

The Isolation and Purification of Trichothecene 3-O-Acetyltransferase for Protection Against T-2 Toxin

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Summary

Mycotoxins are secondary metabolites produced by fungi. T-2 toxin is the most toxic trichothecene mycotoxin and is produced by the *Fusarium* species. T-2 toxin causes significant economic losses to animal and crop producers. The *Tri101* gene has been suggested to serve as a method of self protection against T-2 toxin in the species that produce T-2 toxin. The objective of this study was to prepare and purify recombinant *Tri101*, Trichothecene 3-O-acetyl transferase. SDS-PAGE displayed a band near 49.6 kDa indicating trichothecene 3-O-acetyltransferase had been induced, isolated, and purified.

Introduction

The word mycotoxin comes from the greek words *myco* meaning fungus and *toxicarious* meaning poison (Stearn, 2004). Mycotoxins, toxic secondary metabolites produced by fungi, enter the food chain through feedstuffs (Betina, 1984). The Council for Agricultural Sciences and Technology estimate that mycotoxins result in economic losses of up to 1.66 billion dollars per year (CAST, 2003). Of these fungal toxins, the trichothecene mycotoxin, T-2 toxin, is among the most virulent (Lesson, 1995).

Outside the cluster of genes responsible for trichothecene biosynthesis, an additional biosynthetic gene can be found (Hohn et al., 1993; Kimura et al., 1998a). This gene, *Tri101*, found in *Fusarium* species, is also involved in self defense (Kimura et al, 1998b; McCormick et al., 1999). The *Tri101* gene product exerts protective effects by inserting an acetyl group onto carbon-3 of trichothecenes (Kimura et al., 1998b). The 3-acetyl derivatives of T-2 toxin, deoxynivalenol, and diacetoxyscirpenol are one third less toxic than the 3-hydroxy derivatives (Kimura et al, 1999b). The *Tri101* gene has been inserted into yeast, fungus, rice, and tobacco plants. As a result, each displayed greater livability when challenged with T-2 toxin (Kimura et al., 1998b; McCormick et al., 1999; Muhitch et al., 2000; Ohsato et al., 2007). The objective of this study was to prepare a construct expressing histidine-tagged recombinant *Tri101* and purify the recombinant *Tri101* using Ni-NTA affinity chromatography.

Methods and Materials

Histidine (His)-tagged recombinant Tri101 was produced in JM 109 *E. coli* competent cells (Promega, # L2001, Madison, WI) by using the pQE-30 vector (QIAGEN #32915 Valencia, CA) in the QIAexpressionistTM expression system (Qiagen, 2003). The Tri101 gene, in plasmid pUCSVTri101, was donated by Kimura and coworkers (1998). Based on the complete sequence of Tri101 gene, a pair of primers were designed to amplify full-length of Tri101 consisting of 451 amino acids (Kimura et

al., 1998). Primers Tri101F (AAA GGA TCC ATG GCT TTC AAG ATA CAG CTC GAC) included restriction enzyme Bam HI (Promega catalog # R602A, Madison, WI) and Tri101R (CCC AAG CTT CTA ACC AAC GTA CTG CGC ATA CTT) included restriction enzyme Hind III (Promega catalog #R604A, Madison, WI). Each restriction enzyme was attached to the 5' end of each primer to facilitate the cloning of the amplified PCR product into pQE-30 vector. The amplified PCR product was digested with restriction enzymes Bam HI and Hind III and then purified by using QIAquick PCR Purification Kit Protocol (QIAGEN catalog #28104, Valencia, CA). The pQE-30 vector was digested with the same restriction enzymes and PCR products were ligated with T4 DNA ligase (Promega catalog # M180a, Madison, WI). Transformation and screening for positive recombinants was performed according to the procedures supplied with the pQE30 vector. The resulting plasmid, pQETri101, was sequenced and no mutations were observed within the coding sequence. Recombinant Tri101 was induced in the transformed JM109 *E. coli* competent cells using 2mM isopropyl- β -D-thiogalactoside (IPTG) for 3 hours. His-tagged Tri101 was purified using nickel-nitrilotriacetic acid metal-affinity chromatography (Ni-NTA) (QIAGEN, 2003). The concentration of purified *Tri101* was measured using Pierce BCA Protein Assay Kit (Pierce, 23225, Rockford, IL).

