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Review Article

Comparison of Host Expression Systems used for Efficient Recombinant Proteins Production

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Abstract: The marvels of DNA recombination technology have revolutionized the field of biotechnology. Several hormones, antibody subunits, vaccines, enzymes, and interferons are being produced at the industrial level, in suitable expression systems, under optimized conditions. For recombinant protein production, a range of expression systems are available such as bacteria, yeast, fungi, plant cells, insects and animal cells, etc. All recombinant proteins are naturally different from each other and various challenges are kept into consideration while choosing an expression system for their production. Every expression system has its advantages and limitations on the basis of which it can be considered or rejected for a particular protein production. Therefore, it is very significant to investigate the potential and limitations of several expression systems to choose the suitable one for particular protein production at an industrial scale. The optimization criteria of an expression system is evaluated on several factors such as productivity, efficiency, physiological characteristics, total cost, safety, convenience, and down-streaming conditions. *Escherichia coli* and *Saccharomyces cerevisiae* remained the organisms of choice to produce recombinant proteins for a long time, but now several other microorganisms are also being targeted to evaluate their efficiency toward recombinant protein production. Prokaryotic expression systems can be used to produce eukaryotic proteins as well however, the use of a eukaryotic expression system is preferable because it retains the structural, functional, and regulatory properties of therapeutic proteins. This review illustrates a brief view of a variety of expression systems, their efficiency, and limitations in recombinant protein production.

Keywords: DNA Recombinant Technology, Expression Systems, Cell Lines, Recombinant Proteins

1. INTRODUCTION

Proteins are the basic biological building blocks which play a key role in the metabolic machinery of all life forms. Some proteins perform structural roles while others act as biocatalysts i.e., enzymes which accelerate the metabolic rate. Proteins are the most vital biological molecules that play a key role in almost every function of the cell such as immune responses, cell adhesion, cell signaling, and cell cycle [1]. These biological molecules can now be produced at a commercial scale, all thanks to recombinant DNA technology. Recombinant DNA (rDNA) technology refers to the genetic manipulation in an organism's genetic material to induce desired characteristics in the organism or to produce desired products i.e., proteins. It was in 1973 when the first recombinant DNA molecule was produced at the University of California San Francisco and Stanford University by the combined efforts of Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen [2].

At first rDNA technology was suggested to foster agriculture and drug development, however, several unexpected difficulties hinder the achieving satisfactory results [2]. Till 1980 several products i.e., vaccines, hormones, and therapeutic proteins had been developed. The first ever developed therapeutic recombinant protein was insulin which was approved as safe in 1982 after which it became one of the global scale pharmaceutical products with a continuously increasing demand worldwide [2-4]. Besides the pharmaceutical sector, enzyme and agriculture industries also take benefit from recombinant DNA technology [4].

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At present, several genetically engineered proteins are produced at an industrial scale. These proteins are used in different sectors i.e., enzyme industries, pharmaceutical industries, agriculture industries, etc. The products manufactured in these industries act as raw materials for many other fields such as diagnostics, medicine, nutrition, pharmacy, detergent, paper pulp, textiles, plastics, leather, and polymers [1, 5]. The development and innovation in recombinant DNA technology have evolved the strategy of treatment. Figure 1 demonstrates some key advantages of DNA technology in diagnostics, treatment, and improvement of the health quality of people. According to the Vantage Market Research report, the value of the global recombinant DNA technology market remained at 142.9 Billion USD and is expected to surpass 223 Billion USD by 2018 [6].

To fulfill the required need of production, various expression systems have been established [7]. Expression system refers to the host cell, providing the metabolic machinery for protein synthesis, and a transfected or modified DNA vector, responsible for providing the blueprints of the desired protein. The genetic code of vector DNA is transcripted into mRNA which interacts with host ribosomes and results in the translation of desired amino acid sequence. During the process of translation, transfer RNA and ribosomal RNA also play their key roles of supplying amino acids and catalyzing the linkages between amino acids respectively.

Translation of protein is followed by the addition of protospacer adjacent motifs (PTMs) in the molecules which are complex and different for prokaryotes and eukaryotes [8]. Prokaryotic expression systems can be used to produce eukaryotic proteins as well [9]; however, the use of a eukaryotic expression system is preferable because it retains the structural, functional, and regulatory properties of therapeutic proteins [10, 11].

The present review illustrates the recently available variety of expression systems for the production of beneficial recombinant proteins. Moreover, a comparison of the efficiency and benefits of these expression systems along with their potential to produce recombinant proteins at an industrial scale will also be discussed in this review.

2. CONSIDERATIONS TO CHOOSE AN EXPRESSION SYSTEM

Choosing the right expression system to express the protein of interest is an extremely vital approach as it affects the outcomes. There are several factors to be considered to choose an expression system: the

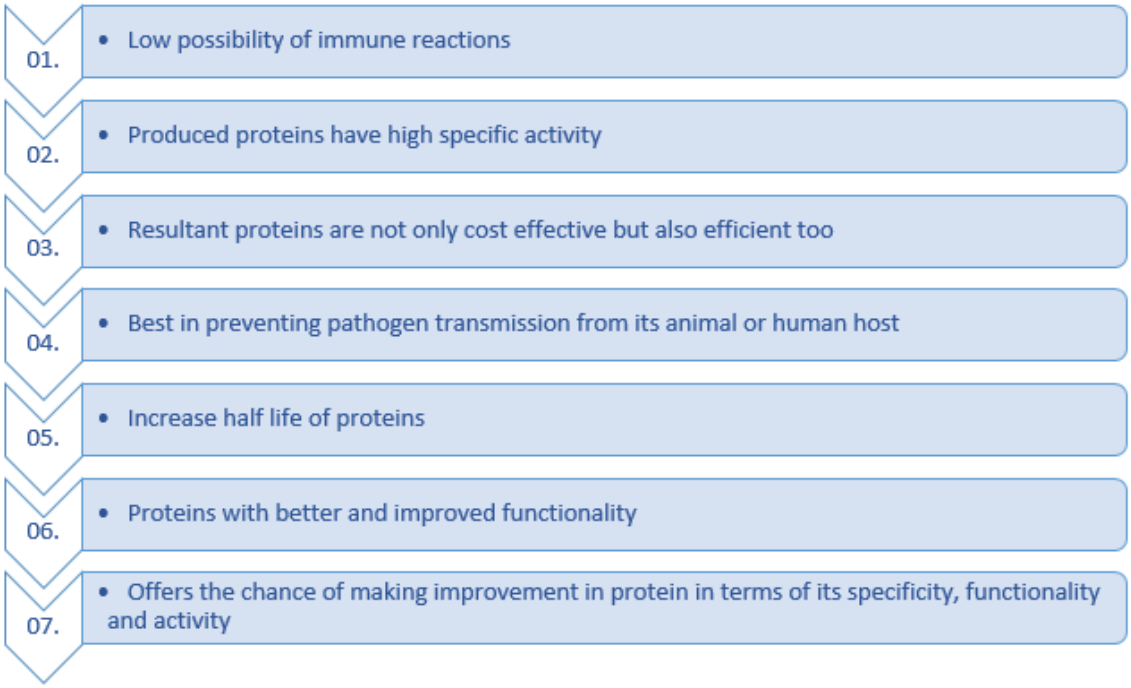

Fig. 1. Some advantages of using recombinant DNA technology in protein production

origin of the protein (prokaryotic or eukaryotic); solubility of the protein (insoluble proteins may form aggregates in the form of inclusion bodies or do not fold accurately); structural complexity, post-transnational modifications, cellular localization, purification, yield and the total investment to produce the protein at commercial scale [12]. Every protein is different and requires different expression systems for proper recovery. Mostly, the recombinant proteins produced in an expression system behave foreign for it, hence the post-translational modifications could be different from the original product. Almost 5 % of the whole cell proteome consists of enzymes which perform several post-translational modifications which are different for genetically different types of cells [13]. It indicates that even if the host cell has a capability of a specific post-translation modification such as glycosylation, still there is a possibility of a difference in the pattern of glycosylation compared to the native protein. To retain the stability, immunogenicity, biological activity, and pharmacokinetic behavior of a therapeutic protein, its N-linked glycosylation pattern should be correct [14]. Some general considerations and requirements for the proficient production of recombinant protein are described in Figure 2.

2.1 Overview of Recombinant Proteins Production

Genetic manipulation involves the insertion of a specific DNA fragment, containing the gene of interest, in an appropriate vector. This process is facilitated by the enzymatic activity of two most important enzymes: endonucleases (for sequence-specific DNA cutting activity) and DNA ligases (to attach the gene of interest with the vector). The vector is further inserted into an expression system, grown to produce several copies of the gene of interest to produce desirable products [15] as demonstrated in Figure 3. There is a diversity of available expression system platforms for recombinant protein production. Different expression systems such as bacteria, yeast, mammalian cells, plants, and insects could be used. Among these expression systems, bacterial (*Escherichia coli*) and yeast expression systems (*Saccharomyces cerevisiae* and *Pichia pastoris*) are more common [16-17].

3. BACTERIA

Bacteria are simple unicellular prokaryotes which proves beneficial protein-producing cell factories. Bacteria are considered an easily available

