AKTINOMISETES PENGHASIL ANTIBIOTIK DARI HUTAN BAKAU TOROSIAJE, GORONTALO

INTISARI

Peningkatan resistensi mikroba patogen terhadap antibiotik dan penemuan kembali antibiotik sejenis yang dihasilkan aktinomisetes terestrial, mendorong upaya eksplorasi aktinomisetes penghasil antibiotik di lingkungan ekstrem. Hutan bakau Torosiaje, Gorontalo berada di ekosistem kars, terdiri atas tipe *overwash* dan *fringe* yang berbeda dalam status hidrologi yang direspon distribusi pohon bakau dalam pola zonasi. Penelitian ini bertujuan untuk mengungkap distribusi, kemelimpahan, dan diversitas aktinomisetes penghasil antibiotik di rizosfer berbagai jenis pohon bakau pada tipe hutan berbeda, dan potensinya sebagai penghasil antibiotik. Tanah rizosfer dikoleksi berdasarkan *purposive sampling method* pada pohon bakau *Rhizophora mucronata* dan *Bruguiera gymnorhiza* di zona *upper overwash; Rhizophora apiculata* dan *Bruguiera gymnorhiza* di zona *middle fringe; Avicennia marina, Soneratia alba, Ceripos tagal,* dan *Xylocarpus* sp. di zona *upper fringe*. Distribusi dan kemelimpahan aktinomisetes di tanah rizosfer berbagai jenis pohon bakau ditentukan melalui tahapan isolasi aktinomisetes berdasarkan teknik kultur diperkaya. Diversitas aktinomisetes penghasil antibiotik dianalisis berdasarkan karakter fenetik *colour grouping*, profil elemen BOX, dan profil ARDRA; dan karakter molekular sekuen gen 16S rRNA. Aktivitas antibiotik ditentukan melalui tahapan percobaan sintesis antibiotik berdasarkan *shake flask culture method,* ekstraksi antibiotik berdasarkan metode solvent, dan uji aktivitas antibiotik berdasarkan Kirby-Bueur test melawan bakteri Gram-positif dan Gram-negatif, serta fungi. Antibiotik diidentifikasi berdasarkan karakter profil KLT, LCMS, GCMS, bioautografi, dan deteksi gen pengkode sintesis antibiotik. Identitas isolat aktinomisetes penghasil antibiotik ditentukan berdasarkan pendekatan sistematika polifasik. Hasil penelitian menunjukkan bahwa aktinomisetes ditemukan terdistribusi pada rizosfer seluruh jenis pohon bakau dengan kemelimpahan yang bervariasi. Kemelimpahan tertinggi pada rizosfer *R.mucronata* di zona *upper overwash*, dan terendah pada rizosfer *R.apiculata* di zona *middle fringe.* Sebanyak 167 isolat aktinomisetes berhasil diisolasi, 77 isolat diantaranya berpotensi penghasil antibiotik. Isolat aktinomisetes penghasil antibiotik menunjukkan keanekaragaman fenetik yang tinggi, baik karakter morfologi, maupun karakter elemen BOX, dan profil ARDRA. Diversitas aktinomisetes penghasil anibiotik berdasarkan karakter molekular 16S rRNA menunjukkan komunitas aktinomisetes penghasil antibiotik tersusun atas genus *Streptomyces*, *Nocardiopsis, Saccharomonospora*, dan *Amycolatopsis.* Anggota genus *Streptomyces* mendominasi seluruh lokasi dan jenis bakau, *Nocardiopsis* dan *Saccharomonospora* spesifik ditemukan di zona *upper overwash*, sedangkan *Amycolatopsis* spesifik ditemukan di zona *upper fringe*. Antibiotik yang dihasilkan isolat aktinomisetes terpilih menunjukkan aktivitas antibakteri bersifat *broad spectrum* dan *narrow spectrum,* juga antifungi. Aktivitas antibiotik tertinggi dihasilkan dua isolat aktinomisetes anggota genus *Streptomyces*, BUFA-2 dan BMFB-9, dengan nilai MIC 0,0625–0,5 mg/ml

terhadap *E.coli, S.aureus, B.subtilis, A.niger* dan *C.albicans*. Antibiotik merupakan senyawa campuran yang tersusun atas β-laktam, aminoglikosida, namun bukan golongan alkaloid. Sebagian antibiotik merupakan asam lemak bebas yang diidentifikasi sebagai asam palmitat, asam Di-n-oktil ptalat, dan asam risinoleat; juga merupakan kelompok poliketida aromatik dan non-ribosimal polipeptida. Isolat aktinomisetes penghasil antibiotik diidentifikasi sebagai *Streptomyces sp.* strain BUFA-2, *Streptomyces sp.* strain BMFB-9, *Streptomyces sp.* strain BMFR-4, *Saccharomonospora sp.* strain BUOB-1, *Amycolatopsis sp.* strain BUFX-4 and *Nocardiopsis sp.* strain BUOR-2, memiliki peluang sebagai spesies baru (*novel species*). Hutan bakau Torosiaje Gorontalo merupakan habitat aktinomisetes anggota genus *Streptomyces* dan *rare actinomycetes* yang berpotensi menghasilkan antibiotik dengan karakter khusus.

