Identification and Cloning of the Gene Involved in the Final Step of Chlortetracycline Biosynthesis in *Streptomyces aureofaciens*

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For chlortetracycline biosynthesis in *Streptomyces aureofaciens*, the final reduction step is essential to give an antibiotic activity to its intermediate, which is catalyzed by tetracycline dehydrogenase with 7,8-dedimethyl-8-hydroxy-5-deazariboflavin (FO) as a cofactor. We identified and cloned the gene, which is essential for the biosynthesis of 6-demethyltetracycline and participates in the final step of its biosynthesis, from the genomic DNA of the 6-demethyltetracycline producer *S. aureofaciens* HP77. DNA sequence analysis revealed that the gene (*tchA*) had an open reading frame of 455 amino acids with an estimated molecular mass of 48.1 kDa. Southern hybridization analysis revealed that the *tchA* gene was located external to the chlortetracycline biosynthetic gene cluster in the genome. A conserved domain search of protein sequence databases indicated that TchA showed a similarity to FbiB, which is involved in the modification of FO in *Mycobacterium bovis*.

**Key words:** chlortetracycline biosynthesis; *tchA*; tetracycline dehydrogenase; coenzyme F420; *Streptomyces aureofaciens*

Chlortetracycline (CTC) is a natural antibiotic produced by *Streptomyces aureofaciens*. The biosynthetic pathway of CTC has been well established by co-synthesis and biotransformation experiments. Briefly, eight molecules of malonyl-CoA and a malonamyl-CoA were condensed by a type II polyketide synthase to make the basic skeleton of tetracycline, and this skeleton was converted into CTC by eleven successive reactions. During these biosynthetic processes, coordinate reactions by biosynthetic enzymes were required for the complete formation of CTC, because a disorder of the coordinated reactions, usually caused by a mutation, resulted in the generation of at least nineteen CTC-related shunt compounds.1–3 Among these CTC-related compounds, only four compounds, tetracycline, CTC, 6-demethylchlortetracycline (6-DCT), and 6-demethyltetracycline (6-DMT), have antibacterial activity. These four compounds were characterized by the presence of a single bond at the 5a,11a-position, in contrast to other shunt products, which have a double bond at this position. This structure was essential for antibacterial activity and was formed by the reduction of the 5a,11a-double bond of 5a,11a-dehydrotetracycline by the enzyme, which was referred to as “tetracycline dehydrogenase (TCDH),” at the final step of its biosynthesis (Fig. 1). This enzymatic reaction requires the FO fragment of coenzyme F420 (formerly referred to as “co-synthetic factor 1”) as a cofactor,4–6 and the rate-limiting step in CTC production by *S. aureofaciens* mutants.4

The biosynthetic gene cluster of CTC was cloned from *S. aureofaciens*, which was approximately a 30-kb DNA fragment.7,8 Although the complete sequence of the 30-kb DNA fragment was determined and twenty-four ORFs were suggested to exist in this fragment, no gene encoding tetracycline dehydrogenase was identified.7 There was little information about the gene(s) participating in the final step of CTC biosynthesis.

To gain a better understanding of CTC biosynthesis, it was essential to clone and identify the gene participating in the final step of the biosynthesis. Thus, in this paper, we report the cloning and sequencing of the gene from *S. aureofaciens*, and discuss the role of the gene product for the final step of CTC biosynthesis in *S. aureofaciens*.

Materials and Methods

**Bacterial strains, plasmids, and media.** *S. aureofaciens* HP77 (6-DMT producer; [chl]) derived from H-75913 was used in this study. *Escherichia coli* JM1109 was used in the preparation of a gene bank of *S. aureofaciens* HP77 and in the isolation of the *dam*
and *dcm* plasmid DNAs that were used for the transformation of *S. aureofaciens* strains. Integrative vector pSE119 and *E. coli*–*Streptomyces* shuttle vector pSE101 were used for integrative transformation and plasmid transformation of *S. aureofaciens* respectively. Plasmid pBR322 was used to recover a recombinant DNA fragment that was integrated into the *S. aureofaciens* genome. Cosmids pGLA2 (38-kb insert) and pGLA11 (37-kb insert), which covered the 30-kb DNA fragment carrying the CTC biosynthetic gene cluster of *S. aureofaciens*, were used for Southern analysis. SK2 medium was used for the growth of *S. aureofaciens* strains. For tetracycline production, SK2 and PK2 medium were used as the seed and the production medium respectively. The culture conditions and procedures have been described previously.

