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AN EFFICIENT METHOD FOR DNA ISOLATION FROM FISH FIN

Haji Muhammad[1,2], Zafar Iqbal[1], Muhammad Usman Iqbal[2], Tayyaba Younas[2] and Qamar Bashir[2,*]

[1]Department of the Zoology, University of the Punjab Lahore, Pakistan; [2]School of Biological Sciences, University of the Punjab Lahore, Pakistan.
[*]Corresponding author's e-mail: qamar.sbs@pu.edu.pk

Genomic DNA isolation is fundamental step to study genetic diversity, phylogeny of species and cloning of desired genes. The preferable protocol needs to be efficient, less time consuming, economic and non-destructive particularly for the endangered species. The current study was aimed at economic isolation of considerable quantity of DNA manually from a particular tissue of the fish without any potential damage to the animal. For this purpose fin tissue was preferably selected over scale due to its universal occurrence in fish. A total of 180 samples of fin tissues belonging to twenty nine species of fresh water fish preserved in different preservatives, were used for DNA isolation. DNA could not be isolated from the fin tissues preserved in formalin, alcohol, air dried and ice preserved fins more than one week by any of two previously described methods including salt extraction and urea treatment. However, introducing some modifications in salt extraction method resulted in large quantity of high quality DNA from those fin tissues that were isolated from the living animal and immediately preserved in absolute ethanol. The modifications include use of low quantity of proteinase K and slight increase in incubation period due to hard nature of fin tissue. The extracted DNA by the modified salt extraction method was very pure, of high quality and resulted in successful PCR amplification by Random Amplified Polymorphic DNA primer and cytochrome oxidase subunit 1 gene specific primers. This modified procedure results in highest yield of isolated DNA reported so far. The method is particularly suited for DNA isolation from endangered and sparse species as it requires minute quantity of fin tissue, without any detrimental effect on fish.

Keywords: DNA, fish fin, salt extraction method, RAPD, CO1

INTRODUCTION

Deoxyribonucleic acid (DNA) not only stores hereditary information's but transfers these information's generation after generation from parents to their offspring's. It is known as hereditary material in all living organisms and massively used in various molecular studies. The principle and isolation techniques play important role for successful extraction of purified and considerable quantity of DNA because cell is the complex of different organelles and biological molecules. Scientifically DNA is used in diagnostic purposes, forensic, genetic, and medical studies. It is also used to manipulate bacterial, plants and animals cells as well as for pathogenic, paternity and organismic identification. (Srividya *et al*., 2011). Contaminants in isolated DNA like lipids, proteins, polysaccharides, different inorganic and organic compounds interfere with its further analysis especially by Polymerase Chain Reaction and decrease the quality and storage life of DNA (Bauer and Patzelt, 2003). The extent of DNA isolation and purity depends upon many factors including sample size, extraction methods and storage of sample.

In the recent years isolation of DNA from the sources that are not destructive for the organism is the most interesting and emerging methodology. This technique is extremely important for DNA isolation from the endangered or threatened species to study their conservation, population, diversity and genetic assessment. DNA isolation can be done from eggshells, feces, sloughed off skin of whale and snake, urine, feathers, hairs and bones in feces pellets of carnivores. These sources provide poor and low quantity DNA thus hardly used for individual identification. On the other hand blood, fins, scales, skin and muscles can be used for successful isolation of good quantity and quality DNA without any potential damage to the animals. The isolated DNA can be used to determine genetic polymorphism between and within the populations, reconstruction of pedigree, related estimates, sex determination and individual identification. In case of fish isolation of DNA from scales and fins is desirable because both tissues are attractive source of DNA isolation and proved to be non-destructive for fish (Wasko *et al*., 2003; Kumar *et al*., 2007). Fins are given preference over scales for DNA isolation because scales are not present in all species of fish. Many researchers (Bruyn *et al*, 2011; Raja *et al*, 2011) tried to isolate DNA from the animals preserved in the natural history museums but they were not successful to recover intact and sufficient quantity of DNA from the preserved specimens. Probably these organisms were collected before advancement in molecular

