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DNA EXTRACTION METHODS FOR FIN OF MACKEREL IN THAILAND

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ABSTRACT: The quality and quantity of DNA are crucial aspects for fish studies based on molecular techniques. The extraction method of genomic DNA is depended on tissue types and organism species. This research aimed to identify an appropriate extraction method for gDNA from the fin of three popular mackerel species in Thailand; short-bodied mackerel (*Rastrelliger brachysoma*), island mackerel (*R. faughni*) and Indian mackerel (*R. kanagurta*). Four different methods for gDNA extraction were compared based on time, quality and quantity of extractable gDNA for PCR technique. Method III showed the highest quantity of gDNA in *R. brachysoma* and *R. kanagurta*. Nevertheless, the highest purity of gDNA for both species was obtained by method II and IV, respectively. The gDNA from method IV was successful to amplify the intense band of *β-actin* fragment. The highest concentration and purity of gDNA from *R. faughni* were received using method II. However, *β-actin* gene fragment amplified from gDNA of method IV showed intense bands. These results indicated that method IV was suitable for gDNA extraction from the fin of three mackerel species because of the fastest, high quality and quantity for PCR amplification.

Keywords: DNA extraction, Fin, Mackerel, Fish

1. INTRODUCTION

Extraction of genomic DNA is an important step of fish studies based on molecular techniques. Several reports related to DNA extraction from various fish suggested quality and quantity of extracted gDNA were depended on fish species, tissues types and extraction methods [1-5]. Nowadays, attempt to extract gDNA from fish tissue without or less invasive sampling method was investigated in many fish. Several reports demonstrated the different protocols of DNA extraction from the external organs of fish, such as fins [1, 5], and scales [3]. The mackerel of the genus *Rastrelliger* are pelagic fish species, belonging to the Scombridae family. The *Rastrelliger* mackerel comprise three species, namely, *R. brachysoma*, *R. faughni* and *R. kanagurta*. Their distribution is widely in the Indo-Pacific Ocean [6]. There is little information related to molecular studies in these popular mackerel species in Thailand. Therefore, the objective of this study was to identify an appropriate method for gDNA extraction from the fin of three mackerel species for PCR-based techniques. The result data will be as a beneficial knowledge for further mackerel research such as genomic analysis, biomarker study, and genetic species identification, etc.

2. MATERIALS AND METHODS

2.1 Fish Sample and Extraction Methods

R. brachysoma, *R. faughni* and *R. kanagurta* (Fig.1) were obtained from the port in Chumphon province, Thailand. Caudal fin was isolated and subjected to gDNA extraction using four different methods composed of method I-V.

Method I was modified from urea treatment method [7]. Fin tissue (20 mg) was mixed with 300 µl of extraction buffer (10 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.5% SDS, 4 M Urea) with 5 µl of RNase (10 mg/ml). The mixture was homogenized and incubated at 42°C with shaking at 200 rpm. After incubation for 1 h, the mixture was gently mixed with 10 µl of Proteinase K (10 mg/ml) and then incubated at 42°C with shaking at 200 rpm for 16-18 h. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was then added into the mixture and inverted several times. The mixture was centrifuged at 11,000 rpm for 15 min. The upper phase was collected to a new tube, and mixed with 1 M NaCl and an equal volume of absolute ethanol. The mixture was centrifuged at 11,000 rpm for 15 min. The supernatant was discarded. The 70% ethanol was added to DNA pellet and then centrifuged at 11,000 rpm for 5 min. The DNA pellet was air-dried and re-suspended in sterile water.

Method II was modified from the report of [1]. Fin tissue (20 mg) was homogenized in 300 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 50 mM EDTA, 1% SDS) with 7.5 µl of Proteinase K (10 mg/ml). The mixture was incubated in the shaking incubator

at 55°C for 16-18 h with oscillation of 200 rpm. Subsequently, 300 µl of 5 M NaCl was mixed with the mixture and centrifuged at 11,000 rpm for 10 min. The supernatant was collected and inversely mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The mixture was then centrifuged at 11,000 rpm for 10 min. The upper phase was collected and mixed with an equal volume of absolute ethanol for DNA precipitation. The mixture was centrifuged at 11,000 rpm for 15 min. The supernatant was discarded. The 70% ethanol was added to DNA pellet and then centrifuged at 11,000 rpm for 5 min. The DNA pellet was air-dried and re-suspended in sterile water.

