Journal of Bioresource Management

Volume 9 | Issue 1 Article 16

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Naz, Z., Kanwal, A., & Tayyeb, A. (2022). A Review on Empirical Approach to Therapeutic Recombinant Protein Production Factories: Applications, Pharmacokinetics and Challenges, *Journal of Bioresource Management, 9* (1).
ISSN: 2309-3854 online
(Received: Aug 16, 2021; Accepted: Sep 28, 2021; Published: Mar 24, 2022)

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A REVIEW ON EMPIRICAL APPROACH TO THERAPEUTIC RECOMBINANT PROTEIN PRODUCTION FACTORIES: APPLICATIONS, PHARMACOKINETICS AND CHALLENGES

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ABSTRACT

Major portion of the proteins in a cell refers to their critical roles in metabolism of the living body. Proteins dysfunction results in the severe disorders which need to be treated in time. For this, respective proteins can be extracted by the external sources and be supplied to the human beings. Such medicinal proteins are known as therapeutic proteins (TPs). This review summarizes some of the key areas about these miracle proteins like their types, classification, production factories, routes of administration, pharmacokinetics, and potential applications. Furthermore, challenges and hurdles faced in getting a potential protein drug, its FDA approval and commercial availability are also highlighted for further research.

Keywords: Recombinant protein, therapeutic protein, production factories, medicinal proteins, pharmacokinetics.

INTRODUCTION

Proteins have diverse and dynamic role in the living body, building of cell structure, formation of cellular channels, receptors, catalyzing biological reactions, molecular transportation and defense mechanisms (Akash et al., 2015). Malfunctioning of this main component of a living body could lead to harmful diseases. Proteins and small peptides have been identified for showing great potential for treatment of syndromes and diseases. These protein-based drugs are named as bio-drugs or biopharmaceuticals (Antosova et al., 2009). These drug candidates can either replace or inactivate the abnormal protein in a particular disorder. Moreover, some TPs can act by augmenting the supply of beneficial protein to the body in order to reduce the impact of disease. That is why, protein-based therapeutics are constantly being worked upon as novel entities in the life sciences and pharmaceutical industries (Gharelo et al., 2016).

The history of protein therapeutics begins with isolation of insulin from bovine in 1922 (Leader et al., 2008). Later the insulin (1983) was obtained using the recombinant DNA technology. This opened the doors for research for developing new therapeutic products and studies regarding quality regulations (Kar 2008, Fuh et al., 2016). Until now, more than 150 protein-based drugs have been approved by FDA for various diseases (Ozgur and Tutar 2013, Lagassé et al., 2017).

Unlike the small biomolecules which are routinely synthesized via chemical methods in lab, synthesis of TPs and peptides have developed using recombinant DNA technology (Kar 2008, Akash et al., 2015). Credit to the molecular biology, these protein products can be expressed and obtained by using genetically modified microbial (bacteria/yeast) or mammalian cell-based host systems, substantially reducing the downstream stages of the process (Niazi, 2002). Moreover, using the genetically modified host and protein engineering techniques, researchers aimed to obtain the protein in its native functional form under optimized environmental conditions. Till the date, recombinant technologies have contributed

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in manufacturing and commercializing various TPs. These have proven effective in treating various disorders (Chennamsetty et al., 2009) such as metabolic disorders (Roberts, 2014), cancer (Adler and Dimitrov, 2012), diabetes (Leader et al., 2008), Phenylketonuria (PKU) (Russell and Clarke, 1999), anemia, hepatitis (Dipti et al., 2006, Xu et al., 2011), heart (Ho et al., 2018) and chronic diseases (Kar 2008, Karvar, 2014).

Recombinant TPs possess several advantages over chemical-based proteins. Proteins possess the property of binding specificity and acting selectivity so there is fewer chance for the protein drug to affect with the normal biological process and cause the damage to body (Fei Wen et al., 2009). In addition, proteins are usually well tolerated by the body so TPs have low potential to induce an immune response. Considering time duration, TPs also dominate chemical drugs by requiring less time for FDA approval. As the protein drugs are unique in function, so it is easy for companies to get patent protection for longer period of time, making protein industry attractive for investors and researchers (Leader et al., 2008, Gharelo et al., 2016,).

Classification of Therapeutic Proteins

TPs exhibit a wide range of variations in terms of their properties and therapeutic applications. Based on these variations, protein-based therapeutics can be differently categorized such as based on their pharmacological activities, molecular type and molecular mechanism as shown in Figure 1. On the basis of pharmacological activities, proteins are categorized into five categories: (i) interfering with the organism or molecule; (ii) providing a novel activity or function; (iii) augmenting an existing pathway; (iv) replacing an abnormal or deficient protein; and (v) conjugation to deliver other proteins or compounds (effector proteins, radionuclide or drugs) to the site of action (Leader et al., 2008, Buchanan and Revell 2015).

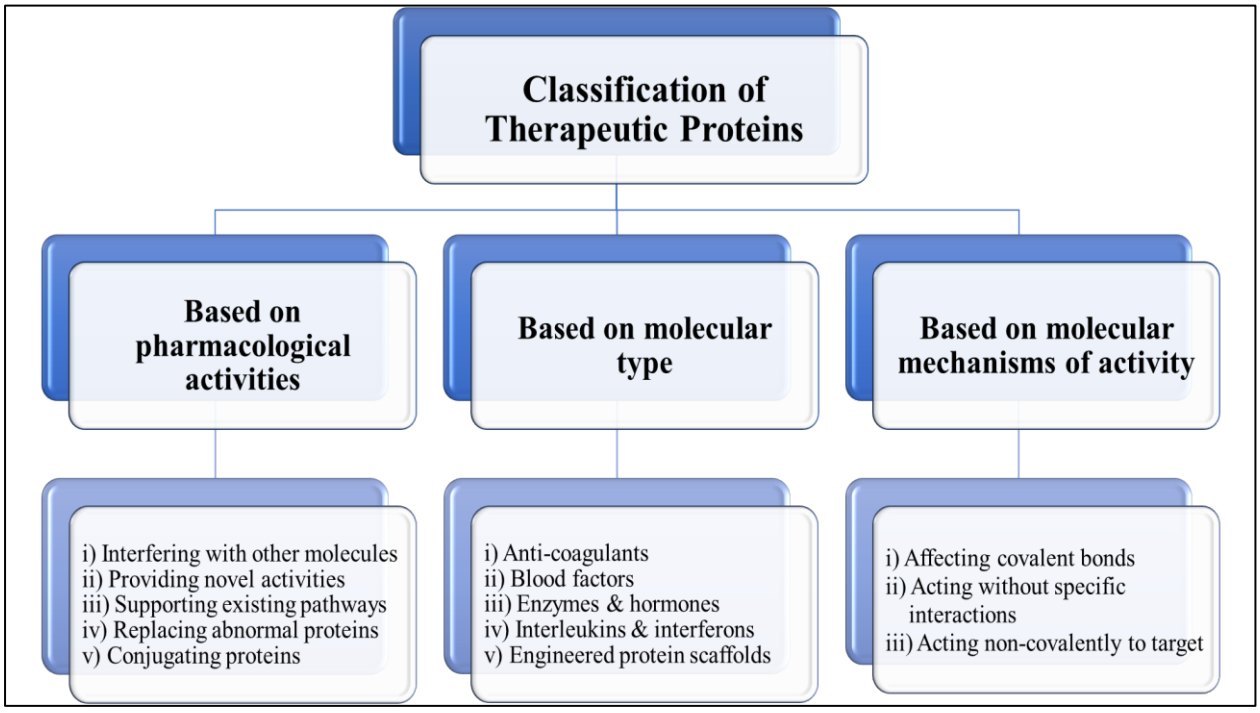

Figure 1: Classifications of TPs based on their activity and type.

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Another way to classify the TPs is based on their molecular types. Here, these drugs can be separated into anti-coagulants, enzymes, hormones, interleukins, blood factors, interferons, engineered protein scaffolds and thrombolites (Nicolaides et al., 2010, Carter, 2011).

TPs are also congregated on the basis of their molecular mechanism as (a) affecting covalent bonds with substrates, e.g., enzymes; (b) activity without specific interactions, e.g., serum albumin and (c) binding non-covalently to target, e.g., monoclonal antibodies (mAbs) (Dimitrov, 2012).

