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The yeast stands alone: the future of protein biologic production

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Yeasts are promising alternative hosts for the manufacturing of recombinant protein therapeutics because they simply and efficiently meet needs for both platform and small-market drugs. Fast accumulation of biomass and low-cost media reduce the cost-of-goods when using yeast, which in turn can enable agile, small-volume manufacturing facilities. Small, tractable yeast genomes are amenable to rapid process development, facilitating strain and product quality by design. Specifically, *Pichia pastoris* is becoming a widely accepted yeast for biopharmaceutical manufacturing in much of the world owing to a clean secreted product and the rapidly expanding understanding of its cell biology as a host organism. We advocate for a near term partnership spanning industry and academia to promote open source, timely development of yeast hosts.

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Introduction

Biologic drugs remain the area of highest growth for the medical industry worldwide, with projected global market growth worth of nearly 400 Billion USD by 2025 [1]. Recombinant proteins currently represent a dominant modality among these therapeutics and will continue in the near future. As the global population grows and the biopharmaceutical industry expands, new challenges are emerging that impact strategies both for the development of new products and for manufacturing. A bifurcation of potential markets by size is placing significant pressure on existing manufacturing strategies. On one hand, there are extremely high-volume demands. Examples include the development of breakthrough monoclonal antibody (mAb) products for very large central nervous system disease indications like the >200 million predicted Alzheimer's patients worldwide by 2050 [2], for cardiovascular disease [3], and for chronic infectious diseases [4]. On the other hand, there are precision medicines designed to treat small cohorts of patients for indications defined by molecular features or biomarkers rather than traditional broad classifiers like tissue-specific cancers [5]. Both directions diverge from a global infrastructure for manufacturing built and optimized over 25 years for 'platform' products like monoclonal antibodies with annual product demands of 100–1000 kg [6]. New strategies for manufacturing are needed to enable cost-effective, high-volume production reaching multiple metric tons. Separately, approaches also are needed that prioritize agility in product development and speed to market for diverse new treatments with limited volume demands at kilograms or less. These future scenarios pose a critical question for the industry now: Is it possible to make investments in innovative technologies that could address both manufacturing challenges?

Many new technologies are being tested for their potential to impact both costs of goods manufactured (COGM) and speed to market, while maintaining product quality [7–9]. Some of these include alternative host expression systems [10••], straight-through operations for purification [11,12], and alternative designs for unit operations and entire facilities [13], including continuous processes [14–17] and single-use technologies [18,19]. Of these, we believe alternative hosts have the greatest likelihood for broad, disruptive impact on the organizational resources required to develop a product in both clinical and commercial stages of manufacturing.

Yeasts are safe and widely accepted

Many reviews have been published recently by us [10••] and others [20,21] carefully outlining the myriad benefits of using alternative hosts — and eukaryotic microbes in particular — given their similar capabilities to secrete products as mammalian hosts. In short, these hosts, including fungi, microalgae, and protozoa, are already proven workhorses for manufacturing industrial enzymes [22••,23], with volumetric productivities on par or greater than those currently achieved in CHO-based manufacturing processes depending on the protein expressed [24•]. Many offer additional benefits beyond standard CHO cell lines, including genomic stability, targeted transgene integration, and fewer contaminating host cell proteins owing to smaller secretomes [10••,25]. These and other

traits, including their simplicity and robustness in cultivation, have enabled the expression of thousands of proteins within hundreds of protein families to date by these microorganisms. The question remaining is not 'Are alternative hosts suitable for manufacturing protein drugs?', but rather 'Could targeted near-term investments in an alternative host accelerate transformative new capabilities to meet patients' needs sooner?'. Although various microorganisms have unique benefits and demonstrated examples for specific classes of proteins, we outline here why yeasts hold the most promise for a disruptive and generalizable solution for manufacturing at all scales in the next 5–10 years. We first address how yeasts already demonstrate widespread utility in biomanufacturing and then discuss their capability to transform manufacturing processes in terms of simplicity, efficiency and productivity.

