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ORIGINAL PAPER

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# Disruption of a gene encoding a putative γ-butyrolactone-binding protein in *Streptomyces tendae* affects nikkomycin production

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**Abstract** A 2.6-kb *Bam*HI fragment from the genome of the wild-type, nikkomycin-producing strain of *Streptomyces tendae* ATCC 31160 was cloned and sequenced. This 2.6-kb *Bam*HI fragment corresponds to the DNA site where transposon Tn*4560* had inserted to create a nikkomycin-nonproducing mutant. A possible ORF of 660 nucleotides was found in this 2.6-kb *Bam*HI fragment, in which the third base of each codon was either G or C in 92% of the codons. The deduced amino acid sequence coded by this ORF (TarA, tendae autoregulator receptor) shows strong homology with several γ-butyrolactone-binding proteins that negatively regulate antibiotic production in other streptomycetes and have a helix-turn-helix DNA-binding motif. A portion (179 nucleotides) of *tar*A that encodes the helix-turn-helix motif was replaced with *erm*E, and wild-type *S. tendae* was transformed with this construct borne in pDH5, a gene-disruption vector. Southern hybridization indicated that *erm*E had inserted in the 2.6-kb *Bam*HI region in one isolate that is erythromycin resistant. Northern hybridization indicated that *tar*A disruption significantly increased the amount of disrupted-*tar*A mRNA. This suggests that TarA negatively regulates its own synthesis. Nikkomycin production by the *tar*A disruptant was delayed but reached the wild-type level after longer incubation in production medium.

## Introduction

Nikkomycins include several nucleoside-peptide inhibitors of chitin synthase and are promising fungicides produced by *Streptomyces tendae* (Bruntner et al. 1999). Bruntner et al. (1999) isolated, sequenced, and identified the first seven genes (*nik*A-*nik*G) with their corresponding enzymes and reactions in the synthesis of hydroxypyridylhomothreonine, the aglycone moiety of nikkomycin Z and nikkomycin X. DNA flanking the site of transposon Tn*4560* (Siemieniak et al. 1990) insertion into the genome of a nikkomycin-nonproducing mutant (Nik6)(Engel and Wright 1993) was used to isolate a 2.6-kb fragment of wild-type genomic DNA. The 2.6-kb fragment, corresponding to the DNA where Tn*4560* had inserted, was sequenced. The deduced amino acid sequence coded by 660 nucleotides (nt) within the 2.6-kb fragment shows strong homology with several γ-butyrolactone-binding proteins (Miyake et al. 1990; Nakano et al. 1998; Waki et al. 1997) that regulate secondary metabolism in other streptomycetes. This 660-nt ORF was designated *tar*A (tendae autoregulator receptor) and codes for a protein (TarA) whose deduced amino acid sequence bears the helix-turn-helix (HTH) DNA-binding motif found in ArpA (Miyake et al. 1990), BarA (Nakano et al. 1998), and FarA (Waki et al. 1997). *tar*A is 1.2 kb from the site where Tn*4560* inserted to generate mutant Nik6 (Engel and Wright 1993).

Replacement of the sequence encoding the HTH in *tar*A with *erm*E resulted in increased disrupted-*tar*A mRNA production, thus, providing evidence that TarA regulates its own synthesis. Nikkomycin production in the *tar*A disruptant was delayed but reached the wild-type level after 72 h.

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## Materials and methods

### Strains, vectors, *Streptomyces* transformation, and culture conditions

*Streptomyces tendae* ATCC 31160, the wild-type, nikkomycin-producing strain, and *Yarrowia lipolytica* ATCC 8661 were purchased from the American Type Culture Collection (Manassas, Va.). The Tn*4560*-bearing, nikkomycin-nonproducing mutant (Nik 6) was described by Engel and Wright (1993). Gene disruption experiments were carried out to generate strain 4A, as described below. pDH5, an *Escherichia coli* plasmid designed for gene disruption/replacement in *Streptomyces* (Hillemann et al. 1991), was provided by W. Wohlleben (University of Tübingen). pIJ4026, bearing *erm*E (Hopwood et al. 1985), was obtained from M.J. Bibb (John Innes Centre).

