

Isolation of the Threonine Dehydratase Gene from a Tylosin-Producing Strain of *Streptomyces fradiae*

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From the plasmid library made from *Sst*I and *Sal*I-digested genomic DNA of *Streptomyces fradiae* NRRL 2702, four positive clones were selected using an oligodeoxynucleotide probe from the N-terminal amino acid sequence of purified threonine dehydratase. The cloned gene for threonine dehydratase was a 2.0 kilobase pair DNA fragment. The deduced amino acid sequence of PCR product (PCR245) was matched to that of the N-terminal part of threonine dehydratase from *S. fradiae* and this showed a high similarity to the threonine dehydratases of other organisms. This indicated that amino acid sequences of threonine dehydratases were highly conserved and the polypeptide product of the PCR245 was likely to be involved in the deamination of threonine.

The biosynthesis of tylosin that is an important 16-membered macrolide antibiotic produced commercially by *Streptomyces fradiae* NRRL 2702 (9) was reported to be repressed by ammonium ion (11). Valine dehydrogenase was repressed by the ammonium ion in a batch culture (12). Recently, we reported that both tylosin biosynthesis and threonine dehydratase (Tdt) synthesis were also repressed by ammonium ions in batch and continuous cultures (7), and Tdt was purified and characterized (8). The excess amount of the ammonium ion could play an important role in regulating the Tdt gene (*tdt*) expression, but this has not been verified yet. As a preliminary work to elucidate the regulation, we cloned and partially sequenced the *tdt* gene from *S. fradiae*.

Escherichia coli DH5 α (13) and JM109 (13), to be used as a host for plasmid and for M13 DNA sequencing, were grown at 37°C on Luria-Bertani (LB) medium (13). *Streptomyces fradiae* NRRL 2702 was aerobically grown in a seed culture medium (14) and a synthetic medium (7 and 15). Genomic libraries of *S. fradiae* were prepared using pGEM-3zf(-) (Promega). pCRTM II (Invitrogen) was used for inserting PCR products by TA cloningTM and excising them at the *Eco*RI site (Invitrogen).

The *tdt* gene was cloned by hybridization with an oligodeoxynucleotide constructed on the basis of the

N-terminal amino acid sequence of the purified threonine dehydratase (Tdt) from *S. fradiae* NRRL 2702 (8). Southern blot analysis (15) showed that the 41-mer oligodeoxynucleotide probe containing inosine (I) substitutions at especially degenerate codon positions (10) (5'-GAGGCCACCGGCCCTIACCCACCGAGTCIGCCGCCCCIGT-3') was hybridized only with the 2.0 kb *Sst*I-*Sal*I DNA fragment of the *S. fradiae* genomic DNA (lane 2 of Fig. 1). Screening of plasmid DNA isolated from the genomic DNA isolated by method of Hopwood *et al* (5) yielded four positive clones (clone B50, D72, D82, and D210) among 1,000 clones produced by colony hybridization (4) (Fig. 1). The sequence of the cloned DNA (clone D210) was sequenced using M13mp18 and M13mp19 (16). CODON PREFERENCE analysis (3) showed that there was no complete open reading frame (ORF) of the cloned DNA (data not shown). Therefore, we are now confirming the sequence and sequencing other cloned DNA fragments of clone B50, D72, and D82.

At the same time, PCR amplification (6) of the *tdt* gene fragments was carried out using consensus sequences of PILEUP (3) in Tdts of several other organisms (Fig. 4) from a search of the GeneBank and EMBL data bases as of July 1994 with TFASTA (3). We expected that the size of the PCR product obtained by using primer Pf-1 (5'ATCTACCT(GC)AAGCG(GC)GAGGAC-3'), the oligodeoxynucleotide of consensus residues of I-Y-L-K-R-E-D) and Pb-4 (5'-GT(CG)CCCTG(CG)CCCG(CG)GCGAT(CG)AC-3'), the

