Supporting Information

Characterization of TioQ, a type II thioesterase from the thiocoraline biosynthetic cluster

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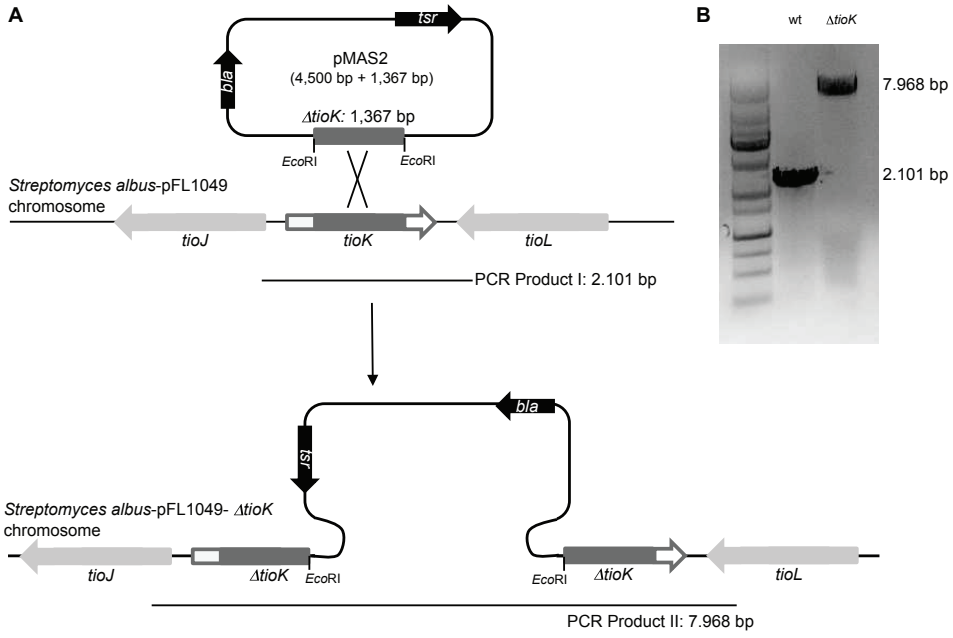

Fig. S1. Preparation of the *tioK* mutant strain (Δ*tioK*). **A.** Transformation of *S. albus*-pFL1049 strain with the plasmid pMAS2 renders a gene disruption mutant in the *tioK* gene after a single recombination event. PCR amplification regions from the wild-type (wt) and mutant strains are shown using the oligonucleotide primers MAS-ΔKup and MAS-ΔKrp for mutant strain confirmation. **B.** Agarose gel electrophoresis showing the PCR reactions using the above primers on the wt and Δ*tioK* mutant strain genomic DNA, confirming the disruption event in the *tioK* region.

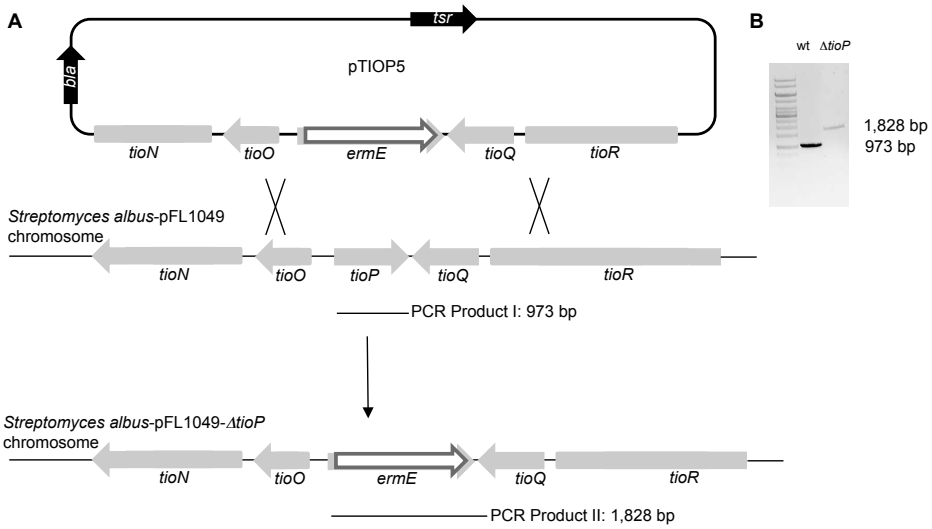

Fig. S2. Preparation of the *tioP* mutant strain (Δ*tioP*). **A.** Transformation of *S. albus*-pFL1049 strain with the plasmid pTIOP5 renders a gene replacement mutant in the *tioP* gene after a double recombination event. PCR amplification regions from wild-type (wt) and mutant strain are shown using oligonucleotide primers Test-ΔPup and Test-ΔPrp for mutant strain confirmation. **B**: Agarose gel electrophoresis of PCR reactions using these primers on wt and mutant strain genomic DNA, confirming the gene replacement event in the *tioP* region.

