteins

controlling the expression of *aveR* is also enhanced in association with *frr* overexpression, leading to the increased production of avermectin biosynthetic enzymes. The finding that elevated *frr* expression promotes cell growth in *S. avermitilis* is consistent with the function of RRF in enhancing protein synthesis. Thus, the observed overproduction of avermectin in *frr*-overexpressing *S. avermitilis* may be attributable to both enhanced cell growth and the efficient expression of avermectin biosynthetic genes.

The biosynthesis of avermectins, similar to that of other antibiotics, is considered to be tightly regulated through a complex cascade of regulatory factors, thereby leading to very low avermectin yield in the wild-type strain. Mutant strain 76-02-e and engineered strain GB-165 both had a notably higher avermectin production than the wild-type strain. In this concern, the genetic backgrounds in the two mutant strains are obscure, since both 76-02-e and the parent of GB-165 underwent continuous mutagenesis. Relative to the wild-type strain, the effect of *frr* on avermectin production in strains 76-02-e and GB-165 was less obvious, probably because most of the negative stimulatory factors that inhibit avermectin production are downregulated in these strains while most of the positive factors that promote avermectin production are upregulated, resulting in relatively limited potential for further improvement of avermectin yield. In this concern, it would be of interest to analyze the expression level of the *frr* gene and a number of regulatory genes involved in avermectin production in the wild-type strain and the high producer.

The strategy for yield improvement based on overexpression of RRF, as suggested by results of this study, can potentially be extended to antibiotic overproduction in other industrially important bacterial strains.

Conclusion

Overexpression of the *frr* gene promotes avermectin production in various *S. avermitilis* strains, thereby providing an efficient approach for antibiotic overproduction in *Streptomyces* and other industrially important bacterial strains.

Acknowledgments This work was supported by grants from the National High Technology Research and Development Program (Grant No. 2006AA10A209), and the National Basic Research Program of China (Grant No. 2009CB118905).

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