

# Isolation and characterization of ice-binding protein from Antarctic microalga, *Chloromonas* sp.

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## **Abstract**

Antarctic microalgae are primary producers in polar environment. *Chloromonas* sp. (*Chlamydomonadaceae*, Volvocales, Chlorophyceae) was isolated from freshwater ponds near the King Sejong Station in King George Island, Antarctica. Morphology and phylogenetic tree of this Antarctic alga was characterized. Antarctic *Chloromonas* sp. was shown to have ice-binding protein (IBP), which prevent from growing ice crystals in freezing condition. cDNA of IBP was cloned and the genomic sequences of IBP was identified as 3,221bp in length which was composed of eleven intron and twelve exon. The open reading frame for IBP gene of *Chloromonas* sp. was 1,062 nucleotides. IBP gene encoded 36.3 kDa protein which included a signal peptide of 23 amino acids. Southern blot analysis suggested that more than two copies of IBP genes exist in the genome of *Chloromonas* sp. Western blot analysis revealed that IBP from this microalga was secreted to growth media. Ice binding activity of the protein was measured by morphological study of single ice crystals using nanolitre-osmometer attached to optical microscopy. In order to analyze the characteristics of *Chloromonas* IBP, this protein has been expressed in eukaryotic heterologous expression systems. Codon-optimized genes of *Chloromonas* IBP had been transformed into *Pichia pastoris* and *Chlamydomonas reinhardtii* for further characterization.

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