Screening and Characterization of Novel Promoters from *Pichia pastoris*

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Abstract

In order to develope novel promoters working in *P. pastoris*, mRNAs were extracted from *P. pastoris* cultivated in various substrate-limited chemostat, and the cDNAs were synthesized by RT-PCR technique. By analyzing the sequences of cDNAs, predominantly expressed genes of 3-phosphoglycerate kinase (*PGK*) and translation elongation factor1-α (*TEF*) were screened, and the potential of its promoter were compared to well-known promoter (P_{GAP})[1)] by using bacterial lipase gene in *P. pastoris*. It was demonstrated that two promoters, 3-phosphoglycerate kinase promotor (P_{PGK}) and translation elongation factor1-α promoter (P_{TEF}), were constitutively expressed, and their strength depended on carbon source for cell growth. The expression level of lipase gene using P_{TEF} was about 1.2 times stronger than that using P_{GAP} in batch culture with glycerol as a carbon source. Concerning the strength of P_{PGK}, it was very feeble comparing to that of P_{TEF}. The results of fed-batch cultures using both promoters in *P. pastoris* will be discussed.

Reference

1. Geoffrey P., Cereghino L., Creeg JM, Application of yeast in biotechnology: protein production and genetic analysis(1999), *Curr Opin Biotechnology*, 10, 422-427.