Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Leishmania: A Promising Expression Host for Eukaryotic Recombinant Proteins

Afshin Abdi Ghavidel[1], Mojgan Bandehpour[2], Bahram Kazemi[2, 3]*

[1]Student Research Committee, Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Cellular and Molecular Biology Research Center, Systems Biology Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Review Article

Keywords: *Leishmania tarentolae*, Iranian lizard *Leishmania*, Recombinant protein, Expression host, Glycosylation, Protein engineering

Received: 04 Nov. 2025
Received in revised form: 22 Aug. 2026
Accepted: 23 Oct. 2026
DOI: [To be assigned]

***Correspondence**
Email: Bahram_14@yahoo.com; kazemi@sbmu.ac.ir

© The Author(s)

ABSTRACT

Recombinant proteins are essential to biomedicine, research, and industry, but expression hosts differ substantially in productivity, folding, glycosylation, cost, and scalability. The lizard-associated trypanosomatid *Leishmania tarentolae* has emerged as a potentially useful eukaryotic expression host that combines relatively straightforward cultivation with the capacity for disulfide-bond formation, complex-protein assembly, and selected mammalian-like post-translational modifications (PTMs). Nevertheless, its glycosylation patterns are not inherently identical to those of human cells, and product quality must be established individually. Iranian lizard *Leishmania* (ILL), an isolate obtained from an Iranian lizard, has also been investigated for producing several recombinant proteins. This narrative review compares major expression platforms, summarizes genetic constructs and cultivation approaches for *L. tarentolae* and ILL, and critically examines reported protein yields and biological activities. Particular challenges include variable or low product titers, proteolysis, strain-specific behavior, incomplete characterization of PTMs, and a lack of directly comparable scale-up and cost data. Vector engineering, improved mRNA processing and stability, protease control, glycoengineering, and systematic process optimization may enhance performance. The evidence currently supports *Leishmania* mainly as a promising research and development platform, rather than an established replacement for industrial mammalian-cell manufacturing.

INTRODUCTION

The development of recombinant DNA technology enabled the production of biologically active proteins in heterologous hosts. In 1977, a chemically synthesized gene encoding the peptide hormone somatostatin was expressed in *Escherichia coli*, establishing a landmark proof of concept for recombinant expression [1, 2]. Recombinant hormones and other therapeutic products were subsequently developed for clinical use [3]. Present-day production systems range from bacteria and yeasts to insect, plant, and mammalian cells. Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) cells are important platforms for several complex biopharmaceuticals, although the optimal host depends on the protein and its intended application [4–7]. This narrative review first compares established expression hosts and then assesses the evidence for *Leishmania tarentolae* and Iranian lizard *Leishmania* (ILL), emphasizing production yields, product quality, expression vectors, and remaining translational barriers.

Expression hosts

Bacteria

Bacterial hosts, especially *E. coli*, are widely used because of their rapid growth, genetic tractability, comparatively inexpensive cultivation, and capacity for high expression of selected products. They are particularly

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

suitable for proteins that do not require complex eukaryotic post-translational modifications (PTMs). Nevertheless, correct protein folding and three-dimensional structure are essential even when glycosylation is not required. Bacterial limitations include the absence of conventional eukaryotic intron splicing and human-type glycosylation, differences in codon usage, endotoxin contamination in Gram-negative hosts, and aggregation or inclusion-body formation for some heterologous proteins [2, 8].

Other bacterial hosts, including *Bacillus subtilis* and *Lactococcus lactis*, can support recombinant protein secretion and provide alternatives to *E. coli* for selected products. Their advantages and limitations depend on secretion pathways, protease activities, product stability, and manufacturing requirements [9].

Unicellular eukaryotic hosts

Unicellular eukaryotes, particularly yeasts, provide eukaryotic folding and secretion machinery while retaining many of the cultivation and scale-up advantages of microorganisms [10]. Their glycosylation pathways differ from those of mammalian cells, but can be engineered for selected products. *Saccharomyces cerevisiae* and *Komagataella phaffii* (formerly referred to as *Pichia pastoris*) are widely studied hosts for recombinant enzymes and biopharmaceutical proteins [11].

Yeast hosts

Saccharomyces cerevisiae is a well-established host for recombinant proteins [12]. However, its native N-glycosylation often generates mannose-rich glycans that may alter the biological properties, clearance, or immunogenicity of particular glycoproteins [13, 14]. Glycoengineering can modify this pathway, although achieving the desired glycoform remains product-dependent [15]. Secretion may also be limited by folding capacity, trafficking, or extracellular proteolysis; engineering the secretory pathway can improve yields [16]. Codon usage and expression-context effects require optimization, but differences from mammalian codon frequencies are not an intrinsic barrier to the expression of every human gene [17].

Komagataella phaffii (*P. pastoris*) is frequently used for high-cell-density cultivation and efficient recombinant protein expression, although titers vary widely among proteins, strains, and production processes [18]. Native yeast glycans may be unsuitable for some therapeutic glycoproteins; humanized glycosylation pathways offer one strategy for improvement [19]. Proteolytic degradation, secretion bottlenecks, and metabolic stress can lower recoverable yields. Process optimization and engineered strains, including protease-deficient variants when appropriate, can mitigate these limitations [20].

Other eukaryotic expression platforms

Filamentous fungi, insect cells, transgenic animals, plants, and mammalian cell cultures offer distinct production options. None can be ranked universally by PTM fidelity, yield, cost, or scalability because these properties vary with the host, strain, product, and production process. More complex systems may also entail demanding development, purification, biosafety, or regulatory requirements [21].