Kata kunci: Aktinomisetes, rizosfer, bakau, antibiotik, analisis molekular.

ANTIBIOTICS-PRODUCING ACTINOMYCETES FROM MANGROVE FOREST OF TOROSIAJE, GORONTALO

ABSTRACT

The increasing of pathogenic-microbial resistance to antibiotics, and rediscovery of antibiotics by terrestrial actinomycetes encourages efforts to explore antibiotic-producing actinomycetes in extreme environments. Mangrove forest of Torosiaje, Gorontalo is karst ecosystem, consisting of overwash and fringe types, in which different in hydrological status. The objective of the study was to reveal the distribution, abundance, and diversity of antibiotic-producing actinomycetes in the rhizosphere of various types of mangrove in different forest types, and to review their potential as antibiotic producers. Rhizosphere soil sample was collected based on purposive sampling method on various types of mangroves *Rhizophora mucronata* and *Bruguiera gymnorhiza* on upper overwash*; Rhizophora apiculata* and *Bruguiera gymnorhiza* on *middle fringe; Avicennia marina, Soneratia alba, Ceripos tagal,* and *Xylocarpus* sp. on *upper fringe*. Distribution and abundance of actinomycetes on rhizosphere soil were determined through the stage of actinomycetes isolation based on enrichment culture technique. Diversity of antibiotics-producing actinomycetes was analyzed based on phenetic characters of colour grouping, element BOX profile, and ARDRA profile; and molecular character of sequence of 16S rRNA gene. The activities of antibiotics were determined through the stage of antibiotic synthesis based on shake flask culture methods, the antibiotics extraction was carried out based on solvent method. The antibiotics activities assay was conducted based on Kirby-Bueur test against Gram-positive and Gram-negative bacteria, also fungi. Antibiotic was identified based on TLC, LCMS, and GCMS profile, bio-autography, and detection of gene encode of antibiotic synthesis. The identity of antibiotics-producing actinomycetes was determined based on polyphasic systematics approach. The result of study show that the actinomycetes were found distributed on rhizozphere of all of mangroves types with the varied of abundance. The highest abundance found on rhizosphere of *R. mucronata* on upper overwash, and the lowest one found on rhizosphere of *R.apiculata* on middle fringe. As much as 167 actinomycetes isolates were successfully isolated from mangrove rhizosphere, and about 77 isolates were potentially as antibiotics producers. Antibiotics-producing actinomycetes show the high-phenetic diversity of morphology, as well as element BOX profile and ARDRA profile. The community of antibiotics-producing actinomycetes consist of genus *Streptomyces*, *Nocardiopsis, Saccharomonospora*, and *Amycolatopsis* based on molecular character of sequence of 16S rRNA gene. The member of genus *Streptomyces* was found dominance on rhizosphere soil of 7 types of mangrove on all of location, *Nocardiopsis* and *Saccharomonospora* were found on *upper overwash*, while *Amycolatopsis* was found in the upper fringe. The antibiotics show *broad spectrum* and *narrow spectrum* antibacterial, and

antifungals activities. The highest antibiotic activity was produced by two actinomycetes isolates belonging to the genera Streptomyces, BUFA-2 and BMFB-9, with MIC values of 0.0625–0.5 $mgml^{-1}$ against *E. coli, S. aureus, B. subtilis, A. niger* and *C. albicans*. Antibiotics were mixed compounds composed of β-lactams, aminoglycosides, but it not alkaloids. Several antibiotics were free fatty acid identified as palimitic acid, Di-n-octyl ptalate acid and risinoleic acid; and as well as polyketida aromatics and non-ribosomal polypeptide groups. The antibiotic-producing actinomycetes isolate was identified as *Streptomyces* sp. strain BUFA-2, *Streptomyces* sp. strain BMFB-9, *Streptomyces* sp. strains BMFR-4, *Saccharomonospora* sp. strain BUOB-1, *Amycolatopsis* sp. strain BUFX-4, and *Nocardiopsis* sp. strain BUOR-21. They were potential as a novel species. Mangrove forest of Torosiaje Gorontalo was the habitat of genus *Streptomyces* and rare actinomycetes which potential as a source of new character of antibiotics.

