CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS FROM MARINE SPONGE-ASSOCIATED *Streptomyces* spp. AGAINST SOME GRAM-NEGATIVE PATHOGENIC BACTERIA

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RINGKASAN

FIRDA SRI EFENDI. Karakterisasi Senyawa Antibakteri dari *Streptomyces* spp. Asosiasi Spons Laut terhadap Beberapa Bakteri Patogen Gram Negatif. Dibimbing oleh YULIN LESTARI dan SRI BUDIARTI

Ancaman yang muncul dari bakteri patogen yang menyebabkan berbagai penyakit infeksi menjadi perhatian penting di seluruh dunia. Kasus ini menimbulkan ancaman serius karena bakteri patogen juga dapat resisten terhadap antibiotik yang biasa diresepkan sehingga saat ini telah banyak antibiotik yang digunakan menjadi tidak efektif dalam mengobati penyakit infeksi. Bakteri patogen yang resisten terhadap antibiotik tidak hanya menyebabkan melonjaknya beban ekonomi pada perawatan kesehatan dan lama tinggal di rumah sakit tetapi juga meningkatkan morbiditas dan mortalitas. Krisis resistensi antibiotik yang terjadi pada bakteri patogen menimbulkan kebutuhan mendesak akan agen antibakteri sehingga diperlukan pendekatan untuk mengendalikan infeksi bakteri patogen. Salah satu pendekatan potensial adalah dengan memanfaatkan potensi antibakteri dari mikroorganisme yaitu *Streptomyces* spp. *Streptomyces* merupakan bakteri Gram positif dari filum aktinobakteri yang dikenal sebagai penghasil senyawa bioaktif yang beragam terutama antibiotik. Sekitar 80% antibiotik yang saat ini digunakan secara medis dan tersedia secara komersial diisolasi dari *Streptomyces*. Saat ini, *Streptomyces* secara berkelanjutan terus dieksplorasi untuk penemuan senyawa antibakteri dengan efikasi yang lebih baik dan biaya produksi yang lebih rendah. Oleh karena itu, penelitian ini bertujuan mengkarakterisasi senyawa antibakteri dari *Streptomyces* spp. asosiasi spons laut Kepulauan Seribu Indonesia terhadap beberapa bakteri patogen Gram negatif.

Penelitian ini dimulai dengan uji resistensi beberapa bakteri patogen Gram negatif, yaitu *Extended Spectrum β-Lactamase* (ESBL)-*producing Escherichia coli*, *Providencia rettgeri*, *Proteus mirabilis*, dan *Pseudomonas putida* terhadap 12 antibiotik komersial untuk menentukan profil resistensi antibiotik pada bakteri patogen tersebut, sehingga dapat memandu dalam mengoptimalkan produksi senyawa antibakteri oleh *Streptomyces* spp. asosiasi spons laut dalam menghambat bakteri patogen. Selanjutnya, dilakukan peremajaan 3 isolat *Streptomyces* spp. asosiasi spons laut, yaitu Car21t, Crc27t, dan Dbi28t pada 3 media pertumbuhan, yaitu *International Streptomyces Project* 2 (ISP2), ISP4, dan media molase yang dimodifikasi, kemudian dilanjutkan dengan proses optimasi produksi senyawa antibakteri meliputi pemilihan media yang sesuai (ISP2, ISP4, dan molase yang dimodifikasi) untuk mendukung ketersediaan nutrisi dan bahan organik yang dapat mempengaruhi karakter dan kemampuan *Streptomyces* spp. dalam menghasilkan senyawa antibakteri terhadap beberapa bakteri patogen. Adapun telaah pustaka menunjukkan bahwa sampai saat ini belum ada laporan penggunaan media molase yang dimodifikasi untuk produksi senyawa antibakteri oleh *Streptomyces* spp. asosiasi spons laut. Selanjutnya, optimasi waktu inkubasi juga dilakukan dengan menginkubasi 3 isolat *Streptomyces* spp. pada 4 waktu yang berbeda, yaitu 5, 10, 15, dan 20 hari dan setiap masa inkubasi selesai masing-masing kultur *Streptomyces* spp. dipanen dan dilakukan uji antibakteri untuk mengetahui waktu pertumbuhan optimal *Streptomyces* spp. dalam menghambat bakteri patogen. Produksi senyawa antibakteri oleh *Streptomyces* spp. dilakukan dengan menumbuhkan setiap isolat

Streptomyces spp. pada medium pertumbuhan dan waktu inkubasi terbaik, lalu dilakukan ekstraksi menggunakan pelarut etil asetat. Ekstrak etil asetat yang diperoleh kemudian dilakukan uji antibakteri terhadap bakteri patogen uji. Ekstrak etil asetat *Streptomyces* spp. yang memiliki aktivitas antibakteri terkuat dipilih untuk dikarakterisasi kandungan senyawa antibakterinya menggunakan *Liquid Chromatography-tandem Mass Spectrometric* (LC-MS/MS).