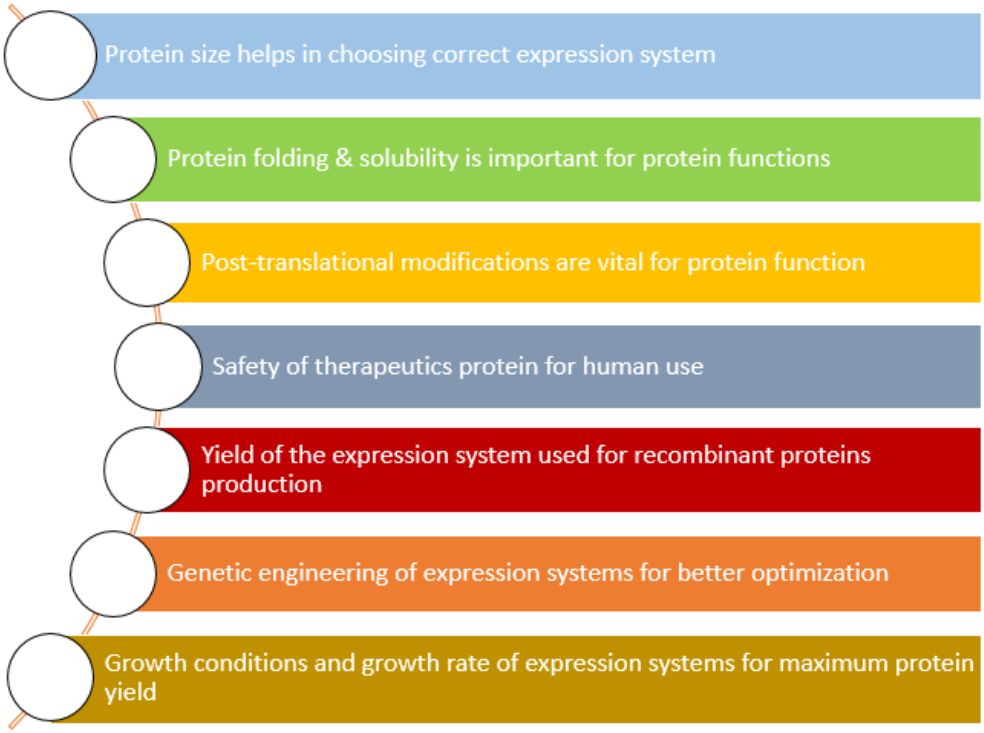

Fig. 2. General considerations for recombinant proteins production

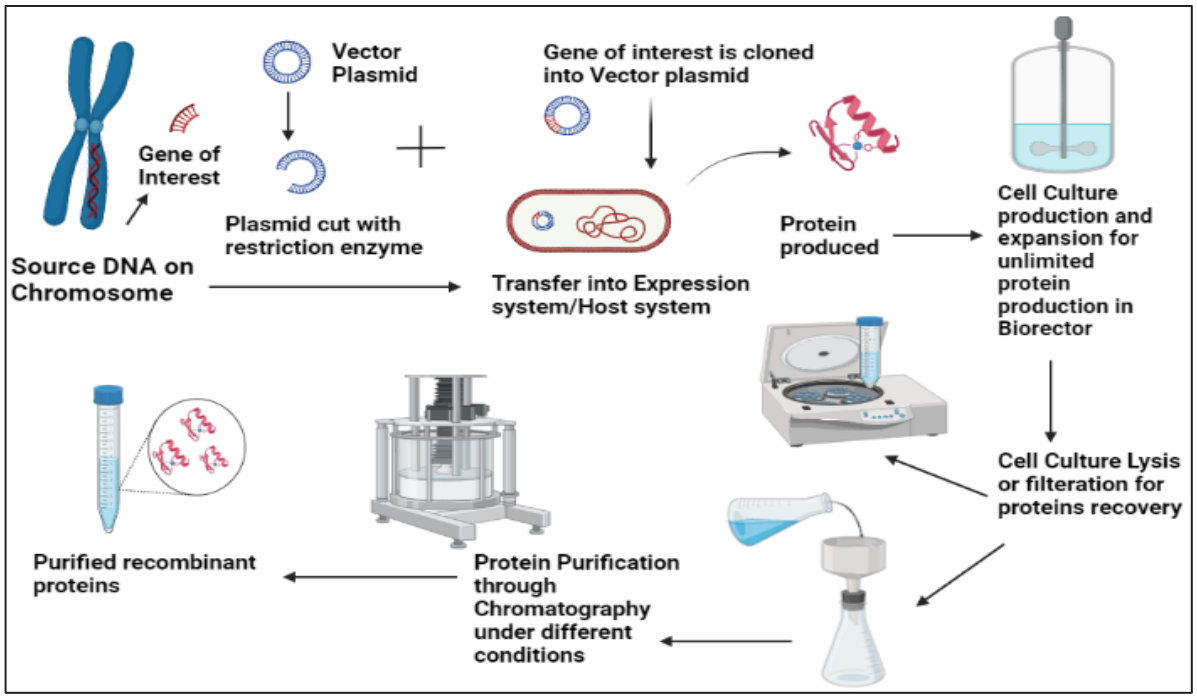

Fig. 3. General methodology for recombinant protein production in expression system and afterward purification

expression system because of certain key factors. The selection of a prokaryotic bacterial expression system is considered an attractive choice because of its low expense cost, medium productivity, and rapid growth of bacteria [17]. Most widely used bacterial expression system has been described as follows:

3.1 *Escherichia coli*

The most widely used bacteria for recombinant protein production is the gram-negative rod, *E. coli* because of its certain significant characteristics i.e., its short replication time and extremely fast growth kinetics enable fast achievement of high cell densities. The reagents and culture media required to grow *E. coli* are quite simple and inexpensive. The genetic manipulation and transformation of *E. coli* are comparatively simple and straightforward due to its genetics, biochemistry, and metabolism [17, 18]. It is comparatively easy to manipulate the genetic information of *E. coli* as compared to other bacterial expression systems such as *Streptomyces* and *Pseudomonas* system [17]. Besides this, therapeutic protein production in *E. coli* eliminates the requirement of optimization step as various standard plasmids can be employed easily. Usually, *E. coli* is used with T7 bacteriophage RNA polymerase. Moreover, it is now considered the most appropriate approach to start the recombinant protein production process [9]. In 1982, the first biopharmaceutical product, approved by Food and Drug Administration (FDA) was biosynthetic insulin, engineered in an *E. coli* expression system [19]. Now a days, the *E. coli* expression system is widely used for several other therapeutic product productions such as tumor necrosis factor, human growth hormone, interleukins etc. [20, 21]. *E. coli* with a T7 RNApol expression system is best suitable for the production of non-glycosylated proteins. *E. coli* is still under research to gain more and more understanding regarding its central dogma and post-translation modifications. *E. coli* is the most suitable expression system because it can tolerate a range of environmental conditions and can store the recombinant proteins in almost 80 % of its dry mass [1]. Figure 4 illustrates the possible strain improvement strategies for *E. coli*.

3.2 Lactic Acid Bacteria

Lactic acid bacteria (LAB), the gram-positive non-sporulating anaerobic rods, have long been used in multiple dairy fermentation processes as recombinant microbial cell factories. Among the other genera of LAB, *Lactococcus* and *Lactobacillus* are most commonly employed either as cell vectors to deliver therapeutic molecules or

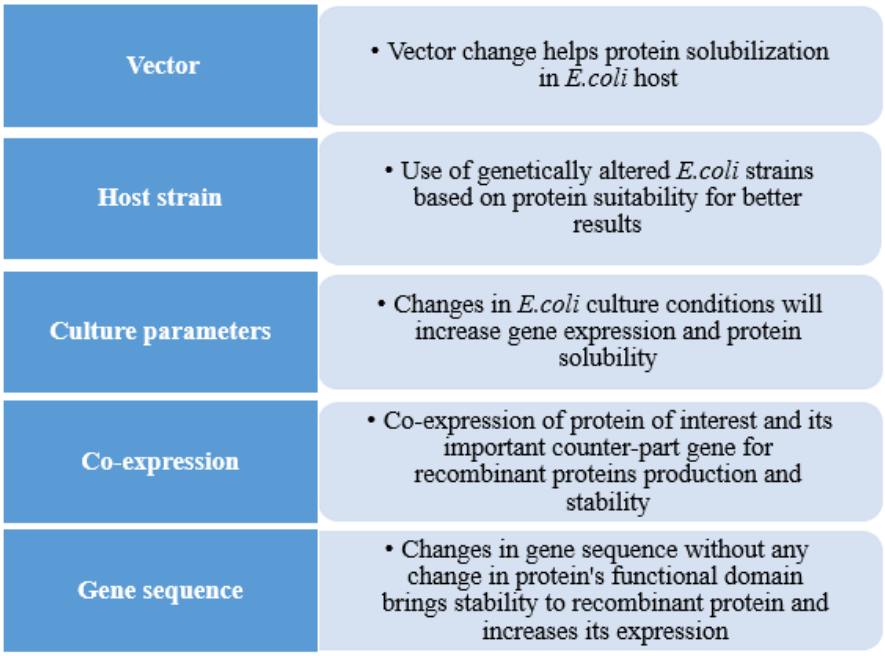

Fig. 4. Strain improvement strategies for *E. coli.*

as producer cells [20]. *Lactococcus lactis* is one of the most promising LAB for recombinant protein production. Different *Lactobacillus* strains such as *L. reuteri, L. gasseri,* and *L. Plantram* are used for the production of green fluorescent proteins, and enzymes such as beta-glucuronidase, beta-galactosidase, and aminopeptidase. Being safe and non-pathogenic, LABS have been declared a Generally Recognized as Safe (GRAS) organism by the food and Drug Administration (FDA) [22]. LABs do not contain exotoxins in their cell membrane hence providing an endo-toxin-free expression system. Other key characteristics of LAB that makes them attractive expression systems include easily scale-able, cheap, safe for food, and production of heterologous membrane proteins. Novel genetic manipulation techniques such as CRISPR-Cas9 has also been used to transform LAB for the production of several therapeutic proteins [23]. LAB has a potential economic impact on the fermentation industry because it is used as an expression system for the production of lactic acid, milk products, wine, meat, high-grade metabolites, and antimicrobial peptides [24].

3.3 *Pseudoalteromonas haloplanktis*

One of the fastest growing and eligible expression system psychrophile is *P. haloplanktis* TAC125 which was isolated from a seawater sample from an Antarctic coast. It is characterized as gram-negative bacteria which has the ability to grow at low temperatures (0–30 °C). The eligibility of *P. haloplanktis* for an expression system has also been improved by certain genetic modifications [10]. Antibody fragment production has been reported by using a cold-adapted platform instead of the conventional mesophilic platform which usually uses *E. coli* [25, 26]. Such platforms have also proved very beneficial for the production of some delicate and heat-sensitive proteins such as alpha-glucosidase and Human Nerve Growth Factor h-NGF [10, 27]. The production of h-NGF in *E. coli* failed as the h-NGF accumulated in inclusion bodies instead of folding accurately. In contrast, h-NGF folded in a proper dimeric form remained soluble and translocated in periplasm when expressed in *P. haloplanktis* TAC125 [10]. Similarly, alpha-glucosidase, a recombinant enzyme of *S. cerevisiae* was recovered efficiently in a soluble and active form from *P. haloplanktis* TAC125 as compared to that of *E. coli* in which alpha-glucosidase became insoluble [27].

3.4 *Pseudomonas*

Pseudomonas can grow rapidly and secrete protein efficiently. Different *Pseudomonas* species i.e., *P. aeruginosa, P. fluorescens*, and *P. putida* have shown the best protein yield and efficient expression

as compared to *E. coli* and hence can be used as the best alternative to *E. coli* for certain proteins [9].

3.4.1 Pseudomonas putida

Pseudomonas putida (strain KT2440) is a gram-negative soil bacterium which is widely used in a cell factory to produce industrially important proteins because of its extraordinary features. These features include versatile metabolism, rapid growth, minimal nutrient requirements, and tolerance to stress conditions. The extensive biochemical network of *P. putida* facilitates extensive production of NADPH instead of ATP. This property enables it to tolerate stress conditions [28].

3.4.2 Pseudomonas fluorescens

A proprietary expression system of *P. fluorescens* has been developed for the efficient production of recombinant proteins. This system is more advantageous as compared to conventional *E. coli*. Protein production in *P. fluorescens* can be carried out even in depleted oxygen concentration and it also prevents the accumulation of acetate in the expression system. However, it shares some characteristics with *E. coli* such as growing ability in saturated cell density (N100 g/L) and over-expression of proteins i.e., half of the total protein [136]. *P. fluorescens* fermentation does need strict control of glucose concentration and aeration passage. The highest production rate of nitrilase, a recombinant enzyme, has been reported as 25 g L^{-1} using *P. fluorescens* as an expression system. Moreover, the *P. fluorescens* expression system proved advantageous for an insecticidal protein with a yield of 3–4 gL^{-1} which is comparatively very high as compared to that of *E. coli* i.e., 100 mg L^{-1} [9, 29].

