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Overproduction of Soluble Trichodiene Synthase from *Fusarium sporotrichioides* in *Escherichia coli*[1]

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Trichodiene synthase is a sesquiterpene cyclase isolated from various fungal species which catalyzes the cyclization of farnesyl diphosphate (FPP) to trichodiene. The trichodiene synthase gene (*Tox5*) of *Fusarium sporotrichioides* has previously been cloned and expressed as 0.05–0.1% of total cell protein in *Escherichia coli*. We have used polymerase chain reaction to amplify the trichodiene coding sequence carried on the plasmid pTS56-1. The resulting DNA, carrying a *Bam*HI restriction site and the T7 *gene 10* ribosome binding site and translational spacer element immediately upstream of the ATG start codon as well as a *Hin*dIII site adjacent to the translational stop codon, was inserted into the corresponding sites of the expression vector pLM1. The latter vector carried the promoter and translational leader sequence from T7 *gene 10* and the *E. coli rmBT$_1$T$_2$* tandem transcription terminator. This construct was cloned into *E. coli* BL21(DE3). The resulting transformants, when induced with isopropyl β-D-thiogalactoside, produced trichodiene synthase as 20–30% of total soluble protein. The recombinant synthase, which could be purified fivefold to homogeneity by ammonium sulfate precipitation, ion-exchange chromatography on Q Sepharose, and gel filtration on Superose 12, was identical to native protein in steady-state kinetic parameters and mobility on sodium dodecyl sulfate–polyacrylamide gel electrophoresis and had the expected MENFP N-terminal sequence.

Sesquiterpene synthases catalyze the cyclization of the universal acyclic precursor farnesyl diphosphate (FPP[3], **1**) to any of 200 known cyclic sesquiterpenes. Although only a relative handful of these enzymes have been isolated and subjected to mechanistic study, they are all operationally soluble proteins, either monomers or homodimers of subunit M_r 40,000–60,000, and require no cofactor other than a divalent metal cation, Mg^{2+} usually being preferred (1–3). By far the best studied of these cyclases has been trichodiene synthase, a fungal protein responsible for the conversion of FPP to trichodiene (**3**), the parent hydrocarbon of the trichothecane family of mycotoxins (4, 5). Extensive mechanistic studies have supported a cyclization mechanism in which FPP is initially rearranged to its tertiary allylic isomer, nerolidyl diphosphate (NPP, **2**), which in turn undergoes further ionization, cyclization, and rearrangement to give trichodiene (6–9) (Scheme I). All the various electrophilic reactions and rearrangements are believed to take place at a single active site. According to this picture, the folding of the acyclic substrate at the active site is a major determinant of the structure and stereochemistry of the eventually formed sesquiterpene product (10).

Trichodiene synthase, which was first isolated from apple mold fungus *Trichothecium roseum* (11), has been purified to homogeneity from the T-2 toxin producer, *Fusarium sporotrichioides* and has been shown to be a homodimer of 45-kDa subunits (12). The closely related trichodiene synthase of *Gibberella pulicaris* (anamorph *F. sambucinum*) has been partially purified as well (13). Screening of a λgt11 genomic library of *F. sporotrichioides* DNA with antibodies to the cyclase resulted in isolation of the structural gene for trichodiene synthase (*Tox5*), encoded in a 1182-bp open reading frame containing a 60-nt in-frame intron (14). *In vitro* excision of the intron and subcloning of the coding sequence into the *Escherichia*

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[3] Abbreviations used: FPP, farnesyl diphosphate; NPP, nerolidyl diphosphate; PCR, polymerase chain reaction; EDTA, ethylenediaminetetracetic acid disodium salt; LB, Luria–Bertani; IPTG, isopropyl β-D-thiogalactopyranoside; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; rbs, ribosome binding site.

