Successful Microbial Expression Systems

by S. Anne Montgomery

The history of the biotechnology industry is literally one of growth: of cells and their ability to express products efficiently, accurately, quickly, consistently — and cost-effectively. Important microbial expression systems for recombinant proteins have been *Escherichia coli* (a prokaryote) and the eukaryotic microbe *Saccharomyces cerevisiae*. A more complete list of systems is offered by Meyer in his article in this special issue (see his "Microbial Expression Systems" box).

The first approved recombinant protein manufactured using transformed *E. coli* was rDNA/Lilly (Humulin) insulin from Eli Lilly & Co. in the 1970s, approved in the early 1980s. The earliest product expressed by yeast was another insulin product developed and approved in that same time frame, rDNA/Novo (Novolin) from Novo Nordisk using transformed *Saccharomyces cerevisiae*.

Other microbial expression systems were also developed in the 1970s: *Pichia* yeast and non–*E. coli* bacteria such as *Bacillus subtilis*, but their adoption for human therapeutics has yet to be fully realized. Nevertheless, a wealth of industry literature reveals ongoing work to optimize familiar expression systems and determine the advantages and optimum conditions of others. Articles in this issue focus on these major areas of exploration and development in the industry — both for microbial and mammalian expression systems. Case studies describe current work involving *Pichia pastoris, P. fluorescens,* CHO, and PER.C6 (among others). Within these articles, comparisons of expression systems illustrate both the complexity of the studies and the increasing sophistication of associated analytical methods.

Optimization Efforts: Examples

Numerous research and industrial uses of *Escherichia coli* bacteria have their origins in the first genetic engineering experiment in 1973, when a gene from an African clawed toad was inserted into laboratory bacteria. Since then, the easily cultivated *E. coli* has been the most common microbial source for recombinant protein production because the most is known about its genetics. In fact, the complete genome sequence of *E. coli* was determined in September 1997 by a team of researchers in the Laboratory of Genetics at the University of Wisconsin-Madison. The 4,639,221 base pairs of the genome contain 4,403 genes (www.accessexcellence.org/WN/SUA11/ecoli997.php).

For many of the same reasons that *E. coli* is popular among bacterial production systems, *S. cerevisiae* (baker's or brewer's yeast) is the fungal species most commonly found in fermentation processes, whether for recombinant or other products. It is the one with which people have the most experience and is thus the best understood (the full genome was sequenced in 1996, but people have been working with it for thousands of years). *S. cerevisiae* has been genetically engineered to produce a wide range of proteins: antigens to hepatitis B, influenza, and polio; human growth hormone and insulin; antibodies and antibody fragments; human growth factors; interferon and interleukin; blood components such as human serum albumin; and tissue plasminogen activator.

As Ron Rader notes in Chapter One, many companies are exploring improvements with these more familiar systems rather than adopting novel systems that might require greater regulatory scrutiny. But tremendous advances are indeed being made, as illustrated by the authors who have written for this issue.

To provide further background, the following list from just our BPI archives alone chronicles some of this evolution of expression systems — a recent history of increasing sophistication. This selection of articles also can point you toward numerous resources and reference lists on the subject of expression systems. You can access these at www.bioprocessintl.com either by searching for an author's name or by opening the issues within our "archives."

Resources

Background History ("the basics") and Predictions for the Future

Scott C. Cell Biology Basics for Discussing Culture Media. *Culture Media. BioProcess Int.* 3(Supplement 4) 2005: 4–14.

Scott C. Microbial Fermentation: The Oldest Form of Biotechnology BioProcess. *Protein Expression in The 21st Century. BioProcess Int.* 2(Supplement 2): 8–20.

Yang X, Cui L. Microbial Process Development for Biopharmaceuticals: From Science and Engineering to Regulatory Requirements. *BioProcess Int.* 2(6) 2004: 32–39.

Gottschalk U. Event report: Biotech Manufacturing Is Coming of Age. *BioProcess Int.* 1(4) 2003: 56–61.

New Applications, Explorations

Vaccine Seed Development

Duncan PA. Characterization of Microbial Seeds Used in the Manufacture of Biopharmaceuticals *BioProcess Int.* 1(2) 2003: 58–62.

Gene Therapies, DNA Vaccines (*E. coli*)

Carnes AE. Fermentation Design for the Manufacture of Therapeutic Plasmid DNA. *BioProcess Int.* 3(9) 2005 36–42.

Evolving Technologies, Optimization

E. Coli

Bowering LC. Recombinant Protein Microbial Systems for the Manufacture of Therapeutic Antibody Fragments. *BioProcess Int.* 2(4) 2004: 40–47.

Chaozhan W, Wang L, Geng X. Refolding Recombinant Human Granulocyte Colony Stimulating Factor Expressed By *E. coli:* A Case Study Using the Unit of Simultaneous Renaturation and Purification for Proteins. *BioProcess Int.* 4(5) 2006: 48–54.

Alexander P, et al. Production in *Escherichia coli:* A Retrospective and Prospective Review. *BioProcess Int.* 5(9) 22–27.

Bentley LK, et al. How Monosodium Glutamate Lot-to-Lot Variability Affects Process Performance of Recombinant *E. coli*. *BioProcess Int.* 4(1) 2006: 34-43

Leonhartsberger S. Vendor Voice: *E. coli* Expression System Efficiently Secretes Recombinant Proteins into Culture Broth. *BioProcess Int.* 4(4) 2006: 64–66.

Pseudomonas fluorescens

Squires CH, et al. Heterologous Protein Production in *P. fluorescens*. *BioProcess Int.* 2(11) 2004: 54–59.

Pichia pastoris

Peacock K, Auton KA. Comparing Shaker Flasks with a Single-Use Bioreactor for Growing Yeast Seed Cultures. *BioProcess Int.* 6(1) 2008: 54–57.

Julien C. Production of Humanlike Recombinant Proteins in *Pichia pastoris*: From Expression Vector to Fermentation Strategy. *BioProcess Int.* 4(1) 2006: 22–31.

Facility Support

Pelin K, Phillips K, and Sarantschin V. Building a GMP Bacterial and Fungal Fermentation Facility: A Case Study in Designing for Evolving Technologies. *BioProcess Int.* 1(6): 56–59.

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