CLONING, EXPRESSION AND PURIFICATION OF ERF8 PROTEIN FROM ARABIDOPSIS THALIANA IN ESCHERICHIA COLI
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Summary
Ethylene-responsive factor 8 (ERF8), a member of AP2/ERF superfamily, is one of the transcription factors that involves in repression of leaf senescence in plants. Leaf senescence is the final stage of leaf development and involves the mobilization of nutrients from old leaves to newly growing tissue. Regulation of leaf senescence depends on the developmental age of plants, and it is also influenced by various external stimuli. Recently, some studies indicated that combination of ERF8 and ERF4 is an important key in signaling pathways related to the progression of leaf senescence. Besides, there is no specific study of ERF8. Therefore, investigating function and interaction of ERF8 is considered effective strategy to control plant’s resistance against stress factors.

In this study, we show the results of cloning, expression and purification of ERF8 protein in Escherichia coli. ERF8 was amplified from cDNA of Arabidopsis thaliana by PCR, cloned into pTZ257R/T vector. This then was subcloned into the expression vector pGEX-5X and expressed in E. coli strain BL21. Besides, the research was carried out to investigate factors and determine the optimal conditions for the expression of ERF8 protein in E. coli train BL21 (at 30°C after 3 hours induction with 0.5 mM IPTG). Finally, ERF8 recombinant protein was initially purified by affinity chromatography for further studies.

Keywords: Arabidopsis thaliana, ERF8, transcription factor, E. coli, protein.