

14

by Hamid Toha

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TIME SUBMITTED	22-APR-2019 09:46AM (UTC+0700)	CHARACTER COUNT	15612
SUBMISSION ID	1116592223		

Genetic relationship of *Sardinella lemuru* from Lombok Strait with fish rich in omega-3 fatty acid

22

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9

Received November 30, 2012; Accepted December 10, 2012; Published December 19, 2012

Abstract:

Lombok Strait has abundance of Sardine, *Sardinella lemuru*, which contains such high amount of omega-3 fatty acid (omega-3). However, the genetic relationship of *S. lemuru* with other commercial fish rich in omega-3 has not been widely studied yet. Studies on genetic proximity of *S. lemuru* with the other marine fish using 12S rRNA gene is very important in order to obtain genetic information of the Sardine. develop an appropriate strategy for future conservation of the fish in Lombok Strait. The aim of this study was to find out the genetic relationship of *Sardinella lemuru* living in Lombok Strait with the economically valuable fish and its correlation with omega-3 production. *Sardinella lemuru* were collected from Lombok Strait, the phylogenetic tree was done based on 12S rRNA gene through a neighbor-joining method to identify the relationship of Sardines and fish rich in omega-3 fatty acid. The phylogenetic tree showed that *Sardinella lemuru* is similar to *Sardinella at* and has a close similarity with *Sardinella maderensis*. However, the relationship did not correspond to omega-3 production. Based on the results of the study, it is suggested that the production of omega-3 is not specifically based on the proximity of the species, but it is more associated with conserved domain of $\Delta 6$ -desaturase. Nevertheless, detailed mechanisms still need to be elucidated.

Keywords: $\Delta 6$ desaturase, conserved domains, *Sardinella lemuru*, omega-3, Lombok Strait

Background:

Omega-3 functions in preventing coronary heart disease, diabetes, cancer, and also plays an important role in the nervous system, brain and eyes [1-3]. The main and the best source of omega-3 food is marine fish [4], i.e. *Sardinella lemuru* that is abundant in Indonesia. Lombok Strait has a variety of *Sardinella lemuru* rich in omega-3 fatty acid. It is indicated that Lombok Strait is very fertile and is appropriate for *S. lemuru* to live and breed. However, the genetic kinship of *S. lemuru* with other marine fish rich in omega-3 has not been widely studied yet. Therefore, we analyzed the genetic relationship of *Sardinella lemuru* from the strait with the economically valuable fish using 12S rRNA gene. The research provided basic information on genetic proximity of *S. lemuru* with other marine fish, and the

information is considered very important for future conservation of main sources of omega-3 in Lombok Strait.

Production levels of omega-3 in various marine fish species are different. Some research reports explained that omega-3 content on fish was affected by the food consumed by the fish [2, 3], the genetic factors, and the environmental characteristics [5]. The other studies reported that the concentration of omega-3 in fish is determined by the activity of groups of desaturase and elongase enzymes [6-12]. A desaturase is a group of enzyme which removes two hydrogen atoms from an organic compound, creating carbon double bonds [13]. The desaturase enzyme is known to be involved in synthesizing of omega-3 [14], the $\Delta 5$ and $\Delta 6$ -desaturases (D5D and D6D) are encoded by

fatty acid desaturase 1 (FADS1) and 2 (FADS2) genes, respectively; and they are key enzymes in the metabolism of omega-3 fatty acids.

21

The $\Delta 6$ -desaturase (D6D) has an important role in the synthesis of poly unsaturated fatty acids (PUFAs), including omega-3, in microorganisms and also in higher animals [15]. A previous study reported that a factor which determines the activity of enzyme to synthesize omega-3 is in the active site [16]. The active site usually lies within a domain that shows a high degree of similarity among protein families or groups [17]. Hence, conserve domain containing active site of $\Delta 6$ -desaturase might have an important role in biosynthesis of omega-3. Furthermore, we examine the correlation between either genetic proximity or $\Delta 6$ -desaturase conserved domain with omega-3 productions on economically valuable fish.

Methodology:

Sardinella lemuru was collected from Lombok Strait. DNA isolation and other analyses were conducted at Molecular Biology, and Bioinformatics Laboratories, Faculty of Sciences, Brawijaya University, from January to September 2012.

