THE FIRST STEP TOWARDS HUMANIZED RECOMBINANT TAU PROTEIN IN P. PASTORIS YEASTS

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Neurodegenerative diseases are among the most common in the world. Despite intensive research, there are few treatment options available [1]. Many studies are done on disease-causing proteins, which are often produced in bacteria and differ from those in humans by post-translational modifications. Here, N-glycosylation is most interesting to us. By producing protein in a humanized expression system, we can glycosylate it similarly to humans [2]. Such proteins would enable more precise research and more effective drug discovery. Partially humanized *Pichia pastoris* M5 yeast, performing modified N-glycosylation, was chosen for our study. The goal of our project is to use gene engineering to change this N-glycosylation to be closer to the human system [3].

Using PCR, *TAU* gene was amplified with an additional sequence encoding 6xHis tag and restriction sites, cloned it into the integrating pPIC3.5K plasmid. Using the SalI REase, cutting the *HIS4* gene, Mut^+ phenotype was obtained, which efficiently uses methanol, grows faster, and produces more proteins. For homologous recombination, digested plasmid was concentrated in 3 ways: column purification, organic DNA extraction, and magnetic particles (the best method). Yeast transformation was performed using chemical and electroporation methods. By the same scheme, the pPIC3.5K-Tau plasmid was cut with BglII, resulting in a Mut^S phenotype with the *AOX1* knocked out, having slower methanol catabolism, but if this will reduce the yeald of protein will be checked in the future.

Pilot cultivation of *P. pastoris* M5 Mut^+ was done, expression induction with methanol and SDS-PAGE was performed to check expression over time. To ensure having the target protein, western blot was done with anti-His monoclonal antibodies. To enhance gene expression a Kozak sequence will be inserted upstream of *TAU*, which should enhance gene expression. Using Mega primers, an Alpha mating factor signaling sequence will be inserted, which will direct protein for secretion into the medium, facilitating protein purification. From the available literature, Tau protein was obtained from partially humanized yeasts with modified N-glycosylation for the first time. This and further glycosylation modifications will allow to test whether these modifications change Tau protein aggregation and it's structure.

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