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Low-temperature-inducible SLTI 182 gene has L-asparaginase activity

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Introduction

L-asparaginase mediates the conversion of asparagine into aspartate and ammonia and play an important role in nitrogen metabolism in plants. It also have a role in biosynthesis of amino acids and nodulation process in legumes. SLTI 182 clone has high homologies with L-asparaginase gene. In this study, the functional analysis of SLTI 182 gene is performed.

Materials and Methods

Materials: plant- *Glycine max* cv. sinpaldal 2; host cell: BL21(DE3); vector: pET

Method: low temperature treatment(4°C); affinity chromatography; northern blot; SDS-PAGE; native-PAGE; western blot

Results and discussions

The function of SLTI 182 gene was analyzed using *E. coli* transformation. The recombinant proteins purified with three-step purification schemes; fractionation, immobilized metal ion affinity chromatography, and histidine affinity chromatography. Recombinant SLTI 182 protein was expressed in *E. coli* cells

(Fig. 1, Lane 2). L-asparaginase activity of SLTI 182 was assayed by measuring ammonia amount released from asparagine. *E. coli* cells expressing SLTI182 showed an increased L-asparaginase activity (Fig. 2).

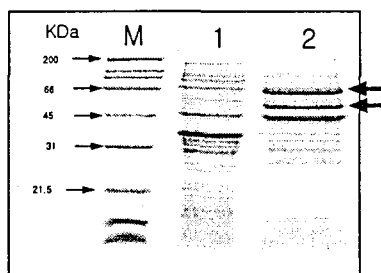


Fig. 1. SDS-PAGE analysis of recombinant SLTI 182 protein expressed in *E. coli* cells.

M; molecular weight marker

Lane 1: pET42(a) vector

Lane 2: pET42-SLTI 182

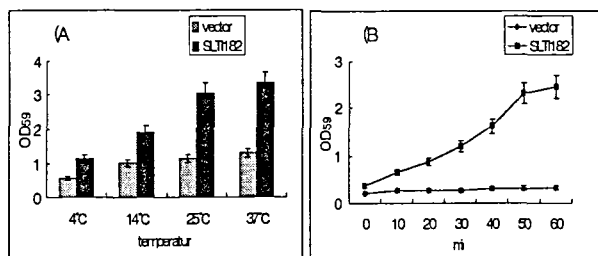


Fig. 2. L-asparaginase activity of recombinant SLTI 182 protein expressed in *E. coli* cells.

(A) Enzyme activity at different temperatures

(B) Enzyme activity at different incubation times.