THE APPLICATION OF PCR-RFLP METHODS USING UNIVERSAL CYTOCHROME B PRIMERS FOR SPECIES IDENTIFICATION OF LOCAL PISCES, AVES AND MAMMALS

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Introduction

Indonesia is called as megadiversity country with very high endemism of wildlife, since she harbors 12 percent of the world's mammals, 16 percent of the world's reptiles and amphibians, 17 percents of all birds and more the one-quarters of all marine and freshwater fish. Nowadays, many Indonesian fauna admitted in categorizing threatened species in the world. However, the biodiversity richness will never remain sustainable if there are no efforts to protect and conserve these valuable resources. Poaching of wildlife and illegal meat has become highly commercialized, lead to the unsustainable. While identification of poached wildlife products as carcassesor meat face big problems when morphological characters are missing, therefore among more reliable techniques for species identifications should be taken.

A mitochondrial DNA (mtDNA) is useful for assesing genetic relationships of individuals or groups within a species and also for identifying the phylogeny {evolutionary relationships) among different species. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) of a mDNA *cytochrome* b (*cyt b*) gene can used to study polymorphism between species in one class, whichever polymorphism is a kind of variation related to biodiversity, genetic variation and adaptation of animal. Polymorphisms function can also to study about population, for examples to assessing the degree of genetic diversity in a population, determining whether two populations represent separate species or races of the same species, and tracking migration patterns of a species (3).

This study is aim to analysis the restriction length fragment polymorphism of the *cyt* b gene amplicon (PCR product) for species identification of local pisces, aves and mammals using cyt b universal primers. This species identification is essential not only for detection and identification of animals carcasses or biological material of any unknown animal origin (included biological materials in feed stuff and food samples), but also biological materials of trace animal sources.

Materials and Methods

Nine local fishes, 8 birds and 7 mammals samples as in Table 1 were used in this study. The 23 muscles (0.1g) of local fishes, birds, and mammals and one sample hair follicles collected from Bogor area were preserved in NaCl containing DMSO until DNA isolation. The mitochondrial DNA were isolated using amonium acetat precipitation method, followed by determination of mtDNA concentration and purity using Spectrophotorneter Absorban-Assay. Universal oligonucleotide cytochrome b primers L14841/H15149 of Kocher et al. (2, 4) were used for amplification of the *cyt b* gene fragment in polymerase chain reaction (PCR), performed in GeneAmp 9600, followed by determinalion of the PCR product by electrophoresis using *2.5%* agarose gel (ScientifiX™). The cyt b gene PCR product were digested using restriction enzymes *HinfI* (1000 U, Roche Diagnostics Gmbh) and *RSA* I (Pure Extreme™, Fermentas) for 1 and 6 hours, respectively. The digested fragments are separated according to the molecular size using 2.5% agarose gel electrophoresis. Ethidium bromide staining is used to reveal the fragments under UV (260 nm) light. Molecular size standards are used to estimate fragment size (1).

Results and Discussion

The amplification of the *cytochrome b* gene fragrnent using the universal

oligonucleotide primers L14841/H15149 yielded a 359 bp amplicon. These amplicons were digested with *Hinf* I and *RSA* I restriction endonuclease and showed high polymorphism in fragments size among species in pisces, aves and mammals.

The amplicon of fish samples digested with *Hinf* I showed 2 fragments of various sizes, except *Trichogaster pectoralis*, *Cyprinus carpio*, *Oreochromis mossambicus* and *Hypostomus sp*, do not show any *Hinf* I restriction site. However, they can be differed by the *RSA* I restriction site, except the *Trichogaster pectoralis* sample.

All of the amplicon of bird samples can be differed each other by the *Hinf* I and *RSA* I restriction sites. *Ploceus manyar* and *Lonchura punctulata* shows similar location of *Hinf* I site but differed by the *RSA* I site. While the *Ploceus manyar* and *Pycnonotus goiavier*, as well as *Gallus gallus domesticus* and *Pycnonotus goiavier* can not be differed by the *RSA* I site.