On the basis of pharmacological activity of proteins, Leaders categorize proteins in four groups (Figure 2). Group I and II contain FDA approved proteins which are used in disease treatments and therapies. While group III and IV are protein drugs which are used as vaccines and diagnostic agents. Group I is further divided in sub-group Ia, Ib and Ic that refer to the proteins which replace deficient protein, change a prevailing pathway or do a unique novel activity, respectively (Dimitrov 2012, Akash et al., 2015).

Group Ia is specified for proteins needed to treat protein deficiency-based endocrine dysfunctions like hemophilia A or type 2 diabetes mellitus. Group Ib includes the proteins that are known to augment body endocrine pathways like interferon-α (Akash et al., 2015). While the group Ic is dominated by the proteins that are utilized to modify pathophysiology of human disorders like lepirudin for heparin-induced thrombocytopenia (Adkins and Wilde, 1998, Petros 2008).

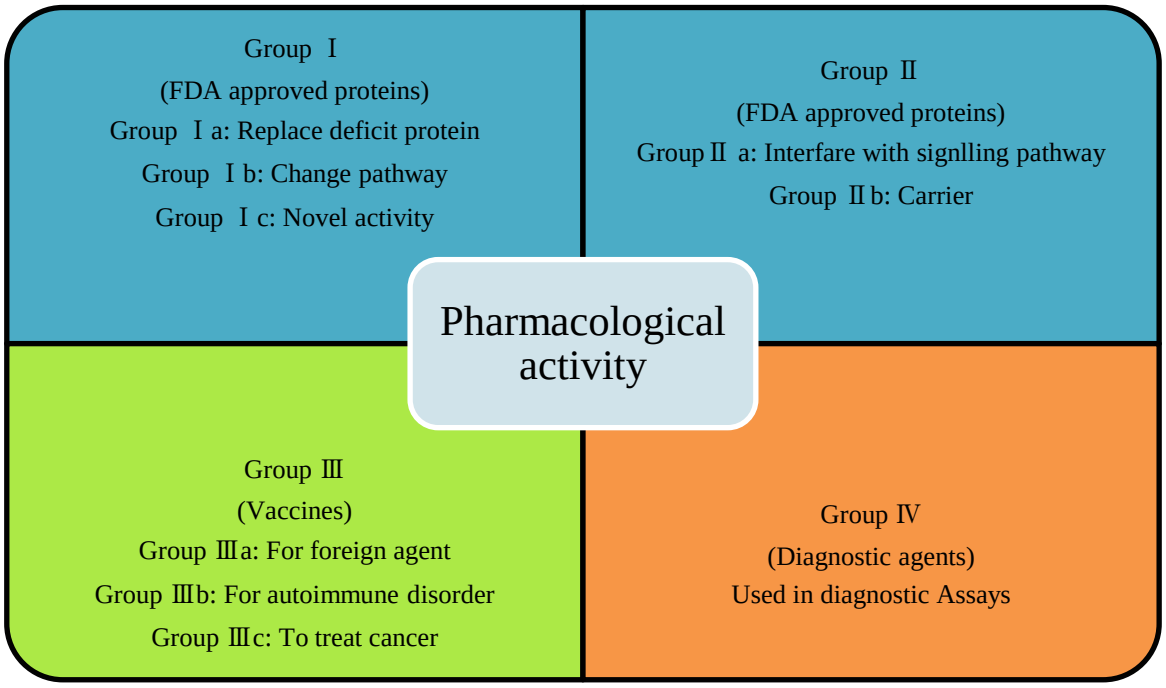

Figure 2: Classification of TPs on the basis of their pharmacological activity.

Group II contains the proteins with special targeting activity. Proteins in sub-group IIa are known to interfere with a molecule in the body. These proteins (i.e., monoclonal antibodies) do so by binding to specific targets and then modifying their function, hence contributing to stimulate or inhibit a signaling pathway. While the proteins in sub-group IIb act as a carrier to deliver other proteins or molecules to the targeted site of action (Sutradhar et al., 2011). The examples include the denileukin diftitox and ibritumomab tiuxetan that are used for treatment of cutaneous T-cell lymphoma and transformed non-Hodgkin's lymphoma, respectively (Leader et al., 2008, Akash et al., 2015).

Group III is a small class that categorizes protein therapeutic and prophylactic vaccines. The sub-groups IIIa, IIIb and IIIc contain proteins that act to protect against a

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harmful foreign agent, overcome an autoimmune syndrome or treat cancer, respectively. Proteins in this class may possess recombinant, purified or synthetic components (Lagassé et al., 2017). Moreover, this class possesses the potential for production of broad-spectrum recombinant vaccines against infectious diseases. List of vaccines approved by FDA are given at: http://www.fda.gov/cber/vaccine/licvacc.htm.

The group IV represents a collection of diagnostic proteins affecting clinical decisions. These can either act as imaging agents to detect cancer or diagnostic agents to detect infectious diseases. These proteins are utilized in a wide range of diagnostic assays including flow cytometry, matrix assisted laser desorption/ionization (MALDI), immunohistochemistry (IHC), protein microarrays and enzyme linked-immunosorbent assay (ELISA) (Lin, 2010; Powers and Palecek, 2012).

Most of the protein drugs are commercially available as recombinant while many of these are in clinical trials to offer differences and better potential over the others. Some of the newly engineered proteins i.e. multispecific fusion proteins are currently under research and development (Carter 2011; Dimitrov, 2012).

Production Factories for TPs

Formulation and production of protein therapeutics have to face various technical and critical challenges from those set by the traditional methods for small molecules or drugs (Fuh et al., 2016). There are a number of possible protein producing systems including plants, microbes, fungi, animal cell cultures and transgenic animals.

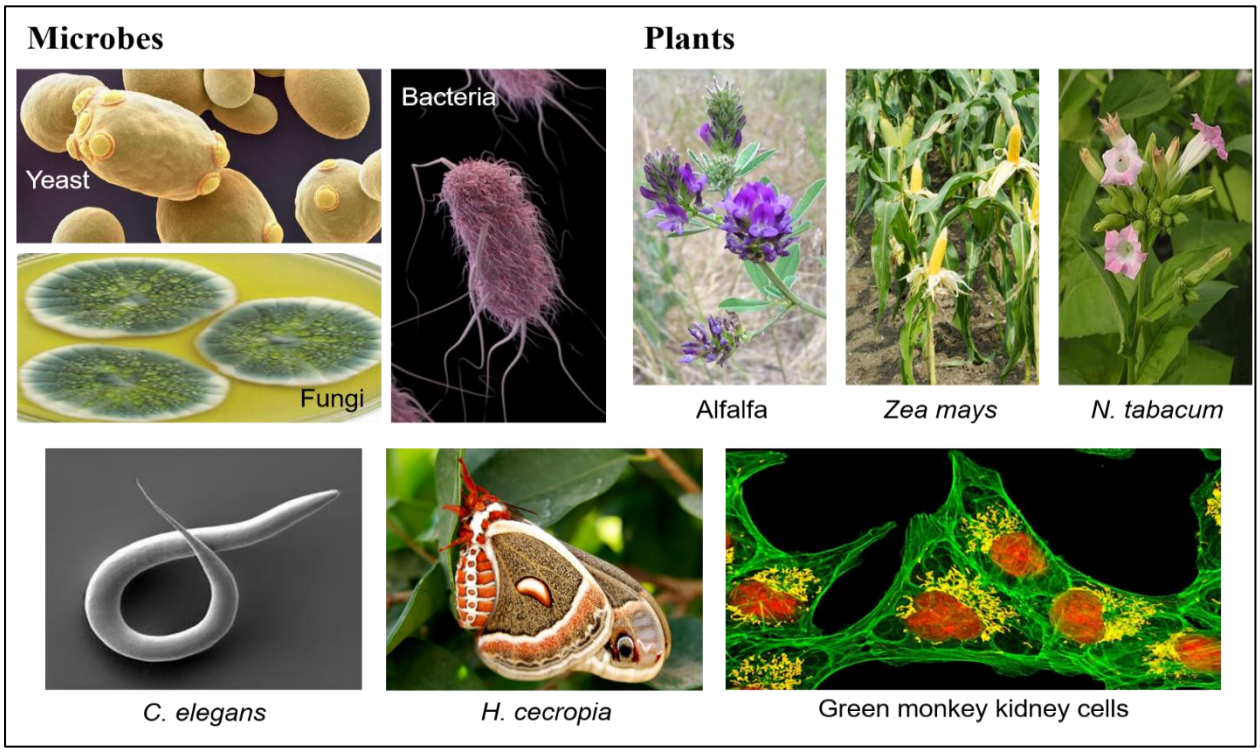

Figure 3: Examples of the organisms used to obtain TPs.

