Isolation of DNA Genomes from the Head and Middle Gland of Silkworms (*Bombyx mori* L)

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Abstract: The quality and quantity of DNA are very much needed for the molecular analysis of organisms. The superior quality of DNA samples can be obtained from an appropriate DNA extraction protocol.This research was conducted to isolate the DNA of silkworm larvae from the head and middle glands. The silkworms used are from three National silkworm breeding centers, namely Perhutani-Bogor, Bili-Bili and Soppeng. Organ samples taken are the head and middle silk glands. Samples are crushed and put into extraction buffers using a Mini Kit (Promega, Madison, WI, USA) in accordance with established procedures.The measurement of DNA quantity was carried out by the spectrophotometric method using a spectrophotometer at wavelengths (λ) 260 and 280 nm.DNA purity was determined by calculating the absorbance ratio at A260 to A280. DNA isolation showed a very thick band (high concentration) and also firm, this indicated that the results of DNA isolation are very good.DNA samples from the middle silk gland from the Bogor region were not obtained because the sample used when extracting was too much hence the extraction buffer used was insufficient to lyse lipids or compounds in the silkworm glands. DNA purity ranges from 1.75-2.2 with an average purity of 1.95 and the concentration of isolated DNA reaches 37.000 ng / µl.

1 INTRODUCTION

Changes in the nucleotides that make up DNA can cause genetic diversity. This change can affect the phenotype of an organism and can affect an individual's reaction to its environment. This genetic diversity can occur because of the recombination, mutation and migration of genes from one place to another. Nucleotides play a central role in many cellular processes, including regulation of metabolism and storage and utilization of genetic information (Bowater & Gates, 2015). According to Rao & Hodgkin (2002), genetic diversity can occur at three levels: (1) ecosystem diversity (species community and environment), species diversity (species richness) and genetic diversity (variations in genes and genotypes).

Genetic diversity begins with the extraction and purification of DNA. DNA extraction and purification is the process of separating DNA from other cell components. Extraction to obtain high-quality DNA as a condition that must be met in molecular analysis. DNA extraction and purification are also one of the success factors in DNA amplification that will be used in genetic character analysis. Hybridization programs require genetic distance, genetic character and not geographical diversity (Maqbool et al., 2015). A good extraction must be supported by the results of the quantity of DNA extract. The measurement of DNA quantity is using a spectrophotometer at wavelengths (λ) 260 and 280 nm. DNA purity was determined by calculating the absorbance ratio at A260 to A280 (A260: A280 ratio). DNA molecules are said to be pure if the absorbance ratio ranges from 1.8 to 2.0. Information on DNA isolation of the Silkworm genome identified by microsatellite markers can be used for breeding programs in encouraging bivoltine silk production (Bukhari et al., 2019).

DNA isolation can also be used to study the properties of DNA in certain parts of the body. In silkworm cocoons (Bombyx mori), fat DNA has been studied. Pupa body fat DNA is separated into three components called α-DNA, β1-DNA, and β2-

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DNA by the Kieselguhr Albumin Column Chromatography (MAK) method. All of these DNA classes are demonstrated as pure DNA, not contaminated or hybridized with RNA, because it is positive for the diphenylamine reaction, sensitive to DNase, resistant to RNase, and includes thymidine-6-3H but not uridine-5- 3j. The GC content was calculated from a Tm value of around 38% for these three components, almost the same as the total DNA. But the α-DNA molecular weight, which is roughly calculated from the sedimentation coefficient on centrifugation gradient sucrose density, is several times greater than β1-DNA.In the cocoon stage, body fat DNA consists mainly of β1- and β2-DNA with small amounts of α-DNA, whereas in the larval stage, only consists of α-DNA. Typical body fat DNA types were observed in silk gland larvae, and in adult pupa tissues such as integuments, muscles, and gonads. On the other hand, cocooned fat DNA is detected in degenerating tissue or in degeneration processes, such as silk glands, cocoons and midgut. These facts show that β1- and β2-DNA are degradation products of α-DNA (Chinzei, 1974).

