Expression of the *E. coli* APPA phytase in the genetically enhanced Enviropig™ breed

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The Enviropig™ breed of pigs expresses the APPA phytase gene from *Escherichia coli* under the control of the mouse parotid secretory protein (PSP) promoter. The phytase enzyme is produced primarily in the salivary glands of the pig (Golovan et al., 2001), and is not detected in tissues normally recovered for human consumption. The level of enzyme detected in the saliva differed among the various lines of the Enviropig™ breed as well as among the different phases of growth. Greatest concentration of enzyme per milliliter of saliva usually occurred during the weanling phase and decreased to a base level during the late growing and finishing phases of growth. The phytases produced by different lines of pigs had a similar size of approximately 51 kDa, which were approximately 6 kDa larger than the *Escherichia coli* phytase due to N-linked glycosylation. The purified enzyme from three different lines of the Enviropig™ breed exhibited similar *K*_m* and V*_max* values for phytase and acid phosphatase activities to that of the APPA phytase overexpressed in *E. coli*. These activities were determined by assaying phytate hydrolysis at pH 4.5 and *p*-nitrophenyl phosphate hydrolysis at pH 2.5. The three enzyme preparations also exhibited similar pH and temperature profiles to the *E. coli* phytase. The phytase produced by the Wayne line of Enviropig™ hydrolyzed phytate and produced inositol phosphate intermediates and final products identical to that of the *E. coli* phytase. Analysis of tryptic peptides of the three different preparations of the Enviropig™ phytase indicated the presence of pyroglutamic acid at the N-terminus and documented N-glycosylation at all three potential N-glycosylation sites. The glycosylated phytase, like that of the *E. coli* enzyme, was unstable below pH 2.0 in the absence of proteases, but was stable at pH values 2.5 and 4.5 in the presence of pepsin. It was inactivated at pH 7.0 in the presence of serine proteases. The differential stability of the phytase in the presence of pepsin and its sensitivity in the presence of trypsin may be explained by the limited number of solvent accessible pepsin recognition sites compared to the higher number of solvent accessible trypsin recognition sites.

These characteristics of the phytase ideally suit the dietary requirements for a genetically enhanced monogastric food animal. When the animal begins eating, this is when most phytase is secreted in the saliva, the pH increases due to the buffering capacity of the food to a pH between 2.5 and 5.0. The phytase is maximally active in this pH range. Once the enzyme reaches the small intestine and is exposed to serine proteases, it is rapidly degraded.

References