biology. One can better understand the knowledge of genetic and molecular evolution of different species by successful extraction of DNA from preserved specimens (Paabo, 1989). Most of the natural museums preserved animals in liquid preservatives like formalin, ethanol and isopropanol. Formalin is the most popular source for organismic preservation. The extraction of high quality DNA from the animals preserved in formalin is problematic. Many physical, chemical and biological processes affects the quality of DNA and results in the formation of strong cross linkage between proteins and DNA. Thus DNA is broken into fragments (Fang *et al*., 2002). Formalin reaction is reversible with DNA if the specimens are stored for short period. Long term storage in formalin causes a variety of chemical reactions and leads to DNA denaturation. It is also reported that nucleic acid extracted from formalin fixed tissues are worse template for PCR amplification (Raja *et al*., 2011).

The present study was designed to isolate large quantities of genomic DNA from fish fin that could be subjected to successful PCR amplification. Moreover, the removal of small size (few centimeters) of fin has non-detrimental effect on fish. Essentially two DNA extraction methods were employed and compared for the quality of isolated DNA, including urea extraction and salt extraction methods. Urea extraction method has been previously adopted for DNA isolation from fin tissue, however it is laborious and results in little quantity of DNA isolation. Salt extraction method has been used for DNA isolation from fish scale and has never been used for fin tissue. We have made some modifications in the salt extraction method which make it a successful method for DNA isolation from fish fin. The modified method gives highest ever reported yield of pure DNA which serves as decent template for PCR amplification of desired sequences. We have also investigated the effect of different preservatives on quality of DNA isolation. Our results demonstrate that absolute ethanol is the best preservative for DNA isolation from fin tissue.

MATERIALS AND METHODS

***Sampling of fish and preparation of tissues*:** Around 200 specimens of fresh water fishes belonging to 29 species were collected by exploiting different types of fish nets from the Indus River at Taunsa Barrage, District Muzaffargarh, South Punjab, Pakistan. All fish specimens were tagged and transported to "Fish Disease and Health Management Lab" Department of the Zoology University of the Punjab Lahore, Pakistan and identified with the help of standard taxonomic key (Mirza and Sandhu, 2007) on the basis of morphometric characteristics. Both paired (caudal, pectoral and pelvic fin) and un-paired fin tissues (dorsal and anal fin) were cut with the help of sterilized scissor from each species of fish. Tissues were immediately preserved by absolute ethanol, 10% formalin or at -20°C after their excision from the fish. Some fin tissues were not preserved in any preservative, were air dried and transported to the Lab for DNA extraction.

***Extraction of DNA*:** At least triplicate specimens of each species were exploited for the isolation of DNA. Two methods were used for DNA isolation from fin tissue. These methods include urea treatment method (Wasko *et al*., 2003) and salt extraction method (Kumar *et al*., 2007). DNA suitable for PCR analysis was only extracted successfully from the fin tissues preserved in absolute ethanol after their excision from the animal by salt extraction method with some modifications.

***DNA isolation by urea treatment method*:** DNA was tried to be isolated from fin tissues of fish according to previously described urea treatment method (Wasko *et al*., 2003) for the extraction of DNA from fish scales and fin. 50-150 mg of air dried and preserved (using different preservatives) fin tissues were preceded for genomic DNA isolation. Each fin tissue was cut into small pieces with the help of sterilized scissor, dried on filter paper and placed in 2ml of lysis buffer (10 mM Tris-HCl pH 8.0; 10 mM EDTA; 125 mM NaCl; 0.5% SDS; 4 M urea) in the 15 ml tube. Thirty microliter of RNAse (10 mg/ml) was also added inside the tube and tissues were incubated in the tube at 42°C for 1h. After this incubation, thirty microliter of Proteinase K (20 mg/ml) was added and contents of the tube were mixed gently and incubated at 42°C for 10h at least. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added in the tube, mixed by gentle inversion for 10-15 minutes and centrifuged at 13000 rpm for 15 minutes. Top aqueous layer was removed very carefully in new sterilized tube. One molar sodium chloride and 2-3 volumes of cooled absolute ethanol were added and tube contents were inverted again. Tubes were placed at -20°C for 1-2h and centrifuge at 13000 rpm for 15 min. The DNA pellets were washed briefly with 70% ethanol, air dried, resuspended in appropriate volume of injection water (Aqua-Pro Injection, Pakistan) and stored at -20°C until further analysis.

