RINGKASAN

Agarase merupakan enzim utama yang mampu mendegradasi polimer agar pada lingkungan laut. Enzim ini memiliki kemampuan untuk menghidrolisis polimer agar menjadi agarooligosakarida (AOS), neoagarooligosakarida (NAOS), neoagarobiosa (NAB), dan 3,6-anhidro-L-galaktosa (L-AHG). Keempat molekul tersebut dapat diaplikasikan sebagai biomaterial fungsional seperti antiinflamasi, antioksidan, antidiabetes, antimikroba, antiobesitas, prebiotik, pemutih kulit, dan pelembab. Enzim agarase sering ditemukan pada mikroorganisme yang bersimbiosis dengan alga penghasil agar. Enzim ini juga didapati pada mikroorganisme yang hidup di air laut, sedimen, air tawar, tanah, moluska laut, dan fungi. Penemuan mikroorganisme penghasil agarase masih sangat dibutuhkan, terutama dari lingkungan laut Indonesia. Oleh karena itu, penelitian ini bertujuan untuk mengetahui adanya bakteri penghasil agarase yang bersimbiosis dengan *marine sponge* asal Selat Lembeh, kemudian mengidentifikasi isolat terpilih berdasarkan tingkat kemiripan sekuens DNA, karakter fenetik dan biokimia.

Kelompok riset *Microbial-Omics*, Pusat Riset Mikrobiologi Terapan, dari Organisasi Riset Hayati dan Lingkungan, BRIN telah melakukan studi pendahuluan dengan mengisolasi mikroorganisme yang bersimbiosis dengan *marine sponge* asal Selat Lembeh, Bitung, Sulawesi Utara. Terdapat 23 isolat yang berhasil diisolasi, namun penelitian mengenai kemampuannya dalam menghasilkan agarase masih belum diketahui. Metode penelitian dilakukan melalui seleksi isolat pada media *double layer agar,* uji DNS, deteksi kemampuan produksi oligosakarida menggunakan TLC, penentuan berat molekul menggunakan SDS-PAGE dan zimogram, dan karakterisasi pH dan suhu optimum enzim. Isolat terpilih yang menghasilkan agarase potensial selanjutnya diidentifikasi secara molekuler dengan sekuensing gen 16S rRNA dan dikarakterisasi secara fenetik dengan pengamatan mikromorfologi dan makromorfologi serta uji biokimia. Data hasil sekuensing DNA disejajarkan menggunakan BLAST dan dikonstruksi pohon filogenetiknya menggunakan *software* MEGA. Data karakteristik fenetik dan biokimia diselaraskan dengan *Bergey's Manual of Determinative Bacteriology*.

Hasil penelitian mendapatkan 8 isolat simbion *marine sponge* mampu menghasilkan enzim agarase. Isolat 26 menjadi isolat terpilih penghasil agarase potensial dengan aktivitas enzim sebesar 0,208 U/mL pada kondisi optimum pH 9 dan suhu 80°C. Identifikasi gen 16S rRNA dan karakterisasi mikrobiologis menunjukkan isolat 26 termasuk genus *Bacillus.* Isolat 26 memiliki kemiripan 99,59% dengan *Bacillus halosaccharovorans* T3H7 dan *Bacillus niabensis* BLB2.

Kata kunci: *agarase, gen 16S rRNA, identifikasi fenetik, identifikasi molekuler, seleksi.*

SUMMARY

Agarase is the main enzyme capable of degrading agar polymers in the marine environment. This enzyme has the ability to hydrolyze polymers become agarooligosaccharides (AOS), neoagarooligosaccharides (NAOS), neoagarobiose (NAB), and 3,6-anhydro-L-galactose (L-AHG). These four molecules can be applied as functional biomaterials such as anti-inflammatory, antioxidant, anti-diabetic, anti-microbial, anti-obesity, prebiotic, skin whitener and moisturizer. The agarase enzyme is often found in microorganisms that are in symbiosis with agar-producing algae. This enzyme also obtained from microorganisms that live in sea water, marine sediment, freshwater, soil, marine mollusks and fungi. The discovery of agarase-producing microorganisms still needed, especially from the Indonesian marine environment. Therefore, this study aims to determine the presence of agarase-producing bacteria from marine sponge in the Lembah Strait through identifying selected isolates based on DNA sequence similarity, phenetic and biochemical characters.

The Microbial-Omics research group, Center for Applied Microbiology Research, from the Biological and Environmental Research Organization, BRIN has conducted preliminary research by isolating microorganisms that are in symbiosis with marine sponges from the Lembeh Strait, Bitung, North Sulawesi. There were 23 isolates that were isolated, but research regarding their ability to produce agarase is still unknown. The research method was carried out through selection of isolates on double layer agar media, DNS test, detection of oligosaccharide production using TLC, determination of molecular weight using SDS-PAGE and zymogram, and characterization of the pH and optimum temperature of the enzyme. Selected isolates that produced agarase were identified molecularly by 16S rRNA gene sequencing and characterized phenetically by micromorphological and macromorphological observations as well as biochemical tests. Data from DNA sequencing were aligned using BLAST and a phylogenetic tree was constructed using MEGA. Data on phenetic and biochemical characteristics were then aligned with Bergey's Manual of Determinative Bacteriology.

The results of the research obtained 8 isolates of marine sponge symbionts which were capable of producing the agarase enzyme. Isolate 26 was the selected isolate as a potential agarase with an enzyme activity of 0,208 U/mL at optimum conditions of pH 9 and temperature 80°C. Identification of the 16S rRNA gene and microbiological characterization showed that this isolate belonged to the genus *Bacillus*. This isolate has 99.59% similarity to *Bacillus halosaccharovorans* T3H7 and *Bacillus niabensis* BLB2.

Key words: *16S rRNA gene, agarase, molecular identification, phenetic identification, selection*