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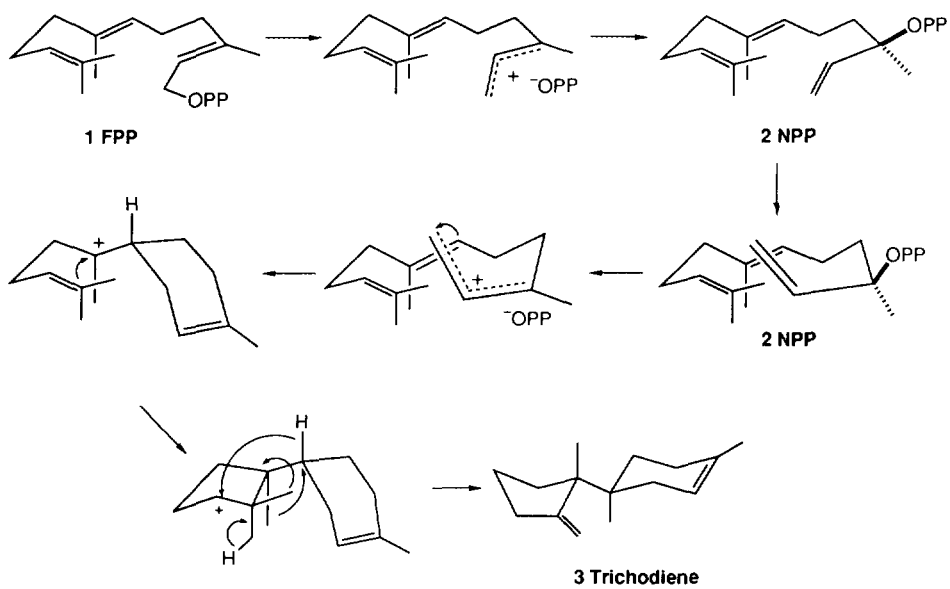


SCHEME I. Cyclization of farnesyl diphosphate (FPP) to trichodiene through intermediacy of nerolidyl diphosphate (NPP).

coli expression vector pDR540 resulted in bacterial expression of trichodiene synthase, albeit at relatively low levels corresponding to 0.05–0.1% of total cellular protein (15). Interestingly, transformed cells produced trichodiene itself at levels of up to 60 μg/liter, presumably due to utilization of endogenous FPP by the recombinant cyclase. The *F. sporotrichioides Tox5* gene has also been cloned into tobacco (*Nicotiana tabacum*), resulting in heterologous expression of trichodiene synthase and the production of detectable quantities of trichodiene in the plant host (16).

The corresponding trichodiene synthase gene of *G. pulicaris* has also been isolated by using the *F. sporotrichioides* gene as a hybridization probe to screen a library of *G. pulicaris* DNA (17). The two sequences showed an 89% homology at the nucleotide level, including the 60-nt intron, and a 96% identity at the amino acid level, the primary difference lying in the presence of an additional nine amino acids at the C-terminus of the *G. pulicaris* synthase. Although trichodiene synthase had no overall homology to any other known gene, significantly, both synthases were found to contain an aspartate rich motif previously noted in several isoprenoid chain elongation enzymes, including FPP synthase (18). Thus the trichodiene synthase of *F. sporotrichioides* had the sequence VLDDSKD starting at amino acid 98, while the *G. pulicaris* cyclase had the sequence VLDDSSD at the same position. Furthermore, both enzymes contained a short sequence rich in basic amino acids (DRRYR—*F. sporotrichioides*, DHRYR—*G. pulicaris*) closely analogous to a presumptive active site peptide previously implicated in pyrophosphate binding by avian FPP synthase (19). Nothing more is known currently about the active site of trichodiene synthase, or indeed any other sesquiterpene synthase. We therefore sought to obtain sufficient quantities of enzyme for further mechanistic and enzymological studies. We now report the high-level overexpression of trichodiene synthase and the purification and preliminary characterization of the recombinant cyclase.

EXPERIMENTAL PROCEDURES

Materials. Plasmid pLM1 and *E. coli* strain BL21(DE3) were gifts from Professor Gregory L. Verdine of Harvard University. *E. coli* strain XL1-Blue was from Stratagene (San Diego, CA) and strain JM109(DE3) was from Promega (Madison, WI). Restriction enzymes *Sca*I and *Bam*HI were from Stratagene and *Hin*dIII was from Promega. T4 DNA ligase was purchased from Promega. *Pfu* DNA polymerase was obtained from Stratagene. Oligonucleotides were synthesized by the phosphoramidite method on a BioSearch 8700 by Charles Sutterland. Bulk matrix Q Sepharose and Superose 12 were obtained from Sigma (St. Louis, MO) and Bio-Gel P-6DG was purchased from Bio-Rad (Richmond, CA). [1-^{3}H]FPP (16.7 μCi/μmol) was synthesized as previously described (7). All other materials used for recombinant DNA manipulations, enzyme assay, and protein purification were analytical grade or higher. All buffers and nutrient broths were prepared with doubly deionized nanopure grade water.

