Kloning dan ekspresi endo-B-1,4 glukanase dari isolat lokal BPPTCC-RK2 = Cloning and expression of endo-B1,4 glucanase from indigenous bacteria BPPTCC-RK2

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Abstrak

Potensi keanekaragaman Indonesia memberikan peluang untuk mendapatkan mikroorganisme penghasil endo--1,4-glukanase yang mampu menghidrolisis selulosa. Bacillus amyloliquefaciens BPPTCC-RK2 telah berhasil diisolasi dari rayap. Gen endo--1,4-glukanase dikloning dari DNA genom B.amyloliquefaciens BPPTCC-RK2 menggunakan metode rekombinatorial dan diekspresikan secara fungsional di dalam E.coli. Didapatkan ORF sepanjang 1500 nukleotida yang menyandikan 499 asam amino dengan berat molekul 55 kDa. Gen dikloning kedalam pDEST14 dan dioverekspresi pada E.coli BL21-Star. Aktivitas tertinggi sebesar 26,05 U/mg protein setelah diinduksi dengan 1mM IPTG selama 24 jam. Enzim optimum pada pH 6,0 dan suhu 65 dan memiliki waktu paruh 90 menit pada suhu 60. Pada konsentrasi etanol 100 g/L, masih memberikan aktivitas hingga 78% setelah 24 jam.

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Indonesia has potential biodiversity that provides opportunities to obtain endo--1,4-glucanase producing microorganism that could hydrolize cellulose. Bacillus amyloliquefaciens BPPTCC-RK2 have been isolated from termites. Endo--1,4-glucanase gene have been cloned from genomic DNA B.amyloliquefaciens BPPTCC-RK2 using recombinatorial method and functionally expressed in E.coli. A full length gene of endo--1,4-glucanase consisting 1500 nucleotides that encoded for a protein 499 amino acids with predicted molecular weight 55 kDa. Highest enzyme activity (26,05 U/mg) achieved after 24 hour induction with 1mM IPTG. The enzyme optimum at pH 6,0 and temperature 65 and 90 minutes half-life at 60. This enzyme give 78% residual activity after 24 hour incubation in 100 g/L ethanol.