Most of mammal samples analyzed in this study do not show differences in the *RSA* I site, except the *Mus musculus albinus*. However, *Oryctolagus cuniculus*, *Capra aegagrus*, *Mus musculus albinus*, *Bos taurus* and *Tragulus javanicus* can be differed each other by using the *Hinf I* restriction enzyme. Neither *Sus scrofa* nor *Felis catus* showed the *Hinf* I and *RSA* I sites, which indicated that other restriction enzymes should be applied for this species identification.

Table 1. Fragment sizes of L14841/H15149 *cyt b* fragment cut ,with *Hinf* I and *RSA* I

No	Spesies	Hinf I		RSA I	
		fragmen 1	fragmen 2	fragmen 1	fragmen 2
1.	*Pangasius sutchi* (Patin)	189 bp	170 bp	189 bp	170 bp
2.	*Cyprinus carpio* (Mas)	359 bp	0 bp	181 bp	178 bp
3.	*Clarias sp* (Lele)	188 bp	171 bp	243 bp	116 bp
4	*Trichogaster pectoralis* (Sepat)	359 bp	0 bp	359 bp	0 bp
5.	*Hypostomus sp* (Sapu-sapu)	359 bp	0 bp	230 bp	129 bp
6.	*Oreochromis mossambicus* (Mujair)	359 bp	0 bp	186 bp	173 bp
7.	*Oreochromis niloticus L* (Nila)	230 bp	129 bp	293 bp	66 bp
8.	*Osphronemus gouramy Lacepede* (Gurami)	302 bp	57 bp	319 bp	40 bp
9.	*Colossoma macropomum* (Bawal)	199 bp	160 bp	335 bp	24 bp
10.	*Gallus gallus domesticus* (Ayam kampung)	198 bp	161 bp	200 bp	159 bp
11.	*Lonchura leucogastroides* (Bondol Jawa)	243 bp	116 bp	189 bp	170 bp
12.	*Lonchura punctulata* (Bondol Peking)	255 bp	104 bp	182 bp	177 bp
13.	*Pycnonotus aurigaster* (Cucak Kutilang)	300 bp	59 bp	209 bp	150 bp
14.	*Pycnonotus goiavier* (Merbah Cerukcuk)	183 bp	176 bp	200 bp	159 bp
15.	*Ploceus manyar* (Manyar Jambul)	255 bp	104 bp	197 bp	162 bp
16.	*Streptopelia bitorquata* (Dederuk Jawa)	271 bp	88 bp	197 bp	162 bp
17.	*Anas sp* (Bebek)	231 bp	128 bp	359 bp	0 bp
18.	*Oryctolagus cuniculus* (Kelinci/Rabbit)	223 bp	136 bp	359 bp	0 bp
19.	*Capra aegagrus* (Kambing/Goat)	211 bp	148 bp	359 bp	0 bp
20.	*Sus scrofa* (Babi/Pig)	359 bp	0 bp	359 bp	0 bp
21.	*Mus musculus albinus* (Mencit/Mouse)	313 bp	46 bp	287 bp	72 bp
22.	*Bos taurus* (Sapi/Cow)	206 bp	153 bp	359 bp	0 bp
23.	*Felis catus* (Kucing/Cat)	359 bp	0 bp	359 bp	0 bp
24.	*Tragulus javanicus* (Kancil)	319 bp	40 bp	359 bp	0 bp

Conclusion

The *Hinf* I and or *RSA* I restriction fragment length of the L14841/H15149 *cyt b* gene of several local fishes, birds and mammals show polymorphism, which indicated that this method can be used for species identification. Species identification of some mammals as *Sus scrofa* and *Felis catus* need further analysis using other restriction enzymes.

References

Anonim. 2005. – Department of Biology, Davidson College, Davidson, NC 28036. How to Calculate the MW of a molecule that has been Separated in a Gel (http://www.bio.davidson.edu/Courses/Molbio/Protocols/molwt.html).

Anonim. 2008. – mtDNA Primers Database (http://www.usc.es/mtdna/doc/primers.html).

Anonim. 2008. – Restriction Fragment Length Polymorphism (http://en.wikipedia.org/wiki/RFLP).

Kochler TD et al. 1989. Natcl. Acad. Sci. USA. 86:6196-6200.