Biodiversity of the Indonesian marine sponge genus *Aptos* with molecular networking approach

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Abstract. Many natural products have been isolated from the cryptic species of the Indonesian marine sponge. Despite this, it remains unclear how sponges produce such a diverse array of metabolites and their relationship to sponge diversity. During our expedition in 2017-2019, we collected the Indonesian marine sponge of the genus *Aptos*, which was found surrounding Weh and Aceh Islands, the northwesternmost part of the Indonesian archipelago. All specimens were subjected to a feature-based molecular networking approach to understand their relationship. The molecular phylogenetic clades of genus *Aptos* were examined by cytochrome oxide I (COI) while the presence of their chemotypes were confirmed by chemical analysis based on bioassay-guided separation with several steps of chromatography. As results, the presence *Aptos suberitoides*, *Aptos aptos*, *Aptos nigra*, *Aptos lobata*, and *Aptos sp.* were confirmed by the molecular phylogenetic. On the other hand, chemical analysis showed the presence of alkaloid-class molecules on all specimens. Interestingly, feature-based molecular networking revealed the whole figure of cryptic species were formed a clear and distinct group together with their chemotypes. Consequently, it confirmed that the relationship between the presence of each species as well as its chemotype can be distinguished clearly. In conclusion, we suspect the chemical analysis of the genus *Aptos* have unknowingly conflated between different cryptic species, resulting in the seemingly idiosyncratic chemical variations.

1 Introduction

Metabolites found in marine ecosystems exhibit wide structural diversity and bioactive properties [1]. Sponge, which belongs to the phylum Porifera, is known to contain a variety of secondary metabolites [2]. Interestingly, compounds from sponges have often been found to be promising pharmaceutical agents [3]. To date, FDA has been approved 3 marine drugs derived from sponges such as anticancer cytarabine (ara-C) and anticancer Eribulin mesylate, and antiviral vidarabine (ara-A) [4-7].

Sponge genus *Aptos* is found in a wide range of ecosystems, including the South China Sea, Japanese, Indonesian, and Caribbean waters [8-11]. The first discovery was conducted by Nakamura and co-workers from an Okinawan sponge *Aptos aptos* which contain aptamine, an alkaloidal molecule, in 1981 [12]. To date, many other research groups throughout the world have also carried out chemical analyses on different *Aptos* species to investigate the structural diversity [13-15].

During our expedition around Weh and Aceh Islands in the early 2017, we discovered the marine sponge of the genus *Aptos*. Since there are no reported papers on the *Aptos* sponges around that area to date, we examined both genetic and chemical contents of them. In this paper, we revealed the whole figure of genetic

and chemical diversity on *Aptos* sponges around Weh and Aceh Islands using the metabolomics approach.

2 Materials and methods

2.1 Materials

Nuclear Magnetic Resonance (NMR) 500 MHz spectroscopy (Bruker Biospin, Billerica, MA, USA) in CDCl₃ (Cambridge Isotope Labs). All extracts were injected into LC-MS/MS data using a reversed-phase analytical HPLC column (5µm, 250mm x 4.6mm). Peaks were detected by positive ion mode of high-resolution MS/MS system.

2.2 Extraction and isolation

Specimens were collected by hand using SCUBA at 10 to 30 m depth off surrounding Weh and Aceh Islands from 2017 to 2019 (Fig. 1). The specimens were kept in the freezer during the trip. Furthermore, all specimens consist of 209 specimens from Weh and 347 specimens from Aceh Islands were extracted by acetone. After partitioning and concentrating the extracts, the organic layer was used for purification and metabolomics purposes. On the other hand, all extracts which contain

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alkaloidal markers were purified to obtain pure material used for the chemical standard.

2.3 Phylogenetic analysis

All crude extracts (10.0 mg) were extracted using Qiagen's DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), followed by PCR amplification using *dmaw* forward (5'-TTGATCTGGAGTGGTTCCGC-3') and reverse (5'-CGTTCATGCCGAAGGTTGTG-3') under the standard condition. Amplicons were analysed by AB3130 with ABI PRISM BigDye Terminator Cycle Sequence Kit v1.1, and sequence alignment used MEGA v5.1.

Fig. 1. The collection sites.

2.4 Metabolomics approach

The presence of the marker alkaloidal was confirmed by a ¹H NMR spectra comparison. On the other hand, For LC-MS/MS analysis, MeOH solution (20 µL) containing each crude extract was prepared. We applied a flow rate of 0.5 mL/min under positive ion mode of high resolution to samples ranging from 50 to 1000 m/z. Furthermore, all raw data were converted to mzXML format, and it was uploaded online with reference number MSV000088027 (<https://massive.ucsd.edu>) [16]. To further analysis, we subjected them to MZmine2 software. On the GNPS website (<http://gnps.ucsd.edu>), quantitative and MS/MS tables have been uploaded for use in a feature-based molecular networking study [17]. A cosine score of 0.6 was applied to the parameters with a maximum of 10 matched peaks. Then, by using Cytoscape 3.7.2 version, the results were visualized [18].

