PRODUCTION AND CHARACTERIZATION OF FUNGAL PHYTASE PRODUCED BY SOLID-STATE FERMENTATION.

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Abstract

A high phytase producing strain, Aspergillus oryzae 9252, used for soysauce fermentation was selected from a stock culture of fungal strains. The ultrafiltrated crude phytase was purified by ion exchange chromatography using Q-Sepharose and hydrophobic interaction using phenyl-sepharose CL-4B resins. The molecular weight of 9252 phytase was 100 kDa. A solid medium, suitable for 9252 phytase production, was composed of 0.3 g of rice flour and 0.65 ml of distilled water per 1 g of soybean meal. General properties of 9252 phytase produced by solid state fermentation were determined. The crude enzyme was stable at pH range 2.0-7.0 and under temperature range of 37-80 °C after 2-hr storage. The optimum activity was found at 75 °C, pH 5.0. Study on the effect of metal ions and EDTA showed that crude 9252 phytase was stable in the presence of Mg2+, Ca2+, Mn2+, and Fe2+, while Zn2+ and EDTA inhibited the phytase activity. Formulation of crude 9252 phytase was done by addition with 0.1% (v/v) each of lactic acid and propionic acid and stored at 4°C. The activity was retained at 1-2 u/mg through to the seventh week. Additionally, crude amylase and protease in the koji were characterized. It was found that amylase worked best under pH 4.5 and at 55 °C. In the case of protease, there seemed to be 2 kinds of protease, one showed maximum activity at pH 5.5 and the other at pH 6.5. Optimum temperature of both proteases was at 55 °C. Koji was dried in order to test the effect of temperature on phytase, amylase and protease activities. It was found that phytase and amylase were stable when the koji was dried at 60-65 °C. For the protease activity, the remaining activity was lower than 50% after 2-hr storage at 60-65 °C. It was found that 9252 also gave other digestive enzyme activities such as xylanase, lipase, cellulase, and endoglucanase activities. A 42-day feeding experiment was conducted with 1-day-old male and female broilers (n=192) to evaluate the effectiveness of phytase supplement on growth performance and on reduction of the Pi excretion to the environment. The basal animal diet contained 0.26% nonphytate P. Three levels of crude 9252 phytase; i.e. 0, 300 and 600 U/kg feed, were added. However, the treatment did not significantly improve the body weight gain and feed intake at all phytase levels. Phytase supplementation at 300 and 600 U/kg fed could reduce the feces weight of male and female chickens by 11 to 14%, when compared to the chickens in the control group.

KEY WORDS: Aspergillus oryzae/ KOJI MOLD PHYTASE/ SOLID-STATE FERMENTATION

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