**Isolation and characterization of 6-DMT non-producing mutants.** The protocols for the nitrosoguanidine mutagenesis of *S. aureofaciens* and the isolation of 6-DMT non-producing mutants used here have been described previously. The amount of 6-DMT and related compounds in the culture of the mutants were determined by reverse-phase HPLC analysis. 6-De-methyl-7-chloranhydrotetracycline and 6-demethyl-anhydro-tetracycline were prepared from a culture broth of strain NP-4. The protocol for the preparation has been described previously.

**DNA isolation and manipulation.** Genomic DNAs were isolated from *Streptomyces* strains by the method of Hopwood et al. For shotgun cloning, the genomic DNA of *S. aureofaciens* HP77 was partially digested with *Sau*3AI, or completely with BamHI. DNAs were ligated with the BamHI-digested pSE119 and introduced into *E. coli* JM110 cells, yielding a genomic DNA library. The plasmids were prepared with a Qiagen plasmid kit (Hilden, Germany).

**Genetic complementation.** Genetic complementation of *S. aureofaciens* mutants was performed by either an integrative transformation or a plasmid transformation. The integrative transformation was performed by homologous recombination using non-replicative plasmids and the same methods as those described by Kormanec et al.

The plasmid integrants were selected by plating transformed cells on RA agar, followed by coverage with soft agar containing thiostrepton at a final concentration of 20 μg ml⁻¹. The plasmid transformation was performed with *E. coli*–*Streptomyces* shuttle plasmids. After transformation with recombinant plasmid DNA, thiostrepton-resistant clones were selected by the same
method as those used for the plasmid integrants, with the exception of the thioestrepton concentration, which was 200 µg ml⁻¹. The protocols for protoplast preparation and transformation of *S. aureofaciens* were the same as those described by Dairi et al.⁹¹

Disruption of the *tcsC* gene. Disruption of the *tcsC* gene encoding anhydrotetracycline oxygenase was carried out by introducing a frame-shift mutation at an NcoI site as described previously.⁹² Briefly, the plasmid carrying the mutated allele was introduced into *S. aureofaciens* NP71, and a mutant generated by a double crossover was obtained by protoplasting and regeneration of the transformant under nonselective culture conditions. After cultivation of these recombinants, the products in the fermentation broth were analyzed by HPLC. Genomic DNA was prepared from a 6-demethyl-anhydrotetracycline producer, NP71ΔtcsC, and the frame-shift mutation was confirmed by Southern analysis using the plasmid DNA as a probe.

Recovery of the cloned DNA from the genome of *S. aureofaciens* TBZ1. After double-digesting the TBZ1 genome with both *Cla*I and *Sph*I, a DNA fragment, the size of which was approximately 4.0-kb, was ligated with a *Cla*I-*Sph*I-digested pBR322, and then analyzed the ligated product was introduced into *E. coli* JM110. A plasmid in which a 1.2-kb *Cla*I-*Ban*HI fragment carried the truncated *lacZ* gene from pSE119 and a 2.8-kb *Saa*3AI-*Sph*I fragment carried the truncated clone was selected by colony hybridization using part of the *lacZ* gene as a probe (oligonucleotide: 5'-TTGGGTAACGC-CAGGGTTTTCCCAGTCACG-3'). Next, the recovered 2.6-kb *Ban*HI-*Sph*I fragment was used as a probe to clone the full length of the cloned gene. Labeling of DNA probes, hybridization, and detection were performed by using the DIG system (Boehringer Mannheim, Germany).

Sequence and ORF analysis. The nucleotide sequences of a 4.4-kb DNA fragment (Fig. 2A) were determined. After construction of a series of plasmids, sequencing was carried out by the dideoxy chain-termination method, using an automatic DNA sequencer (ABI). Frame analysis and multiple alignments of the amino acid sequences were carried out with the GENETYX program (Genetyx Corp., Japan).

Nucleotide sequence accession number. The DNA sequence reported here is available from the EMBL, GenBank, and DDBJ databases under accession number AB125899.

