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Functional Analysis of SLTI182 clone with asparagine catabolism

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Objective

L-asparaginase mediates the conversion of asparagine into aspartate and ammonia, and plays an important role in nitrogen metabolism in plants. It also has 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

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

Method : low temperature treatment(4℃); affinity chromatography; Northern blot; SDS-PAGE; native-PAGE; Westhern blot

Results and discussions

The function of SLTI 182 gene was analyzed through *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. L-asparaginase activity of SLTI 182 was assayed by measuring ammonia amount released from asparagine. *E.coli* cells expressing SLTI182 function showed an increased L-asparaginase activity (Fig. 1). The protein of antisense pET-SLTI182 was largely increased the amount of asparagine compared with sense pET-SLTI182 (Fig. 2).

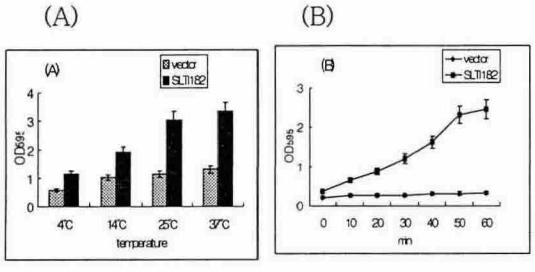

Fig.1 L-asparaginase activity of recombinant SLTI 182 expressed in *E. coli* cells
(A) Enzyme activity at different temperatures.
(B) Enzyme activity at different incubation times.

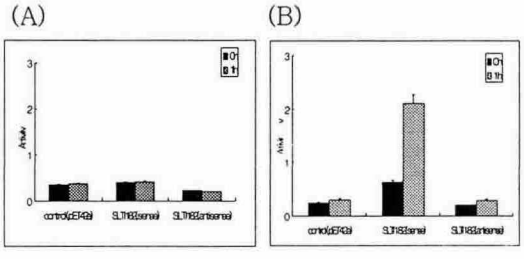

Fig.2 Activity of L-asparagine in sense or antisense of SLTI 182.
(A) (-) asparaginase.
(B) (+) asparaginase.

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