Fungi

Filamentous fungi, including *Trichoderma reesei* and certain *Aspergillus* species, are valuable industrial hosts, particularly for secreted enzymes [22]. Nevertheless, fungal N-glycans do not necessarily reproduce mammalian glycosylation, and some species or strains present product-degradation, metabolite-safety, viscosity, or engineering challenges. These risks should be assessed for each production strain rather than attributed uniformly to all filamentous fungi [23].

Insect cells

Insect cell expression, frequently coupled to baculovirus vectors, can facilitate the production of correctly folded and assembled recombinant proteins and protein complexes [24]. Its performance depends on the product and process, and insect N-glycan processing often differs from mammalian-cell processing. Such differences may matter for glycoproteins whose activity, immunogenicity, or pharmacokinetics depend on specific terminal sugars [25].

Accordingly, baculovirus-insect cell systems should be compared with yeast and mammalian platforms using product-specific measures such as functional yield, glycan profiles, process reproducibility, and manufacturing costs. Insect cells are not intrinsically inferior to glycoengineered yeast; their relative suitability depends on the target protein and required quality attributes [26].

Silkworm (*Bombyx mori*) larvae and pupae have also been investigated as *in vivo* expression hosts, including for recombinant glycoproteins [27]. Their use may be advantageous for certain research or production applications, but production efficiency, batch consistency, purification, and scalability require product-specific evaluation [28].

Transgenic animals

Transgenic animals can produce recombinant proteins in selected tissues under the control of tissue-specific regulatory elements. For example, mammary-gland expression can permit protein secretion into milk, using promoters such as those derived from milk-protein genes.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

The appropriate promoter and host species depend on the production strategy; recombinant products have also been explored in other biological fluids and in avian egg-white systems [29]. Transgenic mammals can provide mammalian folding and PTM pathways, but the resulting product is not necessarily identical to its endogenous human counterpart. Glycosylation, recovery, viral safety, purification from complex biological matrices, animal welfare, and regulatory considerations must be assessed before therapeutic use [30].

Plant expression systems

Plant-based systems support recombinant-protein expression in whole plants or plant-cell cultures under controlled conditions [31, 32]. Plant N-glycans may carry β1,2-xylose and core α1,3-fucose, which differ from common human glycan structures; glycoengineering can reduce some of these differences [33]. Proteases, phenolic compounds, host proteins, and heterogeneous expression may complicate downstream processing, depending on the product and production method [34].

Protein secretion from engineered plant roots into hydroponic medium ("rhizosecretion") has been explored as one route to simplify product recovery. However, secretion signals and specialized cultivation do not guarantee higher purity, lower cost, or an improved overall process. Expression levels, extracellular proteolysis, media complexity, and recovery costs must be determined experimentally [35].

Mammalian cell hosts

Mammalian expression systems remain central to the manufacture of many complex therapeutic proteins because they support sophisticated folding, assembly, and PTM pathways, including glycosylation. These advantages come with demands for process control, cell line development, viral safety testing, and quality assurance, although productivity and costs vary greatly across modern manufacturing platforms [36].

Some mammalian cell lines require adherent growth, whereas widely used CHO production lines have been adapted to suspension culture in bioreactors. Compared with microbial hosts, mammalian cultures often have longer production cycles and more demanding media and process-control requirements; however, optimized industrial processes can achieve high cell densities and high product titers [37].

CHO and HEK293 cells are established expression platforms for diverse recombinant proteins. Nevertheless, their PTMs are not automatically identical to those of native human proteins, and functional activity, glycan distribution, purity, and aggregation must be characterized for each product [38].

***Leishmania* overview**

The genus *Leishmania* was described in the early twentieth century; its taxonomic history is more complex than attribution to a single investigator [39]. The number of recognized species and species implicated in human or animal disease depends on taxonomic definitions and subsequent revisions, so fixed counts should not be presented without a current taxonomic source [40]. *Leishmania* comprises kinetoplastid protozoa in the family Trypanosomatidae. *Leishmania tarentolae* is a lizard-associated species assigned to the subgenus *Sauroleishmania*; the genome of the TARII strain was published in 2012 [41]. Several laboratory isolates and strains, including TARII and LEM-derived lines, have been investigated. *L. tarentolae* is generally regarded as nonpathogenic to humans and is commonly handled under low-containment laboratory conditions, but biosafety classification is strain- and institution-dependent. Experimental uptake or limited persistence in mammalian phagocytes has been described, so the blanket claim that all strains are "strictly non-pathogenic" is not justified [42].

Leishmania tarentolae has been developed as a eukaryotic platform for producing selected recombinant proteins [43]. Relative ease of culture, genetic manipulation, and the capacity to support folding and assembly of some complex proteins are important advantages. Nevertheless, strain performance, protein secretion, folding fidelity, and the cost of purified product depend on the expression construct and manufacturing process. Broad claims of universally human-compatible codon usage or inexpensive purification are not substantiated across proteins [21, 43].

Protein N-glycosylation and O-glycosylation are important quality attributes of many recombinant glycoproteins. N-linked glycans are typically attached to asparagine residues in appropriate sequence contexts, whereas common mucin-type O-linked glycans are attached to serine or threonine residues. Although *L. tarentolae* can generate selected mammalian-like glycan features, its glycosylation does not reproduce all human glycoforms. In particular, native pathways may lack terminal sialylation, which can affect the stability or circulation of some therapeutic proteins. In one study, expression of human sialyltransferase together with suitable culture supplementation improved the sialylation profile of recombinant IFN-β, demonstrating product- and process-specific glycoengineering rather than universal restoration of human-type glycosylation [44]. The functional consequences of altered sialic acid content should be evaluated for each product [45].