***DNA isolation by modified salt extraction method*:** The protocol was followed and modified according to Kumar *et al*. (2007) utilized for DNA extraction from scales of seven species of fresh water fishes. Initially 50mg of fin tissue of each species of fish were cut into small pieces with sterilized scissor and dried on filter paper. Fin tissues were incubated in 15ml tube containing 1.94 ml lysis buffer (200 mM Tris-HCl pH 8.0; 100 mM EDTA; 250 mM NaCl), 10µl Proteinase K (20 mg/ml) and 60 µl of 20% SDS. The tubes contents were incubated at 48°C for 2-3 h in water bath. The DNA was isolated by adding 2 ml of phenol: chloroform: isoamyl alcohol (25:24:1) in the tubes after incubation. The tubes contents were mixed manually by gentle mixing for 10-15min and then centrifuged at 13000 rpm for 15min. The top aqueous layer was removed and transferred in new tube leaving the interphase and lower phase. Equal volume of chloroform was added in the tubes contents, mixed by

inverting the tubes and centrifuged at 13000 rpm for 15min. Again top transparent layer was removed leaving the interphase and lower phase. After this an equal volume of isopropanol and 0.2 volume of ammonium acetate (10 mM) were added in the tube containing aqueous phase and incubated for 30 min at -20°C for good precipitation of DNA. The pellet of DNA was formed by centrifugation of tubes again at 12,000 rpm for 5minutes. The DNA pellet was washed two times by cold 70% ethanol, dried, dissolved in appropriate volume of injection water. Only one microliter of RNase (10 mg/ml) was added to digest RNA, in few samples of DNA. The isolated DNA was then stored at -20°C.

***Purity and yield of isolated DNA*:** Known volume of extracted DNA from each sample was diluted with injection water to make a solution of 100 µl and transferred to separate quartz cuvettes. The impurities in the diluted DNA were assessed and quantified by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) with the help of UV-Visible spectrophotometer (Hamberg Biophotometer; eppendorf AG22331, Germany). The concentration of DNA was calculated by multiplying the dilution factor with the A_{260} measurement and then by 50 on the basis of relationship that an A_{260} of 1.0 equals to 50 µg ml^{-1} pure DNA. By multiplying the value of DNA concentration with the final volume of extracted DNA, yield of DNA was calculated. The purity of DNA was determined by calculating the A_{260}/A_{280} ratios. The comparison of absorbance at 260-280 nm by spectrophotometer provide a DNA/protein relationship of 1.6-2.0, for pure samples and free from proteins or RNA contamination (Cawthorn *et al.*, 2011). The integrity of DNA was checked by loading 10 µl of isolated DNA, 2 µl DNA loading dye on 1% agarose gel stained with ethidium bromide. Total amount of isolated DNA was quantified by direct comparison with 1Kb mix standard marker (Fermentas). Further polymerase chain reaction with specific Random Amplified Polymorphic DNA (RAPD) and cytochrome oxidase subunit 1 (CO1) gene of mitochondrial DNA primers were used to amplify the template DNA. The results of amplified products were recorded by electrophoresis on 2% agarose gel stained with ethidium

Table 1. RAPD and CO1 specific primers sequence.

S. No.	Primer	Sequence	Melting temperature
1	RAPD	5′-AAAGCTGCGG-3′	32.0
2	CO1	F, 5′-TCAACCAACCACAAAGACATTGGAAC-3′	66.3
		R, 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	66.3

Table 2. Comparison of DNA concentration, yield and purity isolated from 29 freshwater species of fish.

