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OPTIMAZING LINKER PEPTIDE FOR EFFECTIVE PRESENTATION OF Aga2 AND *A. baumannii* Blp1 C-TERMINAL FRAGMENT FUSION PROTEIN ON THE SURFACE OF *S. cerevisiae*

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Yeast display (or yeast surface display), a protein engineering technique that involves the expression of recombinant proteins incorporated into the yeast cell wall, offers a versatile platform for expanding the applications of *Saccharomyces cerevisiae* in various scientific, biotechnological, and biomedical applications. This technique leverages yeast cell wall proteins to anchor target proteins onto the cell surface [1]. The yeast display technique was first published in 1997 [2], and until now, there is no data that it has been used in Lithuania. The display of proteins on yeast cell surfaces is facilitated by wall protein a-agglutinin, which mediates cell-cell contacts during yeast cell mating. A-agglutinin consists of an Aga1 subunit linked to an Aga2 subunit by two disulfide bridges. Genetic fusion of the target protein with the Aga2 subunit enables its display on the cell surface through interaction with the Aga1 subunit, which is directly anchored to the cell wall [1].

Concerning the immunostimulatory properties of the yeast wall and GRAS status, yeast surface display technology is a promising tool for developing oral vaccines [3]. In this study, we used a protein from the opportunistic bacterium *Acinetobacter baumannii* as the target antigen. The C-terminal 163 amino acid fragment of Blp1 exhibits conservation among clinically isolates, including those resistant to antibiotics, making it an ideal candidate for vaccine development [4].

In the initial phase of this study, the Aga2 subunit was linked to the C-terminal 163 amino acid fragment of Blp1 via a flexible linker composed of glycine and serine amino acids. The aim of this study was to investigate the feasibility of displaying the C-terminal 163 amino acid fragment of the Blp1 protein on the surface of *S. cerevisiae* yeast cells using different linker peptides. The Aga2 subunit was linked to the target fragment by a double extension of the original linker sequence consisting of glycine and serine amino acids, also by a single and double alpha-helix-forming sequence, and by an alpha-helix-forming sequence with serine and glycine amino acid termini. All constructed fusion proteins were successfully displayed on the surface of yeast cells. The results demonstrated that altering the linker sequence had a positive effect and increased the efficiency of the fusion protein display. Among the tested linker variants, the single alpha-helix-forming sequence exhibited the most pronounced effect, with the greatest effect on anchoring the target protein to the yeast cell surface.

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