The ability to express multiple polypeptide chains and assemble selected protein complexes makes *L. tarentolae* of interest for multisubunit proteins [46]. Recombinant monoclonal antibodies have been produced experimentally, including an anti-IL-2Rα antibody. However, correct assembly or selected structural similarities do not establish therapeutic equivalence or guarantee low immunogenicity. Antibody Fc

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

glycosylation, target binding, effector functions, aggregation, and product-specific safety require direct analytical and functional testing [47].

The platform has also been studied for the production of viral antigens and candidate vaccine components, including influenza and coronavirus-related antigens. The evidence spans recombinant antigen expression and other experimental approaches, including engineered extracellular vesicles; these applications should not be equated with licensed vaccines or clinically validated products [48, 49].

Applications of *Leishmania*

Historically, *Leishmania* and related kinetoplastids have served as experimental systems for investigating mitochondrial kinetoplast DNA and RNA editing. A seminal 1990 study proposed a model of guide-RNA-directed editing in kinetoplastid mitochondria [50]. Earlier investigations of amplifiable DNA circles in *L. tarentolae* were relevant to parasite genetics and drug resistance rather than demonstrating general-purpose recombinant protein production [51]. Subsequently, stable *Leishmania* expression vectors supported investigations of parasite gene products; such work should not be described as a demonstration of a clinically protective vaccine [52].

Cell-free lysates prepared from *L. tarentolae* have also been evaluated for eukaryotic protein synthesis [53]. A 2024 study reported a lyophilized cell-free formulation that retained approximately 76% of protein-expression activity relative to nonlyophilized controls and remained usable after more than two weeks of room-temperature storage under the tested conditions. The reported percentage of activity retention is conditional on the original study design and does not establish generalized room temperature stability or commercial shelf life [54].

Leishmania and *L. tarentolae* for protein expression

Leishmania tarentolae and ILL are distinct lizard-associated research isolates or lines with potentially useful characteristics for recombinant protein expression. The available literature does not establish that they have identical genomes, PTM profiles, biosafety properties, or production capacities. The following sections describe the better-characterized commercial *L. tarentolae* system and the separately investigated Iranian lizard isolates [41-43, 55, 56].

Commercial *Leishmania* strain

Jena Bioscience markets the LEXSY expression platform based on an *L. tarentolae* P10 host and compatible expression vectors. Stable genome integration, selectable markers, and inducible or constitutive expression options can be used according to the vector design. These capabilities derive from platform development, but the specific performance of any product requires experimental validation; commercial availability alone does not establish manufacturing-scale viability [55].

Iranian lizard *Leishmania*

An Iranian lizard-associated *Leishmania* isolate, subsequently referred to as Iranian lizard *Leishmania* (ILL), was obtained from a reptilian host historically identified as *Agama agilis* (also referred to as *Trapelus agilis*) [56]. Preliminary promastigote transcriptome data have been reported [57]. These data may assist in identifying candidate pathways for future genetic engineering, but they do not by themselves demonstrate which genes can be safely disrupted. ILL has been used experimentally to express factor VII, IL-12, IL-29, and amelogenin, as discussed below. Claims of human-equivalent glycosylation or commercially competitive production remain to be established by quantitative glycan analysis, functional testing, and standardized bioprocess comparisons [43, 56, 57].

Expression constructs used in *Leishmania*

A range of *L. tarentolae* expression vectors has been developed for stable integration, inducible expression, intracellular accumulation, and protein secretion. In these kinetoplastids, expression depends on genomic context, RNA processing, translation, and protein stability; describing optimization solely in terms of conventional promoter strength oversimplifies the underlying biology. A 2024 Modular Cloning (MoClo) toolkit based on a modified LEXSY vector provided 34 domesticated genetic parts for standardized assembly of expression modules, including protein tags and targeting signals [58]. These tools facilitate experimental construct development but do not ensure high yields for all recombinant proteins (Figure 1).

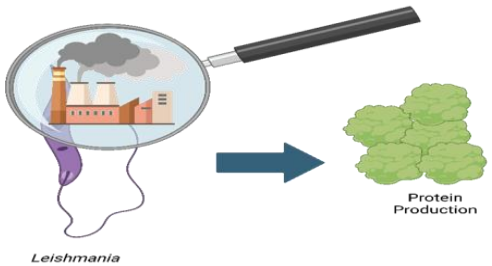


Fig. 1. Schematic illustration of the potential use of *Leishmania* as a recombinant protein expression host. This conceptual diagram emphasizes protein production but does not provide experimental evidence of superior yield, human-equivalent glycosylation, product safety, or industrial-scale performance. Created with BioRender.com.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

Stable *L. tarentolae* expression systems can target defined genomic loci, including ribosomal DNA (rDNA) regions, while some experimental ILL systems have compared expression configurations involving an rDNA-associated locus and a cysteine-proteinase gene locus [59]. Vector architecture, transcription, RNA processing, selection, and gene-copy context can influence expression. The comparison should therefore be interpreted within the particular strain and constructs studied, rather than as proof that an rDNA-targeted construct universally yields more protein [58, 59].

Inducible LEXSY-based expression can employ stable genomic integration or episomal elements, depending on the system. Certain integrative designs target the ornithine decarboxylase (*odc*) locus; this does not apply to every *Leishmania* expression construct. Appropriate controls are required to evaluate vector stability and induction-related effects [55, 60].

In established inducible designs, T7 RNA polymerase can be combined with a tetracycline-responsive regulatory system to control transcription from a T7 promoter. The degree of induction and background expression vary with the construct, host, and experimental conditions [60]. Kinetoplastid mRNA maturation involves spliced-leader trans-splicing and polyadenylation, making suitable intergenic processing sequences important to vector design. CRISPR/Cas9-based genome editing approaches have also been described in *Leishmania* [61]. DNA delivery commonly uses electroporation, although transfection efficiency varies with strain and protocol (Figure 2).

