Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 6, p. 267-274, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Genetic diversity of razor clam (*Solen* sp.) at Pamekasan Beaches and Surabaya East Coast Indonesia based on RAPD markers

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Article published on December 29, 2015

Key words: Genetic diversity, RAPD method, Pamekasan Coast, *Solen* sp., Surabaya East Coast.

Abstract

Razor clam (*Solen* sp.) is one of the species in the class of Pelecypoda on the phylum of Mollusca that is used as the source of food. Overexploitation can lead to the loss or the decline of the genetic diversity of a population. Then, further decrease of genetic diversity may result the reduction in the adaptability, the resilience of the population, and its productivity. This study was aimed to assess the genetic diversity of *Solen* sp. found at Pamekasan Coast, Madura and Surabaya East Coast using RAPD method. This study used a survey by random sampling technique. The observed data includes the molecular characters in the form of RAPD markers that are amplified using OPA 1-20 primers. Genome was isolated from the *Solen* sp tissue. RAPD markers amplification was performed using PCR technique. The method of analysis was conducted using a similarity index that is based on the presence or absence of specific DNA bands on agarose gel. The result of the research showed that the relationship of similarity between *Solen* sp at Surabaya East Coast and beaches in Pamekasan was 13.1%. The identification result showed that *Solen* sp. which were found in both locations is *Solen regularis*.

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Introduction

In Indonesia, *Solen* sp. is known in several different local names. In Madura Island and at the east coast of Surabaya, *Solen* sp. was called '*Lorjuk*', and others call it bamboo clam or knife clam. *Solen* sp. or Razor clam is a Mollusca clam that is found in the world ocean, i.e. *Solen dactylus* in northern coast of the Persian Gulf, Iran (Saeedi *et al.*, 2009). Other clams are *Solen marginatus* in Atlantic coast, Europe, south west of Africa, and Mediterain Ocean (Hmida *et al.*, 2012), and *Solen regularis* in Malaysia (Hamli, 2012). Nutrient concentration of Razor clam in Pamekasan city was stated by Nurjanah *et al.*, (2008) in a dry weight condition respectively: 55.34% of protein, 27.98% of carbohydrate, 1.82% of fat, 14.87% of ash level, and 349.66 kcal of calorie. Overexploitation and coast waters pollution tend to ruin the sustainability and genetic diversity of *Solen* sp. population. Overexploitation causes the disappearance or the decrease of genetic diversity of a population. The decrease of genetic diversity then leads to the decrease of adaptability, population resilience, and its productivity (Andareas, 2011).

Study of genetic diversity on an organism population aims at giving an evaluation on the genetic diversity of a population that is related to the preservation. This matter is needed since the genetic diversity is always needed by each species in its reproduction ability and its adaptation to the changes of environment condition. In other words, the species needs a varied spare genetics so that they can survive in its conditional environment that changes overtime. Besides, the data of the varied genetics is very crucial to support the usage of germplasm. The higher the genetic diversity of germplasm, the higher the chance to get organism with desired characteristics.

Research on genetic diversity on a natural population can be conducted directly using genetic mark such as DNA, or indirectly using enzyme polymorphism. One of the DNA features that were used to analyze the population genetic diversity is Random Amplified Polymorphic DNA (RAPD). RAPD molecule marker is produced by the DNA amplified process using in vitro process that uses polymerase chain reaction (PCR) method. RAPD standard method uses short-single oligonucleotide (10-12 bases) with the random order as the primer to amplify DNA gen in the number of nano-grams in low annealing temperature. The result of PCR amplification is separated by gel colored by ethidium bromide. The analysis of RAPD will be different in the standard PCR condition where it just uses one primer and it doesn't need any information of sequent preliminary DNA (Bardakci, 2001).

RAPD marker that was used in the research on the bivalvia genetic differences are Clam *Polymesoda erosa* (Andareas, 2011), Abalone (*Haliotis Asinina L.*) (Litaay *et al.*, 2012), *Solen marginatus* (Hmida *et al.*, 2012) and *Solen* sp. in Malaysia (Hassan and Kanakaraju, 2013). The use of RAPD marker in the research on the genetic diversity of bivalvia population in Indonesia, especially on *Solen* sp. has not been conducted yet. The data of the variation and the genetic diversity of *Solen* sp. have not been much provided. The observation on the genetics is necessary to be conducted as the source of additional information on the variation and genetic diversity of *Solen* sp. in Indonesia.

