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FTIR Spectroscopic Characterization of Spider Silk of Family *Lycosidae* and *Erasidae*

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ABSTRACT

Spiders are unique in their ability to synthesize and utilize silk for various purposes. The primary structure of silk is its amino acid sequence, mainly consisting of highly repetitive blocks, which is why silks are often referred to as a block co-polymer. This attempt was made to characterize silk of *Lycosiedae* and *Eresidae* for the functional groups amino acids by FTIR spectroscopy. The test results shows that, the silk of *Lycosideae* contain strong –COOH asymmetrical vibration and $-NH_3$ symmetrical bending vibration for Glumatic acid and Lysine, while in *Eresidae,* Argnine amino acid and with $-NH_2$ medium bending vibrations. Both the silk samples show presence of Beta – sheet secondary protein structure. In *Lycocedae,* strong vibrations in Amide-I region, while in *Eresidae,* medium vibrations get observed. Amide-II band are absent in E*residae.* Amide –B band are observed in both the samples.

Keywords: amide, amino acid, eresidae, lycosidae, silk, spider.

INTRODUCTION

The term silk normally refers to a wide range of continuous filament spun by the spider, use for building structures for various purposes including prey capture. Spiders are unique in their ability to synthesize and utilize silk for various purposes. Many species of spider have different glands to produce silk with different properties for different purposes, including housing, web construction, defense, capturing and detaining prey, egg protection and mobility gossamer for ballooning or for a strand allowing the spider to drop down as silk extruded. Different specialized silk have evolved with properties suitable for different uses.

The orb-web spinners are equipped with 5-7 different types of silk secreting glands, each synthesizing its own type of silk to be utilized for a

specific purpose, e.g., construction of dry and sticky parts of the web, construction of egg-sac and swathing silk of captured prey (Lombardi *et al.*, 1990).

Silk is made up of two primary proteins; a fibrous protein known as fibroin and a sticky protein s are sericin with two comprising 70-80% and 20-30% of silk respectively. Silk as well as many other biomaterials have a hierarchical structure (e.g., cellulose, hair etc.). The primary structure is its amino acid sequence mainly consists of highly repetitive glycine and alanine blocks, which is why silks are often referred to as a block co-polymer. The proteins largely made from non-essential amino acids. Most spiders produce a variety of silks from different specialized glands. The polygenetic divergence is reflected in the variability of silk properties in response to varying environmental conditions (Madsen *et al*., 1999). Various compound other than protein is used to enhance the fiber's properties, these are sugars, lipids, ions and pigments that might affects the aggregation behavior and act as a protective layer in the final fibers.

The mechanical properties of spider silk originate from the core of the fibre, which is protected by a series of 'skin' layers. The outer core (≈15% of the radius) is thought to consist mainly of the highly ordered protein Spidroin I, with the inner core (≈80% of the radius) consisting of both Spidroin I and the less-ordered Spidroin II1,2. The intrinsic disorder in Spidroin II occurs due to the presence of proline, which twists away from simple, ordered configurations 3 . This combination of proteins results in a distribution of approximately 40% ordered domains (two hydrogen bonds per amide group), 15% permanently disordered domains (one hydrogen bond per amide group) and 45% intrinsically disordered domains but with potential for order 3 (Brown *et al.* 2011)

However, the most outstanding properties are found in the dragline and radial threads of some giant spiders such as *Nephilla.* Although they are not as strong as some synthetic fibers such as Kevlar, but it is more elastic. This allows it to absorb more energy prior to breaking than any commonly used synthetic material. This fibers combine the advantages of protein structure , including hydrophilic properties, biodegradability, biocompatibility with high strength and high module, comparable to some synthetic high performance fibers but with an extremely high extension of break (Rangaswamy *et al.*, 2005).

Amino acid composition of many ampullate gland silk (dragline) produced by the mature, male golden orb weaving spider, *Nephilla clavipes* was 4. Although the amino acid composition is known for the seven silks from one animal (Anderson, 1970), only two of the seven types have been investigated in any detail. *Nephilla clavipes* is a large orb weaving spider, dispersed in the tropical and subtropical areas of the western hemisphere (Lombardi, S.J., *et al*., 1900). The design principles of dragline silk spider and nature's high performance fiber are still largely unknown in particular for the non crystalline glycine rich domains, which form the bulk of the material. The alanine residues are predominantly found in a β sheet conformation. The glycine residues are partly incorporated into the β sheets and otherwise form helical structures with an approximately 3-fold symmetry (Beek *et al*., 2002).

Lycosidae is a worldwide family of ground living spiders. Most are free living and are the likely to be encountered in the field. The funnel web is a sheet web built under a ground, litter or rocks. It ends in tubular section. The spider stays hidden in the funnel and emerges the moments it detects prey struggling on its web. The victim is pulled into the funnel from where escape is impossible.

