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Characterization of *Antheraea assamensis* Cocoon Proteins as a Potential Candidate against Urinary Tract Infection

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Silk is a biomaterial which is a fibrous protein with remarkable mechanical properties produced in fibre formed by silkworms and spiders. Apart from being a fine biomaterial, it has also been used since past few centuries as sutures in medical surgeries due to its advantages in healing properties and antimicrobial activity to avoid infection. Silk as a material has also been successful in various modern medical areas.[1] Here the researchers attempt to study the antimicrobial properties of silk proteins and their potential utility as medicinal agents against the bacteria of the urinary tract infections. This study was done to analyse the affect of muga silk based protein sericin and fibroin on the pathogenic bacteria causing UTI and its role in antibiotic sensitivity. It was found that there was a potential in both the silk proteins (Sericin and fibroin) in its anti-microbial effects against the UTI causing bacteria and had formidable results when treated against them.

Keywords: Silk, UTI, *Antherae aassamensis*, Fibroin, Sericin.

Urinary tract infection (UTI) is one of the very common category of disease affecting around more than 15 million people annually in India[1]. The treatment includes the use of a wide range of antibiotics along with other medicines. Silk from immune challenged silkworm *(Bombyxmori*) on the other hand is having regenerative properties in injury treatment also can act as an antibacterial agent as reported by Choi et al., 2019[2]. Being an excellent agent for anti- inflammatory and antibacterial properties, as per the studies of Manju Tiwari (2017)[8], it has been assumed that the muga silk already has antimicrobial and anti-inflammatory properties as studied by. The recent development in the study of silk as a medicine has inspired the researchers to find out the usuability of the silk extracted from *the* cocoon *of Antheraea assamensis* as a potential medicine in the treatment of UTI. The primary interest is to investigate the applicability and efficiency of the extracted proteins from the cocoons of *Antheraea assamensis* and examine the anti-bacterial and anti-inflammatory activity of the proteins found in the organisms with respect to the bacteria found in Urinary tract infections. These methods will help to state that whether the silk proteins will be a potential medicinal agent against the UTI bacteria and also if any other benefiting properties with respect to the primary objective.

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METHODOLOGY

Collection of Samples

Samples of patients suffering from Urinary Tract infections and admitted in SRL diagnostics, Ulubari, Guwahati were collected and the study was approved by Assam down town University Ethical Committee. The culturing procedure was done within 3 hours after the sample collection.

Selection of Bacterial Species

For the experiment five bacterial species were selected- *Escherichia coli*, *Staphylococcus aureus, Klebsialla pneumonia, Enterococcus species and Enterobacter species* on the basis of the occurrence in case of urinary tract infected patients. The isolation of the bacteria from urine is followed and explained in the following steps-

Isolation from Urine

The samples were evenly mixed in a peptone solution and was cultured on blood agar and chocolate agar plates respectively followed by quadrant streaking and incubation at 37^0C for 24 hours.

Isolation of Bacteria

Microbial colonies were obtained using serial dilution method.

Bacterial Incubation

After the media was solidified, these plates was placed in an inverted position at 37°C ± 10°C for 24-72 hours in an incubator for incubation.

Bacterial List/Enumeration

Plate selection was conducted in which 30-300colonies in a colony counter.

Discrete bacterial colonies isolation

The selected bacterial colonies were transferred to nutrient agar slant aseptically for further purification.

VITEK® 2 Compact system (Version: 08.01) - For the confirmation of the UTI Bacteria VITEK was done to determine the specificity of the bacteria.

Procedure

1. After primary organism isolation, there is minimal handling with a simple standardized inoculum
2. The inoculum is placed into the VITEK® 2 Cassette at the Smart Carrier Station™
3. The VITEK® 2 Card and sample are linked via barcode
4. Once the Cassette is loaded, the instrument handles all subsequent steps for incubation and reading
5. Results are recorded at the end.

Storage conditions of the bacteria

Storage and culturing of the bacteria was performed with Brain Heart Infusion Agar for the Gram negative bacteria and TSA agar for Gram positive bacteria.

For the Silk Proteins

Fibroin Extraction

Pure Silk Fibroin was prepared following the Degumming, the Dissolution and the Dialysis

Silk Sericin extraction

Small sections of autoclaved silk cocoons were filtrated with filter paper followed by centrifugation. Pelets of sericin were obtained and dried in oven at 100^0C

Antimicrobial Sensitivity Test/Susceptibility Test

Antimicrobial Sensitivity Test was conducted following standard protocol.