RAPD method gives faster result on the genetic diversity than other molecular techniques. This technique is capable of giving unlimited characters number so it is very helpful in analyzing organism varieties with unknown genome background (Suryanto, 2003). Therefore, the aim of this research is to find out the genetic diversity on the clam population, *Solen* sp. at Pamekasan Coast and East Coast of Surabaya Indonesia based on the RAPD marker.

Materials and methods

Study area and sampling

Location of the taken sample was from two different locations, Pamekasan Coast, Madura Island, and East Coast of Surabaya from March to May, 2015. The identification of *Solen* sp. was conducted at Indonesia Science Institute (LIPI), in Cibinong, Bogor, and the

analysis of RAPD of *Solen* sp. was conducted at Science Laboratory Centre of Brawijaya University, Malang. The steps were started by collecting the sample of *Solen* sp. by taking its meat, and then preserved it in 96% alcohol. It was kept at room temperature for the DNA analysis.

DNA extraction

Procedure of extraction stages were referred to Kalinbunga *et al.*(2004). *Solen* sp. meat is cut, weighed for 25 mg, chopped using sterilized scissor, and put into 1.5 mL tube containing PBS 1X using tweezers. Then, it is added by TI buffer, 180 µL, mixed, added by 25 µL pro-K and vortexed in 20 seconds. Then, it is incubated in waterbath shaker 56°C overnight and shaked with shaker 500. After that, the sampel vortexted, and if there is a solid particle, then, it is being centrifuged 11,000 g, on 25°C temperature in 5 min. Then, the extract is taken and is added by 200 µL B3 buffer and being vortexted within 20 seconds. After that, it is incubated in oven in 70°C in 30 min (being vortexted every 10 min). Then, it is added by 210 µL absolute ethanol that is not cold and vortexed within 10 seconds. Then, the liquid in 1.5 mL tube is moved to nucleospin column tube, being centrifuged 11,000 g at 25°C temperature within 1 min. The column is changed and added by 500 µL BW buffer and being centrifuged by 11000 g at 25°C temperature within 1 min. Then, it moved to dry silica membrane and added by 600 µL BS buffer and centrifuged by 11,000 g at 25°C temperature in 1 min. Dry silica membran re-centrifuged by 11,000 g at 25°C temperature in 1 min. Then, silica membran tube moved to 1.5 mL tube, added by 50 µL warm BE buffer (70°C) and incubated at the room temperature for 5 min, centrifused by 11,000 g at 25°C temperature in 1 min. Then, it was added by 50 µL warm BE buffer (70°C), incubated at the room temperature in 5 min and is centrifused by 11,000 g at 25°C temperature in1 min.

DNA replication

DNA replication was processedby using PCR (*Polymerase Chain Reaction*). It was conducted using the following procedures; for the composition of 10 µl on PCR reaction that consists of DNA genome sample 1 µL; Primer (10 pmol.µL^{-1}) 1 µL, ddH_2O 2.75 µL, PCR mix 5 µL and 0.25 µL of BSA 10 mg.mL^{-1}.RAPD program started in hot temperature, 92°C in 4 min with the stages of 2 seconds of denaturalization at 92°C temperature, 90 seconds of primer attachment (*annealing*) at 36°C temperature for 45 times, 120 seconds of DNA synthesis (*Primer extension*) at 72°C temperature and 10 min of DNA extension (*final extension*) at 72°C temperature. The product of DNA replication was analyzed using electrophoresis process in agarose gel of 1.5 %, and then it was visualized byUV transilluminator.

Data analysis

The data analysis, each DNA fragment was analyzed by RAPD method in OPA 1-20 primers. The fragment will be valued by polymorphic analysis if it was found on several samples. The differences on band patterns in each primer will be considered as genotype differences. The document of the electrophoresis result will use Polaroidor digital camera. The resulted ribbon interpreted using the number of 1/0. '0' represents the absence of the ribbon, and '1' represents the presence of the ribbon. The similarity coefficient calculation was conducted to test the object pairs. The pattern of similarity coefficient stated by Nei and Li (1979):

$$S_{xy} = \frac{2N_{xy}}{N_x} + N_y$$

where

S_{xy} = The genetic similarity Coefficient

N_{xy} = The number of lines produced by x and y

N_x = The number of lines produced by x

N_y = The number of lines produced by y

The obtained data was analyzed using *RAP Distance Package Software Version* 1.04 (Amstrong *et al.*, 1994). The Similarity Index (SI) analysis of individuals and samples was conducted using the formula of Nei and Li (1979). The familiarity relationship was described by using dendogram that

is based on *Unweighted Pair-Group Method of Arithmetic (UPGMA)* method.