Hasil penelitian menunjukkan bahwa ESBL-producing *E. coli*, *P. mirabilis*, *P. rettgeri* dan *P. putida* telah resisten terhadap 3 kelas antibiotik (*β-lactam*, *fluoroquinolones*, dan *aminoglycosides*). Hasil optimasi produksi senyawa antibakteri menunjukkan bahwa media molase yang dimodifikasi mampu meningkatkan pertumbuhan dan aktivitas antibakteri *Streptomyces* spp. terhadap ESBL-*producing E. coli*, *P. mirabilis*, *P. rettgeri*, dan *P. putida*. Aktivitas antibakteri tertinggi terlihat pada waktu produksi 15 hari. Hal ini diduga karena pada waktu inkubasi 15 hari, kondisi pertumbuhan terbatas sehingga isolat *Streptomyces* spp. mengeluarkan senyawa antibakteri untuk mempertahankan diri dari kondisi keterbatasan nutrisi. Hal ini membuktikan bahwa media molase yang dimodifikasi mampu menginduksi dihasilkannya senyawa antibakteri oleh *Streptomyces* spp., sehingga dapat digunakan sebagai media pertumbuhan dan produksi senyawa antibakteri dengan waktu inkubasi 15 hari sebagai acuan untuk waktu produksi senyawa antibakteri. Ekstrak etil asetat *Streptomyces* sp. Dbi28t diketahui mampu menghasilkan aktivitas antibakteri terkuat dibanding 2 ekstrak etil asetat lainnya. Ekstrak etil asetat *Streptomyces* sp. Dbi28t sebesar 20 µL 1000 µg/mL menunjukkan zona penghambatan signifikan terhadap ESBL-*producing E. coli* dan *P. rettgeri* kemudian diikuti oleh *P. mirabilis* dan *P. putida* dan kekuatan penghambatan dikategorikan sangat kuat dan kuat, serta aktivitas antibakteri yang dihasilkan lebih tinggi dibandingkan beberapa antibiotik komersial yang diujikan. Oleh sebab itu, ekstrak ini dipilih untuk ditentukan profil senyawa antibakterinya dengan mengkarakterisasi menggunakan LC-MS/MS. Sembilan senyawa antibakteri teridentifikasi, dengan kelimpahan tertinggi *pumilacidin* A, kemudian diikuti oleh *pumilacidin* B, *surfactin* B, *surfactin* A, *phenazostatin* B, *chalcomycin* B, *neopyrrolomycin* C, *saquayamycin* A dan *saphenamycin*. Penelitian ini memberikan laporan pertama dari *Streptomyces* sp. Dbi28t menghasilkan *pumilacidin*, *surfactin* dan senyawa antibakteri lainnya dengan media molase yang dimodifikasi untuk mengoptimalkan karakterisasi senyawa antibakterinya.

Kata kunci: antibakteri, LC-MS/MS, molase, resistensi antibiotik, *Streptomyces*

SUMMARY

FIRDA SRI EFENDI. Characterization of Antibacterial Compounds from Marine Sponge-associated *Streptomyces* spp. against Some Gram-Negative Pathogenic Bacteria. Supervised by YULIN LESTARI and SRI BUDIARTI.

The emerging threat of pathogenic bacteria that cause various infectious diseases is a great concern worldwide. This case poses a serious threat as pathogenic bacteria can develop resistance to commonly prescribed antibiotics, rendering many treatments ineffective against infectious diseases. Antibiotic-resistant pathogenic bacteria not only cause soaring economic burdens on health care and length of hospital stay but also increase morbidity and mortality. The antibiotic resistance crisis that occurs in pathogenic bacteria raises an urgent need for antibacterial agents, so some approaches to control the pathogenesis of these pathogens are required. One potential approach is to take advantage of the antibacterial potential of the microorganism, namely *Streptomyces* spp. *Streptomyces* is a Gram-positive bacterium from the phylum Actinobacteria, well-known for producing a variety of bioactive compounds, mainly antibiotics. Almost 80% of all commercial antibiotics used today are isolated from *Streptomyces*. *Streptomyces* is continuously being explored for the discovery of new antibacterial compounds with better efficacy and lower production costs. Therefore, this study aimed to characterize antibacterial compounds of marine sponge-associated *Streptomyces* spp. from the Seribu Islands of Indonesia against some Gram-negative pathogenic bacteria.

