Penapisan actinomycetes asal Raja Ampat, Papua penghasil antimikroba dan antikanker, dan identifikasi molekuler isolat-isolat terpilih = Screening of antimicrobial and anticancer activities of actinomycetes isolated irom Raja Ampat, Papua and molecular identification of selected isolates

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Abstrak

[ABSTRAK
Penelitian bertujuan memperoleh identitas isolat-isolat actinomycetes dari Raja Ampat, Papua penghasil antimikroba dan antikanker dengan aktivitas tertinggi. Penapisan aktivitas antimikroba dilakukan menggunakan metode difusi agar terhadap bakteri (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus) dan khamir (Candida albicans dan Saccharomyces cerevisiae). Metabolit dari isolat terpilih yang memiliki aktivitas antimikroba tertinggi dianalisis lanjut. Nilai Minimum inhibitor concentration (MIC) dari senyawa murni yang diproduksi isolat terpilih terhadap mikroba diukur menggunakan metode mikrodilusi. Kebocoran asam nukleat dan protein dari mikroba uji dideteksi dengan menggunakan metode spektrofotometri pada panjang gelombang 260 dan 280 nm. Kebocoran urasil dianalisis menggunakan HPLC. Perubahan morfologi diamati menggunakan scanning electron microscope (SEM). Uji sitotoksik dilakukan terhadap 6 sel kanker (MCM-B2, Leukemia, T47D, HeLa, A549, dan WiDr) dan satu sel normal (Vero) menggunakan metode trypan blue dan MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Sebanyak sembilan isolat potensial penghasil antimikroba tertinggi (BL-36-1, BL-20-2, BL-14-2, RC-SS-37-4, RC-SS-37-16, BL-22-1, BL-06-5, BL-22-3, dan BL-22-5) diidentifikasi berdasarkan data sekuen penuh gen 16S rRNA (± 1.500 PB). Hasil penelitian menunjukkan 44 % isolat actinomycetes memiliki aktivitas antimikroba. Senyawa F.5.1 merupakan senyawa murni yang diproduksi oleh isolat terpilih BL-22-5. Nilai MIC senyawa tersebut terhadap mikroba uji berkisar 16 -- 64 µg/ml. Aktivitas antifungi senyawa F.5.1 lebih tinggi dibandingkan antibakteri. Senyawa F.5.1 menyebabkan kebocoran protein, asam nukleat, dan urasil pada mikroba uji. Nilai IC50 senyawa F.5.1 berkisar 0,02 -- 3,81 µg/ml terhadap sel kanker dan ≥ 30.000 µg/ml untuk sel normal. Isolat RCSS-37-4, RC-SS-37-16, BL-22-3, dan BL-22-5 teridentifikasi sebagai Streptomyces costaricanus (100 %), Streptomyces costaricanus (99,8 %), Streptomyces parvulus (98,6 %), dan Streptomyces badius (98,9 %). Lima isolat teridentifikasi sebagai Streptomyces spp. (BL-36-1, BL-20-2, BL-14-2, BL-22-1 dan BL-06-5) dan menjadi kandidat spesies baru karena memiliki nilai homologi yang rendah terhadap spesies terdekatnya (93,9% -- 97,4 %).

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ABSTRACT
The objective of this research was to obtain the identity of actinomycetes isolates from Raja Ampat, Papua-producing antimicrobial and anticancer with the highest activity. Antimicrobial screening was conducted based on agar diffusion method against bacteria (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus) and yeast (Candida albicans and Saccharomyces cerevisiae). Metabolite from selected isolates with the highest antimicrobial activity was chosen for subsequent analysis. Minimum inhibitor concentration (MIC) value against microbial tested of pure bioactive compound was determined using microdilution method. Leakages of nucleic acids and proteins from microbial tested were detected using UV-vis spectrophotometer method on 260 and 280 nm wavelengths. Uracil Leakage was analyzed using HPLC. Morphological changes were observed using a scanning electron microscope (SEM). Determination of cytotoxic activity was conducted in 6 cell lines (MCM-B2, Leukemia, T47D, HeLa, A549, and WiDr) and normal cell (Vero) using trypan blue and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) methods. Nine selected isolates with the highest antimicrobial activity (BL-36-1, BL-20-2, BL-14-2, RC-SS-37-4, RC-SS-37-16, BL-22-1, BL- 06-5, 22-3-BL and BL-22-5) were identified based on full sequence of 16S rRNA gene (1,500 bp). The results showed 44% of isolates of actinomycetes have antimicrobial activity. F.5.1 is a compound produced by the selected isolate (BL-22-5). MIC values of this compound against tested microbes in range of 16 -- 64 µg/ml. Antifungal activity from F.5.1 compound was higher than antibacterial activity. F.5.1 compound caused leakage of proteins, nucleic acids, and uracil on tested microbes. Inhibitor concentration 50% (IC50) value in the range of 0.02 -- 3.81 µg/ml against cancer cells and ≥ 30,000 µg/ml for normal cells. Isolate RC-SS-37-4, RC-SS-37-16, BL-22-3, and BL-22-5 have been identified as Streptomyces costaricanus (100%), Streptomyces costaricanus (99.8%), Streptomyces parvulus (98.6%), and Streptomyces badius (98.9%), respectively. Five isolates (BL-36-1, BL-20-2, BL-14-2, BL-22-1 and BL-06-5) were identified as Streptomyces spp. and can be presumed as candidates for new species because of the low homology value to their closest related species (93.9 % -- 97.4%).;The objective of this research was to obtain the identity of actinomycetes isolates
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