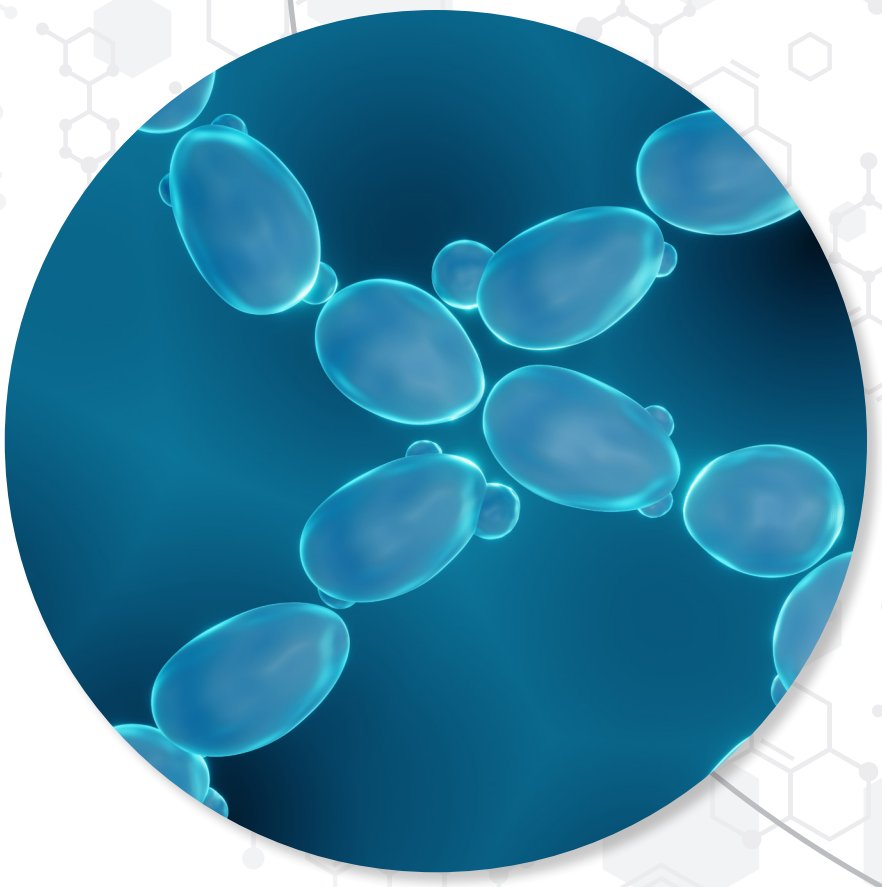

WHITE PAPER

Paveway™ PLUS: Enabling Faster, Better Decisions in Early-Stage Development of Microbial-Based Biotherapeutics

An interview with **STEVE LOFTUS, PhD,**
Microbial Business Steering Group Lead,
FUJIFILM Biotechnologies

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Value from Innovation

Steve Loftus, PhD, Microbial Business Steering Group Lead at FUJIFILM Biotechnologies, describes the benefits of microbial fermentation for biologics production, and the recent improvements introduced to the Paveway™ microbial expression platform.

Market Overview

More than half of the 150+ biopharmaceuticals developed and approved for human use through 2009 were manufactured via microbial fermentation in bacteria or yeast.[1] Although the next decade saw a shift towards the use of mammalian cell culture systems[2] for expression of antibodies and complex proteins that require posttranslational modifications, microbial fermentation has experienced a renaissance in recent years, particularly in the area of next-generation therapies such as antibody and small peptide fragments.[3,4,5]

How is microbial fermentation used at FUJIFILM Biotechnologies?

FUJIFILM Biotechnologies has more than 30 years' experience developing bacterial and yeast fermentation and purification processes, and microbially-derived biologics are the cornerstone of our business. We have developed processes for over 200 molecules, ranging from traditional biologics such as enzymes, interferons, and antigens, to more novel therapeutics like antibody fragments, leading to production of more than 2000 GMP batches and 6 commercially approved products. More recently, we have manufactured three biologics for COVID-19 therapies via our microbial expression systems.

E. coli was the first expression system used in the manufacture of recombinant human insulin in the 1980s. Since then, other expression systems such as yeast, mammalian cells and insect cells have been widely adopted. Does *E. coli* remain a popular host for recombinant protein expression today? What are its pros and cons?

While mammalian-based expression systems have come to dominate the biologics market, microbial systems, and *E. coli* in particular, are still responsible for production of ~20% of the global biologics demand. Much of the growth in mammalian cell culture demand is driven by the rise in monoclonal antibody (mAb) therapies, a class of molecules that are typically difficult to produce in *E. coli* due to the lack of human-like glycosylation pathways. *E. coli* expression systems—like our proprietary Paveway PLUS expression platform—continue to shine is in the rapid growth kinetics and product titers that can be achieved for a wide range of non-mAb biologics.

Traditionally, high product yield has been the primary driver for strain selection. What are other important criteria to consider during *E. coli* strain development?

Achieving the highest titer is especially important during strain selection; however, ensuring that your product is free from any disadvantageous posttranslational modification is also key. Early identification of unwanted protein modifications such as glycation, oxidation, or amino acid misincorporation de-risks downstream process development activities and timelines.

What are some of the unique features of the FUJIFILM Biotechnologies Paveway PLUS expression system?

