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Protein Expression in *Corynebacterium glutamicum*

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Since its beginnings in the 1970s, recombinant DNA technology has become integral in the production of pharmaceutically important proteins such as blood factors, hormones, growth factors, interferons, interleukins, and vaccines. Recombinant technology was developed in *Escherichia coli*, and because of its long history of use and ease of manipulation, the bacteria remains a common expression system. However, *E. coli* is limited in the types of proteins it can express, and production can be hampered by the formation of intracellular inclusion body aggregates which complicate the processes of protein recovery and purification. The non-pathogenic *Corynebacterium glutamicum* can accumulate amino acids extracellularly and has historically been used for the commercial production of amino acids glutamate and lysine.[1]

More recently, *C. glutamicum* has been developed as an expression system with the ability to secrete properly folded, functionally active proteins into the fermentation broth. Production in *C. glutamicum* has the same advantages of scalability and ease of culturing as in *E. coli*, but with more simplified recovery and purification processes.

E. coli as a Model for Heterologous Protein Expression in Bacteria

Among protein-based recombinant pharmaceuticals licensed in the United States or European Union as of January of 2009, 30% were produced in *E. coli*.[2] Only mammalian cell lines, which were used to produce 39% of products, were more common. Examples of proteins produced in *E. coli* are tissue plasminogen activator, insulin, interferon-α, interferon-γ, interleukin-2, and human growth hormone.

The advantages to protein expression in *E. coli* are numerous. Its genome is sequenced, and it has a robust cellular structure suitable for culturing. The cells are fast-growing and can double every 20 minutes in optimal culturing conditions. Culturing medium, which consists of simple carbon and nitrogen sources, and inorganic salts, is relatively inexpensive. In contrast, the cost of medium required for Chinese hamster ovary (CHO) cell production can be approximately ten times greater. A fermentation or bioreactor run to produce protein can be as short as 24 hours in bacteria versus 14–21 days in CHO cells. Many of the advantages and potential limitations of bacteria and mammalian cell culture are shown in Table 1.

In *E. coli*, synthesized protein accumulates internally, and cells need to be ruptured to isolate the target protein. Host protease activity commonly occurs during cell disruption and can substantially decrease yield. Following cell lysis, multiple rounds of chromatography are required to separate the unwanted cell contents from the target protein. Gram-negative bacteria such as *E. coli* contain lipopolysaccharides that function as endotoxins and can cause high fever in humans. The processes of removing host cell contaminants can be extensive, and each step in the process increases the need for resources such as equipment or physical space. Every step of recovery and purification also results in loss of yield, which can reach proportions of 50 – 80% of the original output.

Some proteins expressed in *E. coli* form inclusion body aggregates. Sometimes the proteins can be solubilized by adjusting medium components, growth temperature, inducer concentration, promoter strength, or plasmid copy number. However, proteins native to eukaryotes tend to be resistant to solubilization. And solubilized proteins can become denatured, thus requiring subsequent *in vitro* refolding processes that can be complicated or ineffective. The refolding process is often preceded by a partial purification to remove host cell proteins that can aggregate with the target protein. Refolding techniques vary and are often arrived at through trial and error.

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TABLE 1. Host cell types with their advantages and potential limitations.

Host Cell	Benefits	Potential Drawbacks
Bacteria	• Fast-growing • Inexpensive to culture • Easily scalable • Robust cellular structure • Sequenced genome (select species)	• Limited post-translational modification • Limited protein size and complexity • Protein extraction and refolding usually required • Endotoxins in gram-negative species
Mammalian	• Eukaryotic • Innate secretion mechanisms • Synthesis of complex, multi-subunit proteins • Greatest post-translational modification capabilities	• Slow-growing • Requires expensive, complex media • Sensitive to osmotic shock and shear stress • Can harbor human pathogens

Corynebacterium glutamicum for Heterologous Protein Expression

In addition to being able to secrete heterologous proteins, *C. glutamicum* has several advantages for protein production. The bacteria is gram-positive and therefore lacks endotoxins. The genome of the *C. glutamicum* wild-type strain ATCC 13032 has been fully sequenced and is available in the public domain. Minimal proteolytic activity has been detected in *C. glutamicum* culture supernatants, which contain very small amounts of endogenous protein.

In prokaryotes, most secreted proteins are translocated unfolded across the cytoplasmic membrane via the *sec* pathway, which uses energy provided by *secA*-dependent ATP hydrolysis. However, in some cases, heterologous proteins fold before they reach the *sec* machinery which blocks interaction and translocation. Recently, a second general pathway designated "the *tat* pathway" has been found to be present in *Corynebacterium.* It differs from the *sec* pathway in that it translocates folded proteins, many of which are complexed with cofactors. The *tat* pathway uses the transmembrane proton electrochemical gradient as its energy source. Functional analyses of various *tat* deletion constructs suggest the *tat* pathway is a *bona fide* pathway for secretion of heterologous, folded proteins.[3] Heterologous proteins such as isomaltodextranase from *Arthrobacter globiformis*,[3] green fluorescent protein,[3] and pro-protein glutaminase from *Chryseobacterium proteolyticum*[4] can be secreted via the *tat* pathway in *C. glutamicum*. Overexpression of *tatABC* can increase the amounts of pro-protein glutaminase secretion to amounts useful for industrial-scale protein production.[5]

Use of *C. glutamicum* eliminates the processes of cell disruption, protein refolding, removal of endotoxin, and multiple chromatography runs that are standard in other bacterial expression systems. Commercial-scale production of human IGF-1 requires only half of the downstream processing steps relative to production in *E. coli* (Table 2).