DNA Isolation and Sequencing

DNA was isolated using DNeasy Blood & Tissue Kit (Qiagene). The DNA was used as a template to amplify 12S rRNA gene by using a pair of primers, the 5'GCTTGGTCTGACTTTAGTA as a forward primer and 5'CTTACCATGTTACGACTTGC as a reverse primer. PCR composition consisted of sterile water 2 μ l, 5 μ l PCR mix, 1 μ l forward primer, 1 μ l reverse primer, and 1 μ l DNA samples. The PCR condition was denaturation at 94 $^{\circ}$ C for 1 minute, then 35 cycles of denaturation at 94 $^{\circ}$ C for 1 minute,

annealing at 54 $^{\circ}$ C for 30 seconds, and extension at 72 $^{\circ}$ C for 1 minute. The last extension was performed at 72 $^{\circ}$ C for 10 minutes, and followed by sequencing (First Base, Singapore).

Phylogenetics Construction

The phylogenetic trees were constructed based on 12S rDNA and sequences of $\Delta 6$ -desaturase. The nucleotide sequence of 12S rRNA of *S. lemuru* was obtained by sequencing and other sequences were obtained from GeneBank (www.ncbi.nlm.nih.gov). The whole amino acid sequences of $\Delta 6$ -desaturase were retrieved from GeneBank (www.ncbi.nlm.nih.gov). The alignments were done separately for 12S rRNA gene and sequences of $\Delta 6$ -desaturase. The alignment processes were performed through Clustal-W using MEGA5.05 software, and then followed by construction of phylogenetic trees through a neighbor-joining method.

Conserved Domain Analysis

The conserved domains of $\Delta 6$ -desaturase from 11 kinds of fish were identified by using Conserved Domain Databases (CDD) search. CDD is a software to annotate Conserved Domains within a protein sequence based on a sequence homology with conserved domain database in GeneBank (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [18].

Construction of 3D Model of $\Delta 6$ -desaturase

The tertiary structure of $\Delta 6$ -desaturase protein from the 11 fish were not available yet in the protein databases, then the 3D structures were predicted by threading modeling provided by I-TASSER [19]. The 3D models were aligned to determine structure differences among the proteins. The 3D alignment were done by a super impose method using PyMol software.