Results and discussion

Morphological markings

The result of morphological analysis and taxonomy conducted by LIPI stated that *Solen* sp. found at east coast of Surabaya is identified

Phylum : Mollusca
Class : Bivalva
Ordo : Solenoidea
Family : Solenidae
Genus : *Solen* (Linnaeus, 1758)
Species : *Solen regularis* (Dunker, 1861)
Location : Malaka

The characteristics: 2 clamshells, thin, long with straight posterior side and anterior side that bend toward downward. *Umbo* is located, almost reaches, the down part of the anterior or ¾ of posterior side. The sculpture is crossed thin lines and lays to form straight and curving lines, widened from posterior and narrowed at anterior part, and the shell color is yellowish green. Its soft body consists of a coat with anterior widening peddle legs and thick that is united with ventral part and coat edging parts inside.

Table 1. UPGMA Simple Matching Coefficient.

Group		Similarity	Object
Pamekasan Coast	Pamurbaya		In grup
A	B	0.131	2

The tentacle aims at becoming a censor device, thin gill that is 2 pairs of long plates almost ¾ of its soft body. Short siphon and united with other parts of soft body. The length of the shell is approximately 2.20 – 64.44 mm. *Solen* sp. from South Coast of Pamekasan is identified as identical with *Solen* sp. from East Coast of Surabaya which has smaller shell size of 1.00 – 37.32 mm in length. The color of the shell is similar to one another but the lines is clearer (Fig. 1).

Fig. 1. *Solen* sp. from East Coast of Surabaya (top) and Pamekasan Coast (below).

RAPD

In the research used *Solen* sp. sample, there was 1 primer out of 20 primers (OPA-1 to OPA-20) that showed unsuccessful DNA ribbon to produce band pattern, i.e. primer 14 OPA. Based on Julisaniah *et al.* (2008), Kumar*et al.* (2011), and El-Bayomi *et al.* (2013), some of molecular markings to analyze RAPD are OPA primer, OPB, OPC, OPE, OPF, OPO, OPX. In Litaay *et al.* (2012) research on abalone clam, OPB primer and UBC can produce different

polymorphism.

The result of DNA fragment analyzed by RAPD method using OPA 1-20 primers produce a different band pattern on each primer. The amplification of primer used produces 84 lines with 1 primer that can amplify DNA. DNA amplification happens randomly that causes ribbons that are located between 150 bp to 1600 bp. The success of DNA genome amplification using RAPD method is determined by many factors, one of them is the primer of base order used in every reaction and its quantity (primer content in each reaction). RAPD method principal is based on the primer ability to attach on DNA print. The designed primer is short-single primer that can attach randomly to organism genome DNA. DNA that does not attach to primer causes the absence of DNA ribbon because there is no attachment on the primer. On OPA 14 primer, there is no ribbon appears because there is no primer that is attached to the sample of *Solen* sp. DNA. This matter causes the absence of amplification process. The differences on the ribbon pattern shown on each sample show the varieties of *Solen* sp. The higher the differences, the further the familiarity of *Solen* sp. However, the smaller the differences of the ribbon's pattern shown, the closer the familiarity among the *Solen* sp. samples.

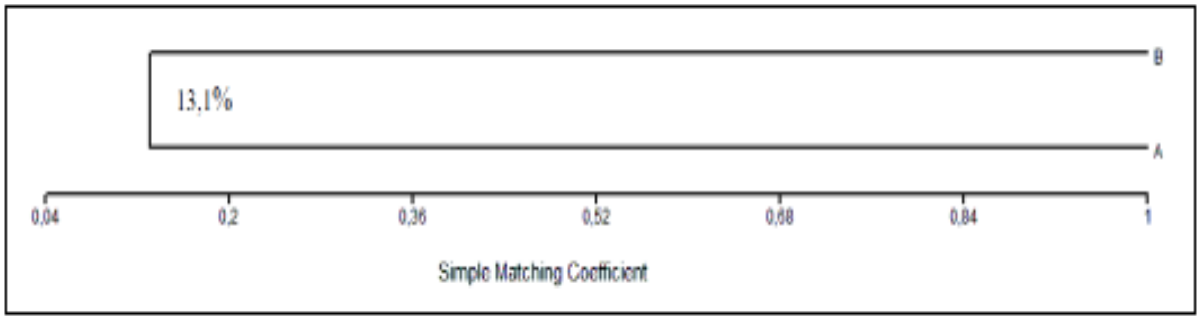

Fig. 2. The similarity of *Solen* sp. sample dendogram based on RAPD result using OPA 1-20 primer.

