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

Tiew-Yik Ting[1], Hoe-Han Goh[1]* Plant Functional Genomics Group, [1]Institute of Systems Biology, University Kebangsaan Malaysia, UKM Bangi 43600 Selangor, Malaysia. *Corresponding author. Email: gohhh@ukm.edu.my.
Funding: FRGS/1/2019/STG05/UKM/02/10

Background

•Protease is a class of enzyme that catalyses proteolysis with wide substrate specificity and biological function. 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).

•Proteases from *Nepenthes* pitcher fluid possess unique properties that are desirable in industrial application such as high optimal temperature, high activity in wide pH range, and high stability against chemicals or denaturing agents (Ravee et al. 2018). Neprosin is 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) and neprosin domain (pfam03080).

•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.

Method

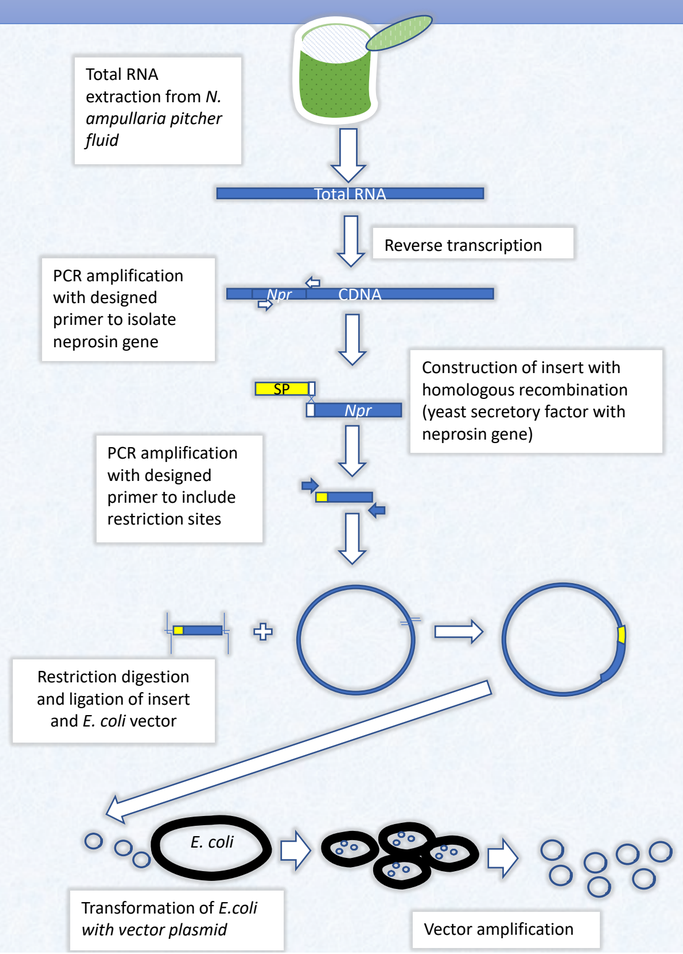


Figure 1: Isolation and cloning of neprosin gene into *E. coli*

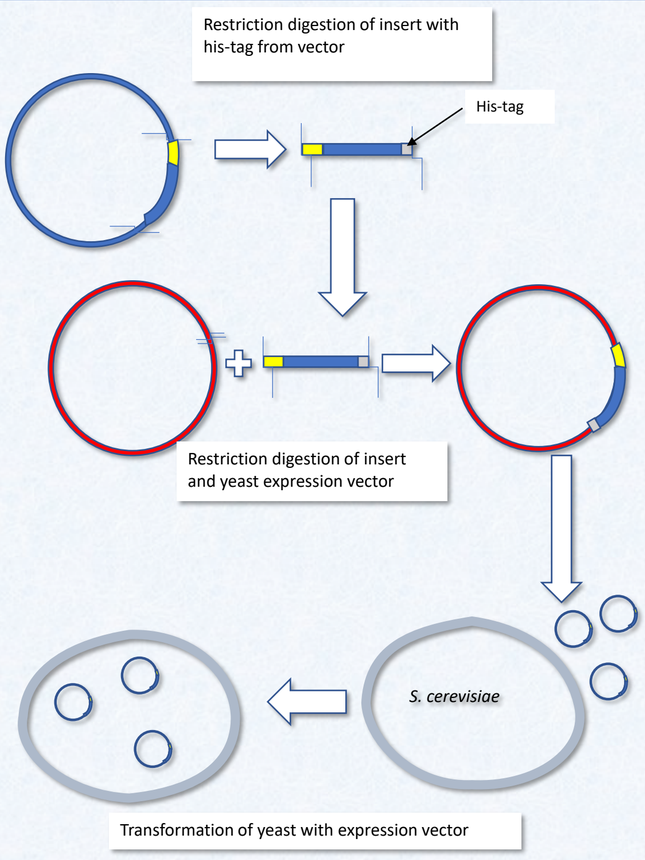


Figure 2: Subcloning of neprosin gene into *S. cerevisiae*

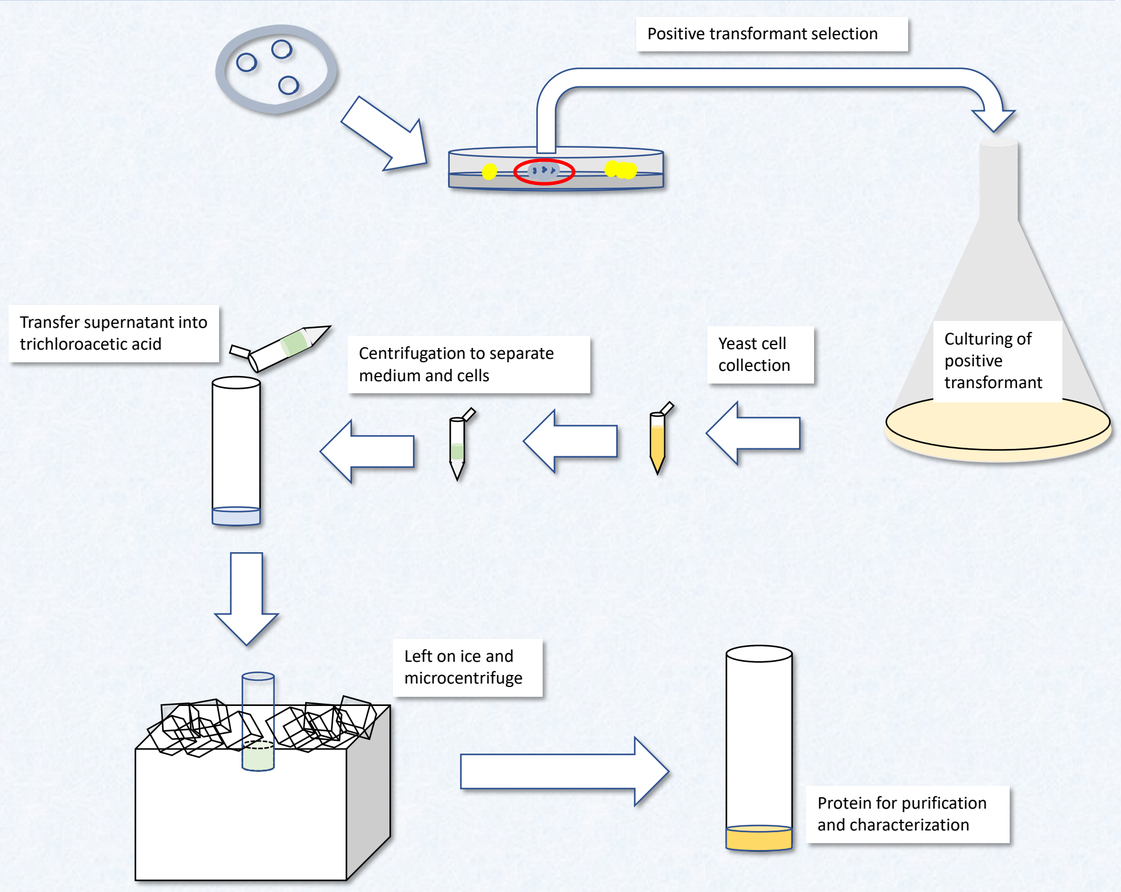


Figure 3: Harvesting of secreted protein from bioengineered *S. cerevisiae*

