P 46 An Application of *E.coli* Ornithine Deacetylase (*argE*) Gene as a Negative Selectable Marker in Plant

Woo Yong Lee, Jung Moo Park, and Won Il Chung*

Department of Biological Sciences, KAIST, Gusung-dong, Yusung-gu, Daejeon 305-701, Korea

Objectives

Negative selection system is a useful tool in plant genetic engineering, especially in gene stacking to acquire multi-gene transformed plant. We successfully developed a novel negative selection system by modifying the herbicide phosphinothricin (Pt) into nontoxic N-acetylated precursor and using *E. coli* ornithine deacetylase (*argE*) gene as a selectable marker gene.

Materials and Methods

1. Materials: *Nicotiana tabacum* L. Samsun NN lines.
2. Methods
   - Phosphinothricin was N-acetylated by organic synthesis.
   - *argE* gene was expressed under the constitutive Cauliflower Mosaic Virus 35S (CaMV 35S) promoter.

Results and Discussion

The herbicide, phosphinothricin was N-acetylated by organic synthesis and purified by vacuum evaporation of acetic acid and water. The structure of this product was revealed to N-acetylated phosphinothricin by nuclear magnetic resonance (NMR) spectroscopy. Then it was confirmed to be nontoxic to wild type tobacco when it is applied to leaf surface.

We isolated *E.coli argE* gene which deacetylates N-acetyl amino acids relatively nonspecifically, and introduced this gene into tobacco under the control of constitutive CaMV 35S promoter by leaf disc transformation method. In transformed tobacco line which contain active *argE* enzyme, the inactive precursor N-acetyl phosphinothricin (N-AcPt) was converted to active herbicide phosphinothricin (Pt) which was sufficient to make the treated leaf area go under necrosis.

So, we could established successful green-house level negative selection system by using *E.coli argE* and synthetic N-acetyl phosphinothricin, which is nontoxic to nontransgenic tobacco but selectively destroys *argE* transgenic tobacco leaves.

*Corresponding author. Tel 042-869-2624 E-mail wichung@mail.kaist.ac.kr