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Review Article

Unlocking Microbial Biosimilars: Strategies, Development and Future Prospects

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Abstract

Biosimilars represent a cornerstone of affordable biopharmaceuticals, with microbial production systems emerging as efficient, scalable alternatives to traditional mammalian cell platforms. The microbial production of biosimilars is examined in this review, along with approvals in developed markets like the US, EU and Japan, where strict regulatory frameworks guarantee comparability to reference products. Additionally, the approvals of microbial biosimilars from emerging markets demonstrate their therapeutic significance. Important factors for choosing expression systems are discussed, along with the crucial roles that bacteria like *Escherichia coli*, yeasts like *Pichia pastoris* and *Saccharomyces cerevisiae* play in the production of biosimilars. After outlining the qualities of an ideal microbial host, such as high yield, post-translational modification capabilities, and genetic tractability, a detailed description of the production processes is given, covering every aspect from fermentation, purification, and quality control to genetic engineering methods like CRISPR-Cas9 and synthetic biology. Microbial systems' benefits such as their affordability, quick development times, and lower risk of contamination are weighed against their drawbacks, such as glycosylation restrictions. Lastly, this review article expands the future directions for next-generation biosimilars for complex biologics as well as innovations like engineered strains for human-like glycosylation and continuous manufacturing. The potential of microbial platforms to democratize access to life-saving treatments is highlighted by this synthesis.

Keywords: Biosimilars; Microbial Production; Expression System; Recombinant Proteins; *E. coli*; *P. pastoris*

Abbreviations

ABL: Advanced Biotech Lab; ATP: Adenosine Triphosphate; Cas: CRISPR-Associated Protein; CD: Circular Dichroism; CFPS: Cell-Free Protein Synthesis; CHO: Chinese Hamster Ovary; CLD: Cell Line Development; CQAs: Critical Quality Attributes; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; DSP: Downstream Processing; *E. coli*; *Escherichia Coli*; EMA: European Medicines Agency; EPO: Erythropoietin; FDA: Food and Drug Administration; GST: Glutathione S-Transferase; HCDNA: Host Cell Deoxyribonucleic Acid; HCPs: Host Cell Proteins; HTS: High-Throughput Screening; LC-MS: Liquid Chromatography-Mass Spectrometry; LPS: Lipopolysaccharides; MCB: Master Cell Bank; *P. pastoris*: *Pichia Pastoris;* PTM: Post-Translational Modification; QC: Quality Control; QTPP: Quality Target Product Profile; *S. cerevisiae*: *Saccharomyces cerevisiae;* SUMO: Small Ubiquitin-like Modifier Tag; STBs: Stirred-Tank Bioreactors; tRNA: Transfer Ribonucleic Acid; US: United States

Introduction

Over the past forty years, there has been a paradigm shift in the pharmaceutical industry, with complex, large-molecule biotherapeutics known as biologics replacing chemically synthesized small molecules. Recombinant proteins, vaccines, and nucleic acids are examples of biologics, which are now the gold standard for treating life-threatening and chronic illnesses like diabetes, growth hormone deficiencies, and a variety of autoimmune diseases. However, patient access is frequently hampered by the high cost of innovator biologics, especially in low and middle-income nations. Biosimilars, which are biological products designed to be extremely similar to an already approved reference medicinal product in terms of quality, safety, and efficacy, have emerged globally as a result of this economic barrier [1].

Unlike generic versions of small molecule drugs, biosimilars cannot be exact 'copies' because they are produced in living cellular systems. The inherent complexity of the production process means that the final product is sensitive to even minor changes in the manufacturing environment [1]. Consequently, the selection of the expression host is the most critical decision in the biosimilar development lifecycle [1]. While mammalian cell lines, such as Chinese Hamster Ovary (CHO) cells, are preferred for complex monoclonal antibodies due to their ability to perform human-like glycosylation, microbial systems remain the 'workhorses' for a vast array of simpler, non-glycosylated therapeutic peptides and proteins [2].

Microbial hosts, particularly the bacterium *Escherichia coli* (*E. coli*) and yeasts such as *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Pichia pastoris* (*P. pastoris*), provide unique benefits such as high volumetric productivity, rapid growth kinetics, and substantially reduced capital investment requirements. For the production of high-volume biosimilars like insulin, filgrastim, teriparatide, and numerous other monoclonal antibodies (mAbs) and fusion proteins, these characteristics make microbial platforms ideal. Microbial production has drawbacks despite these advantages, such as the possibility of immunogenic hyper-mannosylation in yeast and the development of insoluble inclusion bodies in bacteria [3].