Results

Isolation of mutants defective in the final step of CTC biosynthesis from a 6-DMT producing *S. aureofaciens* strain

To identify the gene involved in the final step of CTC biosynthesis, first we attempted to isolate mutants that are defective in this step, then we cloned the gene responsible for the defect by complementation using the mutant as a host. In the selection of mutants, we took advantage of a unique feature of them. McCormick has reported that a 6-DMT-related shunt compound, referred to as “tetramid blue (TB),” must be chemically formed from 5α,11α-dehydro-6-demethyltetracycline (DHDMT) (Fig. 1).¹³ It was already known that TB is a major by-product in 6-DMT production by *S. aureofaciens* HP77 (Table 1). Therefore we screened mutants that were not able to synthesize 6-DMT but did synthesize TB. We isolated three mutants. One of them, which we referred to as NP71, was used for further analysis.

Confirmation of the defective step of 6-DMT biosynthesis in *S. aureofaciens* NP71

To confirm that strain NP71 was indeed defective in the final step of the 6-DMT biosynthetic pathway, a successive coupling reaction using anhydrotetracycline as a substrate was employed. As shown in Table 1, strain NP71 did not convert 6-demethyl-7-chloranhydrotetracycline into 6-DCT. On the other hand, the parental strain, HP77, efficiently formed 6-DCT from 6-demethyl-7-chloranhydro-tetracycline, suggesting that the NP71 strain was defective in the final step. Next we inactivated an anhydrotetracycline oxygenase (*TcsC*) gene of the NP71 strain in order to obtain a mutant that was defective in both the final reduction and the *TcsC* steps. This was done for the following reasons: If the double mutant could produce 6-demethylanhydrotetracycline (DMATC),¹⁴ which is an intermediate just before the formation of DHDMT, it can be confirmed that the NP71 strain had a complete gene set for 6-DMT biosynthesis, except for the gene participating in the final step. In addition, it is thereby demonstrated that TB was formed from DHDMT (Fig. 1). By gene replacement manipulation, we were able to obtain a large number of double mutants. Only DMATC accumulated in the culture broth of all of the double mutants, suggesting that the NP71 strain has a defect only in the gene participating in the final step. The characteristics of the double mutant, which we referred to as NP71ΔtcsC, are shown in Table 1.

Cloning of the gene required for 6-DMT biosynthesis of the NP71 strain from *S. aureofaciens* HP77

We used integrative transformation for the cloning of the gene that complemented the mutation on NP71 in order to avoid serious decreases in antibiotic production caused by the gene dosage mechanism in *Streptomyces* strains carrying replicative plasmid. By screening thousands of integrants, we were able to obtain an integrant, TBZ1, that partially restored 6-DMT production (0.65 g liter⁻¹). To confirm that the restoration was caused by the DNA fragment integrated in the TBZ1 genome, we isolated thioestrepton-sensitive segregants,
in which the integrated DNA fragment had been excised from the TBZ1 genome by a second crossover. If the DNA fragment integrated into the TBZ1 genome indeed encodes the (truncated) gene corresponding to the mutated allele in the NP71 genome, some of the segregants would be expected fully to restore 6-DMT productivity, although almost all of the segregants were associated with lost 6-DMT productivity. By utilizing single crossover events, we obtained 75 thiostrepton-sensitive segregants that excised the integrated DNA from the genome. About 20% (15 colonies) of the segregants restored 6-DMT productivity (4.5 g liter\(^{-1}\)) as expected, showing that the integrated DNA in the TBZ1 genome corresponded to the mutated allele in the NP71 genome.

**Table 1.** Characteristics of *S. aureofaciens* HP77 and Its Derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>Production (g liter(^{-1}))(^{a})</th>
<th>Conversion from Cl-DMATC to 6-DCT(^{c})</th>
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<tbody>
<tr>
<td>HP77</td>
<td>4.5 ± 0.2 4.2 ± 0.3 ND(^d)</td>
<td>0.29</td>
</tr>
<tr>
<td>NP71</td>
<td>ND 4.5 ± 0.3 ND ND</td>
<td>ND</td>
</tr>
<tr>
<td>NP71ΔteC</td>
<td>ND ND + ND ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\)The values are means ± SD based on three determinations.

\(^b\)Production was determined by qualitative analysis with HPLC. +, abundant production.

\(^c\)Crude 6-demethyl-7-chloranhydrotricycline (Cl-DMATC) was added at a final concentration of 0.5 g liter\(^{-1}\) to a 2-d culture. The amounts of 6-demethyl-7-chlorotetracycline (6-DCT) were determined after a further 3 d of cultivation.

\(^d\)ND, not detectable.

Fig. 2. Overview of the *tchA* Region of *S. aureofaciens* HP77.

