

Cloning and characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RbcS) cDNA from green microalga *Ankistrodesmus convolutus*.

Abstract

An initial study on gene cloning and characterization of unicellular green microalga *Ankistrodesmus convolutus* was carried out to isolate and characterize the full-length cDNA of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RbcS) as a first step towards elucidating the structure of *A. convolutus* RbcS gene. The full-length of *A. convolutus* RbcS cDNA (AcRbcS) contained 28 bp of 5' untranslated region (UTR), 225 bp of 3' non-coding region, and an open reading frame of 165 amino acids consisting of a chloroplast transit peptide with 24 amino acids and a mature protein of 141 amino acids. The amino acid sequence has high identity to those of other green algae RbcS genes. The AcRbcS contained a few conserved domains including protein kinase C phosphorylation site, tyrosine kinase phosphorylation site and N-myristoylation sites. The AcRbcS was successfully expressed in *Escherichia coli* and a ~21 kDa of anticipated protein band was observed on SDS-PAGE. From the phylogenetic analysis of RbcS protein sequences, it was found that the RbcS of *A. convolutus* has closer genetic relationship with green microalgae species compared to those of green seaweed and green macroalgae species. Southern hybridization analysis revealed that the AcRbcS is a member of a small multigene family comprising of two to six members in *A. convolutus* genome. Under different illumination conditions, RT-PCR analysis showed that AcRbcS transcription was reduced in the dark, and drastically recovered in the light condition. Results presented in this paper established a good foundation for further study on the photosynthetic process of *A. convolutus* and other green algae species where little information is known on Rubisco small subunit.

Keyword: Green microalgae; *Ankistrodesmus convolutus*; cDNA library construction; Expressed sequence tags.