

**BAKTERI ENDOFIT TANAMAN TAMAN NASIONAL
DI PULAU JAWA SEBAGAI AGENT PENGHASIL ANTIBIOTIK**

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INTISARI

Kebutuhan antibiotik abru selalu meningkat sebanding dengan perkembangan resistensi pathogen bakteri dan selaras peningkatan jumlah penyakit baru. Mikrobia endofit merupakan sumber potensial untuk menemukan antibiotik baru yang belum banyak diteliti. Tujuan penelitian ini adalah untuk mendapatkan bakteri endofit antibiotik baru yang mampu menghasilkan senyawa antibiotik baru dengan target utama patogen kelompok eukariot. Metode penelitian yang dilakukan meliputi isolation, seleksi bakteri, identifikasi pendahuluan antibiotik, optimasi produksi antibiotik, dan identifikasi akhir bakteri dan antibiotik. Hasil penelitian menunjukkan bahwa isolasi bakteri dari berbagai Taman Nasional dan hutan di pulau Jawa yang didasarkan atas daya hambat terhadap mikrobia target didapatkan sebanyak 306 isolat dari 273 tanaman. Seleksi yang didasarkan atas kemampuan isolat memanfaatkan sumber karbon dan daya hambat terhadap mikrobia indikator seperti *Bacillus subtilis*, *Candida albicans*, dan *Fusarium oxysporum* ditemukan 4 isolat dengan daya hambat lebih dari 5 dan memiliki spektrum patogenisitas lebar yakni isolat BIN-1, SLM-1, STG-1, dan OOH-1. Identifikasi antibiotik pendahuluan ditujukan untuk mengetahui karakter yang dilakukan berbasis kromatografi dan berbagai eluen. Dengan harapan antibiotik baru yang diperoleh dapat diaplikasikan pada bidang kesehatan manusia dan pertanian. Hasil seleksi menunjukkan bahwa isolat STG-1 dan OOH-1 dapat dipilih sebagai kandidat isolat unggul. Tahap percobaan selanjutnya isolat STG-1 sebaiknya dipilih sebagai model untuk tujuan optimasi produksi antibiotik pada skala fermentor. Tahap optimasi dilakukan berdasarkan pada kajian terhadap pengaruh faktor lingkungan (pH, agitasi, temperatur), sumber nutrisi (karbon dan nitrogen), sumber mineral (fosfor, potassium dan kalsium), dan konsentrasi inokulum. Hasil optimasi menunjukkan bahwa isolat STG-1 mampu memproduksi antibiotik terbaik pada kondisi konsentrasi inokulum 10%, nilai pH 7, agitasi 150 rpm., $(\text{NH}_4)_2\text{SO}_4$ - 1 g/l, K_2HPO_4 - 1.9 g/l, KCl - 1,5 g/l, dan CaCl_2 - 0.013 g/l. Identifikasi isolat STG-1 dan OOH-1 yang didasarkan atas ciri-ciri fenotip (morfologi, fisiologi, biokimiawi, dan Kid-API) dan genotip (amplikasi menggunakan primer 27F dan sekuen gen 1492R), yang dianalisis berdasarkan atas nilai similaritas menggunakan program Bank Data dari BLAST, dan dikonstruksi lewat pohon filogeni. Identifikasi berbasis fenotip ditemukan bahwa isolat STG-1 dan OOH-1 lebih dekat dengan dengan ciri-ciri *Pseudomonas luteola* dengan nilai similaritas berturut-turut 99% dan 87%, namun apabila diidentifikasi berbasis molekuler (genotip) isolat STG-1 memiliki kedekatan dengan *Pseudomonas brenneri* strain SFML dengan nilai similaritas 99% dan isolat OOH-1 memiliki kedekatan dengan *Enterobacter xiangfangensis*. Identifikasi antibiotik yang dihasilkan oleh isolat STG-1 memakai teknik Kromatografi Lapis Tipis menghasilkan noktah (*spot*) berwarna kekuning-kuningan terang yang menunjukkan adanya senyawa utama antibiotik. Selanjutnya noktah dikerok dan diekstraksi menggunakan campuran chloroform: etanol (1:1). Hasil ekstraksi disaring dan diuapkan sehingga diperoleh residu. Sebagian residu dianalisis menggunakan GC-MS, FTIR, dan NMR. Hasil GC-MS menunjukkan 168 m/z yang merupakan puncak ion inti (*nuclear ion peak*), analisis menggunakan FTIR mengindikasikan adanya pita pada 2900-2800 m^{-1} yang merupakan senyawa karbon, dan menurut analisis NMR, data base spektrum massa senyawa tersebut diidentifikasi sebagai *1,2 Benzene dicarbonyl acid diisooctyl ester*, yang memiliki rumus kimia $\text{C}_{24}\text{H}_{28}\text{O}_4$, berat molekul 390 kD, dan LC_{50} 0,001 – 0,002% terhadap mikrobia indikator *Fosarium oxysporum*.