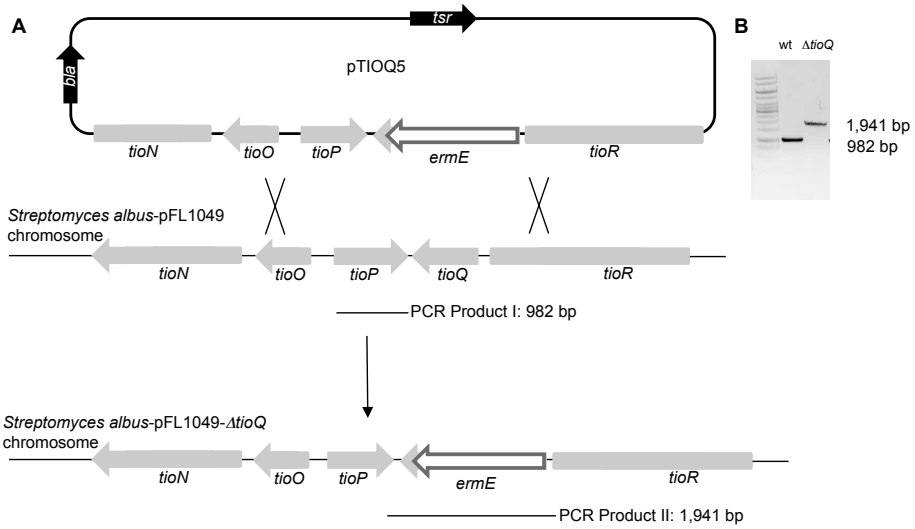

Fig. S3. Preparation of the *tioQ* mutant strain (Δ*tioQ*). **A.** Transformation of *S. albus*-pFL1049 strain with the plasmid pTIOQ5 renders a gene disruption mutant in the *tioQ* gene after a double recombination event. PCR amplification regions from wild-type (wt) and mutant strain are shown using oligonucleotide primers Test-ΔQup and Test-ΔQrp for mutant strain confirmation. **B.** Agarose gel electrophoresis of PCR reactions using these primers on wt and mutant strain genomic DNA, confirming the gene replacement event in the *tioQ* region.

