

Cloning of an alpha enolase enzyme from the oral pathogen streptococcus mutans in *Escherichia coli* strain BL21 (DE3)

N. Hajighahramani^{1,2,*}, Y. Ghasemi², F. Dabbagh^{1,2}

¹Student Research Committee, International Branch, Shiraz University of Medical Sciences, Shiraz, Iran

²Department of Pharmaceutical Biotechnology and pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

Background and Aims: α -enolase belongs to a family of cytoplasmic and glycolytic enzymes. This protein is localized in the cytoplasmic, cell wall and extracellular fractions of *Streptococcus mutans*, a gram-positive bacterium and the etiologic agent of dental caries and infective endocarditis. Enolase plays important roles in pathogenicity, and thus could be a protective antigen, serving as a novel vaccine candidate against infections.

Methods: In the present work, the enolase gene from *S. mutans* was cloned in to an expression vector and expressed in *Escherichia coli* strain BL21 (DE3). Total genomic DNA were isolated and used for PCR amplification of the enolase gene by means of the specific primers. The resulting PCR product was double digested and further ligated into the vector. The obtained recombinant construct was then introduced into the *E. coli*.

Results: A ~1296-bp of the structural enolase gene was amplified using the specific primers; the resulting PCR amplicon was ligated in an expression vector. *E. coli* cells were transformed with the recombinant plasmid to get the expression strain.

Conclusions: The DNA and amino acid sequence alignments resulting from the BLAST search of enolase showed that *S. mutans* protein at the amino acid level had greater than 90% identity with enolases of other streptococcal species. The commercial availability of enolase is of great importance for medical applications and also pharmaceutical purposes as protective antigen. Thus, the characterization of new recombinant enolase and the development of rapid, simple and effective production methods are not only of academic interest but also of practical importance.

Keywords: Gene cloning; *Streptococcus mutans*; *Escherichia coli*; Alpha enolase