Original Research Paper

Volume - 14 | Issue - 04 | April - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biotechnology

ISOLATION OF HIGHER MOLECULAR WEIGHT OF GENOMIC DNA FROM VARIOUS BODY TISSUES OF TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY

Kumari K Department of Zoology, Nirmala College, Ranchi University, Ranchi -834002, India.

Seraphim E R Department of Zoology, Nirmala College, Ranchi University, Ranchi -834002, India.

ABSTRACT In the present study Genomic DNA was isolated from different body tissue of 3rd and 5th instars larva and pupae of Tasar silkworm, *Antheraea mylitta* Drury. The current article focuses to investigate the various samples for higher molecular weight genomic DNA isolation from Tissues commonly used for molecular studies. The modification was targeted to collect the tissue and the primary process of genomic DNA isolation. The cationic detergents used and performance was recorded in gel documentation. This article describes the best sample to obtain higher molecular weight of genomic DNA from Tasar silkworm.

KEYWORDS : Antheraea mylitta, Haemolymph, Fat body, Pupae, DNA isolation, Polyvinyl-pyrrolidone

INTRODUCTION

Silkworm is a domesticated insect having been cultured for a period of over 5000 years (Goldsmith, 1995). It possesses excellent characteristics as an experimental organism. The tropical Tasar silkworm (Lepidoptera: Saturniidae) is a sericigenous insect that produces silk (Tasar silk) of high commercial importance. It is a wild polyphagous insect. There are five larval stages which feed on the primary host plant like *Terminalia arjuna* (Arjun plant) *and Terminalia tomentosa (*Asan plant) (Suryanayana and Srivastava, 2005). The Tasar silkworm, scientifically known as *Antheraea mylitta*, is an esteemed insect species that is renowned for its production of the world-famous Tasar silk. These insects, distributed across diverse ecological and geographical regions, exhibit it mainly culturing in central and eastern parts of India (Lokesh *et al*., 2012). Tasar culture is a traditional livelihood for lakhs of tribal populace in the areas of Jharkhand, Orissa, Chhattisgarh, Maharashtra, Andra Pradesh, West Bengal and Uttar Pradesh (Renuka and Shamitha, 2016). DNA isolation plays a crucial role in genomic study. The isolation of higher molecular weight genomic DNA is of utmost significance. It is essential for many Molecular biology applications including PCR, Endonuclease restriction digestion, Southern blot analysis and genomic library construction (Buhroo *et al*., 2011). As we know that the quality of DNA depends upon the extraction protocol used. Moreover, the suitability of DNA isolation methods depends on the DNA source (sample) because of differences in interfering substances present in biological material. Higher molecular weight of genomic DNA from this sample is not reported yet, the suitability of available methods for DNA isolation from *A. mylitta* is not known. Additionally, the samples used in this experiment are selective, allowing for the isolation of higher molecular weight genomic DNA. The isolation of higher molecular weight genomic DNA was successfully achieved, validating the efficacy of the new protocols and selective sample set.

The successful isolation of higher molecular weight genomic DNA provides significant advancements in the field of genomic study. This finding opens up new avenues for further research and exploration. The innovative protocols implemented in this study demonstrate the potential for enhanced genomic research in the future.

MATERIAL AND METHODS

Experimental Material:

The Tasar silk moth samples were obtained from the Central Tasar research and Training Institute (CTRTI), Piska, Nagri Ranchi, Jharkhand, India.

Sample Collection:

Fresh silk moth, Instars larva, pupae and cocoon were collected with labelled perforated paper boxes and were carried to Genomics Laboratory, Bunshi Bioscience Pvt. Ltd., Ranchi (Jharkhand) for conducting the genomics studies.

Genomic DNA Extraction:

Genomic DNA was extracted from 3rd instars, 5th instars and pupae of silk moths, from the following body tissues.