3.5 Other bacteria

With the advancement in research and technology, many other bacterial expression systems have emerged as successful alternatives. One of the most noteworthy bacterial systems is the gram-positive bacteria *Streptomyces* which has an efficient secretory system. It secretes a high concentration of the desired proteins in the medium which reduces some recovery steps [9]. Another choice of expression system is *Ralstonia.*

4. FUNGI

Filamentous fungi have been used as commercial organisms to produce pharmaceutical and enzymatic products. The versatile metabolic ability of fungi makes it an attractive and outstanding cell factory. Fungi also have the ability to express several prokaryotic as well as lower eukaryotic genes after genetic manipulation. Filamentous fungi have a strong capacity for secretion due to which they are also considered one of the most promising expression systems for recombinant protein production. However, there are a few fungal species (*Aspergillus* sp. and *Trichoderma* sp.) that are used to produce a competitive level of recombinant protein at an industrial scale [27].

4.1 *Aspergillus*

Aspergillus is one of the most extensively studied genera from a research perspective. Its species are considered model organisms i.e., *A. nidulans.* Other species i.e., *A. oryzae* and *A. niger* have great importance in citric acid production even at the industrial scale [33]. Several molecular genome editing tools such as RNA interference-RNAi, selection markers, and CRISPR/Cas9 are also being used to manipulate the *Aspergillus sp*. genome to get the desired level of protein of interest [34, 35]. *A. niger* genome has been successfully edited using CRISPR/Cas9 which incorporate double-strand breaks in the DNA sequence. Moreover, this technology is also being used for other *Aspergillus* sp. Being considered as GRAS, the genetically modified *A. niger* has been employed at an industrial scale to produce very significant proteins i.e., human lactoferrin, calf chymosin, and neoculin, a plant-derived sweetener [36-42] The yield of all these proteins have been increased by optimizing the physiological growth conditions of the fungi.

4.2 *Trichoderma reesei*

Another important fungus is *T. reesei* which also contains an extraordinary secretory system. Its genome can be modified by aggressive mutations to produce extracellular cellulase. A yield of 100 g/L cellulase production was reported by *T. reesei* of which 60 % was characterized as Cel7a (CBHI) while 20 % was Cel 6a (CBHII) [43]. Initially, *T.reesei* was exploited for the production

of calf chymosin [44, 45] later on it was reported to efficiently produce antibody fragments [46]. The higher extent of recombinant protein production was attributed to the taxonomic relatedness of the gene of interest and the host. *Trichoderma* is used to produce recombinant cellulase by incorporating its genes into cassettes [43, 47]. Usually, Cel 7a (CBHI) single peptide is responsible to mediate the high secretion level of this protein [48].

4.3 Other fungi

There is comparatively less information regarding the gene sequencing of filamentous fungi other than *Aspergillus* and *Trichoderma* specie. However, Pectinases, hemicellulases, and cellulases are reported to be produced by several *Penicillium* species such as *P. emersonii, P. funiculosum*, and *P. purpurogenum* respectively [43]. Another fungal system (*N. crassa*), reported to grow at a maximum rate in normal media, has been genetically and biochemically characterized. It has the potential to produce and secrete proteins at a higher level. Its genome size is 40Mb, of which there are 10,000 protein-coding genes. Most of these genes are responsible for secondary metabolite production in *Neurospor*a [43, 49]. Now a days, *N. crassa* has been adopted as an expression system to produce vaccine subunits such as neuraminidase antigens (NA) and influenza hemagglutinin (HA) [50]. Recently, many fungal strains i.e., *T. reesei, N. crassa,* and *Aspergillus* species are being used in the production of antibodies [51-53].

5. YEAST

Yeast, unicellular microorganisms having the ability to process proteins like eukaryotes i.e., assembly, folding, and post-translational modifications, has always remained an organism of interest to be used as an expression system. Due to easy manipulation in genes and efficient growth rates, yeast cells are beneficial hosts. Moreover, it does not possess any oncogenes or endotoxins. *Saccharomyces cerevisiae* has been used to express the majority of recombinant proteins since 1980 [61]. Food and Drug Administration (FDA) has declared *S. cerevisiae* as GRAS (generally regarded as safe). But there is one problem and that is yeast is not good for large-scale productions because it requires efficient machinery for its fermentation. Moreover, products produced by *S. cerevisiae* mostly remain in periplasmic spaces due to hyperglycosylation [62, 63]. These products start degrading after some time and it is very hectic to remove them from our desired product. These drawbacks led to the quest for new species of yeasts for another expression system, most of these efforts were made by using nonconventional Yeasts such as *Pichia pastoris, Hansenula polymorpha, Sarcoscypha occidentalis, Pichia methanolica, Zygosaccharomyces rouxii, Candida boidinii,* and *Kluyveromyces lactis*, etc. [55, 64-66].

5.1 *Saccharomyces cerevisiae*

For almost the last thirty years, *S. cerevisiae,* a eukaryotic microorganism, has been used to express different recombinant proteins [67]. *S. cerevisiae* was used to express the recombinant form of the first vaccine i.e., Hepatitis B and it was produced intracellularly. *S. cerevisiae* also comes in the first row when approval for any recombinant therapeutics is required from FDA and EMEA, recombinant therapeutics produced from it always get green signals from these agencies [68]. Hirudin, Platelet-derived growth factors, Hepa-B surface antigen, insulin, and GM-CSF (Granulocyte macrophage-colony-stimulating factors) are some of the main products of *S. cerevisiae* which are currently available in the market for use [6].

5.2 *Pichia pastoris*

P. pastoris is a methylophilic yeast because it has the ability to use methanol to fulfill its requirement of carbon in the absence of any other carbon alternative [69]. *P. pastoris* has a strong tendency to secrete protein even if they have a high molecular weight. This characteristic makes them better than *S. cerevisiae* in which heavy proteins retain in the periplasm. Moreover, it is comparatively easy to purify the secreted proteins from the extracellular medium. The strains of *P. pastoris* i.e., protease deficient strains (SMD1163, SMD1165, SMD1168) and Auxotrophic mutant (GS115) are commonly used and are derived from wild type NRRL-Y 11430 strain. *P. pastoris* strains have also been characterized on the basis of their ability to utilize methanol i.e., Mut+, MutS, and Mut- [70]. A therapeutic polypeptide of 60 amino acids was produced by *P. pastoris*. It was further approved as safe by Food and Drug Administration FDA in 2009 to treat hereditary angioedema. A Comparison

of the yield of some recombinant protein products produced by bacteria and other expression systems is given in Table 1.

5.3 *Kluyveromyces lactis*

Since the 1950s, the production of β-galactosidase (also known as lactase), as well as the heterologous appearance of rennin (bovine chymosin) is done by using *K. lactis* [9]. Many features of *K. latis* played their role in its popularity for the production of r-protein. Some of these features are as follows, LAC4 which plays the role of such a powerful inducible promoter. This promoter is regulated by even low amounts of glucose. It also has the ability to use whey as well as lactose-like cheap substrates. For its approval as a GRAS strain and its ability to produce proteins with high molecular weights [39], a complete sequence of its genome [67], as well as a kit for protein expression is commercially available from NEB [47]. Previous literature about *K. lactis* is mostly about the MATα CBS 2359 mating strain and GG799 haploid strain which is a wild-type strain that is known as a good host as it is added in the commercially available kit.

5.4 *Yarrowia lipolytica*

For expressing the heterologous proteins, the use of Y. lipolytica expression system is getting popularity due to the following reasons; i) a large amount of proteins with high molecular weight is secreted due to its inherent ability; ii) pathway named as co-translational translocation is analogous to eukaryotes and could be the reason for the secretion of proteins [76]. This system of protein secretion is exactly opposite to the pathway named as post-translational translocation and is mostly present in *S. cerevisiae*; iii) sugar is not fermented by this organism, iv) completely sequenced genome is available [69, 77]; v) fermentation with high density is possible; and vi) many GRAS processes on the industrial level are approved by FDA by using this organism [69].

5.5 Other yeast

Hansenula polymorpha expression systems which include a hepatitis B recombinant vaccine, insulin, phytase, hirudin, and alpha 2a interferon, along with food supplements known as lipase as well as hexose oxidase having GRAS notification by FDA. A dimorphic yeast named as *Arxula adeninivorans* is a temperature-dependent yeast. It has the ability to grow as budding cells as well as mycelium. *A. adeninivorans* also has the ability for secreting extracellular enzymes during cultivation in the surrounding medium. It mostly secretes the proteases, glucosidases which include xylosidase, cellobiose, pectinases, invertase, acid phosphatases, glucoamylase, trehalose, phytase, and β-glucosidases. It also releases RNAse [69]. A fission yeast named as *Schizosaccharomyces pombe* have the ability to grow as preferential

Table 1. Comparison of the yield of some recombinant protein products produced using bacteria and yeast expression systems

Recombinant protein	Example of Expression-system used	Highest product yield (g/Lh)	Reference
Interleukin-6	*E. coli*	7.5mg mL^{-1}	[131]
	P. pastoris	0.28 mg mL^{-1}	[71]
Riboflavin	*C. famata*	0.11 g L^{-1}	[72]
	B. subtilis	0.33 g L^{-1}	[1]
	A. gossypii	0.07 g L^{-1}	[132]
	C. ammoniagenes	0.21 g L^{-1}	[54]
Glutamic acid	*S. cerevisiae*	0.46 mg mL^{-1}	[131, 73]
decarboxylase	*E.coli*	12.5 mg mL^{-1}	[71]
	P. pastoris	0.42 mg mL^{-1}	[73]
	Spodoptera frugiperda cells	0.02 mg mL^{-1}	[71]
Insulin	*S. cerevisiae*	0.075 g L^{-1}	[71]
	E. coli	4.34 g L^{-1}	[1]
	B. subtilis	1 g L^{-1}	[74]
	P. pastoris	3.07 g L^{-1}	[75]

haploids. Having a complete genome sequenced eukaryotic list it is numbered at 6 [78]. Having a complete availability of proteome in Swiss Prot as well as UniProtKB it is numbered at 3 in the eukaryotic list following *Homo sapiens* and then *S. cerevisiae*. For expressing the mammalian proteins *S. pombe* is considered an eye-catching host and a great area of research [79, 80]. A brief list of microorganisms that have applications in recombinant protein production are listed in Table 2.

6. PLANTS

With the discovery of growth hormones in tobacco plants the production of recombinant proteins by using plant expression machinery came into existence. In today's world, three types of methods are used for the production of recombinant proteins: by forming transgenic plants, by using systems relying upon plant-tissue, and by using cell cultures. Bacterial infection and viral infection are used for the transformation methodology. Some direct methods such as biolistic bombardment and PEG-mediated technique can also be used for transformation methodology [17].

Therapeutic recombinant protein production is done by using plant expression systems and the main focus is to enhance the quantity and efficiency of produced recombinant proteins [81, 82]. The main features of plants for their use as recombinant proteins producer are as follows: growth conditions are cheap, the manufacturing procedures are well understood, scalability levels are very high, their high ability for the production of complex proteins, the infrastructure of the already existing industry, the ability for the rapid production, less chances of contamination with human pathogens [83].