Species	Concentration of DNA µg/50 mg of fin sample	Standard deviation	A_{260}/A_{280} ratio	PCR amplification
Labeo rohita	637	±44.8	1.6	+
Labeo calbasu	642	±26.8	1.7	+
Labeo gonius	**604**	±62.9	1.8	+
Catla catla	844	±29.6	1.8	+
Cirrhinus mrigala	831	±89.7	1.7	+
Cirrhinus reba	787	±151.5	1.9	+
Systomus sarana	982.5	±14.7	1.9	+
Ctenopharangodon idella	1066	±591.8	2.0	+
Hypophthalmicthys molitrix	1320	±242.6	1.7	+
Cyprinus carpio	905	±285.3	1.9	+
Oreochromis niloticus	1187	±66.1	1.8	+
Oreochromis mossambicus	1083	±81.4	1.8	+
Oreochromis aureus	1215	±74.6	1.7	+
Colisa fasciata	**1779**	±275.1	1.6	+
Bagarius bagarius	782	±211	2.0	+
Sperata sarwari	1091.5	±134.8	1.8	+
Mytus bleekeri	754.16	±14.3	1.9	+
Mystus vittatis	825	±31.7	2.0	+
Eutropichtyes vacha	901.3	±35.5	1.8	+
Clupisoma garua	962.5	±25.0	1.9	+
Wallago attu	1040	±352.5	1.7	+
Ompok pabda	629	±19.3	1.8	+
Channa punctate	915	±25.2	1.6	+
Channa marulius	879	±85.3	1.7	+
Rita rita	1214	±67.2	1.9	+
Mastacembelus armatus	1131	±249.4	2.0	+
Chitala chitala	1217	±45.3	1.9	+
Gadusia chapra	1028	±51.7	1.8	+
Notopterus notopterus	900	±60	1.9	+

For each fish species DNA was isolated from triplicate samples at least, whose mean values are given in this table.

Table 3. One way ANOVA table of isolated DNA quantity of 29 fish species.

S.No		Sum of squares	Degree of freedom (df)	Mean Square	F	P
1	Between groups	966918	28	34532	0.11	1.000
2	Within groups	1.75	58	301097		
	Total	1.84	86			

Table 4. One way ANOVA of isolated DNA purity at $A_{260/280}$ of 29 fish species.

S.No		Sum of squares	Degree of freedom (df)	Mean Square	F	P
1	Between groups	0.214297	28	0.00765348	0.0036	1.000
2	Within groups	122.79	58	2.11717		
	Total	123	86			

bromide and standard ladder (1Kb mix, Fermentas) was used to make direct comparison.

***Polymerase chain reaction*:** Polymerase chain reactions for amplification of genomic DNA and a conserved region of mitochondrial CO1 gene consisting of 658 base pair fragment were performed by two primers (Hebert *et al*., 2003). Genomic DNA of all the species were amplified by arbitrary decamer primer (Table 1), while CO1 fragment of mitochondrial gene were amplified by gene specific primers (Table 1).

The PCR reaction for RAPD decamer primer was carried out in total volume of 25 µl mixture containing 20 ng of genomic DNA, 2.5 µl of PCR buffer 10X (Fermentas), 2.0 mM $MgCl_2$ (Vivantus), 0.2mM of dNTPs mixture (Vivantus), 20pmol of Decamer primer (Macrogen) and 0.75 U of Taq DNA polymerase (Fermentas). Amplification profile (GeneAMP PCR system 2720; Thermocycler, Germany) was as follows; initial denaturation for 4 min at 95°C; subsequent 45 cycles for 1 min at 95°C, 45 sec at 38°C and 1 min at 72°C; followed by one final step of extension for 5 min at 72°C. The 10µl amplified products were analysed on 2% agarose gel.

The PCR reaction for CO1 gene was performed in total volume of 25 µl reaction mixture containing 20 ng of mitochondrial DNA, 0.75 U Taq DNA polymerase (Fermentas), 2.5 µl PCR buffer (Vivantus), 2 mM $MgCl_2$ (Vivantus), 20pmol of each primer and 0.2 mM of each dNTPs (Vivantus). PCR was carried out (Gene AMP PCR system 2720; Thermocycler, Germany) using the following conditions. Initial denaturation at 94 for 4 min; followed by 30 cycles of denaturation at 94 for 1 min; primer annealing at 55 for 30 sec; elongation at 72 for 1 min and final elongation at 72 for 5 min. PCR products (5 µl) were loaded on 2% agarose gel, containing 0.02 µl ml^{-1} ethidiumbromoide, in 1X TAE electrophoresis buffer. The amplified fragments were visualized under an ultraviolet transilluminator (Wealtec, USA).