This review explores the current state of microbial production for therapeutic biosimilars. It provides a detailed analysis of the characteristics of ideal microbial hosts, the technical hurdles associated with upstream and downstream processing, and the transformative role of genetic engineering tools like CRISPR/Cas9 in optimizing yield and product quality [4]. Furthermore, we discuss the regulatory landscape and the economic implications of utilizing microbial systems to improve global healthcare equity through affordable biotherapeutics.

Approval of microbial biosimilars in developed markets

In recent years there has been a steady growth in the approval of microbial biosimilars worldwide, especially in developed and in semi-regulated countries. This growth originates from the evolution of regulatory strategies by the agencies to improve patient access to affordable advanced therapies. EMA has pioneered and maintained its leadership in approving microbial biosimilars through centralized procedure, a harmonized approach to make the advanced therapies available to patients in the entire Europe under a single marketing authorization. Till date, EMA has authorized (inclusive of CHMP positive opinion) ~34 biosimilars covering various therapeutic areas viz. diabetes (~7), immunostimulants (~16), ophthalmology (~5) and others (~8) [5].

On the other hand, approval of biosimilars by US FDA has steadily increased which led to the approval of ~18 microbial biosimilars by December 22, 2025 which covers similar therapeutic groups like EMA approvals [6]. Table 1 captures the list of microbial biosimilar approvals by US FDA and their manufacturers. As per the approval status, *E. coli* has been the most preferred host system for these therapeutic biosimilars [6].

In Japan, the Pharmaceuticals and Medical Devices Agency (PMDA) initially approved Sandoz's Somatropin biosimilar in June 2009. Later, since 2012, approval of microbial biosimilars has steadily increased, and as of September 2025, 10+ biosimilars are approved by the PMDA which covers diabetes (4), immunostimulants (4), and ophthalmology (1), supportive care and other therapeutic indications (2) [7].

The Health Canada, being one of the global leader biosimilar approvals for patient access, has approved a considerable number of microbial biosimilars to date. As of January 2026, more than 19 microbial biosimilars have been approved by Health Canada across a variety of therapeutic areas. Health Canada's science-

S. No.	Brand name*	Molecule	Therapeutic class	Therapeutic indication	Host system	Manufacturer	Approval date
	Nufymco	Ranibizumab	Ophthalmologicals	AMD, Macular Edema following RVO, DME, DR, mCNV	*E. coli*	Formycon AG	Dec 2025
	Cimerli					Sandoz Inc.	Aug 2022
	Byooviz					Samsung Bioepis Co., Ltd.	Sep 2021
	Armlupeg	Pegfilgrastim	Immunostimulants	Decrease the incidence of infection, as manifested by febrile neutropenia in patients with non-myeloid malignancies	*E. coli*	Lupin Ltd.	Nov 2025
	Stimufend					Fresenius Kabi USA, LLC	Sep 2022
	Fylnetra					Kashiv BioSciences, LLC	May 2022
	Nyvepria					Hospira, Inc.	June 2020
	Ziextenzo					Sandoz Inc.	Nov 2019
	Fulphila					Mylan GmbH	June 2018
	Udenyca					Coherus BioSciences, Inc.	Nov 2018
	Nypozi	Filgrastim				Tanvex BioPharma USA, Inc.	June 2024
	Releuko					Kashiv BioSciences, LLC	Feb 2022
	Nivestym					Hospira, Inc.	July 2018
	Zarxio					Sandoz Inc.	Mar 2015
	Merilog	Insulin aspart	Diabetes	Diabetes mellitus	*E. coli*	Sanofi-Aventis U.S. LLC	Feb 2025
	Rezvoglar	Insulin glargine				Eli Lily Co.	Dec 2021
	Kirsty	Insulin aspart			*P. pastoris*	Biocon Biologics Inc.	July 2025
	Semglee	Insulin glargine					July 2021
*Not limited to, representative examples							

Table 1: List of US FDA approved biosimilars manufactured using microbial host system.

Note: AMD-Neovascular (Wet) Age-Related Macular Degeneration, RVO-Retinal Vein Occlusion, DME-Diabetic Macular Edema, DR-Diabetic Retinopathy, mCNV-Myopic Choroidal Neovascularization.

based review process is reflected in these approvals, which ensure that biosimilars meet the same strict safety, efficacy, and quality standards as their original biologics [8].