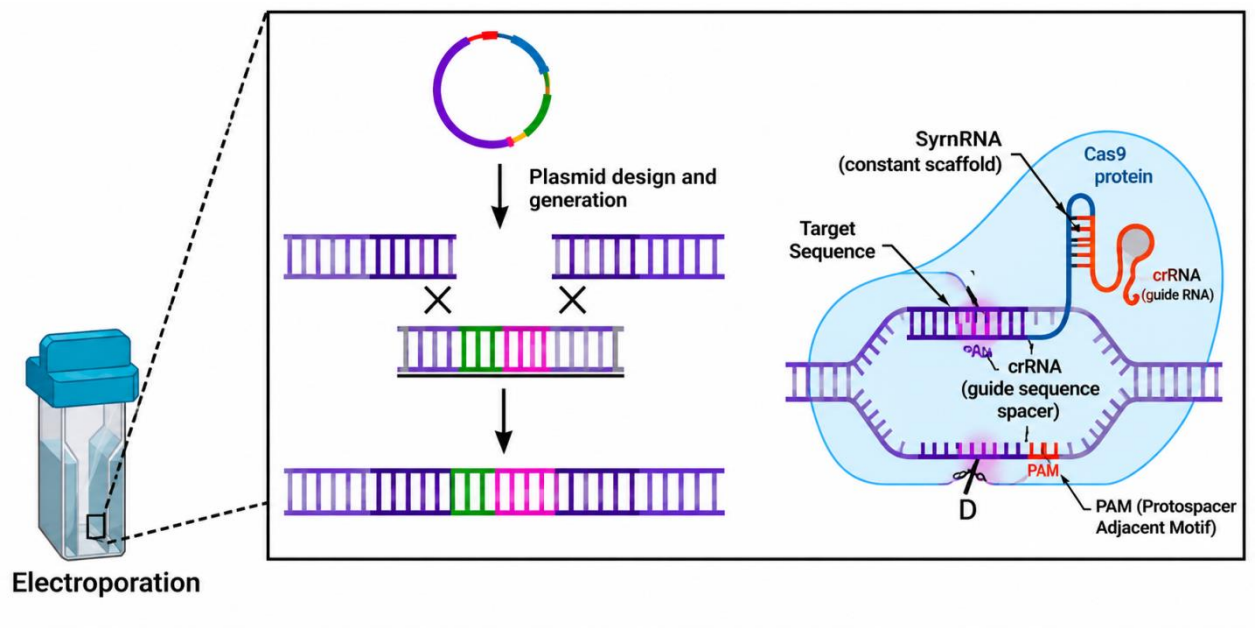


Fig. 2. Conceptual illustration of DNA introduction into *Leishmania* cells by electroporation and the possible integration of introduced DNA by homologous recombination. Electroporation transiently increases membrane permeability; it does not itself guarantee stable integration or precise genome editing. The outcome depends on DNA design, the cellular repair machinery, and selection conditions. Created with BioRender.com.

Culture media for *Leishmania*

Culture medium influences parasite growth, cell physiology, secretion, and downstream processing, and it may represent an important component of production cost. Medium optimization should therefore consider product yield and quality as well as cell density and cost per liter of culture [21, 43, 60].

L. tarentolae can be maintained in several supplemented media, including brain heart infusion (BHI) formulations containing hemin. A commercial LEXSY host may require medium components and antibiotics specified for the relevant expression protocol [62]. Chemically defined medium has also been developed to improve the reproducibility and control of selected *L. tarentolae* cultivation processes [63].

Traditional *Leishmania* cultivation and parasite-propagation protocols have used Novy–MacNeal–Nicolle (NNN) medium and liquid media such as M199, RPMI 1640, and other supplemented formulations. Requirements for serum, hemin, buffers, and additional nutrients differ among strains and experimental purposes [64]. Because nutrient availability, medium stability, and contamination risk are context-dependent, supplementation schedules should follow a validated cultivation protocol rather than a universal prescription for all *Leishmania* strains [64].

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

An experimental study found that ILL could grow in Luria–Bertani (LB) broth at approximately 24–26°C and that recombinant protein expression was measurable under the conditions examined [65]. Growth in LB was broadly comparable to RPMI 1640 in that study, while protein expression findings were specific to the investigated construct and culture conditions. These laboratory results support investigating inexpensive cultivation formulations, but the authors did not establish industrial yields, validated large-scale processes, or a reduction in cost per gram of purified functional product [65].

Expression levels in various strains

Reported recombinant protein titers vary greatly with product identity, host genotype, expression strategy, cultivation mode, and whether yield refers to intracellular expression, secreted protein, purified protein, or biologically active product. High titers have been reported for selected proteins in engineered *E. coli*, but individual results should not be treated as universal host-level maxima [66]. Similarly, modern yeast bioprocesses can be highly productive, but direct comparisons require harmonized definitions of titer, volumetric productivity, purity, and quality attributes [67].

CHO manufacturing platforms can achieve substantial titers under optimized industrial conditions, particularly for established antibody products [68]. However, these results cannot be compared directly with research-scale *Leishmania* studies unless expression constructs, culture duration, product class, assay methods, purification recovery, and functional activity are taken into account.

Mammalian-cell production can entail expensive media, complex facility requirements, and extensive release testing, but these costs should be evaluated at the level of the full manufacturing process. Viral-safety controls and product-quality testing are integral to many regulated biopharmaceutical processes rather than disadvantages unique to mammalian cells [69].

