Abstract

The methylotrophic yeast *Pichia pastoris* has been widely used for the production of human therapeutics, but production of granulocyte colony-stimulating factor (G-CSF) in this yeast is low. In this study, improved extracellular production of G-CSF was carried out by introducing mutations in the α-mating type (MAT) secretory signal using a native cDNA (WT-*GCSF*) and a codon optimized *GCSF* gene (CO-*GCSF*). Mutations in the pro-region of the α-MAT (deletion (△) of the amino acids 57-70), resulted in an increase in extracellular production of G-CSF in both the cases with higher production from the CO-*GCSF*. Response to △30-43 and △47-49 deletions was different from the CO-*GCSF* and the WT-*GCSF* genes indicating higher rates of synthesis in the former to overcome the regulatory control exercised by these segments. The loss of secretion occurring due to △30-43 in the WT-*GCSF* was partially restored (by 60%) when the △57-70 was added. An important role of the 47-49 amino acids was also demonstrated. The role of the P1' position of the kex2 cleavage site in the α-MAT was demonstrated and specific substitutions by smaller amino acids lead to increased production of G-CSF. Secondary and tertiary structure prediction using I-TASSER indicated an important role of the 3rd alpha-helix in the pre-pro peptide. Presence of a minimum loop length and secondary structure on the pro-peptide region allowed enhanced extracellular production of G-CSF. Also, the role of several nutritional factors in controlling the morphology of recombinant *P. pastoris* was shown for the first time and the morphological switch was mediated through quorum sensing molecules.