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TRANSGENIC *BOMBYX MORI* AS A BIOTECHNOLOGICAL PLATFORM TO PRODUCE RECOMBINANT PROTEINS

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Abstract

In recent years, the scientific community has been focused on developing feasible platforms to obtain recombinant proteins in order to meet the high demand. There is a wide range of bioreactors that are currently used for this purpose, as bacteria, yeast, mammalian, plant or insect cells. In this regard, decades of dedicated research have shown that the insect biotechnology field is showing the most promising results. In this direction, Bombyx mori exhibits a great potential as a bioreactor to produce target proteins as it possesses various advantages. As expression host, Bombyx mori cells provide optimal post-translational modifications and display a remarkable ability to produce in a short period a great amount of proteins. In this review we discuss progress and we highlight the potential of using transgenic Bombyx mori as a biotechnological platform to obtain both target proteins and to produce enhanced silk fibres. The versatility and the feasibility of transgenic Bombyx mori have been outlined by studies that reported the successful production of human proteins like adiponectin, animal proteins, virus-derived proteins and enhanced silk threads.

Key words: *Bombyx mori, insect biotechnology, recombinant proteins, transgenic silkworms.*

INTRODUCTION

Proteins are complex molecules that have a wide range of applications in various fields, specifically they are important elements for the food, chemical, textile, agriculture and cosmetic industries. Also, due to its diversity, proteins play a key role in the pharmaceutical field and in the medical area. These complex molecules are used as therapeutic proteins and research reagents, but they are also tools used for diagnosis (Dimitrov, 2012; Puetz & Wurm, 2019). Thus, the demand for recombinant proteins is continually increasing, and currently there are major efforts being made by the scientific community to develop feasible biotechnological bioreactors to produce recombinant proteins. In this regard, the insect biotechnology field is showing the most promising results (Chen et al., 2018).

Therefore, there are described various techniques to successfully engineer host's expression system and to successfully extract and purify the target proteins (Schillberg et al., 2019). In this regard, there are various expression systems that have been described and used, such as bacteria, yeast, mammalian, plant or insect cells. The most used host in order to produce recombinant proteins is *Escherichia coli* (*E. coli*), but there is a major throwback, specifically the limited post-translational modifications needed for proteins proper functionality (Nakaya et al., 2020). Even if mammalian cells are the most suitable hosts for recombinant protein production due to its complex processing mechanisms, there are impediments like the high production costs and high risk of contamination (Kollewe & Vilcinskas, 2013).

Insect cells represent ideal bioreactors to produce complex proteins. *Bombyx mori* is one of the most studied insects due to its great economic impact. Fibroin and sericin are the main proteins that are found in silk fibers. Fibroin is synthesized in the posterior silk gland (PSG) and sericin is synthesized in the middle silk gland (MSG) (Xu, 2014).

Silkworms are not only important for sericulture, moreover *Bombyx mori* is a prominent candidate as a bioreactor for recombinant proteins production and represents an important model organism in life sciences

(Chen et al., 2018; Meng et al., 2017). As expression host, *Bombyx mori* cells provide the post-translational modifications required for recombinant proteins structure and functionality. A great advantage owned by *Bombyx mori* as a bioreactor, is the remarkable ability to produce in a short period, a great amount of silk proteins. Another advantage exhibited by silkworms are the numerous genes that are homologous to human genes and the short generation time (Chen et al., 2018).

We herein, by highlighting the pivotal role of *Bombyx mori* in life sciences, review the literature that brings into focus the use of silkworms as a platform to obtain recombinant proteins with an extraordinary impact in the medical, animal sciences and insect biotechnology areas. Furthermore, the use of *Bombyx mori* as a bioreactor exhibits a great step forward for entomology.

