Short Communication

Rapid Isolation of DNA from the Mucus of Asian Arowana *(Scleropages formosus, Osteoglossidae)*

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Abstract

Owing to arowana being listed in CITES, DNA diagnostics should not rely on conventional methods of obtaining tissue samples. A non-invasive method of obtaining material containing DNA has been developed. Mucus samples for DNA isolation can be obtained by cotton swabbing the arowana body surface with subsequent purification of DNA fragments by using phenol/chloroform. DNA sample quality is assessed by UV absorbancy at wave lengths of 260 nm and 280 nm, as well as by calculating the absorbancy ratio involving 260 nm/280 nm results in order to determine DNA sample quality and quantity. Electrophoresis is employed to assess DNA quantity and quality (DNA molecular weight compared to standard DNA, RNA contaminant, DNA double strand breakage, and protein or phenol contaminants). The DNA isolated from mucus is of good quality with little RNA contamination. Although statistics (hypothesis test) suggest that the DNA quantity of samples taken from gills and mucus is significantly different at the 95% significance level, the amount of DNA contained in mucus is sufficient for analysis.

Keywords : Aquatic animals, arowana, DNA, mucus, phenol-chloroform.

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บทคัดย่อ

การสกัดดีเอ็นเอจากเมือกในปลาอะโรวาน่า (มังกร)

นันทริกา ชันซื่อ

การสกัดดีเอ็นเอจากเมือกปลามังกรหรือปลาอะโรวาน่าโดยการเก็บตัวอย่างด้วย cotton swab แล้วดูดเมือกที่ได้ไปสกัดดีเอ็นเอโดยวิธีฟีนอล-คลอโรฟอร์ม และนำสารละลายดีเอ็นเอที่ได้ไปวัดปริมาณและตรวจคุณภาพดีเอ็นเอโดยการวัดค่าการดูดกลืนแสงที่ 260 นาโนเมตร และ 280 นาโนเมตร และ หาอัตราส่วนค่าการดูดกลืนแสงที่ 260 นาโนเมตรกับ 280 นาโนเมตร เพื่อคำนวณปริมาณดีเอ็นเอและตรวจคุณภาพ และทำอิเล็กโตรโฟเรซิสเพื่อศึกษาด้านคุณภาพ ได้แก่ ขนาดโมเลกุลของดีเอ็นเอโดยเปรียบเทียบกับดีเอ็นเอมาตรฐาน การปนเปื้อนจากอาร์เอ็นเอ การแตกหักของดีเอ็นเอ การปนเปื้อนของสารอื่นๆ และปริมาณ พบว่าคุณภาพดีเอ็นเอที่ได้อยู่ในเกณฑ์ที่ดี มีปริมาณอาร์เอ็นเอเปื้อนเล็กน้อย และจากการทดสอบสมมติฐานทางสถิติ พบว่า ปริมาณดีเอ็นเอจากเหงือกและเมือกมีปริมาณแตกต่างกันทางสถิติที่ระดับความเชื่อมั่น 95 เปอร์เซ็นต์

คำสำคัญ: สัตว์น้ำ ปลาอะโรวาน่า ดีเอ็นเอ เมือก ฟีนอล-คลอโรฟอร์ม

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Introduction

The dragonfish, or arowana *(Scleropages formosus, Osteoglossidae)* (Muller and Schlegel, 1844; Nelson, 1994), is an ancient member of the *Osteoglossidae* family inhabiting South East Asia (Kottelat et al., 1993). It can be categorised into 4 genus and 7 species (Wongkittivechakul, 1988). There are many colour varieties. Due to its spectacular appearance, large scales and scarcity, the arowana is one of the most expensive ornamental fish available (Scott and Fuller, 1976). The arowana has been protected under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) in Appendix I since 1975 (Joseph et al., 1986; Dawes et al., 1999).

Genetic information on aquatic animals, especially on arowanas are limited, due to the complexity of isolated genomic DNA of good quality and high molecular weight. Though the collection of samples from living fish in rare breeds is virtually impossible.An attempt to isolate DNA by standard phenol/chloroform extraction (Blin and Stafford, 1976) from fin clippings is not suitable because insufficient DNA material is extractable. Other reports have been on simple methods for the isolation of DNA, such as salting out (Miller et al., 1988), the silicaguanidinium thiocyanate method (Carter and Milton, 1993; Hoss and Paabo, 1993), CTAB (Tel-Zur et al., 1999), boiling (Valsecchi, 1998), Chelex-base (Walsh et al., 1991) as well as by extraction from muscle tissue (Yue et al., 2000) and fins (Balázs et al., 2001). We focused on DNA isolation from mucus because it contains large numbers of epithelial cells, with each cell

containing a nucleus, which in turn contains genomic DNA.

A number of factors, such as simplicity of method, sample quality and ease of access, have to be considered when collecting material (Piyachokanakul, 1999). This paper presents a method for collecting samples for DNA isolation with the least possible harm to the fish, as well as a protocol for isolating DNA from the surface mucus of arowanas. This method is also feasible for extracting genomic DNA from other expensive, scarce and endangered aquatic animals.