By using plant sources for the production of recombinant biopharmaceuticals the production can be increased and costs can be decreased. The plant factories producing recombinant proteins possess the following qualities mentioned in the literature: safety, insensitivity to changes in temperature as well as pH, low cost, metabolites presence, high stability, easy and cheap storage of produced drugs, and ability to produce proteins named as N-glycosylated [130]. The most important feature of using transgenic plants is that high production is maintained by investing low costs while compared with other expressions of prokaryotic as well as eukaryotic systems it costs lower up to 50 percent [84, 130]. Hypothetically arguing the plant fruits, seeds and leaves might be a rich source of therapeutic proteins. The transgenic plants used for the production of recombinant proteins show an expression ranging from 0.001 % to 46.1 % [85]. The ability of transgenic plants to store recombinant proteins in cell compartments as well as the plant organs makes them different from the other plant

Table 2. Bacterial, fungal, and yeast strains used in recombinant proteins production

Expression systems	Strains used	Reference
E.coli	*E.coli ArcticExpress, E.coli BL21, E.coli BL21-Codonplus (RIL), E.coli M15, E.coli Lemo21(DE3), E.coli C43(DE3)*	[22]
Yeast	*Z. bailii, C. famata, H. polymorpha, P. methanolica, P. pastoris, P. stipites, K. lactis, Z. rouxii, S. occidentalis, S. cerevisiae, Y. lipolytica, C. boidinii*	[54, 55]
Aspergillus	*A. awamori, A. terreus, A. niger, A. sojae, A. nidulans, A. oryzae*	[43, 53, 56, 57]
Bacillus	*B. subtilis KL03, B. subtilis 168, B. megaterium MS941, B. licheniformis BL10GS, B. subtilis 1A751P7, B. subtilis IH6622, B. subtilis BNA, B. brevis, B. licheniformis.*	[1, 58]
Pseudomonas	*P. fluorescens, P. putida, P. aeruginosa*	[1, 9]
Streptomyces	*Streptomyces lividans*	[1, 22, 9]
Corynebacterium	*C. glutamicum, C. ammoniagenes,*	[9]
Trichoderma	*T. reesei, T. altroviride and T. vireus*	[43, 59]
Rhizopus	*Rhizopus oryzae*	[43]
Fusarium	*F. graminearum*	[43, 60]
Ralstonia	*Ralstonia eutropha*	[1]

systems [86, 87].

6.1 Production of Recombinant Proteins by using Plant Species

6.1.1 Tobacco

Recombinant protein production on the laboratory level is mainly done by using Tobacco plants. The Tobacco plant plays a very important role in the mass production of recombinant proteins. The yield with Tobacco plants is "more than 100,000 kilograms/ hectare, especially for the close-cropped tobacco" [88]. It also has the ability for the fast scaling-up process as it can produce large amounts of seeds. Some considerations to be kept in mind while looking for tobacco plants are as follows: protein storage in the aerial parts of the plants especially leaves is unstable which may cause product degradation. To avoid this degradation, product should be extracted as soon as they are expressed, alternatively the leaves can be dried or frozen for product extraction. Due to the presence of alkaloids which are toxic in nature and phenols the tobacco plant can also be used for the downstream process.

6.1.2 Cereals

Cereal seeds act as marvelous storage devices for proteins as they are outfitted with storage vesicles for protein storage. They are also adhering to an intracellular environment with drying conditions which helps them to reduce the activity of protease, and also helps in reducing the hydrolysis by non-enzymatic sources. Among all the food crops, the highest biomass yield is recorded for Maize [89, 90]. Now a days, Maize has been used for the biomass production of some recombinant antibodies, avidin as well as trypsin [82, 88]. Dry cereals such as wheat and rice seeds show high stability for recombinant protein storage and that is why they can be stored at normal temperatures without a noticeable loss in their activity. Due to the self-fertilizing ability of rice, the risk of transfer of transgenes to other plants have been reduced [82, 91].

6.1.3 Legumes

The worth mentioning legumes are alfalfa, soybean, and pea which have been used for the recombinant production of therapeutic proteins. Atmospheric nitrogen is fixed by the legume plants due to which they don't need the nitrogen in their fertilizers and thus leads to the low cultivation cost. The biomass yield of leaves is lower than that of Tobacco plants. Peas are being used as the expression systems due to the high content of proteins in their seeds [88].

6.1.4 Fruits and vegetables

Recombinant proteins especially therapeutic proteins are produced by using the vegetables and fruit crops such as tomato, lettuce, and most importantly potato. One of the main features of this system is that the produced proteins could be used for oral consumption after minimal processing. Conversely, quality maintenance and dose-ranging are still the main challenges to be addressed yet [88, 92, 93].

7. ANIMALS

Transgenic animals have been developed from cows, mice, sheep, goats, and rabbits for recombinant protein production as shown in Figure 5. Aquatic animals are also being explored for the same reason. Human factor IX and AAT (α antitrypsin protein) are being produced by the transgenic sheep in milk as shown in Figure 6. ß-lactoglobulin and tPA by transgenic mice [94]. The amount of recombinant proteins produced in animal milk is as follows: anti-thrombin III is being produced in goat milk around 14 g/L, α-1-antitrypsin is being produced in sheep milk around 35 g/L and α-glucosidase is being produced in rabbit milk 8 g/L. For recombinant proteins production genes are usually taken from human sources. The expression of non-milk foreign proteins is very low than milk proteins [95].

Transgenic mice urine is the main source for the production of growth hormones for humans. The amount produced by the mice is about 0.1 to 0.5 mg/L [1]. One of the most advent features of using the urinary system for recombinant protein production is that the animals start to urinate at an early age rather they lactate at a specific time in their life. About 10,000 L of milk is produced by a cow after hormonal treatment as compared to the amount of urine which is 6000 L. The main disadvantage of recombinant protein production by transgenic animals is the amount of time required for the estimation of production level. This time is

almost 32 months in the case of cows, in mice it is 3.5 months, in sheep it is 28 months and in pigs, it is 15 months [96]. For a cow to keep up, we need almost $ 10,000 per year.

Previously, transgenic animals were being used for the production of lymphokines and vaccines, but now scientist have shifted their focus towards protozoa and are exploring its potential to produce important protein products. Transgenic trypanosome is far better than transgenic animals for the production of heterologous proteins [1]. These benefits include i) with the help of homologous recombination, precise and stable integration is done, ii) the integration can be done on many sites due to which the expression from the multiple unit complexes can come out, iii) high densities growth and easy cells maintenance in the semi-defined medium.

8. INSECTS

An IC (insect cell) system is working as a solution between the two main expression systems which are the mammalian system and the bacterial system. With the development of BEVS (baculovirus expression vector system) recombinant protein production is welcomed by the IC platform [15]. The development of the IC platform includes two steps; first is the multiplication at the desired concentration and the second step is the addition of baculoviruses for infection purposes, this baculovirus also contains GOI [97]. The origin of insect cells used for this purpose came from *Drosophila melanogaster, Autographa californica,* and *Spodoptera frugiperda.* These cells are used as they are susceptible to baculovirus infection [97, 98]. Some other insects can also be used for the development of recombinant proteins such as tPA (plasminogen activator), hGAD65, parasitic proteins as well as viral proteins [17, 97, 98].

8.1 Silkworm

Bombyx mori, a silkworm, produces large quantities of silk proteins in its silk glands which can be used to form cocoons. Silk proteins are the major components of silk and include sericin and fibroin proteins majorly [99]. In the case of transgenic silkworms, the recombinant proteins are expressed in the silk glands. The expression and place of the recombinant proteins can be controlled by controlling the location of the genes that are able to produce the silk proteins. By using PGS (Pre-implantation genetic screening) we can localize the expression of silk proteins in the inner core of fibroin. By using MSG (Monosodium glutamate), the expression of silk proteins can be localized into the outer layer of sericin [99]. Table 3 represents the recombinant proteins obtained from different types of IC.

9. MICROALGAE

Microalgae, also known as photosynthetic microorganisms, are a diverse group of organisms capable of using sunlight to produce proteins, carbohydrates, and lipids [112]. They are usually considered as unicellular eukaryotes in spite of the fact that some cyanobacteria which are prokaryotic organisms also referred to as microalgae [113]. In recent years, interest has been developed in using microalgae for the production of biofuels as well as therapeutic proteins [1]. Some species of microalgae can be used as a substitute for fossil fuels in the biofuel industry due to their astonishing oil content [138]. Another reason for their biotechnical focus might be due to their ability to act as extraordinary bioreactors for the production of recombinant proteins on large scale. Microalgae exhibit the qualities of both prokaryotic and eukaryotic expression systems such as high progression rates as well as post-transcriptional and translational amendments. A major benefit over previously employed expression systems such as insect cells, mammalian cell lines, bacteria, and yeast is that algae have a phototropic lifestyle, making their cultivation CO_2-neutral and simply requiring relatively minimal expenditures [139]. Severe infections can be prevented by the use of vaccines but due to their costly production, the process of vaccination is highly influenced particularly in developing countries. Almost all the time, generation of antigen-grounded vaccines bearing heterologous expression is simpler than the antibody generation procedures. Despite this advantage, these antigen vaccines still required mammalian cell lines' expression system for their intricate post-translational modifications. In addition, they also face high risks of contamination by human pathogens. Microalgae have excellent opportunities in this regard as they do not serve as

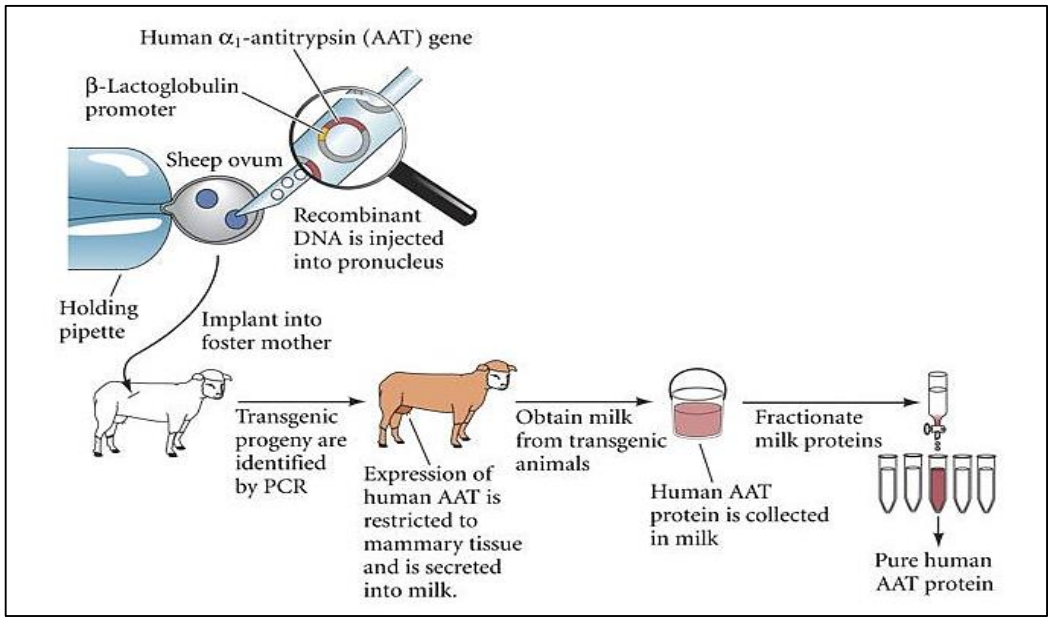

Fig. 5. Proteins pharmaceuticals production by the cloning of transgenic animals [138].

