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Spider Silk from Silk Worm: An Innovative Venture towards Super Silk

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ABSTRACT: Silk production is an age long practice in this world. Recent development in silk industry brings huge potential by exposing the silk industry to genetic engineering, proteomics and medical sciences. Genetic engineering has significant contribution in bringing the best out of silk production by adding non-commercial silks to produce some innovative approaches. Spider silk when mixed or incorporated with the silk producing genes of the silkworm does the miracle of super silk production. In this context, super silk evolution along with its broad genetic usage will be enlightened and the hidden areas are discussed thoroughly. The collection of reviews is emphasized to enlighten the importance of spider silk and its utility in absence of the naturally grown silks obtained from silkworms. As the materials related to spider silk are scanty, this review paper is of utmost effort to provide all the information regarding the same.

Keywords: Silkworm, Spider silk, Genetic engineering, Goat silk.

INTRODUCTION

The combination of sericulture, along with genetic engineering has broadened an innovative way towards a novel biomaterial. In this context, spider silk has gained significant predominance in textile industry due to its attractive mechanical properties. Yet, there were some constraints related to behaviour like cannibalistic behaviour and solitary habit of spiders have made the mass production of spider silk limited. So, in this context, genetical engineering is the only option to obtain higher quality of silk and synthetic spider silk and derived products have been produced from genetically engineered goats, *E. coli*, alfalfa, and silkworms.

Sericogenic Fauna. Silk is commercially produced by silkworms, though there are many diversified insects and non-insects that form silk for their survival. Apart from the silkworms, other animal species which involve themselves in silk production (although not commercial and not exploited for mankind) are listed below:

Sr. No.	Name of the insect	Family and Order	Feature of the silk produced
Insect Silk Producers			
1.	Mulberry silkworm (*Bombyx mori*)	Fam.: Bombycidae Ord.: Lepidoptera	• Most commercial silk produced in the world • Domesticated silk produced by silkworms monophagously fed on Mulberry plant
2.	Tasar Silkworm (*Antheraea* spp.)	Fam.: Saturniidae Ord.: Lepidoptera	• Wild silk, produced entirely on outdoor conditions. • Polyphagous (Feeds on various plants)
3.	Muga Silkworm (*Antheraea assamensis*)	Fam.: Saturniidae Ord.: Lepidoptera	• Endemic to India and produced in Brahmaputra valley of north-eastern part of India • Golden yellow coloured silk produced by semi-domesticated silkworm species
4.	Eri Silkworm (*Samia ricini*)	Fam.: Saturniidae Ord.: Lepidoptera	• Brick-red to white coloured silk produced by the domesticated silkworm • Non-continuous form of silk thread, hence not reeled, only spun
5.	Anaphe Silkworm (*Anaphe panda*)	Fam.: Notodontidae Ord.: Lepidoptera	• Produced by univoltine silkworm • Silk is more elastic and stronger than mulberry silk and used in production of parachutes
6.	Fagara Silkworm (*Attacus atlas*)	Fam.: Saturniidae Ord.: Lepidoptera	• Silk produced by one of the largest silk-moth • Silk is of light brown colour
7.	*Pachypasa otus, P. lineosa*	Fam.: Lasiocampidae Ord.: Lepidoptera	• The insect produces Coan silk which is predominant in Mediterranean region • Used in making of Crimson dyed apparel worn by dignitaries of Rome

8.	Egger moth (*Gonometa* spp.)	Fam.: Lasiocampidae Ord.: Lepidoptera	• Produces *Gonometa* silk which is widely distributed in wild regions of Africa • Unreelable silk that is spun to form lustrous silk
9.	Raspy cricket (*Gryllus* spp.)	Fam.: Gryllacrididae Ord.: Orthoptera	• Crickets use silk to burrow in sand, earth or wood • This silk has many complementary properties to mulberry silk as these are with extended beta sheet structures
10	Hornets (*Vespa orientalis*)	Fam.: Vespidae Ord.: Hymenoptera	• Silk made by the hornets is used to make the "Hornet's nest"
11.	Weaver ants (*Oecophylla smaragdina*)	Fam.: Formicidae Ord.: Hymenoptera	• Silk is produced by the worker ants' larvae through excretion • Silk used for the purpose of protection of nest
12.	Web spinners (*Embidina*)	Fam.: Embiidae Ord.: Embioptera	• Silk galleries has been produced by the glands of fore leg of web spinners • Silk used as the habitat materials
Non-insect Silk Producers			
13.	Bivalve (*Pinna squamosa*)	Fam.: Pinnidae Ord.: Pteriida	• Silk produced by Bivalve is called as Mussel silk • Brown filaments called 'Byssus' spun to form a silk filament popularly known as 'Fishwool'
14.	Spider silk (*Nephila madagascarensis*)	Fam.: Araneidae Ord.: Araneae	• Gossamer silk is produced by spiders which are fine thread used for ballooning by them • Silk is resistant to extremely high temperature and used in optical instruments

List of some important hosts that can be used for spider silk production

The production of spider silk is a tedious job as collection of large number of spiders is very cumbersome process. Scientists failed to set up a spider farm due to their cannibalistic (killing each other in the same group) nature.