Australia and New Zealand, as developed markets, have regulatory systems closely aligned with those of the US FDA, WHO, and EMA. Recent trends include less emphasis on animal and confirmatory clinical efficacy testing, greater regulatory flexibility, and increased reliance on *in vitro* and pharmacodynamic biomarker data to demonstrate biosimilarity. As of January 2026, New Zealand had approved about 8 biosimilar products from microbial hosts for various therapeutic areas, while Australia had approved over 17 biosimilars [9,10].

Approvals of microbial biosimilars in emerging markets

Emerging markets have made significant strides in approving microbial biosimilars, which are typically simpler biologics, such

as insulins, insulin analogs, fusion protein and mAb fragments produced via microbial expression systems. These approvals often follow abbreviated pathways requiring comparability to reference products, though requirements vary by country. Regulatory frameworks in regions like Asia, Latin America, and the Middle East are evolving to balance access and safety. India, South Korea, and Singapore lead with EMA-aligned guidelines, enabling faster approvals (15-18 months) for microbial biosimilars if reference products match local standards. In Latin America, Brazil's ANVISA and Mexico's COFEPRIS streamline processes by recognizing studies conducted by WHO-listed stringent regulators. Middle Eastern countries like Saudi Arabia and the UAE have established pathways, while South Africa and Egypt are advancing among African countries [11-13].

Below is a summary of noteworthy approvals in emerging markets, along with key trends as of late 2025. The global search for biosimilars is a reflection of ongoing healthcare reforms that seek to lower costs and improve patient access to state-of-the-art therapies. Emerging regions demonstrate accelerated pathways, regulatory harmonization initiatives, and strategic alliances to meet the growing demand for biosimilars.

CDSCO has approved multiple biosimilars in the therapeutic areas of diabetes, ophthalmology, immunostimulants, and others under its regulatory framework, established in 2012 and revised in 2016, with continuous modernization efforts evident in the 2025 draft guidelines which align more closely with global standards such as those of EMA, US FDA, and MHRA. More recently, CDSCO's approvals also majorly focus on the detailed analytical and functional characterization to establish 'high similarity' with the reference product (reference biological product). Till date, CDSCO has approved over 24 microbial biosimilars from the key players like Biocon Biologics Ltd. (insulin glargine), Zydus Lifesciences Ltd. (filgrastim, peg-filgrastim), Serum Institute of India Pvt. Ltd. (filgrastim), Intas Pharmaceuticals Ltd. (filgrastim and Ranibizumab), Dr. Reddy's Laboratories Ltd. (filgrastim) and others [11].

In South Africa, due to resource limitations and strict pharmacovigilance requirements by the South African Health Products Regulatory Authority (SAHPRA), approval timelines are irregular and can be lengthy. Till date SAHPRA has approved over 20 microbial biosimilars [12]. Latin American (LATAM) countries, particularly Brazil and Argentina, have pushed for early approvals of microbial biosimilars. The National Health Surveillance Agency (Agÿ Nacional de Vigilância Sanitção, or ANVISA) was the first country to pass biosimilar laws in 2010, closely adhering to WHO guidelines. Later, between 2011 and 2012, the National Administration of Drugs, Foods, and Medical Devices (Administración Nacional de Medicamentos, Alimentos y Tecnológñica, or ANMAT) implemented regulations for biosimilars. By November 2025, ANVISA has approved more than 8 biosimilars for a variety of therapeutic conditions, including diabetes, ophthalmology, immunostimulants and others [13].

Microbial hosts in biosimilar production

The most commonly used microorganisms for biosimilar manufacturing are bacteria (e.g., *E. coli*) and yeasts (e.g. *S. cerevisiae*, *P. pastoris,* etc.). Bacteria are favored for their rapid growth and ease of genetic manipulation. Yeasts are chosen when post-translational modifications (like glycosylation) are required. Each microbial expression system offers distinct advantages and challenges for biosimilar production. *E. coli* is known for its rapid growth, high yield, and cost-effectiveness, making it ideal for large-scale production. However, it lacks the ability to perform complex post-translational modifications, which can limit its application for certain biologics, and proteins often form inclusion bodies, requiring additional processing [14].