Neprosin gene sequences were obtained from the *N. ampullaria* pitcher fluid transcriptome and proteome analyses. Two neprosin genes were cloned into pET-28(b)+ (figure 1) and expressed in the *E. coli* before sub-cloning into yeast expression vector (figure 2). *S. cerevisiae* CEN.PK-2d will be transformed with the yeast expression vector for further analysis (figure 3). 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.

Result

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.

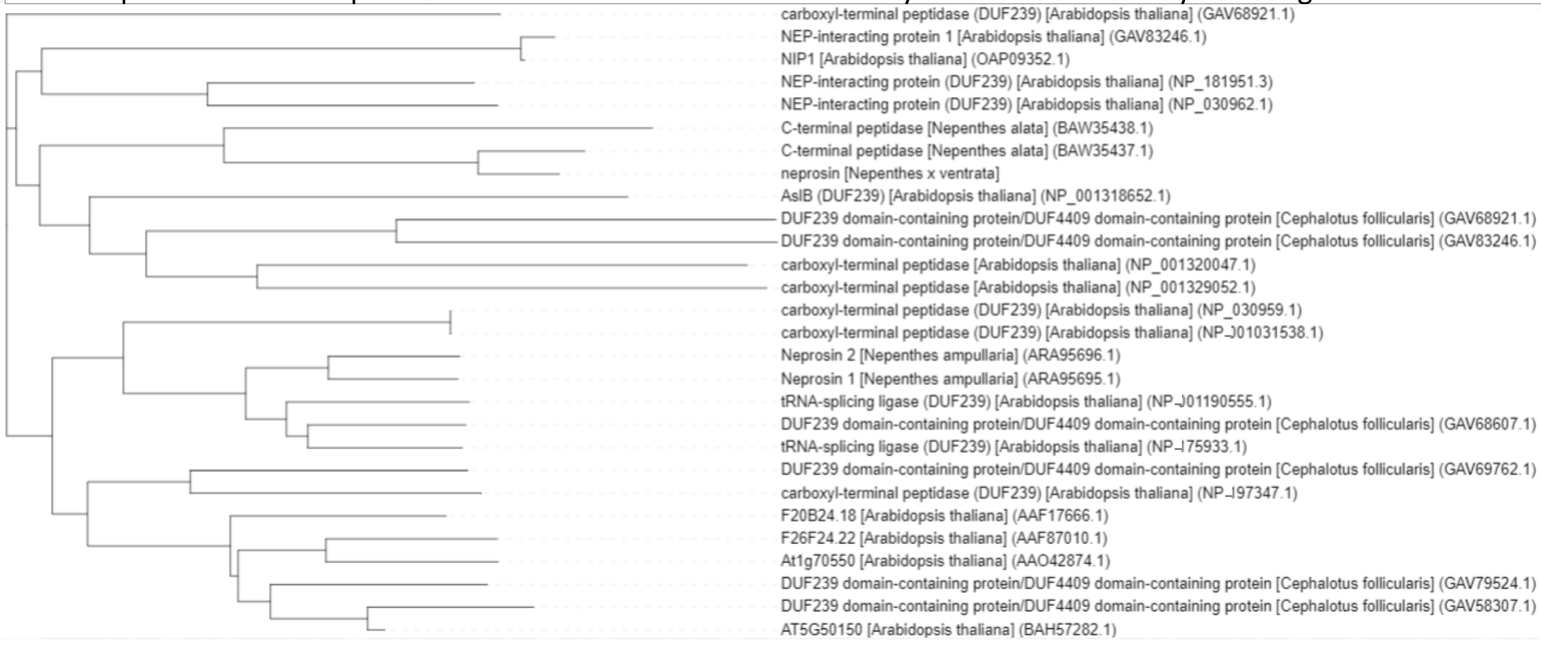
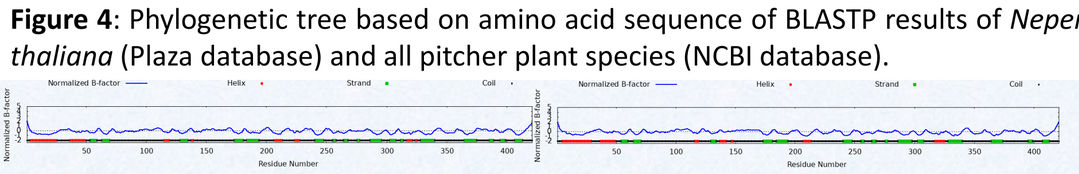