***Statistical analysis*:** The data about the concentration, yield and purity of DNA isolation of all the species was analysed by using one way ANOVA manually (Table 3 and 4).

RESULTS

In our study fin tissues of fish were selected for DNA isolation due to their ubiquitous nature. The two previously reported methods for DNA extraction from various fish tissues were initially employed without any modifications. Although DNA could be isolated using urea treatment method, however it did not prove to be very efficient and resulted in smeared and poor quantity DNA. The modified salt extraction method proved to be efficient method for genomic DNA isolation from fish fin. The quantity and quality of isolated DNA from all the utilized samples was checked on 1% agarose gel which showed non-smeared, high quality DNA free from protein or RNA contamination (Fig. 1). The method was utilized for DNA isolation from fin tissues of more than 180 fish samples belonging to 29 different species. The DNA/Proteins ratio (A_{260}/A_{280}) was calculated by visible spectrophotometer with mean value of 1.8 indicating good quality DNA free from any major protein/RNA contamination (Table 2). The method resulted in high quantity of genomic DNA from a small quantity of fin tissue (50 mg), with the highest yield reported so far. The highest quantity of DNA was isolated from the fin tissue of *C. fasciata* (1779µg/50mg) while lowest quantity of DNA (604µg/50mg) was isolated from fin tissue of *L. gonius* (Table 2). Further the quality of isolated DNA was assessed for its use as template in PCR amplifications of desired sequences. PCR amplification with the help of RAPD primers (Table 1) generated very thick, numerous and clear bands (Fig. 2) as well as cytochrome oxidase gene specific primers (Table 1) also produced thick and clear bands (Fig. 3).

The urea treatment method was also modified by increasing the both incubation temperature and incubation period in lysis buffer from 42-55°C and 10-17h, respectively. Similarly, the concentration of Proteinase k and RNase was also increase up to 40 µl and 30 µl, respectively in lysis buffer but failed to obtained satisfactory results. Modified salt extraction method proves to be more successful in our study for isolation of DNA from fin tissue of 29 species of fresh water. This method was previously used for isolation

of DNA from scales of fish by Kumar et al., (2007). The modifications were made to make it economic, efficient, rapid and highly productive.