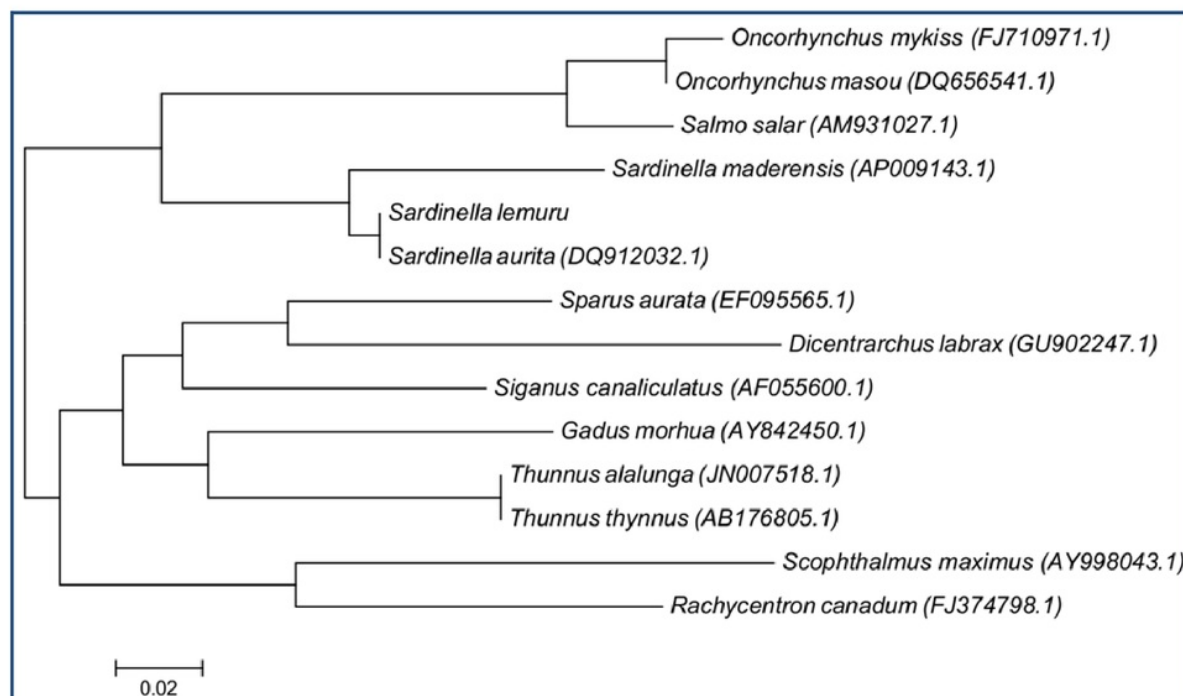


Figure 1: Phylogenetic tree of marine fish by using 12S rRNA gene. The *S. lemuru* from Lombok Strait has some similarities with *S. aurita* and is closely related to *S. maderensis*.

<i>S. aurita</i>	GGAAAGCTTGAACCCCTGACACGAAAGTAGCTTTACCTTCTCCTGAACCCACGATAGCTGGGGCACAACCT	140
<i>S. lemuru</i>	GGAAAGCTTGAACCCCTGATACGAAAGTAGCTTTATCTTCTCCTGAACCCACGATAGCTGGGGCACAACCT	
<i>S. aurita</i>	TAGGAAGAAATGGGCTACATTATCTGAGACAGATTATTCACGGAGGGTCACCTGAAACGGCGACCCGAAG	490
<i>S. lemuru</i>	TAGGAAGAAATGGGCTACATTATCTGAAACAGATTATTCACGGAGGGTCACCTGAAACGGCGACCCGAAG	

Figure 2: Homology 12S rRNA gene between *S. aurita* and *S. lemuru*. The polymorphism occurs only in 3 bases along 575 bases of the gene. Sequence numbering according to *S. aurita* 12S rRNA gene (DQ912032.1)

Results:

We have utilized 12S rDNA sequences to analyze the phylogenetic relationships among the marine fish that were considered economically important or contained high amount of omega-3. The phylogenetic tree showed that *Sardinella lemuru* is similar to *Sardinella aurita* and has a close similarity with *Sardinella maderensis*. The *Sardinella* is more closely related to Salmon and Oncorhynchus than to other species (Figure 1). The data indicated that *S. lemuru* shares genetic similarities with *S. aurita*, and the two probably belong to the same species. Moreover, all of the species were clustered into three groups that did not correspond to omega-3 content. *Thunnus* has an ability to produce high amount of omega-3 and so does *Sardinella*. However, *Thunnus* were clustered with *Siganus*, which has low content of omega-3. The productions level of omega-3 among the 12 economically important marine fish were different, ranged from 1.9% to 32.9%. The difference in omega-3 contents are caused by multifactor i.e. food, species, genetic, climate, enzyme activity, geography and environmental characteristics [1, 2, 20]. Because the concentration of omega-3 is determined by different kind of factors, we just took the minimum levels of omega-3 for each species to eliminate bias from those factors in order to analyze the relationship between genetic kinship with omega-3 concentration levels (Table 1). The minimum level of omega-3 production for *Tunnus* and *Sardinella* were over than 20% of their tissue. The data indicated that among the species, *Tunnus* and *Sardinella* contained the highest amount of omega-3 that warrant them as a main source of omega-3 from marine environment.

Discussion:

The similarity in 12S rRNA gene between *S. lemuru* with *S. aurita* indicated that the two kinds of fish might still belong to the same species; since the polymorphism only occurs in 3 bases along 575 of the gene (Figure 2). Both of the fish share similar genetic factors, characteristics, behaviors and susceptibility to some certain environment factors. The genetic similarity of the two kinds of fish would allow for cross-breeding, that will improve the genetic diversity of *Sardinella*. This information is very beneficial in order to rectify the genetic quality of *Sardinella* and especially to promote a strategy to conserve *S. lemuru* in Lombok Strait. Even though the morphology variety of *S. lemuru* in Bali Strait is diverse, it does not reflect such a high genetic variation. Furthermore, the data on genetic diversity of *S. lemuru* in Lombok Strait is needed to be elucidated to portrait its genetic variation.

