

ABSTRACT

ISOLATION OF POLYKETIDE SYNTHASE (PKS) GENE SEQUENCE FROM THE SPONGE-ASSOCIATED AKTINOBACTERIA

Pseudonocardia carboxydivorans 18A13O1

By

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Drug-resistant bacteria pose a serious risk to people's health, making it necessary to search for new antibacterials from natural sources such as marine sponges. The bioactive compounds of sponges are mostly produced by microbial symbionts, especially actinobacteria, which are well-known for their high G+C content and variety of bioactive compounds. *Pseudonocardia carboxydivorans* is one species of marine actinobacteria that has the potential to produce bioactive compounds. These compounds are often complex polyketides synthesised by the polyketide synthase (PKS) gene. Therefore, screening of PKS genes can reveal the potential for new bioactive compounds. This study aimed to isolate the polyketide synthase (PKS) gene sequence from the marine actinobacteria *Pseudonocardia carboxydivorans* 18A13O1 associated with sponges. Samples were enriched on 1% colloidal chitin agar medium, followed by microscopic observation to ensure the purity of the isolates. Afterwards, the isolates were subcultured on liquid ISP-2 medium and incubated for 7 days. DNA was extracted using PROMEGA Kit and analysed for concentration and purity with a nanophotometer. Subsequently, PKS sequences were amplified using primers PKS1. Amplification results were visualised by 1.2% agarose gel electrophoresis. Sequencing was performed by the Sanger method. Sequencing results were analysed using BioEdit and Open Reading Frame (ORF)-Finder from NCBI. The results showed that the PKS gene sequences obtained encoded hexokinase proteins, which are known to contribute to the production of antibiotic compounds.

Keywords: *Pseudonocardia carboxydivorans*, antibacterial, PKS, PCR, Hexokinase

ABSTRAK

ISOLASI SEKUENS GEN *POLYKETIDE SYNTHASE* (PKS) DARI AKTINOBAKTERI *Pseudonocardia carboxydivorans* 18A13O1 YANG BERSIMBIOSIS DENGAN SPONS

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Bakteri resisten obat menimbulkan risiko serius bagi kesehatan masyarakat, sehingga diperlukan pencarian antibakteri baru dari sumber alami seperti spons laut. Senyawa bioaktif spons sebagian besar dihasilkan oleh mikroba simbiosis, khususnya aktinobakteri, yang terkenal dengan kandungan G+C tinggi dan variasi senyawa bioaktifnya. *Pseudonocardia carboxydivorans* merupakan salah satu jenis aktinobakteri laut yang berpotensi menghasilkan senyawa bioaktif. Senyawa ini sering merupakan poliketida kompleks yang disintesis oleh gen polyketide synthase (PKS). Oleh karena itu, skrining gen PKS dapat mengungkap potensi senyawa bioaktif baru. Penelitian ini bertujuan mengisolasi sekuens gen *polyketide synthase* (PKS) dari aktinobakteri laut *Pseudonocardia carboxydivorans* 18A13O1 yang bersimbiosis dengan spons. Sampel diremajakan pada media 1% koloid kitin agar, dilanjutkan dengan pengamatan mikroskopis untuk memastikan kemurnian isolat. Kemudian, isolat disubkultur pada media ISP-2 cair dan diinkubasi selama 7 hari. DNA diekstraksi menggunakan *Kit PROMEGA* dan dianalisis konsentrasi dan kemurniannya dengan nanofotometer. Kemudian sekuens PKS diamplifikasi menggunakan primer PKS1. Hasil amplifikasi divisualisasi dengan elektroforesis gel Agarosa 1,2%. Sekuensing dilakukan dengan metode *Sanger*. Hasil sekunsing dianalisis menggunakan BioEdit dan laman bioinformatika *Open Reading Frame* (ORF)-Finder NCBI. Hasil penelitian menunjukkan bahwa sekuens gen PKS yang diperoleh mengkodekan protein heksokinase, yang diketahui berkontribusi dalam produksi senyawa antibiotik.

Kata kunci: *Pseudonocardia carboxydivorans*, antibakteri, PKS, PCR, Heksokinase