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Recombinant Insulin Production in *E. coli* and Yeast: A Comparative Review

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Abstract

The production of recombinant human insulin marked a milestone in the history of biotechnology and therapeutic medicine. The introduction of recombinant DNA technology allowed the synthesis of human insulin identical to its natural form, eliminating the limitations of animal-sourced preparations. Over the past four decades, *Escherichia coli* and yeast have emerged as the two primary expression hosts for recombinant insulin production. This review provides a comprehensive comparison of these systems, focusing on genetic engineering strategies, protein expression, post-translational modifications, purification processes, and industrial applications. It further explores technological innovations, recent trends, and future perspectives in optimizing yield and quality. While *E. coli* remains the system of choice for large-scale, cost-effective production, yeast offers superior folding and secretion capabilities, producing insulin analogs with improved therapeutic profiles. Together, these systems continue to shape the evolving landscape of biopharmaceutical manufacturing.

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Introduction

Insulin plays an indispensable role in glucose metabolism by facilitating the uptake of glucose into muscle and adipose tissues and inhibiting hepatic glucose production [1]. A deficiency or resistance to insulin action results in diabetes mellitus, a global health burden that affects hundreds of millions of individuals worldwide. Before the era of recombinant biotechnology, therapeutic insulin was extracted from bovine and porcine pancreases. While effective, these animal-derived insulins carried significant drawbacks, including immunogenic responses, variability in purity, and challenges related to large-scale extraction [2].

The advent of recombinant DNA technology in the late 1970s revolutionized this field [3]. The collaborative effort between Genentech and Eli Lilly led to the production of *Humulin*®—the first recombinant human insulin approved by the U.S. FDA in 1982. This achievement demonstrated that microorganisms could serve as efficient cell factories for producing complex human proteins. Since then, microbial platforms, primarily *Escherichia coli* and yeast, have become the backbone of insulin manufacturing [4].

This review aims to provide a detailed comparative understanding of insulin production in *E. coli* and yeast. The discussion includes molecular design, fermentation technology, downstream processing, and industrial implementation. Moreover, the review highlights innovations in strain engineering and expression optimization that continue to enhance yield and product quality.

Overview of Recombinant Insulin Production

Recombinant insulin production involves multiple integrated stages: gene design, expression in a suitable host, protein folding, post-translational processing, and purification. The human insulin gene encodes a precursor molecule known as preproinsulin, which comprises a signal peptide, the B chain, connecting C-peptide, and A chain. During post-translational maturation, the signal peptide is cleaved, the C-peptide is removed, and the two chains are linked by disulfide bonds, forming the active insulin molecule [6].

The choice of the host organism is critical. It determines not only expression levels but also the biological activity, folding efficiency, and cost-effectiveness of production. Bacterial systems such as *E. coli* offer high yield and simplicity, whereas yeast systems provide eukaryotic processing advantages such as disulfide bond formation and partial glycosylation control. Modern industrial processes combine synthetic biology, bioinformatics, and precision fermentation to enhance the productivity of both systems.

Insulin Production in *Escherichia coli*

Genetic Engineering and Expression Strategies

E. coli was the first host used for recombinant human insulin expression and remains one of the most well-characterized organisms in molecular biology. Its rapid growth rate, availability of diverse genetic tools, and well-understood metabolic network make it a preferred system for producing therapeutic proteins [7].

In the early approaches, separate expression of the A and B insulin chains was carried out. Each chain was fused with a carrier protein such as β-galactosidase or tryptophan synthetase to enhance stability and solubility.

After expression, the fusion proteins were isolated, cleaved chemically using cyanogen bromide, and recombined through oxidative folding to generate active insulin [8].

Subsequently, a more advanced method involving proinsulin expression was developed. In this strategy, a single gene encoding the proinsulin sequence is expressed as an inclusion body, which simplifies recovery. The expressed protein is then refolded and enzymatically processed using trypsin and carboxypeptidase B to yield mature insulin. This method, widely used in industrial settings, minimizes the complexity of folding two separate chains [9].