2.5 Spatial analysis of the Sponge genus *Aaptos*

The specimen data collected at each observation station were then spatially analysed using an interpolation method. Inverse Distance Weighted (IDW) interpolation was employed to perform the distribution model of the detected individual. This method corresponds to the

Indonesian Standard (SNI 7644:2010) for oceanographic-spatial data provider. IDW method can calculate faster than the other methods such as kriging, even though the calculation quality is categorised to "acceptable" [19]. IDW assumes the value resulted from interpolation process as similar as the closer sampled data compared to the distant ones. The distance among the interpolated points toward the reference is going to influence the weight of calculation [20]. The interpolation results were limited to the initial data so that the product will not be exceeded or extrapolated.

3 Results and discussion

All specimens both from Weh and Aceh Islands were examined by ¹H NMR comparison with those reported [21-25]. As results, all extracts were identified as known compounds. However, since there was a methoxy moiety was introduced with those reported, it was likely a typical solvolysis product during chemical works. Careful analysis by LC-MS/MS revealed the presence of phenolic groups on all extracts (Fig. 2). It also confirmed that those likely reported to be artifacts by solvolysis reaction to yield methoxy derivatives [26].

Since there is quite similar morphology on the marine sponge of the genus *Aaptos*, we examined the whole diversity figure using molecular networking approach. After obtaining the MS/MS data, all raw data were subjected to MZmine2 for feature detection, grouping, and alignment. By uploading the results on the GNPS web platform, the feature-based on molecular networking may reveal the whole figure of diversity in the sponge of the genus *Aaptos* relating to their chemotypes. As results, the presence of some clades on the sponge of the genus *Aaptos* were confirmed by the phylogenetic tree. Moreover, feature-based on molecular networking revealed the whole figure of their diversity (Fig. 3).

Fig. 2. Chemotypes of sponge of the genus *Aaptos* around Weh and Aceh islands.

To understand how the sponge was spread, we used the distribution analysis to examine their spread (Figs. 4-5). As results, *A. aaptos*, *A. lobata*, *A. nigra*, and *A. suberitoides* were spread in the northern part while *Aaptos* sp. was spread in the western part of Weh Island (Fig. 4). The characteristic of geography in Aceh Island may introduce unique sponge spread in the area. The presence of *A. nigra*, and *A. suberitoides* were discovered in the northern part of the collection site in

Nasi Island, meanwhile, *Aaptos sp.* was found in the southern part of Nasi Island. On the other hand, the sponge of the genus *A. lobata* was spread in both northern and southern parts of Breueh Island (Fig. 5). We suspected that *A. lobata* is a more tolerant sponge of big waves and strong currents from the Indian Ocean in the western part of Aceh Island. As the sponges contain the potential of secondary metabolite, it also gives us an opportunity to discover the resources for the pharmaceutical sciences purposes.

Fig. 3. Feature-based on molecular networking.

Fig. 4. Distribution of the genus *Aaptos* in Weh Island; (a) *Aaptos aaptos*, (b) *Aaptos lobata*, (c) *Aaptos nigra*, (d) *Aaptos sp.* and (e) *Aaptos suberitoides*.

Fig. 5. Distribution of the genus *Aaptos* in Aceh Island; (a) *Aaptos aaptos*, (b) *Aaptos lobata*, (c) *Aaptos nigra*, (d) *Aaptos sp.*, and (e) *Aaptos suberitoides*.

4 Conclusion

The presence of *Aaptos suberitoides*, *Aaptos aaptos*, *Aaptos nigra*, *Aaptos lobata*, and *Aaptos sp.* from the Indonesian marine sponge of the genus *Aaptos* were confirmed by the molecular phylogenetic. The chemical analysis showed the presence of alkaloid-class molecules on all specimens. Interestingly, feature-based molecular networking revealed the whole figure of cryptic species were formed a clear and distinct group together with their chemotypes. Further analysis revealed that, *A. aaptos*, *A. lobata*, *A. nigra*, and *A. suberitoides* were spread in the northern part while *Aaptos sp.* was spread in the western part of Weh Island. Moreover, the presence of *A. nigra*, and *A. suberitoides* were discovered in the northern part of the collection site in Nasi Island, meanwhile, *Aaptos sp.* was found in the southern part of Nasi Island. On the other hand, the sponge of the genus *A. lobata* was spread in both northern and southern parts of Breueh Island. As the sponges contain the potential of secondary metabolite, it also gives us an opportunity to discover the resources for the pharmaceutical sciences purposes.

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