The result of Similarity Coefficient calculation to test *Solen* sp. sample pairs from Pamekasan Coast and East Coast of Surabaya based on Nei and Li (1979) coefficient formula is 0.131 (Table 1). The result of gel electrophoresis is 1.5% with OPA 1-20 primers as shown at Fig. 3. At the right annealing temperature during the thermal cycles, oligonucleotide primer with the sequent random order attaches on several priming site on a complementary sequence on DNA genomic template and produce products if the priming site is in the area that can be amplified. The amplification pofile of DNA depends on the sequent homogeneous nucleotide between the template of DNA and oligonucleotide primer. The variation of the nucleotide between each DNA template results the absence or presence of the band becasue of the priming site's changes.

The result of the visualization of RAPD method is classified by a cluster analysis. Cluster analysis is a technique used to classify object into a group that tends to be similar to one another and it is very different from one object to another group. Genetic diversity happens because there are changes of DNA nucleotide assembler. These changes might affect the phenotype of an organism that can be observed using our eyes or it affects individual reaction on certain environment. Generally, genetic diversity of a population can happen because of mutation, recombination, or gene migration from one place to another (Suryanto, 2003).

The individual isolated relationship or whole population can be stated using numbers and this analysis can be shown in a dendogram. By using *Unweighted Pair- Gouping Method with Aritmatic Averaging* (UPGMA) method, resulting the genetic distance dendogram (Fig. 2). Dendogram showed the

familiarity relationship between observed *Solen* sp. samples. The familiarity relationship between *Solen*sp.from east coast of Surabaya and Pamekasan coast has a value of 13.1%. This value implied that the genetic diversity is very distance or far between one individual to another. The information obtained from the dendogram showed the correlation between individual of *Solen* sp. samples from Pamekasan coast and east coast of Surabaya. The genetic distance shown at dendogram indicates that the familiarity relationship is in equal balance. The higher genetic distance of each individual, the further familiarity of each individual is. It is shown by *Solen* sp. sample from east coast of Surabaya and Pamekasan coast that have a distant familiarity.

