HAYATI Journal of Biosciences xxx (2017)

HOSTED BY

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

Metagenomic Survey of Potential Symbiotic Bacteria and Polyketide Synthase Genes in an Indonesian Marine Sponge

Nia M. Kurnia,[1] Agustinus R. Uria,[2,6*] Yudi Kusnadi,[2] Lidia Dinawati,[3] Dewi S. Zilda,[2] Tri A. Hadi,[4] Siswa Setyahadi,[5] Feliatra Felix[3]

[1] *Graduate School of Pharmacy, Pancasila University, Jakarta, Indonesia.*
[2] *Laboratory of Marine Biotechnology, Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries, Jalan KS Tubun Petamburan VI, Jakarta 10260, Indonesia.*
[3] *Faculty of Fisheries and Marine Science, University of Riau, Pekanbaru, Indonesia.*
[4] *Research Center for Oceanography, Indonesian Institute of Sciences (LIPI), Indonesia.*
[5] *Center for Bioindustrial Technology, Agency for the Assessment and Application of Technology (BPPT), Jakarta, Indonesia.*

ARTICLE INFO

Article history:
Received 9 October 2016
Received in revised form
12 April 2017
Accepted 26 April 2017
Available online xxx

KEYWORDS:
"*Candidatus* Entotheonella sp.",
marine sponge,
microbiome,
polyketide synthase,
Rhabdastrella sp.

ABSTRACT

There has been emerging evidence that the bacteria associated with marine sponges are the key producers of many complex bioactive compounds. The as-yet uncultured candidate bacterial genus "*Candidatus* Entotheonella" of the marine sponge *Theonella swinhoei* from Japan have recently been recognized as the source of numerous pharmacologically relevant polyketides and modified peptides, as previously reported by the Piel group (Wilson *et al.* 2014). This work reported the presence of "*Candidatus* Entotheonella sp." in the highly complex microbiome of an Indonesian marine sponge from Kapoposang Island, South Sulawesi. We further identified the Kapoposang sponge specimen used in this work as *Rhabdastrella* sp. based on the integrated morphological, histological, and cytochrome oxidase subunit I (COI) gene analyses. To detect the polyketide biosynthetic machinery called type I polyketide synthase (PKS) in this Indonesian *Rhabdastrella* sp., we amplified and cloned the ketosynthase-encoding DNA regions of approximately 700 bp from the uncultured sponge's microbiome. Further sequencing and analysis of several randomly chosen clones indicated that all of them are mostly likely involved in the biosynthesis of methyl-branched fatty acids. However, employing a PKS-targeting primer designed in this work led to the isolation of four positive clones. BlastX search and subsequent phylogenetic analysis showed that one of the positive clones, designed as RGK32, displayed high homology with ketosynthase domains of many type I PKS systems and may belong to the subclass *cis*-AT PKS group.

1. Introduction

Polyketides are a large class of highly diverse natural products, which include many important pharmaceuticals, agrochemicals, and veterinary agents (Cane and Walsh 1999). The enormous diversity of most polyketides are built from simple carboxylic acid monomers through a number of programmed events catalyzed by polyketide synthases (PKSs) (Hertweck 2009; Llewellyn and Spencer 2007). Three types of bacterial PKSs known to date are type I, II, and III. Type I PKSs are multifunctional enzymes that are organized into modules, in which each module harbors catalytic domains that mediate one cycle of polyketide elongation and modification. A module minimally consists of three domains: an acyltransferase (AT) domain that chooses the appropriate acyl-CoA building block, an acyl carrier protein (ACP) domain that serves as an anchor for the building block, and a ketosynthase (KS) domain that catalyzes the polyketide chain elongation. Optional domains perform various functional modifications on the β-position (Piel 2010; Rawlings 2001; Staunton and Weissman 2001). In principle, type I PKS systems are similar to type I fatty acid synthases (FASs) that use simple precursors such as acetyl-CoA and malonyl-CoA monomers. FASs are different from type I PKSs in that they consist of a single module that is

