UNIVERSITI TEKNOLOGI MARA

YEAST SYSTEM FOR SURFACE DISPLAY OF HETEROLOGOUS PROTEIN

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result on my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

An *Escherichia coli*-yeast shuttle vector for the anchoring of heterologous protein to the yeast host's cell wall was constructed using the backbone from pGAD424. A construct comprising the signal sequence from the yeast sucrose isomerase gene (SucSg) , a multiple cloning site sequence and a DNA fragment encoding 67 amino acids from the carboxyl -terminal of the yeast cell wall protein 2 (CWP2) was constructed *in vitro*. The construct was designed such that a gene sequence cloned into the MCS will be translated in-frame with the SucSg and CWP2. The construct was then inserted into the *Hin*dIII site on pGAD424, replacing the GAL4 fusion tag and the original MCS sequence. DNA sequencing confirmed the correct insertion of both signal and anchor proteins in the vector. The newly obtained working plasmid vector was termed pYDSM01. A green fluorescent protein (GFP) was incorporated as a reporter gene into the vector and transformed into a yeast host to test the functionality of the vector. A substantial fraction (60 %) of the cells were observed to fluoresce green, indicating successful expression of the GFP. The green fluorescence was observed to largely concentrate in clusters on the edge of the cells, indicating that the GFP is transported and anchored to the cell surface. To investigate the potential commercial application of the vector, a bacterial α-amylase and the yeast meiosis-specific glucoamylase were later cloned separately into the system. *Saccharomyces cerevisiae* is a glucose feeder therefore by attaching the amylase gene to the surface, *S.cerevisiae* is able to use starch as a feed providing a cost effective and better way of utilizing abundance source of starch. This is valuable for instance in ethanol fermentation for industry or green technology. A total of 30 yeast transformants (amy-E) were recovered indicating successful expression. Transformants A5, B1 and B6 were successfully expressed on the cell surface, but C5 and D2 shows successful expression on the growth medium. Transformants A5, B1 and B6 have fusion protein on the cell wall at 81.3%, 30% and 6.7% respectively. Three transformants were found (yeast glucose isomerase) that differs in qualitative assays compared to amy-E transformants. GA-1 and GA-3 only gave nearly 32.9 % and 22.9% respectively in percentage of glucose released from a cell fraction. This was believed to be due to the catalytic domain of the two amylases despite belonging to the same group of family enzyme. Qualitative assay of the washed cell pellet and supernatant fractions indicate that both activity and anchoring efficiency varies. Anchoring of proteins therefore was not completely achieved.

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