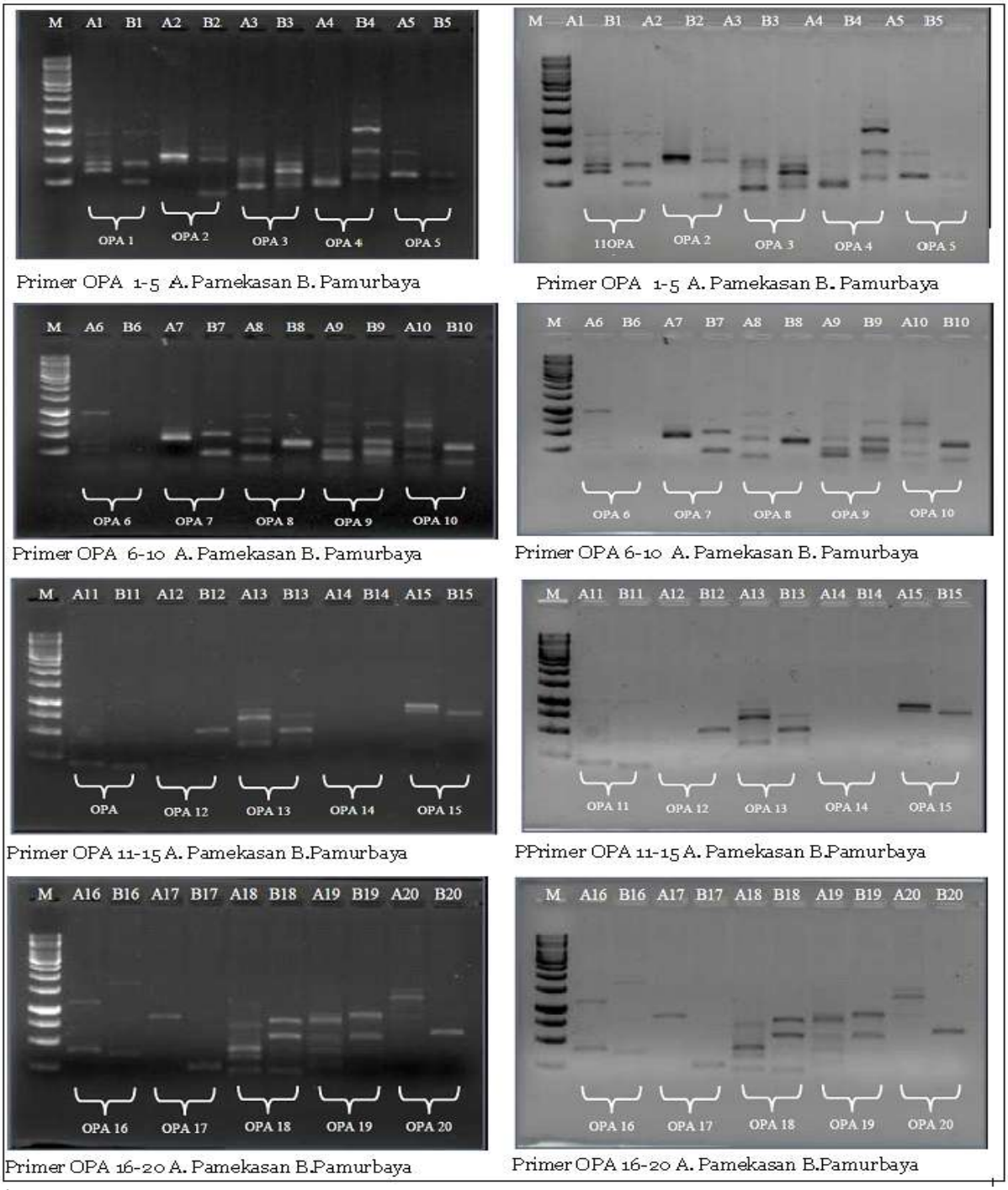

Fig. 3. Electrophoresis Results on Gel Agarose 1.5%.

The familiarity relationship of *Solen* sp. sample that is very far, i.e. 13.1%, is predicted as the cause of predecessor that has a distance familiarity relationship with other predecessor of another variety. Even though coming from the same species, i.e. *Solen regularis*, the sample has different variety. This can be observed from the differences of *Solen*'s size. The size of *Solen* sp. observed in Surabaya during the research is 52.99 – 64.44 mm in length, whereas *Solen sp*. that is from Pamekasan coast is 26.13 – 37.12 mm in length. The size differences are predicted as the cause of environment and capturing intensity. The result of Litaay *et al.* (2012) stated that there is a crossed reproduction between species that results a varied offspring, even it can result identical ones when 2 individuals have similar genes have crossed reproduction. The diversity on abalone clams is caused by the crossed heterozygote on abalone's predecessors.

The result of the primary research on *Solen* sp. gives important information about *Solen* sp. at sea waters in Indonesia. The result states that *Solen* sp. and its different species are found at Madura Coast in Pamekasan district (Nurjanah *et al.*, 2008), East coast of Surabaya (Trisyani and Irawan, 2008; Trisyani and Hadimarta, 2013), and Kejawanan Coast, Cirebon (Subiyanto *et al.*, 2013). The research in molecular biology related to genetic diversity using RAPD method gives an advantage in sustaining and preserving the genetic diversity needed for research and human needs. The understanding of genetic diversity and its relative relationships is needed in genetic conservation activities or its sustainability. For *ex-situ* conservation, this information is needed to determine the number of the population or species collected, so that we can sustain the basic genetic diversity. Moreover, for *in-situ* conservation, this information is needed to determine the number of determined location with its width and the number of the species inside.

Conclusion

Conclusion of this research is the genetic diversity that was shown by the similarity indexes by RAPD method using OPA 1-20 primer on *Solen* sp. from Pamekasan Coast, Madura and East Coast of Surabaya 13.1%. Results of LIPI identification showed that species found at South Coast, Madura and East Coast of Surabaya was *Solen regularis*, but it is different on its variety. Further research is needed to be conducted by taking the sample of *Solen* sp. at Indonesia sea waters to identify its relationship and its genetic diversity in Indonesia and *Solen* sp. in coasts around the world.

Acknowledgement

The author would like to thank sincerely to the Directorate of Higher Education Board for 2015 Competive Research Fund.

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