Katakunci: bakteri endofit, Taman Nasional pulau Jawa, penghasil antibiotik

Plant Endophytic Bacteria Isolated from National Park of Java Island as Antibiotic Producer

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ABSTRACT

The new antibiotic demand are always increase as well as resistancy development on bacterial pathogen and the current increase in the number of new diseases. Endophytic microbes are potential sources of new antibiotics and have not been widely studied. The obyectives research were to find out of new endophytic bacteria which able to produce new antibiotic compund especially with eucaryotic microbes target. The methods of research including isolation,selection of endophytic bacteria and preliminary identification of antibiotic, optimization of antibiotic production, and final identification of antibiotic and isolated endophytic bacteria. Research results showed that isolation of bacteria from several and forest in Java were found out 306 isolates from 273 of plants. Selection was done based on the ability of isolates to utilize carbon source and their ability to inhibit microbial indicator such as *Bacillus subtilis*, *Candia albicans* and *Fusarium oxysporum*. Selection step resulted in 4 among of 306 isolates had inhibitory effect more than 5 and had large spectrum pathogenicity to microbial target, that were BIN-1, SLM-1, STG-1 and OOH-1 isolates. Preliminary identification of antibiotic was done to study on the characters of antibiotic using chromatography technique and many kinds of eluent. Hoping that found new antibiotic could be applied to human healt and agricultural fields. Selection result showed that STG-1and OOH-1 isolates could be choosed as a candidate of selected bacteria. The next step of experiment STG-1 isolate should be selected as a model for optimization purpose on the fermentor scale. Optimization step was done based on the study the environmental conditions (pH value, agitation, temperature), nutrition sources (nitrogen and carbon), minerals sources (phosphor, potassium and calcium), and inoculum concentration. The optimization results showed that STG-1 isolate could produce antibiotic on the conditions such as inoculum concentration 10%, pH value 7, agitation 150 rpm; (NH₄)₂SO₄ was 1 g/l, K₂HPO₄ was 1.9 g/l, KCl was 1,5 g/l, and CaCl₂ was 0.013 g/l. Identification of STG-1 and OOH-1 isolates were based on the phenotypic characters (morphology, physiology, biochemistry, and API Kids test) and genotypic caharacters (amplification using primers 27F and 1492R gene sequence), analyzing was based on the similarity value (using Gene Bank data of BLAST program, and conctructed by phyllogeny tree). The phenotypic identification of STG-1and OOH-1 isolates resulted in close to *Pseudomonas luteola* with similarity values 99% and 87.9%, respectively. Identification based on the genotype resulted in that STG-1 isolate close to the *Pseudomonas brenneri* strain SFML with similarity value 99% and OOH-1 isolate close to the *Enterobacter xiangfangensis*. Identification of antibiotic produced by STG-1 isolate using Thin Layer Chromatography technique result showed that the intence yellowish spot color indicated the major compound. The spot was then removed and extracted by mixtures of chloroform : methanol (1:1). Up on filtration and evaporation was obtained residue, part of it was run in the GC-MS, FTIR, and NMR apparatus. GC-MS analysis indicated that 168 m/z was compound nuclear ion peak, FTIR analysis indicated that 2900 – 2800 cm⁻¹ bands were hydrocarbon compound, and according to mass spectrum data base was then identified as 1.2 *Benzene dicarboxylis acid diisooctyl ester*, with chemical formula C₂₄H₂₈O₄, molecular weight was 390 kD, and LC₅₀ was 0.01 – 0.02% againts to microbial indicaor of *Fusarium oxysporum*.

Keywords : endophytic bacteria, National Park of Java, antibiotic producer