Ice-binding proteins from psychrophilic *Chloromonas* sp. enhance freezing tolerance in transgenic *Arabidopsis thaliana*

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Genus *Chloromonas* is cosmopolitan green algae which is secondary larger group in Chlorophyceae. Chloromonas species was often found in extreme environments such as snow surface, alpine and polar region. Microalgae in polar region have developed their own strategies to protect the cells under repeated freeze-thaw cycles of ice crystals. Ice-Binding Proteins (IBPs) which are widely spread in polar fungi, slim mold, bacteria, diatom and microalgae, possibly play a key role making the microenvironment surrounding cells more habitable. Recent studies have identified that two types of IBPs are more abundantly present in Chlorophyceae in low temperature environments. Among them, type 2 IBP has been found only Antarctic green alga Chlamydomonas CCMP681. To discover more IBP genes, we performed type 2 IBP gene screening using psychrophilic Arctic/Antarctic Chloromonas strains and confirmed that these IBPs are more frequently found in this genus but not in *Chlamydomonas* species. For focusing on cold acclimation, we carried out transcriptome analysis under 4, 8, 12, 16, 20°C conditions using one of the psychrophilic *Chloromonas* (KCCPM KNF032). By comparison, we selected 39 up-regulated transcripts under low temperature specific. More than half of annotated transcripts were revealed as type 2 IBPs and those proteins were shared structural properties: conserved TXT motifs and none predictable domains. Transgenic Arabidopsis plants overexpressing CmIBPs (Chloromonas IBPs) revealed to increase a freezing tolerance through ion leakage assay. Furthermore, these heterogeneous IBPs in plant more stabilized under cold acclimation (CA) than non-cold acclimation (NA) condition. Recombinant protein expressing by E. coli system were used to characterize a freezing inhibition activity. Characterization of these proteins and predictable biological functions will be discussed.