(A) Frame analysis and restriction map. (B) Complementation of the mutant NP71 by replicative plasmids (Plasmid) and single crossover integration (Crossover). Inserted DNAs in the vector, the locations of which can be seen on a physical map. *Restored 6-DMT production, +; not restored, –. A 2.8-kb Sau3AI-SphI fragment recovered from the TBZ1 genome.

Identification of the gene required for 6-DMT biosynthesis of the NP71 strain

By genomic Southern analysis of the TBZ1 genome using pSE119 as a probe, part of the gene integrated into the TBZ1 genome was expected to be recovered as a
4.0-kb Clal–SpH\text{I} DNA fragment (data not shown). Hence, we constructed a plasmid library by inserting an approximately 4.0-kb Clal–SpH\text{I} DNA fragment of the TBZ1 genome into the corresponding sites of pBR322. After colony hybridization, we were able to obtain the 4.0-kb Clal–SpH\text{I} fragment.

Next, independently we obtained five DNA fragments from the genomic library of \textit{S. aureofaciens} HP77 by hybridization using a piece of the 4.0-kb Clal–SpH\text{I} fragment as a probe. These five fragments overlapped with each other (Fig. 2B). To locate the gene corresponding to the mutated allele in the NP71 genome, several fragments prepared from these fragments were introduced into the NP71 strain using the integrative and the replicative plasmids, and recovery of 6-DMT productivity was examined. In consequence a replicative plasmid, pZTDH1, carrying a 2.1-kb ZTDH1-DNA fragment, successfully complemented the NP71 strain. Moreover, another plasmid, in which the ZTDH1-DNA fragment was inserted in the reverse direction, also restored 6-DMT productivity, indicating that the gene responsible for the defect in 6-DMT biosynthesis in the NP71 strain was located in this fragment (Fig. 2B). Plasmid pZTDH1 also complemented the other two mutants isolated from HP77, suggesting that the gene cloned in this study is the only one responsible for the conversion of DHDMT into 6-DMT.

\textit{Nucleotide and amino acid sequence analysis of the \textit{tchA} gene}

We sequenced a 4.4-kb DNA fragment, shown in Fig. 2A. Frame analysis of the nucleotide sequence predicted 3 complete open reading frames (ORFs), ORF2 to ORF4, and two truncated frames, ORF1 and ORF5 (Fig. 2A). Among these ORFs, ORF3 was confirmed to be the gene responsible for the conversion of DHDMT into 6-DMT based on the complementation experiments. It was designated the \textit{tchA} gene. The \textit{tchA} gene encodes a predicted protein of 455 amino acids with a theoretical molecular mass of 48.1 kDa and a pI of 8.26.

A RPS-BLAST search\textsuperscript{[13]} of the NCBI database showed that the gene product of \textit{tchA} had two conserved domains, COG1478 and NfnB, which are located at the N-terminal side and the C-terminal side respectively. This result suggested that the \textit{tchA} gene product is a homolog of FbiB, which is known to modify the N-ribityl part of FO to yield its \textit{N}-ribityl moieties. Indeed, the deduced amino acid sequence of the \textit{tchA} gene product showed 54.2\% identity with FbiB of \textit{M. bovis} \textsuperscript{[20]}. The \textit{tchA} gene also showed high similarity to other genes, SCO3037 of \textit{S. coelicolor} \textsuperscript{[21]} (73.5\% identity) and SAV5039 of \textit{S. avermitilis} \textsuperscript{[22]} (73.0\%), both of which are annotated to be genes encoding putative oxidoreductase. Comparative analysis of the amino acid sequences of \textit{tchA}, SCO3037, and SAV5039 supported the hypothesis that the deduced N-terminal amino acid sequence of the \textit{tchA} gene product is that shown in Fig. 3. The promoter region of the \textit{tchA} gene was suggested to be located within 527bp upstream of the S-end, considering that the 2.1-kb ZTDH1-DNA fragment inserted into replicative plasmids in both directions successfully complemented the mutant. The stop codon of \textit{tchA} was followed by a GC-rich region containing a palindromic sequence, (\textit{S}-\textit{GGCGCGCGGCGC}T\textit{GCCGTCGCCG-GCC-3}), which might act as a transcriptional terminator.