Expression Systems and Vector Design

Various expression vectors have been engineered for high-level expression of proinsulin in *E. coli*. Commonly used promoters include T7, tac, and lac promoters, which ensure strong and inducible expression. The use of optimized ribosome-binding sites, fusion tags, and codon adaptation further enhances translation efficiency [10].

Modern plasmids also incorporate antibiotic resistance markers, origin of replication, and secretion signals to control plasmid copy number and stability. In some cases, signal sequences such as pelB or ompA are used to direct recombinant proteins to the periplasmic space, where disulfide bond formation can occur under more favourable conditions [11].

Fermentation Process

Large-scale fermentation of recombinant *E. coli* is carried out in stainless-steel bioreactors under aseptic, controlled conditions. Fed-batch culture is the standard approach, where nutrients are added gradually to sustain exponential growth and high cell density. Key parameters—such as pH (maintained around 7.0), temperature (usually 30–37°C), dissolved oxygen, and agitation speed—are tightly monitored. Glucose or glycerol often serves as the carbon source, while ammonia or phosphate provides nitrogen and buffering capacity.

Induction of protein expression commonly occurs through the addition of IPTG (Isopropyl β-D-1-thiogalactopyranoside) when the culture reaches the late exponential phase. The expression phase is optimized to balance cell growth and product formation, as overexpression can lead to stress and the formation of insoluble aggregates [12].

Inclusion Body Formation and Recovery

Most recombinant proteins in *E. coli* accumulate as inclusion bodies, especially when expressed at high levels. Although inclusion bodies represent insoluble aggregates of misfolded proteins, they offer the advantage of protecting the target protein from proteolytic degradation. After cell lysis, inclusion bodies are isolated by centrifugation and washing steps to remove contaminants.

The solubilization process involves using strong denaturants such as 6–8 M urea or guanidine hydrochloride. Controlled refolding is then carried out by gradually removing the denaturant in the presence of redox agents that facilitate disulfide bond formation between the A and B chains. Correct folding is critical for achieving biological activity and stability [13].

Purification

Once refolded, proinsulin is enzymatically converted to insulin by the sequential action of trypsin and carboxypeptidase B, which removes the connecting peptide (C-peptide).

Subsequent purification steps, such as ion-exchange chromatography, reverse-phase HPLC, and ultrafiltration, ensure high purity and removal of host cell proteins or endotoxins. The purified insulin is then crystallized, lyophilized, or formulated into pharmaceutical preparations [14].

Advantages and Limitations

E. coli offers several advantages—rapid growth, inexpensive media, and ease of genetic manipulation. However, it lacks the machinery for post-translational modifications and proper protein folding, often resulting in inclusion body formation.

The presence of lipopolysaccharides (endotoxins) also requires extensive purification. Despite these challenges, *E. coli* remains a highly efficient and economical platform for producing biosimilar insulin on a commercial scale [15, 16].

Insulin Production in Yeast Systems

Yeast as a Recombinant Host

Yeasts, particularly *Saccharomyces cerevisiae* and *Pichia pastoris*, represent an intermediate expression system bridging the gap between simple prokaryotes and complex mammalian cells. They combine rapid microbial growth with the eukaryotic ability to perform post-translational modifications and proper protein folding. *Saccharomyces cerevisiae*, a well-established organism in baking and brewing, was initially employed for recombinant insulin production. Later, *Pichia pastoris* gained prominence due to its strong, methanol-inducible AOX1 promoter and high-level secretory expression [17, 18].

Gene Design and Secretion Pathways

In yeast systems, the insulin precursor or proinsulin gene is cloned into a plasmid vector under the control of strong promoters such as AOX1 (*P. pastoris*) or GAL1 (*S. cerevisiae*). A secretion signal peptide, usually derived from the yeast α-factor pre-pro leader sequence, directs the recombinant protein through the secretory pathway. This approach allows insulin to be secreted into the culture medium, simplifying downstream purification [19, 20]. The folding and processing machinery of yeast enables partial disulfide bond formation and assembly of the insulin molecule within the endoplasmic reticulum. This intrinsic capability enhances the biological activity and reduces the need for complex refolding procedures required in *E. coli* systems.

