

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 terglykosilasi ~20 kDa dan tidak terglykosilasi ~18 kDa. Selain itu, terdapat protein dengan berat molekul lebih dari protein target yaitu protein fusi terglykosilasi ~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.