



E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(5): 576-578
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Received: 21-07-2016
Accepted: 22-08-2016

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Evaluation of efficiency of four DNA extraction methods in *Apis mellifera* L. (Hymenoptera: Apidae)

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Abstract

Quality and quantity of DNA extraction is important in molecular studies. In this research, four methods were evaluated in order to choose the most appropriate way in DNA extraction. DNA extraction methods included the optimal salt, phenol – chloroform, CTAB and CTAB + SDS. The quantity and quality of DNA extracted were compared with spectrophotometry and agarose gel respectively. Results indicated that CTAB+SDS method with the average purity of 1.89 (absorption ratio 260nm/280nm) was better than to other methods for DNA extraction in *Apis mellifera*.

Keywords: DNA extraction, bee, quality, CTAB

1. Introduction

Many different methods and technologies are available for the isolation of genomic DNA. In general, all methods involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA [7, 9, 17]. Laboratory procedures for extracting DNA should be optimized to provide the most satisfactory results. For each species and type of tissue, methods should be adopted to maximize output, with minimum cost and time requirements, while maintaining quality. Techniques should have low toxicity reagents for DNA extraction. Initial procedures used phenol, chloroform, and isoamyl alcohol. CTAB method is relatively simple, and has been used successfully with a wide range of monocot and dicot species. This method may be used with either fresh or dehydrated plant material [16]. Most of the purposed methods for DNA extraction are in one way or another modified versions of hexadecyltrimethylammonium bromide (CTAB) extraction [11] having some crop-to-crop limitations and varying in time and cost. The main cause of diversity and modification in CTAB protocol is the composition of cell walls and intra- and extra-cellular components. CTAB is a cationic surfactant providing a buffer solution for dissociation and selective precipitation of DNA from histone proteins [1]. Isolating and analysing an organism's DNA is a key for developing insights into species or strain identification, for uncovering variants useful in breeding or a more thorough understanding of biology, and for discovering the microbes carried by individuals. DNA extraction methods must be robust for small amounts of starting material even if that material has become degraded. They must deliver extracted DNA of sufficient quality, purity, and quantity for downstream efforts ranging from target identification. In many cases changes in composition and extraction buffer pH, is effective in improving the quality [13]. The purpose of this research is to designate the best method for extraction of DNA with good quality and quantity.

2. Material and Method

Sampling from the hive of bees was done in khuzestan province randomly and DNA extracted from the thorax region of workers bees.

2.1. Salting out method: Firstly, bee's thorax was powdered by liquid nitrogen and transferred into a tube with volume of 1.5 ml; then 400 ml lysis buffer added and the samples were laid in warm water bath for 2 hours at 65 °C. After adding 300 ml NaCl, samples centrifuged and upper phase was separated and transferred to new tube; then chloroform added to the samples. After centrifuge and separating the upper phase, cold absolute ethanol and 3M

sodium acetate added to the samples. Then samples centrifuged and were washed with 10% ethanol, so DNA Plate stayed at bottom of tube; finally, 50 µl distilled water was added to each samples [15].

2.2. phenol-chloroform method: firstly, the samples were powdered by liquid nitrogen and transferred into a tube with volume of 1.5 ml; Then 500 ml lysis buffer was added to each tube and was centrifuged at 11000 g for 5 minutes; Then upper solution was separated and added 180 ml buffer (TE 1X), 18 ml SDS 10%, 4 ml proteinase K and placed in warm water bath for 2 hours at 55 °C. Then phenol-chloroform added and centrifuged for 15 min with 11000 g; the upper solution was transferred to the tube and 20 ml 3 M sodium acetate, 500 ml cold absolute ethanol was added and stored at freezer -20 °C for 20 min. then 50 µl distilled water was added in each tube [2].

2.3. CTANB+SDS method [5, 6]: In this method the samples were crushed in 1.5 ml tube by liquid nitrogen then 20 ml CTAB buffer, 100 ml SDS lysis buffer was added to each sample. Samples were placed in warm water bath at 60 °C for 5 hour; Then 500 ml phenol was added to each tube and they were shaken gently for 10 min; The upper phase was transferred into a new tube and was placed in warm water bath at 60 °C for 5 h; 500 ml phenol was added to them and were shaken gently and centrifuged again. 500 ml chloroform was added to each samples and centrifuged with maximum speed and upper phase was transferred into a new tube; Then 20 ml cold absolute ethanol was added to sample and the samples were stored at 70 °C freezer for 20 min; Samples were centrifuged at 12000 g for 2 min; 1000 ml 70% ethanol was added to the tube, then centrifugation was performed in rotating 12000 for 10 min (at this step the samples were washed twice). The sample solutions were poured on paper and sustained for 20 min to dry. Finally, 50 µl distilled water was added to each samples.

