SPECIFIC ACTIVITY INCREASE OF A BETA-PROPELLER PHYTASE

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Introduction. The degradation of phytate (*myo*-inositol hexakisphosphate) is possible by the action of a group of monomeric hydrolases known as phytases (*myo*-inositol hexakisphosphate phosphohydrolases). These enzymes are important in feed formulations, and beta-propeller phytases are especially suitable as feed additives for agastric aquaculture species, since these phytases are active at a broad range of pH values, with optimum activity near neutrality. For enzymes of such biotechnological interest, a high specific activity is particularly desirable.

In this work, we have increased the specific activity of the beta-propeller phytase (FTEII) (1) using a protein engineering approach and the *Pichia pastoris* expression system.

Methods. The amino acid residue candidates to be mutated in FTEII were determined by a structure-guided consensus approach (1, 2). Modification of the nucleotide sequence was performed in a single assay using the QuikChange Lightning multi site-directed mutagenesis kit and the plasmid pGEMfteIIaox, with further confirmation by nucleotide sequencing. The mutated nucleotide sequence (*fteIII*) was subcloned into vector pPIC9 using the XhoI and AvrII sites. *Pichia pastoris* recombinant strains were constructed by transformation of the *P. pastoris* host strain, KM71 (*his4*), with SalI-digested pPIC9*fteIII*. The transformants were selected by histidine auxotrophy, and the integration of the expression cassette into the genomes of the selected strains was verified by PCR. Phytase production from a randomly selected strain was verified in shake-flask cultures in buffered minimal glycerol (BMG) and buffered minimal methanol (BMM) media with analysis of the cell-free culture medium for phytase activity. The cell-free culture medium was concentrated 20-fold and diafiltrated by ultrafiltration, and additional phytase purification was performed using anion exchange chromatography. The highest ratio of phytase activity to protein concentration of a chromatographic fraction was considered as the specific activity of purified phytase FTEIII. The same procedure was used to determine the specific activity of FTEII.

Results. The structure-guided consensus approach led to the selection of three mutations in FTEII, which were performed by simultaneous mutations of five nucleotides at the *fteII* gene. Transformation of *P. pastoris* KM71 gave 18 His^+ transformants. PCR analysis of the genomic DNA from the transformants showed a 1,535-bp band, confirming the correct integration of the expression cassette into the *P. pastoris* genome. The selected *P. pastoris* recombinant strain showed a phytase activity of 1.8 U/mL in the cell-free culture medium from 72 h BMM cultures The new beta-propeller phytase (FTEIII) showed a specific activity at pH 7.5 and 37°C of 19.2 U/mg, while phytase FTEII showed a specific activity of 15.2 U/mg.

Conclusions. Using a structure-guided consensus approach, the specific activity of the beta-propeller phytase FTEII was increased by 26%.

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