Granulocyte colony-stimulating factor (G-CSF) is one of the first cytokines to be discovered that finds wide applications in the treatment of neutropenia, central nervous system disorders, myelosuppression, myocardial infarction, hepatic damage and others. Recombinant G-CSF (Filgrastim obtained from *Escherichia coli*) is currently used for the treatment purpose. The aim of the present work was to enhance the production of G-CSF in *Pichia pastoris* by utilizing different strategies that included creation of a fusion protein and codon harmonization. Two fusion constructs of G-CSF were made with human serum albumin (HSA) domain III, one containing a flexible non-cleavable linker and the other with a cleavable linker. The fusion of *G-CSF* gene with that of the DNA encoding *HSADIII* through a cleavable linker enhanced G-CSF production by 5-8 folds to ~150 mg/L compared to that obtained with a codon-optimized copy of the gene. As a fusion protein, the G-CSF retained its α-helical structure and biological activity in an in vitro cell proliferation assay using THP1 cell lines. Free G-CSF could be obtained on cleavage of the linker with the correct N-terminus sequence. Higher production of the fusion protein was obtained against a methanol utilization (Mut) slow phenotype compared to that with the Mut plus phenotype. Analysis of the transcriptome of the two types of recombinants (the Mut slow and the Mut plus) revealed differential expression of genes involved in methanol metabolism, sterol biosynthesis, β- oxidation, amino acid metabolism, transcription, translation, unfolded protein response and biosynthesis of co-factors. Several new gene targets were identified, the differential transcription of which, controlled extracellular fusion protein production. Codon harmonization, to fine tune G-CSF sequence in light of specie specific codon bias and occurrence of alpha helical and link regions, was used as a strategy and this led to increased production of G-CSF when compared to that obtained with the levels arising out of the native cDNA. The resulting protein showed less aggregation and retained structural and biological integrity.