Methods. Restriction endonuclease digestions, DNA ligations, preparation and transformation of competent cells, plasmid minipreps, and other standard recombinant DNA manipulations were carried out according to published procedures (20), except that after restriction enzyme digestion, a Millipore Ultrafree-Probind filter unit was used to remove protein in place of phenol–chloroform extraction. Maxipreps of plasmid DNA by the alkaline lysis method (20) followed by precipitation with 13% polyethyleneglycol were used to prepare double-stranded DNA for sequencing. DNA sequencing on plasmid products was carried out by the Sanger dideoxy chain termination method using the Sequenase 2.0 kit (U.S. Biochemical) according to the standard protocols and with primers developed earlier for the sequencing of the trichodiene synthase gene (14). PCR was carried out in a Coy thermal cycler (Ann Arbor, MI). SDS–PAGE was performed in 14 cm × 14 cm × 1 mm 12% gels by the method of Laemmli (21) and proteins were visualized by staining with Coomassie brilliant blue R-250. Densitometric analysis of dried SDS–PAGE gels was performed on a LKB Ultroscan XL laser densi-

tometer. Immunoblot detection of trichodiene synthase was carried out as previously described using Immobilon-P PVDF transfer membranes (Millipore) and rabbit antiserum against *F. sporotrichioides* trichodiene synthase (14). Radioactivity measurements were obtained using 10-ml solutions of Optifluor (Packard) in a Beckman LS5801 liquid scintillation counter.

Construction of the pLM1-TS overproducer by PCR. Plasmid pTS56-1, previously described as pTS46-10Δ (470–529) and containing the *F. sporotrichioides* trichodiene synthase coding sequence in pDR540 (15), was linearized by digestion with *Sca*I and the resulting DNA was used as template for the PCR method of Macferrin *et al.* (22). The start primer (Primer 1) was a 56-mer oligonucleotide containing a *Bam*HI restriction site (underlined), the T7 *gene 10* ribosome binding site (rbs) and translational spacer element (italics), and the first nine codons of the trichodiene synthase gene, including the start codon (bold) [5'-d(TGATTACGCCGGATCC*AGGAGATATACAT***ATG**-GAGAACTTTCCCACCGAATACTTC)-3']. The reverse or halt primer (Primer 2) was a 43-mer containing a *Hin*dIII restriction site (underlined) and the complementary sequence corresponding to the stop codon (bold) and the last eight codons of trichodiene synthase [5'-(dGCCAGT-GAATAAGCTTTCACTCCACTAGCTCAATTGAGCTCAG)-3']. A typical 100-μl reaction was made up to contain sterile H_2O (51 μl), 10 mM $MgCl_2$ (10 μl), 10× buffer 1 (10 μl consisting of 200 mM Tris–Cl, pH 8.2, 100 mM KCl, 60 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, 1% Triton X-100, and 100 ng/μl nuclease-free BSA), mixture of four dNTPs, each at 2.5 mM (10 μl), Primer 1 (20 pmol), Primer 2 (20 pmol), linearized pTS56-1 (0.5 ng), and *pfu* polymerase (1 μl, 2.5 units). All reactants except the polymerase were added in the order given to a screw cap 0.5-ml Eppendorf tube which was placed in the thermal cycler before starting the following program: (a) 96°C, 5 min; 65°C, 3 min—add *pfu* polymerase plus 50 μl of mineral oil. (b) 78°C, 3 min (extension); 95°C, 2 min (denaturation); 65°C, 2 min (annealing); 35 cycles. (c) 78°C, 7 min. (d) 4°C, hold. The reactants were first heated to 96°C for 5 min, then removed during the 3-min 65°C step, chilled rapidly in an ice bath before addition of polymerase and mineral oil, briefly centrifuged, and replaced in the cycler at 65°C. The typical yield was 2–5 μg of amplified DNA per 100 μl reaction mixture. The PCR product was analyzed on a 1.5% agarose gel and purified using Millipore Pro-Bind and Ultrafree-MC 30,000 MW filter sets. The recovered DNA was digested sequentially with *Bam*HI and *Hin*dIII and ligated with T4 DNA ligase into pLM1 (23) previously treated with *Bam*HI and *Hin*dIII. The ligation product was used to transform competent cells of *E. coli* XL1-blue and the transformation mix was plated out on LB agar plates containing 60 μg/ml of ampicillin. Plasmid minipreps on several of the resulting single colonies led to isolation of pZW03 containing the desired insert, as established initially by agarose gel electrophoresis of linearized DNA. pZW03 was then used to transform two different hosts carrying the inducible T7 RNA polymerase in a prophage under the control of a *Lac*UV5 promoter, *E. coli* BL21(DE3) and JM109(DE3). From the resulting transformants, 20 single colonies were picked for trichodiene synthase assay, as described below. Most of these colonies were found to have the desired cyclase activity, with colonies of *E. coli* BL21(DE3)/pZW03 having about 10 times higher activity than those of *E. coli* JM109(DE3)/pZW03. One colony giving rise to the highest observed cyclase activity was used for further preparative scale production of trichodiene synthase.

