B103

Cloning and characterization of p450-4 and p450-1 gene involved in GA biosynthesis from *Fusarium proliferatum* KGL0401

Department of Microbiology, [1]Department of Agriculture , [2]Department of Agriculture Chemistry, Kyungpook National University

Soon-Ok Rim, Jin-Hyung Lee, In-Joong Lee[1], In-Koo Rhee[2], Jong-Guk Kim*

Objectives

GAs are secondary metabolites of the fungus *Gibberella fujikuroi*, that control such diverse developmental processes as seed germination, stem elongation, leaf expansion, trichome development, and flower and fruit development. *Fusarium proliferatum* KGL0401 was isolated from *Physalis alkekengi* var. *francheti* plant roots and it showed higher GAs productivity than wild-type *G. fujikuroi*. It was known that the seven genes(*des*, *p450-4*, *p450-1*, *p450-2*, *ggs2*, *cps/ks*, *p450-3)* of GA biosynthesis were clustered in the fungus *G. fujikuroi*. Here we present the structure of *p450-4* and *p450-1* involved in GA biosynthesis from fungus *F. proliferatum* KGL0401.

Materials and Methods

○ Materials

Fusarium proliferatum KGL0401 was isolated from *Physalis alkekengi* var. *francheti* plant roots and it showed higher GAs productivity than wild-type *G. fujikuroi*.

○ Methods

For DNA isolation the *F. proliferatum* KGL0401 were grown in 40 ㎖ Czapek's liquid medium for 7 days at 30℃ on rotary shaker set at 160 rpm. The mycelia were harvested by filtration and lyophilized for 48h. Genomic DNA was isolated from lyophilized mycelium by CTAB buffer.

Genomic DNA of *F.proliferatum* KGL0401 was used as templates for amplification of the *p450-4* and *p450-1* gene. The specific primers, which were synthesized by Bioneer Co.

Results and Discussion

p*450-4*(ent-kaurene oxidase) and p*450-1*(GA14 synthase) genes were cloned and determined from a fungus *F. proliferatum* KGL0401. The deduced amino acid sequences of *p450-4* and *p450-1* showed 91% and 81% similarities with those of *G. fujikuroi*.

Corresponding author : 김종국 E-mail : kimjg@knu.ac.kr Tel : 053-950-5379

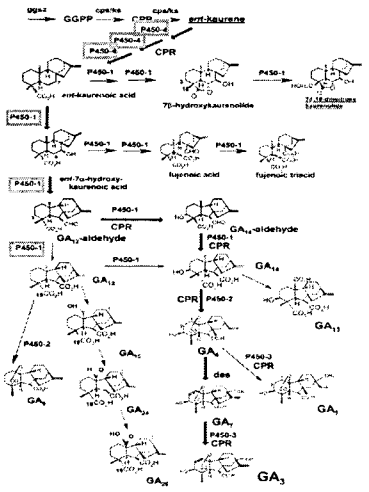

Fig. 1 The Pathawy of gibberellin biosynthesis. The catalyzing points of cloned gene products were designated by rectangulars.

Table 1. Primers designed for the amplification of p450-4 and -1

Primers	Sequences
P450-1UP	5` - AGA ACA GTC GTC CAA GCA TCA GCA - 3`
P450-1DN	5` - GGC TAC ATA TCT CGT GCT AGA CAG - 3`
P450-4UP	5` - CCA AAC TCC TCG GAC ATC ACT TTG - 3`
P450-4DN	5` - GTG TCA GTA GGT CGA ACC CAT AGC - 3`

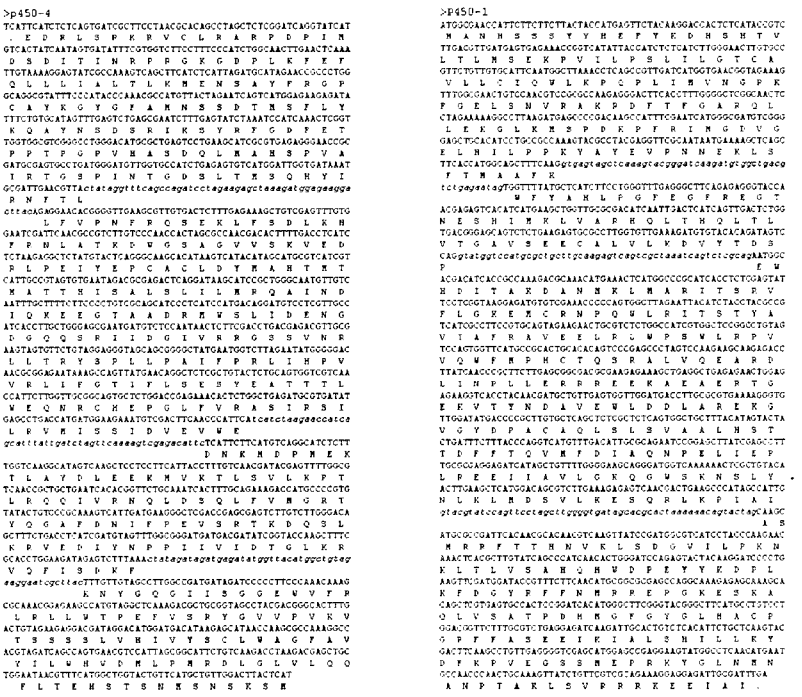

Fig. 2 Nucleotide and deduced amino acid sequences of P450-4, P450-1.