TABLE 2. Human IGF-1 production steps in *E. coli* vs *C. glutamicum*.

E. coli	*C. glutamicum*
Fermentation	Fermentation
Cell harvest	pH adjustment
Cell disruption	Microfiltration (cell removal)
Centrifugation	
Refolding	
Ion exchange chromatography	Ion exchange chromatography
Reverse-phase chromatography	
Ion exchange chromatography	0.22 µm filtration
Gel filtration	
Ultrafiltration	

Production of industrially important human proteins can be done in *C. glutamicum*. Human epidermal growth factor (hEGF) stimulates the proliferation of a number of epithelial tissues both *in vivo* and *in vitro*. The protein is a single-chain polypeptide including six cysteine residues that form three disulfide bonds. Disulfide bonds are often abundant in secreted proteins, but maintenance of the bonds can be complicated and involve overexpression of chaperones or foldases. *C. glutamicum*-produced hEGF is secreted via the *sec* pathway into the culture medium at a rate of up to 156 mg/L/day.[6] The N-terminal amino acid of the secreted hEGF is asparagine (same as in the authentic hEGF) and the retention times of authentic and the secreted hEGF are identical on reverse-phase HPLC. Moreover, the growth-stimulatory activity of the secreted hEGF on MCF-7 cells is concentration-dependent and similar to authentic hEGF (Figure 1). Thus, *C. glutamicum* is capable of producing properly folded, functionally active proteins native to humans.

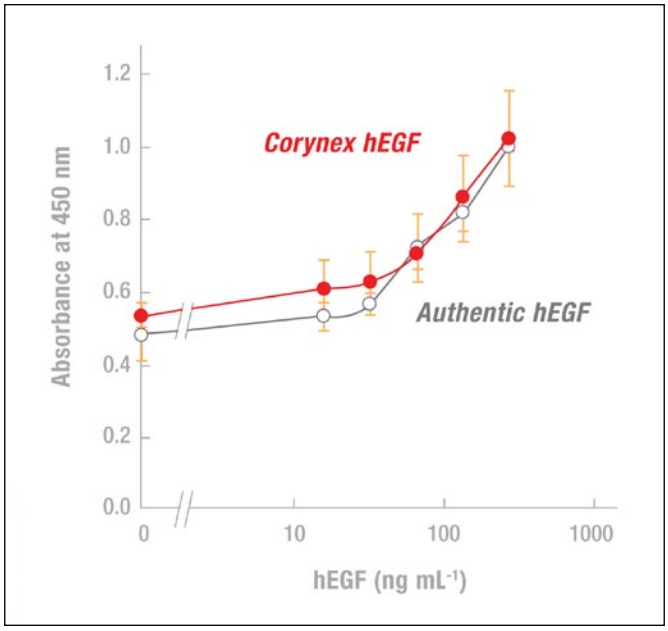

FIGURE 1. Stimulation of the growth of MCF-7 cells by hEGF. Authentic hEGF or hEGF secreted by *C. glutamicum* was added to the cultures at various concentrations (0, 15.6, 31.2, 62.5, 125, and 250 ng/mL). After four days, the numbers of living cells were measured using the WST-1 assay method.

Summary

C. glutamicum has demonstrated capabilities in producing a wide range of prokaryotic and eukaryotic proteins. The use of *C. glutamicum* eliminates many of the complex, empirical processes of recovering and purifying recombinant proteins inherent in bacterial expression systems such as *E. coli* (Figure 2).

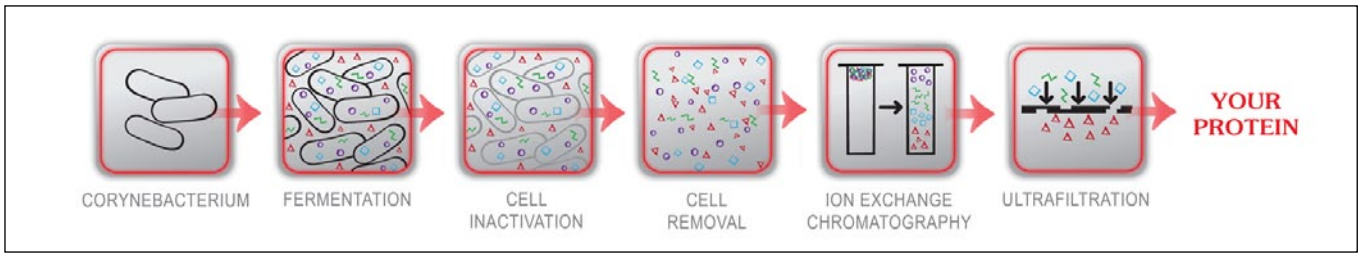

FIGURE 2. The need for cell lysis and target protein refolding is eliminated, streamlining recombinant protein production.

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NOTES

1. *Corynebacterium glutamicum* 13032™ is a trademark of American Type Culture Collection (ATCC®).
2. Corynex™ is a trademark of Ajinomoto.

ABOUT THE AUTHOR

Joel White holds both an MS and BS in Biology from the State University of New York at Buffalo. As Business Manager of Biotechnology at Ajinomoto, Joel is responsible for the sales, marketing, and business development of the company's Corynex™ Recombinant Protein Expression System and related service-oriented business. He drove the launch of the breakthrough system in April 2009. Since that time, Joel has helped Ajinomoto's pharmaceutical and biotech customers realize streamlined and more efficient downstream purification processes and accelerated speed-to-market.