P. pastoris, a yeast system, allows for eukaryotic post-translational modifications like glycosylation, and can achieve high expression levels, but its glycosylation patterns differ from those of higher eukaryotes, and its high oxygen demand can complicate large-scale fermentation. On the other hand, *S. cerevisiae* also performs eukaryotic modifications and has been widely used in the production of biologics, offering a proven track record and cost-effectiveness. However, its lower yield compared to other systems along with differences in glycosylation, can affect the therapeutic efficacy of some proteins. Each system is suited for different types of proteins, and the choice depends on the specific therapeutic requirements, scalability, and cost considerations [15]. Table 2 summarizes the advantages and challenges of both bacterial and yeast expression systems for the production of microbial biosimilars [14,15].

S. No.	System	Advantages	Challenges
1	*E. coli*	Lower cost Well characterized system Simple media requirement Rapid expression and easy scale-up Most popular for recombinant proteins	Inclusion bodies Lack of eukaryotic post-translational modifications Risk of endotoxin contamination Risk of protein secretion/folding issues
2	*P. pastoris* (yeast)	Eukaryotic system Rapid expression and High Yield Ease of genetic manipulation Diverse post-translational modifications Inexpensive media and cost effective	Improper glycosylation Oxygen demand Risk of protein secretion/folding issues
3	*S. cerevisiae* (yeast)	Eukaryotic system Well characterized system Cost effective and proven track record	Improper glycosylation Lower yield Protein secretion and folding issues

Table 2: Considerations while choosing an expression system for biologic product.

Characteristics of ideal microbial host

To achieve commercial and therapeutic success, selecting a microbial host is not merely a matter of convenience but a strategic decision based on the complexity of the target molecule. Below is an expanded analysis of the characteristics of an ideal microbial host for biosimilar production. The 'ideal' host must balance biological capability with industrial robustness [3]. The following parameters are critical for ensuring that the biosimilar is both economically viable and biologically equivalent to the reference product (Table 3) [3,16].

S. No.	Particulars	Details
1	High Protein Expression and Volumetric Productivity	To ensure cost-competitiveness, a biosimilar requires the host to reach high titer levels, measured in grams of protein per liter of culture.
2	Prokaryotic Efficiency	*E. coli* is often preferred because it can reach very high cell densities in short timeframes. The use of strong, inducible promoters (like the *lac* or *T7* systems) allows for 'luxury' production of the target protein, sometimes accounting for up to 30-50% of the total cellular protein.
3	Secretion Titer	In yeast like *P. pastoris*, the focus is on secretory expression. An ideal host should secrete the protein into the culture medium with minimal endogenous protein contamination, which significantly simplifies downstream purification.
4	Proper Protein Folding and Solubility	The biological activity of a biosimilar is entirely dependent on its three-dimensional conformation.
5	The Inclusion Body Challenge	In bacteria, rapid expression often leads to the formation of inclusion bodies dense, insoluble aggregates of misfolded proteins. While these are easy to isolate, the 'refolding' process is technically difficult and yields are often low.
6	Chaperone Integration	An ideal host is often engineered to co-express molecular chaperones (e.g., DnaK-DnaJ-GrpE) that assist in folding the protein correctly *in vivo*, thereby increasing the fraction of soluble, bioactive product.

7	Post-Translational Modification (PTM) Capability	Glyco-engineering	Standard yeast strains naturally perform 'hyper-mannosylation,' which can cause rapid clearance from the body or allergic reactions. An ideal host for the future is one where the native glycosylation pathway has been 'humanized' by deleting yeast-specific genes and introducing human glycosyltransferases to produce complex, sialylated glycans.
		Disulfide Mapping	The host must provide an oxidative environment (such as the endoplasmic reticulum in yeast or the periplasm in bacteria) to ensure the correct formation of disulfide bridges, which are essential for the structural integrity of proteins like insulin.
8	Genetic and Phenotypic Stability	Plasmid Retention	In many microbial systems, the gene for the biosimilar is carried on a plasmid. An ideal host must maintain this plasmid without the continuous need for expensive antibiotics (often achieved through 'auxotrophic' markers).
		Sequence Integrity	The host must have low mutation rates. Any 'sequence drift' or amino acid substitution during long-term cultivation would result in a 'sub-standard' batch that fails regulatory comparability tests.
		Low Proteolytic Activity	A major hurdle in microbial production is the presence of proteases enzymes produced by the host that 'eat' or degrade the therapeutic protein. Ideal industrial strains are often 'protease-deficient' (e.g., *E. coli* BL21 or specific *Pichia* knockouts) to ensure the target protein remains intact throughout the fermentation process.

