Center for Eukaryotic Structural Genomics

Protein Structure Initiative

LARGE-SCALE PRODUCTION OF TEV PROTEASE FROM PSI MATERIAL RESOURCE PLASMID pMHT238Δ

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Abstract

Tobacco etch virus NIa proteinase (TEV protease) is frequently used for removal of fusion tags from recombinant proteins. The Center for Eukaryotic Structural Genomics (CESG) created expression vector pMHT238Δ for the production of TEV protease and provided this vector to the Protein Structure Initiative Material Resource. pMHT238Δ accounts for nearly 40% of all material transfer requests placed through PSI Materials Repository for CESG clones (latest update January 20, 2009). Due to the popularity of this expression plasmid, there is an emerging need for detailed protocols for its use that can be adapted to types of resources available in most academic laboratories. Here we present detailed methods for growing the cell culture in standard flasks, as well as in a 10-L volume fermentor, and compare the results of the two. Initial tests of protein expression in various *E. coli* hosts were carried out in small-scale culture (0.4 mL) using 96-well high-through growth blocks. Three *E. coli* expression strains (*Escherichia coli* BL21, BL21-CodonPlus-RIL, and B834-pRARE2) were characterized for scale-up studies. Large-scale production growths (0.5 L) were performed to optimize and verify the ability of each host strain to express soluble TEV protease. By using 2 L PET bottles in orbital rotation shakers, TEV protease was highly expressed in factorial-evolved auto-induction media (Blommel at al., 2007) and in a Terrific Broth/glucose auto-induction medium. The Terrific Broth/glucose medium was also used for growth in a 10-L instrumented fermentor, where continuous aeration allowed the cells to reach higher cell density than in shaken flasks. Yields of purified TEV protease up to 0.3 g/L were obtained from the fermentor culture. Target proteins prepared from CESG expression plasmids (also available from the Protein Structure Initiative Material Repository) were used to demonstrate catalytic activity of the TEV protease preparations.

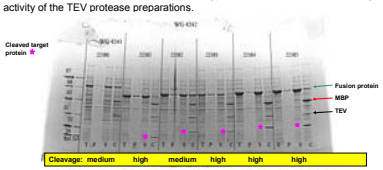


Figure 1. TEV protease preparation is used to cleave the target proteins from MBP. The protocol uses 3 µL of TEV preparation with a 1 h incubation at 30 degrees C. Lane annotation on gel: T-total expression, P-insoluble fraction, S-soluble fraction, C- cleaved fraction.

Tobacco Etch Virus Protease Production

The mission of CESG is focused on research and development of technology aimed at improving the production and purification of eukaryotic proteins for structure determination. Tobacco Etch Virus (TEV) protease is an important component of the CESG research platform, and is used to remove the affinity tag (His 8-tag) or solubility enhancer partner (maltose binding protein, MBP) from the N-terminal of recombinant eukaryotic proteins. Initial mutagenesis has been done on TEV protease by Dr David Waugh (see **Figure 2**). These mutant TEV proteases have a reduced internal cleavage activity (S219V), and therefore do not inactive themselves. We determined that there were several additional challenges with TEV protease that needed to be addressed in order for the protease to be successfully applied to our research platform. The TEV protease has low solubility (precipitates when concentration is greater than 1.4 mg/mL), undergoes a C-terminal autocatalytic deletion and inactivation, has low expression yields, and inefficient purification. This poster presents research and development that show how we improved the performance of TEV protease.

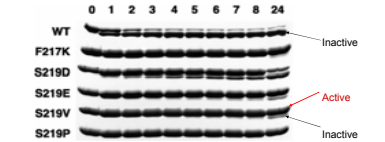


Figure 2. Professor David Waugh identified TEV S219V as particularly resistant to autocatalytic inactivation. This substitution makes this mutant approximately 10 times more resistant to auto-inactivation than the wild-type protease (Kapust at al., 2001).

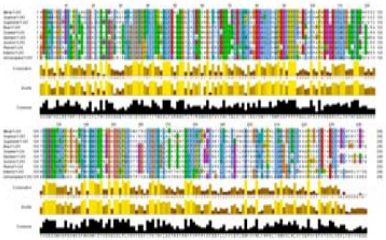
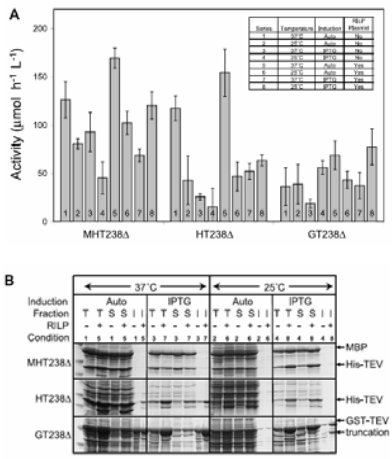


Figure 3 Shows a comparison and alignment of the amino acid sequence of TEV protease analogues from various potyviruses (peptidase family C4) (Waterhouse at al., 2009).

Table 1. Summary of various TEV protease constructs, their solubility profile, and termini designs (Blommel and Fox, 2007). The expression vector pQE30-S219V was obtained from Professor B.F. Volkman and Dr. F.C. Peterson at the Medical College of Wisconsin (Milwaukee, WI) and pRK793 – from Dr. D.S. Waugh at the NCI (Frederick, MD).

