

Poster Presentation

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## *Zea mays* L. transglutaminase expression in *Escherichia coli*

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### Background

Transglutaminase (protein-glutamine:amine  $\gamma$ -glutamyl-transferase, E.C. 2.3.2.13) catalyses acyl-transfer reactions between  $\gamma$ -carboxamide groups of glutamine residues and the  $\epsilon$ -amino group of lysines in proteins, leading to inter- or intramolecular cross-linking. Transglutaminases (TGs) have been found in mammals, plants, fish, nematodes and bacteria. Two maize cDNA clones (*TGZ15* and *TGZ21*) that expressed active transglutaminase localized in chloroplasts were isolated [1,2].

### Results

A TGZ sequence was subcloned into pET (Novagen) vector (named as pET28a+*TGZ4*). The expression assays in *E. coli* BL21 DE3 cells showed that the main fraction of the protein (>80%) was found in the inclusion bodies (see Figure 1). The purification under denaturing conditions in FPLC system followed by a refolding step was a suitable procedure to obtain functional TGZ4p (see Figures 2,3). In addition, a specific antibody against TGZ4p was obtained in the laboratory for the immunolocalization of this protein in *E. coli* cells (see Figure 4).

### Conclusion

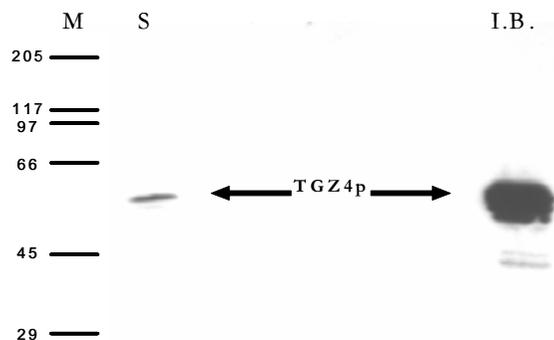
TGZ4p was expressed in *E. coli* mainly as inclusion bodies. The purification under denaturing conditions and refolding *in vitro* was a suitable procedure to obtain functional TG.

### Acknowledgements

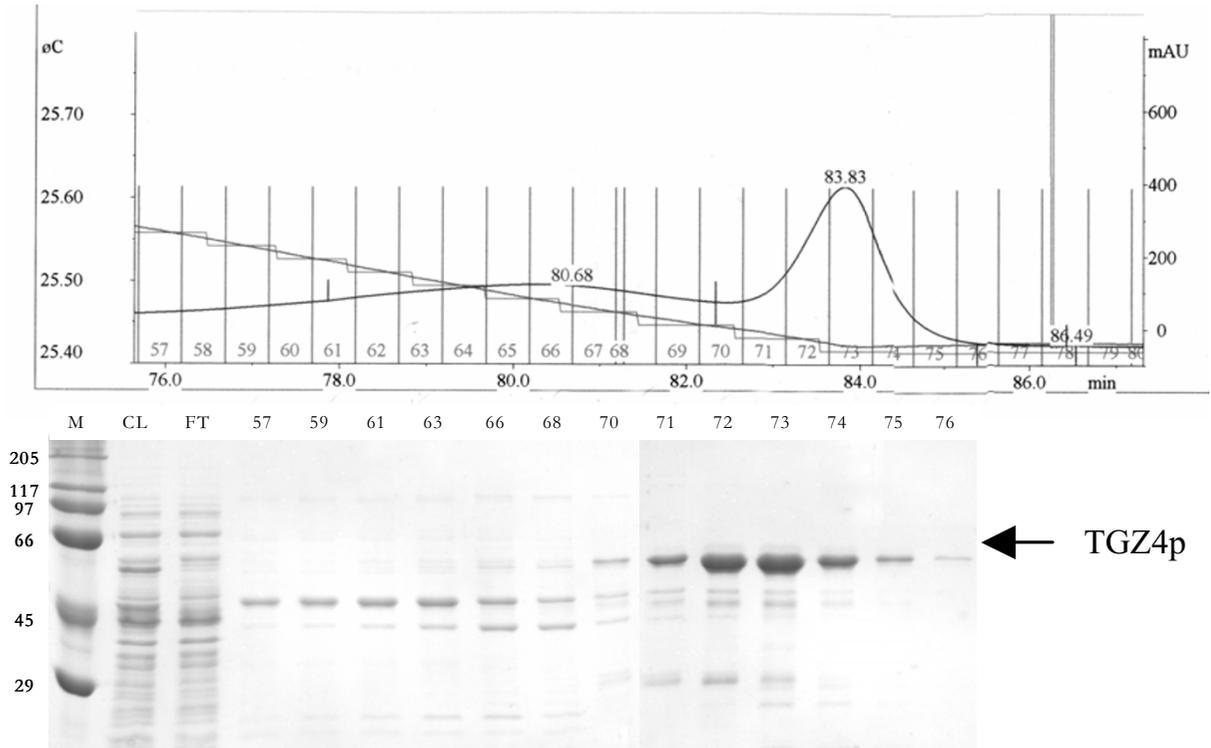
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### References

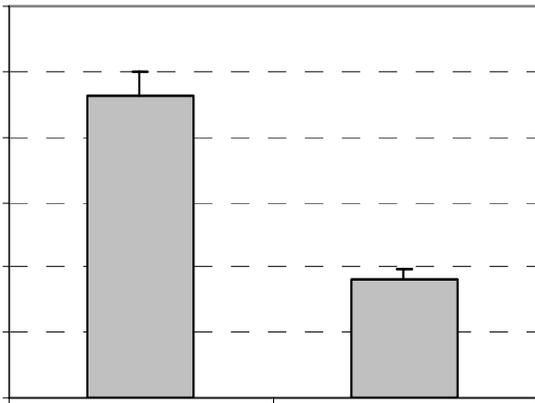
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2. Torné JM, Santos MA, Talavera D, Villalobos E: **Maize nucleotide sequence coding for a protein with transglutaminase activity and use thereof.** *PCT/ES03/00247*. Patent number WO03/02128 2002.



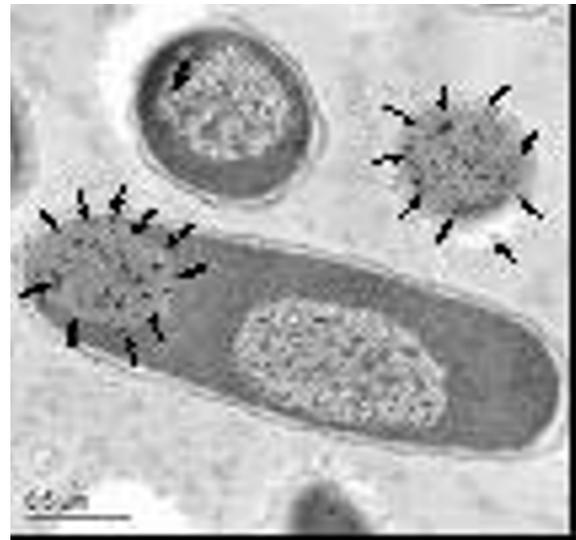
**Figure 1**  
Western blot of TGZ4p. S = soluble, IB = inclusion bodies



**Figure 2**  
TGZ4p FPLC purification under denaturing conditions.



**Figure 3**  
TGase activity of refolded TGZ4p against Tris and phosphate buffer.



**Figure 4**  
TGZ4p TEM Immunolocalization, S=soluble, IB=inclusion body.