CREATING MUTATIONS IN BACTERIAL PHYTASE GENE COMPATIBLE WITH CHICKEN CODON PREFERENCES

Farzad Rashidi Khorasgani1, Shahin Eghbalsaied2*, Kamran Ghaedi3

1Young researchers club, Isfahan (Khorasgan) branch, Islamic Azad University, Isfahan, Iran
2Department of Animal Science, Isfahan (Khorasgan) branch, Islamic Azad University, Iran
3Department of Biology, University of Isfahan, Iran

*corresponding author email address: shahin080@gmail.com

INTRODUCTION
Phytase is a special group of phosphatase enzyme that catalyzes the stepwise hydrolysis of phytic acid and releases a usable form of inorganic phosphorus. Currently, in order to increase the absorption of dietary phosphorus and decreasing the phosphorus pollution in the environment, phytase enzyme supplemented to diet of monogastric animals including, Pig, poultry and fish. Commercially available exogenous phytases are commonly derived from either fungi, yeasts and bacteria, Nevertheless, Ecoli bacteria is one of the main sources of phytase expression, that produces phytase enzyme that resist to pepsin hydrolysis of most animals, and also has a high specific activity of phytic acid. Therefore, the aim of this study was to isolation of bacterial phytase gene, then optimization to its protein coding sequences corresponding to protein coding sequences of chicken through specific primers and finally cloning of this gene to pTG19 vector.

Materials and Methods
Phytase gene from the bacterial Escherichia coli was isolated using specific primers. In order to increase expression of this gene in the gastrointestinal tract of broiler chicken, changing protein coding sequences of Escherichia coli to chicken, specific primers for the 24 mutations in this gene designed and for final enzyme digestion, EcoR1 and Xho1 sites were added to 5’ ends of these primers. Then, using TA cloning system, mutated gene was transfected to pTG19-vector. The presence of a target gene was confirmed in the recombinant vector, using white-blue colony method, enzymatic digestion with EcoR1 and Xho1, and PCR technique.

Results
The result indicated that, it is easy to clone the bacterial phytase gene to cloning vector for creating transgenic chickens.

Keywords: Site direct mutation, Phytase gene, Cloning, Chicken, Escherichia coli