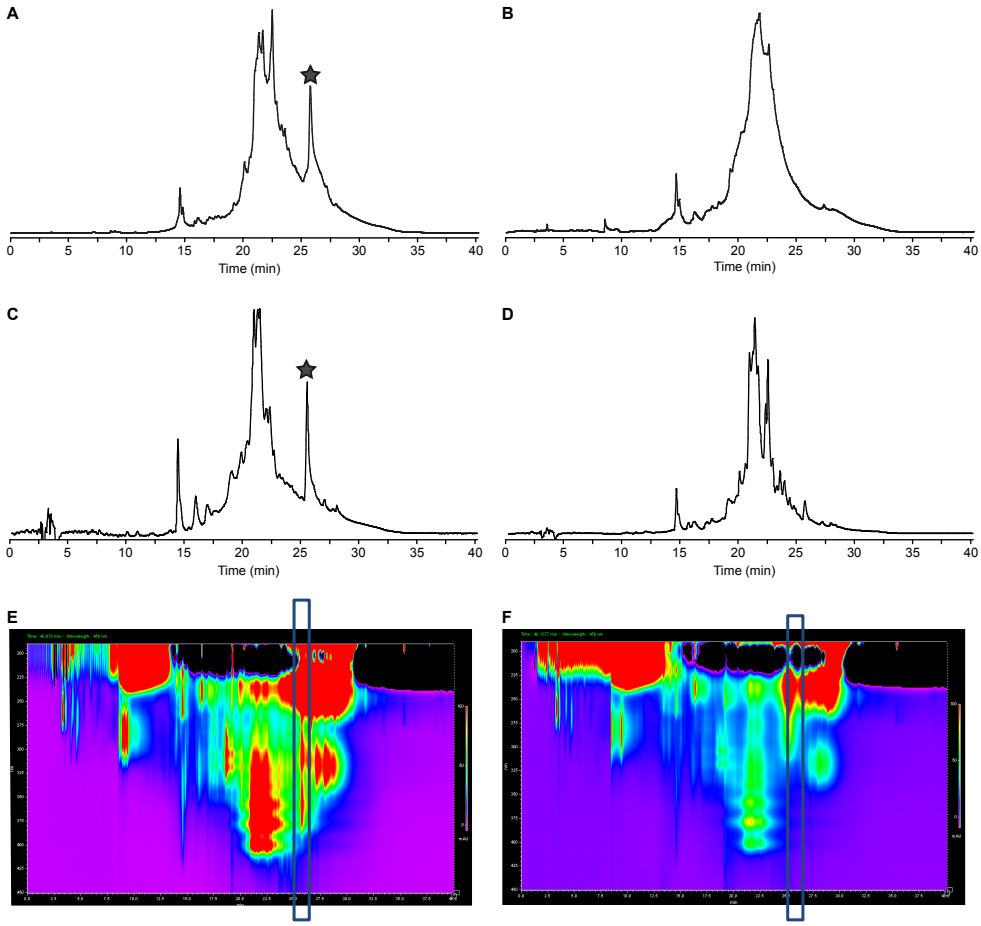

Fig. S4. HPLC chromatograms (with the thiocoraline peak at 25.8 min indicated by a star) of extracts from cultures of strains **A.** *S. albus*-pFL1049, **B.** *S. albus*-pFL1049-Δ*tioK*, **C.** *S. albus*-pFL1049-Δ*tioP*, and **D.** *S. albus*-pFL1049-Δ*tioQ*. As observed in the UV-Vis of the HPLC traces and mass spectrometry, no thiocoraline production was detected in cultures of *S. albus*-pFL1049-Δ*tioK* (panel **F**, with retention time where thiocoraline should elute highlighted by the blue box) and in cultures of *S. albus*-pFL1049-Δ*tioQ* (data not shown), whereas thiocoraline production was detected (at level of production similar to that of the *S. albus*-pFL1049 wild-type strain) in HPLC traces and mass spectrometry in cultures of *S. albus*-pFL1049 (panels **A** and **E**, with thiocoraline highlighted by the star and the blue box, respectively) and in cultures of *S. albus*-pFL1049-Δ*tioP* (data not shown).

***NOTE 1*:** In panels **B** and **D**, the peak at ~25.8 min does not correspond to thiocoraline as confirmed by mass spectrometry, confirming that TioK and TioQ are biosynthetic enzymes involved in the bisintercalator production.

***NOTE 2*:** The complete HPLC bi-dimensional chromatograms of the extract from all deletion mutants (Δ*tioK*, Δ*tioP*, and Δ*tioQ*) were scanned for thiocoraline or any its derivatives or relatives by using the diode array detector and mass spectrometry. No molecules related to thiocoraline or intermediates/shunt products were detected at all for Δ*tioK* and Δ*tioQ*.

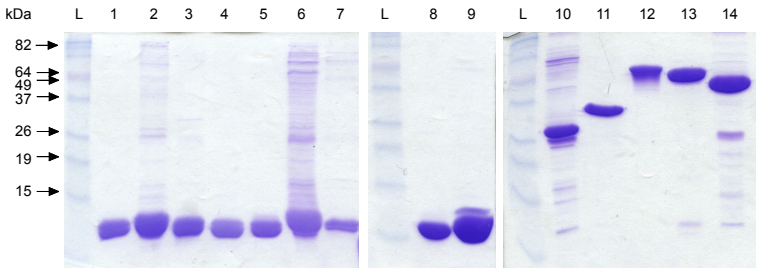

Fig. S5. Coomasie blue-stained 15% Tris-HCl SDS-PAGE gel showing the purified CP domains TioK(T) (11897 Da, lane 1), TioS(T_4) (12647 Da, lane 2), TioO (12190 Da, lane 3), CytC2 (11454 Da, lane 4), CloN5 (12319 Da, lane 5), AsbD (12792 Da, lane 6), CouN1 (12299 Da, lane 7), JamF (11667 Da, lane 8), and CurB (11797 Da, lane 9), the purified TEII domains TioQ (29111 Da, lane 10) and TioP (30244 Da, lane 11), and the purified A domains CytC1 (58939 Da, lane 12), CloN4 (56089 Da, lane 13), and AsbC (48673 Da, lane 14). L indicates the BenchMarkTM pre-stained protein ladder from Invitrogen. 6 μg of each protein were loaded on the gel.