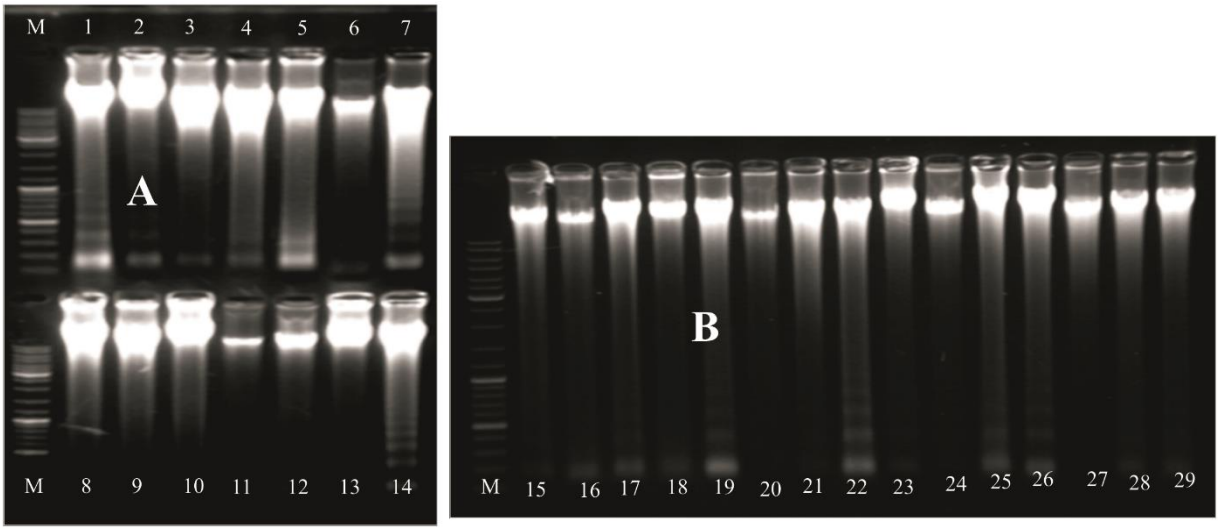

Figure 1A, B. Representative (1%) gel of DNA isolated by modified salt extraction method from 14 different species of the fish. (M) DNA ladder mix (SM 331, Fermentas), (1) *L. rohita,* (2) *L. calbasu*, (3) *L. gonius*, (4) *C. catla,* (5) *C. mrigala,* (6) *C. reba,* (7) *S. seenghala*, (8) *R. rita,* (9) *B. bagarius,* (10) *W. attu,* (11) *O. pabda,* (12) *O.mossambicus,* (13) *M. armatus,* (14) *C. fasciata* (15) *S. sarana,* (16) *C. idella*, (17) *H. molitrix,* (18) *C. carpio,* (19) *O. niloticus,* (20) *O. aureus*, (21) *M. bleekeri*, (22) *M. vitattis,* (23) *E. vacha*, (24) *C. garua,* (25) *C. punctata,* (26) *C. marulius*, (27) *C. chitala,* (28) *G. chapra* and (29) *N. notopterus*

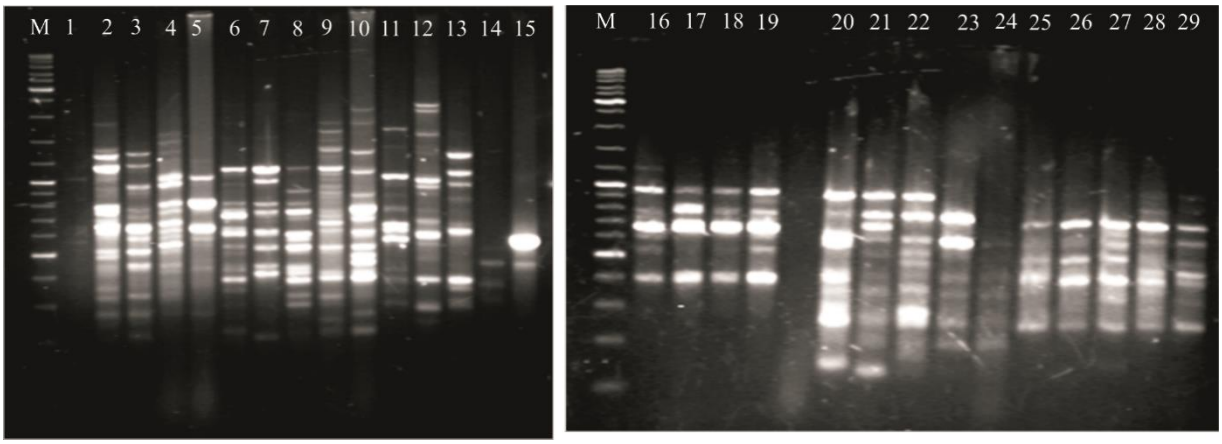

Figure 2. Representative (2%) gel electrophoresis of PCR amplified DNA of 15 species by RAPD primer (M) DNA ladder mix. (SM 331, Fermentas), (1) *L. rohita,* (2) *L. calbasu*, (3) *L. gonius,* (4) *C. catla*, (5) *S. sarana,* (6) *C. mrigala,* (7) *C. reba,* (8) *S. seenghala*, (9) *C. idella* (10) *H.molitrix,* (11) *W. attu,* (12) *O. pabda,* (13) *O.mossambicus,* (14) *M. armatus,* (15) *C. fasciata* (16) *C. carpio*, (17) *O. niloticus,* (18) *O. aureus,* (19) *B. bagarius,*(20) *R. rita,* (21) *M. bleekri,* (22) *M. vitattis,* (23) *E. vacha,* (24) *C. garua,* (25) *C. punctata,* (26) *C. marulius,* (27) *C. chitala,* (28) *G.chapra* and (29) *N. notopterus*

Highest yield and purity of DNA was isolated by using only 10µl of proteinase K in lysis buffer and incubated for 2-3h. High yield of DNA was also isolated by this modified method by adding as low as 5 µl of Proteinase K but this was of poor quality due to proteins contamination. The incubation duration of fin in lysis buffer was slightly increased from 2-3 h due to hard nature of fin (bony or cartilaginous fin rays) as compared to scales. Similarly, absolute ethanol is proved to be best preservative for isolation of high quality DNA from fin tissue in our study. The results of this study shows that a very high yield with best quality DNA can be isolated by modified salt extraction method as represented by the gel (Fig. 1). The amplification of numerous, clear and very thick bands by RAPD primer and CO1 gene specific primer of

mitochondrial DNA strengthened the extent of efficacy of this method to isolate high quality DNA for use in PCR amplification (Fig. 2 and Fig. 3).

