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A new era for *E. coli*: Revolutionizing microbial bioproduction with chemically defined fermentation media

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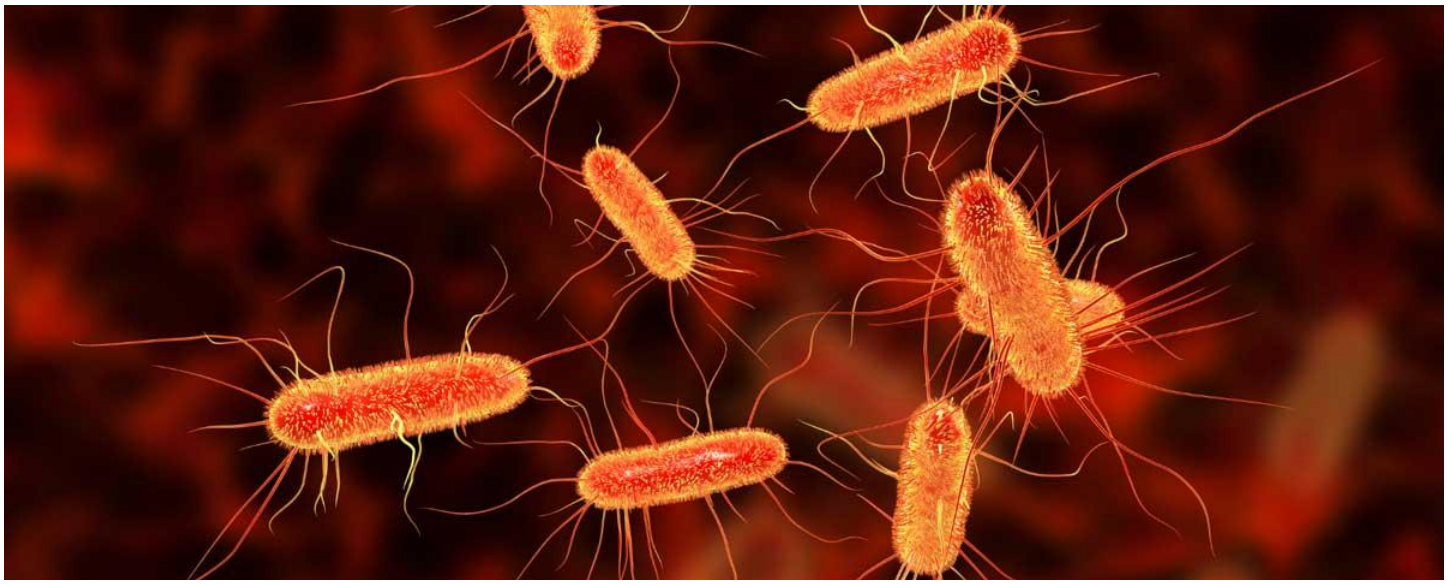

For many years, microbial bioproduction systems have played a major role in the manufacture of life-saving biopharmaceuticals. This is particularly true for systems using Escherichia coli (*E. coli*), which have historically been widely used for both recombinant protein expression and plasmid DNA preparation.

As with the growth of all living cells, *E. col*i cultures require a specialized medium containing all the essential nutrients necessary for cell growth. However, despite the long-standing popularity of *E. coli* for microbial bioprocessing, the options for growth media have been traditionally limited compared to eukaryotic production systems.

Most biopharmaceutical manufacturers working with *E. coli* use lysogeny/Luria-Bertani broth (LB) containing a combination of peptones, with sodium chloride added to provide essential electrolytes for transport and maintaining osmotic balance. Although this can deliver suitable results, the variability introduced by animal origin (AO) components—found in the LB—can reduce batch-to-batch consistency and present safety risks to patients.

To address this issue for mammalian cell culture, media developers began formulating chemically defined (CD) media in the early 2000s. With a well-defined nutritional composition, CD media can reduce variability and improve process consistency. However, until recently, this development was limited to media for mammalian cells and small-scale experimental media, such as M9 Minimal Salts, for *E. coli*. As a result, there was a long-standing lack of commercially available CD fermentation production media designed for use with *E. coli* cultures within bioprocessing workflows.