PRODUCTION OF FOREIGN PROTEIN IN *BOMBYX MORI*, BY USING A BACULOVIRUS BASED SYSTEM

The first recombinant protein obtained by using *Bombyx mori* as a bioreactor, was the human interferon alpha (IFN-α), that exhibits a great importance for the pharmaceutical industry. For this purpose Maeda et al. (1985) used for the first time *Bombyx mori* nuclear polyhedrosis virus (BmNPV) as an expression system. The gene sequence of IFN-α was driven by the polyhedrin gene promoter, in order to achieve a substantial level of expression. After the expression of recombinant proteins and their secretion in the hemolymph, it was observed that the exogenous proteins are degraded by the cysteine protease. To overcome this obstacle, in order to obtain a higher level of recombinant proteins, another vector which was unable to express the cysteine protease was constructed (Kato et al., 2010).

The first step in the production of recombinant BmNPV is cloning the target gene into the transfer vector. For homologous recombination to take place, there is a second step involved, specifically the co-transfection with the virus DNA into the target cells. The most complicated part of this method is to differentiate and to isolate the recombinant baculoviruses from the ones which have not been transformed (Xiang et al., 2010). However, this process is laborious and time consuming (3-6 months) (Kato et al., 2010).

PRODUCTION OF TRANSGENIC *BOMBYX MORI* GERM-LINE BY USING A PIGGYBAC TRANSPOSON BASED SYSTEM

Since Tamura et al. (2000) reported the development of a complex for stable germline transformation in *Bombyx mori* by using a transposon named piggyBac, the interest of the scientific community has been focused on using *Bombyx mori* as a bioreactor to produce recombinant proteins, emphasizing the key role played by silk gland in production of this type of proteins.

The piggyBac transposon was first isolated from *Trichoplusia ni* (Cabbage looper). Being a movable genetic element, piggyBac can transpose its location between vectors and chromosomes, representing a “cut and paste” tool. PiggyBac as a vector has great benefits, specifically it is safer than a viral vector and it can transpose larger DNA fragments (Zhao et al., 2016). Representing an important tool for genome’s manipulation, piggyBac transposon has been carefully studied and has been shown that it possesses certain features, for example in most cases (98%) it prefers as a site for integration, adenine (A) - timine (T) rich sequences (Li et al., 2013; Yusa, 2015). The main components of piggyBac transposon are the inverted terminal repeats (ITRs), which are located to both ends, and an enzyme named transposase (Li et al., 2013).

Tamura et al. (2000) used a piggyBac system containing the green fluorescent protein (GFP). The gene was placed under the control of *Bombyx mori* cytoplasmic actin gene promoter (*BmA3*). The two elements were placed between the ITRs sequences. A non-autonomous helper plasmid which carried the transposase enzyme, was also used. They reported that in the G1 broods about 2% of individuals have been successfully genetically transformed. After these results were reported, many studies have focused on this topic, thus a wide range of recombinant proteins with applicability in the medical field and beyond, were produced.

SILK GLAND EXPRESSION SYSTEMS IN *BOMBYX MORI*

In the process of producing valuable recombinant proteins by using transgenic *Bombyx mori* as a biotechnological platform, the silk synthesis system is involved. However, the fibroin gene and the sericin gene are involved, being powerfully expressed in the silk gland. Up to now, for the production of recombinant proteins by using *Bombyx mori* as a bioreactor, several silk gland expression systems have been described. Each of these systems has advantages and disadvantages, but the target protein determines which kind of system is going to be used (Xu, 2014). Choosing the right system is the most important step for the recombinant proteins production.

Fibroin L chain gene expression system in *Bombyx mori*

The fibroin L chain gene (*FibL*) expression system was the first system of this type used to express an exogenous protein in the silkworms. When using this kind of expression system, the target proteins are secreted into the lumen of PSG as independent macromolecules. This structure has three main elements, specifically a 5′-flanking sequence, a 3′-flanking sequence, and a partial cDNA sequence of the *FibL* (Xu, 2014).