Trichodiene synthase assay. The assay of trichodiene synthase was based on the previously described procedure (1, 12). Enzyme extract (1–5 μl) was added to 500 μl of buffer T (10 mM Tris, pH 7.5, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 15% glycerol) and 10 μl of [1-^{3}H]FPP (1.13×10^5 dpm) in a glass tube; the mixture was vortexed and incubated at 30°C for 15 min. The reaction was terminated by addition of 100 μl of 100 mM EDTA (pH 7.25) and hexane was added to extract the olefinic product. After vortexing for 10 s followed by centrifugation for 3 min, the supernatant was withdrawn and passed through a 4-cm silica gel column in a Pasteur pipet directly into a scintillation vial and the radioactivity was measured. One unit of trichodiene synthase activity corresponds to the formation of 1 nmol of trichodiene/min.

Expression of trichodiene synthase by E. coli BL21(DE3)/pZW03. Growth and production conditions were optimized to give the highest level of trichodiene synthase activity. In a typical growth, a loopful of *E. coli* BL21(DE3)/pZW03 was used to inoculate 50 ml of LB medium containing 100 μg/ml of ampicillin in a 250-ml flask and the culture was incubated overnight at 37°C and 250 rpm. A portion of the resulting culture (10 ml) was used to inoculate 500 ml of the same medium and the culture was grown at 37°C and 250 rpm for ca. 5 h until an OD of 0.7–1.1. IPTG was then added to a final concentration of 0.5 mM and the cultures were incubated further at 30°C. For assay of trichodiene synthase production, 1-ml samples of the culture broth were withdrawn at intervals and centrifuged for 1 min at 12,000*g*. The pellet was taken up in 75 μl of lysis buffer (50 mM Tris, pH 8.0, 1 mM EDTA, and 100 mM NaCl) and 5 μl of a solution of lysozyme (10 mg/μl). After vortexing, the mixture was incubated at room temperature for 20 min. A 10-μl aliquot of the lysate was assayed for trichodiene synthase activity as described above. A profile of trichodiene synthase production as a function of time after IPTG induction is shown in Fig. 1. Portions of the whole cell lysate were mixed with SDS sample buffer, boiled for 3 min, and analyzed by SDS–PAGE. By densitometric analysis of the stained SDS–PAGE gels, the trichodiene synthase band was found to correspond to 30% of total cellular protein. To determine the solubility of the overexpressed protein, the lysate was also centrifuged at 8000*g* for 15 min and the soluble and insoluble fractions were analyzed by SDS–PAGE.

