

An integrated approach to enable rapid scalable upstream production of subunit vaccines with *Pichia pastoris* (*Komagataella phaffii*)

by

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Abstract

Recent experience with the COVID-19 pandemic motivates the development of vaccine designs and manufacturing technologies tailored to enable widespread access in low- and middle-income countries (LMICs). Due to limited supply, vaccines targeting SARS-CoV-2 were initially distributed primarily to high-income countries. The distribution of vaccines which were eventually made available to LMICs was complicated by logistical challenges such as the need to maintain cold-chain integrity for available vaccine modalities. These technological limitations restricted vaccine access, ultimately driving up excess deaths in LMICs and increasing global pandemic risk by enabling SARS-CoV-2 to rapidly accumulate potentially harmful mutations in an unprotected population. Resolving underlying vaccine supply and distribution challenges for LMICs will be essential to controlling the COVID-19 pandemic and to enable better response to future pandemic threats but will require improved vaccine design and manufacturing.

Subunit vaccines produced with the yeast, *Komagataella phaffii*, could significantly improve access to vaccines in LMICs, owing to their potential for rapid development timelines, high productivity in existing manufacturing capacity, thermostability, and strong efficacy. In this thesis, we present an integrated approach to improve vaccine design and manufacturing in *K. phaffii*. In the first part, we demonstrate that *K. phaffii* strains engineered to eliminate the need for methanol-feeding enable improved production of SARS-CoV-2 RBD antigen. Obviating the need for methanol improved cell health and enabled production of clinical material in a 1200 L bioreactor, larger than would have been possible with traditional methanol-feeding. The benefits of methanol-free engineering appear to be generalizable to other proteins of interest. In the second part, we present a novel “modular blending” approach to media development. This new method enabled the design of a soluble medium with 2x higher productivity than our previous best defined production medium and highlighted the importance of lipid supplementation and carbon metabolism for optimal heterologous protein production in *K. phaffii*. Finally, viral antigens typically require multimeric display to induce strong immune responses, but common nanoparticle display technologies are difficult to produce. We present initial design and experimental work towards the secreted production of novel protein nanoparticles tailored for optimal production in *K. phaffii*.

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