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Short communication

Biochemical characteristics of phytases from fungi and the transformed microorganism

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Abstract

Five sources of phytases were used to study their biochemical characteristics. Phytase E was from an original *Escherichia coli* (*E. coli*), phytase PI and PG from the transformed *Pichia pastoris* (*P. pastoris*) with phytase gene of *E. coli*, phytase B and R from *Aspergillus niger* (*A. niger*). The results showed that the relative phytase activities had no significant changes when temperature was below 60 °C ($P>0.05$), and then decreased significantly with temperature increasing ($P<0.01$). The fungal phytase with the phytase gene from *A. niger* had the higher thermostability than the bacterial phytase with the phytase gene from *E. coli*; *i.e.* at 70 °C, 27–58% of phytase activity (compared with 30 °C) was retained for the bacterial phytase, and 73–96% for the fungal phytase; at 90 °C, 20–47% was retained for the bacterial phytase, and 41–52% for the fungal phytase, especially for the most thermostable phytase R ($P<0.01$). The optimum pH ranges were 3.0–4.5 for the bacterial phytases and 5.0–5.5 for the fungal phytases ($P<0.01$). When pH levels were 1, 7 and 8, only 3–7% of phytase activity (compared with the maximum phytase activity at a pH point) was retained for both bacterial and fungal phytases. The amount of inorganic P released from soybean meal was significantly increased when the levels of phytase activity in the soybean meal increased from 0 to 1.0 U/g soybean meal ($P<0.01$), except for phytase PI. The maximum P released was obtained at 1 U/g soybean meal for all five kinds of phytases ($P<0.01$). The most economical phytase concentration for P released was 0.25 U/g for phytase PI and B, and 0.50–1.0 U/g for phytase PG, E and R. In addition, the linear and non-linear regression models were established to estimate phytase activity and its characteristics very easily and economically.

Keywords: Bacterial phytase; Fungal phytase; Expressed phytase; Biochemical characteristics; Regression analyses

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1. Introduction

Phytic acid is present in all practical pig and poultry rations with levels ranging from 0.5 to 1.4%, where it serves as a phosphorus reservoir. It is a powerful chelating agent of positively charged nutrients, so the solubility and digestibility of many nutrients are reduced by the formation of phytate complexes including amino acids (lysine, histidine and arginine, *etc.*) and starch complexes as well as mineral complexes (Selle, 1997). Phytase is able to catalyze the hydrolysis of phytate, and release inorganic phosphorus (Wodzinski and Ullah, 1996) and phytate-bound calcium (Ca), iron (Fe), zinc (Zn) and magnesium (Mg), *etc.* (Murry et al., 1997). Because there is little phytase activity in the digestive tracts of the non-ruminant animals (Bitar and Reinhold, 1972), these animals cannot use these minerals effectively, especially phytate-bound P. P is an important mineral in animal nutrition, but it is always deficient in the diets of non-ruminant animals. The addition of phytase in animal diet can reduce the supplementation of inorganic P in animal diets by releasing phytate-bound P, as a result, P excretion was reduced by 30–50% (Selle, 1997) and P pollution was also reduced (Sweeten, 1992).

Although bean, wheat and corn contain phytase activity, it is not enough to digest phytate-bound minerals effectively. The phytase gene has been expressed in soybean cells (Li et al., 1997) to increase apparent P digestibility by 31%, while microbial phytase increased apparent P digestibility by 54% in pig (Selle, 1997). It was reported that micro-organisms might be a more feasible source of the enzyme (Reddy et al., 1982). This is why more and more researches focus on the microbial phytase production. In the commercial market, there are many kinds of phytases from fungi, bacteria or phytase gene transformation. The different sources of phytases have the different characteristics, which must be considered when they are applied in animal diets. The objective of this research was to study the biochemical characteristics of the original and expressed phytase from fungi, *Escherichia coli* (*E. coli*) and yeast.

2. Materials and methods

2.1. Five sources of phytases

Phytase R—a commercial phytase (3529 U/g) from *Aspergillus niger* (*A. niger*) (F. Hoffmannp-La Roche Ltd., Switzerland), phytase B—a commercial phytase (3507 U/g) from *A. niger* (BASF company, The Netherlands), phytase E—the phytase (663 U/g) from the original *E. coli*, phytase PG (2546 U/g) and PI (4138 U/g) from the transformed *Pichia pastoris* (*P. pastoris*) with pGAPZaA and pPICZaA plasmids containing phytase gene from *E. coli* made in our laboratory. The activities of the above five kinds of phytases were determined in our laboratory under the same conditions. Phytase E, PI and PG were extracted and purified with Rodriguez et al. (1999a)'s protocol. Phytase gene (1.25 kb) was amplified from genomic DNA of *E. coli* by PCR, inserted into the plasmids (pPICZaA and pGAPZaA, Invitrogen Company, USA), and transformed into the wild-type *P. pastoris* by electroporation for phytase production.