

Genetic diversity of *Ulva lactuca* from the intertidal zone in Ulee Lheue Beach Aceh, Indonesia



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Abstract. Ulee Lheue Beach in Banda Aceh was developed for tourism. The macroalgae and seaweed species found in this area have been widespread but never exploited. Among these species is the green seaweed, *Ulva lactuca*, commonly known as sea lettuce. The physiological variations and genetic features of the seaweed can be attributed to the variations in the coastal environment. This study aims to describe the molecular identity of the genetic diversity of *U. lactuca* from the intertidal zone at Ulee Lheue Beach. The development of molecular biotechnology has enabled identification the gene expression through genomic DNA to PCR amplification. Genetic distance was determined using UPGMA. The results from 600-bp fragments were analyze the genetic diversity. A total of 15 (31%) expression identified as polymorphic (>0.500). Heterozygosity (H_e) and allelic differential (N_a) diversity were found of 1.500–3.000. The highest PIC was observed in the *rbcL1*, with a correlation between subpopulations of 0.459. PCR amplification using the degenerate primer *rbcL1* produced fragments ranging from 300 to 460 bp, whereas the expression of UL2 was detected at 448 and 500 bp using the *rbcL2* primer. The phylogenetic identify are shown two (2) clusters. The populations of UL1, UL3, and UL4 were found a close relationship. Furthermore, the UL1 and UL2 populations were further divided into distinct clusters but related to the main branch of UL3. Based on this research, the concern of *U. lactuca* species for industrial and biotechnology destinations, we can describe a suitable method for obtaining he genetic distances between species.

Keywords: Genes, *Ulva lactuca*, Ulee Lheue, Beach, DNA.

INTRODUCTION

The genetic diversity of the marine plant biological resources is explained by the great wealth of marine waters in Indonesia. Genetic diversity was a necessary solution for marine plants to survive for future generations. The higher genetic populations were established for Deep-sea plants like algae to survive. The genes responded distinct to environmental conditions. The characteristics of genetic algae populations were indicated by the spatial heterogeneity and genetic distances [1]. Algae have been found in the coastal waters of Pulo Breuh Aceh, there were 18 species of the Chlorophyta, Rhodophyta, and Phaeophyta divisions. In addition, 18 species of algae were found in Batuputih Nature Park including Rhodophyta, Phaeophyta, and Chlorophyta [2-4]. Recently, the utilization of genetic diversity in the plants of the Chlorophyta division at Ulee Lheue Beach has not been found. The macroalgae

species of the Chlorophyta division were widespread in Ulee Lheue Beach, but they have never been exploited.

U. lactuca from the macroalgae grew through the coast and the depth of the lowest intertidal line was up to 40 meters [5]. *U. lactuca* known as sea lettuce contains a lot of chlorophyll to reproduce quickly. The part of the algae known as the thallus, namely the roots, stems, or leaves was difficult to distinguish. The thallus was shaped like a tube, flat, and round like a bag and hair. Wherein, that is composed of unicellular or multicellular. Macroalgae were mostly attached to rocks, wood, muddy sand, mollusca, and epiphytes on other algae. Chlorophyceae class algae had a green color a higher plants and contained dominant chlorophyll a and b pigments [6]. The presence of algae can affect the diversity of plants and animals in the waters. More diverse species of algae, higher diversity from biota in the sea. The tropics plant of the *U. lactuca* was air shallow or in the upper intertidal



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Table 1. The primer used for polymerase chain reaction (PCR) analysis

No	Primer	Forward (5'-3')	Reverse (3'-5')	Amplification (bp)	Tm (°C)
1	rbcl	ATG TCA CCA CAA ACA GAG ACT AAA GC	GTA AAA TCA AGT CCA CCR CG	600	55
2	rbcl	ATG TCA CCA CAA ACA GAG ACT AAA GC	TGC CAT GTA CCT GCA GTA GC	600	55

zone to a depth of 10 meters the conventional techniques from morphological characters, but the identified species were not used maximally.