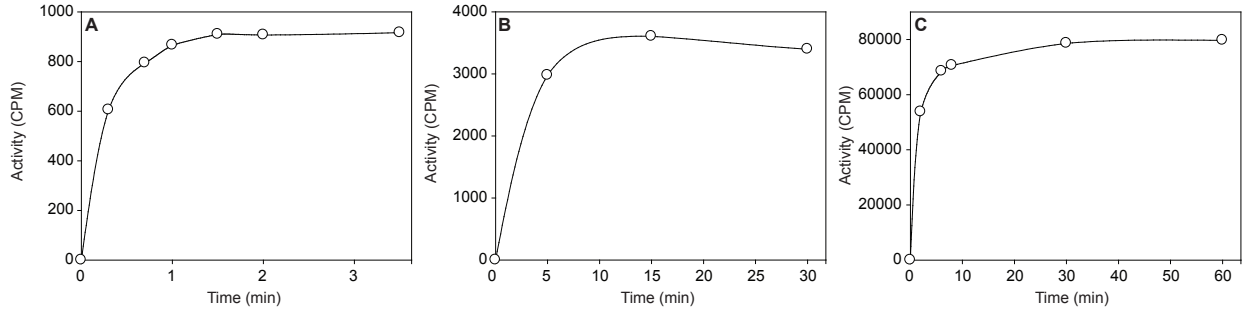

Fig. S6. Conversion of apo- to acyl-*S*-T domains for **A.** TioK(T), **B.** TioO, and **C.** TioS(T_4) via trichloroacetic acid (TCA) precipitation assays using [^{3}H]acetyl-CoA.

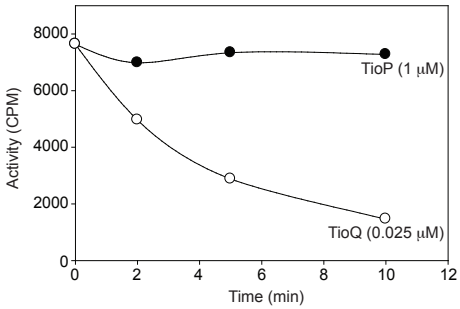

Fig. S7. Unloading of [^{3}H]acetyl from [^{3}H]acetyl-*S*-TioK(T) over a 10 min period using TioP (1 μM, black circles) or TioQ (0.025 μM, white circles). TioP was unable to unload the [^{3}H]acetyl group from TioK(T) whereas TioQ was efficient at performing the unloading of this moiety.

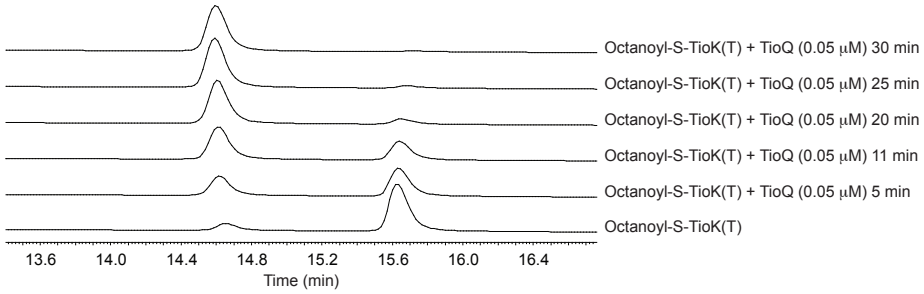

Fig. S8. Time course of the TioQ unloading of the octanoyl moiety from octanoyl-*S*-TioK(T) to form holo-TioK(T) monitored by RP-HPLC.

