

## Enzymatic characterization and Expression of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas fluorescens*

Gun Woong Lee, Jae Eun Ju, Hae Min Kim, Si Nae Lee, Jong Chan Chae, Yong  
Hoon Lee, Byung Taek Oh, Byoung Yul Soh

Division of Biotechnology, College of Environmental & Bioresource Sciences,  
Chonbuk National University, Jeonju, Jeonbuk, 561-756, Korea

Ethylene, known as a stress hormone regulate wide developmental processes including germination, root hair initiation, root and shoot primordial formation and elongation, leaf and flower senescence and abscission, fruit ripening. The acceleration of ethylene biosynthesis in plant associated with environmental and biological stresses. 1-Aminocyclopropane-1-carboxylate deaminase(ACCD) is an enzyme that cleaves ACC into and ammonia, a precursor of the plant hormone ethylene. Plant growth-promoting rhizobacteria (PGPR) having ACCD can decrease endogenous ACC level of tissue, resulting in reduced production of ethylene in plants. ACC deaminase was a key enzyme for protect stressed plants from injurious effects of ethylene. ACCD gene was encoded from *Pseudomonas fluorescens*, PGPR and was cloned in *Escherichia coli*. We expressed the recombinant ACCD(rACCD) containing 357 amino acids with molecular weight 39 kDa that revealed by SDS-PAGE and western blot. The rACCD was purified by Ni-NTA purification system. The active form of rACCD having enzyme activity converted ACC to a-ketobutyrate. The optimal pH for ACC deaminase activity was pH 8.5, but no activity below pH 7.0 and a less severe tapering activity at base condition resulting in loss of activity at over pH 11. The optimal temperature of the enzyme was 30°C and a slightly less severe tapering activity at 15 - 30°C, but no activity over 35°C. *P. fluorescens* ACC deaminase has a highly conserved residue that plays in allowing substrate accessibility to the active sites. The enzymatic properties of this rACCD will provide an important reference for analysis of newly isolated ACCD and identification of newly isolated PGPR containing ACCD.

**Key Word** : 1-Aminocyclopropane-1-carboxylate deaminase, *Pseudomonas fluorescens*, Plant growth-promoting rhizobacteria, Enzymatic characterization, Recombinant Protein Expression