Tomita et al. (2003) applied *FibL* gene expression system for the production of human collagen in *Bombyx mori*. Xue et al. (2012) also used this system to express in *Bombyx mori* a hematopoietic growth factor, named human granulocyte-macrophage colony stimulating factor. In order to be secreted into the PSG lumen, the recombinant protein has to be linked with the fibroin H chain *(FibH)*. The transgene produced in *Bombyx mori* cannot compete with the endogenous normal *FibL* in the process of S-S linking with the *FibH*, since the normal *FibL* chain has higher affinity for the *FibH* chain. As the unmodified *FibL* has a stronger affinity for the *FibH*, there is a lack of the disulfide bond between the target protein and the *FibH*, that explains the low expression level of recombinant proteins (Tatemastu, 2012). To overcome this impediment, to obtain a higher production of recombinant proteins, Inoue et al. (2005) obtained mutant silkworms which did not have a full endogenous *FibL* gene sequence.

Fibroin H chain gene expression system in *Bombyx mori*

The expression system that involves the *FibH* gene is the most used system of this kind in *Bombyx mori* for the production of improved silk, needed for medical purposes. The most important advantage of this kind of system is the promoter, the *FibH* promoter has a stronger activity in *Bombyx mori* than the *FibL* promoter (Tatemastu, 2012). In this category of expression structures are included three systems R1, R2 and R3 (Xu, 2014). The last one is the most efficient expression system and its structure consists of *FibH* promoter and the N- and C-terminal ends of the *FibH* (Tatemastu, 2012). Also, when using this type of system, the recombinant proteins are secreted in the lumen of PSG as independent proteins. Even if the highest level of fibroin is found in the PSG, it is easier to extract and purify the target proteins from the MSG (Xu, 2014).

Teulé et al. (2012) used the *FibH* gene expression system to improve the mechanical properties of the silk thread, specifically they genetically manipulated *Bombyx mori* to produce silk threads containing sequences of spider silk proteins. The transgenic *Bombyx mori* lines produced silk fibers more resistant than the fiber produced by the untransformed silkworm lines. They observed that the silk threads were not only tougher than the silkworm fibers, but even tougher than the original spider silk fibers.

Sericin-1 expression systems in *Bombyx mori*

The three genes that are involved in the sericin synthesis are *ser1*, *ser2* and *ser3*. The proteins encoded by the first and the last genes shape the cocoon's sericin layer. The *ser2* gene encodes proteins which have been shown that are linked with larval silk (Kunz et al., 2016). The promoter activity of *ser1*, *ser2* and *ser3* genes was analyzed by Tatematsu et al., (2010) as the request for the production of recombinant proteins using feasible bioreactors continues to expand.

In order to examine the promoters activity, they used the binary GAL4/UAS expression system for the expression of EGFP. Using the *ser1* gene promoter, strong activity was observed in the PSG and MSG. In the MSG, the *ser3* upstream region showed average activity, but the *ser2* promoter did not show activity in none of the regions of the silk gland.
To increase the expression level of recombinant proteins the promoter's activity has to be improved. Thus, this type of expression system involves the use of an enhancer, specifically *hr3* (baculovirus-derived) and also implicates a trans-regulator, IE1 (Tomita et al., 2007).

SILK FIBERS WITH ENHANCED PROPERTIES OBTAINED BY USING TRANSGENIC *BOMBYX MORI*

Using transgenic *Bombyx mori* to create silk fibers with improved cell proliferation activity