* Corresponding author.
E-mail addresses: agustinus.uria@gmail.com, agustinus.uria@kkp.go.id (A.R. Uria).
Peer review under responsibility of Institut Pertanian Bogor.
[6] Present address: Faculty of Pharmaceutical Sciences, Hokkaido University, Kita 12, Nishi 6, Kita-ku, Sapporo 060-0812, Japan.

http://dx.doi.org/10.1016/j.hjb.2017.04.004

Please cite this article in press as: Kurnia, N.M., et al., Metagenomic Survey of Potential Symbiotic Bacteria and Polyketide Synthase Genes in an Indonesian Marine Sponge, HAYATI J Biosci (2017), http://dx.doi.org/10.1016/j.hjb.2017.04.004

used iteratively and rigidly adhered-to specificities of component enzymes to ensure the faithful production of a saturated long-chain fatty acid (Smith and Tsai 2007). Since the PKS module architecture and function corresponds to the resulting intermediates, a polyketide structure can be predicted from PKS domain sequences. This correspondence is known as the colinearity principles that are usually applied for a subclass of modular type I PKSs called "*cis*-AT PKSs" with an AT domain integrated in each module (Hertweck 2009; Piel 2010). In contrast to *cis*-AT PKSs, the subclass *trans*-AT PKSs lack integrated AT domains and perform unusual enzymatic features to generate diverse complex polyketides. In *trans*-AT PKS systems, a free-standing AT domain acts in *trans* to load acyl building blocks into the assembly line (Nguyen *et al.* 2008).

Marine sponges are a rich source of natural products that include polyketides and modified peptides. There has recently been emerging evidence that many sponge-derived polyketides and peptides are actually produced by the associated symbiotic bacteria (Piel 2010; Uria and Piel 2009). The associated microbial consortia can account for up to 60% of the sponge total biomass and consist of hundreds to thousands of bacterial species (Taylor *et al.* 2007). However, the limitation to cultivate the vast majority (>99%) of these bacterial symbionts (Amann *et al.* 1995; Hugenholtz *et al.* 1998; Webster and Hill 2001) has hampered the attempts to access their biotechnological potential. Cultivation-independent approaches, popularly called metagenome mining, have enabled cloning of biosynthetic gene clusters of interest within diverse genetic mixtures of sponge symbiotic systems providing convincing proof about the bacterial origin of sponge-derived polyketides and modified peptides, as exemplified by onnamide A (Nguyen *et al.* 2008; Piel *et al.* 2004), psymberin (Fisch *et al.* 2009), polytheonamides (Freeman *et al.* 2012), and misakinolide (Ueoka *et al.* 2015). Subsequent heterologous expression of gene clusters in easily culturable bacteria could generate sustainable and large-scale supply of sponge-derived drug candidates for drug development (Piel 2004; Uria and Piel 2009).

Identifying PKS systems in the microbiomes of marine sponges is extremely challenging, because sponge-associated microbial assemblages are highly complex (Scheuermayer *et al.* 2006; Taylor *et al.* 2007) that may contain hundreds of individual genomes with numerous homologous genes from diverse pathways (Schirmer *et al.* 2005). Recent single-cell and metagenomic studies on microbiome inhabiting the Japanese sponge *Theonella swinhoei* have revealed that "*Candidatus* Entotheonella sp." are the true producers of many polyketides and modified peptides (Ueoka *et al.* 2015; Wilson *et al.* 2014). Furthermore, *Entotheonella* symbionts were found to be widely distributed in taxonomically diverse sponge species from distant geographical regions (Wakimoto *et al.* 2014; Wilson *et al* 2014). Such previous studies strongly suggest that different *Entotheonella* variants, either in different sponge species or within the same sponge species from geographically different locations, may produce different biologically active compounds encoded on their genomes.