Results

Sequence analysis revealed that pQETri101 had not experienced any frameshifts during subcloning. Secondary structure using Signal P (www.cbs.dtu.dk/services/SignalP/) and TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) online molecular biology tools strongly suggests that Tri101 does not contain a signal peptide or largely hydrophobic region (Rozen & Skaletsky, 2000; Sonnhammer et al., 1998). Therefore, Tri101 is likely a soluble enzyme in the cytoplasm. However, majority of induced recombinant Tri101 formed non-soluble inclusion bodies, likely due to the high level of expression of Tri101 in the presence of the inducer, IPTG. Empigen BB is a mild zwitterionic detergent and is known for its ability to preserve the antigenicity and functional activity of isolated proteins (Lowthert et al., 1995). Thus, Empigen BB (Calbiochemical #324690, San Diego, CA), was used to facilitate solubilization of recombinant Tri101 during purification. In future studies, lowering the concentration of IPTG and length of the induction period may reduce the formation of aggregates and lead to purification without the use of the detergent, Empigen BB (QIAGEN, 2003).

As shown in Figure 1, His-tagged Tri101 was dramatically induced in the *E. coli* construct and purified by Ni-NTA agarose affinity chromatography. Following induction and subsequent purification, the BCA protein assay indicated that approximately 1 mg of purified recombinant *Tri101* had been obtained from 50 mL of transformed *E. coli* culture.

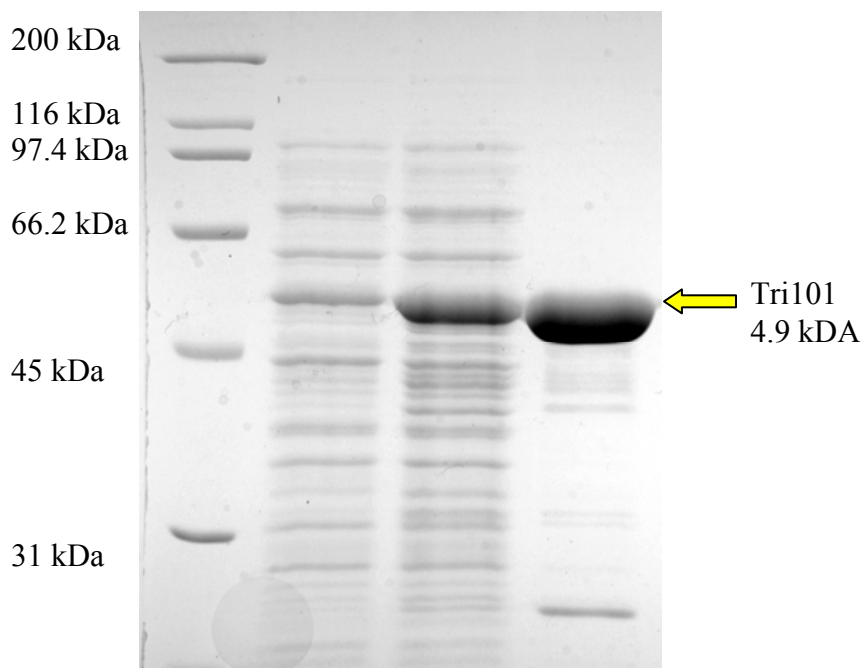


Figure 1. SDS PAGE gel analysis. Lane 1 contains the standard marker. Lane 2 contains the pre-IPTG-induced sample. Lane 3 contains the post-IPTG-induced sample. Lane 4 contains purified Trichotheccene 3-O-Acetyltransferase

Conclusion

High purity trichotheccene 3-O-acetyltransferase was prepared using QIAexpressionist expression system. Future experiments are planned to test its effectiveness in preventing or reducing the toxic effects of T-2 toxin *in vitro*. Pending those results, recombinant *Tri101* will be incorporated *in vivo* system in an attempt to develop an intervention strategy to control the detrimental effects of mycotoxins in animals.

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