Table 3. List of recombinant proteins obtained from different IC (insect cells)

IC (insect cells) of	Recombinant proteins	References
• *Drosophila melanogaster* • *Autographa californica* • *Spodoptera frugiperda*	• **tpA**(plasminogen activator) • hGAD65 • Parasitic proteins • Viral proteins	[97, 98]
• Silkworm cocoons (Transgenic)	• Human collagen α chain • Human-serum albumin • Monoclonal antibody (mouse)	[99, 100, 101]

hosts for human infections and have rapid growth rates. In fact, once the proper bioreactors are in place, growing them is relatively inexpensive as they only require light and water [140].

9.1 *Chlamydomonas reinhardtii*

Chlamydomonas reinhardtii, is known as the model algae and is mostly used as a model for representing the eukaryotic micro-alga. Not only for its metabolic and genetic ability but also for its rapid reproduction. The sexual cycles are not only rapid but they are also controllable [107]. Microalgae as an expression system have expressed almost 20 recombinant proteins, especially in the *C. reinhardtii* [107]. *C. reinhardtii* is being used extensively because it is stable, and easily transformed especially for the transformation of mitochondrial organelles, nucleus, and chloroplast, quick reproduction of transformants, availability of many tool kits at the molecular level, and alternative growth of organisms such as heterotrophic growth and phototrophic growth. 16-5 % is the rate for the expression level of recombinant proteins. Proteins developed in chloroplast have higher levels of expression than expressed in the nucleus. HSV8 is the first protein that could be expressed in this organism [1].

9.2 Other algae

Some other algae are also being explored for recombinant protein production. With time the development of transgenic algae is enhancing. *Charophyte* alga is used for expressing exogenous genes [114]. *Gonium pectoral* which is a volvocine alga has a nuclear genome and it is successfully transformed [115] as it has the *C. Haematococcus pluvialis* [116, 117] because it is co-cultivated with the help of agrobacterium. *Chlorarachniophyte*

Lotharella amoebiformis which is a marine organism is successfully put into the transient transformation [118]. Some other successful transformations are done by using the Ulva *Pertusa chlorophyta* alga [119]. *Cyanidioschyzon merolae* also known as the red alga is used for successful transformation [120]. *Dunaliella salina* which is a previously known alga is also being used for the expression of recombinant proteins [121]. The genetics of cyanobacteria has also been explored for this purpose.

10. MAMMALIAN CELL LINES IN USE FOR RECOMBINANT-PROTEIN PRODUCTION

Mammalian cell lines offer a range of cell lines made from different tissues and support various proteins' growth. These cell lines have all the needed machinery for protein expression and release. Among many reasons for them being a preferred expression system, one reason is their ability to synthesize proteins which is closely related to proteins present in humans in terms of their molecular structure and biochemical properties [122]. They are preferably selected when it comes to glycosylated protein production as these proteins are usually complex [123].

Most of the biopharmaceutical research uses two types of mouse cell lines i.e., myeloma cell lines (NS0) and hybridoma cell lines (SP2-0) as well as two types of hamster cell lines known as CHO cell lines (Chinese hamster cell ovary cells 1) and BHK cell lines (Baby hamster kidney cells) [122]. The major reason why these cell lines are different from other expression systems is due to their ability of N- and O-linked glycosylation as proteins that work in glycosylation encodes 2 % of the human genome [124]. Nearly all mammalian cells have the necessary machinery for recombinant protein production, but only a few meet the criteria of bioreactors such as mouse myeloma cells NS0, CHO, Sp2/0, and BHK. Viral vectors which are helpful for gene therapy are usually formed by using mammalian cell lines. Many vaccines are manufactured by the use of mammalian cell lines such as rabies, measles, rubella, and hepatitis A [141]. FDA has approved 27 biopharmaceutical products and 12 of which are being produced in mammalian cells [123].

10.1 Human cell-lines

In the quest to produce human recombinant proteins, the current major focus for biopharmaceutical industries is to look for an expression system that is not only safe clinically but can also give a high yield of proteins [133]. As human cell lines have glycosylation machinery, a few powerful human lines have emerged as a substitute for human recombinant protein production on a commercial level [108]. HEK293 is the human cell line currently used for the production of different therapeutic products such as human-cl, rFVIIIFc, drotrecogen alfa, etc. Another human cell line HT-1080 also used for the production of pharmaceuticals such as Epoetin delta, Agalsidase alfa, and Idursulfase [141]. Table 4 represents the recombinant proteins produced by different expression systems. Further, Table 5 shows the main advantages and disadvantages of each type of expression system used.

11. CONCLUSION AND FUTURE PERSPECTIVE

A huge extent of therapeutic protein marketing comprises of a variety of products such as antibodies, vaccine subunits, hormones, and enzymes. In order to meet the day-by-day increasing need for these therapeutic proteins, DNA recombination technology is being used. All recombinant proteins are naturally different from each other and various challenges are kept into consideration while choosing an expression system for their production. Therefore, it is very significant to investigate the potential and limitations of several expression systems to choose the suitable one for particular protein production at an industrial scale. The optimization criteria of an expression system is evaluated on several factors such as productivity, efficiency, physiological characteristics, total cost, safety, convenience, and down-streaming conditions. There are certain challenges which are associated with recombinant protein production at a higher scale i. e., maintaining protein production in higher cell densities, separation of cell debris from viable cells and product of interest, separation of yield without causing cell lysis, downstream processing and mass transferring of the products [134]. DNA recombinant technology can also produce recombinant proteins comparatively

Table 4. List of recombinant proteins produced in different expression systems

Expression system type		Recombinant protein	Application	Reference
Insects (Transgenic silkworm cocoon)		Human collagen α chain	Biomaterial, DDS, therapeutics	[100]
		Human-serum albumin	Therapeutics	[101]
		Monoclonal antibody (mouse)	Therapeutics, diagnostics	[99]
Bacteria	Lactic acid bacteria (LAB)	Gamma-aminobutyric acid (GABA)	Anti-hypertensive & antidepressant activities	[102]
	P. fluorescens	Granulocyte colonystimulating factor (G-CSF)	Drug candidate	[103]
Plants	*N. benthamiana*	Protein E envelop from Zika virus	Zika virus	[104]
	Barley seed (*Hordeum vulgare*)	Human Epidermal Growth factor	Burns treatment	[17]
	Arabidopsis thaliana	Recombinant human intrinsic factor	Vitamin B12 defciency	[84]
Algae		High mobility group protein B1	Therapeutics	[105]
		cholera toxin B subunit (CTB-D2)	Oral vaccine	[106]
		Human metallothionine-2 (hMT-2)	Pharmaceutical, UV protection	[107]
Human Cell line	Hek293		Proteglycan	[108, 109]
	Hek293EBNA1		Erythropoietin	[110]
	PERC.C6		IgM	[111]

better than the native ones by manipulating the protein sequence at the genetic level. Therefore, recombinant proteins can be adopted in several applications such as therapeutics, diagnostics, health maintenance, etc. With technological advancement, nanotechnology has also been adopted with recombinant DNA technology for the production of more advantageous and efficient recombinant proteins [135].

12. CONFLICT OF INTEREST

The authors declared no conflict of interest.

13. REFERENCES

1. Y. Gong, H. Hu, Y. Gao, X. Xu, and H. Gao Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. *Journal of Industrial Microbiology and Biotechnology* 38(12): 1879-1890 (2011).
2. S. Khan, M.U. Ullah, R. Siddique, G. Nabi, S. Manan, M. Yousaf, and H. Hou. Role of recombinant DNA technology to improve life. *International journal of genomics* (2016).
3. B. Calo-Fernández, and J.L. Martínez-Hurtado. Biosimilars: company strategies to capture value from the biologics market. *Pharmaceuticals* 5(12):1393-1408 (2012).
4. D. Weinacker, C. Rabert, A.B. Zepeda, C.A. Figueroa, A. Pessoa, and J.G. Farías. Applications of recombinant Pichia pastoris in the healthcare industry. *Brazilian Journal of Microbiology* 44: 1043-1048 (2013).
5. N.K. Tripathi, and A. Shrivastava. Recent developments in bioprocessing of recombinant proteins: expression hosts and process development. *Frontiers of Bioengineering and Biotechnology* 7: 420 (2019).
6. Research Report. Recombinant DNA Technology Market Economy Size Expected a Growth of USD

Table 5. Comparison of advantages and disadvantages of using various expression systems

Expression system	Advantages	Disadvantages & Challenges	Ref.
Bacteria	Simple and low price media, High cell density, Easy to cultivate, Rapid growth rate, Cost-effective, Virus free, Optimized growth procedures, Genetically characterized, Fermentation processes worked out for scale-up, FDA approved SOPs	Post-translational modifications don't occur here, Secreted proteins are of low level, Inclusion bodies formation from protein aggregate, Endotoxin production, Susceptible to proteases degradation, Codon usage is preferential, Chances of misfolding and export issues for proteins larger than (>30kDa)	[1, 71, 76, 82, 125, 126]
Yeast	High cell density, Highly adaptable to fermentation processes, Genetically characterized, No endotoxin production, High-level protein secretion, High yield, Durability, Cost-effective, Maximum yield in chemically defined media, Product processing similar to mammalian cells, Can handle S–S rich proteins, Can assist in protein folding, Can glycosylate proteins, Correct folding in functional recombinant proteins, Low-budget purification processes, FDA approved SOPs	N-or O-linked glycosylation pattern is different from a eukaryote, Hypermannosylation, Proteolytic degradation, Hyperglycosylation, Produced glycoproteins are not found fit for human consumption	[1, 71, 76, 126, 127]

Expression system	Advantages	Disadvantages & Challenges	Ref.
Fungi	Poor growth conditions, Fast growth, High density, Well-characterized, Optimized growth procedures, Scalable, Moderately amenable to genetic engineering, Correct protein folding and processing, Less time consumption for production, Production strains are stable, Existing regulatory bodies' approval	Immunogenic sugars with nonhuman glycosylation	[82]
Insect (Baculovirus)	Post-translational modification is similar to higher eukaryotes, Proper protein folding, Able to produce glycosylated recombinant proteins, Produced protein is of high level, Up to 15 kb insert expressed, Noninfectious, and safe, Easy scale-up, Flexibility in protein-size, Signal peptides cleavage is efficient, and can express multiple genes at a time, FDA-approved SOPs	Slow growth rate, Expensive media, Multi-parallel protein expression is time-consuming and laborious, At late stages protein folding is improper, Stability of recombinant virus, Complex glycosylation not possible, Presence of lipidic envelopes in virions, Polyproteins processing is less efficient, Immunogenic sugars with nonhuman glycosylation, Undesired post-translational modifications	[1, 76, 82, 126]
Plants	Rapid growth, Low-cost purification, Suitable for glycosylated proteins production, Modification of any type is possible, Maximum and cheap scale-up possibility, Low growth costs, Can produce complex proteins, Relatively simple and cheap,	Highly specific to the plant of choice, Universal recombinant production system is not reported yet, Immunogenic sugars with nonhuman glycosylation, Lacks regulatory approval	[1, 82, 126, 128]

