# SLOW GROWTH RATE TRIGGERED TRANSITION TO A PSEUDOHYPHAL LIFESTYLE OF THE PROTEIN PRODUCTION HOST PICHIA PASTORIS

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Specific growth rate is an important process control parameter for industrial protein production. In the widely used yeast protein production host *Pichia pastoris*, growth rate is known to significantly impact protein expression and secretion [1]. In that regard, glucose-limited chemostat cultivations carried out over a wide range of specific growth rates have revealed that slow growth rates can trigger a pseudohyphal phenotype in *P. pastoris* [2]. Such phenotypes are undesirable during large-scale protein production processes since they can lead to foam production. In *Saccharomyces cerevisiae* pseudohyphal growth is controlled by *FLO11*, a member of the *FLO* gene family, which is a group of genes encoding cell surface proteins responsible for conferring a diverse array of adhesion-related phenotypes and reported to be controlled by epigenetic mechanisms. *P. pastoris* also carries a number of *FLO* genes but their functions and regulatory patterns are yet unknown. Thus, we set out to investigate this gene family to shed some light on how pseudohyphal growth and other adhesion phenotypes are triggered and regulated in *P. pastoris*.

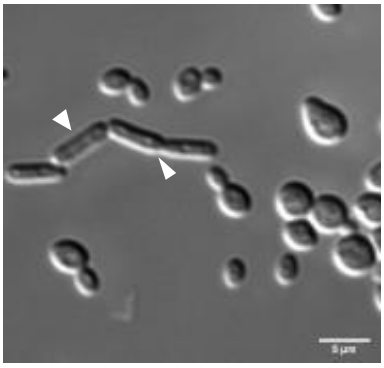

*Figure 1- P. pastoris cells displaying pseudohyphal phenotype*

We ran glucose-limited chemostat cultivation, initiated first at a specific growth rate (μ) of 0.1 $h^{-1}$, then switched to a slower growth rate of 0.05 $h^{-1}$ and finally switched back to the faster growth rate of 0.1 $h^{-1}$. We observed that after two residence times in the slow growth rate condition, some cells in the population took a more elongated form. With increasing residence times in this condition, the phenotype grew stronger, with some cells taking up a branched pseudohyphal appearance, as seen in figure 1. Interestingly, after switching back to the faster growth rate, some pseudohyphal cells persisted, indicating that this phenotype might be under stable epigenetic regulation. Preventing pseudohyphae formation by knocking out the master transcription regulator of many members of the *FLO* family decreased foaming and additionally showed better performance for protein production in fed-batch cultivations.

RT-PCR and RNASeq helped us to identify three *FLO* genes, including *FLO11*, which showed an interesting pattern of regulation at the slow growth rate. We further carried out FAIRE-Seq to analyze open chromatin regions under the different growth rates and two of the *FLO* genes identified before showed stable silencing upon switching to the slow growth rate. Similar chemostat cultivations with knock-outs of these three genes revealed that unlike in *S. cerevisiae*, pseudohyphal growth in *P. pastoris* requires the involvement of not just *FLO11* but also the two other identified *FLO* genes. Investigations using reporter strains expressing green fluorescent protein tagged to these two *FLO* proteins have led us to speculate that these could be acting as signal proteins whose expression might be necessary for expression of *FLO11*, which then triggers the initiation of pseudohyphal growth. Thus our data and observations point to a more complex regulation mechanism of the pseudohyphal phenotype in *P. pastoris* compared to *S. cerevisiae*.

References

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[3] Bester M.C. et al. G3 (Bethesda) 2(1): 131–14