

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

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Abstrak

[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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ABSTRACT

Indonesia has potential biodiversity that provides opportunities to obtain endo--1,4-glucanase producing microorganism that could hydrolyze 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.;Indonesia has potential biodiversity that provides opportunities to obtain endo--1,4-glucanase producing microorganism that could hydrolyze 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., Indonesia has potential biodiversity that provides opportunities to obtain endo-#946;-1,4-glucanase producing microorganism that could hydrolyze cellulose. *Bacillus amyloliquefaciens* BPPTCC-RK2 have been isolated from termites. Endo-#946;-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-#946;-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°C; and 90 minutes half-life at 60°C;. This enzyme give 78% residual activity after 24 hour incubation in 100 g/L ethanol.]