Expression system	Advantages	Disadvantages & Challenges	Ref.
	Human pathogen contamination risk is low, Growth procedures are optimized, Protein complexes (Proper folding and assembly)		
Animals	Scaling up potential is massive, Post-translational modifications are correct, Harvesting is easy, Farming techniques are optimized, Cell lines are stable, Production cost is low, Existing regulatory approval	Costly, Transgenic organisms are difficult to create, Production time period is long, and Ethical as well as regulatory issues, Recombinant protein production system is poorly characterized, Low control,	[1, 82]
Mammalian Cell lines	Post-translational-modifications are very much similar to native protein, Safe, No immunogenicity concerns, Highly adaptive, Properly folding, Can produce glycosylated protein, High yields, Many current products give precedent to regulatory bodies, Active research and industry funding, FDA approved SOPs	Grow very slowly, Difficult to cultivate, Time-consuming, Large-scale culture limitations, Protein yields are low, Nutrient requirements are complex, Fermentation cost is very high, Viral and human pathogen contamination risks are very high, Expensive, Costs increase due to complex growth requirements, Complex cells create problems in cell lines engineering and understanding, Product is heterologous, Cell lines are unstable, Scaling-up is difficult	[1, 71, 76, 126, 129]

223.0 Billion by 2028, According to Vantage Market Research, *Vantage Market Research* (2022). https://www.vantagemarketresearch.com/recombinant-dna-technology-market-1829/request-sample

7. W.H. Brondyk. Selecting an appropriate method for expressing a recombinant protein. *Methods in Enzymology* 463:131-147 (2009).
8. R. O'Flaherty, A. Bergin, E. Flampouri, L.M. Mota, I. Obaidi, A. Quigley, Y. Xie, and M. Butler. Mammalian cell culture for production of recombinant proteins: A review of the critical steps in their biomanufacturing. *Biotechnology Advances* 43:107552 (2020).
9. R. Chen. Bacterial expression systems for recombinant protein production: *E. coli* and beyond. *Biotechnology Advances* 30(5):1102-1107 (2012).
10. N. Ferrer-Miralles, P. Saccardo, J.L. Corchero, Z. Xu, and E. García-Fruitós. General introduction: recombinant protein production and purification of insoluble proteins. *Insoluble Proteins* 1-24 (2015).
11. S.A. Berkowitz, J.R. Engen, J.R. Mazzeo, and G.B. Jones. Analytical tools for characterizing biopharmaceuticals and the implications for biosimilars. *Nature Reviews Drug Discovery*11(7): 527-540 (2012).
12. Gupta, S.K., A.K. Dangi, M. Smita, S. Dwivedi and P. Shukla. Effectual bioprocess development for protein production. In *Applied Microbiology and Biotechnology,* 203-227 (2019).
13. C. Walsh. Posttranslational modification of proteins: expanding nature's inventory. Roberts and Company Publishers (2006).
14. F. Kadir, P. Ives, A. Luitjens, and E. van Corven. Production and purification of recombinant proteins. In *Pharm. Biotechnology* 47-67 (2013).
15. R.S. Felberbaum. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnology Journal* 10(5):702-714 (2015).
16. M. Karbalaei, S. A. Rezaee, and H. Farsiani. Pichia pastoris: A highly successful expression system for optimal synthesis of heterologous proteins. *Journal of cellular physiology* 235(9):5867-5881 (2020).
17. B. Owczarek, A. Gerszberg and K. Hnatuszko-Konka. A brief reminder of systems of production and chromatography-based recovery of recombinant protein biopharmaceuticals. *BioMed Research International* (2019).
18. F.J. Fernández, and M.C. Vega. Choose a suitable expression host: a survey of available protein production platforms. *Advanced Technologies for Protein Complex Production and Characterization* 15-24 (2016).
19. J.R. Ho, and J. Chien. Trends in translational medicine and drug targeting and delivery: new insights on an old concept—targeted drug delivery with antibody–drug conjugates for cancers. *Journal of Pharmaceutical Science* 103(1):71-77 (2014).
20. E. García-Fruitós. Lactic acid bacteria: a promising alternative for recombinant protein production. *Microbial cell factories* 11(1):1-3 (2012).
21. A.F. Jozala, D.C. Geraldes, L.L. Tundisi, V.D.A. Feitosa, C.A. Breyer, S.L. Cardoso, P.G. Mazzola, L.D. Oliveira-Nascimento, C.D.O. Rangel-Yagui, P.D.O. Magalhães, and M.A.D. Oliveira. Biopharmaceuticals from microorganisms: from production to purification. *Brazilian Journal of Microbiology* 47:51-63 (2016).
22. T.V. Plavec and A. Berlec. Engineering of lactic acid bacteria for delivery of therapeutic proteins and peptides. *Applied microbiology and biotechnology* 103(5):2053-2066 (2019).
23. R.A. Börner, V. Kandasamy, A.M. Axelsen, A. T.Nielsen, and E. F. Bosma. Genome editing of lactic acid bacteria: opportunities for food, feed, pharma and biotech. *FEMS microbiology letters* 366(1):291 (2019).
24. J.A. Mora-Villalobos, J. Montero-Zamora, N. Barboza, C. Rojas-Garbanzo, J. Usaga, M. Redondo-Solano, L. Schroedter, A. Olszewska-Widdrat, and J.P. López-Gómez. Multi-product lactic acid bacteria fermentations: a review. *Fermentation* 6(1): p.23 (2020).
25. M. Dragosits, G. Frascotti, L. Bernard-Granger, F. Vázquez, M. Giuliani, K. Baumann, E. Rodríguez-Carmona, J. Tokkanen, E. Parrilli, M.G. Wiebe, and R. Kunert. Influence of growth temperature on the production of antibody Fab fragments in different microbes: a host comparative analysis. *Biotechnology Progress* 27(1): 38-46 (2011).
26. M. Giuliani, E. Parrilli, P. Ferrer, K. Baumann, G. Marino, and M.L. Tutino. Process optimization for recombinant protein production in the psychrophilic bacterium Pseudoalteromonas haloplanktis. *Process Biochemistry* 46(4): 953-959 (2011).
27. V. Meyer, F. Wanka, J. van Gent, M. Arentshorst, C.A. van den Hondel, and A.F. Ram. Fungal gene expression on demand: an inducible, tunable, and metabolism-independent expression system for Aspergillus niger. *Applied and environmental microbiology* 77(9):2975-2983 (2011).
28. C. Batianis, E. Kozaeva, S.G. Damalas, M. Martín-

Pascual, D.C.Volke, P.I. Nikel, and V. A. Martins dos Santos. An expanded CRISPRi toolbox for tunable control of gene expression in Pseudomonas putida. *Microbial biotechnology* 13(2):368-385 (2020).

29. K.X. Huang, M. Badger, K. Haney, and S.L Evans. Large scale production of Bacillus thuringiensis PS149B1 insecticidal proteins Cry34Ab1 and Cry35Ab1 from Pseudomonas fluorescens. *Protein Expression and Purification* 53(2);325-330 (2007).
30. T.W. Overton. Recombinant protein production in bacterial hosts. *Drug Discovery Today* 19(5):590-601 (2014).
31. G.C. Barnard, G.E. Henderson, S. Srinivasan and T.U. Gerngross. High level recombinant protein expression in Ralstonia eutropha using T7 RNA polymerase based amplification. *Protein Expression and Purification* 38(2):264-271 (2004).
32. M. Hansson, P. Samuelson, T.N. Nguyen, and S. Ståhl. General expression vectors for Staphylococcus carnosus enabled efficient production of the outer membrane protein A of Klebsiella pneumoniae. *FEMS Microbiology Letters* 210(2):263-270 (2002).
33. T.C. Cairns, C. Nai, and V. Meyer. How a fungus shapes biotechnology: 100 years of Aspergillus niger research. *Fungal Biology and Biotechnology* 5(1):1-14 (2018).
34. L. Martins-Santana, L.C. Nora, A. Sanches-Medeiros, G.L. Lovate, M.H. Cassiano and R. Silva-Rocha. Systems and synthetic biology approaches to engineer fungi for fine chemical production. *Frontiers in Bioengineering and Biotechnology* 6:117 (2018).
35. V. Meyer, B. Wu, and A.F. Ram. Aspergillus as a multi-purpose cell factory: current status and perspectives. *Biotechnology Letters* 33(3):469-476 (2011).
36. F. Ntana, U.H. Mortensen, C. Sarazin and R. Figge. Aspergillus: A powerful protein production platform. *Catalysts* 10(9):1064 (2020).
37. C.S. Nødvig, J.B. Nielsen, M.E. Kogle, and U.H. Mortensen. A CRISPR-Cas9 system for genetic engineering of filamentous fungi. *PloS One* 10(7): e0133085 (2015).
38. T. Katayama, Y. Tanaka, T. Okabe, H. Nakamura, W. Fujii, K. Kitamoto, and J.L. Maruyama. Development of a genome editing technique using the CRISPR/Cas9 system in the industrial filamentous fungus Aspergillus oryzae. *Biotechnology Letters* 38(4):637-642 (2016).
39. K.K. Fuller, S. Chen, J.J. Loros, and J.C. Dunlap. Development of the CRISPR/Cas9 system for targeted gene disruption in Aspergillus fumigatus. *Eukaryotic Cell* 14(11):1073-1080 (2015).
40. N.S. Dunn-Coleman, P. Bloebaum, R.M. Berka, E. Bodie, N. Robinson, G. Armstrong, M. Ward, M. Przetak, G.L. Carter, R. LaCost, and L.J. Wilson. Commercial levels of chymosin production by Aspergillus. *Bio/Technology* 9(10): 976-981 (1991).
41. P.P. Ward, J.Y. Lo, M. Duke, G.S. May, D.R. Headon, and O.M. Conneely. Production of biologically active recombinant human lactoferrin in Aspergillus oryzae. *Bio/technology* 10(7): 784-789 (1992).
42. K.I. Nakajima, T. Asakura, J.I. Maruyama, Y. Morita, H. Oike, A. Shimizu-Ibuka, T. Misaka, H. Sorimachi, S. Arai, K. Kitamoto, and K. Abe. Extracellular production of neoculin, a sweet-tasting heterodimeric protein with taste-modifying activity, by Aspergillus oryzae. *Applied and Environmental Microbiology* 72: 3716-3723 (2006).
43. O.P. Ward. Production of recombinant proteins by filamentous fungi. *Biotechnology Advances* 30:1119-1139 (2012).
44. A. Harkki, J. Uusitalo, M. Bailey, M. Penttilä, and J.K. Knowles. A novel fungal expression system: secretion of active calf chymosin from the filamentous fungus Trichoderma reesei. *Bio/Technology* 7: 596-603 (1989).
45. J.M. Uusitalo, K.H. Nevalainen, A.M. Harkki, J.K. Knowles, and M.E. Penttilä. Enzyme production by recombinant Trichoderma reesei strains. *Journal of Biotechnology* 17(1): 35-49 (1991).
46. E. Nyyssönen, M. Penttilä, A. Harkki, A. Saloheimo, J.K. Knowles, and S. Keränen. Efficient production of antibody fragments by the filamentous fungus Trichoderma reesei. *Biotechnology* 11(5): 591-595 (1993).
47. M. Penttila. Heterologous protein production in Trichoderma. In: Trichoderma and Gliocladium. G.E. Harman C.P. Kubicek, Taylor and Francis, *London* 365-82 (1998).
48. A. Schuster, and M. Schmoll. Biology and biotechnology of Trichoderma. *Applied Microbiology and Biotechnology* 87(3): 787-799 (2010).
49. H.V. Colot, G. Park, G.E. Turner, C. Ringelberg, C.M. Crew, L. Litvinkova, R.L. Weiss, K.A. Borkovich, and J.C. Dunlap. A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. *Proceedings of the National Academy of Sciences* 103(27): 10352-10357 (2006).
50. S. Allgaier, R.D. Taylor, Y. Brudnaya, D.J. Jacobson,