Proteins smaller in sizes are usually expressed in prokaryotes while larger proteins in eukaryotes. Transgenic plants are utilized to produce various TPs (Tarafdar et al., 2014, Yao et al., 2015). Insect systems, mammalian cells and fungi are better for the proteins requiring glycosylation while prokaryotic systems such as *E. coli* are preferable for the cost effective, easiest and fastest expression of TPs and proteins. However, later is not suitable for the

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expression of either large sized and S-S enriched proteins or for the proteins requiring post translational modifications (PTMs). On the other hand, yeasts can give higher yields of native glycosylated proteins (~50 kDa) at lower costs (Demain and Vaishnav, 2009). Mammalian cell lines are most frequently used for the assembly of recombinant mammalian proteins. Genetically modified animals can also be exploited for the production of TPs which they may secrete in their blood, urine or milk (Jazayeri et al., 2018, Hunter et al., 2019) (Figure 3).

At present, availability of the information regarding the cell's physiology, its metabolism and the factors affecting its protein production and gene expression has endowed the use of altered factories, namely eukaryotic or prokaryotic, plants or animals (Demain and Vaishnav, 2009).

Plants

Plant-based protein production systems include non-transgenic plants with recombinant viruses (Giddings et al., 2000) and transgenic plant cell cultures (Doran, 2000). Using plants reduces the investment which is required for large scale production of proteins like enzymes, biopharmaceuticals, technical materials and TPs (Burnett and Burnett, 2020). Secondary metabolites have major role in chemical defense in plants because of their antimicrobial and antioxidative properties thus molecular farming can be used for their production (Tohidi et al., 2017). Use of *in-vitro* cultured plant cells, tissues or whole plants for the synthesis of recombinant proteins is called molecular farming (Schillberg et al., 2013). For both the stable or transient gene expression, *Agrobacterium* mediated transformation and particle bombardment have been reported (Van Ooijen et al., 1996). Selective expression of gene of interest (GOI) for therapeutic protein can also be done by using tissue specific promoters. These promoters are of greater value because of their higher stability that upon induction results in greater yield (Hofbauer and Stoger, 2013). In this way, proteins can be recruited to specific cellular compartments like plastids, endoplasmic reticulum (ER), cytosol, apoplast or golgi apparatus. Secretory proteins are mainly synthesized by ribosomes on ER. Plant seed is an ideal machinery for the production of desired pharmaceutical proteins, cytokines, antibodies, vaccines and so on (Takaiwa et al., 2017). Advantages of compartment-specific protein expression include extraordinary stability and high yield. A number of seed specific promoters i.e. endosperm specific promoter, whole seed expression promoter, transfer cell specific promoter, and embryo expression-promoter have been utilized to direct the expression to specific tissues (Mohammadinejad et al., 2019).

Recombinant proteins (RPs) can be expressed selectively in *in-vitro* cultures of cells from roots, leaves, seeds, or stems. Targeting RPs to plastids results in a good protein yield but it does not do Post translational modifications (PTMs). That is why RPs can also be directed to endosperm or embryo in seeds (Mohammadinejad et al., 2019).

Lactuca sativa, Musa paradisiaca, Oryza sativa, Solanum tuberosum, Medicago sativa, Zea mays, Nicotiana tabacum, Nicotiana benthamiana, Glycine max, Triticum aestivum, Daucus carota, and Solanum lycopersicum are the preferred systems for therapeutic protein production (Sack et al., 2015). For biomaterial expression, plants can be genetically modified. Moreover, plant molecular farming has increased global interest in the production of nanostructures, pharmaceutical proteins or natural bioactive molecules (Sanchez and Demain, 2010).

Currently available chemical polymerization techniques synthesize variety of polymers as compared to plants which have access to only 20 amino acids (AA). Another drawback of plant based proteins is to prepare a sufficient amount of biomaterial to assess its functionality (Moire et al., 2003). This drawback has significantly been removed with the help of recent advancements in Biotechnology and gene transfer procedures. Now a days, safe transgenic plant proteins are available at reasonable prices but there are still the risks for gene escape and public acceptability

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(Bawa and Anilakumar, 2013). It is expected that in near future, plant-based techniques will undergo advancements making them an efficient alternative for the production of biomaterials which are normally extracted from animal cells.

Therapeutic protein production in plants possesses many advantages like lower production cost, lower risks of animal pathogen contamination and feasibility for large scale manufacturing (Nagels et al., 2012). Apart from this, animal cell expression systems are costly and prone to environmental changes. Mammalian proteins may not be synthesized in microbial cultures correctly because of absence of post translational modifications and codon usage biasness (Fischer and Emans, 2000). Plants also show minor differences in the usage of codons but these can be compensated by transgene sequence adjustment for the production of mammalian proteins (Hood et al., 1997). Protein synthesis can also be directed to seed endosperm to reduce expenses (Wright et al., 2001). Co-extraction of conventional products like oil or starch can also be done with the required protein of interest (Bai and Nikolov 2001). Extraction of edible vaccines from plants may not be feasible because of the short shelf life or need for optimizing antigen doses. For this, cheap and simple food processing procedure can be utilized (Giddings, 2001).

Microbes

Escherichia coli

Escherichia coli is the earliest and most preferred microorganism to express heterologous proteins, as around 30 % of the approved TPs are currently being produced in it. *E. coli* is an expression host of choice in Biotechnology industry for large-scale production of proteins, particularly non-glycosylated proteins (Terpe, 2006). It is used for the massive production of TPs because of improved genetic tools and a good understanding of its transcriptional and translational machinery. This bacterium can accumulate proteins up to 80 % of its dry weight. Proteins can be exported into periplasm or culture medium by increasing cell wall permeability or giving osmotic shocks. Promoter system should be strong and regulated so that they possess low basal expression. The *lac, tac, trc* promoters can be utilized for the enhanced productivity of proteins using simple and inexpensive media ingredients (Maksum et al., 2020).

Easy protein folding mechanisms and bioprocess technologies makes *E. coli* very attractive for industrial applications. However, codon biasness in *E. coli* and absence of post-translational modifications (PTMs), such as glycosylation, phosphorylation, and proteolytic processing, limits its use for production of complex recombinant biopharmaceuticals. Several technological advancements in *E. coli* expression system have been made, such as production of novel engineered strains, modification of *E. coli* to possess capability to glycosylate heterologous proteins, including full-length glycosylated antibodies (Baeshen et al., 2015).

There are a number of ways by which therapeutic protein production can be improved in *E. coli*. Different promoters can be used in different strains for expression regulation. Growth medium can be changed, temperature could be lowered. Further proteins could be secreted into medium or periplasmic space or chaperones and foldases could be co-expressed (Wong et al., 2008). In addition, fusion partner can be added, protein can be denatured or refolded or protein fragment could be expressed (Maldonado et al., 2007). Toxicity and acetate production could be reduced by glucose feeding. TPs produced in inclusion bodies are actually the aggregated, inactive, insoluble proteins possessing inter and intra molecular disulfide bonds and free cysteines. Inclusion bodies must be removed to obtain fully functional proteins. Solubilization can be improved by using denaturants and reducing agents for elimination of S-S bonds (Baeshen et al., 2015). Until 1993, this bacterium was consumed

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for the production of human growth hormone, G-CSF, α, β, γ-interferons and insulin (Swartz 1996). Human IL-3, human IL-6, human BMP-2, murine L1F, murine IL-2, murine SF, murine IL-5, murine IL-4, human M-CSL, human IL-11, and human M1P-l α are products which are synthesized in *E.coli* (Ahmadi and Pfeifer, 2016, Selas Castiñeiras et al., 2018).

Bacillus sp.

Gram-positive bacilli bacteria are also used for heterologous/homologous expression of enzymes. Using *Bacillus sp.*, recombinant proteins are expressed in its native form. Further, it exhibits better growth characteristics and robust metabolism without production of either exo or endo toxins; resulting in cost effectiveness of a therapeutic peptide. Secretion of proteins into medium eliminates the need for cell disruption, chemical processing and helps in downstream processing. *Bacillus*-based protein yields are very high but the formation of proteases may digest the recombinant protein (Pero and Sloma, 1993). About 96-98% extracellular protease activity is due to Neutral protease (metalloprotease) and Subtilisin (serine protease). Until now, six to eight extracellular proteases have been reported. In 2002, a strain lacking these eight extracellular proteases was developed using genetic engineering tools (Murashima et al., 2002). *Bacillus licheniformis* which is an exo-protease lacking host strain is specifically used for heterologous protein expression. *Bacillus brevis* is useful for production of TPs due to production of proteinase inhibitor (Udaka and Yamagata, 1994).