*E. coli* XL1-Blue MRA (P2), the lambda vector EMBL3/*Bam*HI, and pBluescript SK+/– were purchased from Stratagene (La Jolla,

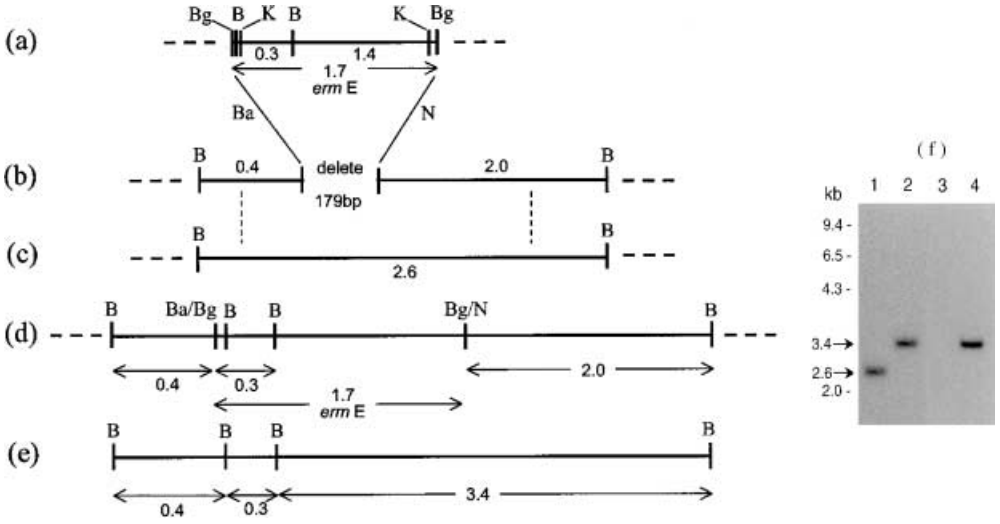


**Fig. 1a–f**. pDH5/B3 and *tar*A disruption. **a** 1.7-kb *Bgl*II *erm*E fragment from pIJ4026; **b** 2.6-kb wild-type *Bam*HI in pDH5 with the helix-turn-helix (HTH) deleted and *erm*E inserted (construct pDH5/B3); **c** 2.6-kb wild-type *Bam*HI portion of wild-type genome; *vertical dashed lines* between **b** and **c**, represent a double crossover. **d** Expected structure in strain 4A genome after double crossover; **e** expected size of *Bam*HI fragments of strain 4A if crossover occurred as shown in **b** and **c**. *Numbers* in **a**–**e** indicate kb between restriction sites. Restriction sites: *B*, *Bam*HI; *Ba*, *Bal*I; *Bg*, *Bgl*II; *K*, *Kpn*I; *N*, *Not*I. **f** Southern hybridization of *Bam*HI digests of genomic DNAs of wild-type (*lanes 1, 3*) and strain 4A (*lanes 2, 4*). *Lanes 1 and 2* probed with the 2.6-kb wild-type *Bam*HI fragment (**c**). *Lanes 3 and 4* probed with the 1.7-kb *Kpn*I fragment of *erm*E (**a**)

Calif.). Competent cells of *E. coli* SCS 110, a Dam- and Dcm-deficient strain, were also obtained from Stratagene. Competent cells of *E. coli* DH5α were purchased from Gibco/BRL (Bethesda, Md.). *E. coli* strains with their cloning vectors were used as directed by the suppliers.

Protoplasts were obtained from overnight cultures of wild-type *S. tendae* as described previously (Engel 1987). The gene-disruption vector, pDH5/B3 (described below), was passed through *E. coli* SCS110 to obtain unmethylated DNA for transformation of wild-type *S. tendae* protoplasts. In addition, the unmethylated DNA of pDH5/B3 was alkali-denatured and then neutralized, as described by Oh and Chater (1997), before transformation of wild-type *S. tendae*. Thiostrepton-resistant (100 µg thiostrepton/ml) transformants were selected as described by Hopwood et al. (1985). Transformants were evaluated for either thiostrepton (100 µg/ml) or erythromycin (200 µg/ml) resistance ($Th^R$ and $Er^R$) by adding individual antibiotics to Hickey-Tresner (HT) medium (Engel 1987).

## Nikkomycin bioassay and HPLC

Separate flasks (25-ml, 125-ml flasks) of production medium (Engel and Wright 1993) inoculated with wild-type and 4A cells were used to obtain 12-, 18-, 24-, 36-, and 72-h-old broths for bioassays and HPLC. Peak fungicidal activity was found in 72-h-old broths.

