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MANGROVE KAWASAN MANDEH MENGGUNAKAN PCR
MULTIPLEX: POTENSI ANTIBIOTIK TERHADAP BAKTERI
MULTIRESISTEN**

TESIS



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**Sebagai Salah Satu Syarat Untuk Memperoleh
Gelar Magister Bioteknologi Pada
Program Pasca Sarjana
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(Dibawah bimbingan: Prof. apt. Marlina, MS, Ph.D dan Prof. Dr. Djong Hon Tjong)

Abstrak

Resistensi antimikroba, khususnya pada bakteri multiresisten seperti methicillin-resistant *Staphylococcus aureus* (MRSA), menjadi tantangan serius dalam terapi infeksi dan mendorong eksplorasi sumber antibakteri baru dari lingkungan alami. Ekosistem mangrove dengan tekanan seleksi ekologis tinggi berpotensi menjadi habitat aktinomiset penghasil metabolit sekunder, terutama genus *Streptomyces*. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri isolat *Streptomyces* dari tanah mangrove kawasan Mandeh terhadap MRSA, *Escherichia coli* ATCC, dan *Cutibacterium acnes*; mendeteksi keberadaan gen biosintetik polyketide synthase (*pks*) dan non-ribosomal peptide synthetase (*nrps*) menggunakan PCR multiplex; serta mengidentifikasi isolat unggulan melalui analisis gen 16S rRNA dan filogenetik. Sebanyak 17 isolat dikarakterisasi secara morfologi dan diuji aktivitas antibakterinya menggunakan metode difusi agar. Hasil menunjukkan variasi spektrum aktivitas antar isolat. Isolat STMMI menghasilkan zona hambat terbesar terhadap MRSA ($23,61 \pm 0,62$ mm), sedangkan STMML menunjukkan aktivitas tertinggi terhadap *E. coli* ATCC ($17,06 \pm 0,63$ mm). Skrining PCR multiplex mengungkap bahwa hanya sebagian isolat membawa fragmen gen *pks* dan/atau *nrps*, serta tidak ditemukan hubungan linier antara keberadaan gen tersebut dengan besarnya zona hambat. Isolat STMML menghasilkan amplicon *nrps* (~600 bp) dan dipilih untuk analisis molekuler lanjutan. Analisis sekuens 16S rRNA menunjukkan kemiripan tinggi dalam kluster genus *Streptomyces* dan kedekatan filogenetik dengan *Streptomyces zaomyeticus*, meskipun identifikasi spesies definitif memerlukan pendekatan genomik lanjutan. Secara keseluruhan, hasil penelitian menunjukkan bahwa tanah mangrove Mandeh merupakan sumber potensial isolat *Streptomyces* dengan aktivitas antibakteri, dan integrasi pendekatan fenotipik serta molekuler efektif sebagai strategi skrining awal dalam bioprospeksi kandidat antibiotik baru.

Kata kunci: *Streptomyces*; mangrove; antibakteri; MRSA; *Escherichia coli* ATCC; *Cutibacterium acnes*; PKS; NRPS; PCR multiplex; 16S rRNA.

MULTIPLEX PCR-BASED SCREENING OF *pks* AND *nrps* GENES IN *Streptomyces* sp. ISOLATED FROM MANDEH MANGROVE SOIL: ANTIBIOTIC POTENTIAL AGAINST MULTIDRUG-RESISTANT BACTERIA

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Abstract

Antimicrobial resistance, particularly in multidrug-resistant pathogens such as methicillin-resistant Staphylococcus aureus (MRSA), represents a major challenge in infectious disease therapy and necessitates the exploration of novel antibacterial sources from natural environments. Mangrove ecosystems, characterized by high ecological selective pressures, are potential reservoirs of actinomycetes capable of producing diverse secondary metabolites, especially within the genus Streptomyces. This study aimed to evaluate the antibacterial activity of Streptomyces isolates obtained from mangrove soil in the Mandeh area against MRSA, Escherichia coli ATCC, and Cutibacterium acnes; to detect the presence of biosynthetic gene fragments (pks and nrps) using multiplex PCR; and to identify the most promising isolate through 16S rRNA gene sequencing and phylogenetic analysis. A total of 17 isolates were morphologically characterized and subjected to antibacterial screening using the agar diffusion method. The results revealed isolate-dependent variation in antibacterial spectra. Isolate STMMI exhibited the strongest inhibition against MRSA (23.61 ± 0.62 mm), whereas STMML showed the highest activity against E. coli ATCC (17.06 ± 0.63 mm). Multiplex PCR screening indicated that only a subset of isolates harbored pks and/or nrps gene fragments, and no linear correlation was observed between gene presence and inhibition zone diameter. Isolate STMML produced a single nrps amplicon (~600 bp) and was selected for further molecular analysis. The 16S rRNA sequence analysis placed this isolate within the Streptomyces clade, showing closest phylogenetic relatedness to Streptomyces zaomyceticus, although definitive species assignment requires genome-based approaches. Overall, these findings demonstrate that mangrove soil from the Mandeh area represents a promising source of antibacterial Streptomyces, and that integrating phenotypic and molecular approaches provides an effective early-stage screening strategy for antibiotic bioprospecting.

Keywords: *Streptomyces*; mangrove; antibacterial activity; MRSA; *Escherichia coli* ATCC; *Cutibacterium acnes*; PKS; NRPS; multiplex PCR; 16S rRNA.