Bioengineering of *Saccharomyces cerevisiae* with *Nepenthes ampullaria* neprosin enzyme

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Background

Endopeptidase is a class of enzyme that catalyzes proteolysis with wide substrate specificity and biological function. Plant proteases are enzymes of interest for industrial applications, such as bromelain from pineapple as food supplement to reduce swelling and body pain and papain from papaya as meat tenderizer. Proteases were secreted into the pitcher fluids of carnivorous plants, such as *Nepenthes ampullaria* which were discovered in transcriptomics and proteomics study (Zulkapli et al. 2017). Due to evolutionary adaptation for extracellular functioning, proteases from *Nepenthes* pitcher fluid possess unique properties that are desirable in industrial application such as high optimal temperature up to 60 °C, high activity in pH range 3-10, and high stability against chemicals or denaturing agents (Ravee et al. 2018). One of the proteases discovered is neprosin, the first plant prolyl endopeptidase with characteristics distinct from previously characterized prolyl endopeptidases of microbial origin. Neprosin contains two novel functional domains, namely neprosin activation peptide pfam14365 (previously DUF4409) and neprosin domain pfam03080 (previously DUF239). A neprosin from *Nepenthes* × *ventrata* with a low molecular weight is shown to exhibit proline-cleaving activity towards protein of any size at low pH and enzyme concentration. Thus, it has the potential as a tool in proteomics for whole-proteome profiling and histone mapping (Schräder et al. 2017). Additionally, Rey et al. (2016) has demonstrated the capability of *N.* × *ventrata* neprosin in degrading gliadin, a subcomponent of gluten which trigger inflammatory response in celiac disease patients. Hence, it has the potential for application in gluten detoxification as enzyme therapy. Our study explores the possibility to bioengineer the common baker's yeast, *Saccharomyces cerevisiae* for recombinant neprosin expression.

Methods

Neprosin gene sequence was obtained from the *N. ampullaria* pitcher transcriptome and fluid proteome analyses. Two neprosin genes were cloned into pET-28(b)+ and expressed in the *E. coli* before sub-cloning into yeast expression vector pAG423GPD-ccdb-Cerulean. *S. cerevisiae* CEN.PK-2d will be transformed with the yeast expression for further analysis. A secretory signal such as alpha-mating factor will be engineered for the recombinant Neprosin enzyme to be secreted extracellularly into culture medium for harvesting and purification by using His-tag affinity chromatography. Enzyme functional characterization will be conducted to determine the enzyme activity, optimal temperature and pH, as well as substrate and inhibitor specificity of neprosin.

Alternatively, a genome engineering approach with CRISPR-Cas will be taken to integrate the gene sequence into the yeast genome for more stable expression.

Results

Phylogenetic analysis of neprosin genes will enable the study on the evolutionary function of the protein in other plant species. This study is expected to produce recombinant neprosin enzymes from *N. ampullaria* in *S. cerevisiae*. Functional characterization will identify optimal temperature, optimal pH, specific inhibitor, and specific substrate for the recombinant neprosin enzymes. Various parameters and optimizations will be tested on the feasibility of the transformed yeast for gluten detoxification in bakery.

Conclusion

Bioengineering of baker's yeast *S. cerevisiae* with heterologous neprosin holds great promises for applications in the food industry with ease of use in baking to produce gluten-free flour-based products.

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