Table 3: Characteristics of an ideal microbial host for biosimilar production.

Steps in microbial production of biosimilars

Microbial production of biosimilars is not much different than the biosimilars in mammalian cells (viz. CHO cells, etc.) other than the host expression system. The production flow is also highly regulated, multi-stage pipeline is designed to ensure that the microbial-derived product is 'highly similar' to the innovator molecule in terms of structure, purity, and potency [2]. Below are the critical steps for the development of a typical microbial biosimilar [17-20].

Reference product selection and target profiling

The first step in development is to establish a Quality Target Product Profile (QTPP) based on the target reference product. Multiple batches of the innovator or reference product (e.g., Neupogen® for Filgrastim, Lucentis® for Ranibizumab) are procured to assess lot-to-lot variability and define acceptance criteria for process optimization. Critical Quality Attributes (CQAs), including molecular weight, primary amino acid sequence, disulfide bridge patterns, and biological activity, are prioritized.

Host cell line development (CLD)

Once the target is defined, the gene of interest is synthesized, often with codon optimization to match the host's tRNA abundance, and inserted into an appropriate plasmid expression vector (e.g. pUC or pET vector series for *E. coli*; pPIC9 for *P. pastoris*; pYeDP60 for *S. cerevisiae*).

- **Clone Selection:** Thousands of microbial clones are screened using high-throughput systems to identify the top producer with high growth rates and stable protein expression.
- **Master Cell Bank (MCB):** The selected clone is used to create a validated cell bank, ensuring all future production batches originate from the same genetic source.

Upstream process (USP): Fermentation

This stage involves cultivating microbial cells in shake flasks and stirred-tank bioreactors (STBs) while optimizing growth conditions for biosimilar production.

- **Inoculum Expansion**: Cells are grown from a vial in shake flasks to generate sufficient inoculum for the production bioreactor (5L).
- **Media and Feed Optimization:** Various media (e.g., Terrific broth, Luria-Bertani broth) and feeds (yeast extract, soy peptone) are screened in the production bioreactor to optimize target protein production. The optimal combination is selected based on growth and productivity profiles.
- **Process Parameters:** Key parameters including pH, dissolved oxygen, temperature, agitation, and aeration are optimized to maximize yield while preventing protein degradation or stress-induced misfolding.

Downstream process (DSP): Purification

Downstream processing represents the largest portion of manufacturing costs because it must eliminate all host-derived impurities.

- **Harvesting:** For *E. coli*, this step involves cell lysis and recovery of inclusion bodies. For yeast, the protein is typically collected from the culture supernatant.
- **Chromatography:** Multiple steps including affinity chromatography, ion exchange, and size-exclusion, are used to achieve over 99% purity.
- **Refolding (if applicable):** If the target protein is present as inclusion bodies, it is chemically denatured and then refolded into its bioactive state.

Formulation development

Once purified, the biosimilar protein is formulated into a stable drug product (DP). This requires selecting appropriate excipients, such as stabilizers, buffers, and surfactants, to maintain protein stability during storage and delivery. Key concerns include preventing aggregation, denaturation, and preserving the correct protein conformation.

- **Buffer and pH Optimization:** The formulation includes buffers to maintain the desired pH throughout the product's shelf life. Maintaining optimal pH is essential for protein stability during storage and administration.
- **Container and Delivery System:** The selection of containers (e.g. vials or prefilled syringes) and delivery methods (e.g. injection or infusion) must be optimized to minimize protein degradation, aggregation, and potential adverse reactions.

Many protein-based biologics are freeze-dried to enhance stability during storage and shipment. The lyophilization process must be optimized to preserve protein activity and prevent damage during drying.

Analytical Characterization and biosimilarity establishment (The 'Fingerprinting' Stage)

Analytical characterization is essential to demonstrate that microbial biosimilars are highly similar to their reference biologics. This process evaluates critical quality attributes (CQAs) such as structure, purity, potency, and stability, using orthogonal methods to build a fingerprint profile. Regulatory agencies, including the FDA, EMA, and WHO, require extensive analytical similarity data to reduce the burden on clinical trials. The drug product is analyzed with a range of orthogonal assays to compare it with the reference product and establish biosimilarity. The following section describes the traditional orthogonal assays used for biosimilarity assessment.