A FASTA homology search\textsuperscript{[23]} of the DDBJ protein database showed that the deduced amino acid sequences of ORF1, ORF2, ORF4, and ORF5 have high similarity to the unidentified genes SCO3035 (61.1\% identity), SCO3036 (77.4\%), SCO3038 (61.4\%), and SCO3040 (57.1\%) respectively of \textit{S. coelicolor},\textsuperscript{[21]} and to SAV5041 (62.7\%), SAV5040 (78.1\%), SAV5038 (60.3\%), and SAV5036 (56.4\%) respectively of \textit{S. avermitilis}.

The deduced amino acid sequence of ORF2 showed 53.3\% identity with the \textit{fbiA} gene,\textsuperscript{[20]} which is known to be involved in the conversion of FO to F\textsubscript{420}-5,6 by FbiB, and constitutes the \textit{fbiAB} operon in \textit{M. bovis} \textsuperscript{[19]}.

The gene organization of the ORF2-\textit{tchA} genes in \textit{S. aureofaciens} was also conserved in their homologs in both \textit{S. coelicolor} and \textit{S. avermitilis}.

Southern analysis of two cosmids, pGLA2 and pGLA11, which covered the 30-kb DNA fragment carrying the CTC biosynthetic gene cluster, revealed that no DNA fragments shown in Fig. 2B hybridized to these cosmids (data not shown), indicating that the \textit{tchA} gene is located external to the CTC biosynthetic gene cluster in the \textit{S. aureofaciens} genome.

\textbf{Discussion}

In this study, we succeeded in the cloning and characterization of the \textit{tchA} gene, which is essential for the conversion of DHDMT into 6-DMT. Unexpectedly, the cloned \textit{tchA} gene was located at the site shown in Fig. 2, external to the CTC biosynthetic gene cluster. Apparently, our result is not in contradiction with the finding of a report by Ryan\textsuperscript{[7]} that \textit{S. lividans} harboring a cosmid (LP\textsuperscript{2,127}, which carries a CTC biosynthetic gene cluster of \textit{S. aureofaciens}) produced a small amount of CTC (6 mg\ liter\textsuperscript{-1}), because the homolog of the \textit{tchA} gene exists in \textit{S. coelicolor} A3(2), a species closely related to \textit{S. lividans}. Poor CTC production by the transformant of \textit{S. lividans} may be caused by inadequate functioning of the \textit{tchA} homolog in the \textit{S. lividans} genome or poor expression of the genes in LP\textsuperscript{2,127}.

Our homology search indicated that the organization of ORF2-\textit{tchA} genes was highly similar to that of the \textit{fbiAB} operon, which encodes the proteins involved in the modification of the N-ribityl moiety of FO to yield F\textsubscript{420}-5,6 in \textit{M. bovis}. Among these proteins, FbiB is essential to convert FO into F\textsubscript{420} in \textit{M. bovis}.\textsuperscript{[19]} This gene cluster was also conserved in \textit{S. coelicolor} and
S. avermitilis. These results suggest that the tchA gene is not a specific functional gene in CTC biosynthesis, but is involved in the conversion of FO into F420. But the relationship between F420 and the final step in CTC biosynthesis remains unclear. The simplest explanation for this homogeneity is that FO is not the true cofactor of the corresponding enzyme, TCDH, and that F420 is the true cofactor. This hypothesis does not, however, agree well with the results of previous reports, because FO is catalytically active in all the F420-dependent reactions, including the S. aureofaciens F420: NADPH oxidoreductase reaction. In M. bovis, no influence of fbiB-deficiency on native metabolism has been identified, although the ΔfbiB-mutant was resistant to the antituberculosis drug PA-824, which probably accounts for the lack of F245,5,6 synthesis. Again, our preliminary experiments showed that the addition of cell-free supernatants of the S. aureofaciens cultures examined (in which some suitable F420 derivatives must have been present) never restored the production of 6-DMT in the NP71 strain (data not shown).

Another account of the function of the tchA gene is that the gene product is involved directly in the TCDH reaction. Some tchA homologs, such as SCO3037 of S. coelicolor, were annotated to be genes encoding putative oxidoreductase, which suggests the possibility that the tchA gene product is the enzyme involved in the TCDH reaction. Despite the efforts of Novotná et al., Fig. 3.

Comparison of the Deduced Amino Acid Sequence of the tchA Gene of S. aureofaciens.