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| | | | | | |
|---------------------|------------|-------------|-------------|-------------|-------------|
| | 451 | | | | 500 |
| pileup. {Humserdhy} | QpsgSFKiRG | ighFckrwa. | ...kqG..ca | hfVcsSAGNa | gmaaAYAarq |
| pileup. {Rnsdh} | QpsgSFKiRG | ighLckmka. | ...kqG...ck | hfVcsSAGNa | gmatAYAarr |
| pileup. {Yscserthr} | QpsaSFksRG | ignLimksai | riqkdGkrsp | qVfasSgGNa | gfaaAtAcqr |
| pileup. {Sttdmrna} | | | | | |
| pileup. {Tomilv} | QrvfSFKlRG | aynMmsnlr | eeldkG.... | .VItaSAGNH | AQGVALAgqr |
| pileup. {Pcr245} | | | |AGNH | AQGVALAask |
| pileup. {Styilva} | QpvhSFKlRG | ayaMmtglte | eqkahG.... | .VItaSAGNH | AQGVAFssar |
| pileup. {Scilv1} | lpvfSFKlRG | aynMiakldd | sqrnqG.... | .VIacSAGNH | AQGVAFaakh |
| pileup. {Bacilva} | QvvrSFKlRG | ayhkmqlss | eqtenG.... | .VVcaSAGNH | AQGVAFsckh |
| pileup. {Ecotdc} | QrtgSFKiRG | afnklsstld | aekrkG.... | .VVacSAGNH | AQGVsLscam |
| pileup. {Corilva} | rkvqe*Wlae | rs*Fvpptfk | rrrhcfppsl | hqlhcSialv | flrkperkst |
| pileup. {Ecoilva} | hsgearrspa | saqL*aa..r | rirhdGgpdg | rtesarrdhc | fcG*prAgrr |
| Consensus | Q---SFK-RG | ----- | -----G---- | -VV--SAGNH | AQGVA-A---- |
| | 501 | | | | 550 |
| pileup. {Humserdhy} | LGvPA..... |tIVv | PgtTPaltIE | rlkneGa... | tckvvGElLD |
| pileup. {Rnsdh} | LGlpA..... |tIVv | PstTPaltIE | rlkneGa... | tVevvGEmLD |
| pileup. {Yscserthr} | Lslpc..... |tVVv | PtaTtkrmVD | kIrtGa... | qVIvsGayWk |
| pileup. {Sttdmrna} | | | | | |
| pileup. {Tomilv} | LncvA..... |kIVM | PttTPqiKID | aVralGg.. | dVVLyGktFD |
| pileup. {Pcr245} | LGltA..... |tIVM | PrtTPsiKVE | aVrarGg... | daVlFGDgFD |
| pileup. {Styilva} | LGvks..... |lIVM | PkaTadiKVD | aVrglGg... | eVlLhGanFD |
| pileup. {Scilv1} | LkIpA..... |tIVM | PvcTPsiKyq | nVsrlGs... | qVVLyGndFD |
| pileup. {Bacilva} | LGihg..... |kIFM | PstTPrqKVs | qVelFGkgfi | dIILtGdtFD |
| pileup. {Ecotdc} | LGIdg..... |kVVM | PkgaPksKVA | atcdysa... | eVVLhGdnFn |
| pileup. {Corilva} | LsVricrmfv | ptrsavr*tL | ershPkssam | qVslphlqvt | mprawpmcas |
| pileup. {Ecoilva} | vffcAvrreg | pdryanr... | hrrhqsrapa | rlrrrsaapr | rel**sEtqs |
| Consensus | LG--A----- | -----IVM | P--TP--KV- | -V---G----- | --VL-G--FD |
| | 551 | | | | 600 |
| pileup. {Humserdhy} | EafelAkaLa | knnpgw.... | .vYIPPFDDP | liweGhasiv | kelketLw.. |
| pileup. {Rnsdh} | EaiqlAkaLe | knnpgw.... | .vYIsPFDDP | liweGhtslv | kelketLs.. |
| pileup. {Yscserthr} | EAdtflktnv | mnkidsqvie | piYVhPFDDP | dIweGhssmi | dEIVqdLksq |
| pileup. {Sttdmrna} | | |PFDAp | gVIkGQGTig | tEInrqLk.. |
| pileup. {Tomilv} | EAqthAleLs | ekd.....G | lkYIPPFDDP | gVIkGQGTig | tEInrqLk.. |
| pileup. {Pcr245} | DAlahAlaLv | eer.....G | ltFVPPYDDP | dVIAGQG... | |
| pileup. {Styilva} | EAKakAieLa | qqq.....G | ftWVPPFDhP | mVIAGQGTla | lEllqq...d |
| pileup. {Scilv1} | EAKaecakLa | eer.....G | ltnIPPFDDhP | yVIAGQGTva | mEIlrgvrtta |
| pileup. {Bacilva} | DvyksAaecc | eae.....s | rtFIhPFDDP | dVmAGQGTla | vEIlndidte |
| pileup. {Ecotdc} | Dtiakvseiv | eme.....G | riFIppyDDP | kVIAGQGTig | lEImedL... |
| pileup. {Corilva} | DwafrdasMf | lclrlqsksvt | asWftaesls | pwwslaiTst | khrlqrMkmq |
| pileup. {Ecoilva} | DrtvtAagvh | lgaavrpsdG | dCR,..... | arhAGAGTap | agrprsrpic |
| Consensus | EA---A--L- | -----G | --YIPPFDDP | -VIAGQGT-- | -EI---L--- |

Fig. 4. PILEUP (3) comparison of the deduced amino acid sequence of the PCR product (PCR245) with those of biosynthetic Tdts (Sttdmrna of *Solanum tuberosum*, Tomilv of *Lycopersicon esculentum*, Styilva of *Salmonella typhimurium*, Scilv1 of *Saccharomyces cerevisiae*, Bacilva of *Bacillus subtilis*, Corilva of *Corynebacterium glutamicum*, and Ecoilva of *Escherichia coli* K-12), biodegradative Tdt (Ecotdc of *E. coli* KL-227), and serine dehydratase (Humserdhy of human, Rnsdh of rat, and Yscserthr of yeast).

The consensus line was determined with the PRETTY program (3). A capital letter indicates conservative residue in each of the vertical columns.

binding site.

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