Silk glands in the larval stage of silkworms, producing thread as silk material to form a cocoon. Silk glands are divided into three parts: anterior, middle and posterior silk glands, each of which plays a different role in silk secretion. Two new proteins are identified in the middle silk gland, and to a lesser extent in the posterior gland, which is thought to be involved in the regulation of proteolytic activity and protection of silk proteins from degradation (Hou et al., 2007).

2 MATERIALS AND METHODS

The silkworms used originated from three National silkworm breeding centers, which is *Pusat Penelitian dan Pengembangan Hutan Bogor, Balai Perhutanan Sosial dan Kemitraan Lingkungan (BPSKL)* of the Sulawesi, Bili-bili and Soppeng region, Sulawesi Selatan. The research begins by hatching and rearing silkworms until the caterpillar reaches instar V.

2.1 DNA Isolation

Silkworm head and glands are used as samples for DNA isolation of the genome. Samples are crushed and put into extraction buffers using a Mini Kit (Promega, Madison, WI, USA) in accordance with established procedures. Samples of silkworms that have been homogenized are incubated for 15 minutes at 65ºC then for 5 minutes at 29-30ºC. Subsequently added 200 µl of protein precipitation solution and applied vortex for 5 seconds then centrifuged at 13000 x g for 3 minutes.The supernatant was taken and put into a micro-tube containing 600 µl of isopropanol and then centrifuged at a speed of 13,000 x g for 2 minutes. The supernatant was removed and the pellet was washed using 600 µl - 70% ethanol and centrifuged at a speed of 13,000 x g for 2 minutes. The supernatant is removed and then dried, then add 50 µl of DNA rehydration solution, applied vortex for 5 seconds then 0.5 µl of RNAse solution is added. DNA was incubated for 15 minutes at 37ºC. DNA can be incubated at 4ºC for 24 hours for further work. The results of DNA extraction are stored at -2ºC until it used. DNA concentrations were calculated using a nano-photometer (IMPLEN, Munich, Germany, serial no. 6042). Next, DNA was analyzed by electrophoresis (SCIE-PLAS. Ltd, Cambridge, England) on 1.2% agarose gel stained with 1 µl ethidium bromide. The electrophoresis results were visualized using Gel Doc (Uvitec, Cambridge, Serial no. 13200263) under UV light with a wavelength of 303 nm.

3 RESULTS AND DISCUSSION

3.1 Genomic Isolation and Measurement of DNA Concentration

The results of silkworm DNA isolation originating from 3 units of National Natural Silkworm-breeding, namely Perhutani Bogor, Bili-bili and Soppeng, and the isolated parts are the head and middle silk glands can be seen in Figure 1.

Genome isolation showed a very thick band (high concentration) and also firm, this showed that isolation of the genome using the Promega kit can already isolate DNA well. According to Bukhari et al, (2019), for molecular analysis of any organism, superior quality DNA samples obtained from a suitable DNA extraction protocol is needed. In the Bg2 sample, there was no genomic or DNA band because the sample used when extracting was too much hence the extraction buffer used was insufficient to lyse lipids or compounds in the silkworm glands resulting in failure to extract the DNA (the lysis reaction failed).DNA extraction and purification is basically a series of processes of separating DNA from other cell components.

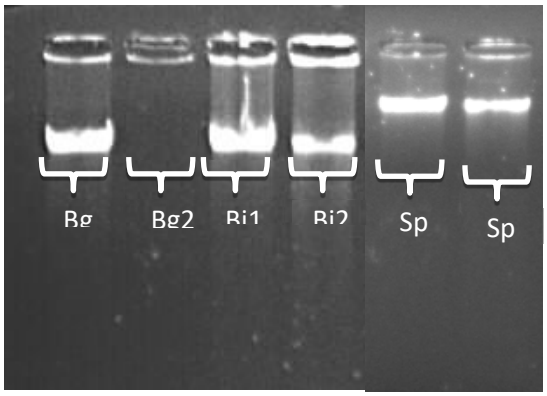

Figure 1: The results of the extraction of silkworm DNA genomes from 3 National silkworm nurseries. Bg1: Silkworm comes from the Bogor area, isolating the sample from the head; Bg2: Silkworm comes from the Bogor region, isolating the sample from the gland; Bi1: Silkworm comes from Bili-bili, isolating the sample from the head; Bi2: Silkworm comes from Bili-bili, isolating samples from glands; SP1: Silkworm comes from Soppeng, isolating the sample from the head; SP2: Silkworm comes from Soppeng, isolating samples from the glands.