Sample Processing

Haemolymph

The Haemolymph sample was collected in a micro centrifuge tube from different larval stages by puncturing the prolegs and from pupae by puncturing rudimentary wing pad after surface sterilization. Haemolymph 500 µl along with 1ml lysis buffer mixed properly and incubated at 65º for 30 min followed with mechanical grinding using mortar pestle. Additionally, homogenization can be performed for 30 min to improve lysis.

Fat Body And Skin

Insect was dissected dorsally; the yellow color fat body was isolated and skin is also taken as a sample from different stages, by dissecting the different instars larva (3rd, 5th) and pupae. Mechanical grinding of sample in mortar and pestle was performed using Liquid Nitrogen for 5mins.

The powdered content was transferred to fresh autoclaved micro centrifuge tubes (1.5ml) containing DNA extraction buffer.

DNA Isolation:

Genomic DNA was extracted from different larval stages and pupae using conventional DNA isolation kit (Bunshi Biosciences Pvt. Ltd.) with modification in sample processing depend upon appearance of polyphenols and protein. Various percentages of PVP and Phenol chloroform ratio were tried with addition of proteinase K and RNase treatment. Such parameter alteration increases the quality and quantity of higher molecular weight genomic DNA Isolation from *A. Mylitta* (Sambrook and Russel, 2023) and as per manufactures protocol with slight modification.

Sample were Centrifuged at 5000 rpm for 5min, supernatant was transferred to new centrifuge tube without disturbing the pellet. Enzyme cocktail of 5 µl was added and incubated for 30min at 37ºC. Phenol-chloroform isoamylalcohol of 150 ml was added, gently mixed to get milky appearance of the sample and centrifuge at 10000 rpm for 10 min at 4ºC. Aqueous layer was carefully pulled in new micro centrifuge tube and added 200 µl of chloroform isoamylalcohol and mixed gently until the solution turns milky. Centrifugation at 10000 rpm for 10min at 4°C was performed and carefully aqueous layer was collected in new micro centrifuge tube. Ammonium acetate (3M), 1/10th volume was added and mixed properly. Absolute alcohols (1:2) ratios were added and mixed, sample were transfer to -20°C for 30 min and centrifuge at 10000 rpm for 10 min. pellet was washed with70 % ethanol and air dried. Pellet was suspended with 100 µl of TE buffer (Tris EDTA, pH -8.0). Agarose gel electrophoresis was performed to check the quality of isolated Genomic DNA. Gel documentation was performed to capture the image of isolated DNA.

RESULT AND DISCUSSION

The importance of DNA isolation for genomic study cannot be overstated. Our study introduces new protocols and samples for the isolation of higher molecular weight genomic DNA. The significance of this contribution to the scientific community cannot be overlooked, as it paves the way for future advancements in genomic research. In conclusion, the isolation of higher molecular weight genomic DNA from tasar silkworm opens various area of genetic research. The methodology performed in this paper provides an easy solution for scientist to obtain higher molecular weight of Genomic DNA.

Understanding the genetic makeup of tasar silkworm can lead to significant discoveries and advancements in the field of genetics. Scientific community uses genetic makeup of tasar silkworm to improve the production of silk and the advancement in the field of genetics. The current methodology explain in this research can ease many researcher to achieve various area of genetically research using higher molecular weight of genomic DNA from *A. mylitta*.

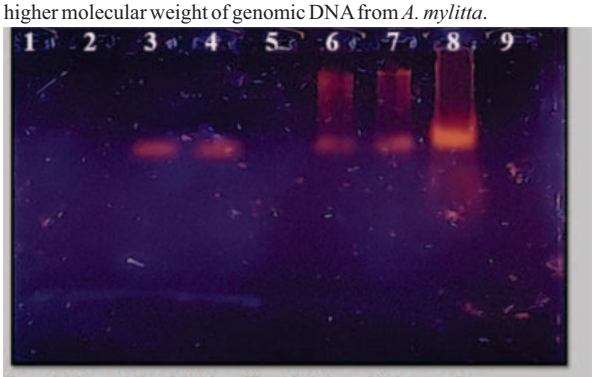

Figure : Gel documentation of DNA from different body tissue of *Antheraea mylitta*
Lane1--Haemolymph of 3rd instars larva, Lane 2-Haemolymph of 5th instars larva, Lane 5-Haemolymph of pupa ,Lane 3-Fat body of 3rd instars larva , Lane 4- Fat body of 5th instars larva, Lane 9- Fat body of pupa
Lane 6- High molecular weight of DNA from skin of 3rd instars larva ,Lane 7- High molecular weight of DNA from skin of 5th instars larva ,Lane 8- High molecular weight of DNA from skin of Pupa .