To solve this problem, a professor of molecular biology at the University of Wyoming, Randy Lewis along with other researchers decided to introduce the gene of interest *i.e.,* the spiders' dragline silk gene into any lactating animals like goats in such a way that they would only make the protein in their milk. Only a certain percentage of the goats ended up with the spider gene like any other genetic factor. Out of seven goat kids born in February 2010, merely only three have tested positive for having the spider silk protein gene. Researcher Lewis found out that when these transgenic goats had kids and started lactating, much higher quantities of spider silk could be purified from the milks collected from the lactating goats. The key finding was that, the goats did not seem to have any changes in their health, morphology or behaviour compared to goats without the gene, other than being able to produce the spider silk protein. Spider goats are genetically engineered goats whose milk contains spider-silk protein. Professor Randy Lewis genetically modified a part of the goat DNA by substituting it with a spider gene and created spider-goats that helped to ace the spider silk production. A silk-spinning gene of the spider is added to the DNA of a goat, while the goat is still an egg in its mother's womb. At the initial stage, the nucleus and chromosomes from the original egg are taken out and the chromosomes from the genetically modified cell are put into the egg by the help of genetic engineering. The genes divide and multiply with the gradual growth of developing eggs. Thus, the body of the goat produces spider-silk protein during the course of its growth. This gene is passed on to the next generation when the goat reproduces. The spider-goats have adorable names such as "Pudding," "Sweetie," and "Freckles." at Utah State University. They are reared for their milk which contains spider-silk protein. The silk is turned into powder, processed and spun into silk fibre, after separating from the milk (Anonymous, 2019).

Scientists plan to incorporate the silk genes into protein rich plants like alfalfa which contains 20-25% of protein, making it a suitable plant for silk protein inoculation. The genetically modified spider-goats look just like any normal goat with the only difference of possessing of spider-silk proteins. Spider silk belongs protein-based materials called "biopolymers" which is of biological origin. The goat milk is separated, and the left-over product is refined, washed, freeze-dried several times before turned into powder. The final processed powder can be spun into a fibre or can be transformed into a coating or adhesive. Although the fibre from spider-goat's milk is only one-half to two-thirds as strong as the spider silk, its elasticity remains same as that of original spider silk (Anonymous, 2019).

There are three layers present in human skin *viz.*, epidermis, dermis, and hypodermis. Cells from the dermis and epidermis layers were used in order to create bulletproof skin. Then, synthetic spider silk was sandwiched between these 2 human skin layers made of bio-engineered skin cells experimented at Leiden University Medical Center in the Netherlands. Usages of spider-goat silk is manifold in practical, most prevalent being in the creator of bulletproof skin. Utah State University got a $1 million contract with the US Army to produce this bio-engineered silk in order to use for defence purpose (Anonymous, 2019).

Even though, the synthetic spider silk proteins can be produced from different hosts, these are usually very minute than original spider silk proteins and purification of this silk is a difficult process. Furthermore, the resulting synthetic fibre is having very low mechanical properties which needs to be improved. Unlike the other hosts, eventhough, in a manner similar to spiders, silkworms are capable of generating large quantities of a fibrous product, the light chain (LC) region of the silkworm genome was incorporated with Minor ampullate spider silk (MiSp) genes through CRISPR/Cas9 induced non-homologous end joining, lead to production of super silk. Similarly, transgenic silk worm genes also incorporated in the heavy chain (HC) region of the silkworm genome to create hybrid, which further was cross-bred with the LC- genome modified silk worm to produce dual transgenic silkworm.

Why spider silk ?