Eresidae family, all the species of *Stegodyphus* are plant-living either solitary or living in community nests, e.g., *S. dumicola* and *S. mimosarum.* A retreat is made by the solitary species usually in grass heads with trip lines extending outwards (South African National Survey of Arachanida, Technical Report, 2010, version.Pp.244). Present attempt is made to compare the protein and functional group characteristics of silk of family *Lycosidae* and *Eresidae*. The infrared spectra usually have sharp features that are characteristics of specific types of molecular vibrations making the spectra useful for sample indication.

MATERIAL AND METHODS

The clean silk samples were collected from the natural habitat of the spiders of *Lycosidae* and *Eresidae* family from basin of Chhatri Tank region near Amravati locality. The silk samples carried to Central Instrumentation Cell of Arts, Science and Commerce College, Kiran Nager, Amravati, then clean fibers were cut in to small pieces and analyzed on Agilent Technology Model CARY & 630 FTIR.

RESULTS & DISCUSSION

Fourier Transform Infra-Red Spectroscopic results for silk of *Lycosidae* sample (Fig.-1) shows that, very strong N-H stretching vibration at 3282 cm^{-1} for Amide –A group and medium stretching vibrations at 1625 cm^{-1} for C=O (70-85%) and C-N (10-20 %) for Amide –I group. Strong N-H bending vibrations (40-60 %) at 1625 cm^{-1} for C-N (18-40 %) and C-C (10 %) occurred. Medium vibrations occur at 1337 cm^{-1} for Amide-III group. Strong absorption at 1560 cm^{-1} for –COOH asymmetrical stretching vibration for glutamic amino acid was observed while $-NH_3$ symmetrical bending vibration at 1622 cm^{-1} explains β-sheet secondary structure of protein. Strong vibrations in 3923-3282 cm^{-1} indicates Amide-B group.