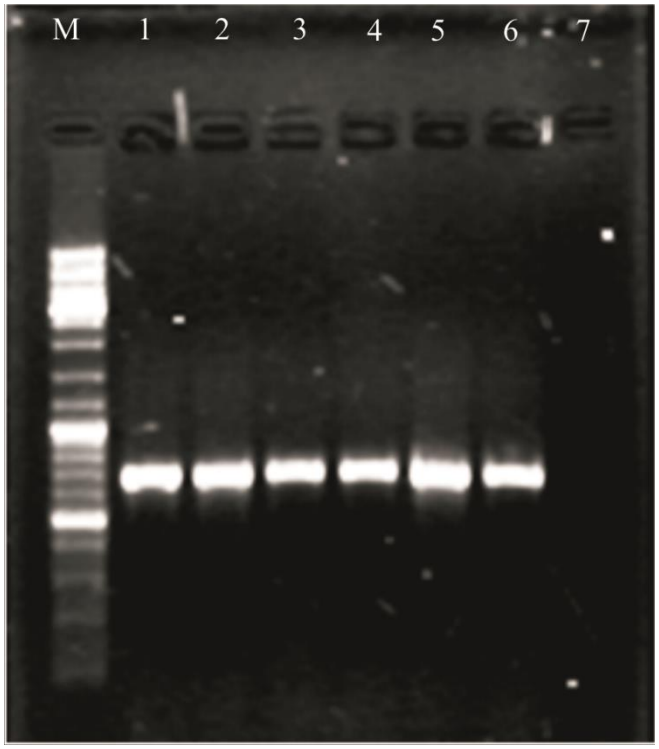

Figure 3. Representative (1%) agarose gel electrophoresis of isolated mitochondrial DNA by modified salt extraction method by with CO1 gene specific marker. (M) DNA ladder mix. (SM 331, Fermentas), (1) *E. vacha*, (2) *C. garua*, (3) *W. attu*, (4) *O. pabda*, (5) *M.armatus*, (6) *N. notopterus* and (7) control without DNA template.

DISCUSSION

Genetic studies mainly based on high quality DNA isolation based upon its isolation techniques from the source. Soft tissues are given preference for this purpose for their easy and rapid breakdown in lyses buffers. Hard tissues are avoided by the workers because they are not easily lysed and take long time for their complete breakdown in the lysis solution. In case of fish DNA is mostly isolated from soft tissues like blood, muscles, liver and etc. but the isolation of DNA from these tissues is achieved with the sacrifice of animals. These tissues are not desirable in case of endangered species or for the individuals of small population for nucleic acid based studies. Fins and scales seem to be attractive to isolate DNA. However, a very poor and low quantity of DNA can be isolated from these tissues due to their small size and hard nature. Some workers successfully isolated the high quality of DNA from these both tissues from different species (Taggar *et al*., 1992; Zhang *et al*., 1994; Estoup *et al*., 1996; Nielson *et al*., 1999; Adcock *et al*., 2000; Wasko *et al*., 2003; Kumar *et al*., 2007). In our study fins were given preference over scales for DNA isolation because scales are not universal in their occurrence in fishes as compared to fins.

Use of liquid nitrogen for tissue homogenization to isolate sufficient amount of DNA form hard tissue is suggested an efficient method by some authors (Chen *et al*., 1995; Strassmann *et al*., 1996; Pinto *et al*., 2000) did not give any further improvement in isolation of DNA in our experiments. Similarly addition of 4-8 M urea and thirty µl Rnase in lysis buffer were suggested very important by Wasko *et al*. (2003) for breakdown of fins and scales, proteins denaturation and RNA degradation. In our experiment 1µl Rnase was added in few DNA samples when they were dissolved in sterile water at the last step. Initial use of 30 µl Rnase and Urea treatment is useless and costly according to our study.