Till present, a number of proteins have been expressed in *Bacillus* systems including amylases, xylanases and interleukin-3EGF for the treatment of disorders. Trimeric TNF-α has been produced by *Pseudomonas fluorescens* in *Pf*enex system (Squires and Lucy, 2008).

Yeasts

To overcome the problems in re-folding and glycosylation of proteins in *E. coli*, single celled fungi are used for the expression of TPs. Yeast strains are well characterized and genetically stable. These give high density growth and good protein yield resulting in cost effectiveness. Its product processing is similar to that of mammalian cells. Post translational modifications can easily be done in these systems. Yeasts can handle S-S rich proteins, assist in glycosylation and protein folding (Gerngross, 2004). Rapid productivity using low-cost media makes the use of these small manufacturing facilities applicable. *Pichia pastoris* is used widely for the production of biopharmaceuticals due to understanding of its cell biology and ability to give clean product (Love et al., 2018). Various other yeast species such as *Saccharomyces cerevisiae* have also been utilized for therapeutic purposes. Yeasts have been proven useful for the production of insulin, glarigine and other monoclonal antibodies (mAbs) (Mengdai et al., 2020). As a cloning host, *S. cerevisiae* possesses certain advantages over bacteria including that it can carry glycosylation and can secrete heterologous proteins into medium by attaching signal sequences. But glycosylation with *S. cerevisiae* is unacceptable for mammalian proteins as O-linked oligosaccharides possess mannose only (Gellissen et al., 1992). This yeast may also cause immunological problems and possess reduction in activity and receptor binding over glycosylated N-linked sites. Therapeutic products which are synthesized using *S. cerevisiae* include urate oxidase, granulocyte macrophage colony stimulating factor (GM-CSF), insulin, glucagons, platelet-derived growth factor, hepatitis B surface antigen and hirudin (Gellissen et al., 1992). Recombinant yeast, mold, mammalian cells or insects can be used for glycosylation of human chorionic gonadotropin or erythropoietin (Saul et al., 1985). Fungal TPs show additional carbohydrates linked to oxygen moiety of threonine or serine (Nunberg et al., 1984). *Pichia pastoris* has been genetically modified and is used to produce anti-microbial peptides (AMP). This

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synthetic protein has been successfully isolated and proved useful against *E. coli*-based infections (Cao et al., 2018).

Filamentous Fungi

High levels of post translationally modified TPs can be achieved by using filamentous fungi such as *Aspergillus niger* (Ward et al., 2004). These fungi are attractive hosts for the production of synthetic proteins because of their ability to secret protein in its native state. In filamentous fungi, stable incorporation of the gene of interest (GOI) can be done via plasmids by its integration as tandem repeats into chromosome. Large quantities of effective antibodies (Abs) are produced in fungi (Matthews et al., 2017). *A. niger* is a genetically amenable model organism for the production of TPs. Despite using different approaches from engineering secretion pathway, gene knock out and knock in strategies, use of strong promoters and multi omics-based tactics to the insertion of multiple copies of a gene, yields are lower than desired in these fungi. Industrial approaches are more effective and direct like reverse genetics, testing of new expression constructs, engineering novel strains, use of automated fermenters and optimization of bioprocessing conditions (Zhang et al., 2018).

Trichoderma reesei is an important host for the synthesis of enzymes. It shows high production efficiency and good yields of cellulases and hemicellulases. Bovine chymosin, Ab Fab fragments, interferon α2b and IgG Abs has recently been synthesized in this fungus by engineering protease deletion strains (Belén et al., 2020).

Aspergillus unguis has been used as a host to develop a platform for protein expression using glyceraldehyde-3-phosphate promoter. High levels of secreted protein were seen using heterologous signal peptides. Human interferon β is expressed using this expression system (Madhavan et al., 2017).

Aspergillus oryzae is used for the synthesis of human lactoferrin, mucor rennin and proteinases (Matthews et al., 2017). *Acremonium chrysogenum* is effective for the synthesis of alkaline protease and hirudin (Zhang and Lan, 2018). *Chrysosporium lucknowense* is a low protease producing strain giving high yields of (TPs) (Legastelois et al., 2017).

Insects

Insect cells can carry out complex PTMs than can't be accomplished by other organisms. Mammalian proteins can be best folded and secreted in soluble form in insects. Baculovirus is pathogenic for lepidopteran cells and its natural host is army worm. Cultures of larvae are cheaper and reported to yield more than 400 TPs. Baculovirus-assisted expression of proteins in insect cell possess a number of advantages like PTMs including acylation, signal peptide cleavage, myristylation, phosphorylation, prenylation, carboxymethylation, amidation, proteolytic processing, N- and O-glycosylation and palmitylation. Polyhedron promoter is used for the expression of foreign gene. This expression system is also safe because baculovirus can attack invertebrates but not vertebrates (Dautzenberg et al., 2017).

In 1980, first anti-microbial peptides (AMPs) were separated from pupae of silk moths; *Hyalophora cecropia*. Until now, more than 1500 AMPs have been identified in different species of animals, plants, fungi and bacteria. Insects show natural anti-bacterial defense mechanism and can be supplied as complex of different AMPs, single peptides or form of proteins present in livestock (Park et al., 2020). *Caenorhabditis elegans* is proven useful for the production of different AMPs against *Staphylococcus aureus* and *Acinetobacter baumanii* and also for rational design of peptidomimetic drugs (Mylonakis et al., 2016).

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Mammalian cells

TPs requiring mammalian PTMs are expressed in mammalian cell-based expression systems. Chinese hamster ovary (CHO) cells are immortalized for the production of tissue plasminogen activator (TPA) and erythropoietin (EPO). Mammalian cells are preferred for the production of mAbs. More than 50 % of the approved synthetic proteins are being produced in mammalian cell lines (Jazayeri et al., 2018, Hunter et al., 2019). Examples include baby hamster kidney (BHK) cells, human embryonic kidney (HEK), SF-9, green monkey kidney cells. Mammalian cell cultures are often preferred because proteins are synthesized in glycosylated, phosphorylated and folded form with fatty acids added. A number of TPs such as human chorionic gonadotropin, activase for acute myocardial infarction, epogen for anemia, human luteinizing hormone (LH), Adalimumab, Trastuzumab, Bevacizumab, Etanercept, and Rituximab have been synthesized in mammalian cells (Kojima et al., 2020). A number of anti-cancer TPs have also been produced in mammalian cell culture systems. These include Darbepoetin-α (enhances erythropoiesis), Lenograstim (stimulates activation of neutrophilic granulocytes), Epoetin-α (enhances production of RBCs), Ziv-aflibercept (for Metastatic colorectal cancer), Thyrotropin-α (for Thyroid cancer), Trastuzumab biosimilar (for Gastric cancer and breast cancer), Rituximab biosimilar (Non-Hodgkin lymphoma) and Interferon-α (for Kaposi's sarcoma, multiple myeloma and non-Hodgkin lymphoma) (Lee et al., 2016, Sanchez-Garcia et al., 2016). To increase productivity of mammalian expression systems, protein-free and chemically defined media can be used (Legastelois et al., 2017).

Transgenic Animals

Transgenic animals may produce TPs in their blood, milk, plasma, urine, silk worm cocoons or egg white. These were developed in 1980s using random genomic insertions but with the passage of time, site directed insertions have become more prevalent (Bosch et al., 2015). New technologies are based on Clustered, Regularly Interspaced, Short Palindromic Repeat (CRISPR)/CRISPR associated protein (Cas 9) system (Redel and Prather, 2016) and transcription activator-like effector nucleases (TALENs) (Ousterout and Gersbach, 2016). A revolutionary example of using transgenic animals as an expression system is the production antithrombin (AT). Transgenic animal bioreactor is selected on the basis of a number of factors like age of sexual maturity of animal, annual dairy production, and reproductive performance (Konkle et al., 2003). Generation of transgenic animal bioreactor is quite laborious, complex and requires lengthy timeline but it can synthesize complex proteins which other systems cannot. Major products of animal-based protein production systems are factor VIII for the treatment of hemophilia A (Soukharev et al., 2002), ß-lactoglobulin, albumin, recombinant human protein C, glucosylceramide for Gaucher disease (Tavares et al., 2016), human hemoglobin, malaria major surface protein (MSP-1) antigen for vaccine production (Behboodi et al., 2005),and human growth hormone. Recombinant sheep, rabbit, pigs, cows, goats, mice have been developed as therapeutic protein production system. Proteins produced in transgenic animals often, possess longer half-life without adverse reactions.