A key role in tissue regeneration is played by the fibroblast growth factors. An important member of the fibroblast growth factors family is the basic fibroblast growth factor (FGF2) which is encoded by *FGF2* gene and for the first time it was isolated from the pituitary gland. FGF2 is involved in cell growth, differentiation, being an important element for tissue regeneration, including skin, muscle, cartilages etc., but also several studies have shown that it has an important role in postnatal neurogenesis, dendritic plasticity (Coffin et al., 2018; Simard et al., 2018; Yun et al., 2010). The transforming growth factor β1 (TGF- β1) is a cytokine which plays a pivotal role in the healing process, also is essential for the maturation of lymphocytes, neutrophils and macrophages (Lodyga & Hinz, 2020).
Wang et al. (2019) used transgenic *Bombyx mori* to obtain silk thread containing FGF2 and TGF- β1, in order to improve the silk fibers for medical uses, specifically for improving the cell proliferation and the anti-inflammatory activity. To construct the transgenic vector, they used the *ser1* promoter, nuclear polyhedrosis virus enhancer *hr3* and the 3′-UTR of *ser1*. GSG-P2A self-cleaving peptide was used to associate the target genes sequences with the *Bombyx mori* codon preference, and also the 3xp3-DsRed-SV40 cassette was integrated. Microinjection is the technique which has been used for delivering the transgenic vector into non-diapausing eggs. They successfully genetically engineered *Bombyx mori* to co-express the recombinant proteins in the silk fibers and MSG.
Acidic fibroblast growth factors (FGF1s) are heparin binding macromolecules and play a major role in cell growth and proliferation. Aiming to increase cell's proliferation activity, there was reported the integration of FGF1s in the *Bombyx mori*'s silk threads (Davis et al., 2018; Kerr et al., 2019).
Another protein involved in cell proliferation, with numerous applications in the medical area, is the human connective tissue growth factor (CTGF) (Wang et al., 2020). It plays a key role in extracellular matrix remodeling, being a cysteine-rich protein (Tsai et al., 2018; Wang et al., 2020). Due to its role in the medical field, specifically in wound healing and implantation, there is a need to produce this protein by using feasible bioreactors. Wang et al. (2020) used two modified *Bombyx mori* strains to produce recombinant CTGF. One of them carried *CTGF-8ht* gene, and the other one contained the *pepCTGF-8ht* gene (transdermal peptide), also an enhanced His-tag was included in both strains. *Bombyx mori* was successfully used as a bioreactor as the target proteins were both expressed in the silk gland and cocoon. In this case, an advantage of using silkworms as a bioreactor was the ease of extraction and purification process. Comparing the proliferation activity of the two recombinant proteins, *pepCTGF-8ht* displayed a better activity.

Using transgenic *Bombyx mori* to produce silk fiber with improved mechanical properties

One of the biopolymers that have superior mechanical features is the spider silk. The most important advantages of spider silk are the strength and the elasticity. These features make it a great candidate for medical applications, but farming the spiders encounters certain obstacles, thus the large-scale production must be done by using a feasible bioreactor (Tokareva et al., 2013; Xu et al., 2018). In order to produce spider fibers, numerous

bioreactors were used (bacteria, yeast, etc.), but the expressed proteins did not meet expectations (Xu et al., 2018). It has been shown that due to the most important spider silk macromolecule, specifically the major ampullate silk protein encoded by *MaSp* gene, the silk fiber exhibits superior mechanical features. This protein has a highly repetitive structure composed of glycine and alanine rich-regions (Santos-Pinto et al., 2016; Jun Xu et al., 2018). Being able to produce proteins that have highly repeated blocks in their structure, *Bombyx mori* is a suitable bioreactor to produce silk containing spider silk polypeptides.

Kuwana et al. (2014) obtained high-toughness silk by using transgenic *Bombyx mori*. They cloned a spider dragline protein fragment (SpA) and for the recombinant protein expression, the *FibH* gene expression system was applied. Along with SpA, the enhanced green fluorescent protein (EGFP) was placed between the N- and C- terminal ends of the *FilH.*

Xu et al. (2018) applied the TALEN-mediated HDR technology for editing *Bombyx mori* to replace *FibH* with *MaSp1.* They performed this study to obtain mass-production of spider silk due to its great mechanical properties. The results showed that the transgenic silk threads did not have the same strength as the original silk fibers, but the elasticity was improved.

In order to increase the tenacity and the extension of silk threads, another study was performed by Wang et al. (2015). Aiming to reduce the calcium content in silk and to increase the α-helix and β-sheet structures, they successfully transformed silk threads not by changing the gene sequence but by overexpression ion-transporting proteins.