Method III was followed by Rapid MT method [4]. The fish fin (20 mg) was homogenized in 660 µl of extraction buffer (100 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) and then 1 µl of Proteinase K (10 mg/ml) was added. The mixture was incubated in the shaking incubator at 55°C for 24 h with oscillation of 200 rpm. After incubation, the sample was centrifuged at 12,000xg for 15 min. The supernatant was taken in a new tube and inversely mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). After centrifugation at 12,000xg for 10 min, the upper phase was collected and mixed with an equal volume of isopropanol, and then centrifuged at 12,000xg for 10 min. The supernatant was decanted and the DNA pellet was washed with 70% ethanol. The DNA pellet was air-dried and re-suspended in sterile water.

Method IV was modified from the report of [5, 8]. Fin sample (20 mg) was homogenized in 400 µl of extraction buffer (200 mM Tris-HCl pH 8.0, 100 mM EDTA, 250 mM NaCl, 1.2% SDS, 200 µg/ml Proteinase K) and incubated in the shaking incubator at 48°C for 3 h. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added into the mixture and mixed by inversion several times. After centrifugation at 11,000 rpm for 15 min, the upper layer was taken into a new tube. An equal volume of isopropanol and 0.2 volume of 10 M ammonium acetate was added and mixed. The sample was centrifuged at 11,000 rpm for 15 min. The supernatant was removed and the DNA pellet was washed in 70% ethanol, air-dried and re-suspended in sterile water.

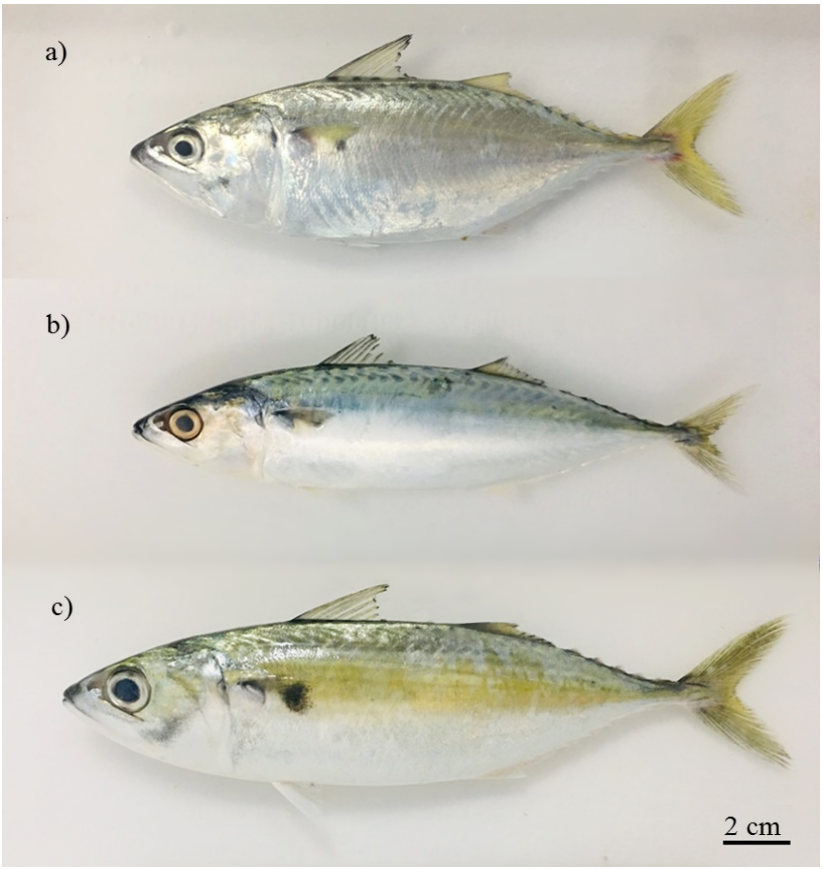

Fig. 1 Mackerel species of this study a) *R. brachysoma*, b) *R. faughni*, and c) *R. kanagurta*

2.2 Quality and Quantity of gDNA Analysis

The quality and quantity of gDNA from each method were determined by agarose gel electrophoresis and spectrophotometer. The DNA sample was visualized in 1% agarose gel stained with SYBR Safe DNA Gel Stain. The concentration and purity were evaluated using UV/visible spectrophotometer with absorbance of 260 and 260/280 nm, respectively. The data were presented as a mean ± standard deviation (mean±SD; n=5). The statistical significance was examined using the One-Way Analysis of Variance followed by a Tukey's HSD test at a significance level of ≤ 0.05.