Key words: Actinomycetes, rhizosphere, mangrove, antibiotics, molecular analysis

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ABSTRACT

The increasing pathogenic-microbial resistance to antibiotics, and rediscovery of antibiotic by terrestrial actinomycetes encourages a study to explore antibiotic-producing actinomycetes in extreme environments. Mangrove forest of Torosiaje in Gorontalo is located in a karst environment. This forest consists of two types, i.e., overwash and fringe mangrove, with different hydrological status represented by the distribution of mangrove tree in a zonation. The objectives of this study were to reveal the distribution, abundance, and diversity of antibiotic-producing actinomycetes in the rhizosphere of various mangroves, as well as the potential of the actinomycetes as an antibiotic producer. The rhizosphere soil as source of actinomycetes was collected based on purposive sampling method. The sampling involved selected mangrove tree species in several zonations, such as *Rhizophora mucronata* and *Bruguiera gymnorhiza* in upper overwash zonation*; Rhizophora apiculata* and *Bruguiera gymnorhiza* in middle fringe zonation, and*; Avicennia marina, Soneratia alba, Ceripos tagal,* and *Xylocarpus* sp. in upper fringe zonation. The distribution and abundance of actinomycetes in the rhizosphere soil of several species of mangrove were determined through several steps of actinomycetes isolation based on enrichment cultures technique. The diversity of the antibiotic-producing actinomycetes was analyzed based on the phenetics character of color grouping, BOX element profile, and ARDRA profile, as well as the molecular character of the 16S rRNA gene sequence. The activity of antibiotics was determined through stages of antibiotics synthesis assay based on shake flask culture method, antibiotic extraction based on solvent method, and antibiotic activity tests based on the Kirby-Bauer test against Gram-positive and Gram-negative bacteria, and fungi. The antibiotics were identified based on TLC, LCMS, and GCMS technique, bio-autography, and detection of gene encoding of antibiotics synthesis. Moreover, the identity of antibiotic-producing actinomycetes was determined based on polyphasic systematics approach. The result of the study shows that the actinomycetes were found distributed on the rhizosphere of all mangroves types with varied abundance. The highest abundance level was in the rhizosphere of *R.mucronata* in the upper overwash zonation, and the lowest one was *R.apiculata* in middle fringe zonation. Out of 167 actinomycete isolates that have been successfully isolated, 77 isolates were potential antibiotic-producers. Those isolates have significant phenetics variety, in terms of its morphological character, BOX element, and ARDRA profile. The diversity of antibiotic-producing actinomycetes based on the character of 16S rRNA gene reveals that the actinomycetes consist of several genera, namely *Streptomyces*, *Nocardiopsis, Saccharomonospora*, and *Amycolatopsis.* The *Streptomyces* genus was mostly in all sites and every species of mangrove. Both *Nocardiopsis* and *Saccharomonospora* were exclusive to upper overwash zonation, while *Amycolatopsis* was specifically found in upper fringe zonation. The antibiotics produced by the selected actinomycetes isolate have a broad and narrow spectrum antibacterial activity, and also antifungal activity. The highest antibiotic activity was produced by two actinomycetes isolates of Streptomyces, BUFA-2 and

BMFB-9, with MIC values of 0.0625–0.5mgml^{-1} against *E. coli, S. aureus, B. subtilis, A. niger* and *C. albicans.* Antibiotics were mixed compounds composed of β-lactams, aminoglycosides, but the compounds are not alkaloids. Several antibiotics were free fatty acid identified as palmitic acid, Di-n-octyl phthalate acid, and ricinoleic acid; those antibiotics were also polyketide aromatics and non-ribosomal polypeptide groups. Antibiotic-producing actinomycetes isolates that identified as *Streptomyces* sp. strain BUFA-2, *Streptomyces* sp. strain BMFB-9, *Streptomyces* sp. strain BMFR-4, *Saccharomonospora* sp. strain BUOB-1, *Amycolatopsis* sp. strain BUFX-4, and *Nocardiopsis* sp. strain BUOR-21, were a potential as novel species. Mangrove forest of Torosiaje, Gorontalo is a habitat of *Streptomyces* and *rare* actinomycetes in which both hold potential to produce antibiotics with unique characteristics.

Keywords: Actinomycetes, rhizosphere, mangrove, antibiotic, molecular analysis.