ABSTRACT

The recent diagnostic tools for peanut allergy showed low predictive value due to the use of crude extract or native peanut allergen. Minimizing the chance of getting low predictive value (e.g. false positive) is necessary to prevent any misdiagnosed. Ara h 2.02 is an isoform of the major peanut allergen Ara h 2 that responsible for causing allergic reaction. It has three IgE epitope recognition sequence with 'DPYSPS' and may increase the affinity towards specific IgE that could be a promising candidate for diagnostic reagent. *Pichia pastoris* was chosen to be the host for expressing this protein due to high-biomass, strong promoters lead to the high-level expression, and efficient protein secretion and purification. In order to increase the expression level in *Pichia pastoris* host, codon optimisation was used to increase the protein production. Therefore, the objective of this research is to generate codon optimised Ara h 2.02 (yAra h 2.02) peanut allergen and the expression construct in *Pichia pastoris.* The synthesized Ara h 2.02 gene was codon optimized and the Codon Adaptation Index (CAI) value has succeed to be increased from 0.57 to 0.84, considered good for expression. Then, the yAra h 2.02 gene was cloned into pPICZαA expression vector and transformed into X-33 *Pichia* strains. Before the transformation into the yeast cell, the gene was sequenced for confirmation test. The sequence showed 100% identical to the reference gene which proved that the cloning was succeed. The transformation was performed via electroporation and single colony was obtained. In conclusion, the yAra h 2.02 gene was successfully made and the expression construct in *Pichia pastoris* has successfully generated. However, further confirmation tests are still needed to validate the integration of pPICZαA in the X-33 *Pichia* genome along with the expression level.

Keywords: peanut allergy, Ara h 2.02, codon optimization, *Pichia pastoris*