

Structure and Function of a Novel Cold Regulated Cold Shock Domain Containing Protein from an Obligate Psychrophilic Yeast, *Glaciozyma antarctica*

ABSTRACT

Cold shock domain (CSD)-containing proteins are one of the groups of the evolutionarily conserved nucleic acid-binding proteins in all three domains of life consisting of an ancient beta-barrel fold that serves to bind nucleic acids. The cDNA of a novel protein-coding gene containing CSD was cloned from *Glaciozyma antarctica* designated as Ga16676. The full length of *Ga16676* gene with the size of 1335 bp encodes for an N-terminal CSD with conserved nucleic acids binding motif RNP1 and RNP2. The *Ga16676* gene was cloned in pET30 Ek/LIC, sequenced, expressed and its resistance towards cold was characterized. Protein expression of recombinant Ga16676 showed overexpressed soluble expression in both supernatant and pellet forms at 20°C. The effects of CSD protein overexpression on colony formation shows that *E. coli* cells were able to grow at 37°C and 20°C but not at 4°C while *E. coli_Ga16676* cells were able to grow at all temperatures tested. In addition, *E. coli_Ga16676* cells showed higher growth rate compared to empty *E. coli* cells at 10°C. Structural analysis of Ga16676 reveals some interesting findings such as more aromatic interactions for efficient binding in low energy environment, a longer loop that may contribute to structural flexibility and clustering of charged amino acids on the protein surface that is important for protein stability and flexibility.