Purification of overexpressed trichodiene synthase. Purification of recombinant trichodiene synthase was adapted from the method previously used for the native *F. sporotrichioides* enzyme. All data in the procedure described below are based on 500 ml of culture. The procedures were carried out at 4°C and all tubes and pipets were either polystyrene or polypropylene. At each stage of enzyme purification, synthase activity was assayed on 50-μl aliquots according to the standard procedure. Protein concentrations were determined by the method of Bradford (24) using the Bio-Rad protein assay kit and bovine serum albumin as standard.

Fermentation of *E. coli* BL21(DE3)/pZW03 was halted 3 h after IPTG induction and the cells were harvested by centrifugation at 8000*g*. The pellet from 500 ml of culture was placed in an 360-ml chamber of a Bead-Beater (Biospec Products, Bartlesville, OK) containing 180 ml of buffer T supplemented with 0.1 mM phenyl methylsulfonyl fluoride and 180 ml of 0.1-mm glass beads. The cells were ruptured using three rounds of a 30-s on, 30-s off cycle. The contents of the chamber were partially decanted and the remaining beads were rinsed with 40–50 ml of buffer T. Any beads which were carried over were removed and the cell homogenate was centrifuged at 8500*g* for 10 min. The supernatants were carefully decanted to avoid collecting the lipid, then recentrifuged at 150,000*g* for 65 min. The resulting supernatant was adjusted to 75% saturation with ammonium sulfate and the precipitated protein was collected by centrifugation at 20,000*g* for 15 min. The pellet, which was redissolved in 2 ml of buffer T, could be used directly for the next purification step or stored at −80°C.

The redissolved pellet was desalted by passage through a column of Bio-Gel P-6DG (1.6 × 25 cm) and the protein-containing fractions were directly loaded onto a Q Sepharose anion-exchange column (1.6 × 19 cm). The column was washed with 25 ml of buffer T and then eluted with 200 ml of a linear gradient of 100 to 350 mM KCl in buffer T. Protein eluted in a broad peak between 260 and 310 mM KCl and the highest activity fractions having a trichodiene synthase specific activity of 164 units/mg were pooled. The latter fractions were applied to a column of Superose 12 (1.6 × 45 cm) which was eluted with buffer T. The highest purity fractions were judged to be homogeneous by SDS–PAGE.

N-terminal sequencing. A portion of the Superose 12-purified protein was subjected to SDS–PAGE then transferred to a Immobilon-P PVDF membrane (25) and stained with Coomassie blue. The portion of the membrane carrying the 45,000-Da protein band was cut out and subjected to automated Edman degradation in an ABI Model 477A protein sequencer at the Harvard Microchemistry Facility by Dr. William S. Lane.

RESULTS

To improve the yields of recombinant trichodiene synthase, we initially examined the effect of the cloning host and culture conditions on total cyclase activity. A modest improvement to 0.2% of soluble protein was observed when pTS56-1 was cloned into *E. coli* XL1-blue and a further twofold increase in yield resulted from lowering the incubation temperature to 25°C following induction with IPTG. On the other hand, the enzyme thus obtained still proved difficult to purify and we therefore explored the use of alternate expression systems. A preliminary examination of several commonly used expression vectors, however, initially gave little or no improvement in synthase expression. Thus subcloning into either pKK223-3 (Pharmacia) (26) or pRIT2T (Pharmacia) (27) resulted in levels of trichodiene synthase less than or no greater than those observed for the parent pDR540 clone. No expression at all was observed for the T7-based, T7-7 expression system of Tabor and Richardson (28), while attempted use of the secretion vector pIN-III-(1pp)-A2 (29) resulted in cell lysis. We therefore turned to the use of the expression cassette PCR (ECPCR) method in which PCR is used to position a strong rbs immediately upstream of the coding sequence of the gene of interest and the resulting amplified construct, containing suitable restriction sites at each terminus, is inserted into an engineered expression vector containing a set of appropriate transcriptional control elements (22). One such recently developed vector is pLM1 (Scheme II), containing a strong T7 promoter and translational leader sequence from T7 *gene 10* placed upstream of the pUC19 multiple cloning site which in turn is followed by the *E. coli* $rmBT_1T_2$ tandem transcription terminator (23).

