Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

# Cloning of arginine deiminase from *Pseudomonas aeruginosa* in *Escherichia coli*

**N. Afrasiabi[1,*], N. Ebrahimi[2], F. Dabagh[2], Y. Ghasemi[3]**

*[1] Student Research Committee, International Branch, Shiraz University of Medical Sciences, Shiraz, Iran / Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran / Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*
*[2] Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran / Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*
*[3] Department of Pharmaceutical Biotechnology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background and Aims:** Arginine deiminase (ADI) catalyzes the hydrolysis of arginine to citrulline and ammonia. ADI gene exists in different microorganisms such as: Pseudomonas aeruginosa and Mycoplasma arginini. This enzyme can be used as a potential anti-cancer agent for the treatment of arginine-auxotrophic tumors, such as: hepatocellular carcinomas (HCCs) and melanomas. In this study ADI from pseudomonas aeruginosa was cloned in the expression vector, pET28a, for further expression in Escherichia coli (E. coli).
**Methods:** Pseudomonas aeruginosa was cultured and genomic DNA was extracted. ADI gene was amplified with PCR, using specific primers containing cut sites for restriction enzymes. The PCR amplicon was digested with NdeI and HindIII restriction enzymes and cloned into expression vector, pET-28a. The recombinant plasmid can be used to transform the expression host E.coli BL21. Expression results can be analyzed by SDS-PAGE.
**Results:** In this study we amplified ADI gene from pseudomonas aeruginosa, using PCR. This gene is 1254bp and encodes 417 amino acids. By digesting with suitable enzymes and ligating by T4 DNA ligase, we had a recombinant plasmid which could be expressed in E. coli.
**Conclusions:** This enzyme is in the clinical trials for the therapy of HCCs and melanomas. ADI has several advantages including high specificity for targeting malignant cells and low toxicity for patients. The cloned ADI gene can provide a suitable source for further studies of this enzyme.

**Keywords:** Arginine deiminase; *Pseudomonas aeruginosa*; Cloning