Antimicrobial susceptibility tests /Disk Diffusion

Disk diffusion method was conducted to determine the antimicrobial substances against UTI causing bacteria.

RESULTS AND DISCUSSION

Antimicrobial Results (Susceptibility Tests)

24 hours later the results are obtained; a zone of inhibition was seen in the samples, the. Silk fibroin and Sericin micro particles a without the drug was taken as negative control and had a good anti-bacterial results (susceptibility). Also with the positive control of two of the drugs a large zone of inhibition was observed. Olflaxin & ciproflaxin were used as Controls during the test.

It was seen during the results that the Silk proteins (namely fibroin and sericin) had

Table 1. Bacterial Inhibition Zone Diameter with Crude Sericin and Fibroin

No.	Bacteria	Crude Fibroin	Crude Sericin
1	*E. Coli*	18mm	20mm
2	*S.aureus*	27mm	30mm
3	*Klebsialla*	28mm	28mm
4	*Enterobacter*	21mm	29mm
5	*Enterococcus*	23mm	25mm

antimicrobial capabilities when they were made to react against the pathogenic bacteria and had formidable results against the UTI.A special emphasis was given upon the anti-microbial properties found in the proteins and the anti-bacterial activities of the silk proteins found in the *Antheraea assamensis*. From the previous studies it was found that *Bombyxmori* cocoon Silk consists of two kinds of proteins i.e. Sericin and Fibroin which constitutes the overall protein content of the Silkworm cocoon.[17] It was seen that the silk proteins namely fibroin and sericin showed antimicrobial properties against some bacteria and the same resultswere again obtained when protein was reacted against the pathogenic UTI bacteria.[7] The core of the protein which constitutes around 70-80% of the total protein content is known as Fibroin and the remaining 20-30% constitutes of Sericin which is surrounded by other layers like inorganic components, colour pigments and wax.[15] Overall there are three layers of sericin which covers up the core protein, i.e. Fibroin and is composed of the inner, outer and middle layer which holds 4.5% , 10.5% and 15% . In cold water Sericin is not soluble.[20] But because of the long protein molecules due to its solubility in hot water as the protein polymers break up as smaller fragments easily gets dispersed and hydrolyzed in the hot condition. Sericin possesses various biological properties like anti-bacterial, resistant Ultraviolet light,antitumor, antioxidant, and absorption of moisture;It has a potential to be utilized as an finishing agent for fibers (natural/ man-made)in textile industries, in cosmetics industry and forpolymeric biomedical products.[16] As per previous studies, Sericin has biological properties like Anti-bacterial, antioxidant, antitumor activities, UV resistant and moisture absorbing properties; it can be used as a finishing agent for natural or man-made fibers of textile industries, in cosmetics industries as skincare, and used for biomedical polymeric products.[38]

It was seen with the accordance with the Kirby disk Bauer test that Sericin had higher antimicrobial properties as compared to fibroin, with the highest inhibition found in *Staphylococcus aureus* with 30 mm and the lowest zone was found in fibroin with 18mm in *Escherichia coli* though it was still considered to have sensitive properties when reacted with E. Coli as per the standard Bacterial Inhibitor scale [Table 1].

Overall the results against the UTI with respect with Silk proteins of the Muga silk proved quite positive as both the sericin and fibroin was showing antimicrobial properties and can be considered as an antimicrobial agent for UTI infections.

CONCLUSION

It was observed that there was a pattern of Antibiotic sensitivity for the UTI bacteria when it was made to react with the silk based proteins in Muller Hinton agar along with the controls, it was observed that the Silk proteins namely fibroin and sericin has antibiotic properties in the crude form and has the potential to be used as an antibiotic in UTI Infections. Nearly all the bacteria shows susceptible ranges within the protocolsof Kirby-Bauer disk diffusion susceptibility test, the zone measuring sizes can be utilized as a potential microbial drug if further research is done.

This is the first report to illustrate the antimicrobial effects of the muga silkworm Antheraeaassama cocoon proteins and its effect as a potential treating agent against the Urinary tract infection bacteria (s). The sericin obtained from the cocoon of the silk was found to have more antimicrobial effects against the pathogenic bacteria compared to fibroin. However, extensive research is still needed to investigate the in vivo behavior and exploit the material withfurther modifications to make it a multi-functional agent against the UTI.

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Conflict of interest

Authors report no conflict of interests.

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