Extraction to obtain high quality DNA is a basic principle that must be fulfilled in molecular analysis and is one of the success factors in DNA amplification that will be used in genetic character analysis. According to (Kawamotoa et al., 2019), incorrect genome assembly sometimes leads to incorrect gene prediction. Good extraction is supported by the results of the number of DNA extracts obtained through spectrophotometric methods using spectrophotometers at wavelengths (λ) of 260 and 280 nm. UV analysis on DNA has become a benchmarking method for rapid quantification and testing of sample purity for various purposes (Pachchigar et al., 2016). The purity and concentration of DNA that has been isolated can be seen in Table 1.

Table 1: Quantitative analysis of Bombyx mori L. silkworm DNA genomes from 3 National silkworm nursery sites.

No	Sample Code	Absorbance (A260/280)	Concentration (ng/ul)
1	Bg1	2,2	13.000
2	Bi1	1,9	30.000
3	Bi2	2,0	37.000
4	Sp1	1,75	2.360
5	Sp2	1,9	7.120

Based on Table 1, it can be seen that DNA Purity ranges from 1.75-2.2 with an average purity of 1.95 with the conclusion that the isolated DNA is pure (Wilson & Walker, 2010). DNA purity was determined by calculating the absorbance ratio at A260 to A280 (A260: A280 ratio). DNA molecules are said to be pure if the absorbance ratio ranges from 1.8 to 2.0 (Sambrook et al., 1989). DNA concentrations were very high, especially in the Bi1 sample code, which was 37000 ng/ul, while the lowest in Sp2 was 7.120 ng/ul, but this concentration was very sufficient to continue further analysis.DNA concentrations that are too low will produce fragments that are very thin on the gel or even not visually visible, on the contrary, DNA concentrations that are too high will cause the fragments to appear thick hence it is difficult to distinguish between fragments from other fragments. The concentration of DNA extraction results is influenced by 2 factors: the extraction rate at the time of extraction and the composition of the buffer lysis addition. The extraction speed factor is the most influential factor because it is present in the stages of cell lysis and precipitation.The application of isolation is for structural analysis and functional analysis of the desired gene. The application of isolation by combining labels can be used to monitor enzymatic reactions, nucleic acid processing and hybridization experiments. The interaction of proteins and nucleic acids plays a major role in all aspects of gene expression. Proteins in the larvae and silk glands, DNA and RNA in the larvae and silk glands are separated and presented with the help of PAGE, SDS-PAGE, and PFGE respectively. This has been a pioneer in the field of silkworm sericulture (Brindha et al., 2012).

The silk glands of Bombyxmori are very different tissues where DNA replication continues without cell division during larval development. DNA polymerase-delta activity is increased in the posterior and middle silk glands during the development period, reaching maximum levels in the middle silk gland of the fifth instar larvae.There are many ways to preserve insect specimens that will protect their DNA from degradation during the collection period for use in molecular genetic studies. However, techniques vary among groups of insects, suggesting that preservation must be carried out before working at the molecular level with large numbers of individuals from certain protein groups. Protein in the silk glands and at each larval stage is estimated quantitatively.

4 CONCLUSION

The quality and quantity of DNA is very much needed for the molecular analysis of organisms. The superior quality of DNA samples can be obtained from an appropriate DNA extraction protocol. Isolation of silkworm DNA (B. mori L.) can be done from the head and middle silk glands.In silk-gland samples must be adjusted to the concentration of the buffer because if there is not enough amount of extraction buffer used, it will fail the process of lipid lysis or compounds in the silkworm gland. DNA purity ranged from 1.75-2.2 with an average purity of 1.95 and the concentration of isolated DNA reached 37,000 ng / µl.

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