Bakteri endofit tanaman bengang [neesia altissima bl. bl.] dan potensi senyawa bioaktif antibakteri penyebab diare. = Endophytic bacteria from bengang (neesia altissima bl.bl.) and their antibacterial compound against diarrhea causing bacteria

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Abstrak

Penelitian bertujuan untuk memperoleh dan mengidentifikasi isolat-isolat bakteri endofit yang potensial senyawa bioaktif antidiare dari tanaman N. altissima; mendeteksi, memurnikan dan mengidentifikasi senyawa bioaktif antidiare yang dihasilkan; serta menganalisis mekanisme kerjanya dalam menghambat pertumbuhan bakteri uji. Bakteri endofit diisolasi dari bagian akar, kulit batang, dan daun tanaman N. altissima. Bakteri endofit diisolasi dan dimurnikan menggunakan medium Nutrient Agar NA dan Luria Bertani LB agar. Aktinomisetes endofit diisolasi dan dimurnikan menggunakan medium Starch Casein Agar SCA dan International Streptomyces Project ISP 2 agar. Identifikasi bakteri dan aktinomisetes endofit dilakukan secara molekuler dengan melakukan analisis filogenetik sekuen nukleotida bakteri dari daerah 16S rRNA dengan metode Neighbour Joining NJ . Isolasi dan purifikasi senyawa dilakukan dengan metode maserasi menggunakan pelarut etil asetat dan kromatografi kolom. Senyawa bioaktif dideteksi dengan teknik Kromatografi Lapis Tipis KLT bioautografi. Senyawa bioaktif yang dihasilkan oleh bakteri dan aktinomisetes endofit diidentifikasi dengan menggunakan KLT, spektroskopi Resonansi Magnetik Inti NMR dan Spektroskopi Massa LC-MS . Mekanisme aksi dari senyawa bioaktif antidiare dianalisis dengan menggunakan mikroskop elektron scanning SEM . Dari 185 isolat bakteri endofit yang diperoleh, 104 isolat 56,21 dari bagian daun; 51 isolat 27,56 dari bagian kulit batang; dan 30 isolat 16,21 dari bagian akar. Sedangkan dari 33 isolat aktinomisetes endofit yang diperoleh, dua isolat 6,06 dari bagian kulit batang, 31 isolat 93,94 dari bagian akar, dan tidak diperoleh isolat aktinomisetes dari daun. Spesies bakteri endofit potensial ialah Pseudomonas aeruginosa strain UICC B-40, P. aeruginosa strain UICC B-93, dan P. azotoformans strain UICC B-91. Sedangkan aktinomisetes endofit potensial diidentifikasi sebagai Streptomyces sp. strain UICC B-92 dan Nonomuraea sp. strain UICC B-94. Hasil identifikasi senyawa menunjukkan bahwa senyawa bioaktif yang diperoleh dari P. aeruginosa strain UICC B-40 diduga merupakan senyawa metabolit baru, terdiri atas 2E,5E -phenyl tetradeca-2,5-dienoate C20H28O2 . Senyawa bioaktif aktinomisetes Streptomyces sp. strain UICC B-92 ialah 4-O-glucocyl, 1-carboxyl-phenazine C19H18N2O8 . Senyawa turunan phenazine dengan adanya gugus gula dari isolat Streptomyces sp. strain UICC B-92 diduga merupakan senyawa bioaktif baru. Hasil bioassai aktivitas antibakteri menunjukkan baik senyawa bioaktif dari P. aeruginosa strain UICC B-40 maupun Streptomyces sp. strain UICC B-92 menghambat bakteri Gram positif Bacillus cereus strain ATCC 10876 dan Staphylococcus aureus strain ATCC 25923. Mekanisme penghambatan dari kedua senyawa menunjukkan adanya aktivitas lisis terhadap membran sel bakteri uji, ditunjukkan dengan terjadinya pemanjangan ukuran sel, kerusakan dan kebocoran membran sel sehingga mengganggu permeabilitas membran sel dan akhirnya menyebabkan kematina sel. Senyawa metabolit P. aeruginosa strain UICC B-40 lebih potensial sebagai senyawa antidiare dibandingkan senyawa metabolit dari Streptomyces sp. strain UICC B-92.Kata kunci : antidiare, bakteri endofit, 16S rRNA, lisis, Neesia altissima, spektroskopi.

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The aims of this study were to obtain potential endophytic bacteria and actinomycetes from N. altissima as anti diarrhea bioactive producer and to screen and identify their anti diarrhea bioactive compound and to investigate the mechanism of action of the bioactive compound in inhibiting the growth of diarrhea causing bacteria. Media for endophytic bacteria isolation and purification were NA and LB agar, while media for endophytic actinomycetes isolation and purification were SCA and ISP2 agar. Identification of endophytic bacteria and actinomycetes was carried out based on phylogenetic analysis of DNA sequence generated from 16S rRNA region. Isolation, purification, and detection of bioactive compounds were carried out using maceration process, column chromatography and Thin Layer Chromatography TLC bioautography, respectively. Identification were elucidated using Nuclear Magnetic Resonance NMR and Liquid Chromatography Mass Spectroscopy LC MS analyses. The mechanism of action of bioactive compound were morphologically observed using scanning electron microscope SEM . In this study, from a total 185 endophytic bacteria obtained, 104 isolates 56.21 obtained from leaves, 30 isolates 16.21 from roots, and 51 isolates 27.56 from stem barks. From a total 33 endophytic actinomycetes isolates obtained, 31 isolates 93.94 from roots, two isolates 6.06 from stem barks, and no isolates obtained from leaves. Based on phylogenetic analysis of nucleotide sequence generated from 16S rRNA region, two isolates of endophytic bacteria determined as P. aeruginosa strain UICC B 40 and one isolate belongs to P. azotoformans strain UICC B 91 two isolates of endophytic actinomycetes determined as Streptomyces sp. strain UICC B 92 and Nonomuraea sp. strain UICC B 94 . On the basis of 1H NMR spectral data and supported with molecular weight data from LC MS analysis, bioactive compound from P. aeruginosa strain UICC B 40 was identified as growth associated metabolite, and determined as 2E,5E phenyl tetradeca 2,5 dienoate C20H28O2 . In addition, bioactive compound from Streptomyces sp. strain UICC B 92 was identified as 4 O glucocyl, 1 carboxyl phenazine C19H18N2O8 . The bioactive compound from Streptomyces sp. strain UICC B 92 is suggested as novel type of phenazine derivative. All of bioactive compounds showed high in vitro antibacterial activity against two Gram positive bacteria, Bacillus cereus strain ATCC 10876 and Staphylococcus aureus strain ATCC 25923. The bioactive compounds from P. aeruginosa strain UICC B 40 and Streptomyces sp. strain UICC B 92 showed membrane cell walls lysis mechanism. The cell walls of S. aureus strain ATCC 25923 and B. cereus strain ATCC 10876 were apparently damaged after treated by the antibacterial compound. Occurrence of local rupture or pore formation in the cell membranes was also found and causing leakage of essential intracellular constituents from the cells. The bioactive compound from P. aeruginosa strain UICC B 40 is more potential as anti diarrhea compound than that from Streptomyces sp. strain UICC B 92.Key words antidiarrhea, endophyte bacteria, 16S rRNA, lysis, Neesia altissima, spectroscopy.