U. lactuca provides a habitat for marine biota including mollusks, fish, and small algae as well as echinoderms. That has been reported a fairly high protein content, namely 14.9% and 50.6% powdered sugar [7]. In addition, this algae contains vitamin B1 and vitamin C. The potential for the development of *U. lactuca* into food products such as nori was needed. *U. lactuca* was beneficial in producing the melatonin and phytochemical compounds (part of alkaloid compounds) that function to inhibit cancer activity. Specific cytotoxic properties swelling (proliferation) of cancer cells, and carcinogenic epigenetics were the most important to study. Recent developments in molecular biotechnology showed the expression of coding genes through the amplification of genomic DNA using the polymerase chain reaction (PCR) method [8]. The molecular analysis of algae was carried out from other Researcher (Wal et.al., 2013) using the rbcl gene. DNA extraction often yields different results depending on the type of algae. The limiting factors was obtain the quality of genomic DNA were the character of the cell wall, the complex components of polysaccharides, polyphenol content, and the secondary metabolites produced from each type of algae. The cell wall of the algae genome was composed of cellulose and lignin, wherein the shape consisted of sheets, threads and colonies. The systematics of DNA barcoding of the genome will influence the approach to algae phylogenetics with the PCR amplification markers. This technique can be used to analyze polymorphism in plants that live on land and sea. Advances in research on *U. lactuca* marine plants will have a major impact on the exploitation of marine biological resources in the future [9]. The genetic study supported advances in molecular techniques to detect the gene expression from plant DNA to solve problems in molecular biology. This research aims to molecularly identify the genetic diversity of *U. lactuca* from the intertidal zone of Ulee Lheue Beach. The genetic diversity study of *U. lactuca* provided the information for the application and development of seaweed.

METHODOLOGY

Data collection

Identification of algae species was carried out using the conventional method based on the morphological characteristics, but it was not optimally. DNA-based on the molecular identification was able to provide more

accurate information and provide the solution to the diversity of *U. lactuca* species. This research aims to determine the genetic diversity of *U. lactuca* found along the coast of Ulee Lheue, Beach, Aceh, Indonesia. The data was collected from 4 location points, namely UL1, UL2, UL3, and UL4 to the DNA extraction. A total of twelve (12) collected the data to sample identification (plant material) for the DNA extraction [10]. Furthermore, Polymerase Chain Reaction method used to the data analysis. Sampling was carried out based on an exploratory survey. Direct collection of sample along the coast of Ulee Lheue Beach (5°36'N 95°18'E) was carried out using the purposive sampling.

DNA analyzed

The DNA genome of *U. lactuca* was extracted using the cetyl trimethyl ammonium bromide (CTAB) method [11]. DNA was included in the base pairs Adenine (A), Guanine (G), Thymine (T) and Citocina (C). The quantity of DNA was identified from the nanophotometer method, and the quality of the DNA was calculated from the agarose gel (1%). Then, the analysis of the DNA band data used the UV-TEX method [12].

Furthermore, the PCR reaction used the algae-specific rbcl primers. The amplification is described in Table 1. A denaturation process was followed (95°C 3 minutes 35 cycles), annealing (50°C 40 seconds) and elongation (72°C 2 minutes). PCR results of DNA amplification visualized from the Gel Doc electrophoresis on UV transilluminator [12]. The genetic diversity was analyzed using the Trychotomy method to data clustering phylogenetic of unweighted pair group method of arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Frequency statistics analyzed of *U. lactuca*

We analyzed the *U. lactuca* sampling from 4 (four) location points collected in Ulee Lheue Beach, Aceh, Indonesia, namely UL1, UL2, UL3 and UL4. Several gene exchanges occurred between the species originating from the coast of Ulee Lheue Beach, Aceh. Thus, the comparison essentially was independent species, and we were found to have undergone certain genetic differentiation. The percentage between populations was found 31% and within individuals 69%. The estimates of variance and sources of variance showed the level of difference between populations values of 0.282 and 2.944 respectively, in the other values showed 0.625 and 10 within individuals (Table 2).

Table 2. Percentages of molecular variance from *Ulva lactuca* using the AMOVA method

Source	Df	MS	SS	%	Est. Var.
Among Populations	3	2.944	8.833	31	0.282
Among Individu	8	1.250	10	69	0.625
Within Individu	12	0	0	0	0
Total	23		18.33	100	0.907

Note: Matrix for F-Statistics Analysis, df = Degree of Freedom; MS= Mean squares; SS= Source of variation; Est.Var= Estimation of variance.

