

(05-1-122)

CSP (cold shock protein) gene isolated from *Arthrobacter* sp. A2-5 can confer freezing tolerance to transgenic tobacco plant

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Objectives

To isolate the *AoCspA* encoding cold shock protein gene from low temperature resistant *Arthrobacter* sp. and to analyze the function of the gene for cold tolerance in yeast and plant cells.

Materials and Methods

1. Material

Plant - tobacco, Yeast strain INVSc1, *Agrobacterium* strain - LBA4404

2. Methods

Arthrobacter sp. A2-5 screen, *AocspA* gene isolation, yeast transformation, *Agrobacterium* transformation, tobacco chloroplastic and nucleic transformation, Northern and Southern blot.

Results and Discussion

Arthrobacter sp. A2-5 isolated from soil in the South Pole was selected as a low temperature resistant strain because it can grow at 15°C. The cold shock protein gene (designated as *AocspA*) was isolated from the strain by screening a genomic library with PCR amplified fragments of the cold shock protein gene. The *AocspA* contained 207 bp long open reading frames. The deduced amino acid sequence of *AocspA* was homologous to those of bacterial cold shock protein genes (60-71.9% identities). The RNA binding motifs, RNP-1 and RNP-2, which are identical domains of bacterial cold shock protein genes, were conserved in cloned genes. Northern blot analysis demonstrated that *AocspA* was highly expressed at low temperature condition. To identify the function, the *AocspA* gene was introduced into yeast (INVSc1). Bio-functional assay showed that the *AocspA* overexpressed yeast can survive at low temperature (15°C), indicating that cold shock protein plays an important role in cold tolerance in microorganism cells. In order to check whether this protein also work in plant cell for cold tolerance, we generate two kinds of *AocspA* overexpressing transgenic tobacco plants, chloroplast transformation one and nuclei transformation one. Southern blot analysis confirmed insertion of *AocspA* in chloroplast of the chloroplast targeted transgenic tobacco plant. Northern blot analysis showed that *AocspA* was expressed in transgenic tobacco plant with significantly high expression level. Result of bioassay showed that *AocspA* overexpressed tobacco plants can be survived under seavior cold shock such as -20°C for 1hr. Confirmation of *AocspA* gene insertion and expression in nuclei targeted transgenic tobacco plant was under the performing now. Bioassay results of the nuclei targeted transgenic tobacco plant will elucidate more detailed function of the *AocspA* gene for cold tolerance in plant cell.

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