Fermentation Optimization

Yeast fermentation is typically conducted in aerobic conditions at temperatures between 28 and–30°C. For *P. pastoris*, a two-phase fermentation process is used—first, a glycerol-fed batch phase for biomass accumulation, followed by a methanol induction phase for protein expression. Methanol serves both as a carbon source and as an inducer of the AOX1 promoter. Parameters such as dissolved oxygen, pH, and methanol feed rate are carefully optimized to prevent cell stress and ensure high productivity [21].

Purification and Processing

Secreted insulin precursors are collected from the culture supernatant using microfiltration or centrifugation. Downstream purification involves chromatographic steps like affinity, ion-exchange, and reverse-phase chromatography.

Because yeast does not produce endotoxins, purification is simpler than in bacterial systems. The proinsulin is enzymatically cleaved to generate active insulin, and final polishing steps ensure pharmaceutical-grade purity [22].

Advantages and Limitations

Yeast-based expression offers multiple advantages—proper folding, secretion into medium, reduced endotoxin contamination, and high product quality. However, expression levels may be lower than in *E. coli*, and hyperglycosylation can occur in some yeast species, potentially affecting product consistency. Optimization

of glycosylation pathways and secretion signals has been an area of ongoing research to mitigate these limitations [23].

Recent Advances and Innovations

Recent years have witnessed significant improvements in the recombinant production of insulin. Advances in synthetic biology, metabolic engineering, and bioprocess optimization have dramatically enhanced yield and efficiency in both *E. coli* and yeast.

Synthetic Biology and Gene Optimization

Codon optimization tailored to the host organism's codon usage bias has greatly improved translation efficiency. Synthetic promoters, fusion tags, and regulatory sequences are now designed using bioinformatics tools to achieve balanced expression without overwhelming the host machinery.

Strain Engineering

Advanced strain engineering has produced *E. coli* and yeast variants with enhanced folding capacity, reduced proteolytic degradation, and improved secretion pathways. Co-expression of molecular chaperones and foldases assists in proper protein folding, while deletion of protease genes minimizes degradation.

Process Intensification

High-cell-density fermentation, perfusion bioreactors, and continuous manufacturing have revolutionized insulin production. Real-time monitoring using sensors and process analytical technology (PAT) ensures consistency and quality control. Single-use bioreactors have further enhanced flexibility and reduced contamination risk.

Biobetters and Insulin Analogs

Recombinant technology now allows the creation of insulin analogs with modified pharmacokinetic profiles. Rapid-acting insulin analogues contain slight amino acid modifications that inhibit the formation of polymers or hexamers, thereby allowing faster absorption and quicker onset of action [24]. The dosage varies depending on the patient's appetite and needs. Common rapid-acting analogues, such as aspart, glulisine, and lispro (Humalog), exhibit comparable glycemic control and pharmacokinetic profiles. Long-acting insulin, also known as basal insulin, helps maintain steady blood glucose levels during fasting and is typically administered once daily, independent of meal timing [25]. Yeast-based expression is particularly advantageous for such analogs due to its superior folding and secretion control [26].

Insulin degludec

Insulin degludec is an ultra-long-acting insulin analogue designed to provide a prolonged and stable glucose-lowering effect lasting over 42 hours. It exhibits a flat pharmacokinetic profile with minimal day-to-day variability, providing more consistent glycemic control than other long-acting insulins, such as insulin glargine. Clinical studies have shown that insulin degludec provides equivalent glycemic regulation to insulin glargine in both type 1 and type 2 diabetes patients, but with a reduced incidence of nocturnal hypoglycemia [27].