An important factor when considering any alternative expression system is its potential safety for patients. Yeasts, including *Saccharomyces cerevisiae*, both *Pichia pastoris* species *Komagataella phaffii* and *Komagataella pastoris*, *Kluvomyces lactis*, *Hansenula polymorpha*, and *Yarrowia lipolytica*, are already designated Generally Recognized As Safe (GRAS). They are routinely used to manufacture products in other industries including food and beverage, agriculture, and consumer products like detergents, bulk biochemicals, and biofuels [22••]. Numerous regulatory agencies worldwide, including the FDA, EMA, PMDA, CDSCO, CFDA, have approved parenteral drugs produced in *S. cerevisiae* and *P. pastoris*, including vaccines, insulin products, enzyme replacements and cytokines; some of these products have more than 25 years of clinical experience [26]. One key safety benefit to using yeasts for biologic expression is that they do not harbor adventitious viruses; viral testing has even been eliminated for some yeast-produced drugs through risk-based assessments and process validation [27•]. Overall, the risks to patients when using these organisms to express biologic drugs for parenteral use are well-known and easily mitigated today by genetic engineering, process control, or both (Table 1). This compilation of historical clinical experience vastly exceeds other eukaryotic microorganisms to date, and provides a solid regulatory foundation and safety profile on which to build future products now.

Simplicity in facility design and strain engineering

Key approaches to reduce costs of manufacturing or to increase throughput in facilities for multiple products typically include shortened production timelines and simplified logistics of operation and quality testing. Fermentation timelines with yeast are fast — typically 7–10 day cycles reach ~100 g/L dry cell weight compared with 14–21 day cycles of only ~0.5 g/L dry cell weight for CHO in fed batch [24•]. This difference in speed is because yeasts' doubling times are 2–3 h, or ~5–10× faster than mammalian cells or filamentous fungi. Faster fermentation directly impacts volumetric productivity. The ability to quickly accumulate biomass, especially when combined with inducible promoters for de-coupling production from growth, has enabled cost effective and uncomplicated fed-batch fermentation processes with predictable product quality for numerous proteins [35•]. Reducing the number of operations overall for production, product testing, and quality assurance is an added benefit of using yeast. Although scale-up remains a challenge for process development with any host, bioreactor platforms designed to be similar across volumetric scale present a unique opportunity early on to identify yeast strains that are tuned for a specific large scale process. Such scale-up was demonstrated recently with glyco-engineered *P. pastoris* using the Sartorius Ambr platform [36]. Manufacturing facilities based on yeast fermentation are generally more modest than those operating mammalian bioprocesses owing to the lower risks of viral contaminations with microbes [37]. Overall, yeast hosts enable fast and significant throughput using simple facilities.

In addition to simplifying facility requirements and manufacturing operations, yeasts also provide an easier means for the process development required for a product to be 'manufacturing-ready'. Shortening the time required to design, build, and test production-ready strains reduces the time and resources required to translate molecules from discovery to manufacturing. Yeast again stand out compared to mammalian cells and even filamentous fungi. They typically have smaller genomes, fewer chromosomes, and shorter doubling times [10••]. These features can improve the rate of learning biological knowledge, which in turn improves biological

Table 1

Known safety risks of yeast hosts for production of protein biologic drugs.

Known risk	Reported mitigation
Hypermannosylation of *N*-linked glycans	Knock out OCH1 and ALG3 genes [28,29]
β-Glucans present in *N*-linked glycans	Knock out β-mannosyl transferase (BMT) genes [30]
Unpredictable *O*-linked glycosylation	Add PMT inhibitors during fermentation [31]; knock out protein-O-mannosyltransferase (PMT) genes [32]; co-express α-1,2-mannosidase [33]
Challenges detecting host-cell proteins	Use multiple orthogonal methods including ELISA and LC/MS [34]