Penapisan dan karakterisasi L-glutaminase ekstraselular dari bakteri laut = Screening and characterization of extracellular L-glutaminase from marine bacteria

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Abstrak

L-glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) telah menarik perhatian para peneliti karena manfaatnya dalam industri farmasi dan makanan. Bakteri laut merupakan sumber penghasil L-glutaminase yang paling diminati, terutama untuk memperoleh L-glutaminase yang tahan garam. Pada penelitian ini telah dilakukan penapisan dan karakterisasi L-glutaminase ekstraselular yang dihasilkan oleh bakteri laut dari perairan Sangihe-Talaud, Sulawesi Utara, Indonesia. Penapisan L-glutaminase secara kualitatif menggunakan media cair (Padma, et.al., 2009) dan metode pengukuran aktivitas L-glutaminase dilakukan secara spektrofotometri berdasarkan metode Imada, et.al (1973). Identifikasi isolat murni dengan aktivitas L-glutaminase paling tinggi dilakukan menggunakan sekuensing gen 16S rRNA. Terdapat 7 isolat menunjukkan hasil positif L-glutaminase, satu diantaranya dengan aktivitas 147,99 U/L atau setara dengan aktivitas spesifik 62,32 Unit/mg dipilih untuk diidentifikasi lebih lanjut.
Hasil sekuensing gen 16S rRNA isolat bakteri menunjukkan kemiripan 96% dengan Pseudomonas aeruginosa strain CG-T8. Parameter fisika yang mempengaruhi produksi L-glutaminase menunjukkan produksi optimum pada suhu 30 0C, kecepatan rotasi 100 rpm, pH media 6, dan konsentrasi starter inokulum 5%. Karakterisasi aktivitas L-glutaminase ekstraselular dari Pseudomonas aeruginosa strain CG-T8 (isolat II.1) menunjukkan kondisi optimum aktivitas enzim pada suhu 37-45 0C, dan pH 7. Aktivitas enzim stabil pada penambahan larutan NaCl hingga 8% dan aktivitasnya mulai berkurang pada penambahan larutan NaCl 16% dan 20% dengan aktivitas relatif berturut-turut mencapai 79,00% dan 74,22%. Pengaruh penambahan ion-ion logam seperti Mn2+, Mg2+, dan Co2+ menunjukkan kenaikan aktivitas, sedangkan pada penambahan ion logam Zn2+, Fe3+, dan Ca2+ aktivitas enzim menurun. Bobot molekul L-glutaminase berkisar 42 kDa dan 145 kDa.
.....L-glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) has attracted much attention with respect to proposed applications in both pharmaceuticals and food industries. Salt-tolerant L-glutaminase produced by marine bacteria become the most desirable in food industry. The current work details the screening of L-glutaminase producing marine bacteria from Sangihe-Talaud Sea, in North of Sulawesi, Indonesia. Screening of L-glutaminase was done using a broth medium (Padma et.al., 2009) and measurement of L-glutaminase activity carried out by spectrophotometry (Imada, et.al., 1973). Identification of selected isolate was performed by analysis of 16S rRNA gene sequence. There are seven isolates showed positive results of L-glutaminase, one of them with the activity 147.99 U/L, equivalent to the specific activity of 62.32 units / mg was selected for further study.
Bacterial identification based on 16S rRNA gene sequencing has revealed the isolate 96% similarity as Pseudomonas aeruginosa strain CG-T8. Optimization of physical parameters that affect the production of L-glutaminase production showed an optimum at 30 0C, 100 rpm, pH of medium 6, and with 5% of starter inoculum. Characterization of extracellular L-glutaminase from Pseudomonas aeruginosa strain CG-T8 (II.1 isolates) showed the enzyme activity was optimum at temperature 37-45 0C, and pH 7. The enzyme activity

was stable in the addition of NaCl solution up to 8% and began to decrease on addition of NaCl solution 16% and 20% with relative activity consecutively 79.00% and 74.22%. The effect of metal ions such as Mn^{2+}, Mg^{2+}, and Co^{2+} showed increased enzyme activity, whereas the addition of others metal ions (Zn^{2+}, Fe^{3+}, and Ca^{2+}) decreased the activity. The molecular weights of L-glutaminase was found around 42 kDa and 145 kDa.