Dashes indicate gaps introduced to maintain alignment. Perfectly conserved positions in the alignment are indicated by inverted letters. An arrow indicates the predicted second initiation codon of the tchA gene.

sau 1: -----------------------MELIEILGVSDSDPVDAIDVLALPGLKLVAKGTVRCILLLLTVTVTVS 46
sco 1: MSTDERTADAAGRGRDGKRVWALPDPVOSRDGLKLLAAEPLALCQVLLVYISVSAK 60
sav 1: -----------------------MPGDLVPAPDGDKLTLKAVEFPGVLGVRVTVTVSVSKA 40
mbo 1: -----------------------MTGEPHSGASTIELPIVLIUPFPFRPDCOSLAAVAPWRLGHKVTVTSAK 56

sau 47: EGRGPLHL----AD--DAATDAGVWRGPRPVKLVIENGPAVAAAGNDASNTAPG 98
sco 61: EGRGPLHL----AD--EAAATDAGVWRGPRPVKLVIENGPAVAAAGNDASNTAPG 112
sav 41: EGRGPLHL-------DD--DAATDAGVWRGPRPVKLVIENGPAVAAAGNDASNTAPG 92
mbo 57: EGRGPLHL-------DD--DAATDAGVWRGPRPVKLVIENGPAVAAAGNDASNTAPG 116

sau 99: TVLLPEPDPASARLRAISRLQOLIQLCAIYTVDTGPRFRGSCGTLVAILGCLMVDDH 158
sco 113: TVLLPEPDPASARLRAISRLQOLIQLCAIYTVDTGPRFRGSCGTLVAILGCLMVDDL 172
sav 93: TVLLPEPDPASARLRAISRLQOLIQLCAIYTVDTGPRFRGSCGTLVAILGCLMVDDL 152
mbo 117: ELALPEPDPASATRLGAIRLLPRLGVTALVTGPRFRGSCGTLVAILGCLMVDDY 176

sau 159: RGPYKSGNLVGLVWPAADERAAADLVKGCATGTGVLValCGLHVTAEVAGGTFPPL 218
sco 173: RGPYKSGNLVGLVWPAADERAAADLVKGCATGTGVLValCGLHVTAEVAGGTFPPL 232
sav 153: RGPYKSGNLVGLVWPAADERAAADLVKGCATGTGVLValCGLHVTAEVAGGTFPPL 212
mbo 177: AGVRPGYNLVTGVWPAADERAAADLVKGCATGTGVLValCGLHVTAEVAGGTFPPL 233

sau 219: RAAADMQLRGLGSLPSE--------RQTVVVRRVRSFDTDPPAAVRKAVVAMVTFPHART 274
sco 233: RAAADMQLRGLGSLPSE--------RQTVVVRRVRSFDTDPPAAVRKAVVAMVTFPHART 288
sav 213: RAAADMQLRGLGSLPSE--------RQTVVVRRVRSFDTDPPAAVRKAVVAMVTFPHART 268
mbo 234: RAAADMQLRGLGSLPSE--------RQTVVVRRVRSFDTDPPAAVRKAVVAMVTFPHART 293

sau 275: RPERGVSAGTGLHLLLNLGLAGORQELRLDGDARIAAATG reproductive diversity of F420: NADPH oxidoreductase reaction. 28) In M. bovis, no influence of fbiB-deficiency on native metabolism has been identified, although the ΔfbiB-mutant was resistant to the antituberculosis drug PA-824, which probably accounts for the lack of F425,5,6 synthesis. 19) Again, our preliminary experiments showed that the addition of cell-free supernatants of the S. aureofaciens cultures examined (in which some suitable F420 derivatives must have been present) never restored the production of 6-DMT in the NP71 strain (data not shown).

Another account of the function of the tchA gene is that the gene product is involved directly in the TCDH reaction. Some tchA homologs, such as SCO3037 of S. coelicolor, were annotated to be genes encoding putative oxidoreductase, which suggests the possibility that the tchA gene product is the enzyme involved in the TCDH reaction. Despite the efforts of Novotná et al., 28)
TCDH activity was not detected in the defined system, which was assembled from anhydrotetracycline oxygenase, F_{520}-NADPH oxidoreductase, co-factors, and each protein fraction of FPLC that was purified from the crude extract of \textit{S. aureofaciens} cells. Hence they suggested that more than two components are involved in the TCDH system. The \textit{icaH} gene product may be one of the hitherto unidentified proteins in the TCDH system.

This is the first report of the cloning of the gene participating in the final step of CTC biosynthesis, which is indispensable for the biosynthesis of tetracycline antibiotics. We believe that this work will extend our understanding of CTC biosynthesis and will also encourage the metabolic engineering of tetracycline antibiotics.

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References