Materials and Methods

Sample Collection

Collecting mucus cells and gills from 9 live arowanas was performed utilising a cotton swab and brushing it along the fish body and gill clip. Storing was at 4 C for further analysis, or at -20 C for long term storage.

DNA Isolation

DNA was extracted from the mucus and gills using phenol/chloroform method by Blin and Stafford (1976).

Quantification and quality of DNA

Spectrophotometry using a spectrophotometer (GeneQuant pro, Amersham Biosciences, Sweden) was used to determine the DNA quality by testing the UV absorbancy of the extracted DNA from the mucus and gills of arowanas at wave lengths of 260 nm and 280 nm. . The fact that a solution containing 50 µg concentrated DNA has an absorbancy of 1 at a wave length of 260 nm (UV can permeate for 1 cm) was employed to detect DNA quantities.

A gel electrophoresis was carried out using the *ThermoEC MINICELL PRIMO EC320* and 0.8% agarose, 1 X TBE buffer under 100 volts for 30 to 45 min, ethidium bromide staining: 0.5 µg/ml), comparing it to defined Standard DNA marker (100 bp DNA ladder; Promega, USA).

Results and Discussion

The UV absorbancy and DNA concentration of isolated DNA from the gills and mucus by the phenol/chloroform method at wave lengths of 260 nm and 280 nm (using spectrophotometer) is shown in Table 1.

Table 1 The average of UV absorbancy and DNA concentration of isolated DNA from 9 arowanas.

	Gill	Mucus
OD 260	1.88	0.45
OD 280	1.24	0.24
OD260/OD280	1.64	1.83
DNA Concentration (µg/ml)	940.78	223.89

Statistical analysis with the Independent sample t-test using SPSS program, suggested that the concentration of DNA from the gills and mucus was significantly different ($p<0.05$). The average concentrations of DNA from mucus and gills were 223.89 µg/ml and 940.78 µg/ml, respectively.

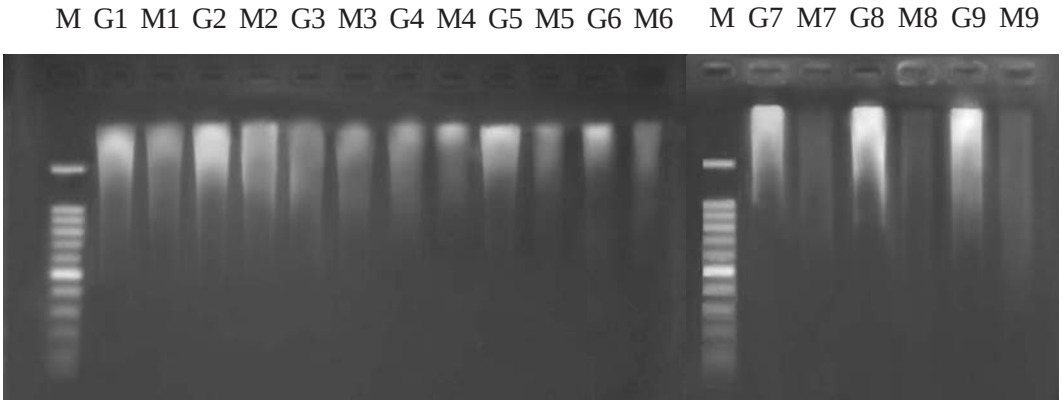

Figure 1 DNA strands from 9 arowanas. Lane M was 100 bp ladders, lane G1- 9 were gill DNA and M 1- 9 were mucus DNA.

The quality of mucus DNA material was 1.83. This differs slightly from the ratio of 1.8 (variance 1.7-1.8) obtained by Piyachokanakul (1999) for purified two-stranded DNA. A ratio above this range suggests that RNA contaminant is present. Ratios below this range would indicate phenol or protein contaminants. We also used the gill to compare the DNA quantity and quality with mucus because the gill is a popular organ for DNA extraction because it has a high molecular weight and quantity of DNA, but it can cause injury to the aquatic animal, so we used mucus to compare with the gills. Jenkins and LaPeyre (2006) used gills for comparing the DNA FCM by flow cytometry (FCM). The result shows they were significantly different at the 95% significance level, but the amount of DNA contained in mucus is sufficient for analysis. The DNA isolated from mucus is of good quality with little RNA contamination (OD260/OD280 = 1.83). Electrophoresis results suggest only a slight degree of contaminant, since there is little evidence of fast-moving particle (RNA contamination) and the molecular weight of DNA is higher than 15,000 bp, which is similar to the mitochondrial genomic sequence (mtDNA) of arowana which is reported to be 16,650 bp. (Yue et al., 2006).

The result of this study indicated that the DNA isolation from arowana mucus can be a safe method to obtain DNA samples of the same quality and quantity as obtained from other organs (Sunarto et al., 2005). This method may be applied to other expensive, scarce and endangered fish as well.

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