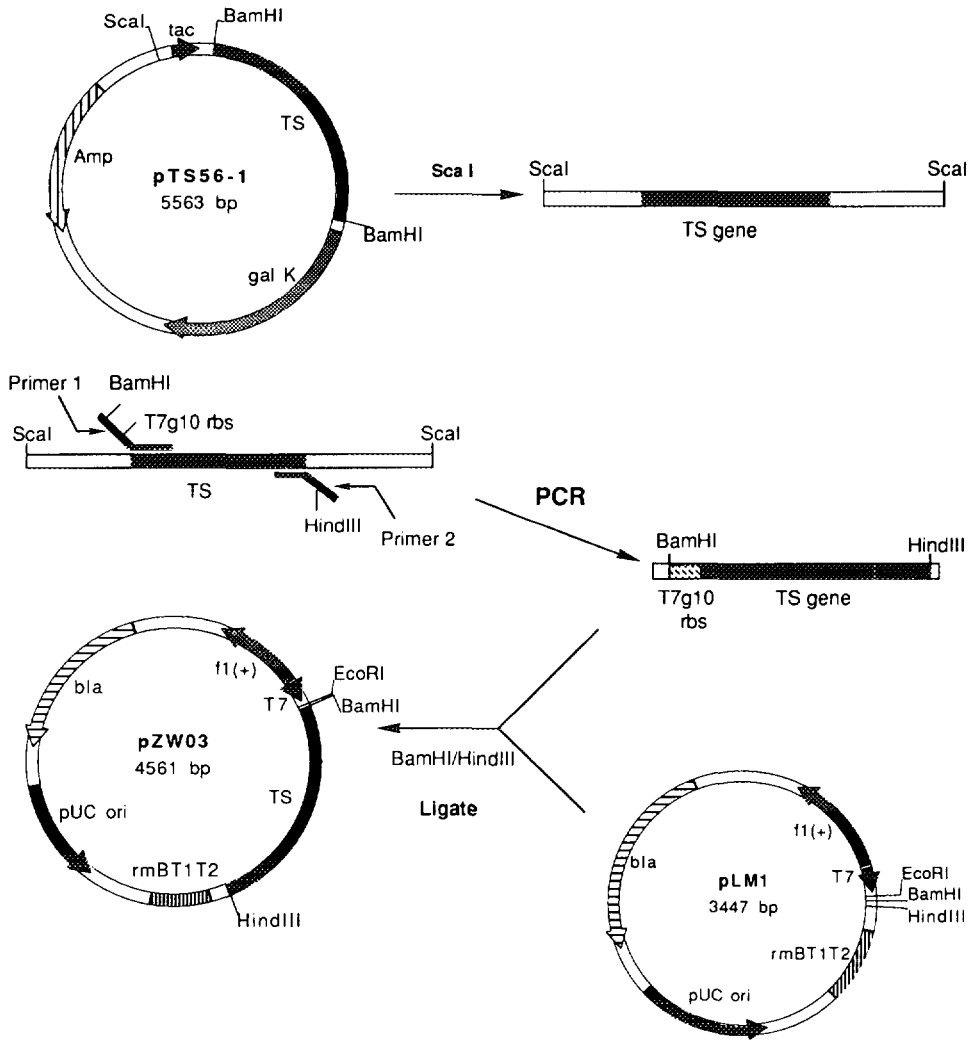
The start primer (Primer 1) for PCR was synthesized so as to contain a *Bam*HI site, a T7 *gene 10* rbs and spacer (30), and the first 27 bases of the trichodiene synthase gene, including the normal ATG start codon. The corresponding halt primer (Primer 2) contained a *Hin*dIII sequence followed by the complementary set of bases for the stop codon and eight C-terminal amino acids of trichodiene synthase. Linearized pTS56-1 DNA was used as the template to generate the desired expression cassette by PCR (Scheme 2). The PCR product was digested with *Bam*HI and *Hin*dIII, ligated into a similarly digested sample of pLM1, and used to transform competent cells of *E. coli* XL1-blue. The resulting expression plasmid was designated as pZW03 and was used to transform a suitable expression host, *E. coli* BL21(DE3), which harbored a prophage carrying the gene for T7 RNA polymerase behind the *lac*UV5 promoter (30, 31). *E. coli* BL21(DE3), which lacks the *lon* protease, was found to be a superior host to the alternative T7 lysogen JM109(DE3). Two colonies from among the resulting transformants were found to have the highest levels of trichodiene synthase activity and one was selected for further study. Sequence analysis of the trichodiene synthase gene in pZW03 confirmed that no base changes had occurred in the PCR amplification and subcloning from the parent pTS56-1 vector.