Primary structure

Peptide mapping by LC-MS/MS confirms the protein structure, while amino acid sequencing verifies the molecular identity and detects modifications such as oxidation or deamidation. Intact mass spectrometry verifies molecular weight and Edman degradation supports N-terminal sequencing for smaller peptides. These methods ensure no sequence variants exist between the biosimilar and the reference product.

Tertiary structure

Advanced techniques such as Circular dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), and hydrogen-deuterium exchange MS (HDX-MS) to assess secondary and tertiary structure. Analytical ultracentrifugation and differential scanning calorimetry evaluate stability, and aggregation. For microbial products, which are non-glycosylated, conformational similarity is prioritized over complex glycan analysis.

Purity and impurities

Size-exclusion chromatography (SEC), capillary electrophoresis (CE-SDS), and dynamic light scattering detect aggregates and fragments. Ion-exchange chromatography and isoelectric focusing characterize charge variants. Host cell proteins and host cell DNA are quantified by ELISA and qPCR, which is especially important for microbial hosts such as *E. coli*.

Functional assays

Cell-based potency assays (e.g. GLP-1 receptor assays for insulin and insulin analogs), receptor binding ELISA (VEGF inhibition for Ranibizumab) and binding studies by surface plasmon resonance (SPR), confirm biological activity and link analytical to functional similarity. Multi-attribute methods (MAM) using high-resolution MS allow monitoring of multiple CQAs in a single run. Using a range of orthogonal tools reduces the risk of variability during approval.

Scale up and commercial manufacturing

The lab-scale process (5L–10L) is scaled up to commercial volumes (200L–500L) using criteria such as tip speed and P/V ratio to replicate the optimized process. Downstream purification is also linearly scaled-up to maximize recovery of the target microbial protein, resulting in the drug substance (DS). The DS is then formulated at commercial scale using standardized buffer systems to produce the drug product (DP), which is then filled and packaged.

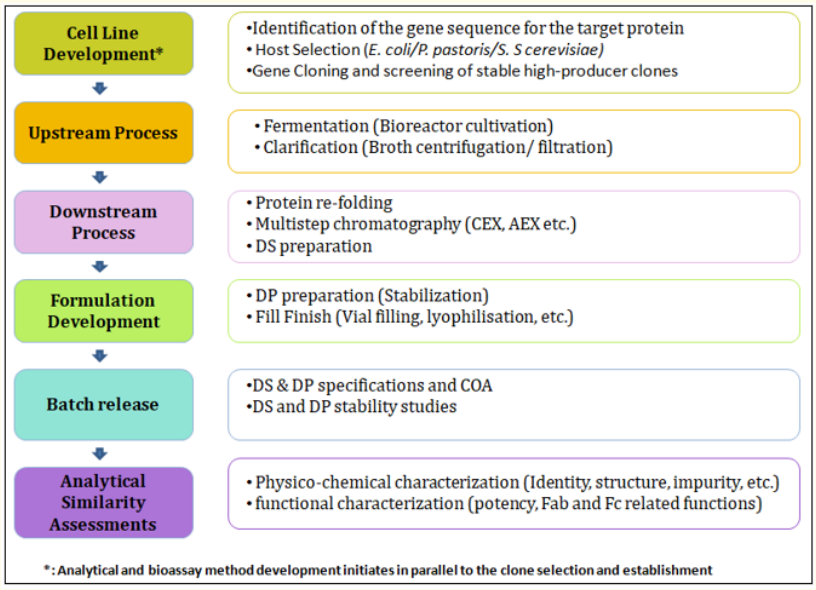

Figure 1: Key steps of production of biosimilars in microbial systems.

Genetic engineering techniques

Modern genetic engineering does not just include 'inserting a gene'; it involves rewriting the host's metabolic landscape to prioritize the production of the biosimilar while maintaining cellular health. Below are examples of technological strategies employed to improve microbial host systems and enhance production of recombinant therapeutic proteins.

CRISPR/Cas9: Precision genome editing

The advent of CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) has replaced older, less efficient methods of genome modification in microbial hosts.

- **Targeted Knockouts:** CRISPR is used to permanently inactivate (knock out) genes that encode endogenous proteases. This prevents the microbial host from degrading the therapeutic protein once it is expressed.
- **Metabolic Rewiring:** By knocking out competing metabolic pathways, researchers can 'funnel' the cell's energy and raw materials (amino acids and ATP) exclusively toward the synthesis of the biosimilar molecule.
- **Integration Sites:** Unlike traditional plasmid-based expression, which can be unstable, CRISPR allows for the precise integration of the biosimilar gene into 'genomic safe harbors' regions of the host DNA, where expression is high and stable over many generations [4].

