

RINGKASAN

Amilase merupakan enzim ekstraseluler yang memecah pati menjadi gula sederhana seperti glukosa. Pada bidang industri, sebagian besar amilase diperoleh dari mikroorganisme. Bakteri amilolitik sebagai salah satu mikroorganisme penghasil amilase dapat diisolasi dari berbagai habitat, salah satunya perairan. Sungai Logawa merupakan sungai yang mengalir dari hulu yang berlokasi di Desa Baseh, Kecamatan Kedungbanteng hingga hilir yang berlokasi di Patikraja, Banyumas. Kawasan hulu Sungai Logawa memiliki aliran yang berarus deras dan jernih. Kawasan hulu Sungai Logawa tidak terlepas dari aktivitas masyarakat sekitar seperti pertanian, pemancingan ikan, peternakan ayam, dan pemukiman yang dapat memengaruhi kondisi perairan dan keragaman mikroorganisme di kawasan tersebut. Penelitian terkait isolasi dan identifikasi molekuler bakteri di kawasan hulu Sungai Logawa belum pernah dilakukan dan potensi bakteri khususnya dalam menghasilkan amilase belum diketahui. Oleh karena itu, penelitian ini bertujuan untuk melakukan isolasi dan skrining bakteri amilolitik dari air dan sedimen kawasan hulu Sungai Logawa dan melakukan identifikasi bakteri amilolitik berdasarkan gen 16S rRNA.

Penelitian dilakukan menggunakan metode survei. Pengambilan sampel dilakukan di kawasan hulu Sungai Logawa, Kecamatan Kedungbanteng, Banyumas pada tiga titik yang berbeda. Variabel bebas berupa isolat bakteri asal air dan sedimen kawasan hulu Sungai Logawa. Variabel terikat berupa aktivitas amilolitik dari isolat bakteri. Parameter utama yang diamati adalah indeks amilolitik dan persentase similaritas sekuens gen 16S rRNA bakteri amilolitik dengan sekuens pembanding. Parameter pendukung yang diamati adalah karakteristik morfologi, fisiologi, dan biokimia isolat bakteri amilolitik potensial. Hasil skrining aktivitas amilolitik dan karakteristik isolat bakteri amilolitik terpilih dianalisis secara deskriptif. Isolat bakteri amilolitik potensial diidentifikasi berdasarkan pensejajaran sekuens gen 16S rRNA.

Sebanyak 22 isolat bakteri telah berhasil diperoleh dari kawasan hulu Sungai Logawa. Skrining aktivitas amilolitik menunjukkan bahwa 8 dari 22 isolat bakteri berhasil mendegradasi 0,1% substrat amilum. Isolat SH 8 menunjukkan indeks amilolitik paling tinggi dengan nilai 1,08. Hasil pensejajaran gen 16S rRNA di NCBI menunjukkan bahwa isolat SH 8 memiliki similaritas tertinggi dengan *Streptomyces lactacystinicus* strain OM-6519^T dengan persentase kemiripan sebesar 99,71%. Konstruksi pohon filogenetik menggunakan metode *neighbor-joining* dengan model *Kimura 2-parameter* menunjukkan bahwa isolat SH 8 memiliki hubungan kekerabatan paling dekat dengan *Streptomyces lactacystinicus* strain OM-6519^T, keduanya membentuk klad monofiletik dengan nilai *bootstrap* sebesar 93 dan jarak genetik sebesar 0,001. Berdasarkan konfirmasi karakter morfologi, fisiologi, dan biokimia, isolat SH 8 termasuk ke dalam genus *Streptomyces*. Hasil penelitian menunjukkan bahwa sebanyak delapan bakteri amilolitik telah diperoleh dari air dan sedimen kawasan hulu Sungai Logawa dan berdasarkan identifikasi gen 16S rRNA, isolat SH 8 sebagai bakteri amilolitik potensial memiliki similaritas tertinggi dengan *Streptomyces lactacystinicus* strain OM-6519^T.

Kata kunci: amilase, bakteri amilolitik, gen 16S rRNA, identifikasi molekuler, Sungai Logawa.

SUMMARY

Amylase is an extracellular enzyme that breaks down starch into simple sugars such as glucose. In the industrial field, amylases are mostly obtained from microorganisms. Amylolytic bacteria as one of the amylase-producing microorganisms can be isolated from various habitats, such as water. Logawa River is a river that flows from upstream located in Baseh Village, Kedungbanteng District to downstream located in Patikraja, Banyumas. The upstream area of the Logawa River has a fast-flowing and clear stream. The upstream area of the Logawa River is closely connected to the surrounding community's activities, such as agriculture, fishing, and poultry, as well as settlements. These activities can affect the area's water conditions and the diversity of microorganisms. Research related to isolation and molecular identification of bacteria in the upstream area of the Logawa River has never been done and the potential of bacteria, especially in producing amylase, is unknown. Therefore, this study aims to isolate and select of amylolytic bacteria from the water and sediment from the upstream area of the Logawa River and identify amylolytic bacteria based on the 16S rRNA gene.

The research was conducted using a survey method. Sampling was conducted in the upstream area of the Logawa River, Kedungbanteng District, Banyumas at three different stations. The independent variables were bacterial isolates from water and sediment from the upstream area of Logawa River. The dependent variable is the amylolytic activity of bacterial isolates. The main parameters observed were amylolytic index and percentage similarity of 16S rRNA gene sequences of amylolytic bacteria with reference sequences. Supporting parameters observed were morphological, physiological, and biochemical characteristics of potential amylolytic bacteria. Screening results of amylolytic bacteria and characteristics of selected amylolytic bacterial isolates will be analyzed descriptively. Amylolytic bacterial isolates with the highest amylolytic index will be identified based on 16S rRNA gene sequence.

A total of 22 bacterial isolates have been successfully obtained from the upstream area of the Logawa river. Screening of amylolytic showed that 8 out of 22 bacterial isolates successfully degraded 0.1% amylum substrate. Isolate SH 8 became a bacteria with the highest amylolytic index with a value of 1.08. The results of the 16S rRNA gene alignment at NCBI showed that isolate SH 8 had the highest similarities with *Streptomyces lactacystinicus* strain OM-6519^T with a similarity percentage of 99.71%. Phylogenetic tree construction using the neighbor-joining method with the Kimura 2-parameter model shows that isolate SH 8 has the closely related to *Streptomyces lactacystinicus* strain OM-6519^T, both forming a monophyletic with a bootstrap value of 93 and a genetic distance of 0,001. Based on the confirmation of morphological, physiological, and biochemical characters, isolate SH 8 belongs to the genus *Streptomyces*. The results showed that eight amylolytic bacteria were obtained by water and sediment from the upstream area of the Logawa river. Based on 16S rRNA gene identification, isolate SH 8, as a potential amylolytic bacteria, had the highest similarity to *Streptomyces lactacystinicus* strain OM-6519^T.

Keywords: *amylase, amylolytic bacteria, Logawa River, molecular identification, 16S rRNA gene.*