INTISARI

Isolasi, Karakterisasi, Aktivitas anti-*Vibrio* dan Klaster Gen Penyandi Senyawa Bioaktif dari *Pseudoalteromonas xiamenensis* STKMTI.2

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Vibriosis adalah ancaman serius bagi budidaya laut sehingga perlu dikendalikan agar tidak menimbulkan kerugian besar. *Pseudoalteromonas* merupakan bakteri laut yang dapat menghasilkan senyawa bioaktif untuk kepentingan manusia seperti senyawa antibakteri. Penelitian ini bertujuan untuk mengidentifikasi secara fenotipik dan molekuler, produksi senyawa anti-*Vibrio* melalui fermentasi cair, isolasi, karakterisasi dan elusidasi struktur kimia senyawa anti-*Vibrio*, uji aktivitas anti-*Vibrio* secara *in vitro* melawan *V. alginolyticus* (strain S4UA, S5UC, BCSA, and BSCP 1C), *V. harveyi* (strain GK18, SB 25, BT1H, SL2, SH2, A1, and A2), dan *V. parahaemolyticus* (strain SMI1A, CJ10, and CJ5), uji biokontrol *V. harveyi* GK18 oleh strain STKMTI.2 dan analisis genom lengkap serta gen penyandi senyawa bioaktif pada bakteri laut STKMTI.2. Isolat STKMTI.2 diidentifikasi menggunakan kit Kb007 dan analisis sekuen gen 16S rDNA. Isolat STKMTI.2 difermentasi pada medium Zobell 2216E agar dan cair dengan lama inkubasi 0-120 jam untuk menghasilkan senyawa antibakteri. Setelah diketahui medium dan lama inkubasi, selanjutnya dilakukan proses fermentasi pada skala yang lebih besar. Etil asetat dan ammonium sulfat digunakan untuk ekstraksi senyawa dari supernatan dan etanol untuk ekstraksi senyawa dari sel pellet. Permurnian senyawa anti-*Vibrio* menggunakan metode kromatografi lapis tipis, kolom kromatografi, dan *Preparative Layer Chromatography,* sedangkan karakterisasi senyawa menggunakan metode kromatografi lapis tipis dan *Gas Chromatography Mass Spectrometry*. Uji aktivitas antibakteri terutama anti-*Vibrio* dilakukan dengan metode difusi cakram pada medium *double layer agar*. Uji bioautografi dilakukan menggunakan kromatografi lapis tipis. *Minimum Inhibitory Concentration* dilakukan dengan metode pengenceran pada *Microplate* 96 *well.* Aktivitas penghambatan *in vitro P. xiamenensis* STKMTI.2 terhadap *V. harveyi* dilakukan dengan metode *co-culture* pada medium cair Zobell 2216E selama 48 jam. Analisis sekuen genom lengkap dilakukan dengan teknologi Oxford GridION. Analisis Klaster gen penyandi senyawa bioaktif dianalisis menggunakan aplikasi antiSMASH dan BAGEL4. Hasil analisis sekuen 16S rDNA menunjukkan bahwa STKMTI.2 memiliki kemiripan dengan *P. xiamenensis*. Senyawa anti-*Vibrio* dari isolat STKMTI.2 dihasilkan ketika difermentasi menggunakan medium cair Zobell 2216E selama 96 jam. Senyawa tersebut merupakan produk ekstraseluler. Isolat STKMTI.2 dengan kepadatan 10^5 dan 10^6 CFU mL^{-1} mampu menurunkan pertumbuhan *V. harveyi* hingga 50%. Kolom kromatografi memisahkan ekstrak partisi kloroform menjadi 148 fraksi. Senyawa bioaktif dari isolat STKMTI.2 secara spesifik menghambat *Vibrio* spp. pada dosis 2.500 µg $disc^{-1}$ untuk ekstrak kasar, 1.300 µg $disc^{-1}$ untuk partisi kloroform, dan 1.000 µg $disc^{-1}$ untuk fraksi kolom kromatografi. Analisis GC-MS pada senyawa aktif subfraksi PLC memprediksi 4 senyawa, yaitu trietoksiboran; 1,3-difenil-1,3,5,5-tetrametil-siklotrisiloksana; 1,6-diazaspiro-4.4-nonan-2,7-diona; and 1,3-bis-4-metoksifenil-5-fenil-1,3,5-triazinan-2-tion. Nilai MIC dari senyawa subfraksi adalah ≤0,78 µg mL^{-1}, lebih

rendah dibandingkan antibiotik komersial. Isolat STKMTI.2 memiliki genom berukuran 4.563.326 bp (*GC content*: 43,2%), yang terdiri dari 1 kromosom, 2 plasmid sirkuler (unnamed1 dan unnamed2), dan 2 plasmid linear (unnamed4 and unnamed5), 4,824 sekuen penyandi protein dan 1 *clustered regularly interspaced short palindromic repeated* (CRISPR). Genom bakteri STKMTI.2 memiliki beberapa gen penyandi senyawa antibakteri, yaitu bmp8, bmp9, *non ribosomal peptide synthase, polyketide-like butyrolactone*, *Lant class I*, *RiPP-like* terdeteksi pada kromosom dan prodigiosin terdeteksi pada plasmid linear Unnamed5. Penelitian ini berhasil mengeksplorasi potensi dari isolat STKMTI.2 sebagai bakteri laut penghasil senyawa anti-*Vibrio* dan kandidat biokontrol untuk mengatasi vibriosis.

