KANOKTIP PACKDIBAMRUNG : IDENTIFICATION AND CHARACTERIZATION OF RESTRICTION ENZYME IN AZOSPIRILLUM LIPOFERUM A_{12}. THESIS ADVISOR : ASSOCIATE PROFESSOR, SIRIPORN SITTIPRANEED, PH.D., 144PP. ISBN 974-576-9169

Azospirillum sp. A_{12} was classified by morphological, physiological and biochemical properties. It was identified as Azospirillum lipoferum. One of the interesting properties of the bacteria is its low resistance to kanamycin and tetracyclin (2 and 6 µg/l respectively). Such property provided the advantage of using this bacteria as host cell to plasmid pCK3 which harbor nif A gene. The resulting transformants should have higher nitrogen fixing ability. However, effort to transform the bacteria by various methods was not successful. One of the possibilities is that the bacterial cell contains rectriction enzymes. Early investigation supported this hypothesis.

Restriction enzymes from A.lipoferum A_{12} were isolated and partially purified by DEAE cellulose column. It was found that there are 2 restriction enzymes and were named Ali I and Ali II respectively.

Ali I, when further purified through hydroxylapatite column until devoid of nonspecific endonuclease, produced 2 cuts on λ DNA, yielding DNA fragments of approximatedly 10, 19 and 20 kb. It could also cleave, ϕX174 at 6 sites, and cleaved pBR322, pSA30 and pCK3 at 1 site each. The optimum condition for this enzyme is, pH 7.5, 30-37°C

Ali II could be purified by Sephadex G-100 and hydroxylapatite columns. This enzyme cleaved ϕX174, pBR322, pSA30 and pCK3 at 1 site each, but could not cleave λ DNA. The optimum condition for this enzyme is 50 mM NaCl, pH 8.0-8.5 and 37-50°C.