*Y. lipolytica*, grown in Difco YM broth (25 ml, 125 ml flasks) at 28 °C with shaking, was used in bioassays. Six-h-old cells of *Y. lipolytica*, diluted in YM to $A_{600nm}$=0.5, were used to inoculate (0.25 ml inoculum) 25 ml of YM containing 0.25 ml of filter-sterilized broth from *S. tendae* cultures. Flasks were incubated overnight with shaking at 28 °C. No culture broth was added to the control flask. The $A_{600nm}$ of the cultures was read after the overnight incubation and dilutions (into YM) were made as necessary to obtain readings between 0.1 and 1.0. To relate the fungicidal activity in culture broths to a known quantity of nikkomycin, one flask with *Y. lipolytica* and 0.1 µg of nikkomycin Z/ml was cultured overnight for each experiment. HPLC was done using two-fold concentrated broths (Engel and Wright 1993).

## Reagents

Restriction endonucleases, T4 DNA ligase and the random primer, DNA-labeling kit were purchased from Gibco/BRL. Gigapack III Gold packaging extracts were purchased from Stratagene. $\alpha^{32}$P dCTP was purchased from Amersham (Arlington Heights, Ill.). The one-step RT-PCR kit was purchased from Qiagen (Valencia, Calif.). ULTRAhyb hybridization buffer was purchased from Ambion (Austin, Tex.). All reagents and kits were used following the suppliers' directions. Primers 1224/1225 (corresponding to Tn*4560* nt 8–1,351) were described previously (Engel and Lax 1997). The *tar*A-based primer pair, 5′-CGG TCT TCG CCG AGC GCG GAT A-3′ (nt 1,980–2,001) and 5′-TCA GCA GGC GAT GCC CGA CA-3′ (nt 2,584–2,565) (GenBank no. AF156161), was from Gibco/BRL. A PCR product derived from this *tar*A-based primer pair was used to probe Northern blots. These primers were also used for RT-PCR.

Nikkomycin Z and thiostrepton were purchased from CalBiochem (LaJolla, Calif.). Erythromycin was purchased from Sigma (St. Louis, Mo.).

## DNA manipulation, sequencing, and identification of potential ORFs

Tn*4560* lacks *Bam*HI sites (Siemieniak et al. 1990); consequently, *Bam*HI digestion of Nik6 genomic DNA gave a 12-kb fragment bearing Tn*4560* (8.8 kb) and its flanking DNA. This 12-kb fragment was cloned into EMBL3/*Bam*HI. DNA flanking Tn*4560* and including the ends of Tn*4560* was subcloned into pBluescript. Plaque and colony hybridizations were carried out with a 1.3-kb PCR product of primers 1224/1225, as described by Engel and Lax (1997).

Approx. 0.6 kb of Nik6 genomic DNA that flanks the left-inverted repeat of Tn*4560* was used to probe plaques and colonies bearing wild-type genomic DNA. A 2.6-kb *Bam*HI fragment of wild-type *S. tendae* genomic DNA that corresponds to the DNA where Tn*4560* inserted in Nik6 was subcloned from lambda into pBluescript. Both strands of the 2.6-kb wild-type *Bam*HI fragment were sequenced at the Iowa State University (Ames, Iowa) DNA sequencing facility. The FramePlot program (version 2.3)

