CREATING MUTATIONS IN BACTERIAL PHYTASE GENE COMPATIBLE WITH CHICKEN CODON PREPERENCES

Farzad Rashidi khorasgani[1], Shahin Eghbalsaied[2*], Kamran Ghaedi[3]
[1]*Young researchers club, Isfahan (Khorasgan) branch, Islamic Azad University,Isfahan, Iran*
[2]*Department of Animal Science, Isfahan (Khorasgan) branch, Islamic Azad University, Iran*
[3]*Deaprtment 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