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Synthetic auto-inducible promoter for protein production: An alternative to T7 promoter

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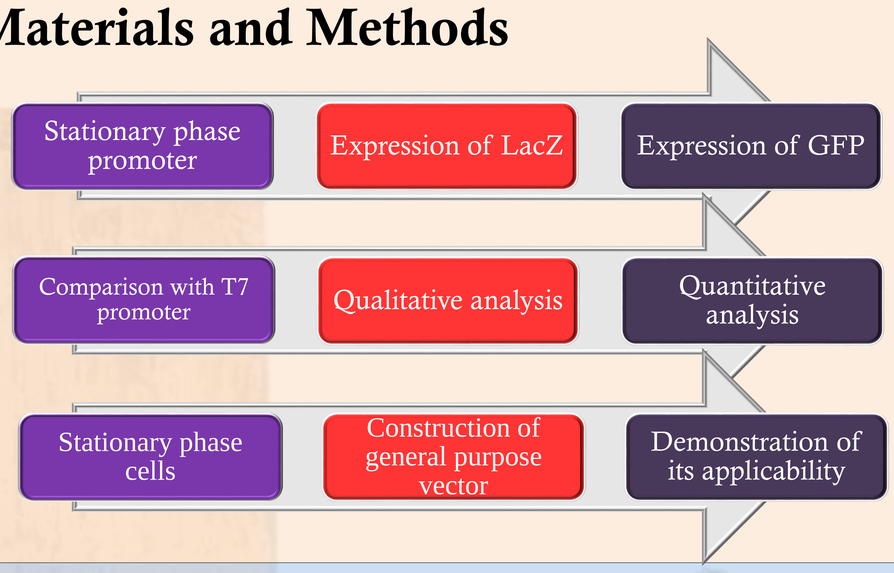
Abstract

Developing a strong promoter for enhanced gene expression is a requisite for production of proteins. The gene expression systems commonly used rely on constitutive or inducible promoters for this purpose. The T7 promoter is widely used for protein production in *E. coli*. However, the requirement of a specific host for producing proteins is a major drawback of its applicability. Here, we report a strong synthetic stationary phase promoter, that produces proteins at par with T7 promoter. The promoter is auto-inducible at stationary phase and results in high level production of proteins. The promoter resulted in ~16,000 Miller units of β-galactosidase activity and ~3,500 RLU/OD_{600} fluorescence intensity of GFP_{uv}. The stationary phase inducibility of the promoter does not affect the growth and metabolism of bacteria and thus uncouples the growth phase and protein production phase. The general purpose vector created using the synthetic promoter can be used for cloning any gene if interest.

Introduction

- Promoter identification is important for developing gene expression systems
- To develop expression systems in bacteria, it is necessary to ensure proper selection of a promoter that would drive the expression of genes at the right time and with maximum amount
- Auto-inducible promoters offer the advantage of saving the cost of additional inducers and absence of requirement of growth monitoring

Materials and Methods



Conclusions

- ✓ Promoter strength was found to be at par with T7 promoter
- ✓ Promoter results in uniform expression across all cells as observed by microscopy and flow cytometry
- ✓ A vector containing the synthetic promoter and MCS is available which can be used for cloning any gene of interest
- ✓ Two heterologous genes have been expressed using the constructed expression vector

Industrial Significance

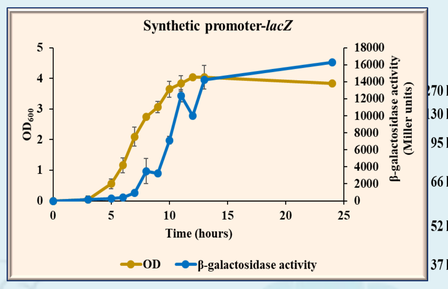
The synthetic promoter developed can be used for cloning genes for producing industrially important enzymes, therapeutic proteins, etc.

Technology Readiness Level

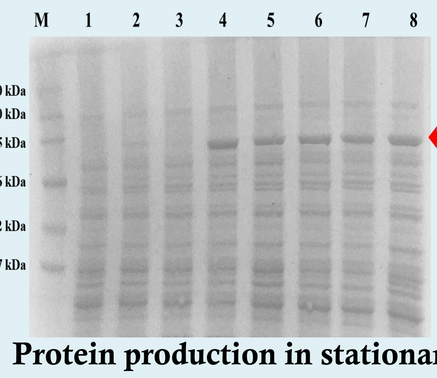
A patent application has been filed on the promoter and auto-inducible gene expression system constructed in the present study (Indian patent application number 201911019476)