3. Result and Discussion

We opted for methods that had traditionally been used to process honey bee tissues [13, 14], involving the use of phenol, chloroform, and isoamyl alcohol. These techniques gave good results. Results indicated that CTAB+SDS method with the average purity of 1.89 was better than other methods. All DNA extraction of four methods can be used in polymerase chain reaction, but DNA quantity of CTAB+SDS method was more than other methods; additionally, it seemed that the cost and time of this method was more appropriate and extraction of DNA was easier and faster than other methods (table 1). On the other hand results showed that twice washing can affect considerable effects on reducing protein and phenol-chloroform pollution.

Quantity and quality of extracted DNA was examined by spectrophotometry (based on the absorption ratio 260/280) and electrophoresis on agarose gel to view of quantity and quality of DNA (table 1).

Table 1: Comparison of absorption ratio DNA in four extraction methods

Extraction method	Absorption ratio 260nm/280nm
Salting out	1.50
Phenol-chloroform	1.59
CTAB	1.75
CTAB+SDS	1.89

Statistical analysis of data showed that there was a significant difference between DNA extraction methods (table 2). Mean comparisons with Duncan test showed that CTAB+SDS method is the best method for DNA extraction.

Table 2: Variance analysis of absorption ratio DNA in four extraction methods

Pr>F	F	Mean of squares	SUM of squares	df	Exchange resource
0.004	6.615	0.146	0.439	3	Treatment
		0.022	0.354	16	Error
			0.793	19	Total

Optimum absorption ratio by spectrophotometry (260nm/280nm) is 1.8 to 2 that indicating good quality and purity of DNA. The results indicated that CTAB+SDS method had Optimum absorption ratio. Researchers [15] were compared three methods of DNA extraction on bee; they obtained salting out method as optimal method and introduced it as a suitable method for DNA extraction. Spectrophotometry and agarose gel (Figure 1) in our experiment showed that CTAB+SDS method had average purity of 1.89 in comparison to three other methods (table 1). It was demonstrated that CTAB method was found to be suitable for PCR [3].

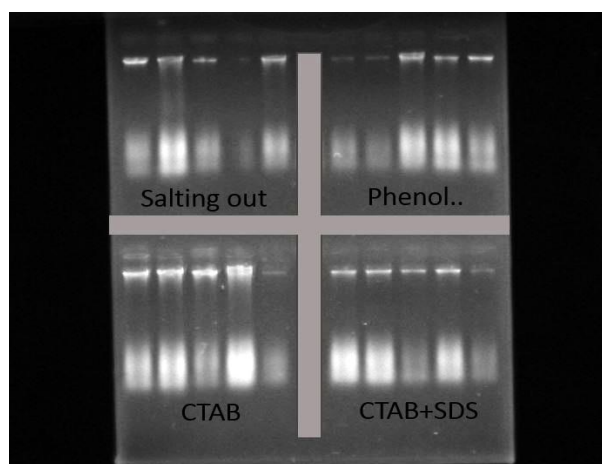


Fig 1: Comparison of four DNA extraction methods on 1% Agarose gel

It is also worthwhile to say that DNA extraction all four methods can be used to perform polymerase chain reaction, but CTAB+SDS method created higher DNA quality and quantity. These result showed that thick bands of DNA on gel was because of lower quality of DNA (figure 1). Also, it seems that CTAB + SDS method has the ability to extract DNA with good quality and quantity. Additionally, this method has high safety and can be advisable for researchers. It was indicated that addition of PVP along with CTAB may bind to the polyphenolic compounds by forming a complex with hydrogen bonds and may help in removal of impurities to some extent [10].

Since we wanted to extract mitochondrial DNA, we chose the thorax because of the large muscles it contains [8]. We referred to this thorax tissue as "thoracic muscle mass", and considered it to be a tissue from which we could obtain larger quantities of DNA [9]. We used thoracic muscle mass as it has the lowest risk of contamination from the environment, since it was collected from inside the thorax with sterilized instruments.

A study was conducted on the efficiency of three modified methodologies for DNA extraction of six sweet potato landraces using the CTAB extraction buffer in regard to quantity and purity of DNA quantification and microsatellite band patterns. All methodologies yielded satisfactory results [4].

A simple, reliable and labor-effective cetyltrimethylammonium bromide-polyvinylpyrrolidone (CTAB-PVP) method for isolation of high quality DNA from xylophagous insects was described [12]. This method was successfully applied to PCR and restriction analysis, indicating removal of common inhibitors. DNA isolated by the CTAB-PVP method could be used in most molecular analyses. Phenolics and other secondary compounds cause damage to DNA and/or inhibit restriction endonucleases and Taq polymerases. Higher concentrations of CTAB and the addition of antioxidants such as polyvinyl-pyrrolidone (PVP) and β -mercaptoethanol to the extraction buffer could help to remove phenolics in DNA preparations [12].

4. Acknowledgment

The authors thank the Entomology Department, Faculty of Agriculture, University of Kurdistan, Iran, for their financial assistance.

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