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Identification and evaluation of the expression and secretion levels of the yeast genome-derived alpha-amylase enzyme *Komagataella phaffii* by HPLC-MS method

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Motivation and Aim: Currently, enzyme preparations that hydrolyze starch, which constitutes a significant proportion of carbohydrates in the feed weight but has a low percentage of digestibility, are in demand in agriculture to increase the nutritional value of feed. Alpha-amylase (α-1,4-glucan-4-glucanohydrolase) is an endoamylase and catalyzes the hydrolysis of α-1,4-glycosidic bonds within the chain of starch and related carbohydrates to form substances with a low degree of polymerization such as glucose, maltodextrin, and oligosaccharides of various lengths. Research in the field of α-amylase production is related to the search for strains of highly efficient thermostable enzymes, improvement of various characteristics of synthesis, secretion of recombinant proteins, protection against protease degradation, etc., as well as the search for inexpensive substrates for industrial cultivation of strain-producers. α-amylases from bacteria of *Bacillus subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, and *B. stearothermophilus* species are of industrial importance. Expression systems based on bacteria including *B. subtilis* and *Brevibacillus choshinensis*, the fungus Aspergillus oryzae and the yeast *Pichia pastoris (Komagataella phaffii)* have been used to produce recombinant α-amylases. The aim of the present work was the qualitative identification and relative quantification of the alpha-amylase enzyme derived from the genome of the yeast *Komagataella phaffii*.

Methods and Algorithms: During the study, proteins were purified by quantitative precipitation and concentration followed by tryptic hydrolysis. The prepared and purified peptide mixture was separated using a Thermo Fisher Scientific Ultimate 3000 Series HPLC system (Nano/CapSystemNCS-3500RS) followed by tandem mass spectrometric analysis on an Orbitrap Fusion Lumos. The primary data were processed using the Proteome Discoverer 2.4 software package to obtain highly accurate information on the composition and ratio of proteins in the samples

Results: In samples of recombinant strain *K. phaffii* T07, peptide sequence fragments identifying the structure of α-amylase with the indicated putative amino acid sequence were detected with 88.09 % convergence (Fig. 1). Quantitative ratios of α-amylase enzyme in 4 clones of K. phaffii T07 were obtained and are shown in Fig. 2.

In addition, a comparison of α-amylase secretion in the original α-amylase-producing strain *B. licheniformis* 47018 and secretion of the target α-amylase enzyme into the culture fluid of the α-amylase-producing strain *K. licheniformis 47018* and the secretion of the target enzyme α-amylase into the culture fluid of the α-amylase-producing strain

K. phaffii T07 (Fig. 3, 4). The ratio of amounts (in culture fluid/proteome) of secreted enzyme for *K. phaffii* T07 clones: clone 10 – 220.7/7.1; clone 45 – 82.4/6.9; clone 5 – 209.7/7.1; clone 7 – 245.7/20.4 (normalization was performed for clone 45).

Fig. 1. Identification of the structure of α-amylase with the putative amino acid sequence of α-amylase from *B. licheniformis* 47018

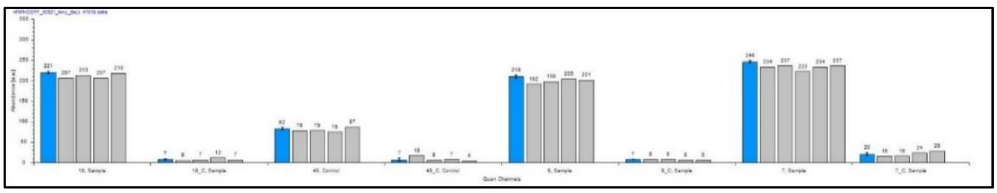

Fig. 2. Quantification of α-amylase enzyme in the proteome (Control) and secretome (Sample) of *K. phaffii* T07 (clones 10, 5, 7) and in the proteome (Control) and secretome (Sample) of *K. phaffii* T07 (clone 45)

