Production of phytase enzyme by a bioengineered probiotic for degrading of phytate phosphorus in the digestive tract of poultry

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Objective: Probiotics are beneficial microorganisms and have long been used in food production as well as health promotion products. Bioengineered probiotics are used to express and transfer native or recombinant molecules to the mucosal surface of the digestive tract to improve feed efficiency and promote health. *Lactococcus lactis* is a potential probiotic candidate to produce useful biological proteins. The purpose of this study was to test the new vector, aimed to clone and express the phytase gene (appA) isolated from *E.coli* into *L.Lactis* and to determine the enzyme activity in the supernatant of recombinant bacterium (in vitro). Also, improvement of digestibility of phytate phosphorus in gastrointestinal of chickens because of the use of recombinant *L.lactis* was examined in this study.

Materials & methods: To enhance the efficiency of expression and secretion of recombinant phytase, usp45 signal peptide was added to the expression vector pBU003 by ligation then transformed to *E.coli (MC1061)*. The recombinant plasmid named pFUM003. Then the appA gene was inserted in plasmid pFUM003 and transferred to *E.coli MC1061*. A Plasmid containing usp45 and appA2 electrotransferred into *Lactococcus lactis* and Zymogram with polyacrylamide gel and SDS-PAGE were done.

Efficiency of recombinant probiotic on degradation of phytate phosphorus was determined by a digestibility experiment on 20 male broiler chickens 18-day-old. A basal corn-soybean diet were supplemented by 3 feed supplements include 1) *E.coli* commercial phytase 2) recombinant *L.lactis* (109 cfu/kg) 3) recombinant *L.lactis* + *Lactobacillus plantarum* (109 cfu/kg) 4) non recombinant *L.lactis* (109 cfu/kg) and 5) a group of birds was fed by without any supplement as control. Total

Results & Conclusion: Sequencing of recombinant plasmid containing appA2 showed the correct construction of plasmid. Total length of the phytase insert was 1.25 kbp. A Blast search of the cloned fragment showed 99% similarity to the reported *E. coli* phytase sequence in the GenBank (accession number: AM946981.2). Zymogram with polyacrylamide gel revealed that the protein extract from the supernatant and the cell pellet of recombinant bacteria had phytase activity.

Enzyme activity of 4U/ml was obtained in cell extracts and supernatant maximal phytase activity was 19 U/ml. To supplement of recombinant *L.lactis* in broiler chicken feed showed that the apparent digestibility of phytate phosphorus increased in the digestive tract and it was like performance of *E.coli* commercial phytase.

Keywords: *Lactococcus lactis*; Phytase; Probiotic; Recombinant; poultry