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

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From the plasmid library made from Sstl and Sall-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 16membered 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 DH5α (13) and IM109 (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). pCR™ II (Invitrogen) was used for inserting PCR products by TA cloning[™] and excising them at the *Eco*RI site (Invitrogen).

oligodeoxynucleotide constructed on the basis of the

The tdt gene was cloned by hybridization with an

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)AAG-CG(GC)GAGGAC-31, the oligodeoxynucelotide of consensus residues of I-Y-L-K-R-E-D) and Pb-4 (5'-GT

(CG)CCCTG(CG)CCCG(CG)GCGAT(CG)AC-31, 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'-GAGGCCACCGGCCCICTIACCACCGAGTCI-

GGCGGCCCIGT-3') was hybridized only with the 2.0

kb Sstl-Sall DNA fragment of the S. fradiae genomic

DNA (lane 2 of Fig. 1). Screening of plasmid DNA iso-

lated 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 pro-

duced by colony hybridization (4) (Fig. 1). The se-

quence of the cloned DNA (clone D210) was

sequenced using M13mp18 and M13mp19 (16). CO-

DON PREFERENCE analysis (3) showed that there was

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oligodeoxynucelotide of those of V-I-A-G-Q-G-T) could be 363 bp (lane 2 of Fig. 2), that of PCR product between primer Pf-2 (5'-GC(GC)GG(GC)AA-CCACGC(GC)CAGGG-3', the oligodeoxynucelotide of those of A-G-N-H-A-Q-G) and Pb-4 245 bp (lane 3 of Fig. 2), that of PCR product between primer Pf-1 and Pb-3 (5'-CCCTG(CG)GCGTGGTT(CG)CC(CG)CC-3', the oligodeoxynucelotide of those of G-Q-A-H-N-G-A) 138 bp (lane 4 of Fig. 2). As shown in Fig. 2, the 245 bp-PCR product (PCR245) showed the same size as expected but sizes of the other PCR products were less than the expected values. When the temperature

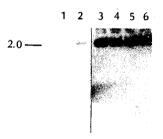


Fig. 1. Southern blot hybridization of *S. fradiae* genomic DNA doubly digested with *Sst*1 and *Sal*1 (lane 2) and plasmid DNA (doubly digested with *Sst*1 and *Sal*1) of clones obtained by colony hybridization (lane 3, clone B 50; lane 4, clone D72; lane 5, clone D82; lane 6, clone D210). Lane 1 is the pGEM size marker.

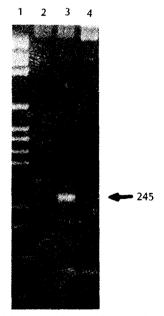
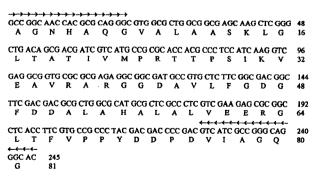


Fig. 2. PCR amplification of the *tdt* gene fragments. The PCR products were separated on a 2% low melting temperature agarose (SeaPlaque).

Lanes; 1, pGEM size marker; 2, PCR product between primer Pf-1 and Pb-4; 3, PCR product between primer Pf-2 and Pb-4; 4, PCR product between primer Pf-1 and Pb-3.

of the PCR reaction was shifted from 42°C to 69°C. PCR245 was more efficiently amplified. As a result of sequencing all the PCR products, it was found that only the PCR245 had proper primers (Pf-2 and Pb-4). Therefore, PCR245 was considered to be a correct PCR product. The nucleotide sequence of PCR245 is shown in Fig. 3. CODON PREFERENCE analysis showed that PCR245 had a ORF and the characteristic codon usage pattern for Streptomyces DNA (1) (data not shown). BESTFIT analysis (3) between PCR 245 and each nucleotide sequence of Sttdmrna (Solanum tubersorum), Tomily (Lycopersicon escultentum), Styilva (Salmonella typhimurium), Scilv1 (Saccharomyces serevisiae), Bacilva (Bacillus subtilis), Corilva (Corynebacterium glutamicum), Ecoilva (Escherichia coli K-12), and Ecotdc (E. coli KL-227) showed more than 60% identity in the N-terminal portion of all the eight Tdts (data not shown). Therefore, it was thought that PCR245 is the N-terminal portion of tdt gene coding for threonine dehydratase of S. fradiae. PILEUP comparison of the deduced amino acid sequence of PCR245 with those of the other Tdts showed that many residues of the PCR245 product were the same as the consensus residues of the other Tdts (Fig. 4). Since some serine dehydratases such as Yscserthr of yeast were reported to have also the function of threonine dehydratase (2), three serine dehydratases (Yscserthr, Humserdhy of human, and Rnsdh of rat) were compared with the Tdts. It was reported that the homology in the N-terminal portion of Styilva, Ecoilva, Ecotdc, and Scilv1 indicated that the N-terminal part was involved in catalysis and the Cterminal region of Styilva, Ecoilva, and Scilv1 was involved in either binding or interaction of the allosteric effector isoleucine with the enzyme, or subunit interactions (2). Therefore, the PCR245 product is likely to be involved in the deamination of threonine and may be the presumptive pyridoxal phosphate