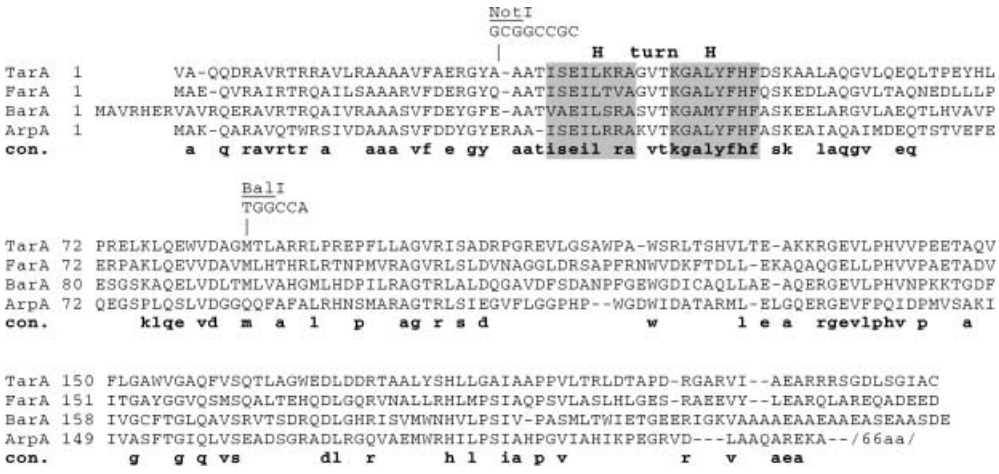


**Fig. 2** Amino acid alignment of TarA with the γ-butyrolactone-binding proteins ArpA, BarA and FarA (Waki et al. 1997). *Not*I and *Bal*I restriction sites used to delete DNA coding for the HTH (*shaded* amino acids) motif are indicated. Consensus indicated when amino acid residues in two or more γ-butyrolactone-binding proteins were identical with the amino acid in TarA. ArpA has an additional 66 residues, signified by /66aa/. Spaces were introduced to optimize alignment

(Ishikawa and Hotta 1999) was used to identify potential ORFs and deduce the amino acid sequence encoded by ORFs. BLASTp was used to search for homologies in the all non-redundant GenBank CDS translations+PDB+Swiss Prot +PIR+PRF peptide sequence database.

### Disruption of *tar*A, Southern and Northern hybridization

The 2.6-kb wild-type *Bam*HI fragment (Fig. 1c) was cloned into the *Bam*HI site of pDH5. The single *Not*I and *Bal*I sites in the 2.6-kb fragment occur 179 bp apart in *tar*A with the region coding for the HTH DNA-binding motif between them (Fig. 1b and Fig. 2). pDH5 lacks *Not*I and *Bal*I sites. To create a 179-bp deletion in the HTH region of *tar*A, the 2.6-kb wild-type *Bam*HI fragment was digested with *Not*I and *Bal*I (Figs. 2, 1b) (Fig. 1c). The *Not*I end was made blunt, as were the *Bgl*II ends of the 1.7-kb *erm*E (Fig. 1a) fragment. The blunt *Not*I and *Bal*I ends were ligated with the blunt *Bgl*II ends to generate the gene-disruption vector pDH5/B3 (Fig. 1d). This vector was sequenced to find the *tar*A and *erm*E junctions and to determine the orientation of *erm*E (Fig. 1d) within *tar*A.

To transform wild-type *S. tendae*, 1–5 µg of pDH5/B3 DNA, prepared from *E. coli* SCS110, was used. $Th^R$ and $Er^R$ transformants were homogenized in tryptic soy broth and sown on HT plates lacking thiostrepton and erythromycin. After 3–5 days, these plates were replicated to HT plates; this was repeated after another 3–5 days. Spores were scraped off the surface of HT plates into tryptic soy broth, vortexed, filtered through sterile cotton, diluted, and sown on HT containing erythromycin. Colonies were replicated to determine whether $Th^R$ was lost. Southern hybridization on *Bam*HI digests of genomic DNA of a thiostrepton-sensitive and $Er^R$ isolate (strain 4A) showed that *erm*E had inserted into the wild-type genome at the 2.6-kb *Bam*HI region with the orientation shown in Fig. 1d.

Total RNA was prepared from cells grown in nikkomycin production medium for various lengths of time using the modified Kirby method (Hopwood et al. 1985). Southern and Northern hybridizations were done using standard methods (Hopwood et al. 1985) and ULTRAhyb hybridization solution. RT-PCR was carried out as directed in the Qiagen handbook using RNA (1.7 µg/50 µl reaction) from 12- and 18-h-old wild-type cultures and the *tar*A-based primer pair.

### GenBank accession number

The sequence of the 2.6-kb wild-type *Bam*HI fragment was deposited in GenBank under accession no. AF156161.

## Results

### Characterization of DNA flanking Tn*4560* and the 2.6-kb wild-type *Bam*HI fragment

The 2.6-kb wild-type DNA has a G+C content of 75.4%. Earlier preliminary sequencing (data not shown) of nucleotides flanking the ends of Tn*4560* in Nik6 showed that this wild-type 2.6-kb *Bam*HI fragment corresponds to the DNA where Tn*4560* inserted. These data also showed that a 5-bp GAGGA site (nt 626–630) occurs as a direct repeat at the ends of Tn*4560* in Nik6.