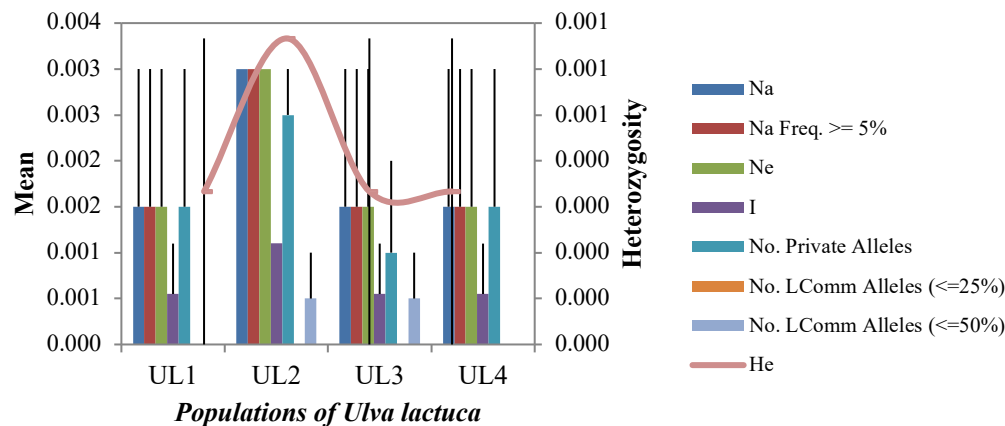


Figure 1. The allelic patterns across populations from *Ulva lactuca*

The UL1, UL3 and UL4 species of *U. lactuca* were tightly related with a heterozygosity value of 0.400, but the UL2 showed a higher He value of 0.800 (Figure 1). This suggests the presence of certain relationships within the UL1, UL3 and UL4 populations, then it indicates a historical expansion of the species. Due to differences in the rbcL1 and rbcL2 gene sequence, we used a 600-bp co-fragment to statistically analyze among species. A total of 3.000 (UL2) variable numbers of different alleles were identified, including 1.500 values of UL1, UL3 and UL4. There was no variation in Na values between the populations. This study revealed a high degree of intra-specific genetic diversity, and AMOVA revealed a higher degree of within-species or population variance. The species *Ulva* from Zhao *et al.*, (2015) research was investigated the genetic variation from *U. prolifera* species because of the green wave in China from the inter-simple sequence repeats (ISSR) method. The lowest level of genetic diversity in the *Ulva* sample He = 0.1663 was found for Shannon index I = 0.2608 in a sample of *U. prolifera* populations [13].

We calculated and analyzed the polymorphisms in the species of *U. lactuca* (Table 3). Results showed Fis and Fit values for the same value (1) from both loci. The difference in Fst values in the rbcL1 locus was identified as 0.459, and the rbcL2 locus showed 0.652. The difference in the migrant value (Nm) at the locus describes a different value. The rbcL1 to Nm locus was found a value of 0.295, and rbcL2 to the lowest value of 0.133. The highest PIC value was obtained for the rbcL1

locus (0.578), whereas it showed a 0.456 value in the rbcL2 locus.

AMOVA method resulted in significant genetic diversity for the quality of gene characteristics among *U. lactuca*. Liu *et al.* (2020) analyzed the genetic variation within and among *U. australis* based on the ISSR marker [14]. They reported to Nei gene diversity range of He from 0.0729 to 0.1496, and a Shannon index (I) to 0.1072. In addition, AMOVA showed the largest variance within populations at 68.57%, but the variance was the lowest value between populations at 22.63% and between regions at 8.79. Furthermore, Zhang *et al.* (2018), used EST-SSR markers (microsatellite marker-based sequence tags), to verify the genetic diversity and population relationships in *U. prolifera* [15]. There were genetically different samples in the UPGMA Dendrogram. Steinhagen *et al.* (2019), reported 68.57% of the total variants in the United States to the *U. australis* population [16]. The population's genetic diversity was thought to be due to the predominant sexual reproduction in this species. The significant relationship between the genetic distance and geography was a variation from the sampling [17].

DNA sequencing analyzed of *ulva* seaweed species has been reported to be feasible and delivered data that partially matched the rbcL barcode [18]. Likewise, Fort *et al.* (2021) reported the complete cytoplasmic used the genome (mitochondria and chloroplasts) to compare species and estimate intra and interspecific genetic diversity [19]. The molecular markers of this type

Table 3. F-statistics and polymorphism to population of *U. lactuca*

Locus	Fis	Fit	Fst	Nm	PIC
rbcL1	1	1	0.459*	0.295	0.578
rbcL2	1	1	0.652*	0.133	0.456
Mean	1	1	0.555	0.214	0.517
SE	0	0	0.097	0.081	

Frequency locus and correlations among individuals (Fis); Frequency population from both factors (Fit); Frequency locus also correlations about subpopulation (Fst); A total migrants (Nm); PIC (Polymorphism Information Content).

provide information about spatial patterns to genetic diversity and biogeography, as exemplified by *U. australis* on a world scale [20-21]. In other places in coastal France, these markers could be assisted to identify the species of *U. lactuca* and other seaweeds [22-24]. Disparate that introduced specimen of *U. laetevirens* from Australia [25]. Recently research into *U. lactuca* released new hypotheses for identity because of species was mostly described from the Aceh marine, Indonesia.