The chemically defined media revolution

By the early-2000's, the transition toward the use of CD media for mammalian cell-based bioprocessing led to the development of a variety of commercially available CD products specialized for different cell types. This innovation introduced a new era of

mammalian bioprocessing consistency and has helped biopharmaceutical manufacturers working with mammalian cells increase confidence in the long-term productivity of their process.

Using a CD medium also has the additional benefit of enabling manufacturers to avoid the use of AO components, particularly removing the need for fetal bovine serum and bovine-derived peptones. This has a significant advantage from a regulatory standpoint, as it eliminates the risk of bovine spongiform encephalopathy (BSE) and other Transmissible Spongiform Encephalopathies (TSE) contamination. Moreover, it is also advantageous from a supply security perspective, as there is no longer a reliance on serum where price and supply can be affected by externals factors, such as weather patterns, cattle cycles, or natural disasters.

New opportunities for *E. coli*

Limitations caused by the lack of complex cell structures present in *E. coli,* combined with the emergence of CHO cells for bioproduction, meant that the need for a CD *E. coli* media was a relatively low priority for the broader industry. Despite the lower production costs and higher levels of productivity that can be achieved with *E. coli* cells compared to mammalian cells, their lack of complex post-translational modification pathways significantly restricted their use. However, the development of next-generation biotherapeutics over recent years, such as cutting-edge monoclonal antibody therapies and new vaccine types, renewed interest.

One key example of this is the advancement of conjugated monoclonal antibodies. While *E. coli* cells are usually unable to produce full-length antibodies, they are able to produce antibody fragments that can be conjugated used in therapeutic products. These treatment option could be especially transformative, as targeted therapies have the potential to increase efficacy, as well as reduce side effects by limiting off-target activity.

New biotherapeutic modalities have also led to rising demand for DNA plasmids that can be met using *E. coli*. In addition to cell and gene therapy applications, plasmids have become instrumental in the manufacture of mRNA vaccines. Given the success of both Pfizer-BioNTech and Moderna's vaccines against SARS-CoV-2, there is an industry-wide expectation that demand will grow as this technology is applied to create new vaccines and increase the efficacy of older vaccines.

As a result of this renewed interest in *E. coli*, significant effort from across the industry was now focused on developing the first commercially available CD microbial media. However, before this could be achieved a significant number of technical challenges had to be overcome.

Finding a recipe for success

One of the biggest challenges for media developers desiring CD media has been replacing the traditional media components such as peptones. Most notably, in addition to AO peptones, a chemically defined medium could not contain animal origin–free peptones as they are inherently variable. Without the use of yeast peptones—a common supplement to LB—creating a medium nutritionally rich enough to avoid an excessively long lag and achieve high levels of cell growth was difficult.

There were also considerations surrounding the growth characteristics of *E. coli* that needed to be kept in mind. For example, *E. coli* bioproduction systems tend to produce acetic acid. Over time, this can lead to a reduction in pH and negatively impact the production process. To solve this, the medium needed to have a suitable buffering capacity that can reduce fluctuations in pH during production cycles.

In addition, the medium was required to achieve optimal performance while remaining as user-friendly as possible and fit into existing processes. A key capability in this area was giving manufacturers the flexibility to use either filter sterilization or autoclaving without compromising on performance. The latter method presented a major challenge, as it required the use of specific components that are resistant to high temperatures and pressures.

Finally, ease-of-use considerations also called for the medium to be available as a single-part solution. This was crucial as eliminating the logistical difficulties of shipping, storing, and mixing multiple components could help enable manufacturers to streamline their workflows and maximize productivity.

A game-changing breakthrough

After much research and development, the goal of a commercially available CD microbial medium was finally achieved in early 2021 by Thermo Fisher Scientific with the release of Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM). Through this innovation, manufacturers can now advance their process into the CD-era and finally gain the improvements in consistency and reduction in manufacturing risk that contemporaries working with mammalian cells have benefited from for decades.

Additionally, by offering a CD option, manufacturers who may not have previously worked with *E. coli* can now do so with confidence. Facilitating improved accessibility is particularly vital to increase the production of essential components for the manufacture of next-generation biopharmaceuticals—especially DNA plasmids and mRNA constructs.

Overall, the innovation of CD microbial media has created a new paradigm of microbial bioprocessing. One which has the potential to not only continue the role of *E. coli* in the manufacture of life-saving biotherapeutics, but also to enhance its performance and safety and help transform the lives of even more patients.

For more information, please visit thermofisher.com/bactocdsupreme.