Some sponges belonging to the class Demospongiae (*e.g. Theonella swinhoei*, *Discodermia dissoluta*) are known to harbor "*Candidatus* Entotheonella sp.", as-yet uncultivable filamentous bacteria living outside the sponge cells mostly in the mesohyl part (Brück *et al.* 2008; Schmidt *et al.* 2000; Wilson *et al.* 2014). Their large unusual morphology and extracellular occurrence made easy to isolate or enrich them by simple mechanical separation (Bewley *et al.* 1996). In this preliminary study, we reported identification of "*Candidatus* Entotheonella sp." in an Indonesian marine sponge from Kapoposang Island, South Sulawesi. A targeting primer pair previously reported by Wilson *et al* (2014) was applied in this work to detect such as-yet uncultivable symbiont in the complex sponge's microbiome. We subsequently reported the presence of a PKS system in a highly complex sponge-microbe symbiotic interaction. The taxonomic status of the Indonesian sponge specimen was investigated in this work by integrated morphological, histological, and DNA analyses. Finding of KS-encoding sequences that belong to PKS systems is expected to become a basis for isolating entire gene clusters encoding the biosynthesis of rare novel pharmacology-relevant polyketides.

2. Materials and Methods

2.1. Sponge collection and microbiome preparation

A sponge specimen was collected from reefs at the depth range of 5–10 m in Kapoposang Island, Indonesia on September 2015 through Coral Triangle Initiative (CTI) program. Seawater (1 L) was sterilized by filtration on 0.45-μm and 0.22-μm membranes. To prepare *Entotheonella*-harboring microbiome, the sponge sample (10 gram) was sliced into small pieces and stored in 70% ethanol diluted with such sterile seawater. A small sample piece (2.5 grams) was squeezed with 10 mL of sterile seawater in a 50-ml falcon tube, mixed by vortex, and let at room temperature for a few minutes. The resulting supernatant was transferred to five 2-ml eppendorf vials and centrifuged at 500 rpm for 10 minutes using Microfuge 222R (Beckman Coulter). The microbial cell pellet in each vial was resuspended with 1 mL of ddH_2O and stored at −20°C for being used later in PCR detection of *Entotheonella* and cloning of KS-encoding fragments. The resulting precipitate (microbial cell-free sponge tissue) was rinsed 3× with ddH_2O for being used in DNA barcoding.

2.2. Sponge identification

Sponge identification in this work involved morphological observation, histological analysis, and DNA barcoding. Histological analysis of the sponge tissue was conducted using light microscopy (Hooper 2003) which consisted of spicule preparation and section preparation. Small fragments of the tissue from inner and outer sides were placed into a 10-ml bottle. A small portion of bleaching agent containing sodium hypochlorite was added to the fragment and waited for a short period to dissolve the organic tissues, leaving only mineral skeleton (spicules). Then, it was washed by replacing the bleaching agent with water for several times. Next, clean spicule suspensions were sucked and pipetted onto an object glass. Finally, it was covered using cover glass carefully to keep the spicule in their original shapes. Sponge was cut in a cross section and then sliced about 1-mm thick. Then the thin slice was placed onto an object glass. To make it observable, several small drops of water were added to the slice and then covered with the cover glass. For sponge identification based on DNA barcoding, genomic DNA was prepared from the squeezed sponge tissue using CTAB method (Piel *et al.* 2004) and then used as the template for PCR amplification using two primer pairs targeting the 5′ end of the mitochondrial cytochrome oxidase subunit 1 (COI): dgLCO1490 (5′-GGTCAACAAATCATAAAGAYATYGG-3′) and dgHCO2198 (5′-TAAACTTCAGGGTGACCAAARAAYCA-3′) (Meyer *et al.* 2005). The PCR master mix consisted of 12.5-μL of 2× KAPA Taq Extra HotStart ReadyMix with dye containing 2-mM $MgCl_2$, 1.25-μL DMSO, 1.5-μL of 10-mM dgLCO1490 primer, 1.5-μL of 10-mM dgHCO2198, 1-μL of sponge DNA, and 7.25-μL ddH_2O. The PCR program was set up at 35 cycles consisting of denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 46.2°C for 30 seconds, elongation at 72°C for 1 minute, and final elongation at 72°C for 1 minute. The target PCR product of approximately 659 bp was separated on the

Please cite this article in press as: Kurnia, N.M., et al., Metagenomic Survey of Potential Symbiotic Bacteria and Polyketide Synthase Genes in an Indonesian Marine Sponge, HAYATI J Biosci (2017), http://dx.doi.org/10.1016/j.hjb.2017.04.004