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Flo8 – A versatile regulator for improving recombinant protein production in Pichia pastoris

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FLO8 – A VERSATILE REGULATOR FOR IMPROVING RECOMBINANT PROTEIN PRODUCTION IN PICHIA PASTORIS

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Flo8 is a main transcriptional regulator of pseudohyphal growth in yeast. Recently, it was shown that disruption of *FLO8* in the popular recombinant protein production host *Pichia pastoris* (syn *Komagataella* spp) abolishes pseudohyphal growth and significantly reduces cell-to-surface adherence, making the mutant an interesting base strain for research and industry [1,2]. However, knowledge on the physiological impact of the mutation remains scarce and comprehensive studies employing *FLO8*-deficient strains for recombinant protein production are lacking. Here, we re-analysed published RNAseq data of the *P. pastoris* wildtype and Δ*flo8* mutant cultivated in glucose-limited chemostats at a “fast” (0.1 h^{-1}) and “slow” (0.05 h^{-1}) growth rate setpoint [2], revealing that Flo8 affects the expected flocculation targets, but also mating, respiration, cell cycle genes as well as catabolite repression and that its actions are specific to the respective growth condition. Furthermore, we tested the Δ*flo8* mutant in combination with the strong glucose-regulated (methanol-independent) *GTH1* promoter (P_{G1}) [3] and its engineered derivative $P_{G1\text{-}3}$ [4] for recombinant protein production in small scale screenings and bioreactor cultivations. It was demonstrated that P_{G1} and $P_{G1\text{-}3}$ expression strength was significantly elevated in the Δ*flo8* mutant, resulting in substantially enhanced recombinant protein titers. Consistently, secreted protein yields of several different products were strongly enhanced as well, establishing the *flo8* mutant as an ideal background strain for recombinant protein production.

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