The Paveway expression platform was launched in 2007 and continues to yield exceptional product titers for a wide range of soluble and insoluble molecules. One of the key benefits is its extremely tight control of pre-induction product expression. It also allows for product targeting to either the cytoplasm or periplasm, depending on client requirements. In addition, fermentations can be run without antibiotic selection pressure while maintaining high plasmid retention, helping avoid regulatory concerns around potential antibiotic resistance and clearance. The improved **Paveway PLUS** service adds a flexible suite of initial process development options targeted towards early product quality readouts and capture chromatography identification.

How does the FUJIFILM Biotechnologies Paveway PLUS process development and expression system help clients establish goals and overcome challenges typically encountered during strain screening and development?

Clients that come to us for strain development can usually be placed into one of the following two categories. Clients in early-phase or pre-clinical stages might need support establishing their first GMP-suitable strains or improving their current strain. These clients typically want a high titer producing strain in a short timeline. This is ideally suited to our core Paveway PLUS strain development workflow, which can identify a lead strain in only four weeks. The second category of clients require more than just high titer. These clients are often keen to build quality by design and manufacturability into their processes early on, while still requiring short timelines. Our enhanced **Paveway PLUS** workflows meet this need by identifying a lead strain based on both titer and product quality, while also providing valuable information about initial purification and capture chromatography. By utilizing our high-throughput robotics and analytics, this can be achieved in as little as 12 weeks.

What kind product analysis can be performed?

One of the new workflows leverages our state-of-the-art high-throughput robotics and analytics in an Ambro® 250®-based screen to generate small quantities of partially purified product from up to 8 candidate strains. This enables strain selection to be made based on product titer and product quality readouts using techniques such as CE-SDS/SDS-PAGE, icIEF/IEX-H/UPLC, SE-H/UPLC, electrospray and mass spectrometry.

How is automation used and how has it benefitted the Paveway PLUS workflow?

Our core strain selection workflow was refined in 2015 by replacing the original, 2-step screen that involved a combination of shake flask cultures and benchtop fermentations with a single-step, micro fermentation-based procedure using an Ambro® 250 system. This shortened the time it takes to go from gene to identification of a lead strain to as little as four weeks.

Following the acquisition of Tecan Evo Freedom® robotic workstations a few years ago, we have continued to develop more applications to effectively harness the power of this technology. For instance, we implemented a high-throughput chromatography resin screening procedure which dramatically reduces the time and amount of feedstock required to identify a capture resin and set of conditions which may then be incorporated into a preparative protein purification procedure.

Are there other benefits to the Paveway PLUS workflows?

The modular **Paveway PLUS** workflows use proprietary FUJIFILM Biotechnologies approaches to fermentation that are scalable from Ambro® 250 mini bioreactors to 5000L (3000 L w/v) fermenters. They also facilitate downstream process development by identifying preliminary primary recovery conditions and a suitable product capture chromatography step. In addition, we offer a solubilization and refold screen for insoluble products that can be used to quickly generate folded product as well as valuable data for refold optimization work.

What are some significant challenges that you have helped clients overcome?

We have successfully generated strains for more than 130 individual molecules since the launch of Paveway in 2007. We have substantially improved production titers for many clients, but two cases stick out. One client came to us with a particularly challenging molecule that can lyse gram-negative bacteria like *E. coli*. They had tried various lab-scale expression systems but found that it was difficult to get viable cultures through to induction due to autolysis. We cloned the gene into our standard suite of Paveway vectors and strains and screened in the Ambro® 250 and were able to achieve a product titer of 13 g/L. Another client needed help with a gene sequence that when cloned produced no detectable expression. Leveraging our expertise in codon optimization with the Paveway expression system, we managed to achieve ~18 g/L.

> "We have successfully generated strains for more than 130 individual molecules since the launch of Paveway in 2007."
>
> — Steve Loftus, PhD

Tell us about how FUJIFILM Biotechnologies' investments in microbial space will benefit the industry.

Large-scale microbial capacity is a particular crunch point for the industry, so our investments have focused on expansion of our large-scale facilities in both Billingham, UK and Research Triangle Park, North Carolina, USA to address this need. Our Research Triangle Park facility was recently expanded to add extra downstream cleanrooms, increasing throughput, and maximizing fermenter utilization. Our UK microbial facility is currently undergoing optimization and expansion with the addition of two new 2000L fermenters. We also recently announced further expansion of the UK facility to add new downstream clean rooms that will boost the capacity of the plant by approximately 70%.

Your mantra is a CDMO "Partner for Life." How do you partner with clients and how does that add value?

FUJIFILM Biotechnologies offers complete end-to-end capabilities for development, scale-up and manufacture. Our mission is to Advance Tomorrow's Medicines. From supplying material for pre-clinical studies to process validation and commercial large-scale manufacture, we support our clients through every step of their journey with our scientific expertise, technical leadership, and customer-first approach to project management.

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About the Author

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Steve Loftus, PhD, leads the Business Steering Group for Microbial Services at FUJIFILM Biotechnologies, a group that looks to define the strategic growth strategy of the offering. Dr. Loftus has over 15 years of experience in the development and manufacture of microbial-based biologics at FUJIFILM Biotechnologies. He has a doctorate in Biochemistry and Biophysics from the University of York.

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MKT-0180-WP-Rev01