PRODUCING L-ASPARAGINASE OF ERWINIA CHYSANTHEMI IMPROVED BY SYNTHETIC EVOLUTION OF PROTEINS.

CUSTODIO, D. F.¹, PESSOA, A. ¹, MONTEIRO, G.¹

¹ Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo – USP, Brazil.

Abstract: L-Asparaginase (ASNase), discovered in 1953 by Kidd is a tetrameric enzyme, efficient inhibitor of tumor growth, used in chemotherapy sessions to deplete asparagine (Asn) and glutamine (Gln), transforming them into aspartate or glutamate, respectively, and ammonia. However, ASNase can induce immune response leading to the production of anti-asparaginase antibody, the main cause of drug resistance. An ideal ASNase would be one with high activity, high stability and low allergenic potential, but unfortunately the commercial available ASNase obtained from E.coli or Erwinia chrysanthemi do not have these three characteristics simultaneously. For this reason, the present work uses random mutagenesis techniques in order to create a new ASNase from Erwinia chrysanthemi variant with improved activity and stability. The gene encoding L-asparaginase from Erwinia chrysanthemi was cloned and expressed in Escherichia coli strain BL21 (DE3). This construction was used as a standard to create a mutant library by error prone PCR, with GeneMorph® Random Mutagenesis Kit. The activity properties of the recombinant enzyme and of the mutants were tested using the method of Nessler and the quantification of protein was performed through the method of Bradford. It was created a library with 1,056 mutants. The specific activity of standard enzyme was 455 U/mg. Putative mutants were selected based on the enzyme activity. Thirty clones were selected, whose activity ranged from 80 to 200% of the specific activity value obtained for the standard enzyme. Four mutants presented no amino acid substitution, seven mutants had only silent mutation; corresponding to 36% of the selected clones. 64% of the selected mutants have at least one amino acid mutation, and one clone had five amino acid mutation preserving the ASNase activity. All these variants will be tested regarding the resistance to human serum proteases, one of the main reasons to ASNase side effects in patients.

Financial Support: This work is supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), grant (Projeto Temático) 2013/08617-7, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).