A summary of advantages, disadvantages and examples of therapeutic protein production factories is given in Table 1.

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Table 1: Summary of advantages and disadvantages of using different cells or organisms for the production of TPs.

TPs Production factories	**Advantages**	**Disadvantages**	**Examples**
Plants	Lower production cost, molecular farming, stable or transient gene expression, tissue specific promoters, extraordinary stability, high yield, low animal pathogen contamination, large scale manufacturing	Large amount of biomaterial, gene escape, public acceptability	*Lactuca sativa, Musa paradisiaca, Oryza sativa, Solanum tuberosum, Medicago sativa, Zea mays, Nicotiana tabacum, Nicotiana benthamiana, Glycine max, Triticum aestivum, Daucus carota, and Solanum lycopersicum*
E. coli	Rapid growth, product high yield, cost effective, easy scale-up process, *lac, tac, trc* promoters, easy protein folding mechanisms	Codon biasness, absence of PTMs,	*Escherichia coli*
***Bacillus* sp.**	Better growth characteristics, robust metabolism, cost effective, native protein production, high yield	Proteases may digest protein	*Bacillus licheniformis, Bacillus brevis*,
Yeast	High density growth, good protein yield, cost effective, suitable for PTMs & S-S rich proteins, rapid productivity, signal sequences can be attached	Immunological problems, reduction in activity, receptor binding over glycosylated N-linked sites	*Saccharomyces cerevisiae, Pichia pastoris*
Filamentous fungi	Native protein production, stable GOI incorporation, genetically amenable	Mode of glycosylation	*Aspergillus niger, Trichoderma reesei, Aspergillus unguis, Aspergillus oryzae, Acremonium chrysogenum, Chrysosporium lucknowense*
Insects	Complex PTMs, proper folding, secretion in soluble form, high-density suspension culture, multiple genes expression, good yields, S-S bond formation, anti-bacterial defense system	Pesticides & herbicides hindrance, lead poisoning, adverse allergic reactions	*Hyalophora cecropia, Caenorhabditis elegans, Staphylococcus aureus* & *Acinetobacter baumanii*
Mammalian cells	Undergo PTMs	Costly, susceptible to environmental changes, absence of PTMs & codon usage biasness	Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK), human embryonic kidney (HEK) cells, SF-9, green monkey kidney cells
Transgenic animals	Transgenic animal bioreactor, complex proteins are synthesized, longer	laborious, complex and lengthy timeline,	Ostrich, hens, rats, mouse, sheep, cows, rabbit, pigs, goats

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Vaccines

Charles Amtzen gave the idea of transgenic plant-based vaccines in early 1990s. In 1992, children vaccine initiative was developed to provide cheap edible vaccines for children (Walmsley and Arntzen, 2000). A number of vaccines for hepatitis B, foot and mouth disease, and Norwalk virus have been developed (Richter et al., 2000).

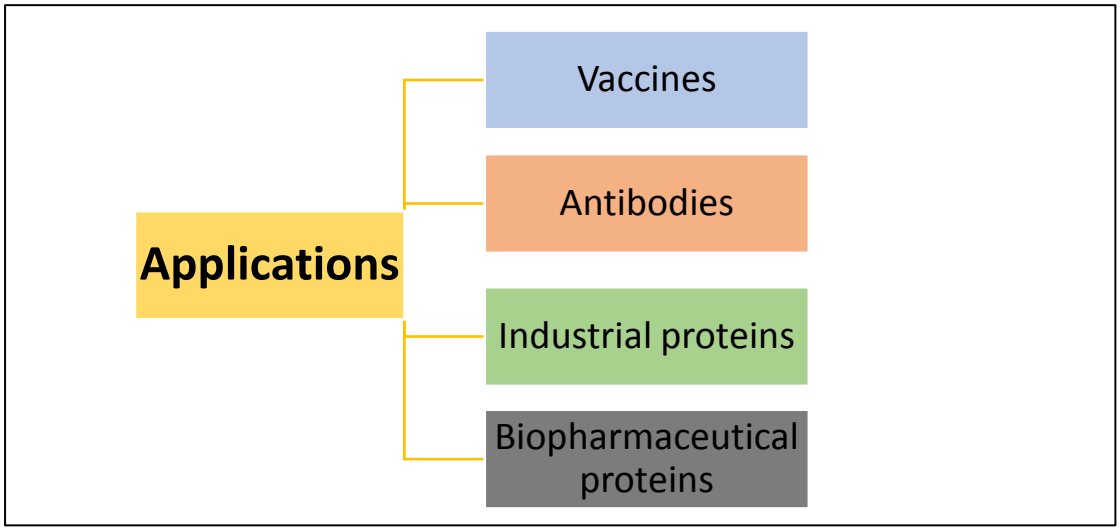

Figure 4: Multiple applications of Therapeutic proteins.

Pharmacokinetics of TPs

Pharmacokinetic (PK) properties have a significant impact on therapeutic efficacy of a therapeutic drug so it is very important to focus these during the development of TPs. The development procedure faces problems in (1) immunogenicity (ability to evoke an immune response); (2) physiochemical instability (degradation or aggregation) and (3) suboptimal time of action or circulation half-life (Fei Wen et al., 2009, Awwad et al., 2018).

Applications

TPs are fast acting medicines that are diverse in their applications (Awwad et al., 2018). TPs can be used as DNase for pulmonary treatment, erythropoietin for anemia, interferon-β and interferon-γ for cancer, interleukin-2 for AIDS, insulin for diabetes, PR-urokinase for heart attacks, tissue plasminogen activator for strokes, vaccines for Hepatitis B and monoclonal antibodies (mABs) for diagnosis (Leung 2007) (Figure 3).

Vaccines produced in transgenic plants comprise between 0.01 %-0.4 % of total soluble protein (TSP) contents. Expression of different proteins depends on the choice of 3′ polyadenylation signals (Richter et al., 2000). Oral tolerance is a hurdle for the development of oral vaccines. Careful dose managements and adjuvants use might be helpful to avoid such problems (Santambrogio et al., 2019).

Now, plant-based vaccine is a well understood concept in medicine but their immunogenicity and efficacy is still in doubt. A number of candidate vaccines that have been tested in animal models include vaccines against mouth disease virus, *Salmonella, E. coli, Yersinia pestis*, bovine, rabbit and canine papillomaviruses, foot and, rabbit haemorrhagic disease virus, porcine circovirus, mink enteritis and bluetongue virus (Rybicki, 2018).

Antibodies

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For diagnosis, management and treatment of a number of disorders, antibodies have been used since many years (Stöger et al., 2000). Recombinant antibodies (rAb) as compared to monoclonal antibodies (mAbs) are easy to detect due to increased biological activity and reduced immunogenicity. Bacterial rAbs are cheaper but difficult to fold, modify or assemble correctly while plant-based rAbs produced have long term storage capacity. The rAbs produced in plants are referred as plantibodies and are successfully stored in seeds, leaves, and tubers such as potatoes (Frigerio et al., 2000).

Plantibodies were first prepared by Planet Biotechnology, Inc (Mountain View, CA). Drug CaroRx^{TM} was targeted against *Streptococcus mutans*, responsible for tooth decay in humans. CaroRx^{TM} was secretory IgA antibodies taken from transgenic tobacco plant. Field peas, rice and wheat have been used for the production of Abs and proved helpful for immune-diagnosis and imaging cancers. Expression of these TPs can be increased 10-100 fold when its location is targeted to ER (Stöger et al., 2000).

Now a day, an important class of medicine has been evolved from therapeutic mAbs which can be used for the treatment of a number of disorders. Abs consist of two heavy chains (HC) and two light chains (LC) which are encoded by two different genes that are specific for assembly of functional recombinant mAb. Currently, it is a big challenge to produce functional mAbs using plant-based expression systems. In 2018, Ebola virus monoclonal antibody (EBOV mAb) was synthesized in transgenic tobacco plants which carried a transcription unit containing HC and LC equal quantities. This gave very good, fully assembled yield of EBOV mAb in transformed tobacco plants (Lin et al., 2018). In 2018, human anti-rabies mAbs were also synthesized from transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* transformed using *Agrobacterium tumefaciens*. Arabidopsis showed 2-fold higher protein expression as compared to that of tobacco proving it a useful system for human anti-rabies mAbs production (Song et al., 2018). In 2019, plant based diagnostic TM43-E10 Abs were synthesized using OmpD protein of *S. typhimurium* as antigen in *Nicotiana benthamiana*. Plant based Abs showed same antigen binding specificity as produced by mammalian or microbial cells (Kopertekh et al., 2019).

