

Pavinee Suttiviriya 2010: Screening indole-3-acetic acid Producing Endophytic Actinomycetes and Cloning Genes Involved in its Production. Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Associate Professor Arinthip Thamchaipenet, Ph.D. 90 pages.

Fifty-seven isolates of actinomycetes from Genetics-Microbiology Kasetsart University (GMKU) Culture Collection were tested for plant growth promoting properties such as phosphate solubilization and the production of indole-3-acetic acid (IAA), ammonia and siderophore. The results indicated that only strain GMKU 103 carried all properties of plant growth promoting agents whereas, strain GMKU 134 had none. Strain GMKU 118 was selected as one of the high IAA producers to study gene involved in IAA production. Partial 16S rRNA gene sequencing of strain GMKU 118 revealed that it belongs to genus *Streptomyces*. Primers were specifically designed from amino oxidase gene which was similar to tryptophan-2-monooxygenase gene (*iaaM*) in Indole-3-acetamide (IAM) pathway. The amino oxidase gene was amplified by PCR and the nucleotide sequences revealed 926 basepairs encoding 308 amino acids. Its protein was similar to tryptophan-2-monooxygenase of *Pseudomonas syringae* pv. T1 (46 % identity, 62 % similarity) and *Agrobacterium tumefaciens* str. C58 (22 % identity, 43 % similarity) and similar to amino oxidase of *Streptomyces* sp. C (79 % identity, 85 % similarity). The amino oxidase gene was then verified its function via gene disruption by transferring pATT502 (containing PCR fragment of amino oxidase gene) into *Streptomyces* sp. GMKU 118 chromosome. Intergeneric conjugation was employed using optimum condition by conjugated heated spore of *Streptomyces* sp. GMKU 118 at 40° C for 10 minutes with *E. coli* ET12567 (pUZ8002/pATT502) on MS containing 10 mM MgCl₂. The mutants were characterized and showed the level of IAA production similar to that of wildtype. It can be concluded that *Streptomyces* sp. GMKU 118 is likely to produce IAA via other pathways not IAM.

Student's signature

Thesis Advisor's signature