The relationship based on 12S rRNA gene among the fish did not correspond with omega-3 productions. Therefore, we analyzed the relationship between omega-3 production with the conserved domain of $\Delta 6$ -desaturase among the fish. The results showed that the similarities in the conserved domains were

clustered into 6 groups associated with omega-3 content (Figure 3). According to the phylogenetic tree of $\Delta 6$ -desaturase, *Thunnus thynnus* and *Salmo salar* has a closed similarity, which corresponded to their omega-3 production level. Hence, the genetic distance between *Thunnus thynnus* and *Salmo salar* was far, based on 12S rRNA gene (species relationship). Based on the results, we suggest that the production of omega-3 is not specified based on the proximity of the species, but is associated with conserved domain of $\Delta 6$ -desaturase. The phenomenon indicated that activity of $\Delta 6$ -desaturase is very influential on fish ability to produce omega-3.

Generally, the ability of marine fish to synthesize long-chain HUFAs, including omega-3 from C18 precursors, is very low. It is caused by the occurrence of various splicing variants of $\Delta 6$ -desaturase mRNA [21]. The variants caused different activity of $\Delta 6$ -desaturase to bind with the substrate. The alignment of the conserved domain of $\Delta 6$ -desaturase from 11 kinds of marine fish species has revealed three conserved sites located at position 180-185; 217-221 and 362-367 – these results are similar with the results from the previous report [22]. We also have analyzed the 3D structure of $\Delta 6$ -desaturase, residues 40-450, which showed that the active site (red color) is located in residues 362-367, that is same in all of the species, although they have different amino acids composition (Figure 3). The 3D model demonstrated that all fish have similar backbones in terms of the enzyme structure, but they are different in their surface. These data suggested that the residues formed conserved structure to maintain their ability to bind with substrates. It corresponds with the previous study showing that binding sites of protein are conserved to maintain competence for binding with ligands/substrates [23].

Therefore, we have classified the fish into 6 groups according to 3D model and motif of active site of $\Delta 6$ -desaturase, namely: group 1 consists of *O. masou*, *O. mykiss* and *S. salar* (active site, HERHQ); group 2 consists of *G. morhua* (HEKQQ); group 3 consists of *T. thynnus* and *T. maccoyii* (HEKHQ); group 4 consists *S. canalicuatus* (YENHN); group 5 consists of *Rachycentron canadum* (HEKHR) and *S. maximus*(HEKHK); and group 6 consists of *D. labrax* and *S. aurata* (HEKHH). Groups 1, 2 and 3 contain higher amount of omega-3 (over than 10%) compared to the other three groups. The variation of the active site and protein surface might be involved in determining the ability of the enzyme to bind and catalyze a substrate. The active site motif indicated to have a relationship with the production of omega-3. The last amino acid of the active site motif might be a crucial factor to support catalytic activity of the enzyme. Sifting from Q to other amino acids might decrease the activity of the enzyme as indicated by the decreasing omega-3 production levels. However, detailed mechanisms are still needed to be elucidated.