Tn*4560* insertion (nt 626–630) occurred approximately 1.2 kb upstream of *tar*A (nt 1,922–2,584). The putative ORF disrupted by Tn*4560* is on the opposite DNA strand from *tar*A and has 1,239 nt, with an ATG translation start codon (nt 1,758–1,756) 164 nt from *tar*A. The deduced amino acid sequence encoded by this ORF shows the best homology with a putative transmembrane efflux protein of *Streptomyces coelicolor* A3(2) (33% identity, 48% similarity). The mutation in Nik6 may affect nikkomycin or γ-butyrolactone transport from cells.

### Identification of an ORF specifying a putative γ-butyrolactone-binding protein

A possible TGA translation stop codon (nt 2,582–2,584) occurs in frame and 660 nt downstream from a potential GTG translation start codon (nt 1,922–1,924) (Fig. 2). Ninety-two percent of the 220 codons in the *tar*A gene have either G or C in the third position of each codon. The deduced amino acid sequence of TarA has the following amino acid identities and similarities with other γ-butyrolactone-binding proteins (Fig. 2): FarA 48% identity, 56% similarity; ArpA 35% identity, 50% similarity; BarA 40% identity, 58% similarity.

GTG (Fig. 2, nt 1,922–1,924) was chosen as the probable start codon of *tar*A because homology searches indicated that this start codon allows the deduced amino

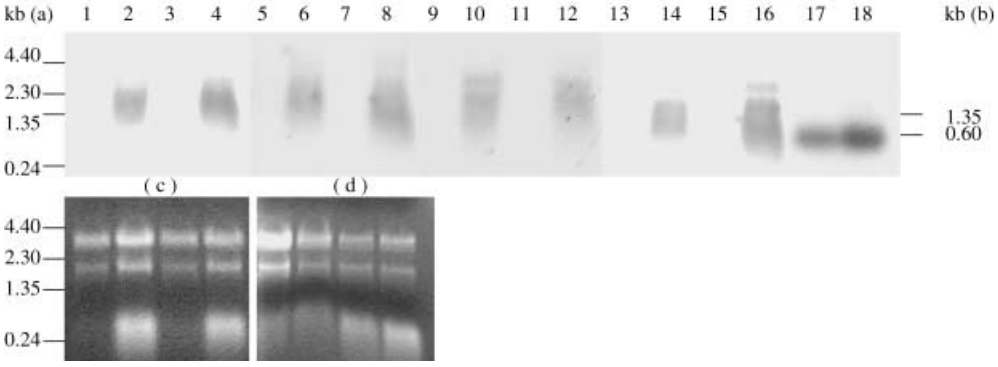


**Fig. 3** **a** Northern hybridization (*lanes 1–16*) and **b** Southern hybridization of DNA derived from RT-PCR of wild-type RNA (*lanes 17–18*). *Lanes 1–8* and *17–18* probed with *tar*A; *lanes 9–16* probed with *erm*E after blots corresponding to *lanes 1–8* were stripped. Total RNA from wild type (od*d-numbered lanes 1–15*) and from 4A (*even-numbered lanes 2–16*). Age (h) of cultures when RNA prepared: *lanes 1, 2, 9, 10* (12 h); *lanes 3, 4, 11, 12* (18 h); *lanes 5, 6, 13, 14* (24 h); *lanes 7, 8, 15, 16* (36 h); *lane 17* (18 h) and *lane 18* (12 h). **a** RNA size markers for *lanes 1–16*; **b** DNA size markers for *lanes 17–18*. **c, d** RNA gels before blotting: **c** corresponds to Northern hybridization, *lanes 1–4 and 9–12*; **d** corresponds to Northern hybridization, *lanes 5–8 and 13–16*

**Table 1** Comparison of nucleotide sequences upstream of *bar*A, *far*A and *tar*A[a]

| | |
|---|---|
| *tar*A[b] | AG-TACGTACTGTTTGGTTTGGTTTCCGAC |
| FARE[c] | -GATACGAACGGGACGGAC-GGTTTGC-AGC |
| BARE-3[d] | AGATACATACCAACCGGTT-CTTTTGA |
| Consensus | AG TACGTAC G GGTT GGTTT C A |

[a] Gaps were introduced to optimize alignment
[b] Nucleotides 1,874–1,902 (GenBank accession no. AF156161) upstream of *tar*A
[c] Kitani et al. (1999)
[d] Kinoshita et al. (1999)

acid sequence of TarA to align well with the conserved amino acids near the N-terminal end of ArpA and FarA (Fig. 2). Also, the GGAGG sequence (nt 1,913–1,917) that is four nucleotides upstream from the putative GTG start codon may be a ribosome-binding site.