Expression levels in P10

Published reviews of *L. tarentolae* report recombinant protein titers commonly within approximately 0.1–30 mg/L for certain older expression configurations, while some optimized protein-specific examples have reached much higher levels. These figures describe heterogeneous studies and must not be interpreted as a validated typical range or performance guarantee for P10 [70]. In one experimental study, an assembled anti-IL-2Rα antibody of approximately 150 kDa was produced in the P10 expression system; the reported concentration was about 2 µg/mL after 48 h under the studied conditions [71]. Production and preliminary functional evaluation of this antibody do not establish clinical efficacy or regulatory suitability.

Expression levels in ILL

Recombinant human coagulation factor VII (FVII) was expressed in ILL in an early study, with a reported concentration of approximately 10 µg per 10 mL (1 µg/mL) [72]. Other studies have reported FVII production in insect and mammalian cell systems [73–75], but differences in protein design, harvest timing, culture format, analytical assay, and specific activity prevent reliable ranking of the hosts from these isolated values. Importantly, active FVII depends on vitamin K-dependent γ-carboxylation of glutamate residues in its Gla domain. An early ILL study estimated a functional fraction of approximately 9% and noted that γ-carboxylation required further characterization [72]. Consequently, detection of FVII antigen, an apparently appropriate molecular mass, or a nominal titer does not establish correct PTMs or therapeutic potency. In hosts with insufficient endogenous γ-carboxylation capacity, engineering of the relevant carboxylation and vitamin K recycling pathways may be necessary, but the exact requirements must be determined experimentally.

Because direct side-by-side comparisons of ILL and commercial *L. tarentolae* strains under standardized conditions are lacking, available reports cannot establish equivalence or superiority. The platform's potential should be assessed across multiple product classes using matched constructs and cultivation conditions, with measurements of purified functional titer, specific activity, PTM profiles, yield consistency, and downstream recovery [59, 65, 70, 72].

Human IL-12 expression has been investigated in ILL using an engineered secretion construct, with one study reporting an estimated concentration of approximately 0.247 ng/mL (247 pg/mL) [76]. A subsequent study investigated transgenic ILL expressing human IL-12 in a BALB/c mouse model of experimental cutaneous leishmaniasis and reported partial protection, with reduced lesion progression and parasite burden but without sterile immunity, under its experimental conditions [77]. This finding should not be generalized to human vaccination or therapeutic protein manufacture. Recombinant IL-29 and amelogenin have also been reported in ILL at approximately 75 ng/mL [78] and 4 µg/mL [79], respectively. These substantially different titers illustrate the product dependence of ILL expression. Further work should test optimized vectors and strains, quantify product activity and glycoforms, and determine whether improvements persist in controlled scale-up studies.

CONCLUSION

A successful recombinant protein expression host must deliver adequate quantities of a product with the required identity, folding, purity, biological activity, and PTM profile. Bacterial and yeast hosts are higly

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

effective for many products, including numerous proteins that do not require human-type glycosylation. Mammalian cells remain important for complex biopharmaceuticals, although no host guarantees native-equivalent glycosylation or immunological safety without product-specific evaluation.

Leishmania tarentolae offers an experimentally useful combination of eukaryotic folding machinery, relatively accessible cultivation, and genetic tools. Nevertheless, its naturally produced glycans differ in important respects from human glycoforms, and the available literature contains considerable product-to-product variation in expression yield. The commercial maturity of CHO production cannot be assumed for *Leishmania*.

ILL provides an additional research model, with preliminary evidence of expression of several recombinant proteins. Current publications do not demonstrate that ILL produces a consistently higher yield or lower cost per gram of active protein than established microbial or mammalian hosts. In particular, between-study concentration comparisons do not compensate for differences in protein identity, analytical methods, scale, and biological activity.

Priorities for further development include standardized strain and vector characterization, appropriate RNA-processing elements, targeted control of proteolysis, secretion optimization, product-specific glycoengineering, and reproducible comparisons of functional titers. For proteins such as FVII, verification of specialized PTMs is indispensable. Biosafety, batch consistency, purification, and downstream economics should be evaluated alongside expression levels.

In conclusion, *L. tarentolae* and ILL are promising platforms for selected laboratory-scale recombinant proteins and may support future specialized manufacturing applications. Their wider adoption requires rigorous product-quality testing, directly comparable bioprocess data, and evidence of reproducible scale-up. Their current promise should be presented as an experimentally testable opportunity rather than an established industrial advantage.

ACKNOWLEDGMENT

This review was developed from work associated with Afshin Abdi Ghavidel's Ph.D. thesis at the Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors thank the Department of Development and Regeneration, Stem Cell Biology and Embryology, KU Leuven Stem Cell Institute, Leuven, Belgium.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest associated with this manuscript.

FUNDING

This work was supported by the Vice-Chancellor's Office for Research, Shahid Beheshti University of Medical Sciences (grant 18369).

AI DISCLOSURE

The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

DATA AVAILABILITY

No new experimental datasets were generated or analyzed for this narrative review. The article summarizes previously published literature; relevant source information is available in the cited publications.

AUTHORS' CONTRIBUTIONS

AAG drafted the manuscript; MB reviewed the manuscript; BK conceived and supervised the project.

ETHICS STATEMENT

This study received clearance from the Institutional Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1398.092). This narrative review did not involve new experiments on human participants or animals.