Industrial Proteins

A number of proteins i.e. β-casein and human milk proteins lactoferrin have been synthesized industrially in transgenic plant-based bioreactors. These are used as a part of human infant formulas and baby foods due to antimicrobial properties and enhanced digestibility (Karav et al., 2017).

Biopharmaceutical Proteins

Glucocerebrosidase and GM-CSF are two of the world's most expensive drugs and are produced in transgenic plants. A number of human hormones i.e. somatotropin have been synthesized in transgenic tobacco plant which has been found useful for treating HIV, chronic renal failure and Turner syndrome (Tiwari and Sahu, 2017). Proteins encoded from chloroplast do not possess disulfide bonds so no post-extraction chemical processing is required. For the treatment of adenosine deaminase (ADA) deficient severe combined immunodeficiency syndrome (SCID), ADA production has been reported in transgenic plants (Doshi et al., 2016).

Challenges/bottlenecks for TPs

Although TPs have acquired central point in drug discovery and development with enhanced safety profiles for human, there are certain issues and challenges that still need to

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be met. A few of the TPs have been reported to cause immune responses and negative side reactions (Buchanan and Revell, 2015).

Immunogenicity

Recombinant plant-based pharmaceuticals may induce undesirable immune responses in mammals. As plant derived mAbs possess great variety of glycan structures so immunogenicity cannot be ruled out in case of systematic delivery (Merlin et al., 2017).

Poor Yield

Despite the availability of a number of filamentous fungi and bacterial strains, yields of some TPs are very low. Yield can be improved by using strong homologous promoters, protease deficit host strains, random mutagenesis, and fusion of genes with highly secreted protein or by increasing gene copy number. Transcription limitations are also found with filamentous fungi. Increasing gene copy number may tremendously increase protein expression but not equivalently. Further, heterologous protein can be attacked by fungal proteases (Legastelois et al., 2017).

Production Expenses

In mammalian cell culture systems, secretion is poor with longer process duration. Costs of materials are high especially for media. Moreover, manufacturing cost as well as construction and validation are expensive. In addition, FDA approval is time taking requiring minimum 4-5 years. These systems can also be easily contaminated by viruses (Arena et al., 2019).

Aggregated Proteins

In insect cell systems, scientists have to determine patterns of PTMs and expression. Insect secretion signals are useful for efficient protein production. Intracellular aggregates of proteins may be formed due to late expression in infection cycle and may lead to improper folding so earlier harvesting could be useful. Using insects for the expression of TPs also shows decreased expression which can be enhanced by optimization of time and conditions. Improper glycosylation has also been a problem. Tissue specific modifications are difficult to occur (Dautzenberg et al., 2017).

Toxicity

There are some challenges with *E. coli* system which has to be overcome for good expression of TPs. Acetate formation can lead to toxicity when we perform with high cell densities. To avoid this, oxygen level needs to be regulated in the culture. Proteins produced in the inclusion bodies may be inactive, or require refolding. If a therapeutic protein possesses many disulfide bonds then its refolding is also difficult. This bacterium produces non-glycosylated proteins that is why some antibodies are unable to recognize mammalian antigens (Jenkins and Curling, 1994).

Time Consumption

Transgenic animals require longer period of time to assess protein production along with the high cost of their maintenance in farms. It takes about 32 months in cows and 28 months in sheep for getting a desired protein (Behboodi et al., 2005).

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To overcome above mentioned problems, therapeutic proteins can be optimized by increasing understanding about protein rational design, structure-function relationship, and mechanism of action to enhance functionality and targeting. For future, we recommend pharmacokinetics information inclusion in drug development as this has been proved helpful in understanding mechanism of drug action and targeting. In future, neo-sequences in proteins may harbor immunogenicity problems so there is ultimate need for immunogenicity mitigation and risk assessment technologies. Further, careful evaluation would be required for proteins with synonymous codon optimization. Experimental methods with good throughput and computational technologies have tremendously increased diversity in therapeutic drug development but some risks are also associated with them which can be managed with modern technologies (Add reference?).

Commercially Available TPs

Since the approval of recombinant insulin by FDA, there is a considerable increase in the number of TPs (Leader et al., 2008). Until now, over 400 biopharmaceuticals have entered the commercialization zone with a valid license for being used for humans in USA and European Union (EU) from FDA. Among these, a significant portion of ~52 % comprises the genuine TPs products while the remaining contains the recombinant proteins, bio-similar products and synthetic peptides (Walsh, 2018). At present, over 1300 peptides and proteins are in clinical trials (Buchanan and Revell, 2015, Sanchez-Garcia et al., 2016).

Online Resources

A manually curated repository of TPs and peptides approved by Food and Drug Administration (FDA) named THPdb: Database of FDA-approved peptide and protein therapeutics (http://crdd.osdd.net/raghava/thpdb/) is available online (Usmani et al., 2017).

CONCLUSION

From this review, it can be conferred that protein engineering is not restricted to primary sequence changes only but it has improved codon biasness. Codon optimization is done by altering with synonymous codons for better yield but these can affect protein function and folding so careful evaluation is required for these TPs. It can also be anticipated that more proteins will be engineered in the near future with new sequences that are not present in nature. In future, risks of immunogenicity and side-reactions will also increase demanding effective methods for immunogenicity risk assessment. Protein engineering in different production factories has opened up unprecedented opportunities for the development of convenient, effective and safe TPs. New risks are associated with these opportunities but these risks can be managed by using recent advancements in Biotechnology.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ZN and AK wrote the original manuscript. AT supervised the manuscript and helped in writing.

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REFERENCES

Adkins JC, Wilde MI (1998). Lepirudin: a review of its potential place in the management of thrombotic disorders. Bio Drugs., 10(3):227-55.

Adler MJ, Dimitrov DS (2012). Therapeutic antibodies against cancer. Hematol Oncol Clin North Am., 26(3):447-vii.

Ahmadi MK, Pfeifer BA (2016). Recent progress in therapeutic natural product biosynthesis using Escherichia coli. Curr Opin Biotechnol., 42:7-12.

Akash MSH, Rehman K, Tariq M, Chen S (2015). Development of therapeutic proteins: advances and challenges. Turk J Biol., 39(3):343-358.

Antosova Z, Mackova M, Kral V, Macek T (2009). Therapeutic application of peptides and proteins: parenteral forever? Trends Biotechnol., 27(11):628-635.

Arena TA, Chou B, Harms PD, Wong AW (2019). An anti-apoptotic HEK293 cell line provides a robust and high titer platform for transient protein expression in bioreactors. Taylor & Francis., 11(5): 977-986.

Baeshen MN, Al-Hejin AM, Bora RS, Ahmed M, Ramadan H, Saini KS, Baeshen NA, Redwan EM (2015). Production of biopharmaceuticals in E. coli: current scenario and future perspectives. J Microbiol Biotechnol., 25(7):953-962.

Bai Y, Nikolov Z (2001). Effect of processing on the recovery of recombinant β-glucuronidase (rGUS) from transgenic canola. Biotechnol prog., 17(1):168-174.

Bawa A, Anilakumar K (2013). Genetically modified foods: safety, risks and public concerns—a review. J food sci technol., 50(6):1035-1046.

Behboodi E, Ayres S, Memili E, O'coin M, Chen L, Reggio B, Landry A, Gavin W, Meade H, Godke R (2005). Health and reproductive profiles of malaria antigen-producing transgenic goats derived by somatic cell nuclear transfer. Cloning stem cells., 7(2):107-118.

Belén LH, Lissabet JFB, de Oliveira Rangel-Yagui C, Monteiro G, Pessoa A, Farías JG (2020). Immunogenicity assessment of fungal l-asparaginases: an in silico approach. SN Appl Sci., 2(2):222.

Bosch P, Forcato DO, Alustiza FE, Alessio AP, Fili AE, Nicotra MFO, Liaudat AC, Rodríguez N, Talluri TR, Kues WA (2015). Exogenous enzymes upgrade transgenesis and genetic engineering of farm animals. Cell Mol Life Sci., 72(10):1907-1929.

