Isolation and molecular characterization of promoters from *Gordonia*

Abstract

Novel regulatable promoters serve as effective tools for the construction of gene expression systems. In this aspect, promoters with varying strength and regulatability are required for use in different process-specific applications. Understanding the promoter functionality and characteristics is essential for their exploitation. In this study, a previously isolated stationary phase promoter from *Gordonia* sp. IITR100 was engineered by repeated rounds of random mutagenesis to obtain a strong synthetic promoter that offers stationary phase inducibility and higher strength as compared to the wild-type promoter in *E. coli*.

To isolate additional novel promoters from *Gordonia* sp. IITR100, the whole-genome sequence was determined, which facilitated the identification of core promoter sequences. Based on this, the consensus sequence of the core promoter regions was deduced, which revealed the presence of conserved extended -10 region, which has also been reported in other members of Actinobacteria such as *Corynebacteria* spp., *Rhodococcus* spp. and *Mycobacteria* spp. Differential expression profiles of the genes were detected by microarray profiling, which led to the isolation of two promoters: P*glx* and P*dsbA*, which drive the expression of glyoxalase and disulfide bond formation protein, respectively.

Microarray profiling also served as the basis for determining the expression profile of divergent gene pairs in *Gordonia* sp. IITR100. Several patterns of expression were observed and one gene pair was selected for further studies. Detailed characterization of two promising divergent promoters: P*maiA* and P*hyd*, driving the expression of genes encoding maleate cis-trans isomerase and hydantoinase, respectively, was carried out. A dual-reporter promoter probe vector has been constructed in the study, which was utilized to demonstrate the co-expression ability of the divergent promoters. Key aspects of their regulation were also studied, which revealed their biological significance. The evidence of the transcription of genes driven by the divergent promoters containing truncated promoter fragments under different conditions serves as useful tools for fine-tuning their expression levels.

Based on the results of promoters obtained in the study, two strong promoters: P*hyd* and synthetic promoter were used to develop useful gene expression systems in Actinobacteria. An expression vector using these promoters was constructed to demonstrate the expression of single-chain analog of insulin (SCI-57). A novel method for the expression of two subunits of Human Chorionic Gonadotropin (hCG) was developed using the divergent promoters working in opposite orientations, which pave the way for using Actinobacterial hosts for therapeutic protein production.

Ultimately, this study has led to the isolation of novel promoters with different efficacy. The characterization of divergent promoters is noteworthy and provides several insights into their applicability. More studies on the identification of such systems will offer alternative approaches for co-expression of genes.