

Ratchaniwan Jeamseang 2008: Cloning and Studying Function of *aroE* Gene in Shikimate Pathway of *Streptomyces venezuelae*. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Arinthip Thamchaipenet, Ph.D. 91 pages.

*Streptomyces venezuelae* produces chloramphenicol which is synthesized via shikimate pathway. Shikimate dehydrogenase (encoded by *aroE* gene) is one of the seven important enzymes in shikimate pathway. The *aroE* gene of *S. venezuelae* was amplified by PCR and used as a probe to hybridize the genomic fragments. Positive fragment of 1.5 kb was subsequently cloned and sequenced. The C-terminal *aroE* gene of *S. venezuelae* consists of 525 basepairs encoded 175 amino acids and its protein was closely similar to that of *S. pristinaespiralis* ATCC 25486. Intergeneric conjugation between *S. venezuelae* and *E. coli* ET12567 (pUZ8002/pSET 152) was conducted in order to develop a plasmid transfer system for gene disruption. The optimum condition for conjugation to occur was when either pre-germinated spores at 40 °C for 10 minutes or 12-24 hours old mycelium of *S. venezuelae* was used as recipient to mate with *E. coli* on TSA (Oxoid) containing 10 mM MgCl<sub>2</sub>. Maximum frequency of conjugation was observed at 10<sup>-4</sup> exconjugants per recipients. The exconjugants showed identical integration pattern of pSET152 into their chromosome and maintained stable morphology and antibacterial activity as the wild type. The attempt to verify the *aroE* gene function was achieved by gene disruption using the optimal conjugal condition to transfer pATT803 (pSET151 harbouring *aroE* PCR fragment) into *S. venezuelae* chromosome. The mutants could not grow on minimum medium lacking of tryptophan, tyrosine and phenylalanine. Furthermore, the mutants could not inhibit *E. coli* JM109 which is sensitive to chloramphenicol. The cultures of mutants were then characterised by HPLC and revealed that chloramphenicol was drastically reduced comparing to that of wild type. The *aroE* gene disruption was essentially verified by Southern blot hybridization. The results of this study indicated that *aroE* gene is involved in shikimate pathway that leads to the production of chloramphenicol in *S. venezuelae*.

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Thesis Advisor's signature