

## **Karakterisasi Molekular (gen *polyketide synthase* dan gen *non-ribosomal peptide synthetase*) Isolat Aktinobakteria Symbion Sponge, Tulamben, dan Aktivitas Anti *Vibrio harveyi***

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### **INTISARI**

Salah satu sumber antibakteri adalah *sponge*, yang pada umumnya berasosiasi dengan aktinobakteria. Aktinobakteria penghasil antibakteri khususnya terhadap *Vibrio harveyi* mempunyai senyawa yang termasuk poliketida disandi oleh gen *polyketide synthase* (*pks*) dan peptida non-ribosomal disandi oleh gen *non-ribosomal peptide synthetase* (*nrps*). Penelitian bertujuan untuk menyeleksi isolat aktinobakteria dari spons penghasil antibakteri khusus yang memiliki gen *pks* dan *nrps*, serta menguji aktivitas antibakteri isolat terhadap *Vibrio harveyi*.

Penelitian ini menggunakan 15 isolat aktinobakteria symbion *sponge* yang berbeda diseleksi berdasarkan keberadaan gen *pks* dan gen *nrps*. Uji kemampuan isolat terpilih menghasilkan senyawa bioaktif melalui kultivasi skala plat kecil 7ml (*6-wellplate*) dengan menggunakan enam media berbeda (GYMM, ISP-2, M-17, M-29, M31, M-51). Senyawa bioaktif (antibakteri) pada masing-masing kultur dideteksi secara kualitatif menggunakan kromatografi lapis tipis (klt). Ekspresi gen senyawa bioaktif diuji berdasarkan kemampuan untuk menghambat pertumbuhan bakteri *Vibrio harveyi* menggunakan format *96-wellplate*. Isolat terseleksi diuji lebih lanjut melalui kultivasi skala 100ml pada medium cair yang sesuai, kemudian diekstrak dengan resin HP-20 menggunakan metanol. Kehadiran senyawa dalam *crude* ekstrak dideteksi menggunakan KLT. Analisis sekuen gen 16S rRNA serta analisis sekuen gen *nrps* dilakukan untuk mendapatkan prediksi potensi metabolit sekunder.

Hasil penelitian menunjukkan bahwa dari 15 isolat yang digunakan, hanya 11 (73%) dari 15 isolat yang positif amplifikasi gen 16S rRNA aktinobakteria. Sepuluh (10) dari 11 isolat (91%) memiliki gen *nrps*, serta tidak ada isolat yang memiliki gen *pks*. Empat isolat aktinobakteria memiliki aktivitas antibakteri terhadap *V. harveyi*. *Crude* ekstrak yang didapatkan dari kultivasi medium cair terpilih masih dapat memacu produksi poliketida dibuktikan warna ungu dengan nilai Rf 0.36 pada isolat CG-5-613-2. Hasil analisis sekuen gen 16S rRNA menunjukkan isolat CG-5-613-2 memiliki similaritas dengan *Nocardiopsis alba* strain Tata-5 sebesar 99% dan isolat CG-17-613-2 memiliki similaritas dengan *Brevibacterium salitolerans* strain TRM 415 sebesar 96%. Analisis sekuen gen *nrps* diperoleh similaritas isolat CG-5-613-2 dengan *Streptomyces venezuelae* ATCC 15439 sebesar 87%, dan isolat CG-17-613-2 dengan *Streptomyces capillispiralis* sebesar 94%. Isolat dapat dijadikan kandidat untuk proses mendapatkan senyawa bioaktif metabolit sekunder lebih lanjut.

Kata kunci: *anti bakteri, gen pks, gen nrps, aktinobakteria, sponge*

## Molecular characterization (*polyketide synthase and non-ribosomal peptide synthetase gene*) of sponge-associated actinobacteria from Tulamben, and anti *Vibrio harveyi* activity

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### ABSTRACT

Sponge-associated actinobacteria known as a prolific source of bioactive compounds especially polyketide and non-ribosomal peptide compounds. These two compounds were synthesized using *polyketide synthase* and *non-ribosomal peptide synthetase* gene and known to produce antibacterial against *Vibrio harveyi*. Hence, the aim of this research was to select actinobacteria which contained *pks* and *nrps* gene and its activity against *Vibrio harveyi*.

The research conducted through several step; actinobacteria reculture, 16S rRNA, *pks* and *nrps* gene detection, microscale cultivation 7ml using 6 different media to obtain a variant of supernatant which contains bioactive compounds. Supernatants were subjected to detection of the compound using TLC and also qualitative antibacterial bioassay in 96-wellplate format against *Vibrio harveyi*. Selected isolate then cultivated and extracted with HP-20 resin using methanol solvent. Crude extract then employed to compound detection using TLC. Furthermore, sequence analysis of 16S rRNA gene and *nrps* gene were done to obtain secondary metabolite prediction.

Among 15 isolates used in the research, 11 isolates (73%) successfully amplified with actinobacteria 16S rRNA gene. However, none of the isolates were amplified for *pks* gene while 10 isolates (91%) were successfully amplified for *nrps* gene. In addition, four isolates contain antibacterial activity against *V. harveyi*. According to compound detection on crude extract, selected cultivation condition still carried out the production of polyketide compound. Showed through violet spot with Rf 0.36 on isolate CG-5-613-2. 16S rRNA gene sequence analysis showed isolate CG-5-613-2 similar with *Nocardiopsis alba* strain Tata-5 (99%) while isolate CG-17-613-2 similar with *Brevibacterium salitolerans* strain TRM 415 (96%). Furthermore, *nrps* gene sequence analysis showed isolate CG-5-613-2 similar with *nrps* sequence of *Streptomyces venezuelae* ATCC 15439 (87%) while isolate CG-17-613-2 similar with *Streptomyces capillispiralis* (94%). Those isolates were found to be a potential candidate for further isolation of bioactive compounds.

Keywords: *antibacterial, pks gene, nrps gene, actinobacteria, sponge*