REFERENCES

1. Itakura K, Hirose T, Crea R, Riggs AD, Heyneker HL, Bolivar F, et al. Expression in *Escherichia coli* of a chemically synthesized gene for the hormone somatostatin. Science. 1977; 198(4321):1056-63.
2. Zhang ZX, Nong FT, Wang YZ, Yan CX, Gu Y, Song P, et al. Strategies for efficient production of recombinant proteins in *Escherichia coli*: alleviating the host burden and enhancing protein activity. Microb Cell Fact. 2022; 21(1):191.
3. Menon RK, Trucco M, Majzoub JA, Stratakis CA. Molecular endocrinology, endocrine genetics, and precision medicine. In: Sperling MA, editor. Sperling pediatric endocrinology. 5th ed. Philadelphia: Elsevier; 2021. p. 9-29.
4. Puetz J, Wurm FM. Recombinant proteins for industrial versus pharmaceutical purposes: a review of process and pricing. Processes. 2019; 7(8):476.
5. Zhang J, Du C, Pan Y, Zhang Z, Feng R, Ma M, et al. Optimization of a novel expression system for recombinant protein production in CHO cells. Sci Rep. 2024; 14(1):24913.
6. Jain T, Suntharalingam P, Cooney JM, Davidowitz RA. Mammalian cell culture in biopharmaceutical manufacturing: a historical perspective. Biotechnol Prog. 2022; 38(6):e3302.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

7. Puente-Massaguer E, Gòdia F. Recombinant protein production in suspension mammalian cells using the BacMam baculovirus expression system. Methods Mol Biol. 2024; 2829:329-39.
8. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. Front Microbiol. 2014; 5:172.
9. Neef J, van Dijl JM, Buist G. Recombinant protein secretion by *Bacillus subtilis* and *Lactococcus lactis*: pathways, applications, and innovation potential. Essays Biochem. 2021; 65(2):187-95.
10. Kim H, Yoo SJ, Kang HA. Yeast synthetic biology for the production of recombinant therapeutic proteins. FEMS Yeast Res. 2015; 15(1):1-16.
11. De Brabander P, Uitterhaegen E, Delmulle T, De Winter K, Soetaert W. Challenges and progress towards industrial recombinant protein production in yeasts: a review. Biotechnol Adv. 2023; 64:108121.
12. Vieira Gomes AM, Souza Carmo T, Silva Carvalho L, Mendonça Bahia F, Parachin NS. Comparison of yeasts as hosts for recombinant protein production. Microorganisms. 2018; 6(2):41.
13. Tang H, Wang S, Wang J, Song M, Xu M, Zhang M, et al. N-hypermannose glycosylation disruption enhances recombinant protein production by regulating secretory pathway and cell wall integrity in *Saccharomyces cerevisiae*. Sci Rep. 2016; 6:25654.
14. Hamilton SR, Gerngross TU. The glycosylation potential of yeast: using yeast species to produce human glycoproteins. Appl Microbiol Biotechnol. 2007; 74(4):735-50.
15. Gerngross TU, Smith KA. Posttranslational modifications in *Saccharomyces cerevisiae* and *Pichia pastoris*. Methods Enzymol. 2003; 373:154-74.
16. Huang M, Wang G, Qin J, Petranovic D, Nielsen J. Engineering the protein secretory pathway of *Saccharomyces cerevisiae* enables improved protein production. Proc Natl Acad Sci U S A. 2018; 115(47):E11025-32.
17. Sharp PM, Cowe E. Synonymous codon usage in *Saccharomyces cerevisiae*. Yeast. 1991; 7(8):657-76.
18. Pan Y, Yang J, Wu J, Yang L, Fang H. Current advances of *Pichia pastoris* as cell factories for production of recombinant proteins. Front Microbiol. 2022; 13:1059777.
19. Gerngross TU. Advances in the production of human therapeutic proteins in yeasts and filamentous fungi. Nat Biotechnol. 2004; 22(11):1428-37.
20. Zahrl RJ, Peña DA, Mattanovich D, Gasser B. Systems biotechnology for protein production in *Pichia pastoris*. FEMS Yeast Res. 2017; 17(7):fox097.
21. Tripathi NK, Shrivastava A. Recent developments in bioprocessing of recombinant proteins: expression hosts and process development. Front Bioeng Biotechnol. 2019; 7:420.
22. Nevalainen H, Peterson R. Making recombinant proteins in filamentous fungi—are we expecting too much? Front Microbiol. 2014; 5:75.
23. Liu D, Garrigues S, de Vries RP. Heterologous protein production in filamentous fungi. Appl Microbiol Biotechnol. 2023; 107(16):5019-33.
24. Yee CM, Zak AJ, Hill BD, Wen F. The coming age of insect cells for manufacturing and development of protein therapeutics. Ind Eng Chem Res. 2018; 57(31):10061-70.
25. Jarvis DL, Jarvis JA. Baculovirus expression vectors: a retrospective and prospectus. Protein Expr Purif. 2017; 125:1-12.
26. Yadav M, Sarkar S, Kumar A. Diversifying expression platforms for biopharmaceuticals and industrial enzymes. In: Verma P, editor. Industrial microbiology and biotechnology: a new horizon of the microbial world. 1st ed. Singapore: Springer Nature Singapore; 2024. p. 573-603.
27. Yagi H, Yanaka S, Yogo R, Ikeda A, Onitsuka M, Yamazaki T, et al. Silkworm pupae function as efficient producers of recombinant glycoproteins with stable-isotope labeling. Biomolecules. 2020; 10(11):1482.
28. Xu H. The advances and perspectives of recombinant protein production in the silk gland of silkworm *Bombyx mori*. Transgenic Res. 2014; 23(5):697-706.
29. Shepelev MV, Kalinichenko SV, Deykin AV, Korobko IV. Production of recombinant proteins in the milk of transgenic animals: current state and prospects. Acta Naturae. 2018; 10(3):40-7.
30. Maksimenko OG, Deykin AV, Khodarovich YM, Georgiev PG. Use of transgenic animals in biotechnology: prospects and problems. Acta Naturae. 2013; 5(1):33-46.
31. Feng Z, Li X, Fan B, Zhu C, Chen Z. Maximizing the production of recombinant proteins in plants: from transcription to protein stability. Int J Mol Sci. 2022; 23(21):13516.
32. Warzecha H, Mason S, Stöger E. Recombinant protein expression in plants for medical applications. Curr Opin Biotechnol. 2023; 84:102977.
33. Strasser R, Gomord V. Potential of plant-based systems for recombinant protein production. Essays Biochem. 2021; 65(2):241-53.
34. Moon KB, Park JS, Park YI, Song IJ, Lee HJ, Cho HS, et al. Development of systems for the production of plant-derived biopharmaceuticals. Plants (Basel). 2020; 9(1):30.
35. Capell T, Christou P. Progress in plant molecular farming. Curr Opin Biotechnol. 2004; 15(2):172-6.
36. Mark JKK, Lim CSY, Nordin F, Tye GJ. Expression of mammalian proteins for diagnostics and therapeutics: a review. Mol Biol Rep. 2022; 49(11):10593-608.
37. Griffiths AD. Mammalian cell culture: fundamentals and applications. 2nd ed. Berlin: Springer; 2000.
38. Khan KH. Gene expression in mammalian cells and its applications. Adv Pharm Bull. 2013; 3(2):257-63.
39. Bari AU. Chronology of cutaneous leishmaniasis: an overview of the history of the disease. J Pak Assoc Dermatol. 2017; 27(1):24-27.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

40. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. Leishmaniasis: a review. F1000Res. 2017; 6:750.

41. Raymond F, Boisvert S, Roy G, Ritt JF, Légaré D, Isnard A, et al. Genome sequencing of the lizard parasite *Leishmania tarentolae* reveals loss of genes associated to the intracellular stage of human pathogenic species. Nucleic Acids Res. 2012; 40(3):1131-47.

42. Mendoza-Roldan JA, Votypka J, Bandi C, Epis S, Modry D, Ticha L, et al. *Leishmania tarentolae*: a new frontier in the epidemiology and control of the leishmaniases. Transbound Emerg Dis. 2022; 69(5):e1326-37.

43. Basile G, Peticca M. Recombinant protein expression in *Leishmania tarentolae*. Mol Biotechnol. 2009; 43(3):273-8.

44. Senra RL, Pereira HS, Schittino LMP, Fontes PP, de Oliveira TA, Ribon AOB, et al. Co-expression of human sialyltransferase improves N-glycosylation in *Leishmania tarentolae* and optimizes the production of humanized therapeutic glycoprotein IFN-β. J Biotechnol. 2024; 394:24-33.

45. Chia S, Tay SJ, Song Z, Yang Y, Walsh I, Pang KT. Enhancing pharmacokinetic and pharmacodynamic properties of recombinant therapeutic proteins by manipulation of sialic acid content. Biomed Pharmacother. 2023; 163:114757.

46. Abdi Ghavidel A, Jajarmi V, Bandehpour M, Kazemi B. Polycistronic expression of multi-subunit complexes in the eukaryotic environment: a narrative review. Iran J Parasitol. 2022; 17(3):286-95.

47. Jones JD. *Leishmania tarentolae*: an alternative approach to the production of monoclonal antibodies to treat emerging viral infections. Infect Dis Poverty. 2015; 4:8.

48. Zimna M, Krol E. *Leishmania tarentolae* as a platform for the production of vaccines against viral pathogens. NPJ Vaccines. 2024; 9 (1): 212.

49. Medina AC, Acevedo Ospina H, Descoteaux A. Immunomodulatory properties of *Leishmania tarentolae* extracellular vesicles containing the spike protein of SARS-CoV-2. Front Parasitol. 2024; 3:1306478.

50. Blum B, Bakalara N, Simpson L. A model for RNA editing in kinetoplastid mitochondria: "guide" RNA molecules transcribed from maxicircle DNA provide the edited information. Cell. 1990; 60(2):189-98.

51. White TC, Fase-Fowler F, van Luenen H, Calafat J, Borst P. The h circles of *Leishmania tarentolae* are a unique amplifiable system of oligomeric DNAs associated with drug resistance. J Biol Chem. 1988; 263(32):16977-83.

52. LeBowitz JH, Coburn CM, McMahon-Pratt D, Beverley SM. Development of a stable *Leishmania* expression vector and application to the study of parasite surface antigen genes. Proc Natl Acad Sci U S A. 1990; 87(24):9736-40.

53. Johnston WA, Alexandrov K. Production of eukaryotic cell-free lysate from *Leishmania tarentolae*. Methods Mol Biol. 2014; 1118:1-15.

54. Alfaro-Palma J, Johnston WA, Behrendorff J, Cui Z, Varasteh Moradi S, Alexandrov K. Development of lyophilized eukaryotic cell-free protein expression system based on *Leishmania tarentolae*. ACS Synth Biol. 2024; 13(2):449-56.

55. Breitling R, Klingner S, Callewaert N, Pietrucha R, Geyer A, Ehrlich G, et al. Non-pathogenic trypanosomatid protozoa as a platform for the production of eukaryotic proteins. Protein Expr Purif. 2002; 25(2):209-18.

56. Kazemi B, Tahvildar-Bideroni GH, Hashemi Feshareki SR, Javadian E. Isolation of a lizard *Leishmania* promastigote from its natural host in Iran. J Biol Sci. 2004; 4:620-3.

57. Ehya F, Kalantari S, Bandehpour M, Kazemi B. Preliminary information of Iranian Lizard *Leishmania* promastigote transcriptome sequencing by next-generation sequencing (NGS) method. Iran J Parasitol. 2023; 18(3):362-8.

58. Hieronimus K, Donauer T, Klein J, Hinkel B, Spänle JV, Probst A, et al. A modular cloning toolkit for the production of recombinant proteins in *Leishmania tarentolae*. Microb Cell. 2024; 11:128-42.