The time course of trichodiene synthase production was studied under a variety of conditions. Cultures of *E. coli* BL21(DE3)/pZW03 incubated at 30°C reached a maximum cyclase activity within 3 h after IPTG induction (Fig. 1). The maximum specific activity of the crude extracts corresponded to ca. 20% of the activity of purified trichodiene synthase (see below). The level of trichodiene synthase activity was roughly correlated with the intensity of a 46.3-kDa protein by SDS–PAGE (Fig. 2) which was absent from extracts of cells harboring only the parent expression vector pLM1. Western blot analysis using specific antiserum to the *F. sporotrichioides* synthase confirmed the identity of this band (data not shown) as trichodiene synthase. Laser densitometric analysis of stained SDS–PAGE gels run on cells boiled in SDS loading buffer led to an estimate that this band corresponded to 30% of total cellular protein.

The recombinant synthase from a 500-ml culture was readily purified fivefold to homogeneity by a two-step procedure involving ion-exchange chromatography on Q Sepharose of the 75% ammonium sulfate pellet followed by gel filtration on Superose 12 to remove a very minor 66-kDa protein contaminant (Table I). The overall yield of the purification procedure was 12%, corresponding to 6 mg of purified protein from 500 ml of culture. The homogenous synthase had a measured V_{max} of 169 units/mg, compared to a previously determined V_{max} for the native fungal enzyme of 102 units/mg (12). The measured K_m for recombinant cyclase was 83 ± 4 nM, in close agreement with the value of 75 ± 4 nM for the native enzyme (8). N-terminal sequencing of the recombinant protein gave a sequence of MENFP, in complete agreement with the established DNA sequence and consistent with the previously observed N-terminal ENFP sequence of the native fungal enzyme.

DISCUSSION

Although by now the structural genes for several sesquiterpene cyclases have been cloned and sequenced (32), prior to the current work only one, trichodiene synthase, had been reported to be expressed in a bacterial host (15). Unfortunately, the maximum levels of cyclase expression achieved with the originally reported construct, pTS56-1 (0.05–0.1% of total protein) (15), were actually lower by a factor of more than 10 than those previously observed for high-producing cultures of *F. sporotrichioides* (12). We now have achieved the expression of trichodiene synthase at levels ranging from 20 to 30% of soluble protein. Examination of cell lysates for insoluble components by SDS–PAGE indicated insignificant amounts of protein with a mobility corresponding to the characteristic 46.3-kDa trichodiene synthase band. This observation is in



SCHEME II. Use of PCR to construct expression vector pZW03 from pTS56-1, containing trichodiene synthase (TS) gene, and pLM1, with T7 *gene 10* promoter and translational leader (T7).

contrast to the formation of large amounts of inclusion bodies which frequently accompanies or even dominates attempts to attain high-level overexpression in bacterial hosts.

The success of the expression strategy described above probably rests on the interaction of several important factors. By placing the trichodiene synthase gene under the control of the T7 promoter, one can simultaneously suppress unwanted expression by the RNA polymerase of the host organism, XL1-blue, in primary transformants, while allowing expression from a strong promoter in hosts harboring an inducible T7 RNA polymerase system (28, 30, 31). In this regard, the reasons for the failure of attempts to express trichodiene synthase with the widely used T7-7 expression system are unclear. Although the precise influence of the T7 *gene 10* translational leader sequence in pLM1 is uncertain, it is likely that the mRNA for this highly expressed gene product is exceptionally stable. Similarly, the T7 *gene 10* rbs and spacer introduced in the expression cassette has previously been shown to support high-level expression in pLM1 and related systems (22, 23, 33). Interestingly, insertion of the same PCR-generated trichodiene synthase expression cassette into two related vectors with different promoters and transcriptional sequences, pHN1$^+$ (*tac* promoter) (22) and pKEN2 (*src* promoter),[4] resulted in extremely low expression of the desired synthase activity. Finally, the greater observed levels of production of trichodiene synthase in *E. coli* BL21(DE3)/pZW03 in comparison to those of JM109(DE3)/pZW03 may reflect the absence of the *lon* protease in the former host, resulting in greater stability of the protein product.

