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Screening for phytases using a metagenomic approach

R. Greiner*[1], A. Sadjidan[2]

[1]*Max Rubner-Institut, Germany,* [2]*Sebelas Maret University, Indonesia*

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 phytase have been purified and characterized in respect to their biotechnological application and some are commercially available in the meantime. However, especially for food applications a higher temperature stability of the enzyme would be of advantage.

Expression of environmental DNA in bacteria (metagenomic screening) has proven highly useful for identification of novel enzymes or enzymes with improved properties. Genomic DNA was obtained from agricultural soil using a method based on direct lysis and purified by gel filtration. The recovered DNA was of high molecular mass and sufficiently pure for subsequent cloning. Following enzymatic digestion, the DNA was cloned into the expression vector pBluescript SK+. The resulting expression plasmid library comprised a total of 25000 clones. The library was screened for phosphatase activity using X-phosphate as a substrate. 27 clones with improved phosphatase activities were identified. Using sodium phytate as a test substrate revealed that 15 clones showed also an improved phytase activity. Compared to many phytases described in the scientific literature so far, 4 of the phytases identified by metagenomic screening exhibited an improved temperature stability. Residual activity after exposure to 80°C for 10 min was determined to be higher than 70%.

In summary, is was shown that direct cloning of environmental DNA is a suitable strategy to utilize the metabolic diversity in a given habitat for biotechnical innovations.

Keywords: phytase, phytate, metagenomics, thermal stability