Thesis Title	**Heterologous Transformation of *Aspergillus niger***
Name	Ladapan Saengklai
Degree	Master of Science (Microbiology)
Thesis Supervisory Committee	
	Apinya Assavanig Ph.D.
	Watanalai Panbangred D. Eng.
	Chuanpit De-eknamkul Ph.D.
	Vithaya Meevootisom Ph.D.
Date of Graduation	21 November B.E. 2534 (1991)

ABSTRACT

Aspergillus niger (capable of citric acid production), designated wt or wild type strain and its uridine auxotrophic mutant, designated *pyr*$^-$ mutant (or mutant 501) strain were used as recipients in the transformation experiment. The protoplasts of both strains were transformed with plasmid pDJB2 containing orotidine 5'-monophosphate decarboxylase *(pyr4)* gene of *Neurospora crassa* and plasmid pOBT containing bacterial bleomycin resistance *(ble)* gene, respectively. The transformants obtained from pDJB2 *(pyr*$^+$*)* were screened on Aspergillus minimal medium (AMM) agar lacking uridine while those obtained from pOBT *(phle*^{R} or *ble*^{R}*)* were screened on Czapex dox agar added with phleomycin. The results revealed that the average of transformation frequency/μg DNA obtained from pDJB2 was 1 transformant/μg DNA and those obtained from pOBT were 0.6 and 0.5 transformant/μg DNA for transformation of wt and *pyr*$^-$ mutant strain 501,

respectively. No free plasmids were found in the transformants obtained from both plasmids but the integration of the plasmids into host chromosome had occurred. Southern hybridization analysis of *pyr*$^{+}$ transformants showed no appearance of *N. crassa pyr4* gene and only pBR325 fragment of the pDJB2 presented on the nitrocellulose paper (only 2 out of 30 transformants showed the hybridization bands on the paper). However, one of two *pyr*$^{+}$ transformant that showed pBR325 hybridization signal exhibited no further requirement of uridine for growth, and it showed 100 % mitotic stability.

Southern hybridization analysis of *phle*^{R} transformants indicated that integration of pOBT into the recipient chromosome mainly occurred as tandem head-to - tail repeats. Some *phle*^{R} transformants showed significantly increased resistance to phleomycin upto 1,000 μg/ml after 10 consecutive transfers on drug-free medium and they were 100 % mitotic stable. Heterologous transformation of *A. niger* with plasmid pOBT was accomplished and was more effective than that with pDJB2. The establishment of transformation system of *A. niger*, especially citric acid-producing strain, will be very useful in strain improvement of *A. niger* to enhance citric acid production.