Plasmid or coding sequence	Anticipated N-terminus	Anticipated C-terminus	C-terminal abbreviation	Solubility enhancing mutations
pQE30-S219V	MRGSHHHHHHGS …	…TQLMNELVYSQ	Full-length	No
pQE30-S219V-pR5	MRGSHHHHHHGS …	…TQLMNRRRRR	pR5	No
pRK793 (S219V)	GHHHHHHHGE…	…TQLMNRRRRR	pR5	No
MHT	AIAHHHHHHHGE…	…TQ …TQLMNE …TQLMNELVYSQ …TQLMNELVYSQ	234Δ 238Δ Full-length Full-length	Yes Yes Yes No
HT	MGSHHHHHHHHGE …	…TQ …TQLMNE …TQLMNELVYSQ …TQLMNELVYSQ	234Δ 238Δ Full-length Full-length	Yes Yes Yes No
GT	Glutathione-S-transferase-MGILG…	…TQ …TQLMNE …TQLMNELVYSQ …TQLMNELVYSQ	234Δ 238Δ Full-length Full-length	Yes Yes Yes No



MHT238Δ- MBP fusion

HT238Δ - His tag

GST238Δ - GST (glutathione-S-transferase-tagged TEV protease)

Figure 4. The construct pMHT238Δ was found to give the highest expression of soluble and active TEV protease (Blommel and Fox, 2007). Tested conditions included growth in TB+g medium with auto-induction or with IPTG induction. Expression testing was performed at 25 and 37 degrees C. The strain with plasmid RILP provided a constitutive expression of rare tRNAs, found to be important for TEV protease expression (Kapust et al., 2002). TEV protease activity was determined using a fluorescence anisotropy-based protease assay (Blommel and Fox, 2005).

Figure 5. Sequence alignment of the wild type TEV NIa proteinase and TEV 238Δ. Shown are mutation improving solubility (T17S, N68D, I77V) (van den Berg et al., 2006) and conferring resistance to auto-inactivation (S219V) (Kapust et al., 2001). The C-terminal truncation at residue 238-242 enhances protease solubility (Blommel and Fox, 2007).

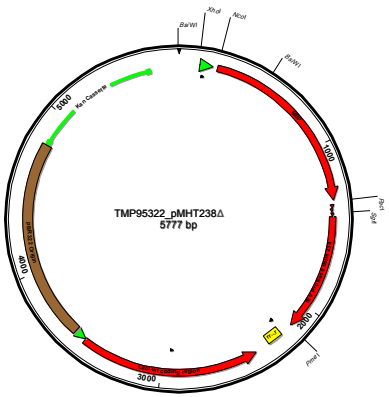


Figure 6. Map of the expression vector pMHT238Δ used for efficient production of TEV protease (Blommel and Fox, 2007).

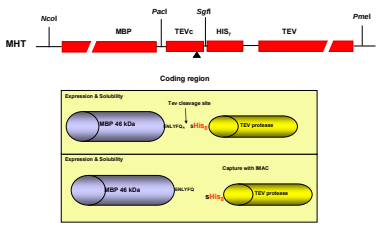


Figure 7. TEV protease is produced from pMHT238Δ as an MBP fusion and undergoes self-cleavage *in vivo* to release a His-8-tagged TEV protease. The protease is then purified using IMAC and Mono-Q FPLC techniques.

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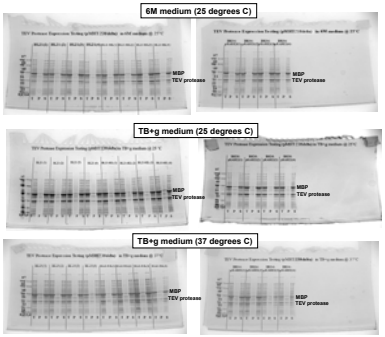

Figure 8. TEV expression testing (pMHT238Δ) in auto-induction unlabeled minimum medium (6M) and TB+g/ glucose medium (Blommel and Fox, 2007). All growths were performed in four replicates in 0.5 L cultures in PET bottles for 24 h at 25 or 37 degrees C. 0.1 g samples of cell pastes were analyzed for TEV protease expression and solubility. Lane annotation on gels: T-total expression, P-insoluble fraction, S-soluble fraction.

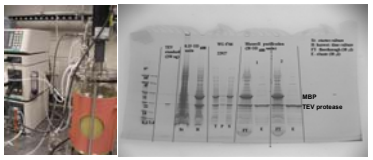

Figure 9. Large-scale TEV protease production in 10 L fermentor BioFlo110 (New Brunswick Scientific, NJ). 500 mL of starter culturer, in MDAG medium (Studier, 2005) was prepared (pMHT238Δ in expression strain B834-pRARE2) and fermentation process was performed in auto-induction TB+g / glucose medium at 25 degrees C for 18 hours to OD_{600}~40 (363 g total cell paste yield). This fermentation yielded 36 g of wet cell paste per liter of culture medium with ~480 mg of purified TEV protease obtained per L of culture as estimated by purification on Maxwell 16, automated polyhistidine protein purification system (Promega, Madison, WI).

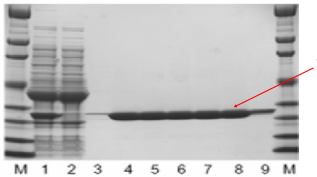

Figure 10. Purification of TEV protease from expression vector pMHT238Δ in BL21-CodonPlus-RILP strain (Blommel and Fox, 2007). TEV protease purification employs two-step procedure consisting of IMAC followed by cation exchange chromatography. The average yield is 15 mg of purified TEV protease obtained per gram of cell paste.

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