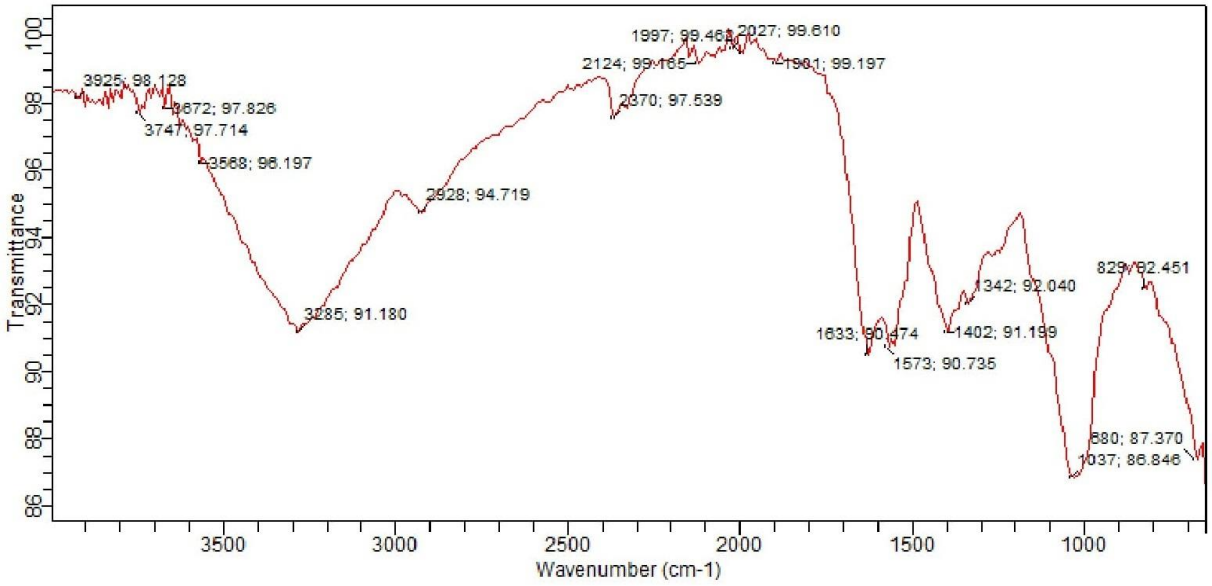

Figure 1- FTIR spectra of Lycosidae

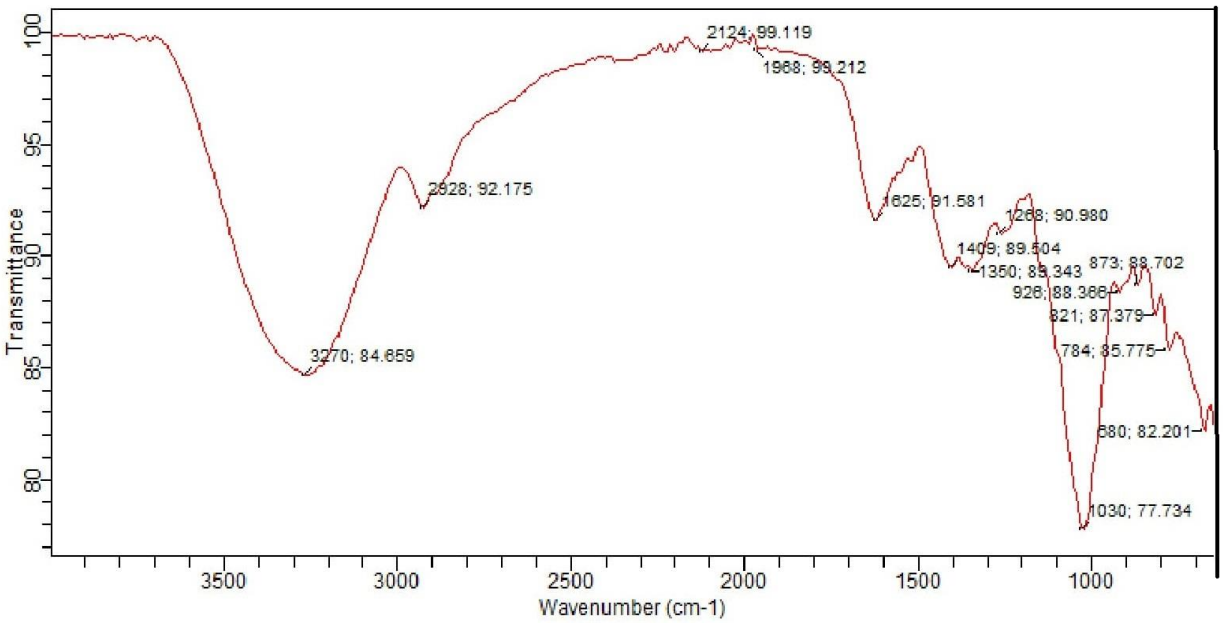

Figure 2- FTIR spectra of Eresidae

Fourier Transform Infra Red Spectroscopic results for silk of *Eresidae* sample (Fig.-2) shows that, a strong N-H stretching vibration(95%) at 3265 cm^{-1} for Amide –A group and C=O stretching (70-80 %) and C-N stretching (10-20 %) vibration at 1622 cm^{-1} indicate Amide-I group. Strong $-NH_2$ bending vibration for arganine protein at 1622 cm^{-1} was observed and it also indicates β- sheet secondary structure of protein. Presence of Amide-B absorbance at 2926-3265 cm^{-1} was observed.

DISCUSSION

The FTIR spectrum showed number of peaks that are characteristics of specific types of molecular vibrations. Silk protein or spidroin largely consist of repeated sequence of amino acids. These repetitive regions of the spidroin are confined in highly organized secondary structure which is responsible for semi crystalline nature of spider silk. The presence of bulky glutamine residues limit the growth of β-sheet and force the loops, tie chains and the surrounding amorphous matrix (Saravanan, 2016). It is however generally concluded that spider silk is made from different motif; (1) an elastic β-sheet spiral that is composed of multiple GPGXX, X varies in protein, (2) crystalline β-sheet , (3) tight amino acid repeats form a helical structure and (4) space region (Gosline *et, al.*, 1999).

Beard (1998) reported that the spider silk drag lines show a distinct CO amide –I band in the range of 1610-1660 cm^{-1}. Below 1200 $cm^{-1,}$ spider silk exhibits distinct bands at 1170 cm^{-1} ,1100 $cm^{-1}$and 1020 cm^{-1} for absorption due to bending vibrations. If the polyptide chains are in the form of α helix, it appears at 1100 cm^{-1} and if they are in β helix, it appears at 1080 cm^{-1}. Mid IR spectra from all types of spider silk showed protein peaks in the amide I and II regions. The widow silk had a peak around the 3250 cm^{-1} wave number that was not present in other silk types. There was also a reversal of alpha helix and β sheet content between two structurally different types of silk from the orb weaver (Normandeau, 2014).

Female spiders in super family *Araneoidea* (orb- spinning spiders and their close relatives) spin six different kinds of silk (three fibroins and three fibre protein glues) that differ in amino acid content and protein structure. In addition to this diversity in silk produced by different glands, we found that individual spiders of the same species can spin dragline silk (drawn from the spider's ampullate gland) that vary in content as well (Catherine, *et al*., 2000).

CONCLUSION

After FTIR spectroscopic characterization of silk samp0les of families *Lycosidae* and *Eresidae* it is to conclude that, Amide-I and Amide-A groups are present in both samples. Amide-II is present in *Lycosidae* while absent in sample of *Eresidae.* In sample *Lycosidae* , glutamic amino acid and lycine is present while asparagines amino acid present in *Eresidae.* The β- sheet secondary structure of protein is present in both the samples.

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