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Improving temperature stability of Escherichia albertii phytase by error-prone PCR

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Phytase [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate phosphohydrolase], a phytate-specific phosphatase, is already used as a supplement in diets for simple-stomached animals to improve phosphate utilisation from phytate [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate], the major storage form of phosphate in plant seeds and grains. In recent years, this class of enzymes has also found increasing interest to be used in food processing and manufacturing, particularly because reduction in dietary phytate is seen as a possibility to combat zinc and iron deficiencies by enhancing their bioavailability in plant-based foods. Several enzymatic properties of the *Escherichia albertii* phytase make this enzyme attractive for biotechnological applications. For many applications however, a higher temperature stability of the enzyme would be of advantage.

Directed evolution was applied to improve temperature stability of the *Escherichia albertii* phytase. Error-prone PCR was performed using a plasmid containing the wild-type *Escherichia albertii* phytase encoding gene cloned into a *Saccharomyces cerevisiae* expression vector as a template. Approximately, 1500 clones were screened for increased temperature stability. Compared with the wild-type enzyme, two variants (K46E and D144N/V227A) showed a significant increase in temperature stability. Compared to the wild-type phytase, the mutants showed a 33% (K46E) and a 95% (D144N/V227A) higher residual activity at 80°C after 10 min incubation. Overall catalytic efficiency (k_{cat} /K_m) of K46E and D144N/V227A was improved by 36% and 97% compared to the catalytic efficiency of the wild-type phytase at pH 4.5, respectively. Thus, the catalytic efficiency of these enzymes was not inversely related to their temperature stability. From an economic point of view it is worth mentioning that the mutants still exhibit excellent high specific activities.

In summary, *Escherichia albertii* phytase variants with a better overall catalytic efficiency and improved temperature stability were obtained. These improvements make these enzymes better suited for the intended biotechnological applications.

Keywords: phytase, error-prone PCR, phytate, thermal stability