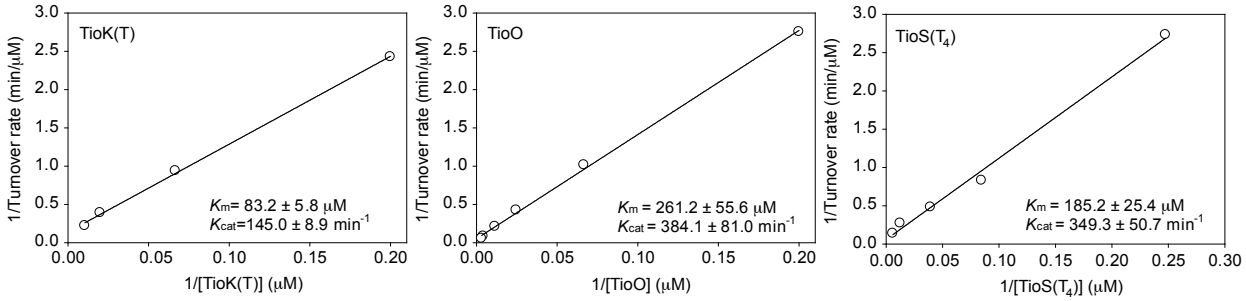

Fig. S9. Lineweaver-Burk plots for the kinetic parameters determined by RP-HPLC for TioQ with octanoyl-*S*-TioK(T), -TioO, and -TioS(T4).

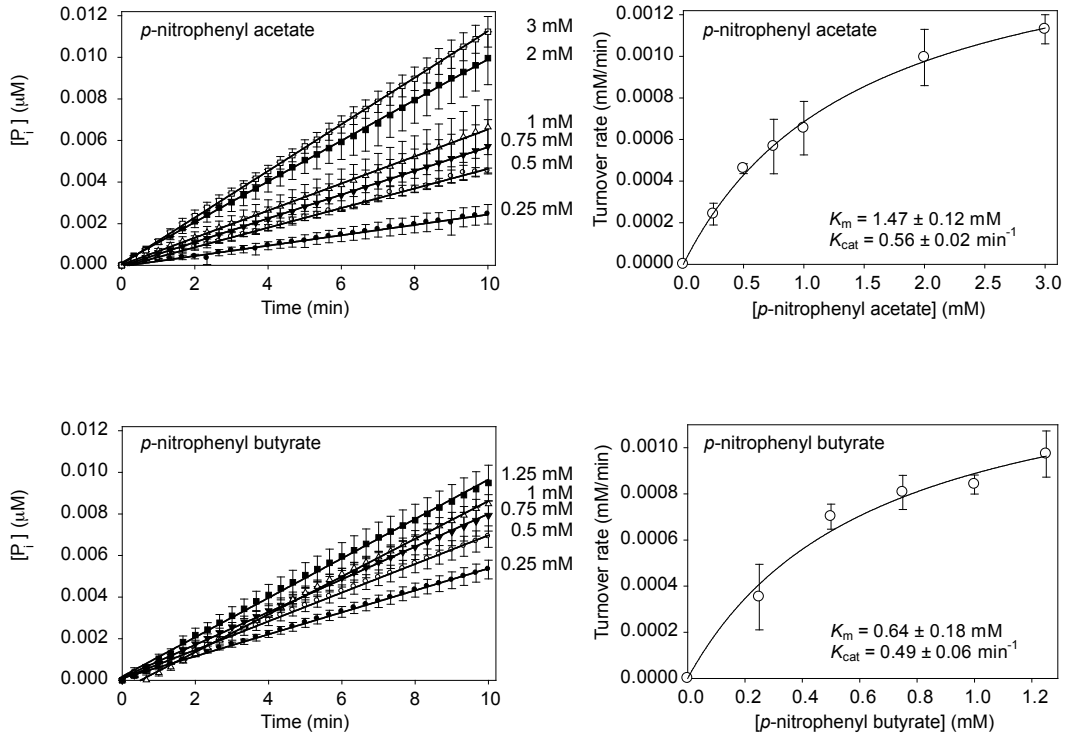

Fig. S10. Michaelis-Menten plots for the kinetic parameters for the TioQ-catalyzed hydrolysis of *p*-nitrophenyl derivatives by spectrophotometric assay.