Codon optimization

Because the genetic code is redundant, multiple codons can code for the same amino acid. However, different organisms prefer different codons based on the availability of corresponding tRNAs.

- **Synonymous Substitution:** To produce a human protein in *E. coli*, the human gene sequence is 'recoded' to use codons that the bacteria can read most efficiently. This prevents 'ribosomal stalling' and dramatically increases the speed and volume of protein production [21].

Protein engineering and rational design

In some cases, therapeutic proteins require minor modifications to enable successful production in microbial hosts.

- **Solubility Tags:** Small peptides such as SUMO or GST are genetically fused to the biosimilar to maintain solubility and prevent inclusion body formation. These tags are removed during downstream purification using specific enzymes.
- **Signal Peptides:** In yeast production, specific leader sequences such as the Alpha-mating factor in *S. cerevisiae* are engineered at the protein's N-terminus. These sequences direct the cell to secrete the protein into the culture medium instead of retaining it internally.

High-throughput screening (HTS)

Engineering often requires screening large numbers of variants. Microfluidics and robotic systems enable simultaneous evaluation of thousands of genetically modified microbial strains to identify those with optimal growth and yield.

Advantages of microbial production systems

Microbial platforms provide clear advantages for producing certain biosimilars, including insulin, filgrastim, and various growth factors. Their main benefit is cost-effectiveness, as microbes grow on simple, inexpensive media such as glucose, glycerol, and ammonium salts [16]. In contrast, mammalian cell cultures require complex, serum-free formulations with added growth factors. Microbial fermenters are also less complex than mammalian bioreactors because microbes are more resilient to mechanical stress, reducing infrastructure needs and lowering costs. Microbial systems are efficient and scalable: *E. coli*, for example, doubles every 20 minutes, allowing production batches to finish in days instead of the 2–3 weeks required for CHO cells. High cell densities, up to 100 g/L dry cell weight, enable substantial protein yields per liter [22,23].

Limitations and challenges

Although microbial systems offer advantages, they face significant biological limitations in producing complex human protein biosimilars due to cellular constraints. The main challenge is the lack or improper execution of post-translational modifications (PTMs). For example, bacteria such as *E. coli* cannot perform the glycosylation required for biosimilars like monoclonal antibodies (mAbs) or erythropoietin (EPO), leading to rapid hepatic clearance and reduced therapeutic efficacy [3]. Yeasts can provide partial glycosylation, but often generate hyper-mannosylated structures with non-human sugar patterns that can trigger severe immunogenicity in patients. Rapid protein synthesis in *E. coli* can also overwhelm folding machinery, resulting in insoluble inclusion bodies that require expensive and risky refolding steps. Incomplete refolding may alter the protein's 3D structure and cause failure in bio-comparability tests. Additionally, Gram-negative hosts such as *E. coli* pose endotoxin (LPS) contamination risks, requiring highly stringent purification to parts-per-billion levels to prevent severe adverse events such as septic shock [14]. Regulatory agencies, including the FDA and EMA, require comprehensive analytical data to ensure that PTM differences do not affect clinical outcomes. Figure 2 summarizes the challenges associated with microbial production of biosimilars.

Innovations and future directions

The next generation of microbial biosimilar production is moving beyond the natural limitations of the host through radical bioengineering.

Glyco-engineered 'Humanized' Yeast

A key innovation in this field is modifying yeast, specifically *P. pastoris*, to perform human-like N-glycosylation. By deleting genes responsible for hyper-mannosylation, such as OCH1, and introducing human glycosyltransferases and transporters, researchers have produced proteins with complex, sialylated glycans. This 'GlycoSwitch technology' enables microbes to produce complex glycoproteins and monoclonal antibodies that were previously limited to mammalian cells [24].

