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CRISPR-CAS9 GENOME ENGINEERING IN *KLUYVEROMYCES MARXIANUS* FOR ENHANCED SECRETION OF RECOMBINANT ANTIBODIES

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Recombinant antibodies (RAbs) are important in diagnostics, research, biotechnology, and therapeutics due to their high specificity, stability, and ease of modification [1]. Yeasts, which are easily genetically modified and cultivated, are often preferred as a cost-effective system for RAbs production. The species *Kluyveromyces marxianus* is known for its efficient production and secretion of properly folded and active native and recombinant proteins, including RAbs [2]. To improve RAbs production technologies, yeasts can be genetically modified to create mutant strains with enhanced protein secretion properties.

Protein glycosylation, a crucial post-translational modification, involves attaching a glycan to a protein, ensuring its proper folding, activity, and stability. Dolichol kinase (DK), encoded by the essential *SEC59* gene, plays a role in glycosylation processes within the endoplasmic reticulum [3]. The reduced activity of DK, along with changes in glycosylation levels and the activity of other proteins in the secretory pathway, may lead to enhanced recombinant protein secretion [4]. Enhanced RAbs secretion can also be achieved by reducing the activity of intracellular peptidases, as RAbs are highly prone to proteolysis and often undergo degradation [5].

The aim of this study was to apply efficient CRISPR-Cas9 genome editing technology to construct a *K. marxianus* strain that displays improved secretion of RAbs. In this investigation, a *K. marxianus* yeast WSS-Δ*pep4* strain was created by introducing mutations encoding G418S and I432S changes in the DK amino acids, and by disrupting the gene encoding vacuolar peptidase (*PEP4*). The introduction of mutations in the DK-encoding gene led to changes in DK activity, as indicated by reduced glycosylation efficiency of carboxypeptidase Y in the WSS strain. Additionally, the disruption of the *PEP4* gene in yeast resulted in a decrease in the proteolytic degradation of RAbs. A secretion assay of the single-chain antibody fragment (scFv) linked to an antibody fragment crystallizable Fc (scFv-Fc) against *Gardnerella vaginalis* vaginolysin was performed and detected in yeast growth medium by Western blot. The results indicated that the constructed *K. marxianus* WSS-Δ*pep4* strain secreted recombinant scFv-Fc protein more efficiently compared to the wild-type *K. marxianus* strain. However, the secretion of RAbs in yeast also depends on the specific properties of the recombinant protein, and further studies are necessary. The newly constructed *K. marxianus* WSS-Δ*pep4* mutant strain could be beneficial for future research aimed at enhancing RAbs production technologies.

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