2.3 Polymerase Chain Reaction

The quality of gDNA was further confirmed by amplification in the conserved region of *β-actin* gene, consisting of 1244 bp, using specific primers: Forward 5' ATGAAATCGCCGCACT GG 3' and Reverse 5' TGGATGGCAACGTACA TGGC 3'. PCR reaction was performed in the total volume of 50 µl mixture consisting of 100 ng of gDNA, 1X Standard Taq Reaction buffer, 1 mM $MgCl_2$, 0.2 mM dNTPs mixture, 0.4 µM of forward and reverse specific primers, and 1.25 U Taq DNA polymerase. Amplification conditions were 95°C for 3 min followed by 30 cycles at 95°C for 30 s, 53°C for 30 s, 72°C for 30 s and then subjected to a final extension of 72°C for 3 min. The amplified products were analyzed through 1.5% agarose gel stained with SYBR Safe DNA Gel Stain.

3. RESULTS AND DISCUSSION

This study identified the appropriate extraction method for gDNA from the fin tissue of three popular mackerel species in Thailand based on extraction time, quality and quantity of gDNA. The gDNA extraction method should be rapid and efficient for achieving the high quantity and quality of gDNA [9, 10]. The fin gDNA was extracted from four different methods: method I-IV. For the extraction time, the method I-III needed more than 20 h for the whole process, whilst the time requirement for method IV was not more than 6 h. Extraction buffer of all methods was generally used SDS and proteinase K as a detergent for tissue digestion process, which was varied the concentration of those. Moreover, urea and RNase were added into the extraction buffer of method I for protein and RNA denaturation [5, 7], respectively. The extraction buffer of method II-IV was additionally mixed with NaCl used for extracting protein [4]. The temperature in tissue lysis step in all methods was carried out at 42-55°C. Subsequently, phenol:chloroform: isoamyl alcohol was required to remove the proteins and DNA was precipitated by either absolute ethanol or isopropanol. Interestingly, DNA precipitation step of all did not take additional time.

A characteristic pattern of DNA bands observed by 1.0% agarose gel electrophoresis was shown in Fig. 2. The gDNA bands of each species were distinct among different methods. The quantity and quality of gDNA from the fin of *Rastrelliger* sp. obtained from different four methods was summarized in Table 1. The extractable gDNA concentration from different methods varied in each species. The concentration of gDNA from *R. brachysoma* was shown in a range of 0.45-0.98 µg/µl. The highest oncentration of gDNA from *R. brachysoma* was found from method III (0.98±0.10 µg/µl), followed by method IV (0.74±0.10 µg/µl), method II (0.64±0.12 µg/µl), and method I (0.45±0.18 µg/µl), respectively. The concentration of gDNA of *R. faughni* was obtained in a range of 0.62-1.10 µg/µl. The gDNA of *R. faughni* was shown the highest concentration obtained from method II (1.10±0.07 µg/µl). This was followed by

Table 1 Quantity and quality of extracted DNA from different extraction methods

Extraction Method	*R. brachysoma*		*R. faughni*		*R. kanagurta*	
	Concentration (µg/µl)	Purity (A260/A280)	Concentration (µg/µl)	Purity (A260/A280)	Concentration (µg/µl)	Purity (A260/A280)
Method I	0.45 ± 0.18^{a}	1.48 ± 0.03^{a}	0.62 ± 0.08^{a}	1.49 ± 0.07^{a}	0.49 ± 0.03^{a}	1.67 ± 0.08^{a}
Method II	$0.64\pm0.12^{a,b}$	1.61 ± 0.07^{b}	1.10 ± 0.07^{b}	1.77 ± 0.05^{b}	0.70 ± 0.08^{b}	$1.75\pm0.06^{a,b}$
Method III	0.98 ± 0.10^{c}	$1.54\pm0.02^{a,b}$	0.94 ± 0.12^{b}	1.66 ± 0.09^{b}	0.84 ± 0.07^{c}	1.70 ± 0.02^{a}
Method IV	0.74 ± 0.10^{b}	1.59 ± 0.03^{b}	1.05 ± 0.14^{b}	1.74 ± 0.08^{b}	0.70 ± 0.07^{b}	1.83 ± 0.04^{b}

Note: Concentration and purity were represented as mean±SD (n=5). The different letters indicated the significant difference between extraction methods within same species. The significant difference was analyzed by Tukey's HSD at $P\leq0.05$.

