ABSTRAK

Eksplorasi mikrob penghasil antibiotik terus menerus dilakukan bersamaan dengan perkembangan penyakit infeksi saat ini. Masalah utama dalam skrining empiris antibiotik adalah penemuan kembali (replikasi) molekul yang sudah dikenal setelah rangkaian penelitian panjang dilakukan. Oleh karena itu perlu penerapan pendekatan sistematis untuk dereplikasi. Penelitian ini bertujuan menerapkan metode kombinasi analisis molekuler dan spektroskopi infra merah untuk seleksi bakteri penghasil senyawa antibakteri.

Penelitian dilakukan dengan menentukan aktivitas ekstrak etil asetat cairan kultur dan hasil fraksinasi dengan metode sumuran dan bioautografi. Fraksi aktif dianalisis keragaman dengan spektroskopi IR. Keragaman genetik isolat bakteri dilakukan terhadap produk PCR gen PKS I (*Polyketide Synthase*) dan NRPS (*Non Ribosomal Peptide Synthetase*). Analisis keragaman dilakukan dengan statistik multivariat. Bakteri penghasil yang terpilih diidentifikasi berdasarkan sekuen gen 16S rRNA dan fraksi aktif dianalisis dengan LC-TOF-MS untuk mengetahui perbedaan kandungan kimia di dalamnya.

Hasil penelitian menunjukkan bahwa 12 dari 27 isolat bakteri menunjukkan aktivitas antibakteri baik pada ekstrak maupun hasil fraksinasi. Hasil analisis keragaman profil RFLP gen NRPS diperoleh 3 klaster yaitu isolat KP13; J4, LP6 & TL; serta J3, J5, J7,JS, LP, P301, P302 & T25A. Adapun hasil analisis keragaman spektra IR diperoleh 3 klaster yaitu J4, J7, P302, TL; JS, LP, LP6 & P301; serta J3, J5, KP13 & T25A. Ketiga isolat (KP13, J4 dan P301) yang terpilih mewakili tiap klaster menunjukkan perbedaan takson dan kandungan kimia. Berdasarkan analisis BLAST terhadap sekuen gen 16S rRNA isolat terpilih, isolat KP13 diidentifikasi sebagai *Stenotrophomonas sp*, isolat J4 adalah *Burkholderia sp*, sedangkan isolat P301 merupakan *Achromobacter sp*. Profil spektra massa senyawa yang terkandung dalam fraksi aktif isolat KP13 menghasilkan *basepeak* dengan nilai m/z sebesar 164, 274, 358, 331 dan 376, pada isolat J4 sebesar 270, 404 dan 274, sedangkan pada isolat P301 sebesar 103, 127, 123, 100, 146, 187, 190, 398, 915, 312 dan 404.

Dengan demikian, penerapan pendekatan kombinasi spektroskopi IR dan molekuler, telah memilih 3 isolat yaitu KP13, J4 dan P301 yang berbeda takson dan kandungan kimia.

Kata kunci : PKS I, NRPS, antibiotik, spektra IR, keragaman

ABSTRACT

Nowadays, the exploration of microbial sources producing antibiotic was carried out continuously following the growth of infection deseases. The main problem in empiric antibiotic screening is refinding (replication) the known molecule after a long research done. Hence, it needs a systematic approach for dereplication. This study aims to apply a combination method of molecular analyses and infrared spectroscopy for selecting antibacterial-producing bacteria.

Research was performed by examining the antibacterial activity of ethyl acetate extracts of culture broth and their fractions using cup-plate method and bioautography. The active fractions were then analysed using IR spectroscopy to determine their diversity. More over, the genetic diversity was determined using PCR products of PKS I and NRPS genes. The both diversities were analysed by multivariate statistic. The selected bacteria was identified based on the 16S rRNA gene sequence and the active fractions were characterized by LC-TOF-MS to explore their chemical contents.

Result showed 12 of 27 bacteria isolates revealed antibacterial activity of both extracts and their fractions. Based on the RFLP profiles of NRPS genes, the 12 isolates were classified into 3 groups of which KP13; J4, LP6 & TL; as well as J3, J5, J7,JS, LP, P301, P302 & T25A. While based on IR spectra, there were 3 groups i.e. J4, J7, P302, TL; JS, LP, LP6 & P301; as well as J3, J5, KP13 & T25A. The 3 selected isolates (KP13, J4 and P301) which represent each cluster show distinct in taxon and chemical content. The BLAST of 16S rRNA sequence revealed that KP13 was *Stenotrophomonas sp*, J4 was *Burkholderia sp*, while P301 was *Achromobacter sp*. The m/z values appeared in mass spectra profile of chemical contents in the active fraction of KP13 were 164, 274, 358, 331 and 376, the m/z values in J4 were 270, 404 and 274, while m/z values in P301 were 103, 127, 123, 100, 146, 187, 190, 398, 915, 312 and 404.

Thus, the application using a combination approach of IR spectroscopy and molecular, has selected three isolates with distinct taxon and chemical contents.

Keywords : PKS I, NRPS, antibiotic, IR spectra, diversity