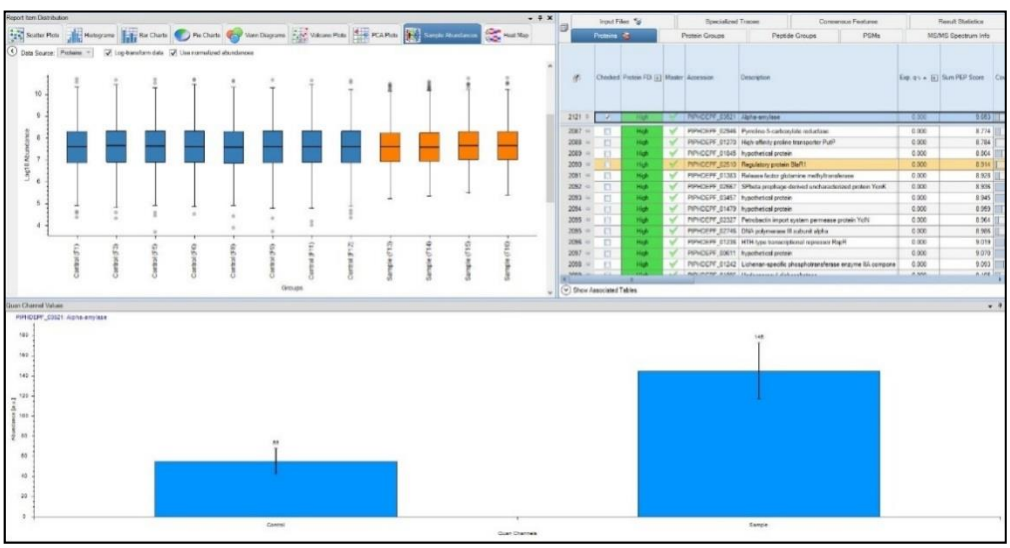

Fig. 3. Identification and quantitative comparison of α-amylase enzyme in the proteome (Control) and secretome (Sample) of *B. licheniformis* 47018

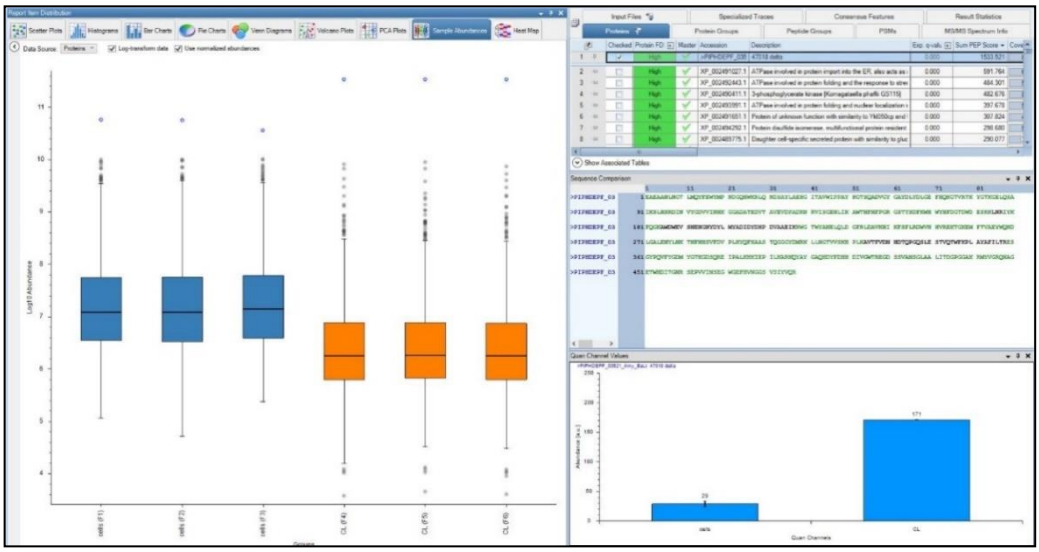

Fig. 4. Identification and quantitative comparison of α-amylase enzyme in the proteome (cells) and secretome (CL) of *Komagataella phaffii* T07

Conclusion: The established basic methods for qualitative and quantitative analysis of molecules of the target enzyme α-amylase from *B. licheniformis* 47018 allow to reliably determine their presence in culture fluids and preparations based on cells of *Komagataella phaffii* strain T07.

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