Using transgenic *Bombyx mori* to improve cell-adhesive properties of silk fibers

Collagen and fibronectin are the most used proteins as layers for the surface of cell culture plates. Due to low availability of the two proteins, silk of *Bombyx mori* is a feasible alternative for this purpose (Yanagisawa et al., 2007). Though, the adhesive feature of silk fibers is not as strong as adhesive ability of collagen and fibronectin. It has been shown that using fibroin for coating the surface of fibroblast or endothelial cells cultures, the adhesion level was weak (Jacobsen et al., 2017). In order to increase the level of adhesion when using the silk fibers on the surface of cell cultures plates, Yanagisawa et al. (2007), inserted into the *Bombyx mori*'s genome, partial sequences of collagen and fibronectin. The results showed that the transgenic silk fibers which contained a fragment of recombinant fibronectin had stronger adhesion activity than the ones which had the partial sequence of collagen.

Enhanced silk with antimicrobial and anti-inflammatory activities by using transgenic silkworms

In order to obtain silk with enhanced properties, particularly with antimicrobial and anti-inflammatory activities, Xu et al. (2019) genetically engineered silkworms to produce recombinant human lactoferrin. This nutrient is a cationic glycosylated protein and has two main lobes, each one owning an iron-binding domain. It is found mainly in mammalian milk, but it is also found in other exocrine secretions like saliva, tears or serum (Kell et al., 2020; Sill et al., 2016). Lactoferrin plays a critical role in the innate immune response, owning antibacterial, antifungal and antiviral properties. Even if lactoferrin is well-known for the bactericidal activity, it also has anti-inflammatory and anti-carcinogenic effects (Kell et al., 2020; Xu et al., 2019).

Due to its great therapeutic activities, the demand for lactoferrin is continually increasing. Xu et al. (2019) used the *Ser-1* expression system to obtain human lactoferrin in silkworms. Their data showed that the level expression of lactoferrin is influenced by the transgene's insertion position. The results also confirmed the inhibitory effect of recombinant human lactoferrin. However, their findings showed that *Bombyx mori* is a promising candidate and a cost-effective bioreactor to produce recombinant proteins.

Gloverin2 (Glv2) is an antibacterial glycine-rich protein, being a key player in the lepidopteran insects innate immune response. Even if the silk threads from *Bombyx mori*'s cocoons exhibit antimicrobial activity, it is not able to combat the infections, thus it cannot be

used for medical purposes. Wang et al., (2019) developed transgenic silkworms by overexpressing the Glv2 protein. They reported the success of developing silk threads that exhibit an increased antimicrobial activity against various species of bacteria and fungi, by using transgenic *Bombyx mori*.

RECOMBINANT PROTEINS OBTAINED BY USING TRANSGENIC *BOMBYX MORI* AS A BIOREACTOR

Recombinant animal proteins

Nakaya et al. (2020) used the expression system of *Bombyx mori* to obtain a nuclear receptor, specifically the thyroid hormone receptor. This family of receptors has two main elements, respectively TRα1 and TRβ1. In the process of fulfilling the role of thyroid hormone function, the two receptors play a crucial role, being ligand-dependent transcription elements. However, these receptors moderate the expression of certain genes and control several main processes as metabolism or growth (Anyetei-Anum et al., 2018). The authors successfully obtained recombinant mice TRβ1 by using *Bombyx mori* as a bioreactor. To obtain the target receptor they used the piggyBac vector and the *FibH* gene promoter that were put together with glutathione S-transferase from *E. coli*. The entire fragment was controlled by the GAL4/UAS system and the EGFP was used to confirm subsequently the success of transformation. However, another transgenic vector was constructed in order to obtain PSG specific expression (Nakaya et al., 2020).

The main component and the most abundant protein of royal jelly is the major royal jelly protein-1 (MRJP1). MRJP1 received great attention from the scientific community due to its therapeutic properties in humans (Tian et al., 2018).

