

GEN INTERVIEW

Joachim Klein, PhD, Head of Microbial Strain Development and Cell Banking, Lonza Biologics

Nikolay Krumov, PhD, Senior Project Leader, Upstream Development Microbial, Lonza Biologics

Lonza's XS[®] *Pichia* Expression System

GEN: *If a Biotech company wants an alternative to traditional CHO or E. coli-based expression platforms, what are some other host options that have proven to be successful in getting products to the market?*

Dr. Krumov: At Lonza, we say that our yeast-based platform, XS *Pichia*, combines the best of both worlds. The key advantages



Nikolay Krumov, PhD

of our *Pichia* system include easier and faster strain development, short fermentation times, pure products secreted into the culture supernatant, and no endotoxin. So, if you want to move away from a mammalian or an *E. coli* approach, *Pichia* is an excellent alternative.

One also has to be mindful that the diversity of biotherapeutic products requires different expression options. That's the reason why Lonza works with and offers CHO, *E. coli*, and

Pichia host systems, while simultaneously providing combinatorial screening that can allow you to choose the right clone and expression system that works best for your molecule.

GEN: *Pichia isn't new. There are incumbent competitors on the market. How is Lonza's XS Pichia different?*

Dr. Klein: You're absolutely right that *Pichia* is not new, and it's been around for 30+ years. Most people associate *Pichia* with methanol-based processes and approved drugs from *Pichia* are on the market.



Joachim Klein, PhD

The use of methanol at commercial scale will require explosion-proof facilities which is a significant cost investment. Also, the toxicity of methanol and its metabolites reduces the cell viability and therefore negatively impacts the product quality in terms of product and/or host cell-related impurities. Constitutive promoters can eliminate some of these problems, but the titers in general are not as high as with



XS *Pichia* system can secrete soluble proteins into the supernatant. As a result, the produced protein is highly pure. No endotoxins are formed with *Pichia* and there is no need for viral clearance.

methanol-based platforms. In addition, due to the induction properties of the AOX1 promoter, methanol-driven fermentations have a low space time yield with fermentation times over 120h.

Lonza's Glucose-Regulated promoter system is at the core of our *Pichia* expression system. We have achieved titers comparable to or even higher than methanol-based processes for many products, including a number of novel scaffolds. It is readily scalable from 50L to 15,000L because the complexity is low, and the processes are relatively simple. Product quality is better with XS *Pichia* because there are no adverse effects from methanol and its metabolites. The entire process is highly customizable by changing the fermentation parameters to better suit your production requirements. All this is of great benefit if you want to take your process toward manufacturing at larger scale and ultimately commercialization.

GEN: *So compared to E. coli systems that secrete protein, what advantages does Lonza's Pichia platform possess?*

Dr. Krumov: *E. coli* is not a secreting microorganism per se. For a protein to be secreted in *E. coli*, it is first secreted into the oxidizing periplasm where the necessary disulfide bridges are built. However, because the physical space of the periplasm is limiting, it restricts the overall titer of generated material.

Pichia can secrete soluble proteins into the culture supernatant. As a result, the produced protein is highly pure. No endotoxins are formed with *Pichia*, and there is no need for viral clearance!

GEN: *For what types of molecules is Pichia ideally suited?*

Dr. Klein: *Pichia*, as a eukaryotic organism, is ideally suited to meet the expression requirements of eukaryotic proteins such as complex proteins or enzymes. New classes of molecules like antibody mimetics are also produced with good titers and desired product quality attributes in *Pichia*.

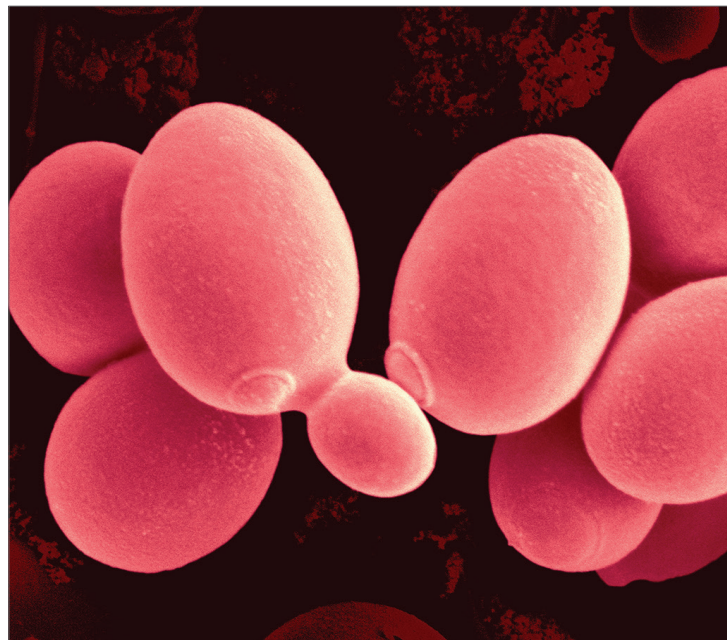
Many novel biotherapeutics such as single-domain antibodies are screened and selected in *Pichia*. Only well-produced lead candidates make it through development. In addition, fusion proteins, such as HSA fusion proteins for half-life extension, are ideal for expression in *Pichia*.

GEN: *How are molecular tools helping solve challenges with protein expression?*

Dr. Klein: Lonza has continuously expanded the *XS Pichia* toolbox addressing the key issues explained earlier. To improve product quality and titer, the *Pichia* toolbox contains a powerful set of novel tools that can help in generating more target protein homogeneity and higher titer. A common concern with other *Pichia*-based systems is around N-terminal heterogeneity due to misprocessing of the secretion signal. The *XS Pichia* Toolbox includes Signal Sequences that can help with precise cleavage of the N-terminus. The *XS System* contains improved host strains for better product quality. You also get access to Helper Factors which, as the name says, can help improve expression.

GEN: *What are the typical issues that an early-stage company faces when choosing an expression system?*

Dr. Krumov: Getting products to the IND phase is crucial for early-stage companies, and timelines are always tight. If they do not select an appropriate expression system, there could be issues around obtaining enough material for developmental activities. It is also important to think about scale-up early on and to choose



XS Pichia Toolbox comes with a range of molecular biology tools that help solve challenges with protein expression.

a system that is adequate. Selecting an industry-trusted platform like the *XS Pichia* will help de-risk any regulatory concerns. Another important piece of advice that we give to such companies is to pay more attention to manufacturing issues, such as post-translational modification or immunogenicity. You can listen to more about this topic from my colleagues in this webinar: www.genengnews.com/resources/webinars/right-first-time-successfully-progressing-biotherapeutics-from-discovery-to-the-clinic/

Lonza can help you overcome these challenges and our technical experts can support strategy optimization by balancing risks against opportunities, while ensuring your processes meet regulatory requirements. ■

To find out more about how *XS*® Microbial Expression Technologies could benefit you, please visit

pharma.lonza.com/xstechnologies

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