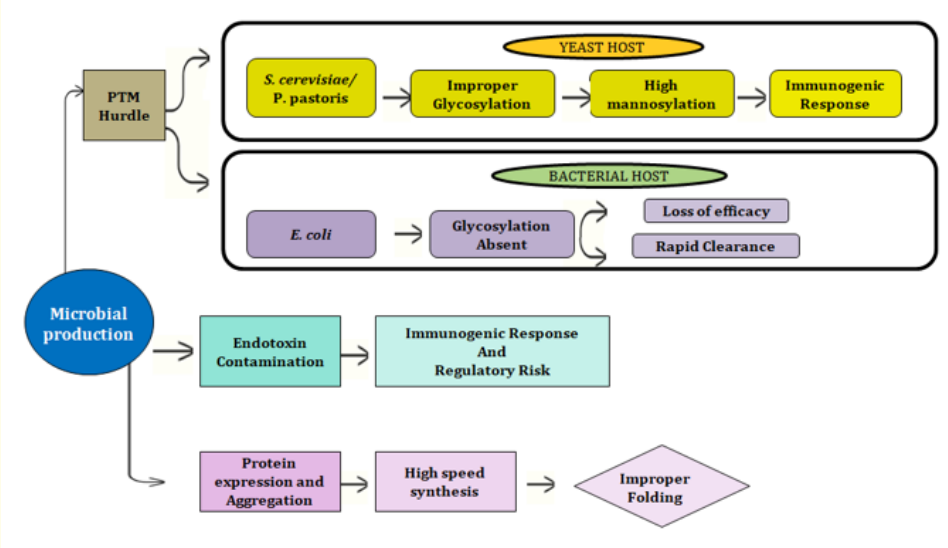

Figure 2: Challenges in the microbial production of biosimilars.

Periplasmic expression and chaperone co-expression

In *E. coli*, recent innovations focus on shifting protein production to the periplasmic space. This oxidative environment favors correct disulfide bond formation, reducing the need for *in vitro* refolding. Furthermore, the co-expression of 'helper proteins' (molecular chaperones like GroEL-GroES) within the bacteria is being used to prevent protein aggregation at the source, significantly increasing the yield of soluble, active biosimilars [24].

Cell-free protein synthesis (CFPS)

A radical shift in the industry is the move toward 'cell-free' systems. By using only the extracted 'machinery' of *E. coli* (ribosomes, enzymes, and tRNAs) *in vitro*, manufacturers can produce biosimilars without the metabolic burden of maintaining a living cell. This allows for faster production cycles and incorporation of non-natural amino acids to improve the drug's stability [25].

Continuous manufacturing

Beyond fed-batch fermentation, the present decade has also seen 'continuous manufacturing' rapidly coming up for the production of microbial biosimilars. Continuous manufacturing improves biosimilar production by enabling steady-state processes that are more efficient than traditional batch methods. This method is well-suited to microbial systems like *E. coli* or yeast due to their rapid growth and scalability. It also helps ensure consistent quality for complex proteins, including monoclonal antibodies and insulin analogs [26-28].

Key benefits

- Reduces equipment footprint by up to 70% and increases volumetric productivity three- to five-fold compared to batch processes.
- Lowers facility costs by 30 to 50%, improves biosimilar affordability, and maintains consistent product quality through stable bioreactor conditions.
- Applies perfusion techniques in microbial cultures to minimize impurities and degradation, making the process suitable for unstable biosimilars [26].

Process overview

- Upstream perfusion bioreactors deliver continuous nutrient supply and product harvest, integrating with downstream chromatography.
- The end-to-end setup supports continuous operation for weeks, as demonstrated in recombinant protein case studies.
- Microbial hosts reach higher cell densities and incur lower media costs than mammalian cells.

Microbial advantages

- Enables rapid scale-up for biosimilars, by reducing production time and costs through continuous fermentation.
- Perfusion in *E. coli*-derived biosimilars produces higher titers and fewer variants than fed-batch methods [27,28]
- Receives regulatory support from ICH Q13 guidelines, facilitating commercial adoption.

Concluding Remarks

Microbial production systems remain the foundation of the biosimilar industry, particularly for non-glycosylated therapeutics. Their unmatched speed, scalability, and cost-effectiveness continue to drive down the prices of life-saving medications like insulins, insulin analogs, filgrastim, mAb fragments, etc. and many others. While biological constraints, specifically the lack of complex post-translational modifications, have historically limited their scope, modern innovations in CRISPR-mediated genome editing and glyco-engineering are rapidly blurring the lines between microbial and mammalian capabilities. As analytical 'fingerprinting' techniques become more sensitive, the ability of microbial platforms to deliver high-quality, highly similar, and affordable biotherapeutics will only expand. The future of biosimilars depends on advancing their development to improve accessibility to complex biological treatments worldwide.

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Conflict of Interest

SB, AK, RK, AJM, NT and SG were employed by Ipca Laboratories Ltd., Mumbai, India. The review research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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