The transgenic silk also known as super silk was found with increased and advanced mechanical properties compared to original silkworm fibres (Morgan, 2016). But, the transgenic silk gained the strength of the spider silk by retaining the elasticity of the original silkworm silk. Spider silk was used for wound covering, preparation of fishing line and used as crosshairs in optical devices by the ancient Greek people. So, attempts have been made to immobilise the spiders for harvesting the respective silk and also by collecting the webs and spider web-sacs. But in long term, these attempts have failed (Lewis, 1996). Recently, along with a team of 80 people, two artists in Madagascar have been observed to collect spider silk from the spiders of species, *Nephila madagascariensis* over five years. *Nephila* silk is obtained to create luxurious textile materials. The mechanical properties and silk spinning process of spider silk and silk worm-produced silks were similar. There are various silks up to six different types produced by spiders (Rising and Johansson, 2015; Tokareva *et al.*, 2013; Colgin and Lewis, 1998; Lewis, 2006) and each silk filament has a biological role. Despite having origin of different glandular systems, spider silks have common features that they contain high amounts of amino acids like serine, alanine and glycine, thus becoming insoluble fibres. This combination finally turns into secondary and tertiary structures that give spider silk its unprecedented mechanical tensile strength (Colgin and Lewis, 1998; Lewis, 2006).

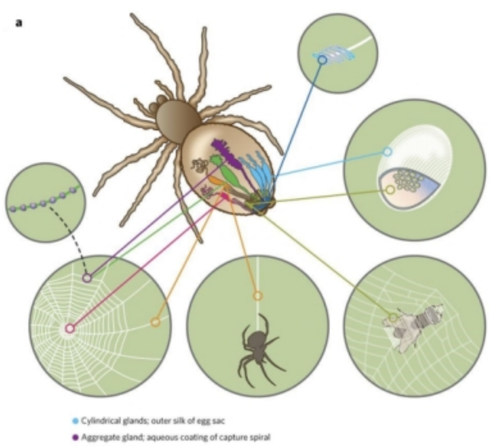

Fig. 1. Depiction of the types of spider silk and originating silk glands.

Dragline silk which is known as one of the major ampullate silk (MaSp) is the largest and most accessible of the spider silk glands, is the strongest and toughest biological materials known till today (Xu and Lewis, 1990; Gosline, 1999; Fahnestock and Irwin, 1997). MaSp is used as the skeletal frame of the orb webs and safe guard the spiders during its borderline movements in the webs. Flagelli-form silk is a highly elastic silk and is used as the main component of capture spiral in an orb web (Adrianos *et al.*, 2013). The combination of strength and extensibility allows this fibrous biomaterial to absorb a large amount of energy with a very small amount of material. The flagelli-form silk in the capture spiral of a web will absorb around 65% of the kinetic energy of an insect flying into it (Gosline, 1999). Overall, the aggregate spider silk serves perfect trap for the spider's pray without failing to gain food for the spider. Thus, in spider silk, flagelli-form silk is comprising the important part, while Major ampullate spider silk proteins (MaSp1 and MaSp2) and Minor ampullate silk proteins (MiSps) have highly repetitive regions rich in glycine and alanine (Opell and Hendricks, 2010) however, MiSpare lower in tensile strength due to the lack of β-spirals leading to decreased hydrophobic interactions (Hayashi *et al.*, 1999; Tatham and Shewry, 2000; Holland *et al.*, 2008). MiSphas a specific region rich in amino acids like serine and alanine. Though the amorphous regions in the silkworms' silk are comprised of 30 amino acids while, the MiSp spacer is comprising of 137 amino acids (Opell and Hendricks, 2010).

Despite having a large range of mechanical properties, the large abdomens of large orb weaving spiders allow for easier research, but the collection process of spider silk fibers is laborious process (Xu and Lewis, 1990). The cannibalistic, territorial behaviour and forced reeling resulted in uneven silk strands (Breslauer *et al.*, 2009). These obstacles impede the development of innovative spider silk products (Opell and Hendricks, 2010).

Under natural conditions, orb weaver spiders have a tendency to attach the spun silk to the substratum and slowly move away to draw the silk filament out. In this context, forced spinning is a good option to get the spider silk but it significantly impacted the mechanical properties and tensile strength of silk (Koeppel and Holland, 2017; Vollrath *et al.*, 2001). Certain artificial methods of silk spinning, such as wet spinning, the silk fibres were put into the coagulation bath which further treated to increase mechanical properties, but the most of wet-spun fibers were still having lower mechanical properties. Furthermore, *E. coli* has been genetically engineered to produce anoriginal sized (284.9 kDa) spider silk which resulted in silk fibers with high mechanical properties. But, the total quantity of bacterial silk is negligible as compared to the spider silk (Kato *et al*., 2010).

