Ekspresi gen CSF3syn dengan promotor konstitutif PGAP pada Pichia pastoris

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Abstrak

Gen CSF3syn adalah gen sintetik yang menyandi protein G-CSF. Protein G-CSF dapat diproduksi secara rekombinan. Sel inang alternatif yang dapat digunakan yaitu Pichia pastoris. Penelitian bertujuan untuk menyeleksi P. pastoris transforman yang stabil, mendapatkan P. pastoris transforman yang terintegrasi dengan gen CSF3syn, dan menganalisis ekspresi protein G-CSF pada P. pastoris transforman dengan promotor konstitutif GAP. Sebanyak 47 transforman berhasil diseleksi pada konsentrasi zeosin 1000 µg/ml. Analisis PCR menunjukkan gen CSF3syn sebesar 567 pb berhasil terintegrasi dalam genom P. pastoris. Analisis SDS PAGE, slot blot, dan western blot menunjukkan protein G-CSF berhasil diekspresikan. Analisis western blot menunjukkan G-CSF terglikosilasi ~20 kDa dan tidak terglikosilasi ~18 kDa. Selain itu, terdapat protein dengan berat molekul lebih dari protein target yaitu protein fusi terglikosilasi ~40--60 kDa.

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CSF3syn gene is a synthetic gene that encodes G-CSF protein. G-CSF protein can be produced by recombinant technique. Pichia pastoris can be used as an alternative host. The objectives of this study were to select stability of the P. pastoris transformant, to obtain P. pastoris transformants which were integrated with CSF3syn gene, and expressed G-CSF recombinant in P. pastoris using the constitutive GAP promoter. A total of 47 transformants were selected in YEPD medium with 1000 µg/ml zeocin.
Analyses by PCR confirmed the inserted CSF3syn gene in P. pastoris genome of 567 bp. Analyses of SDS PAGE, western blot, and slot blot showed that the G-CSF protein was expressed successfully. Western blot analyses showed that the bands of ~20 kDa as glycosylated G-CSF and ~18 kDa as non glycosylated G-CSF. The result also showed that the band with higher molecular mass ~40--60 kDa which was probably glycosylated fusion protein.