Table S1: RP-HPLC retention time[a] of apo-, holo-, and loaded-CPs

protein	product	retention time (min)	protein	product	retention time (min)
AsbD	Apo-AsbD	18.7	**JamF**	Apo-JamF	15.8
	Holo-AsbD	19.6		Holo-JamF	15.3
	3,4-Dihydroxybenzoic-acyl-*S*-AsbD	19.5		Decanoyl-*S*-JamF	17.9
	4-Hydroxybenzoic-acyl-*S*-AsbD	19.6		Octanoyl-*S*-JamF	17.0
	Octanoyl-*S*-AsbD	19.4	**TioO**	Apo-TioO	15.0
CloN5	Apo-CloN5	20.0		Holo-TioO	14.5
	Holo-CloN5	18.2		Decanoyl-*S*-TioO	16.9
	Octanoyl-*S*-CloN5	19.9		Octanoyl-*S*-TioO	16.1
	trans-4-Hydroxy-L-prolyl-*S*-CloN5	17.2	**TioK(T)**	Apo-TioK(T)	14.4
	L-Prolyl-*S*-CloN5	17.5		Holo-TioK(T)	14.6
CouN1	Apo-CouN1	17.3		Acetyl-*S*-TioK(T)	14.6
	Holo-CouN1	15.5		Acetoacetyl-*S*-TioK(T)	14.6
	Acetyl-*S*-CouN1	15.6		Benzoyl-*S*-TioK(T)	15.0
	6-Fluoropicolinyl-*S*-CouN1	15.7		Crotonyl-*S*-TioK(T)	14.2
	Octanoyl-*S*-CouN1	16.8		Decanoyl-*S*-TioK(T)	16.1
	Pyrrolyl-*S*-CouN1	15.7		Glycinyl-*S*-TioK(T)	14.5
CurB	Apo-CurB	16.7		β-Hydroxybutyryl-*S*-TioK(T)	14.5
	Holo-CurB	16.1		Hexanoyl-*S*-TioK(T)	15.2
	Decanoyl-*S*-CurB	18.7		Isovaleryl-*S*-TioK(T)	14.9
	Octanoyl-*S*-CurB	17.9		Lauroyl-*S*-TioK(T)	16.8
CytC2	Apo-CytC2	17.1		Octanoyl-*S*-TioK(T)	15.5
	Holo-CytC2	16.7		*n*-Propionyl-*S*-TioK(T)	14.7
	D-Cysteinyl-*S*-CytC2	16.4		Thiaoxazole-4-carbonyl-*S*-TioK(T)	14.6
	L-Cysteinyl-*S*-CytC2	16.4		L-Tryptophanyl-*S*-TioK(T)	14.4
	D-Valinyl-*S*-CytC2	16.4	**TioS(T_4)**	Apo-TioS(T_4)	15.8
	L-Valinyl-*S*-CytC2	16.4		Holo-TioS(T_4)	15.2
				Octanoyl-*S*-TioS(T_4)	16.6

[a]For CouN1, CurB, JamF, TioK(T), TioO, and TioS(T_4) a 20-60%B gradient over 15 min was used (solvents: A = H_2O (0.1% TFA), B = MeCN). For CloN5 and CytC2 a 20-60%B gradient over 20 min was used.

Table S2: Primers used for the PCR amplification of the *tioK(T), O, P, Q and S(T_4)* genes[a]

gene	5' primer	3' primer
tioK(T)	CCCGACCATATGGCCTCGGCCCGTCCTC	TCCTCGCTCGAGTCAGGGGCGCCCGTTGGGGATGGATG
tioO	TCGGCCCATATGGCGAACCCGGATCGCGACGG	CTGGCGCTCGAGTCATCGGGCCAGCGACCCGAACG
tioP	CTCACGCATATGGGAACGAGAACGAAGAATCGGTGG	GTGGGCCTCGAGCTACCGGTGATCATGGAGTCGGTTG
tioQ	ATGAGCCATATGCGGAAGGGCTGGATACGCACG	CAGGGACTCGAGTCACGCCCACTCCCGGCCCATGATG
tioS(T_4)	GACCACCATATGCTTCCGGAGGTGGAGCAGGCCG	GAAGGGAAGCTTTTACTCGACGTCCTCCCTGCCG

[a]The introduced restriction sites are underlined for each primer. All 5' primers introduced an *Nde*I restriction site, except for the *tioS(T_4)* that introduced an *Hind*III restriction site. All 3' primers introduced an *Xho*I restriction site. The vector used for cloning was pET28a.