E. Cambareri, and W.D. Stuart. Vaccine production in Neurospora crassa. *Biologicals.*, 37: 128–32 (2009).

51. D. Havlik, U. Brandt, K. Bohle, and A. Fleißner. Establishment of Neurospora crassa as a host for heterologous protein production using a human antibody fragment as a model product. *Microbial Cell Factories* 16(1): 1-15 (2017).
52. D. Magaña-Ortíz, F. Fernández, A.M. Loske, and M.A. Gómez-Lim. Extracellular expression in Aspergillus niger of an antibody fused to Leishmania sp. antigens. *Current Microbiology* 75(1): 40-48 (2018).
53. M.P. Zubieta, F.J. Contesini, M.V. Rubio,A.E.D.S.S. Gonçalves, J.A. Gerhardt, R.A. Prade, and A.R.D.L. Damasio. Protein profile in Aspergillus nidulans recombinant strains overproducing heterologous enzymes. *Microbial Biotechnology* 11(2): 346-358 (2018).
54. D. Porro, B. Gasser, T. Fossati, M. Maurer, P. Branduardi, M. Sauer, and D. Mattanovich. Production of recombinant proteins and metabolites in yeast. *Applied Microbiology and Biotechnology* 89(4): 939-948 (2011).
55. D. Porro, M. Sauer, P. Branduardi, and D. Mattanovich. Recombinant protein production in yeast. *Microbial Biotechnology* 31(3): 245-259 (2005).
56. A. Fleißner, and P. Dersch. Expression and export: recombinant protein production systems for Aspergillus. *Applied Microbiology and Biotechnology* 87(4): 1255-1270 (2010).
57. R. Mora-Lugo, M. Madrigal, V. Yelemane, and M. Fernandez-Lahore. Improved biomass and protein production in solid-state cultures of an Aspergillus sojae strain harboring the Vitreoscilla hemoglobin. *Applied Microbiology and Biotechnology* 99(22): 9699-9708 (2015).
58. K. Zhang, L. Su, and J. Wu. Recent advances in recombinant protein production by Bacillus subtilis. *Annual Review of Food Science and Technology* 11: 295-318 (2020).
59. A. Rantasalo, M. Vitikainen, T. Paasikallio, J. Jäntti, C.P. Landowski, and D. Mojzita. Novel genetic tools that enable highly pure protein production in Trichoderma reesei. *Scientific Reports* 9(1): 1-12 (2019).
60. P. Wong, M. Walter, W. Lee, G. Mannhaupt, M. Münsterkötter, H.W. Mewes, G. Adam, and U. Güldener. FGDB: revisiting the genome annotation of the plant pathogen *Fusarium graminearum. Nucleic Acids Research* 39(suppl_1): D637-D639 (2010).
61. R.A. Hitzeman, F.E. Hagie, H.L. Levine, D.V. Goeddel, G. Ammerer, and B.D. Hall. Expression of a human gene for interferon in yeast. *Nature* 293(5835): 717-722 (1981).
62. J. Reiser, V. Glumoff, M. Kälin, and U. Ochsner. Transfer and expression of heterologous genes in yeasts other than *Saccharomyces cerevisiae. Advances in Biochemical Engineering/ Biotechnology* 43: 75-102 (1990).
63. M.A. Romanos, C.A. Scorer, and J.J. Clare, J.J. 1992. Foreign gene expression in yeast: a review. Yeast 8(6): 423-488 (1992)
64. D.R. Haan, and W.V. Zyl. Differential expression of the Trichoderma reesei β-xylanase II (xyn2) gene in the xylose-fermenting yeast Pichia stipitis. *Applied Microbiology Biotechnology* 57(4): 521-527 (2001).
65. L. Brambilla, B.M. Ranzi, M. Vai, L. Alberghina, and D. Porro. Production of heterologous proteins from *Zygosaccharomyces bailii.* Tate and Lyle Ingredients Americas LLC, U.S. Patent 7,041,477 (2006).
66. P. Branduardi, D. Porro, M. Valli, and L. Alberghina. Process for expression and secretion of proteins by the non-conventional yeast *Zygosaccharomyces bailii.* U.S. *Patent Application* 10/534,171 (2007).
67. A. Goffeau, B.G. Barrell, H. Bussey, R.W. Davis, B. Dujon, H. Feldmann, F. Galibert, J.D. Hoheisel,C. Jacq, M. Johnston, and E.J. Louis. Life with 6000 genes. *Science* 274: 546–567(1996).
68. C.J. Huang, A.J. Lowe, and C.A. Batt. Recombinant immunotherapeutics: current state and perspectives regarding the feasibility and market. *Applied Microbiology and Biotechnology* 87(2): 401-410 (2010).
69. E. Çelik, and P. Çalık. Production of recombinant proteins by yeast cells. *Biotechnology Advances* 30(5): 1108-1118 (2012).
70. D. Mattanovich, N. Callewaert, P. Rouzé, Y.C. Lin, A. Graf, A. Redl, P. Tiels, B. Gasser, and K. De Schutter. Open access to sequence: browsing the Pichia pastoris genome. *Microbial Cell Factories* 8(1): 1-4 (2009).
71. N.K. Tripathi. Production and purification of recombinant proteins from Escherichia coli. *ChemBioEng Reviews* 3(3): 116-133 (2016).
72. D. Heefner, C. Weaver M. Yarus, and L. Burdzinski. 1992. Method for producing riboflavin with *Candida famata. US Patent No*. 5164303 (1992).
73. T. Papakonstantinou, R.H. Law, P. Gardiner,

M.J. Rowley, and I.R. Mackay. Comparative expression and purification of human glutamic acid decarboxylase from Saccharomyces cerevisiae and Pichia pastoris. Enzyme and Microbial Technology 26(9-10): 645-652 (2000).

74. J. Olmos-Soto, and R. Contreras-Flores. Genetic system constructed to overproduce and secrete proinsulin in *Bacillus subtilis. Applied Microbiology and Biotechnology* 62(4): 369-373 (2003).
75. C. Gurramkonda, S. Polez, N. Skoko, A. Adnan, T. Gäbel, D. Chugh, S. Swaminathan, N. Khanna, S. Tisminetzky, and U. Rinas. Application of simple fed-batch technique to high-level secretory production of insulin precursor using *Pichia pastoris* with subsequent purification and conversion to human insulin. Microbia. *Cell Factories* 9(1): 1-11(2010).
76. R. Arya, A. Bhattacharya, and K.S. Saini. *Dictyostelium discoideum*—a promising expression system for the production of eukaryotic proteins. *The FASEB Journal* 22(12): 4055-4066 (2008).
77. B. Dujon, D. Sherman, G. Fischer, P. Durrens, S. Casaregola, I. Lafontaine, J. De Montigny, C. Marck, C. Neuvéglise, E. Talla, and N. Goffard. Genome evolution in yeast. *Nature* 430(6995): 35-44 (2004).
78. V. Wood, R. Gwilliam, M.A. Rajandream, M. Lyne, R. Lyne, A. Stewart, J. Sgouros, N. Peat, J. Hayles, S. Baker, and D. Basham. Erratum: The genome sequence of *Schizosaccharomyces pombe. Nature* 415: 871-880 (2002).
79. Y. Giga-Hama, H. Tohda, K. Takegawa, and H. Kumagai. Schizosaccharomyces pombe minimum genome factory. *Biotechnology and Applied Biochemistry* 46(3):147-155 (2007).
80. S. Kjærulff, and M.R. Jensen. Comparison of different signal peptides for secretion of heterologous proteins in fission yeast. *Biochemical and Biophysical Research Communications* 336(3): 974-982 (2005).
81. Y. Tekoah, A. Shulman, T. Kizhner, I. Ruderfer, L. Fux, Y. Nataf, D. Bartfeld, T. Ariel, S. Gingis–Velitski, U. Hanania, and Y. Shaaltiel. Large-scale production of pharmaceutical proteins in plant cell culture—the protalix experience. *Plant Biotechnology Journal* 13(8):1199-1208 (2015).
82. M.J. Burnett, and A.C. Burnett. Therapeutic recombinant protein production in plants: Challenges and opportunities. *Plants, People, Planet* 2(2): 121-132 (2020).
83. K. Moustafa, A. Makhzoum, and J. Trémouillaux-Guiller. Molecular farming on rescue of pharma industry for next generations. *Critical Reviews in Biotechnology* 36(5): 840-850 (2016)
84. J. Yao, Y. Weng, A. Dickey, and K.Y. Wang. Plants as factories for human pharmaceuticals: applications and challenges. *International Journal of Molecular Sciences* 16(12): 28549-28565 (2015)
85. N. Yan, C. Fan, Y. Chen, and Z. Hu. The potential for microalgae as bioreactors to produce pharmaceuticals. *International Journal of Molecular Sciences* 17(6): 962 (2016).
86. R. Biłas, K. Szafran, K. Hnatuszko-Konka, and A.K. Kononowicz. Cis-regulatory elements used to control gene expression in plants. *Plant Cell, Tissue and Organ Culture (PCTOC)* 127(2): 269-287 (2016).
87. A.W. Smagur, K.H. Konka, A. Gerszberg, T. Kowalczyk, P. Luchniak, and A.K. Kononowicz. Green way of biomedicine–how to force plants to produce new important proteins. *Transgenic Plants-Advances and Limitations* 63-90 (2012).
88. J.K. Ma, P.M. Drake, and P. Christou. The production of recombinant pharmaceutical proteins in plants. *Nature Reviews Genetics* 4(10): 794-805 (2003).
89. O.O. Obembe, J.O. Popoola, S. Leelavathi, and S.V. Reddy. Advances in plant molecular farming. *Biotechnology Advances* 29(2): 210-222 (2011).
90. T. Kawakatsu, and F. Takaiwa. Cereal seed storage protein synthesis: fundamental processes for recombinant protein production in cereal grains. *Plant Biotechnology Journal* 8(9): 939-953 (2010).
91. E.P. Rybicki. Plant-made vaccines for humans and animals. *Plant Biotechnology Journal* 8(5): 620-637 (2010)
92. H. Daniell, M. Kulis, and R.W. Herzog. Plant cell-made protein antigens for induction of Oral tolerance. *Biotechnology Advances* 37(7): 107-13 (2019).
93. J. Marsian, and G.P. Lomonossoff. Molecular pharming—VLPs made in plants. *Current Opinion in Biotechnology,* 37: 201-206 (2016).
94. N.S. Rudolph. Biopharmaceutical production in transgenic livestock. *Trends in Biotechnology* 17(9): 367-374 (1999).
95. G. Dutton. Transgenic animal-based protein products move toward clinical trials. *Genetic Engineering News* 16(9): 37-37 (1996).
96. N.J. Chew. Emerging technologies: transgenic therapeutics. *Biopharm Eugene* 6:24-24 (1993)
97. E. Gecchele, M. Merlin, A. Brozzetti, A. Falorni, M. Pezzotti, and L. Avesani. A comparative analysis

of recombinant protein expression in different biofactories: bacteria, insect cells and plant systems. *Journal of Visualized Experiments,* 23: 97 (2015).