Figure 4: Phylogenetic tree based on amino acid sequence of BLASTP results of *Nepenthes ampullaria* against *Arabidopsis thaliana* (Plaza database) and all pitcher plant species (NCBI database).



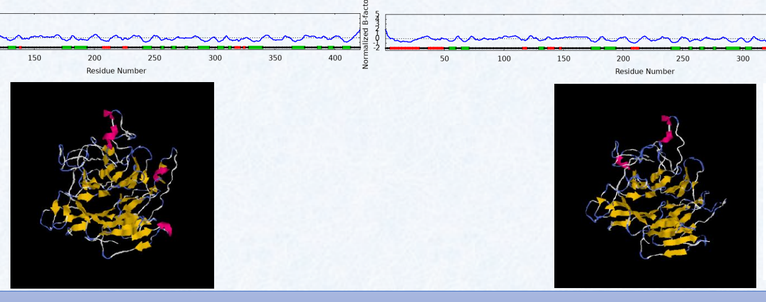


Figure 6: Protein structure prediction of NaNprs using I-TASSER. Left image is predicted 3D structure of NaNpr1 whereas right image is that of NaNpr2, with C-score of -1.99 and -2.05 respectively.

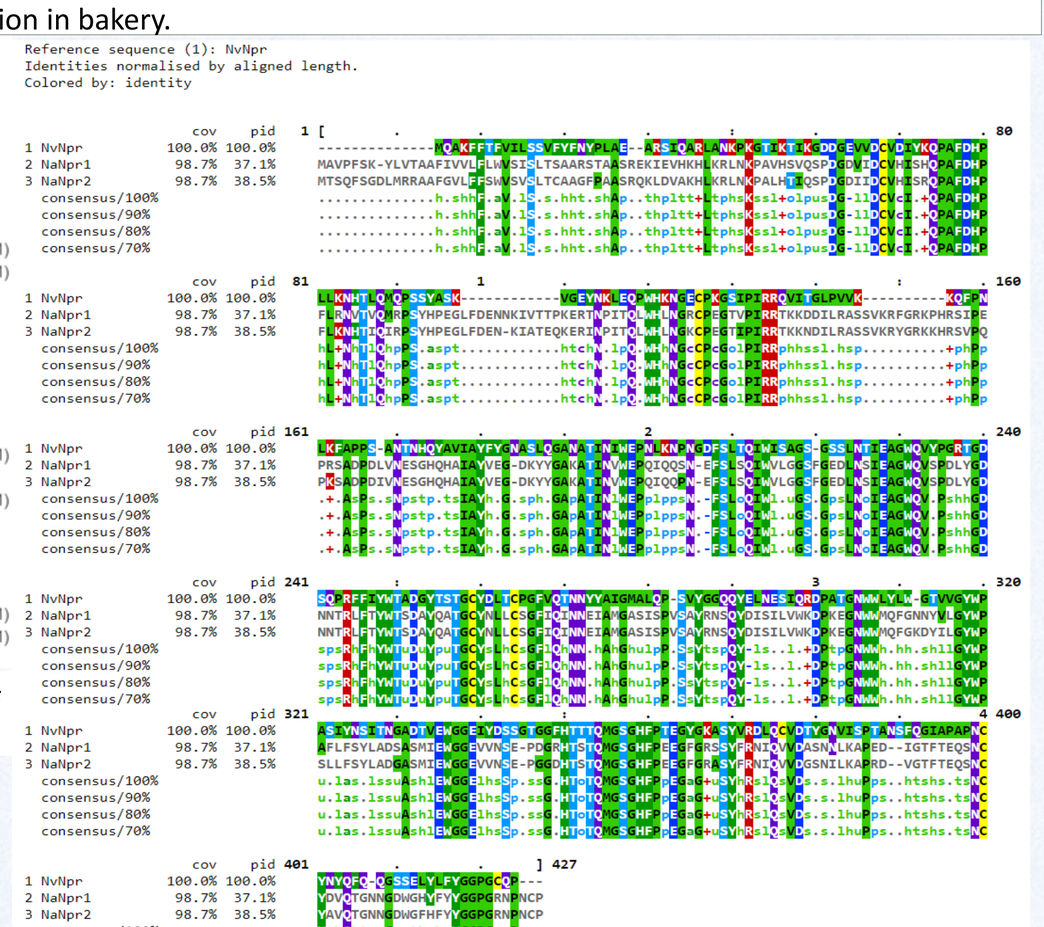


Figure 5: Result of multiple sequence alignment of *N. ampullaria* neprosin proteins (NaNpr) to *N. × ventrata* neprosin (NvNpr). NaNprs and NvNpr have 98.7% consensus, NaNpr1 has 37.1% percentage identity while NaNpr2 has 38.5% percentage identity when compared to NvNpr.

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.

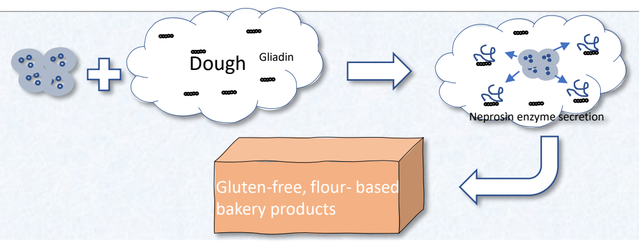


Figure 7: Illustration of application of the bioengineered baker yeast in bakery industry

Reference

Ravee, R., Salleh, F.I.M. and Goh, H.H. (2018) Discovery of digestive enzymes in carnivorous plants with focus on proteases. *PeerJ*, *6*, p.e4914

Rey, M., Yang, M., Lee, L., Zhang, Y., Sheff, J.G., Sensen, C.W., Mrazek, H., Halada, P., Man, P., McCarville, J.L. and Verdu, E.F. (2016) Addressing proteolytic efficiency in enzymatic degradation therapy for celiac disease. *Scientific reports*, *6*, p.30980

Schräder, C.U., Lee, L., Rey, M., Sarpe, V., Man, P., Sharma, S., Zabrouskov, V., Larsen, B. and Schriemer, D.C. (2017) Neprosin, a selective prolyl endoprotease for bottom-up proteomics and histone mapping. *Molecular & Cellular Proteomics*, *16*(6), 1162-1171.

Zulkapli, M.M.I., Rosli, M.A.F., Salleh, F.I.M., Noor, N.M., Aizat, W.M. & Goh, H.H. (2017) Iso-Seq analysis of *Nepenthes ampullaria*, *Nepenthes rafflesiana* and *Nepenthes× hookeriana* for hybridisation study in pitcher plants. *Genomics data*, 12, 130-131.