Results

Synthetic promoter

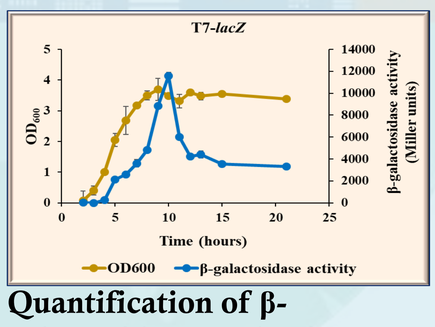


Quantification of β-galactosidase production using the synthetic promoter

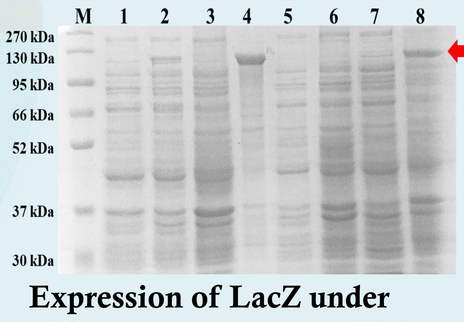


Protein production in stationary phase

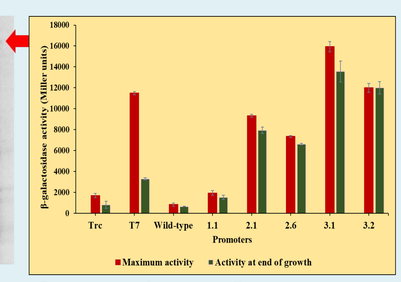
Comparison with T7 promoter



Quantification of β-galactosidase production using the T7 promoter

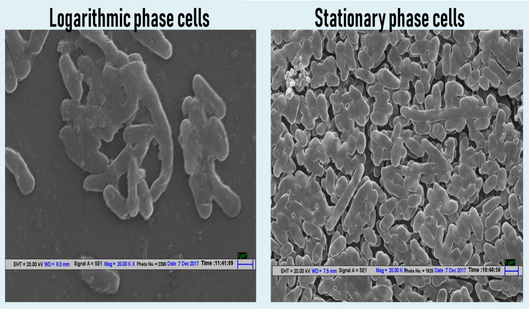


Expression of LacZ under different promoters on SDS-PAGE (Lane 1,2-P*trc*, 3,4-T7, 5,6-wild-type and 7,8-synthetic promoter)

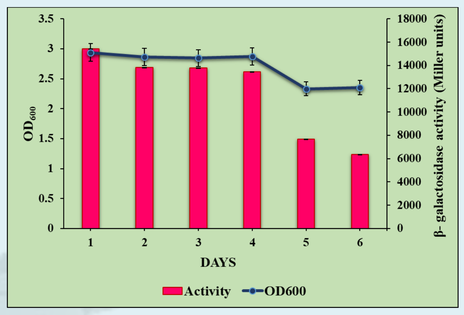


Comparison of promoter activities

Advantages of stationary phase

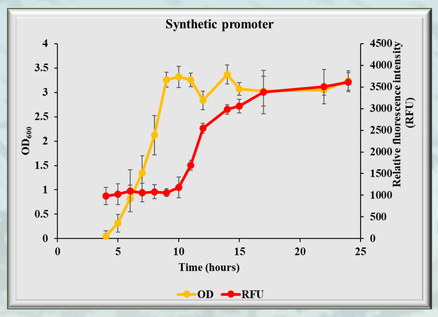


Maximum cell density in stationary phase

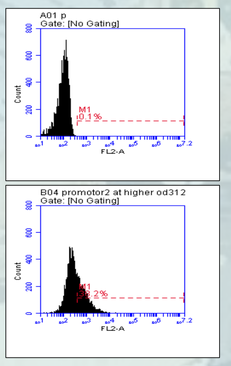


CASP phenomenon

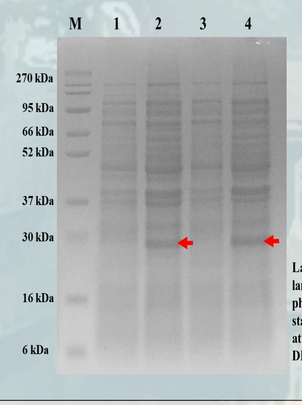
Stationary phase promoter based gene expression system

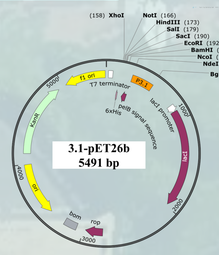


Quantification of GFP production using the synthetic promoter



Demonstration of GFP expression in individual cells





General purpose vector for cloning any gene of interest

Lane M: Broad range protein marker, lane1: lane1: P3.1-pET26b-luxR at exponential phase; lane2: P3.1-pET26b-luxR sample at stationary phase; lane3: P3.1-pET26b-DBRR at exponential phase; lane4: P3.1-pET26b-DBRR at stationary phase.

References

- **Jaishankar, J**., & Srivastava, P. (2017). Molecular basis of stationary phase survival and applications. *Frontiers in microbiology*, *8*, 2000.
- Singh, P., Chachan, S., Singhi, D., & Srivastava, P. (2016). Isolation and molecular characterization of a stationary phase promoter useful for gene expression in *Gordonia*. *Gene*, *591*(1), 153-160.

Acknowledgement

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