This study confirmed the existence of 12 *U. lactuca* species identified using genetic diversity statistics. This result finding similar to Tran et al. (2022) research has been reported some molecular support for the taxonomy review [26]. Furthermore, the species *U. australis* was introduced from Northeastern Asia, wherein previous results were in Brittany [27]. Identification of *U. armoricana* was challenged by sequencing the rbcL marker for *U. capillata* specimens [28]. Additional sampling during the autumn season (summer and winter season) would advance the study of the specific composition in intertidal greenery along the French coast, and the role of individual *Ulva* species in the phenomenon [29]. In distinction to the coast of Aceh, Indonesia the most common *U. lactuca* species were found during the rainy season. New investigations based on molecular analysis might conclude this problem.

The expression of PCR product

Meanwhile, the PCR amplification with the degenerate primer rbcL1 to DNA isolation from *U. lactuca* produced 300 – 460 bp fragments, and 340 – 500 bp rbcL2 primers (Figure 2). However, the data was found shortly after the 600 bp target. Nonetheless, several PCR products were effectively expressed in UL2 species with a value of 500 bp (in the rbcL2 gene analyzed based on the UV-text method). The species identified as UL4 did not show gene expression in the rbcL1 primer, but the UL1, UL2 and UL3 species showed strong fluorescence and obvious expression.

Similarly, the UL1 and UL3 species used to compare the presence of *U. lactuca* did not show a clear fluorescence and expression in the rbcL2 gene after the PCR product was analyzed. The expression of UL2 was detected at

448 - 500 bp in rbcL2 primer. The lowest expression in UL4 was found at 340 bp (rbcL2) in the PCR product identified (Figure 3). Recently, another case was reported regarding 180 sequences of *U. lactuca* a total of 32 PCR products of *U. fenestrata* spaced from substituted species with alignments of 500 and 774 bp [30].

The genetic distance from *U. lactuca* populations

As shown in Figure 4, two (2) clusters were detected. The alga species *U. lactuca* analyzed from the intertidal zone of Ulee Lheue Aceh coastal were closely related, but the populations of UL1, UL3, and UL4 showed a close relationship (Figure 4). In the first cluster, each represents a species from UL3, UL2 and UL1 have been found a certain relationship to the *U. lactuca* population. Wherein, it represents a historical expansion of the species.

The phylogenetic tree revealed that species cluster mutually into two main branches in the intertidal zone Ulee Lheue Beach Aceh, and we hypothesized that it a collectively classified as a geographic population in the Indian Ocean bordering the Malacca Strait. The result was confirmed by the genetic distance information. The UL1 and UL2 populations were further divided into distinct but related to the main branch of UL3.

Another study was conducted to identify algae species *Ulva* from Hormozgan Province, Sistan and Baluchesta Province, Bandar Lengeh port, Iran as well as Persian Gulf waters [31]. The Eastern *Ulva* species identified to species were also confirmed from rbcL and ITS gene sequencing [32]. Both in terms of molecular, morphological and anatomical features that were currently used by characteristics. Some differences from the results obtained for *U. lactuca* now appear distinct to *U. linza*, *U. prolifera*, *U. lactuca*, and *U. ohnoi* [33]. This assumption was supported by an NJ phylogenetic tree of ISSR-SCoT markers differentiated based on the molecular data. Several studies showed that a dual approach using molecular data is combined with morphological and anatomical features to determine the taxonomic complexity of the *Ulva* genus [34]. Favot et al. (2023) based on other ITS markers identified six species of *Ulva*, from which *U. flexuosa* was a new record for Southern Portugal [35]. Previously, only *U. lactuca* was reported as a dichromatic species for this area [36]. In Japan, using ITS2 gene sequencing,

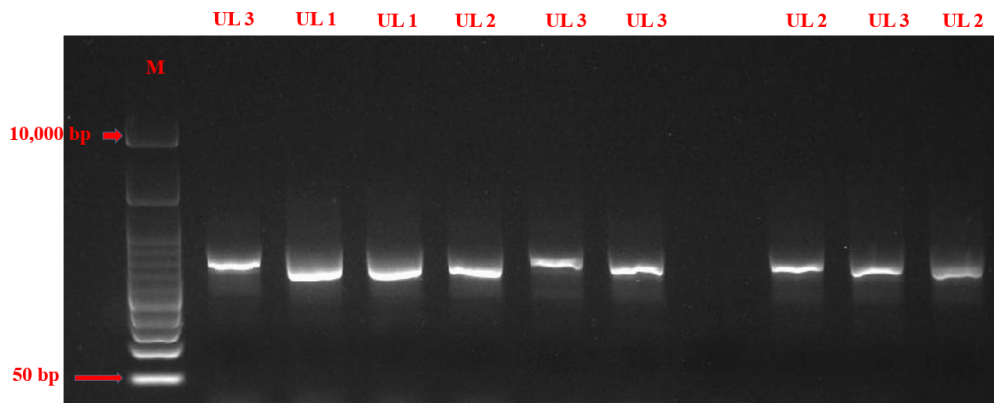


Figure 2. The electrophoresis gel result for the product of PCR from the primer rbcL1

