

Expression of *Escherichia coli* gene transcripts at various stages of growth, under the control of stationary phase promoter.

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Objectives :

These studies have been done to utilize the power of sensitivity and accuracy of real time RT-PCR in determining the level of induction for bacteriocin release protein gene (*kil*), *rpoS* and *rpoD*. Several proteins of prokaryotic and human origin have been released by *E.coli* using a system encoding both the colicin E1 BRP (referred as Kil hereafter) and the target protein. High level expression of BRPs causes quasi-lysis and lethality, so BRP expression has to be optimised. In our studies, BRP is under the control of stationary phase promoter, so kinetics of appearance of Kil protein shall be checked in relation to the activity of sigma S factor. Since Rpo D is involved with the normal housekeeping genes, we have also considered it for studying.

Materials and Methods :

Bacterial strain and Plasmids :

The *Escherichia coli* strain JM109 [*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 Δ(lac-proAB) F'(traD36 pro AB+ lacIqZ lacZ Δ M15)* (Stratagene) was used as a host strain for plasmids.

pBC SK(+) (Stratagene) was used at first to derive the plasmid pLF3 (Miksch, *et al.*, 1997). The plasmid pLF3 has a Kil-Km-Bgl cassette (Fig. 1) containing the gene for β-glucanase (*bgl*) as a reporter gene upstream of the interposon. The *bgl* gene used was a hybrid gene from *Bacillus amyloliquefaciens* (Amino terminus 107 amino acid residues) and *Bacillus macerans* (Carboxy terminus 107 amino acid residues), because of its increased thermostability as compared to the wild types (Borriß *et al.*, 1989). The *kil* gene is under the control of the *fic* promoter, which is chiefly recognised by the σ^s subunit (Tanaka *et al.*, 1993).



Fig. 1: The Kil-Km-Bgl cassette located on pLF3. *bgl* is the gene for β-glucanase, *km* is kanamycin resistance gene as a marker for selection and *kil* codes for bacteriocin release protein from ColE1 and is under the control of stationary phase promoter *fic*.

Batch Cultivation in Bioreactor :

E.coli strain JM109 cultures were grown in a LABFORS fermenter (Fig. 2), with a working volume of 2 L of LB medium. Operating conditions include- temp: 37° C; pO₂ was controlled by stirrer set point 60%; pH of 7 was controlled by 10 % phosphoric Acid and 2 M NaOH ; Aeration rate: 2 L/min; with automatic dosing of antifoam.



Fig. 2: LABFORS fermenter

Growth was monitored spectrophotometrically at 600 nm. The samples were withdrawn after every hour till first 13 hours and last sample after 24 hours.

Total RNA isolation :

Cells were harvested at different stages of growth. QIAGEN RNAprotect reagent was used as prescribed by the manufacturer. Qiagen RNeasy columns were used for Total RNA isolation, with treatment by DNase twice.

RNA quantitation, purity determination and integrity estimation were performed by standard procedures

Comparative attributes for the primer pairs used for Real Time RT PCR:

Gene name	Primer	Length	Sequence	T _m	GC %	Product size
<i>kil</i>	L-Primer	22	ATATTCCGGATAAACCTCTTG	59.50	40.91	100
	R-Primer	21	TCCCCGTCAGTFFAGAAGAGG	60.61	52.38	
<i>rpoS</i>	L-Primer	20	GCACGTGAGTTGTCCATAA	59.57	50.00	103
	R-Primer	20	TAAGACGAAGCATACGGCTG	59.09	50.00	
<i>rpoD</i>	L-Primer	20	TCGTATGCCATCCGGTGAAG	59.52	50.00	106
	R-Primer	20	GCCACGGTGGTGTATTTCT	59.86	50.00	

Real Time RT PCR :

For analysing mRNA transcript levels, Real-time RT-PCR using SYBR Green I detection system was performed in LightCycler. The LightCycler system measures fluorescence at pre-programmed intervals during PCR, typically once every cycle to monitor the level of PCR product formation.

Results & Discussion :

Figures 3-5 show the relative induction levels for *kil*, *rpoS* and *rpoD* transcripts after every hour of growth in the region of interest, with respect to the wild types (Borriß *et al.*, 1989). The *kil* gene is under the control of the *fic* promoter, which is chiefly recognised by the σ^s subunit (Tanaka *et al.*, 1993).

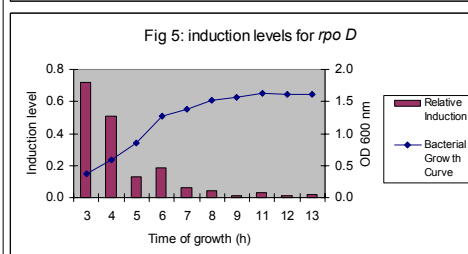
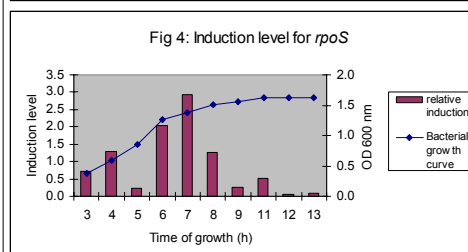
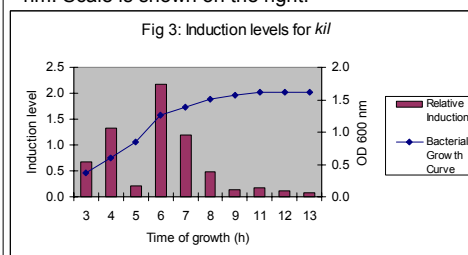


Fig. 3 shows that the levels of the *kil* transcript peak during transition from exponential to stationary phase.

Fig. 4 shows the induction levels for *rpoS* gene transcript. Similar profile is obtained as for the *kil* under the control of stationary phase specific *fic* promoter. It can be commented that the highest level of induction is after 7 h of growth. Activation of *fic* promoter seems to follow the induction profile of Sigma S factor.

Similarly, Fig. 5 shows the relative induction level for *rpoD* gene. It can be noted that the expression level decreases through first 7 hrs of growth and then the level remains more or less constant.

Conclusion :

Kil level rises more than two-fold transiently. We have standardised the methods and now extending the work on already available artificial stationary phase promoters.

The pattern of induction shown by *rpoS* shows that the highest level is observed after 7 h. It can be implied that this increase of induction level has a potential role during the transition from exponential phase to stationary phase. As has been evident from literature, the RpoS protein is responsible for stationary phase specific proteins.

For the *rpoD* transcript, whose level decreases through first 7 hrs, it can be commented it plays key role before achievement of stationary phase. During stationary phase RpoS is also transcribing many genes, partially decreasing *rpoD* utility.

It can be commented that after running into stationary phase the RNA level in general may go down, therefore all the mRNA levels which we observe follow this general profile.

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