In the same direction, You et al. (2017) used transgenic silkworms to produce this glycoprotein. They successfully used the *FibL* expression system to secrete recombinant MRJP1 into the cocoons. In terms of post-translational modification, which is the main reason for not using bacteria or yeast as bioreactors, their data shown that glycosylation of exogenous protein occurred in *Bombyx mori*, highlighting the feasibility of using the transgenic silkworms to produce target proteins.

Antimicrobial peptides (AMPs) are indispensable proteins which are implicated in host's innate immune response to bacteria, fungi or viruses. AMPs are included in numerous organisms like plants or animals (Lei et al., 2019). It has been shown that the black soldier fly (BSF) owns a great potential to live in microbe-rich, hostile environments. However, BSF is currently studied in order to reduce the waste amount due to its extraordinary ability to transform the waste into valuable biomass (Mertenat et al., 2019). Thus, owning this great ability, BFS is one of the most important sources of AMPs (Moretta et al., 2020). Another direction of approach and use of transgenic silkworms, was reported by Xu et al. (2020). They aimed to use transgenic *Bombyx mori* to reduce the incidence of pathogen infections, thus to help the sericulture industry. By using a piggyBac vector, the authors introduced an AMP cassette derived from the BSF. In the cassette named HiAMP4516, the authors incorporated three AMPs, specifically *Hidefensin-1, Hidiptericin-1* and *HiCG13551*. Their results proved that *Bombyx mori*'s susceptibility to bacterial pathogen infection could be reduced by using genetic engineering.

Iizuka et al. (2009) reported the successful use of *Bombyx mori* as a bioreactor to produce recombinant mouse monoclonal antibodies (mAbs) in the cocoons. The use of mAbs is a promising therapeutic method to prevent the infectious disease (Jahanshahlu & Rezaei, 2020). They highlighted the ease of mAbs extraction and purification, thus the feasibility of using *Bombyx mori* as a bioreactor to produce recombinant mouse mAbs (Iizuka et al., 2009).

In addition to mAbs role in the prevention of the infection diseases, the use of recombinant mAbs is one of the most promising methods against cancer. As a bioreactor, Chinese hamster ovary (CHO) cells are the most used cells to obtain recombinant mAbs.

Even if there is a wide range of therapeutic mAbs that have been approved for medical purposes, the process of obtaining therapeutic

mAbs by using this type of cells is not cost effective. Due to the high costs involved in obtaining therapeutic mAbs, Tada et al. (2015) used transgenic *Bombyx mori* to produce chimeric human-mouse anti-CD20 mAbs in order to provide successful target therapy for different types of cancers. In this study the expression level of anti-CD20 mABs produced by using transgenic silkworms was compared with the level of expression observed by using CHO cells. The mAbs used contained an analogous sequence to rituximab, that is one of the most used agents against cancer. Their data revealed a unique feature owned by recombinant mAbs obtained by using transgenic *Bombyx mori*, specifically the mAbs containing N-glycan structures. Compared with the antibody-dependent cellular cytotoxicity level observed in the mAbs produced by using Cho cells, the level observed in mABs derived from transgenic *Bombyx mori*, was higher. Comparing the complement-dependent cytotoxicity activity observed in the mAbs obtained by using both type of cells, the mABs derived from transgenic silkworms present a lower activity that the mAbs obtained by using CHO cells (Aoyama et al., 2018; Tada et al., 2015).

Recombinant human proteins

Vascular endothelial growth factors (VEGFs) play a crucial role in angiogenesis. Angiogenesis represents a critical step in cancer development, precisely in the process of changing the cancer state from benign to malignant. The VEGFs effect on tumor cells results in protecting them from apoptosis (Shibuya, 2011). There is a massive demand for VEGFs due to its applicability in the medical field, specifically the scientific community is focused on developing strategies to inhibit the VEGF in order to treat cancer. Another reason besides the research for developing anti-cancer treatments, is the therapeutic induced angiogenesis by VEGF. On this purpose Zhang et al. (2019) constructed transgenic silkworms that expressed recombinant VEGF165 in MSG and secreted it into the cocoons. This study underlines the feasibility of using transgenic silkworms to produce target recombinant proteins.