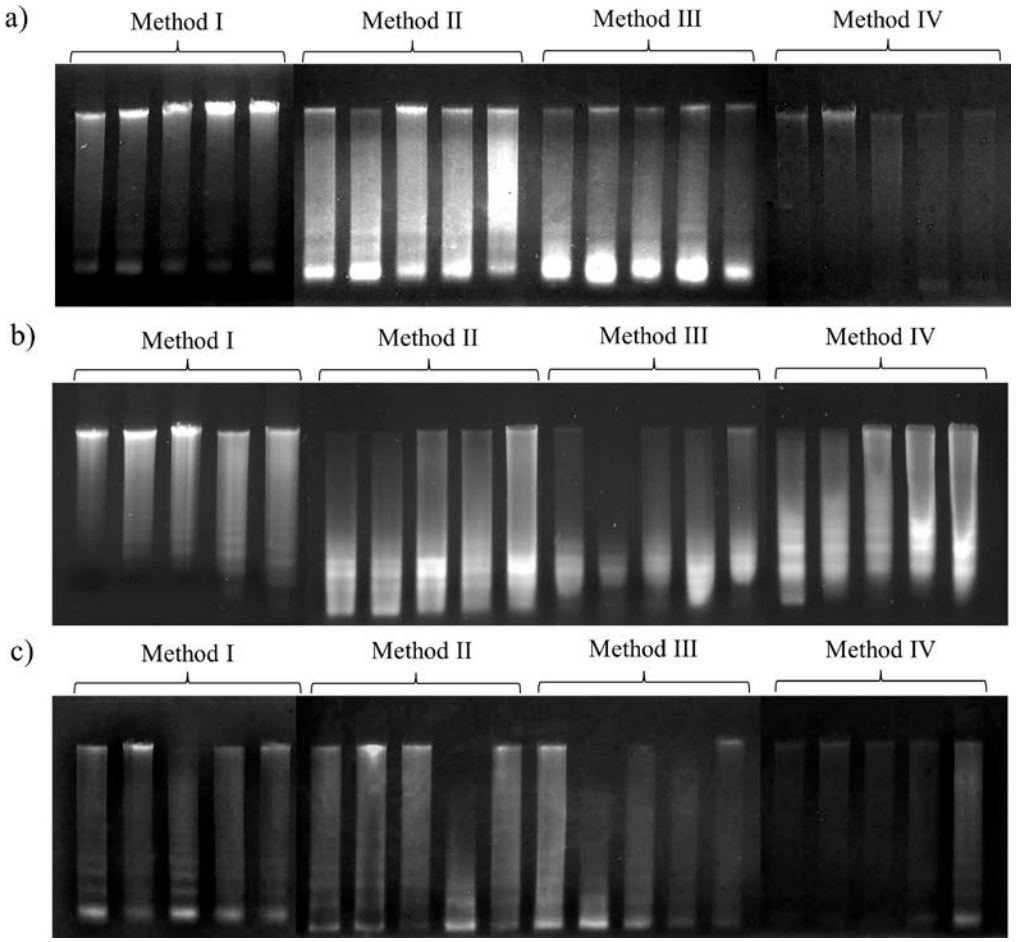

Fig. 2 Genomic DNA extracted from fin of a) *R. brachysoma*, b) *R. faughni*, and c) *R. kanagurta* by using different methods (method I-IV).

method IV (1.05±0.14 µg/µl), method III (0.94±0.12 µg/µl), and method I (0.62±0.08 µg/µl), respectively. The concentration of gDNA of *R. kanagurta* was extractable in a range of 0.49-0.84 µg/µl. The highest concentration of gDNA from *R. kanagurta* was found in method III (0.84±0.07 µg/µl), followed by method IV (0.70±0.07 µg/µl) and method II (0.70±0.08 µg/µl). The lowest concentration of extracted gDNA was observed from method I (0.49±0.03 µg/µl). This study found the significantly lower values were attained from method I supplemented with urea in extraction buffer, suggesting this method did not suitable for DNA extraction from the fin of three mackerel species.