59. Abdi Ghavidel A, Bandehpour M, Noori E, Jajarmi V, Kazemi B. Comparing the yield of recombinant human factor VII protein expressed by the rDNA-promoter with the CMV-promoter in Iranian Lizard *Leishmania*. Iran J Parasitol. 2024; 19(2):192-202.

60. Klatt S, Simpson L, Maslov DA, Konthur Z. *Leishmania tarentolae*: taxonomic classification and its application as a promising biotechnological expression host. PLoS Negl Trop Dis. 2019; 13(7):e0007424.

61. Abdi Ghavidel A, Aghamiri S, Raee P, Mohammadi-Yeganeh S, Noori E, Bandehpour M, et al. Recent advances in CRISPR/Cas9-mediated genome editing in *Leishmania* strains. Acta Parasitol. 2024; 69:121-34.

62. Kushnir S, Gase K, Breitling R, Alexandrov K. Development of an inducible high-yield protein expression system for *Leishmania tarentolae*. Protein Expr Purif. 2005; 42(1):37-46.

63. Cattaneo GM, Varotto-Boccazzi I, Molteni R, Ronchetti F, Gabrieli P, Mendoza-Roldan JA, et al. A novel chemically defined medium for the biotechnological and biomedical exploitation of the cell factory *Leishmania tarentolae*. Sci Rep. 2024; 14(1):9562.

64. de Oliveira Filho VA, Garcia MSA, Rosa LB, Giorgio S, Miguel DC. An overview of *Leishmania* in vitro cultivation and implications for antileishmanial screenings against promastigotes. Parasitologia. 2024; 4(4):305-18.

65. Abdi Ghavidel A, Aghamiri S, Jajarmi V, Bandehpour M, Kazemi B. The influence of different culture media on the growth and recombinant protein production of Iranian Lizard *Leishmania* promastigote. Iran J Parasitol. 2022; 17(4):543-53.

66. Choi J, Keum KC, Lee SY. Engineering of *Escherichia coli* for enhanced production of recombinant proteins. Biotechnol Adv. 2006; 24(5):480-90.

67. Lv W, Cai M. Advancing recombinant protein expression in *Komagataella phaffii*: opportunities and challenges. FEMS Yeast Res. 2025; 25:foaf010.

68. Li ZM, Fan ZL, Wang XY, Wang TY. Factors affecting the expression of recombinant protein and improvement strategies in Chinese hamster ovary cells. Front Bioeng Biotechnol. 2022; 10:880155.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

69. Hamdi A, Széliová D, Ruckerbauer DE, Rocha I, Borth N, Zanghellini J. Key challenges in designing CHO chassis platforms. Processes. 2020; 8:643.

70. de Oliveira TA, Silva WD, da Rocha Torres N, Badaró de Moraes JV, Senra RL, de Oliveira Mendes TA, et al. Application of the Lexsy *Leishmania tarentolae* system as a recombinant protein expression platform: a review. Process Biochem. 2019; 87:164-73.

71. Jalali A, Bandehpour M, Chegeni R, Ghanbarian H, Mardani-Dashti Y, Zarkesh-Esfahani SH, et al. Expression, purification, and evaluation of anti-IL-2Rα antibody secreted by *Leishmania tarentolae*. Int J Pept Res Ther. 2021; 27(1):301-7.

72. Mirzaahmadi S, Asaadi-Tehrani G, Bandehpour M, Davoudi N, Tahmasbi L, Hosseinzadeh N, et al. Expression of recombinant human coagulation factor VII by the lizard *Leishmania* expression system. J Biomed Biotechnol. 2011; 2011:873874.

73. Masroori N, Halabian R, Mohammadipour M, Roushandeh AM, Rouhbakhsh M, Najafabadi AJ, et al. High-level expression of functional recombinant human coagulation factor VII in insect cells. Biotechnol Lett. 2010; 32(6):803-9.

74. Berkner KL, Busby S, Davie E, Hart C, Insley M, Kisiel W, et al. Expression of high levels of functional human vitamin K-dependent protein in mammalian cells: factor VII. Cold Spring Harb Symp Quant Biol. 1986; 51 Pt 1:531-41.

75. Wajih N, Owen J, Wallin R. Enhanced functional recombinant factor VII production by HEK 293 cells stably transfected with VKORC1 where the gamma-carboxylase inhibitor calumenin is stably suppressed by shRNA transfection. Thromb Res. 2008; 122(3):405-10.

76. Donyavi T, Bandehpour M, Kazemi B. Preparation of transgenic Iranian Lizard *Leishmania* coding HIL-12. Iran J Microbiol. 2017; 9(5):305-11.

77. Donyavi T, Chegeni R, Bandehpour M, Mohammadi-Yeganeh S, Zahedi-Zavaram H, Kazemi B, et al. Evaluation of transgenic Iranian lizard *Leishmania* encoding human IL-12 as a candidate vaccine against experimental cutaneous leishmaniasis in susceptible BALB/c mice. J Med Microbiol Infect Dis. 2026; 14(2):115-23.

78. Taromchi AH, Kazemi B, Mahmazi S, Bandehpour M. Heterologous expression of human IL-29 (IFN-λ1) in Iranian Lizard *Leishmania*. Iran J Biotechnol. 2013; 11(3):168-74.

79. Yadegari Z, Bandehpour M, Kazemi B, Sharifi-Sarasiabi K. Expression of recombinant human Amelogenin in Iranian Lizard *Leishmania* and its biological function assay. Iran J Public Health. 2015; 44(7):987-96.

Cite this article:
Abdi Ghavidel A, Bandehpour M, Kazemi B. *Leishmania*: A Promising Expression Host for Eukaryotic Recombinant Proteins. J Med Microbiol Infect Dis, 2026; 14 (3): 211-220.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]