An A-T-rich (55%) region (nt 1,874–1,902; Table 1) 18 nt upstream of the putative GTG start codon of *tar*A (Fig. 2) is similar to upstream regions of *bar*A (Kinoshita et al. 1999) and *far*A (Kitani et al. 1999). Included in this region are two potential –35 promoter sequences, the TTGGTT (nt 1,886–1,891 and nt 1,891–1,896; Table 1) sequences. A-T-rich regions are characteristic of –10 and –35 promoter regions and γ-butyrolactone-binding protein binding sites (Kinoshita et al. 1999; Kitani et al. 1999). Kinoshita et al. (1999) and Kitani et al. (1999) demonstrated that BARE-3 (BarA responsive element) and FARE (FarA responsive element) are DNA sequences 70–100 bp upstream of *bar*A and *far*A, respectively, that bind BarA and FarA. BARE-3 (61% A-T) and FARE (39% A-T) have strong nucleotide identities with the nt 1,874–1,902 region upstream of *tar*A (Table 1). FARE and the upstream region of *tar*A share 39% nucleotide identity, while BARE-3 has 30% nucleotide identity with the upstream region of *tar*A (Table 1). FARE and BARE-3 have 56% nucleotide identity (Table 1).

A highly conserved peptide region (Fig. 2, AATIS EILRA-VTKGALYFHF) near the N-terminal end of the γ-butyrolactone-binding proteins includes the HTH DNA-binding motif (shaded amino acids, Fig. 2). Another conserved peptide region (Fig. 2, RGEVLPHV-P) occurs between amino acids 134 and 143 of TarA.

## Disruption of *tar*A

The role of TarA in nikkomycin biosynthesis and regulation of transcription of *tar*A was evaluated by disruption of *tar*A. A strain (4A) with the expected thiostrepton-sensitive and $Er^R$ phenotype was shown to have *tar*A disrupted as follows: Genomic DNA from 4A was digested with *Bam*HI, and blotted and probed with *erm*E (Fig. 1a) and the 2.6-kb wild-type *Bam*HI fragment (Fig. 1c). Southern hybridization showed that the 2.6-kb wild-type *Bam*HI probe no longer hybridized with a 2.6-kb *Bam*HI fragment from 4A (Fig. 1f, lane 2) but hybridized with a 3.4-kb *Bam*HI fragment (Fig. 1f, lane 2, see Fig. 1e). A faint 0.3-kb band (Fig. 1e) from 4A that hybridized with *erm*E (not shown in Fig. 1f, lane 4) was also seen after several days exposure of the X-ray film. *erm*E did not hybridize with wild-type genomic DNA (Fig. 1f, lane 3) but hybridized with a 3.4-kb *Bam*HI fragment from 4A (Fig. 1f, lane 4). A third blot of *Bam*HI-digested wild-type and 4A genomic DNA was probed with a mixture of the two probes (wild-type 2.6-kb *Bam*HI and *erm*E). This Southern hybridization gave the same results (data not shown) as seen in Fig. 1f, lanes 1 and 2. The results in Fig. 1f are consistent with the diagram in Fig. 1e, which confirms that crossovers on both sides of *erm*E (represented by vertical dashes; Fig. 1b, c) between pDH5/B3 and within the 2.6-kb *Bam*HI portion of the wild-type genome generated a recombinant genome in 4A as diagrammed in Fig. 1d.

A large amount of disrupted-*tar*A mRNA was present in 4A at all sample times (Fig. 3, lanes 2, 4, 6, 8). Northern hybridization of total RNA with *tar*A failed to reveal the *tar*A mRNA of wild-type (Fig. 3, lanes 1, 3, 5, 7). The *tar*A mRNA of wild-type is probably below the level of detection by Northern hybridization. RT-PCR with

wild-type RNA was used to detect the presence of *tar*A mRNA (Fig. 3, lanes 17–18).