The recombinant trichodiene synthase could be purified by a convenient two-step procedure. The N-terminal se-

[4] G. Verdine, personal communication.

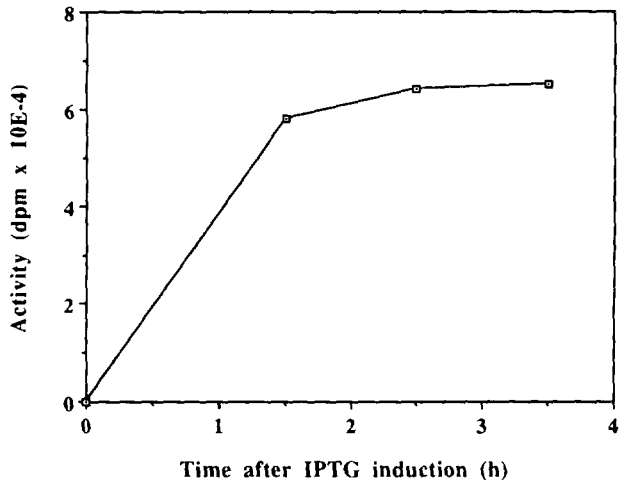


FIG. 1. Time dependence of trichodiene synthase activity of *E. coli* BL21(DE3)/pZW03 as a function of time after IPTG induction and incubation at 30°C. Assays were carried out on 10-μl aliquots of cell lysate for 15 min at 30°C in the presence of 1.13×10^5 dpm of [1-^{3}H]FPP as described under Experimental Procedures. Activity refers to activity of hexane elutable product.

quence of the homogenous protein was identical to that of the native synthase save for the additional Met at the N-terminus. Purified recombinant trichodiene synthase was also essentially indistinguishable from the native fungal enzyme in gel mobility and steady-state kinetic parameters. The small increase in observed V_{max} for the recombinant synthase probably reflects differences in the particular protein standards used to determine protein concentration. Trichodiene synthase is now available in substantial quantities and is currently being used in studies designed to identify the nature of the active site and overall protein structure of this important cyclase.

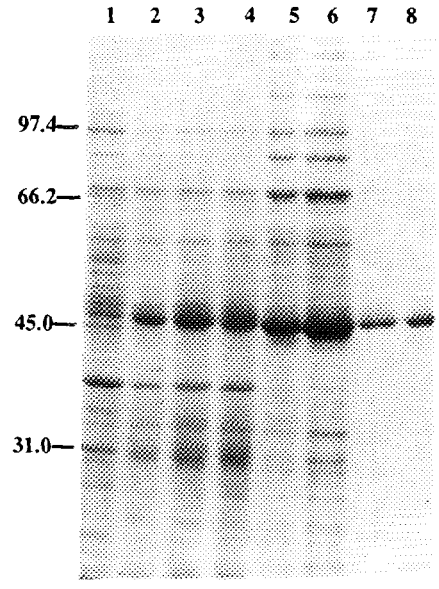


FIG. 2. Ten percent SDS–PAGE analysis of trichodiene synthase production: (molecular weight standards in kDa). Lane 1, total cell protein from BL21(DE3)/pLM1; Lanes 2–4, total cell protein of BL21(DE3)/pZW03 1.5, 2.5, and 3.5 h after IPTG induction (30°C); Lane 5, supernatant after 150,000g centrifugation of extracts (Table I); Lane 6, pellet after 75% ammonium sulfate precipitation; Lane 7, pooled fractions from Q Sepharose column; Lane 8, pooled fractions from Superose 12 column.

TABLE I

Purification of Trichodiene Synthase from *E. Coli* BL21(DE3)/pZW03[a]

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)
150,000g supernatant	277	8600	31	—
$(NH_4)_2SO_4$ precipitation	100	5600	55	65
Q Sepharose	11	1800	164	21
Superose 12	6.1	1031	169	12

[a] Data are based on protein from a 500-ml culture.

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