The quality of the gDNA was indicated by the A260/A280 ratio. The purity of extracted DNA is generally accepted a yield of A260/A280 ratio in a range of 1.8-2.0 [11]. The gDNA of *R. brachysoma* from all extraction methods expressed a low purity, which had a value in a range of 1.48-1.61. The highest purity in this species was exhibited in the extracted DNA obtained by method II (1.61±0.07). This was followed by the gDNA attained from method IV (1.59±0.03), method III (1.54±0.02), and method I (1.48±0.03), respectively. The gDNA of *R. faughni* showed the purity values in a range of 1.49-1.77. The gDNA obtained from method II showed the highest purity for *R. faughni* (1.77±0.05), following by method IV (1.74±0.08), method III (1.66±0.09), and method I (1.49±0.07), respectively. Meanwhile, the gDNA of *R. kanagurta* showed the purity values in a range of 1.67-1.83. The highest purity in this species was shown in the extracted DNA obtained by method IV (1.83±0.04), following by method II (1.75±0.06), method III (1.70±0.02), and method I (1.67±0.08), respectively. These results suggested the method II produced the highest quality of gDNA for *R. brachysoma* and *R. faughni* compared to other methods. The method IV was able to extract the highest quality of gDNA for *R. kanagurta*. Corresponding with quantity result, the quality of gDNA extracted from method I was lowest for all species. Report of [1] revealed the purity of the fin DNA extracted from modified salt method showed in a range of 1.83-2.19. These results showed

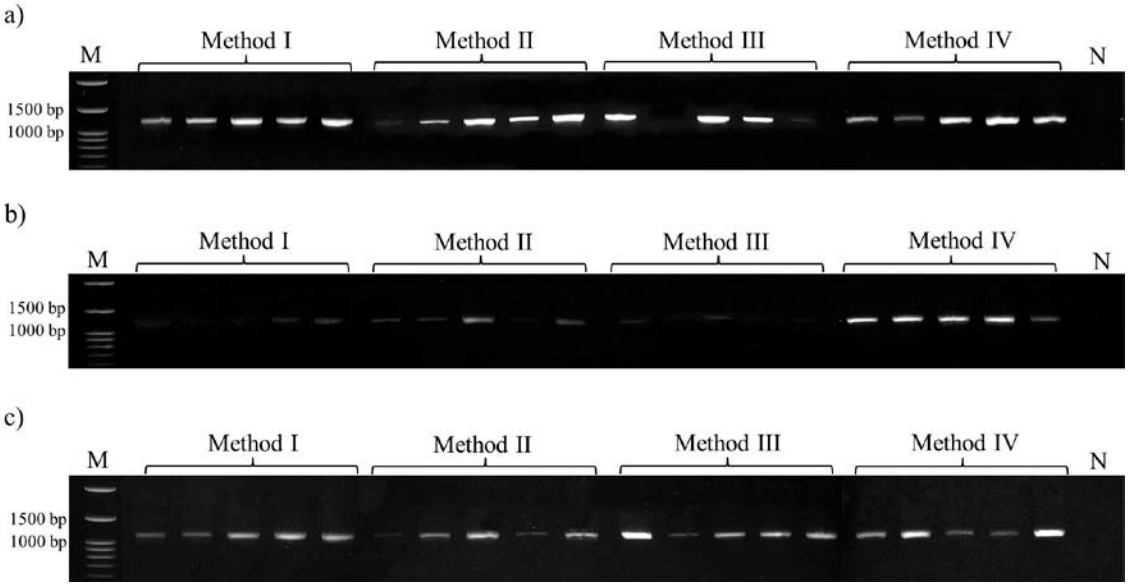

Fig. 3 *β-Actin* fragments amplified from gDNA extracted from fin of a) *R. brachysoma*, b) *R. faughni*, and c) *R. kanagurta* by using different methods (method I-IV). M = 100 bp DNA ladder marker and N = negative control.

similar results of reports from [4, 5] demonstrated the purity of fish DNA was in a range of 1.6-2.0.

Subsequently, the quality of gDNA was considered by using as a template for PCR amplification of *β-actin* gene fragment. The amplified products of *β-actin* gene analyzed by 1.5% agarose gel electrophoresis were shown in Fig. 3. The PCR products in *R. brachysoma* as well as *R. kanagurta* were achieved from gDNA obtained from all methods. The intense band of PCR products was represented from the gDNA obtained from method I and IV. Whereas, some of gDNA obtained from method II and III showed the light band of the amplified PCR fragment. The PCR amplification in *R. faughni* was attained by using gDNA extracted from all methods. The PCR products of gDNA from method IV showed the intense band. The gDNA from method I, II and III were amplified the PCR products with a light band.

4. CONCLUSION

The quality and quantity of gDNA from the fin varied according to fish species and extraction methods. The method IV was the appropriate method for DNA extraction from the fin of three mackerel species in Thailand. It indicated that the advantage of method IV was rapid, good quantity and quality of gDNA for PCR amplification.

5. ACKNOWLEDGMENTS

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