Blots probed with *tar*A (Fig. 3, lanes 1–8) were stripped, exposed to X-ray film to verify that all radioactivity had been removed, and then probed with *erm*E (Fig. 3, lanes 9–16). The presence of *erm*E-specific RNA was evident in 4A (Fig. 3 lanes 9–16). The size of the Northern hybridization bands (Fig. 3, even-numbered lanes 2–16) agreed with the expected size of the disrupted-*tar*A mRNA from 4A (660 nt–179 nt=481 nt)+(1.7 kb *erm*E)=2.1 kb.

### HPLC and bioassay of wild-type and strain 4A culture broths

Using the effect of 0.1 µg nikkomycin Z/ml on *Y. lipolytica* as a basis of comparison, wild-type and 4A broths had the equivalent of 20 mg/l of fungicidal activity in 72 h. Fungicidal activity was detected in wild-type broth at 18 h but was not detected in 4A broth until 36 h. At 36 h, 4A broth had 15% of the activity of 36-h-old wild-type broth. Measurement of protein from sonicated cells of 36- and 72-h-old cultures showed that the cell mass of wild-type and 4A (wild-type: 290 µg/ml and 310 µg/ml at 36 and 72 h, respectively; 4A, 273 µg/ml and 284 µg/ml at 36 and 72 h, respectively) was virtually the same.

HPLC was done on broths from 72-h-old cultures to determine whether there were qualitative differences between the types of nikkomycins produced by wild-type and 4A. There are four major nikkomycins (I, J, X, and Z) with fungicidal activity and distinct peaks on HPLC (Engel and Wright 1993). The HPLC profile of wild-type and 4A broths appeared the same.

## Discussion

The Southern (Fig. 1f) and Northern hybridizations (Fig. 3, lanes 10, 12, 14, 16) confirmed that *erm*E had inserted into *tar*A. *tar*A disruption significantly increases the amount of disrupted-*tar*A mRNA and delays the onset of nikkomycin biosynthesis as compared with wild-type. TarA homologues (ArpA, Miyake et al. 1990; BarA, Nakano et al. 1998; and FarA, Waki et al. 1997) negatively regulate transcription of the genes that encode their respective proteins (autoregulation). Initiation of gene expression by γ-butyrolactones depends on the binding of a γ-butyrolactone to a specific γ-butyrolactone-binding protein; the γ-butyrolactone-binding-protein then dissociates from DNA allowing transcription (Kinoshita et al. 1999; Kitani et al. 1999). The increase in disrupted-*tar*A mRNA in 4A suggests that the putative TarA protein regulates its own synthesis. A specific γ-butyrolactone that induces nikkomycin production in *S. tendae* has not been found. Discovery of a γ-butyrolactone that binds TarA and induces nikkomycin biosynthesis is needed to define the role of TarA in nikkomycin production.

Miyake et al. (1990) isolated double mutants of *S. griseus* deficient in ArpA and A-factor (the ArpA-specific γ-butyrolactone). The mutants produced ten times more streptomycin than the wild-type strain and sporulated at an earlier stage of growth than the wild-type (Miyake et al. 1990). Nakano et al. (1998) deleted 99 in-frame bp of the *bar*A gene coding for the second helix of the HTH and, as a consequence, virginiamycin synthesis began 6 h earlier in this strain of *S. virginiae* than in wild-type. This deletion also abolished production of the BarA-specific γ-butyrolactone virginiae-butanolide C (VB) (Nakano et al. 1998); thus a functional BarA is also involved in synthesis of VB. Interestingly, the strain with the HTH-coding portion of *bar*A partially deleted produced only 10% of the virginiamycin produced by wild-type (Nakano et al. 1998). It was important to determine whether deletion of the HTH-coding portion of *tar*A would affect nikkomycin production given the disparity in the results with *S. griseus* and *S. virginiae* relative to antibiotic production. It is not known why deletion of the HTH of TarA delayed nikkomycin biosynthesis but had no effect on the amount of nikkomycin produced in 72 h, which is contrary to the observations in the VB-BarA system (Nakano et al. 1998). BarA (Kinoshita et al. 1999) and FarA (Kitani et al. 1999) repress their own synthesis in the absence of their specific γ-butyrolactone (VB for BarA; IM-2 for FarA). How derepression of *bar*A and *far*A, resulting in more BarA and FarA production, respectively, promotes antibiotic synthesis is not known (Kitani et al. 1999).

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