Kata Kunci: Anti-*Vibrio*, Kromatografi, *Pseudoalteromonas*, WGS

ABSTRACT

Isolation, Characterization, anti-*Vibrio* Activities and Biosynthetic Gene Clusters of *Pseudoalteromonas xiamenensis* STKMTI.2

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Vibriosis is a prevalent disease in almost all mariculture spesies worldwide caused by *Vibrio* spp. *Pseudoalteromonas* is a marine bacterium that can produce secondary metabolites for human uses, such as antibacterial compounds. This study aimed to identify the STKMTI.2 isolate, conduct the isolation, purify and characterize the anti-*Vibrio* compounds, conduct *in vitro* anti-*Vibrio* activity tests on *V. alginolyticus* (strain codes S4UA, S5UC, BCSA, and BSCP 1C), *V. harveyi* (strain codes GK18, SB 25, BT1H, SL2, SH2, A1, and A2), and *V. parahaemolyticus* (strain codes SMI1A, CJ10, and CJ5), perform a biocontrol test against *V. harveyi* GK18, and analyze the complete genome and the biosynthetic gene cluster of STKMTI.2 isolate. STKMTI.2 isolate was identified using the Kb007 biochemical test kit and 16S rDNA sequencing analysis. STKMTI.2 was inoculated on the Zobell 2216E agar and broth medium with incubation time of 0-120 hours to produce antibacterial compounds. After knowing the medium and incubation time, a large-scale fermentation was processed. Isolation of anti-*Vibrio* compound was carried out using ethyl acetate and ammonium sulphate of supernatan and etanol of the cell pellet. Characterization of anti-*Vibrio* compounds was carried out by thin layer chromatography, column chromatography, preparative layer chromatography and gas chromatography mass spectrometry methods. Antibacterial activity test was conducted by *disc* diffusion on the double layer agar medium. Bioautography was carried out by the TLC. Minimum Inhibitory Concentration test was carried out by microdilution method on the 96 well microplate. In vitro test of STKMTI.2 was conducted by co-culture test on the Zobell 2216E broth medium for 48 hours. Complete genome sequence analysis was conducted by Oxford Nanopore GridON technology. Clusters of genes encoding secondary metabolites synthesis was analysed using antiSMASH and BAGEL4. The results showed that STKMTI.2 was closest to *P. xiamenensis* based on the 16S rDNA sequencing analysis. Anti-*Vibrio* compounds from STKMTI.2 were produced after 96 hours of incubation on the Zobell 2216E broth medium. These compounds are extracellular products. The isolate of STKMTI.2 with 10^5 and 10^6 CFU mL^{-1} inhibited 50% of *V. harveyi* growth. Column chromatography separated the crude extract into 148 fractions. Bioactive compound from STKMTI.2 inhibited *Vibrio* spp. at a dose of 2.500 µg $disc^{-1}$ for crude extract, 1.300 µg $disc^{-1}$ for the chloroform extract, and 1.000 µg $disc^{-1}$for column chromatography fraction. The results of GC-MS analysis on the active compound sub-fraction PLC were predicted 4 compounds, namely triethoxy-borane; 1,3-diphenyl-1,3,5,5-tetramethyl-cyclotrisiloxane; 1,6-diazaspiro-4.4-nonane-2,7-dione; and 1,3-bis-4-methoxyphenyl-5-phenyl-1,3,5-triazinane-2-thione. The MIC value of the partially purified compound was ≤ 0.78 µg mL^{-1}, lower than commercial antibiotics. The genome size of STKMTI.2 was 4,563,326 bp (GC content: 43.2%) consisted of 1 chromosome, 2 circular plasmids, and 2 linear plasmids, 4,824 protein-coding sequences and 1 clustered, regularly interspaced, short palindromic repeated (CRISPR). The genome of STKMTI.2

has several biosynthetic gene clusters, namely bmp8, bmp9, non ribosomal peptide synthase, polyketide-like butyrolactone, lant class I, RiPP-like was detected in chromosome1 and prodigiosin was detected in plasmid linear unnamed5. This research is a pioneer to explore further the potential of STKMTI.2 isolate as a marine bacterium producing anti-*Vibrio* compounds and a candidate for vibriosis biocontrol.

Keywords: Anti-*Vibrio*, Chromatography, *Pseudoalteromonas*, WGS