Cloning of a novel phytase from an anaerobic rumen bacterium, Mitsuokella jalaludinii, and its expression in Escherichia coli

ABSTRACT

The full length phytase gene of Mitsuokella jalaludinii was successfully cloned and was found to be 1047 bp in length, with 348 amino acids, and was designated as PHY7 phytase gene. A comparison of the sequence of PHY7 phytase gene of M. jalaludinii with various microbial phytase gene sequences showed that it was not similar to those from other bacteria except Selenomonas ruminatium, thus suggesting that they may both express a new class of phytase. The PHY7 phytase gene was subsequently subcloned into bacterial expression vector, pET32a, for expression in Escherichia coli strain Rosetta-gami. Expression of the recombinant phytase gene was optimized and characterized. The recombinant phytase was estimated to be approximately 55 kDa by SDS-PAGE analysis. The recombinant phytase exhibited optimum activity at 55℃, pH 4.5 and showed good pH stability from pH 3.5 to 5.5 (>78% relative activity). Metal ions such as Ca2+, Mg2+, and K+ were found to exert significant stimulatory effect on the recombinant phytase activity while Cu2+, Fe3+, and Zn2+ greatly inhibited the enzyme activity. The recombinant phytase showed moderate resistance to trypsin proteolysis, but susceptible to pepsin proteolysis. The results of the study showed that several characteristics of recombinant phytase were slightly different from the native enzyme. Unfavourable characteristics such as reduced pH stability and metal ion effects should be taken into consideration during feed enzyme formulation.

Keyword: Phytase; Cloning; Recombinant; Escherichia coli; Mitsuokella jalaludinii