The research started with antibiotic susceptibility testing using 12 commercially available antibiotic discs, to determine the antibiotic resistance profile of the Gram-negative pathogenic bacteria including *Extended Spectrum β-Lactamase* (ESBL)-*producing Escherichia coli*, *Providencia rettgeri*, *Proteus mirabilis*, and *Pseudomonas putida*, so that it can guide in optimizing the production of antibacterial compounds by marine sponge-associated *Streptomyces* spp. inhibits pathogenic bacteria. Furthermore, the rejuvenation of 3 isolates of marine sponge-associated *Streptomyces* spp., namely Car21t, Crc27t, and Dbi28t on 3 growth media, International Streptomyces Project 2 (ISP2), ISP4, and modified molasses media, then continued with the process of optimizing growth conditions which include the selection of suitable media to support the availability of nutrients and organic matter that can affect the character and ability of *Streptomyces* spp. in producing more optimal antibacterial compounds. Optimization of the incubation time of marine sponge-associated *Streptomyces* spp. was also carried out to determine the optimal growth time of *Streptomyces* spp. in inhibiting pathogenic bacteria. The literature review shows that no data is available regarding the use of a modified molasses medium for the production of antibacterial compounds by marine sponge-associated *Streptomyces* spp. Furthermore, the incubation time optimization was also carried out by incubating 3 isolates *of Streptomyces* spp. at 4 different times, 5, 10, 15, and 20 days and each incubation period was completed, each culture of *Streptomyces* spp. was harvested, and an antibacterial test was carried out to determine the optimal growth time of *Streptomyces* spp. in inhibiting pathogenic bacteria. The production of antibacterial compounds by *Streptomyces* spp. is carried out by growing each *Streptomyces* spp. isolate at the best growth medium and incubation time, then extraction is carried

out using ethyl acetate solvent. The ethyl acetate extract obtained was then subjected to antibacterial tests against test pathogenic bacteria. The ethyl acetate extract of *Streptomyces* spp. with the strongest antibacterial activity was selected to characterize the content of antibacterial compounds using *Liquid Chromatography-tandem Mass spectrometry* (LC-MS/MS).

The result of the study showed that the Gram-negative pathogenic bacteria including ESBL-producing *E. coli*, *P. rettgeri*, *P. mirabilis*, and *P. putida* were multidrug-resistant pathogens to the three antibiotic classes including β-lactam, fluoroquinolones, and aminoglycosides. The result of the optimization of antibacterial compounds production showed that the modified molasses medium was able to increase the growth and antibacterial activity of *Streptomyces* spp. against ESBL-producing *E. coli*, *P. mirabilis*, *P. rettger*, and *P. putida*. The highest antibacterial activity was observed after a 15-day incubation period. This observation is attributed to the limited growth conditions during the incubation period, which stimulates *Streptomyces* spp. isolates to secrete antibacterial compounds as a defense. This proves that the modified molasses medium can induce the production of antibacterial compounds by *Streptomyces* spp., so that it can be used as a medium for the growth and production of antibacterial compounds with an incubation time of 15 days as a reference for the production time of antibacterial compounds. The ethyl acetate extract of *Streptomyces* sp. Dbi28t is known to be able to produce the strongest antibacterial activity compared to two other ethyl acetate extracts. The ethyl acetate extract of marine sponge-associated *Streptomyces* sp. Dbi28t of 20 µL in 1000 µg/mL exhibited a significant inhibitory zone against pathogenic bacteria including ESBL-producing *E. coli*, and *P. rettgeri*, then followed by *P. mirabilis* and *P. putida* whereas the strength of inhibition is categorized as very strong and strong, as well as the results of antibacterial activity against these pathogenic bacteria were higher than some commercial antibiotics. Therefore, this extract was selected to determine the profile of its antibacterial compounds by characterizing them using LC-MS/MS. This study has identified nine antibacterial compounds in Dbi28t, with the most abundance belonging to pumilacidin A, then followed by pumilacidin B, surfactin B, surfactin A, phenazostatin B, chalcomycin B, neopyrrolomycin C, saquayamycin A and saphenamycin. This work provides the first report from a *Streptomyces* sp. Dbi28t produced pumilacidin, surfactin, and other antibacterial compounds with the modified molasses medium for optimization of characterization of its antibacterial compounds.

Keywords: antibacterial, antibiotic resistance, LC-MS/MS, molasses, *Streptomyces*

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The author hopes that this thesis can be useful for readers, especially those who have an interest in microbiology. The author realizes that in the preparation and writing of this thesis, there are still many shortcomings, so the author hopes for suggestions. Hopefully, this thesis can be useful for those who read it.

Bogor, August 2024

Firda Sri Efendi

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