Sericulture, a century long practicesthe farming of silkworms for silk cultivation (Barber, 1991; Murugesh Babu, 2017). Generally, silk worms belong to Bombycidae and Saturniidae grow in climatic favourable regions. The silk fibre is composed of dual fibre and composed of fibroin and sericin, where sericin acts like a glue to hold the 6 fibroin fibers together that provide higher tensile strength. Fibroin is the major silk protein part of silk worm composed of three main components, the light chain (LC), the heavy chain (HC), and a glycoprotein (P25). A disulphide bond connects the large protein part HC (~350 kDa) with the LC (~26 kDa). This disulphide bond provides tensile strength to the silk filament. A fibroin elementary unit is prepared by the P25 (~30 kDa) which also serves as a stabilizer of the six HC-LC units through non-covalent interactions. Like spider silk, the HC region of fibroin is composed of highly repetitive and hydrophobic regions rich in Glycine, Alanine and Serine which form beta sheet structures which are responsible for the mechanical strength of silk (Sehnal and Žurovec, 2004; Malay *et al.*, 2016; Craig and Riekel, 2002). The LC of the fibroin silk is giving the silk its elastic properties by its amorphous, with less crystalline regions and no repetitive motifs. Combined with P25, the fibroin unit results in a fiber that is composed of 2/3 crystalline regions and 1/3 amorphous regions (Koh *et al.*, 2015; Zafar *et al.*, 2015). Both the silk worms and spiders use similar silk spinning methods (Anderson *et al.*, 2016; Omenetto and Kaplan, 2010). Silkworm silk glands originate from salivary glands whilethe spider silk glands originate from ectodermal invaginations in their abdomen. However, despite difference in their origins, the morphological and functional properties of the glands are very similar (Omenetto and Kaplan, 2010).

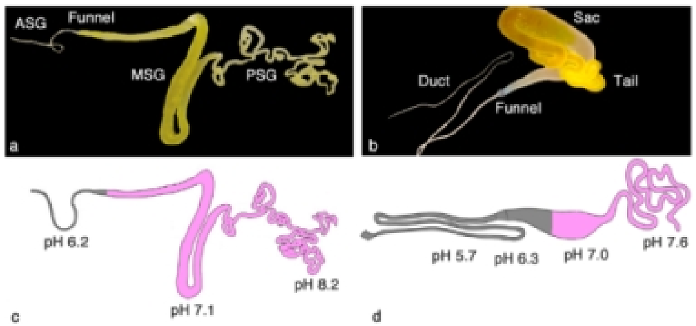

Fig. 2. Macroscopic appearance of silkworm silk gland (a) and spider major ampullate gland (b) with pH gradients highlighted in purple for the silkworms silk gland (c) and spider major ampullate gland (d).

The posterior silk gland (PSG) of silkworm helps to produce the silk proteins particularly fibroin. Similarly, spider silk proteins are produced in the tail of the major ampullate gland which is analogous to the PSG (Anderson *et al.* 2013; Asakura *et al.,* 2007). Silkworm silk proteins travel through the silkworm middle silk gland (MSG), funnel, and finally are transformed into a silk fiber in the anterior silk gland (ASG) before being spun through a specialized spinneret at the bottom of their mouth (Asakura *et al.*, 2007). Spider silk proteins travel from the tail through the sac, funnel, and duct, and the fiber is spun through pores called anal spinnerets at the end of the spider's abdomen (Anderson *et al.*, 2013). Both glands contain a pH gradient where the environment becomes more acidic as fiber formation begins and the silk duct proceeds (Askarieh *et al.*, 2010; Domigan *et al.*, 2015). The ASG and the duct have a constant decrease in diameter as the fiber is formed, to 50 µm and <10 µm respectively. The decreasing diameter as the silk passes through the tail of the glands induces shear force on the proteins which results in the proteins self-assembling and forming a fiber (Anderson *et al.*, 2013; Asakura *et al.,* 2007).

CONCLUSION

Silk culture is a year long practice that has gone through many ups and downs. Conventional way of silk production is achieved with the help of the silkworms. In this modern era, genetic engineering is ushering science and technology to a great level. In this contract, silk and fibre technology is not lagging behind. Genetic engineering in spider silk is becoming popular due to its durable and ever lasting properties. It is used for the higher and diversified usage of silk, but focus should be given for its optimum beneficial utilization to the mankind with sustainability on a long run. Silk in textile industry is making magic in the financial stability of a country by earning a higher foreign return but it should be sustainably exploited to expose the hidden qualities in proteomics and medical science.

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