In silico designing and molecular cloning of flavanone-3-hydroxylase gene from *Arabidopsis thaliana*

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Abstract

Background: Anthocyanins are natural antioxidants properties by quenching free radicals, peroxides and reactive oxygen by stopping the chain reactions that causes oxidative damage. Flavanone-3-hydroxylase (F3H) is one of the anthocyanin biosynthesis pathway key enzymes which can be introduced to yeast (*Saccharomyces Cerevisiae* or *Pichia pastoris*) strains to produce flavonoid precursors of anthocyanin.

Results: The *in silico* analysis results revealed that F3H gene has identity with other plant species. The modelling was performed using SWISS MODEL, I-TASSER, and Modeller9.22. The top four template models were selected from protein-protein BLAST for modeling of F3H protein by Modeller9.22 version. A total of five models were generated by Modeller9.22 for F3H protein and the model with lowest DOPE value was selected among five models. The most stable and validated structures of F3H was obtained from Modeller9.22 and used for molecular docking studies. F3H protein was further predicted for physicochemical characterization, structure prediction, functional prediction, and validation using various online bioinformatics tools. The 3D structure of F3H was validated, the model was found to be stable, reliable and consistent. The F3H protein was reverse translated into nucleotide sequence, codon optimized for yeast (*Saccharomyces Cerevisiae*) and F3H gene was synthesized by Life Technologies Corporation (a part of ThermoFisher scientific). The chemically synthesized F3H gene was cloned to pYES2.1V5-His-TOPO vector and transformed into TOP10 *E. coli* strain.

Conclusions: This study reports the *in silico* analysis and molecular cloning of F3H gene from *Arabidopsis thaliana*. Thus, here we have shown that the *in silico* designed protein was successfully modeled and cloned to pYES2.1V5-His-TOPO vector.