Burnett MJ, Burnett AC (2020). Therapeutic recombinant protein production in plants: Challenges and opportunities. Plants People Planet., 2(2):121-132.

Cao J, de la Fuente-Nunez C, Ou RW, Torres MDT, Pande SG, Sinskey AJ, Lu TK (2018). Yeast-Based synthetic biology platform for antimicrobial peptide production. ACS Synth Biol., 7(3):896-902.

Carter PJ (2011). Introduction to current and future protein therapeutics: A protein engineering perspective. Exp Cell Res., 317(9):1261-1269.

Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL (2009). Design of therapeutic proteins with enhanced stability. Proc Natl Acad Sci., 106(29):11937.

Dautzenberg IJ, van den Hengel SK, de Vrij J, Ravesloot L, Cramer SJ, Hong S-S, van den Wollenberg DJ, Boulanger P, Hoeben RC (2017). Baculovirus-assisted Reovirus Infection in Monolayer and Spheroid Cultures of Glioma cells. Sci Rep., 7(1):17654.

Demain AL, Vaishnav P (2009). Production of recombinant proteins by microbes and higher organisms. Biotechnol Adv., 27(3):297-306.

Dipti CA, Jain SK, Navin K (2006). A novel recombinant multiepitope protein as a hepatitis C diagnostic intermediate of high sensitivity and specificity. Protein Expr Purif., 47(1):319-28.

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Doran PM (2000). Foreign protein production in plant tissue cultures. Cur Opin Biotechnol., 11(2):199-204.

Doshi KM, Loukanina NN, Polowick PL, Holbrook LA (2016). Seed specific expression and analysis of recombinant human adenosine deaminase (hADA) in three host plant species. Transgenic Res., 25(5):629-637.

Fischer R, Emans N (2000). Molecular farming of pharmaceutical proteins. Transgenic Res., 9(4-5):279-299.

Frigerio L, Vine ND, Pedrazzini E, Hein MB, Wang F, Ma JK-C, Vitale A (2000). Assembly, secretion, and vacuolar delivery of a hybrid immunoglobulin in plants. Plant Physiol., 123(4):1483-1494.

Fuh MM, Steffen P, Schlüter H (2016). Tools for the analysis and characterization of therapeutic protein species. Biosimilars., 6:17.

Gellissen G, Janowicz ZA, Weydemann U, Melber K, Strasser AW, Hollenberg CP (1992). High-level expression of foreign genes in Hansenula polymorpha. Biotechnol Adv., 10(2):179-189.

Gerngross TU (2004). Advances in the production of human therapeutic proteins in yeasts and filamentous fungi. Nature Biotechnol., 22(11):1409.

Gharelo RS, Oliaei ED, Bandehagh A, Khodadadi E, Noparvar PM (2016). Production of therapeutic proteins through plant tissue and cell culture. J BioSci & Biotechnol., 5(1)

Giddings G (2001). Transgenic plants as protein factories. Curr Opin Biotechnol., 12(5):450-454.

Giddings G, Allison G, Brooks D, Carter A (2000). Transgenic plants as factories for biopharmaceuticals. Nature Biotechnol., 18(11):1151.

Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, Hwang SJ, Massaro JM, Larson MG, Levy D (2018). Protein biomarkers of cardiovascular disease and mortality in the community. J Am Heart Assoc., 7(14):e008108.

Hofbauer A, Stoger E (2013). Subcellular accumulation and modification of pharmaceutical proteins in different plant tissues. Curr Pharm Des., 19(31):5495-5502.

Hood EE, Witcher DR, Maddock S, Meyer T, Baszczynski C, Bailey M, Flynn P, Register J, Marshall L, Bond D (1997). Commercial production of avidin from transgenic maize: characterization of transformant, production, processing, extraction and purification. Mol Breed., 3(4):291-306.

Hunter M, Yuan P, Vavilala D, Fox M (2019). Optimization of Protein Expression in Mammalian Cells. Curr Protoc Protein Sci., 95(1):e77.

Jazayeri SH, Amiri-Yekta A, Bahrami S, Gourabi H, Sanati MH, Khorramizadeh MR (2018). Vector and cell line engineering technologies toward recombinant protein expression in mammalian cell lines. Appl Biochem Biotechnol., 185(4):986-1003.

Jenkins N, Curling EM (1994). Glycosylation of recombinant proteins: problems and prospects. Enzyme Microb Technol., 16(5):354-364.

Karav S, German J, Rouquié C, Le Parc A, Barile D (2017). Studying lactoferrin N-glycosylation. Int J Mol Sci., 18(4):870.

Karvar S (2014). The role of ABC transporters in anticancer drug transport. Turk J Biol., 38(6):800-805.

Kojima R, Aubel D, Fussenegger M (2020). Building sophisticated sensors of extracellular cues that enable mammalian cells to work as “doctors” in the body. Cell Mol Life Sci., 1-15.

Konkle BA, Bauer KA, Weinstein R, Greist A, Holmes HE, Bonfiglio J (2003). Use of recombinant human antithrombin in patients with congenital antithrombin deficiency undergoing surgical procedures. Transfusion., 43(3):390-394.

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Kopertekh L, Meyer T, Freyer C, Hust M (2019). Transient plant production of Salmonella Typhimurium diagnostic antibodies. Biotechnol Rep., 21:e00314.

Lagassé HAD, Alexaki A, Simhadri VL, Katagiri NH, Jankowski W, Sauna ZE, Kimchi-Sarfaty C (2017). Recent advances in (therapeutic protein) drug development. F1000Res., 6:113-113.

Leader B, Baca QJ, Golan DE (2008). Protein therapeutics: a summary and pharmacological classification. Nat Rev Drug Discov., 7(1):21-39.

Lee CM, Cradick TJ, Bao G (2016). The Neisseria meningitidis CRISPR-Cas9 system enables specific genome editing in mammalian cells. Mol Ther., 24(3):645-654.

Legastelois I, Buffin S, Peubez I, Mignon C, Sodoyer R, Werle B (2017). Non-conventional expression systems for the production of vaccine proteins and immunotherapeutic molecules. Hum Vaccines Immunother., 13(4):947-961.

Lin JC (2010). Protein microarrays for cancer diagnostics and therapy. Med Princ Pract., 19(4):247-54.

Lin Y, Hung C-Y, Bhattacharya C, Nichols S, Rahimuddin H, Kittur FS, Leung T, Xie J (2018). An Effective Way of Producing Fully Assembled Antibody in Transgenic Tobacco Plants by Linking Heavy and Light Chains via a Self-Cleaving 2A Peptide. Front Plant Sci., 9:1379.

Love KR, Dalvie NC, Love JC (2018). The yeast stands alone: The future of protein biologic production. Curr Opin Biotechnol., 53:50-58.

Madhavan A, Pandey A, Sukumaran RK (2017). Expression system for heterologous protein expression in the filamentous fungus Aspergillus unguis. Biores Technol., 245:1334-1342.

Maksum IP, Nabiel A, Ishmayana S, Soedjanaatmadja UM (2020). Safety Aspect of Recombinant Protein Produced by Escherichia coli: Toxin Evaluation with Strain and Genomic Approach.

Maldonado LMP, Hernández VEB, Rivero EM, de la Rosa APB, Flores JLF, Acevedo LGO, Rodríguez ADL (2007). Optimization of culture conditions for a synthetic gene expression in Escherichia coli using response surface methodology: the case of human interferon beta. Biomol Eng., 24(2):217-222.

Matthews CB, Wright C, Kuo A, Colant N, Westoby M, Love JC (2017). Reexamining opportunities for therapeutic protein production in eukaryotic microorganisms. Biotechnol Bioeng., 114(11):2432-2444.

Mengdai X, Xiaoxing M, Hao H, Liegang L, Wei Y (2020). Yeast beta-Glucan effects on gut microbiota for regulating insulin signaling system on alzheimer's disease. Proc Nutr Soc., 79(OCE2).

Merlin M, Pezzotti M, Avesani L (2017). Edible plants for oral delivery of biopharmaceuticals. Br J Clin Pharmacol., 83(1):71-81.

Mohammadinejad R, Shavandi A, Raie DS, Sangeetha J, Soleimani M, Hajibehzad SS, Thangadurai D, Hospet R, Popoola JO, Arzani A (2019). Plant molecular farming: Production of metallic nanoparticles and therapeutic proteins using green factories. Green Chem.