Acknowledgment

The Authors sincerely acknowledges Dr. Jay Prakash Pandey (Scientist D) Central Tasar Research and Training Institute (CTRTI), Piska-Nagri, Ranchi, Jharkhand, India for providing the sample of different larval stages of Tasar silkworm. The author greatly acknowledges Director, Bunshi Bioscience Pvt. Ltd., Dr. Deepak Kumar for providing Laboratory related help to conduct the genomic work.

REFERENCES

1. Asghar U, Malik M, Anwar F, Javed A and Raza A (2015) DNA Extraction from Insects by Using Different Techniques: A Review. *Advances in Entomology, 3, 132-138.*
2. Buhroo ZI, Ganai NA and Malik MA (2016) An efficient protocol for silkworm DNA extraction suitable for genomic fingerprinting and genetic diversity analysis. *Journal of Cell and Tissue Research. Vol.16 (3), 5921-5928.*
3. Buhroo ZI, Nagoo SA, Rafiq I, & Bhat MA (2019) Biotechnological advances in silkworm improvement: current trends and future prospectus. *J. Entomol. Zool. Stud*, *7*, 100-106.
4. Cortes CN, Quesada M, Camacho CM and Paramo GZ (2010) A Simple and Rapid Method for DNA Isolation from Xylophagous Insects *International Journal of. Molecular. Sciences. 11, 5056-5064.*
5. Dillon N, Austin AD and Bartowasky E. (1996) Comparison of Preservation techniques for DNA extraction from hymenopterous insects. *Insect molecular biology 5(1), 21-24.*
6. Datta A, Ghosh KA, Kudu CS (2001) Differential expression of the fibroin gene in developmental stages of silkworm, *Antheraea mylitta (*Saturniidae).*comparative biochemistry and physiology part B: Biochemistry and Molecular biology ,vol.129(1), 197-204.*
7. Henry MJ, Raina AK, Ridgway RL, (1989) Isolation of high-molecular-weight DNA from insects *Analytical Biochemistry185, 147-150.*
8. Lokesh G, Pao PP, Madhusudhan KN, Kar PK, Srivastava AK, Sinha MK, and Prasad B (2012) Study of phenotypic variability in silk gland characters in three ecoraces of tropical tasar silkworm Antheraea mylitta Drury. *Asian Journal of Animal and Veterinary Advances*, *7*(1), 80-84.
9. Mason CJ, Felton GW, and Hoover K (2019). Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. *Scientific reports, 9(1),* 1-11.
10. Mahendran B, Acharya C, Dash R, Ghosh KS, Kundu SC (2006) Repetitive DNA in tropical tasar silkworm *Antherae mylitta. Science direct Gene,vol 370,51-57.*
11. Renuka G and Shamitha G (2016) Genetic variation in ecoraces of tropical tasar silkworm, *Antheraea mylitta* using SSR markers. *J. Genet.95*, 777-785.
12. Shi J, Heckel DG and Goldsmith MR (1995) A genetic linkage map for the domesticated silkworm, Bombyx mori, based on restriction fragment length polymorphisms. *Genetics Research*, *66*(2), 109-1
13. Suryanarayana N and Srivastava AK (2005) Monograph on tropical tasar silkworm. *Central Tasar Research and Training Institute, Central Silk Board, Ranchi, India*, 1-87.
14. Suzuki Y, Gage LP and Brown DD (1972) The gene for silk fibroin in *Bombyx mori. Journal of Molecular Biology, 70: 637-649.*