Another improvement was obtained by the addition of less quantity of Proteinase K (10 µl) and less incubation time of the fin tissues in lysis buffer. Use of low quantity of Proteinase K without RNase in lysis as suggested by Wasko *et al*. (2003) did not affect the quantity and quality of isolated DNA. On the other hand Wasko *et al*. (2003), *Kumar et al*. (2007), and Srividya *et al*. (2011) use thirty microliter, twenty microliter, forty microliter and twenty microliter of proteinase k respectively in equal volume of lysis buffer as used in our study. Wasko *et al*. (2003) suggested that time, temperature and concentration of proteinase k were very important for high quality DNA isolation. They suggested that incubation of fin and scales tissues at 42°C for 10 hrs is very essential. Temperature less than 42°C and duration of incubation less than 10 hrs may not properly dissociate the tissues for proper DNA isolation. Similarly, they also urge that the use of lower quantity of Proteinase k than the thirty µl in lysis buffer failed to digest the tissue completely and leads to poor quality of DNA isolation. We used only 10 µl of proteinase k in lysis buffer and incubate the fin tissue for 2-3hrs at 48°C and obtained very high quality and quantity DNA as compared to Wasko *et al*., (2003) and Kumar *et al*. (2007). Use of low quantity of proteinase k and short incubation period save both money and time.

According to our study the quantity and quality of DNA to be isolated depends its immediate storage in 95% ethanol after their separation from the animal. Quantity and quality of DNA to be isolated directly depends upon the preservation time of fin tissues in 95% ethanol. Further if the fins are separated from the living animals and immediately preserved in the 95% ethanol gave best results for isolation of DNA. As the death period of animal and preservation gap in absolute ethanol increases the quality and quantity of DNA to be isolated decreases. Phenol-chloroform-isoamyl alcohol step is very necessary for proteins separation from the DNA in the lysed tissue in the digestion buffer. After this another treatment of chloroform to the separated DNA is also essential

from any residual proteins contamination during pipetting. The addition of ammonium acetate salt in the isopropanol is also important for DNA threads condensation up to visible level that is later removed by giving double wash to DNA molecule with 70% ethanol.

Spectrophotometer comparison of absorbance at 260-280 nm was subjected on all samples of twenty nine species of fish provide results of DNA/proteins relationship of (1.6-2.0) showing good quality of isolated DNA (Table 2). The concentration of DNA ranged from 604-1779 µg/50mg of fin) with an average concentration of (970.7 µg/50mg). One eighty samples of fins tissue belonging to 29 species of fish were subjected to isolate DNA by applying this methodology and very high quality DNA was isolated from all the samples. It is suggested that this technique can be successfully applied for isolation of DNA from fish fins with less consumption of time and low cast without scarifying the animal. This technique was also applied on fin tissues preserved in ice, formalin and dried tissues. DNA was not isolated from the fins preserved in the formalin, because it promotes the formation of stable multicomplex bond between nucleic acid and proteins within the cell. A very low quality DNA was also isolated from the fin tissues preserved in the ice and air dried only for 1-2 days. This is because of the degradation of nucleic acid within the tissue cells due to activity of nucleases in the cell. DNA was not isolated from the fins older than few days kept in ice and air dried. The isolated DNA was successfully amplified by using arbitrary primers of Randomly Amplified Polymorphic DNA technique and CO1 gene of mitochondrial DNA to be sequenced.

***Conclusion*:** This study conclude that isolation of DNA from fin tissue of freshwater fishes by modified salt extraction method is more suitable than urea treatment method due to the advantage of its economic, rapid and high yield and quality DNA. This methodology can be successfully applied for the isolation of DNA from the fin of other species of fish. Similarly, absolute ethanol seems to be good preservative for isolation of DNA. Formalin is not suitable preservative for fin tissues to be utilized for DNA isolation. Ice preserved and air dried fin tissues are not reliable resource of good quality and quantity DNA especially after one weak.

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