98. A. Contreras-G´omez, F. S´anchez-Mir´on, Garc´ıa-Camacho, E. Molina-Grima, and Y. Chisti, Protein production using the baculovirus-insect cell expression system. *Biotechnology Progress* 30(1): 1–18 (2014).
99. M. Tomita. Transgenic silkworms that weave recombinant proteins into silk cocoons. *Biotechnology Letters* 33(4):645-654 (2011)
100. T. Adachi, X. Wang, T. Murata, M. Obara, H. Akutsu, M. Machida, A. Umezawa, and M. Tomita. Production of a non-triple helical collagen α chain in transgenic silkworms and its evaluation as a gelatin substitute for cell culture. *Biotechnology and Bioengineering* 106(6): 860-870 (2010)
101. S. Ogawa, M. Tomita, K. Shimizu, and K. Yoshizato. Generation of a transgenic silkworm that secretes recombinant proteins in the sericin layer of cocoon: production of recombinant human serum albumin. *Journal of Biotechnology* 128(3):31-544 (2007).
102. Y. Cui, K. Miao, S. Niyaphorn, and X. Qu. Production of gamma-aminobutyric acid from lactic acid bacteria: A systematic review. *International Journal of Molecular Sciences* 21(3):995-1005 (2020).
103. D.M. Retallack, H. Jin, and L. Chew. Reliable protein production in a Pseudomonas fluorescens expression system. *Protein Expression and Purification* 81(2): 157-165 (2012).
104. M. Yang, H. Sun, H. Lai, J. Hurtado, and Q. Chen. Plant-produced Zika virus envelope protein elicits neutralizing immune responses that correlate with protective immunity against Zika virus in mice. *Plant Biotechnology Journal* 16(2): 572-580 (2018).
105. B.A. Rasala, M. Muto, P.A. Lee, M. Jager, R.M. Cardoso, C.A. Behnke, P. Kirk, C.A. Hokanson, R. Crea, M. Mendez, and S.P. Mayfield. Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of Chlamydomonas reinhardtii. *Plant Biotechnology Journal* 8(6): 719-733 (2010).
106. I.A. Dreesen, G. Charpin-El Hamri, and M. Fussenegger. Heat-stable oral alga-based vaccine protects mice from Staphylococcus aureus infection. *Journal of Biotechnology* 145(3):273-280 (2010).
107. E. Specht, S. Miyake-Stoner, and S. Mayfield. Micro-algae come of age as a platform for recombinant protein production. *Biotechnology Letters* 32(10):1373-1383 (2010).
108. K. Swiech, V. Picanço-Castro, and D.T. Covas. Human cells: new platform for recombinant therapeutic protein production. *Protein Expression and Purification* 84(1):147-153 (2012).
109. E. Adam, S. Sarrazin, C. Landolfi, V. Motte, H. Lortat-Jacob, P. Lassalle, and M. Delehedde. Efficient long-term and high-yielded production of a recombinant proteoglycan in eukaryotic HEK293 cells using a membrane-based bioreactor. *Biochemical and Biophysical Research Communications* 369(2):297-302 (2008).
110. X. Sun, P.E. Goh, K.T. Wong, T. Mori, and M.G. Yap. Enhancement of transient gene expression by fed-batch culture of HEK 293 EBNA1 cells in suspension. *Biotechnology Letters* 28(11): 843-848 (2006).
111. A. Tchoudakova, F. Hensel, A. Murillo, B. Eng, M. Foley, L. Smith, F. Schoenen, A. Hildebrand, A.R. Kelter, L.L. Ilag, and H.P. Vollmers. High level expression of functional human IgMs in human PER. C6® cells. In *MAbs* 1(2): 163-171 (2009).
112. J.A.V. Costa, and M.G. De Morais. The role of biochemical engineering in the production of biofuels from microalgae. *Bioresource Technology* 102(1): 2-9 (2011).
113. T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, and F. Bux. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology* 102(1):57-70 (2011).
114. J. Abe, Y. Hiwatashi, M. Ito, M. Hasebe, and H. Sekimoto. Expression of exogenous genes under the control of endogenous HSP70 and CAB promoters in the Closteriumperacerosum–strigosum–littorale complex. *Plant and Cell Physiology* 49(4): 625-632 (2008).
115. L. Kai, and H. Armin. Stable nuclear transformation of Gonium pectorale. *AGRIS* (2009).
116. S. Kathiresan, and R. Sarada. Towards genetic improvement of commercially important microalga Haematococcuspluvialis for biotech applications. *Journal of Applied Phycology* 21(5):553-558 (2009).
117. S. Kathiresan, A. Chandrashekar, G.A. Ravishankar, and R. Sarada. Agrobacterium-mediated transformation in the green alga Haematococcuspluvialis (Chlorophyceae, Volvocales) 1. *Journal of Phycology* 45(3):642-649 (2009)
118. Y. Hirakawa, R. Kofuji, and K.I. Ishida. Transient transformation of a chlorarachniophyte alga,

Lotharella amoebiformis (chlorarachniophyceae), with UIDA and EGFP reporter genes 1. *Journal of Phycology* 44(3):814-820 (2008).

119. M. Kakinuma, M. Ikeda, D.A. Coury, H. Tominaga, I. Kobayashi, and H. Amano. Isolation and characterization of the rbcS genes from a sterile mutant of Ulvapertusa (Ulvales, Chlorophyta) and transient gene expression using the rbcS gene promoter. *Fisheries Science* 75(4):1015-1028 (2009).
120. M. Ohnuma, T. Yokoyama, T. Inouye, Y. Sekine, and K. Tanaka. Polyethylene glycol (PEG)-mediated transient gene expression in a red alga, Cyanidioschyzonmerolae 10D. *Plant and Cell Physiology* 49(1): 117-120 (2008).
121. S. Feng, L. Xue, H. Liu, and P. Lu. Improvement of efficiency of genetic transformation for Dunaliellasalina by glass beads method. *Molecular Biology Reports* 36(6):1433 (2009).
122. J. Zhu. Mammalian cell protein expression for biopharmaceutical production. *Biotechnology Advances* 30(5):1158-1170 (2012).
123. T. Rose. Alternative strategies and new cell lines for high-level production of biopharmaceuticals. *Modern Biopharmaceuticals* 761-777 (2008).
124. C.T. Campbell, and K.J. Yarema. Large-scale approaches for glycobiology. *Genome Biology* 6(11): 1-8 (2005).
125. S.K. Gupta, and P. Shukla. Advanced technologies for improved expression of recombinant proteins in bacteria: perspectives and applications. *Critical Reviews in Biotechnology* 36(6):1089-1098 (2016).
126. S. Fahad, F.A. Khan, N.S. Pandupuspitasari, M.M. Ahmed, Y.C. Liao, M.T. Waheed, M. Sameeullah, S. Hussain, S. Saud, S. Hassan, and A. Jan. Recent developments in therapeutic protein expression technologies in plants. *Biotechnology Letters* 37(2): 265-279 (2015).
127. M. Haon, S. Grisel, D. Navarro, A. Gruet, J.G. Berrin, and C. Bignon. Recombinant protein production facility for fungal biomass-degrading enzymes using the yeast Pichia pastoris. *Frontiers in Microbiology* 6:1002 (2015).
128. R.A. Rader. FDA biopharmaceutical product approvals and trends in 2012. *BioProcess International* 11(3): 18-27 (2013).
129. O. Flaherty, A.B. Adam, and M. Butler. Mammalian cell culture for production of recombinant proteins: A review of the critical steps in their biomanufacturing. *Biotechnology Advances* 107552 (2020).
130. E. Łojewska, T. Kowalczyk, S. Olejniczak, and T. Sakowicz. Extraction and purification methods in downstream processing of plant-based recombinant proteins. *Protein Expression and Purification.* 120: 110-117 (2016).
131. M. Merlin, E. Gecchele, S. Capaldi, M. Pezzotti, and L. Avesani. Comparative evaluation of recombinant protein production in different biofactories: the green perspective. *BioMed Research International* (2014).
132. E.Y. Park, J.H. Zhang, S. Tajima and L. Dwiarti. Isolation of Ashbya gossypii mutant for an improved riboflavin production targeting for biorefinery technology. *Journal of Applied Microbiology* 103(2):468-476 (2007).
133. E. Langer. On the Horizon: New Expression Systems to Become Common Industry Platforms, *BioPharm International* 2-4 (2009).
134. S.R. Rudge, and M.R. Ladisch. Industrial challenges of recombinant proteins. *Current Applications of Pharmaceutical Biotechnology* 1-22 (2019).
135. L. Gifre, A. Arís, A. Bach, and E. Garcia-Fruitós. Trends in recombinant protein use in animal production. *Microbial cell factories* 16(1): 1-17 (2017).
136. K.X. Huang, M. Badger, K. Haney, and S.L. Evans. Large scale production of Bacillus thuringiensis PS149B1 insecticidal proteins Cry34Ab1 and Cry35Ab1 from Pseudomonas fluorescens. *Protein Expression and Purification* 53(2): 325-330 (2007). (Font)
137. J. B. Cibelli, K. H. Campbell, G. E. Seidel, M. D. West and R. P Lanza. The health profile of cloned animals. *Nature Biotechnology* 20(1):13-14 (2002).
138. Y. Chisti. Biodiesel from microalgae. Biotechnology Advances 25(3):294-306 (2007).
139. G. Potvin and Z. Zhang. Strategies for high-level recombinant protein expression in transgenic microalgae: a review. *Biotechnology advances* 28(6):910-918 (2010).
140. N. ERGEN and H. TÜFEKÇİ. Mammalian cell lines used in bioprocessing. *Journal of Experimental and Clinical Medicine* 39(3) (2022).
141. J. Dumont, D. Euwart, B. Mei, S. Estes and R. Kshirsagar. Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives. *Critical reviews in biotechnology* 36(6):1110-1122 (2016).