A notable advantage of insulin degludec is its dosing flexibility, allowing patients to adjust injection times without compromising efficacy or safety. It is also available in a concentrated 200 U/mL formulation, suitable for individuals requiring larger basal insulin doses. Furthermore, a co-formulation with rapid-acting insulin aspart, known as insulin degludec/insulin aspart, offers the benefit of fewer daily injections.

Clinical trials demonstrate that this combination is non-inferior to insulin detemir for type 1 diabetes and may serve as an effective starting therapy for type 2 diabetes patients inadequately managed by oral hypoglycemics. Both insulin degludec and its combination formulation are generally well tolerated, marking them as significant advancements in diabetes management [28].

Insulin Glargine

Insulin Glargine is a long-acting basal insulin analogue administered once daily subcutaneously to provide a steady insulin level throughout the day for patients with type 1 or type 2 diabetes. After injection, the molecule forms microprecipitates in the subcutaneous tissue due to its modified solubility profile, which results in the slow and consistent release of insulin, thereby avoiding a pronounced peak and providing up to approximately 24 hours (and in some studies 24–36 hours) of effect. Because of this flat and prolonged profile, glargine is well-suited to serve as a basal insulin that maintains fasting and between-meal glucose levels with lower variability in action [29].

Figure.1 Structure of human insulin

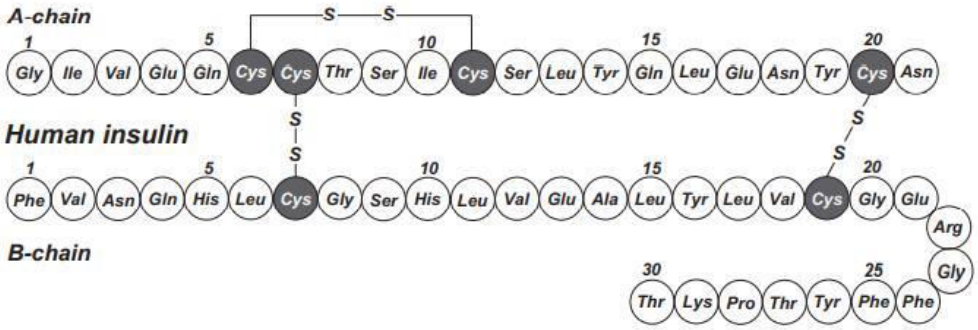

Table.1 Comparative Overview of Insulin Production in *Escherichia coli* and Yeast Systems

Parameter	Insulin Production in *Escherichia coli*	Insulin Production in Yeast Systems (*Saccharomyces cerevisiae*, *Pichia pastoris*)
Host Type	Prokaryotic expression system	Eukaryotic expression system
Historical Development	First recombinant host used for human insulin	Adopted later for improved folding and secretion efficiency
Expression Strategy	Separate A and B chain expression (initial); later proinsulin inclusion body expression	Expression of insulin precursor or proinsulin with a secretion signal
Promoters	T7, tac, and lac (strong, inducible promoters)	AOX1 (*P. pastoris*), GAL1 (*S. cerevisiae*), inducible and high-level
Fusion or Signal Tags	Fusion with β-galactosidase or tryptophan synthetase for stability	α-factor pre-pro leader or similar signal peptide for secretion
Protein Localization	Cytoplasmic (inclusion bodies)	Secreted into the periplasmic space or the extracellular medium
Disulfide Bond Formation	Achieved during *in vitro* refolding after solubilization	Occurs *in vivo* in the endoplasmic reticulum during secretion
Induction Method	IPTG induction during the late exponential phase	Methanol (*P. pastoris*) or galactose (*S. cerevisiae*) induction
Fermentation Conditions	Fed-batch culture at 30–37 °C; pH ≈ 7.0	Aerobic fermentation at 28–30 °C; two-phase process (glycerol + methanol)
Protein Form	Insoluble inclusion bodies	Soluble secreted form
Refolding Requirement	Extensive requires urea/guanidine solubilization and redox refolding	Minimal correct folding is largely achieved intracellularly
Processing to Mature Insulin	Chemical cleavage (cyanogen bromide) and enzymatic conversion (trypsin, carboxypeptidase B)	Enzymatic cleavage post-secretion to obtain active insulin
Purification	Multiple steps: ion-exchange, RP-HPLC, ultrafiltration (endotoxin removal)	Simplified purification: affinity or ion-exchange chromatography; no endotoxins
Endotoxin Contamination	Present must be removed due to *E. coli* lipopolysaccharides	Absent yeast is endotoxin-free
Yield	High expression levels but risk of misfolding	Moderate yield with a higher proportion of active protein
Cost and Scalability	Low-cost media, highly scalable	Moderate cost; scalable but requires methanol handling (*P. pastoris*)