Moire L, Rezzonico E, Poirier Y (2003). Synthesis of novel biomaterials in plants. J Plant Physiol., 160(7):831-839.

Murashima K, Chen C-L, Kosugi A, Tamaru Y, Doi RH, Wong S-L (2002). Heterologous production of Clostridium cellulovorans engB, using protease-deficient Bacillus subtilis, and preparation of active recombinant cellulosomes. J Bacteriol., 184(1):76-81.

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Mylonakis E, Podsiadlowski L, Muhammed M, Vilcinskas A (2016). Diversity, evolution and medical applications of insect antimicrobial peptides. Philos Trans R Soc B: Biol Sci., 371(1695):20150290.

Nagels B, Weterings K, Callewaert N, Van Damme E (2012). Production of plant made pharmaceuticals: from plant host to functional protein. Crit Rev Plant Sci., 31(2):148-180.

Niazi SK (2002). Handbook of biogeneric therapeutic proteins: regulatory, manufacturing, testing, and patent issues. CRC Press.

Nicolaides NC, Sass PM, Grasso L (2010). Advances in targeted therapeutic agents. Expert Opin Drug Discov., 5(11):1123-40.

Nunberg JH, Meade JH, Cole G, Lawyer FC, McCABE P, Schweickart V, Tal R, Wittman V, Flatgaard J, Innis M (1984). Molecular cloning and characterization of the glucoamylase gene of Aspergillus awamori. Mol Cell Biol., 4(11):2306-2315.

Ozgur A, Tutar Y (2013). Therapeutic proteins: A to Z. Protein Pept Lett., 20(12):1365-72.

Park SR, Ko K, Lim S, Cha SY, Chung HJ, Park SJ, Myung SC, Kim MK (2020). In vitro wound healing: Inhibition activity of insect- derived mAb CO17- 1A in human colorectal cancer cell migration. Entomol Res., 50(4):199-204.

Petros S (2008). Lepirudin in the management of patients with heparin-induced thrombocytopenia. Biol : Targets Ther., 2(3):481-490.

Redel BK, Prather RS (2016). Meganucleases revolutionize the production of genetically engineered pigs for the study of human diseases. Toxicol Pathol., 44(3):428-433.

Richter LJ, Thanavala Y, Arntzen CJ, Mason HS (2000). Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nature Biotechnol., 18(11):1167.

Roberts CJ (2014). Therapeutic protein aggregation: mechanisms, design, and control. Trends Biotechnol., 32(7):372-380.

Russell CS, Clarke LA (1999). Recombinant proteins for genetic disease. Clin Genetics., 55(6):389-394.

Sack M, Hofbauer A, Fischer R, Stoger E (2015). The increasing value of plant-made proteins. Curr Opin Biotechnol., 32:163-170.

Sanchez-Garcia L, Martín L, Mangues R, Ferrer-Miralles N, Vázquez E, Villaverde A (2016). Recombinant pharmaceuticals from microbial cells: a 2015 update. Microb Cell Factories., 15(1):33.

Sanchez S, Demain AL (2010). Enzymes and bioconversions of industrial, pharmaceutical, and biotechnological significance. Org Process Res Dev., 15(1):224-230.

Santambrogio L, Berendam SJ, Engelhard VH (2019). The Antigen Processing and Presentation Machinery in Lymphatic Endothelial Cells. Fron Immunol., 10:1033.

Saul R, Ghidoni JJ, Molyneux RJ, Elbein AD (1985). Castanospermine inhibits alpha-glucosidase activities and alters glycogen distribution in animals. Proc Natl Acad Sci., 82(1):93-97.

Schillberg S, Raven N, Fischer R, M Twyman R, Schiermeyer A (2013). Molecular farming of pharmaceutical proteins using plant suspension cell and tissue cultures. Curr Pharm Des., 19(31):5531-5542.

Selas Castiñeiras T, Williams SG, Hitchcock AG, Smith DC (2018). E. coli strain engineering for the production of advanced biopharmaceutical products. FEMS Microbiol Letters., 365(15):fny162.

Song I, Park S-A, Han D, Lee HK, An HJ, Ko K (2018). Expression, glycosylation, and function of an anti-rabies virus monoclonal antibody in tobacco and Arabidopsis plants. Hortic Environ Biotechnol., 59(2):285-292.

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Soukharev S, Hammond D, Ananyeva NM, Anderson JA, Hauser CA, Pipe S, Saenko EL (2002). Expression of factor VIII in recombinant and transgenic systems. Blood Cells Mol Dis., 28(2):234-248.

Squires CH, Lucy P (2008). Vendor voice: a new paradigm for bacterial strain engineering. BioProcess Int., 6(Suppl 4):22-7.

Stöger E, Vaquero C, Torres E, Sack M, Nicholson L, Drossard J, Williams S, Keen D, Perrin Y, Christou P (2000). Cereal crops as viable production and storage systems for pharmaceutical scFv antibodies. Plant Mol Biol., 42(4):583-590.

Sutradhar KB, Khatun S, Al Mamun A, Begum M (2011). Distribution and elimination of protein therapeutics: a review. Stamford J Pharm Sci., 4(2):1-12.

Swartz J (1996). Escherichia coli recombinant DNA technology. Escherichia Coli.,1693-1711.

Takaiwa F, Wakasa Y, Hayashi S, Kawakatsu T (2017). An overview on the strategies to exploit rice endosperm as production platform for biopharmaceuticals. Plant Sci., 263:201-209.

Tarafdar A, Kamle M, Prakash A, Jasdeep A, Padaria J (2014). Transgenic Plants: Issues and Future Prospects.

Tavares KCS, de Oliveira Dias AC, Lazzarotto CR, Neto SG, de Sá Carneiro I, Ongaratto FL, Pinto AFM, de Aguiar LH, Calderón CEM, Toledo JR (2016). Transient expression of functional glucocerebrosidase for treatment of Gaucher's disease in the goat mammary gland. Mol Biotechnol., 58(1):47-55.

Terpe K (2006). Overview of bacterial expression systems for heterologous protein production: from molecular and biochemical fundamentals to commercial systems. Appl Microbiol Biotechnol., 72(2):211.

Tiwari P, Sahu PK (2017). Plants altering hormonal milieu: A review. Asian Pac J Reprod., 6(2):49.

Tohidi B, Rahimmalek M, Arzani A (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem., 220:153-161.

Udaka S, Yamagata H (1994). Extremely efficient protein secretion system in Bacillus brevis. Bioprocess Technol., 19:71-79.

Usmani SS, Bedi G, Samuel JS, Singh S, Kalra S, Kumar P, Ahuja AA, Sharma M, Gautam A, Raghava GPS (2017). THPdb: Database of FDA-approved peptide and protein therapeutics. PLOS ONE., 12(7):e0181748.

Van Ooijen AJ, Rietveld K, Hoekema A, Pen J, Sijmons PC, Verwoerd TC, Quax WJ (1996). Production of enzymes in seeds and their use.

Walmsley AM, Arntzen CJ (2000). Plants for delivery of edible vaccines. Curr Opin Biotechnol., 11(2):126-129.

Walsh G (2018). Biopharmaceutical benchmarks 2018. Nature Biotechno., 36:1136.

Ward M, Lin C, Victoria DC, Fox BP, Fox JA, Wong DL, Meerman HJ, Pucci JP, Fong RB, Heng MH (2004). Characterization of humanized antibodies secreted by Aspergillus niger. Appl Environ Microbiol., 70(5):2567-2576.

Wong MS, Wu S, Causey TB, Bennett GN, San K-Y (2008). Reduction of acetate accumulation in Escherichia coli cultures for increased recombinant protein production. Metab Eng., 10(2):97-108.

Wright K, Prior F, Sardana R, Altosaar I, Dudani A, Ganz P, Tackaberry E (2001). Sorting of glycoprotein B from human cytomegalovirus to protein storage vesicles in seeds of transgenic tobacco. Transgenic Res., 10(2):177-181.

Xu J, Ge X, Dolan MC (2011). Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. Biotechnol Adv., 29(3):278-99.

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Yao J, Weng Y, Dickey A, Wang KY (2015). Plants as Factories for Human Pharmaceuticals: Applications and Challenges. Int J Mol Sci., 16(12):28549-28565.
Zhang J, Lan N (2018). Hirudin variants production by genetic engineered microbial factory. Biotechnol Genet Eng Rev., 34(2):261-280.

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