Evolution constrains large-scale bioproduction

February 22 2018

Lab photo from the Novo Nordisk Foundation Center for Biosustainability. Credit: Michael Barrett Boesen

The transition toward sustainable biobased chemical production is important for green growth, but productivity and yield of engineered cells frequently decrease in large industry-scale fermentation. This barrier to commercialization of more bioprocesses is largely ascribed to the physical inefficacies of large cubic-meter steel tanks.

Bioengineers have long debated whether it was realistic for evolution to eliminate production of engineered production cells during the relatively short time courses of large industrial-scale production. According to a new study published in *Nature Communications*, the role of evolution has been underestimated in limiting bioprocesses.

"Using a new ultra-deep DNA-sequencing approach, we found that evolution constrains bioproduction. We can use this insight to redesign our cell factories to produce more efficiently at an industrial scale," says Morten Sommer, professor at the Technical University of Denmark.

The study from the Novo Nordisk Foundation Center for Biosustainability suggests that the mechanisms of evolution are constraining the scale-up by a variety of mutations wider and faster than previously expected. Findings indicate that evolution is a more important threat to scale-up, and it cannot be ruled out that it limits the efficiency of commercialized large-scale fermentation.

"Evolution of non-productive cell subpopulations leads to loss of production in our case studies. The speed of evolution depends on the biochemical product, but it can definitely happen within industrial time-scales. This makes it difficult to scale-up biobased processes," says Peter Rugbjerg, postdoc at the Novo Nordisk Foundation Center for Biosustainability.

By using ultra-deep sequencing of thousands of different cells, it is now possible to score the different error modes of scale-up and design against evolution earlier. The researchers are now validating their bioinformatics approach and testing how widespread the problem is in the actual industry by collaborating with fermentation companies.

Results hint that engineered production genes in chemical producing bacteria were mainly mutated by non-expected disruptions and genetic

rearrangements, rather than the slower, classical point mutations known as single nucleotide polymorphisms (SNPs). The mutations make the nonproducing cells more fit in the competition for nutrients of a fermentation tank.

"We discovered that a wide diversity of genetic disruptions turned producing cells into nonproducing when we deep-sequenced thousands of production organisms over time. Cells have many built-in ways to remove unneeded genes, and it turned out the most important is overlooked in standard analysis tools," says Peter Rugbjerg, postdoc at the Novo Nordisk Foundation Center for Biosustainability.

The evolutionary mutants that eventually led to complete loss of production could be detected very early at frequencies below 0.1 percent, which may permit future identification of failing fermentations earlier.

Residing at the ultra-low frequency when grown in the laboratory, the mutants played no negative role in production in shake flasks and were traditionally difficult to detect. However, the advantage of lost production means these early mutations had already determined the magnitude of production decline that happens as the process is scaled up to industrial growth durations. "Based on these findings, I would encourage companies running industrial scale fermentations to deploy deep sequencing of the fermentation populations to assess the extent of detrimental evolution," says Morten Sommer. He expects that addressing this detrimental evolution would substantially improve commercial bioprocesses.

More information: Peter Rugbjerg et al, Diverse genetic error modes constrain large-scale bio-based production, *Nature Communications* (2018). DOI: 10.1038/s41467-018-03232-w

Provided by Technical University of Denmark