Another research has been focused on using transgenic silkworms to evaluate the response of target human protein to certain drugs. The authors developed transgenic *Bombyx mori* that synthetized the human insulin receptor (hIR) (Matsumoto et al., 2014). Insulin is a pancreatic hormone that has a crucial function in the organism due to its role in the metabolism of glucose and lipids. Glucose homeostasis depends on the insulin level; the hormone controls the conversion of glucose into glycogen. To regulate the metabolism, the insulin must bind to its specific receptor, hIR (Hall et al., 2020). Matsumoto et al. (2014) obtained recombinant hIR by using transgenic silkworms and demonstrated its ability to reduce the hemolymph sugar level. Their data highlighted the similarity between the silkworms and humans, in terms of drug pharmacokinetics, however, the transgenic lines could be used to examine the curative outcome of hIR agonists.

Adiponectin is a peptide secreted by adipocytes being an important factor for glucose and lipid metabolism. However, low concentration level of adiponectin is associated with obesity related disorders, like diabetes or various cardiovascular affections. This protein has an important insulin-sensitizing activity and represents a pivotal player in the process of decreasing the insulin resistance, in order to combat type 2 diabetes (Achari & Jain, 2017; Luo et al., 2020). Being a potential versatile therapeutic agent there is a high demand for human adiponectin in the medical area. Shin et al. (2014), in an effort to combat the obesity rate, used *Bombyx mori* as a bioreactor to obtain human adiponectin. Their findings showed that the target recombinant protein was successfully produced in silkworms and the recombinant adiponectin is a promising future therapy for type 2 diabetes patients.

Recombinant virus-derived proteins

Hepatitis B virus (HBV) causes liver infection that leads to cirrhosis or hepatocellular carcinoma. In order to develop a new effective vaccine against Hepatitis B virus, Abdurakhmanov et al. (2019) used *Bombyx mori* to obtain recombinant PreS2-S protein. This protein is a family member of a surface

antigen of HBV. Their aim was to develop a method that is cost effective and not time-consuming to obtain the PreS2-S protein in silkworm larvae. The recombinant PreS2-S protein obtained by using BmNPV as an expression vector, is a potential candidate for developing a new vaccine against HBV.

The use of recombinant subunit vaccines is a promising approach to avoid the safety concerns of using cell-culture based vaccines. The recombinant subunit vaccines do not involve the use of a virus but the use of virus-like particles (VLPs). The transgenic silkworms were used as a platform to produce recombinant target protein by Deo et al. (2011), specifically they used this type of bioreactor to obtain Rous sarcoma virus-gag virus-like particles. Their data highlight the feasibility of using transgenic silkworms to obtain VLPs in order to produce vaccines.

CONCLUSIONS

Although there are numerous expression platforms that are used to produce target proteins that own a great impact for animal science area but also for the human health and beyond, transgenic *Bombyx mori* is one of the most promising candidates in this regard. This expression system is feasible due to *Bombyx mori*'s remarkable ability to satisfy the extensive post-translational modification required for recombinant proteins structure and functionality. There are various studies in which *Bombyx mori* was manipulated to enhance the silk threads quality for medical purposes. Numerous studies reported the success of developing silk threads that possessed improved cell-adhesive properties, better mechanical features or silk fibers that owned antimicrobial and anti-inflammatory activities. These findings highlight the key role of *Bombyx mori* in obtaining biomaterials with enhanced properties. Furthermore, there is a broad variety of recombinant proteins with clinical importance that have been developed by using silkworms as expression hosts. The versatility and the feasibility of using this type of bioreactor have been highlighted by the studies that reported the successful production of human proteins, animal proteins and virus-derived proteins. This review highlights the feasibility and the importance of *Bombyx mori* as a powerful biotechnological platform for large-scale production of recombinant proteins.

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