Table.2 Comparison of Pharmacokinetic and Therapeutic Profiles of Long- and Rapid-Acting Insulin Analogues

Parameter	Insulin Degludec	Insulin Glargine	Insulin Aspart
Type	Ultra-long-acting insulin analogue	Long-acting insulin analogue	Rapid-acting insulin analogue
Duration of Action	>42 hours	~24 hours	3–5 hours
Onset of Action	Slow and steady	Slow	Rapid (10–20 minutes)
Glycemic Profile	Flat and stable	Relatively steady	Quick peak followed by a decline
Dosing Frequency	Once daily; flexible timing possible	Once daily at a fixed time	Taken with meals or immediately before
Variability (Day-to-day)	Minimal	Moderate	High (due to meal dependence)
Risk of Nocturnal Hypoglycemia	Lower	Moderate	Low (short duration)
Formulations Available	100 U/mL and 200 U/mL; also co-formulated with aspart	100 U/mL	100 U/mL
Use in Diabetes Type	Type 1 and Type 2	Type 1 and Type 2	Type 1 and Type 2 (postprandial control)
Special Advantage	Flexible dosing; suitable for high-dose needs; lower hypoglycemia risk	Stable basal control	Rapid mealtime glucose control
Combination Therapy	Available as Insulin Degludec/Insulin Aspart	Often combined with rapid-acting insulin	Can be used in combination with basal insulin

Insulin Aspart

Insulin Aspart is a rapid-acting insulin analogue used at mealtimes to cover prandial glucose. It is absorbed more quickly than regular human insulin, with an onset typically in about 10–15 minutes, a peak effect around 60–90 minutes, and a duration of action of approximately 3–5 hours. This faster pharmacokinetic profile allows better post-prandial glucose control and greater flexibility in timing relative to meals [30].

Future Prospects and Challenges

The future of recombinant insulin production lies in achieving a balance between cost efficiency and product sophistication. Research is moving toward novel hosts such as plant systems, insect cells, and cell-free synthesis platforms. These systems may offer enhanced scalability and reduced dependency on complex fermentation infrastructure.

Another promising direction involves the integration of AI-driven design, machine learning–based optimization, and automated bioprocessing to fine-tune expression and purification parameters. Sustainability and green manufacturing are also gaining attention, aiming to minimize energy consumption and waste. However, several challenges persist—ensuring global affordability, maintaining product quality, and navigating complex regulatory pathways for biosimilars. Continuous innovation in strain design, process control, and formulation science will be crucial to address these challenges [24, 31].

In conclusion, the production of recombinant human insulin stands as a landmark achievement in biotechnology. *E. coli* and yeast remain the two cornerstone systems for their industrial manufacture, each with distinct advantages and constraints.

While *E. coli* offers unmatched simplicity, speed, and cost-effectiveness, yeast provides superior folding, secretion, and product quality—particularly suited for advanced insulin analogs. Continuous progress in genetic engineering, synthetic biology, and process optimization has refined both systems to a level of remarkable efficiency. Looking ahead, hybrid and novel expression technologies, combined with sustainable manufacturing approaches, will further enhance the global accessibility of this life-saving therapeutic

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