# CHAPTER 5

# Nuances in the ppGpp dependent phenotypes in *Escherichia coli* MC4100 and MG1655 in relation to their genotypes.

## 5.1 Musings on the two strains used in this study, MG1655 and MC4100: Strain variation and their phenotypes.

Intrastrain variation

It is matter of concern and caution that the commonly used strains of *Escherichia coli* the world over, MG1655, W3110 and MC4100, maintained in different labs differ in their DNA sequence at genetic loci that matter in physiological studies and stress response adaptation (Freddolino *et al.*, 2012, Soupene *et al.*, 2003, Spira *et al.*, 2008, King *et al.*, 2004).

Reports below list interstrain and intrastrain genetic differences that can potentially affect interpretations of several studies. We mean here mutations in genes besides those that are indicated in the genotype of the strain. The interstrain differences indeed affected our studies too. The work reported in the thesis describes a genetic mutation manifesting the phenotype in only *Escherichia coli* strain, MC4100. The same mutation is without an effect in MG1655 background. Though a large phenotypic effect can be attributable to one mutational difference between the two strains, there is indication of other genes involvement as well.

(i) The sequence variations have been noted in strains depending upon their source, the time of storage, and medium of growth. For example, Freddolino *et al.*, (2012) noted a set of variations in several MG1655 stocks that include strains from the American Type Culture Collection (ATCC) and Yale Coli Genetic Stock Center (CGSC). Variations at *glpR* (repressor in glycerol metabolism), *crl* (affects balance of RNA polymerase holoenzyme with different sigma factors) and *gatC* (affect ability to grow on galactitol as a sole carbon source) genes may have physiological implications under various laboratory conditions. These and other differences at regulatory loci will directly confound the results of experiments using the affected strains. A reason to exercise caution in interpretation of the results in the article by Fabich *et al.*, (2011) is that some of the variations identified in the work involving laboratory evolution experiments have apparently been reported as novel mutations in their work since the evolved strains'

sequence were compared to reference sequence rather than to that of the founder strain (Fabich *et al.*, 2011).

(ii) Soupene *et al.*, (2003), Freddolino *et al.*, (2012) carried out comprehensive analysis of MG1655 strains from several sources and listed important differences between them that can compound physiological studies.

(iii) Another commonly used sequenced strain, W3110 (Jishage & Ishihama, 1997), differs in important respects in various stocks.

(iv) Strain MC4100 is adopted by laboratories that worked particularly on gene regulation and also in protein export, physiology, and cell division studies (Ferenci *et al.*, 2009 and references therein). This strain genotype according to the *E. coli* Genetic Stock Center is: $F^-$ (*araD139*)Δ(*argF-lac*)*169* $\lambda^-$ e14$^-$*flhD5301* Δ(*fruK-yeiR*)*725*(*fruA25*) *relA1 rpsL150*($Str^r$) *rbsR22* Δ(*fimBfimE*)*632*(::IS*1*) *deoC1*]. It is derivative of HfrC strain MO of S Brenner (genotype according to the *E. coli* Genetic Stock Center: $F^-\lambda^-$e14$^-$*relA1 rspL150 spoT1*). The latter strain has mention of *spoT1* mutation; it is however missing in the former's genotype description (Ferenci *et al.*, 2009). MC4100 differs from MG1655 at quite a few loci. The two strains have been subjected to extensive comparative studies revealing macromutations by restriction mapping (Heath *et al.*, 1992), using microarrays based on the MG1655 sequence (Peters *et al.*, 2003) and by pulsed-field electrophoresis, and finally at the level of sequence analysis (Ferenci *et al.*, 2009).The studies have been carried out to better understand genetic makeup with phenotypic correlates. Some of the important differences demarcating the two strains are – (i) Differences in the positions of insertion sequences in MG1655 and MC4100 have been important and affects anaerobic gene regulation (Sawers, 2005); (ii) Another far-reaching difference likely to affect physiological adaptability is the level of sigma factor $\sigma^S$ in the two strains (King *et al.*, 2004), levels in the strain MC4100 being higher than in MG1655. (iii) There are also differences in central metabolism between the two K-12 strains (Maharjan *et al.*, 2005). The genetic mutations unique to MC4100 and not present in MG1655 are available in the paper by Ferenci *et al.*, (2009).