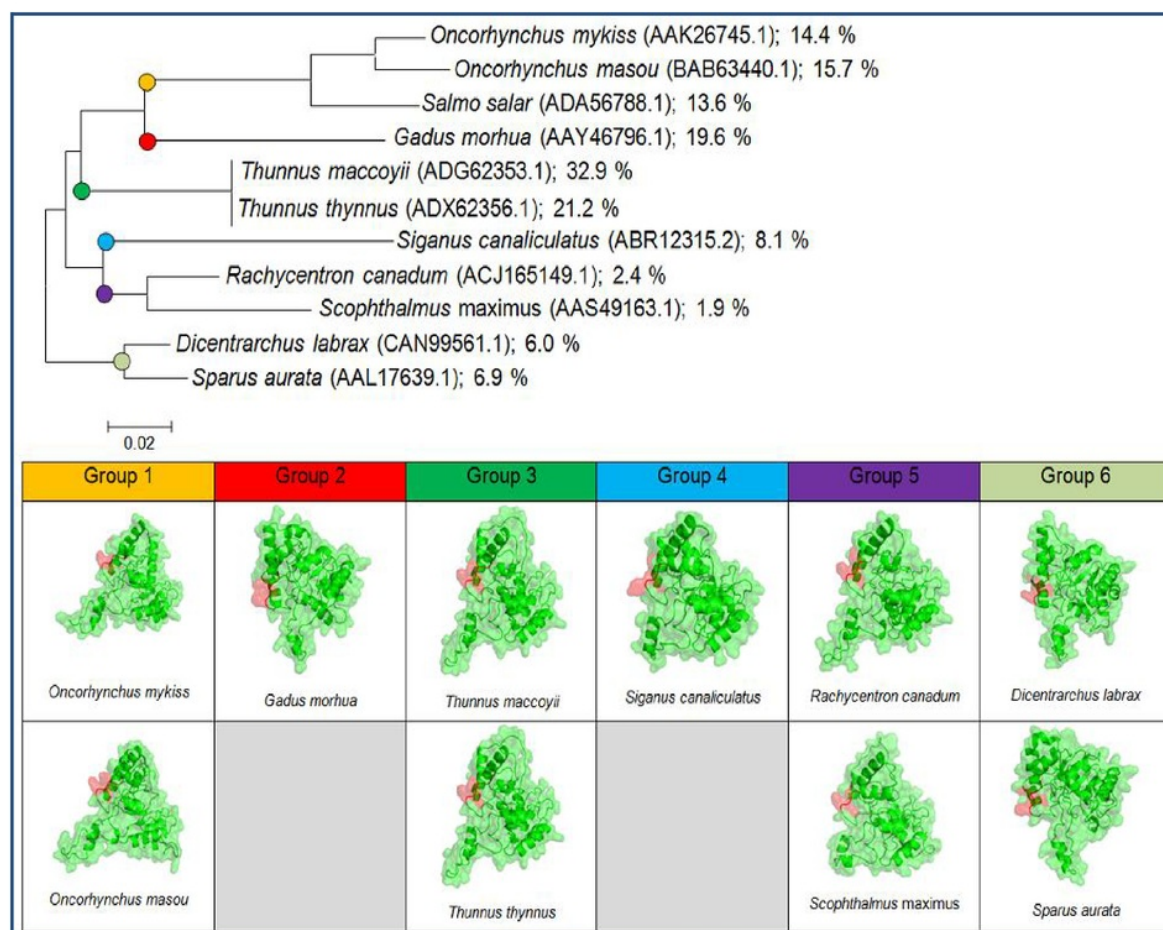


Figure 3: Phylogenetic tree of marine fish by using $\Delta 6$ -desaturase protein. The fish were clustered into 6 groups associated with omega-3 content. Groups 1-3 contain higher amount of omega-3 (over than 10%) compared to the other three groups. Active sites were indicated by red color.

Conclusion:

Sardinella lemuru from Lombok Strait possesses some similarities with *S. aurata*. The relationship based on 12S rRNA gene among the fish did not correspond to the omega-3 productions. The similarity found in the coserved domains indicated a relationship with omega-3 content. However, further study is needed to elucidate the mechanism of $\Delta 6$ -desaturase activity and other parameters that correlated with omega-3 production of marine fish.

5

Acknowledgement:

The authors would like to thank the Directorate General of Higher Education, Ministry of Education and Culture of Indonesia for providing the funding to carry out this study. Thanks to Brian Rahardi as the Head of Bioinformatics Laboratory, Biology Department, Brawijaya University, for providing the facilities to conduct this study.

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Edited by P Kanguane

Citation: Mahrus *et al.* Bioinformation 8(25): 1271-1276 (2012)

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Supplementary material:

Table 1: Concentrations of omega-3 in the marine fish

Number	Name of species	Omega-3 (%)	References
1	<i>Thunnus maccoyii</i>	32.9	24
2	<i>Sardinella lemuru</i>	23.0	*
3	<i>Thunnus thynnus</i>	21.2	25
4	<i>Gadus morhua</i>	19.6	20
5	<i>Oncorhynchus masou</i>	15.7	26
6	<i>Oncorhynchus mykiss</i>	14.4	10
7	<i>Salmo salar</i>	13.6	27
8	<i>Dicentrarchus labrax</i>	8.1	28
9	<i>Siganus canaliculatus</i>	7.3	29
10	<i>Sparus aurata</i>	6.9	30
11	<i>Rachycentron canadum</i>	2.4	31
12	<i>Scophthalmus maximus</i>	1.9	32

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