pileup. {Humserdhy} pileup. {Rnsdh} pileup. {Yscserthr} pileup. {Sttdmrna} pileup. {Tomilv} pileup. {Pcr245} pileup. {Styilva} pileup. {Scilv1} pileup. {Bacilva} pileup. {Ecotdc} pileup. {Corilva} pileup. {Corilva} pileup. {Ecoilva} Consensus	QpsgSFKiRG QpsaSFKsRG QrvfSFKlRG QpvhSFKlRG lpvfSFKlRG QvtSFKlRG QrtgSFKiRG rkvqe*wlae hsgearrspa	ighLckmka. ignLimksai	kqGck riqkdGkrsp eeldkG eqkahG sqrnqG eqtenG aekrkG rrrhefppsl rirhdGgpdg	.VItaSAGNH	gmatAYAarr gfaaAtAcqr
pileup. (Humserdhy) pileup. (Rnsdh) pileup. (Yscserthr) pileup. (Sttdmrna) pileup. (Tomilv) pileup. (Pcr245) pileup. (Styilva) pileup. (Scilv1) pileup. (Bacilva) pileup. (Ecotdc) pileup. (Corilva) pileup. (Ecoilva) Consensus	LGlpA Lslpc LncvA LGltA LGVks LkIpA LGIdg LsVricrmfv vffcAvrreg	tIVvtVVvkIVMtIVMtIVMkIfMkVVM ptrsavr*tL pdryanr	PttTPqiKID PrtTPsiKVE PkaTadiKVD PvcTPsiKyq PstTPrqKVs PkgaPksKVa ershPkssam hrrhqsrpaa	rlkneGa kIrntGa aVralGg aVrarGg aVrglGg rVsrlGs qVelfGkgfi	eVVLhGDnFn mprawpmcas
pileup. (Humserdhy) pileup. (Rnsdh) pileup. (Yscserthr) pileup. (Sttdmrna) pileup. (Tomilv) pileup. (Pcr245) pileup. (Styilva) pileup. (Scilv1) pileup. (Bacilva) pileup. (Ecotdc) pileup. (Corilva) pileup. (Ecoilva) Consensus	EAiqlAkaLe EAdtflktnv EAqthAleLs DAlahAlaLv EAkakAieLa EAkaecakLa DvyksAaecc Dtiakvseiv pwafrdasMf DrtvtAagvh	knnpgw mnkidsqvie	.vYIsPFDDP piYVhPFDnP PFDaP lkYIPPFDDP ltFVPPYDDP ftWVPPFDhP ltnIPPFDhP rtFIhPFDDP riFIPPYDDP asWftaesls dcR	llweGhasiv llweGhtslv dlweGhssmi gVIkGQGTig gVIkGQGTig dVIAGQG mVIAGQGTla yVIAGQGTla kVIAGQGTla kVIAGQGTig pwwslaiTst arhAGaGTap -VIAGQGT	kElketLsdEIvqdLksq tEInrqLk tEInrqLk lEllqqd mEIlrqvrta vEIlndidte lEImedL khrlqrMkmq agrpsrpric

Fig. 4. PILEUP (3) comparison of the deduced amino acid sequence of the PCR product (PCR245) with those of biosynthetic Tdts (Sttdmrna of Solanum tubersorum, Tomilv of Lycopersicon escultentum, Styilva of Salmonella typhimurium, Scilv1 of Saccharomyces serevisiae, Bacilva of Bacillus subtilis, Corilva of Corynebacterium glutamicum, and Ecoilva of Escherichia coli K-12), biodegrative 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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