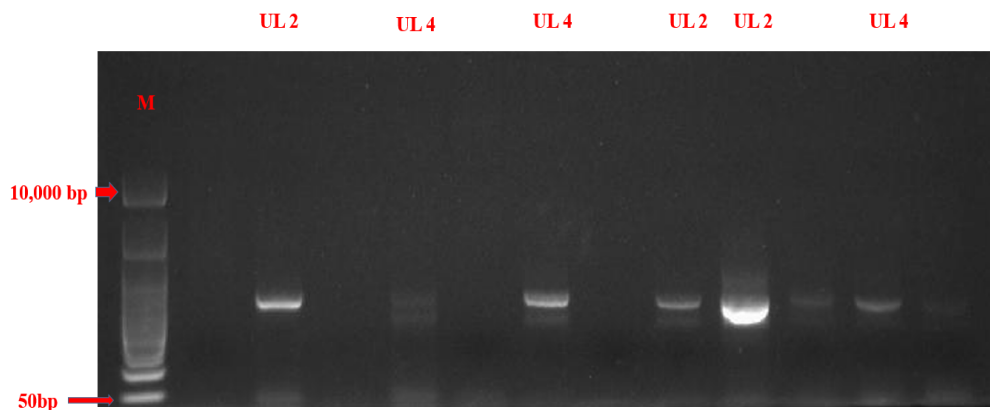


Figure 3. The electrophoresis gel result for the product of PCR from the primer rbcL2

Masakiyo and Shimada (2014) identified 164 *Ulva* gene specimens with 29 distinct fragments composed of several species known also as the novel gene sequences [37]. Melton and Lopez-Bautista (2021) studied reports of *U. flexuosa*, to collect the species from two sampling locations (Uf1 and Uf2) that differed in the main two cluster branches. Then, it was observed molecular differences to associated with the presence of two subspecies in Persian Gulf waters [38].

The nucleotide sequence was rbcL as ISSR markers to suitable for study to the genetic diversity. In this research, we obtained most similar the genetic diversity based on molecular markers. Moreover, the level of genetic diversity in the *U. prolifera* population was lowest based on the microsatellite markers (SSR) [39]. This occurs because the asexual reproduction of *U. prolifera* has been reported a low diversity of *U. prolifera* [40]. The genetic differentiation measured from the quantitative traits was compared with the genetic differentiation measured using the neutral traits of frequency (Fst) analyzed of *Ulva* species. Fst was taken as an index of local morphological adaptation through natural selection, but it was influenced by the environment [41]. Genetic selection is when one extreme phenotype is favored over another extreme phenotype among populations [42]. Kang et al. (2019) used the ITS

phylogeny which has been reported to provide a high resolution for several clusters of species from *U. ohnoi*, and other closely related species [43]. However, it was detailed a morphological assessment along with the molecular analysis using chloroplast and nuclear markers, especially rbcL genes could be applied as an efficient and reliable approach for accurate delineation and identification of *Ulva* species. Based on molecular approach data of *Ulva* species in the intertidal area of the Ulee Lheue marine zone, Aceh, the level of genetic diversity and AMOVA revealed that these species were genetic diversity from each other and showed a higher level of intraspecific genetic variability. The phylogenetic trees obtained from distinct molecular markers delivered to similar clusters and relationships of species.

CONCLUSION

Based on the concern of *U. lactuca* species for industrial and biotechnology destinations, we can describe a suitable method for obtaining the genetic distances between species. This study was the presence of 4 species of *Ulva* (UL1, UL2, UL3, UL4) identified based on GenAlex. DNA research to PCR products obtained the heterozygosity value of 0.333 - 0.667 with polymorphic within 15 bands produced from 4

populations of *U. lactuca* in Indonesia. PCR analysis found a polymorphism of 0.456 and 0.578 (PIC). This finding added several molecular supports to the genetic distance of *U. lactuca* Ulee Lheue beach was closely related between species with a genetic distance of 0.82 in two main clusters.

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