(v) Intrastrain differences in MC4100 isolates from two labs have also come to the fore in the studies of Spira *et al.*, (2008). MC4100BS from B. Spira lab and MC4100TF from T. Ferenci lab

differ from each other in some very important respects. Each of the two strains contained the same pair of changes in the *spoT* gene, have raised intracellular levels of ppGpp and yet the level of RpoS is different in them; its level being high in MC4100TF than in MC4100BS. Given that the two MC4100 stocks have the same ppGpp level and the same *spoT* allele, and that MC4100TF and MC4100BS also have the same *rpoS* sequence, it is apparent that other extragenic differences between these two strains affect *rpoS* expression.

The purpose of highlighting the comparison studies in the context of the studies here is the confirmation of *spoT1* mutation in MC4100 in the sequence analysis studies not otherwise mentioned in the genotype description of MC4100. In fact MC4100 was considered *spoT*$^{+}$ in some of the earlier studies (Sarubbi *et al.*, 1988). The two mutations *relA1* and *spoT1* apparently compensate each other's compromised function such that the strain MC4100 though *relA*$^{-}$ , has high intracellular ppGpp levels, more than in MG1655, and is better suited for growth under different stress conditions than on different types of nutrients (Spira *et al.*, 2008, King *et al.*, 2004). The fact that *spoT1* mutation is possible to be introduced in *relA*$^{+}$ strain background means that *spoT1* mutation only partially affects ppGpp hydrolytic function. The stronger alleles of *spoT* are not possible to be introduced in *relA*$^{+}$ strains (Sarubbi *et al.*, 1988).

## 5.2 Materials and Methods

**Table 5.1: Bacterial strains and plasmids used in this chapter**

| ***E. coli* strains/plasmids** | **Relevant genotype/Description** | **Reference/Source** |
|---|---|---|
| **Strains** | | |
| MG1655 | *λ*$^{-}$, *rph-1* | lab collection |
| MC4100 KP | *F*$^{-}$ *araD139 (argF-lac)U169 rpsL150 deoC1 relA1 thiA ptsF25 flbB5301 rbsR* | lab collection |
| CAG12182 | *λ, cysI3152*::Tn*10kan, rph1* | Singer, M. et al,1989 |
| JW2757-1 | *Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ*$^{-}$, *ΔbarA784*::*kan, rph-1, Δ(rhaD-rhaB)568, hsdR514* | Keio Collection, Baba et al, 2006 |
| JW2758-5 | *Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ*$^{-}$, *ΔgudD785*::*kan, rph-1, Δ(rhaD-rhaB)568, hsdR514* | Keio Collection, Baba et al, 2006 |
| JW3617-1 | *Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ*$^{-}$, *ΔpyrE748*::*kan, rph-1, Δ(rhaD-rhaB)568, hsdR514* | Keio Collection, Baba et al, 2006 |
| KP1.1 | JM101 Δ*rumA1 relA2009* | This study |
| KP3 | MC4100 *relA1 cysI3152*::Tn*10kan* (transduction from CAG12182 ) | This study |

| | | |
|---|---|---|
| KP4 | MC4100 *relA*$^+$ *cysI*$^+$ | This study |
| KP5 | MC4100 *relA*$^+$ *ΔbarA784*::*KAN* (transduction from JW2757-1) | This study |
| KP6 | MC4100 *relA*$^+$ *ΔgudD785*::*KAN* (transduction from JW2758-5) | This study |
| KP7 | MC4100 *relA2009* Δ*rumA1* by P1 transduction using KP1.1 | This study |
| KP11 | MC4100KP *spoT1ΔpyrE748*::*kan* (transduction from JW3617-1) | This study |
| KP22 | MG1655 *relA1 rumA*$^+$ (transduction from KP3) | This study |
| KP23 | MG1655 *relA2009* Δ*rumA1* (P1 transduction using KP1.1) | This study |
| KP29 | MG1655 *spoT1 ΔpyrE748*::*KAN* (transduction from KP11) | This study |
| KP31 | MG1655 *spoT1pyrE*$^+$ | This study |
| KP35 | KP4 *spoT*$^+$ *ΔpyrE748*::*KAN* (transduction from JW3617-1) | This study |
| KP37 | KP4 *spoT*$^+$ *pyrE*$^+$ | This study |
| **Plasmid** | | |
| pTE4 | Full length *relA* gene (2289 bp) cloned at *Eco*RI - *Kpn*I sites of pBAD18Kan. | This study |

### 5.2.1 P1 lysate preparation

0.150 ml of overnight grown culture of the donor strain in LB broth was mixed with $10^7$ pfu (75 µl) of P1 phage. Adsorption was allowed to occur at 37°C for 20 min and the lysate was prepared in one of the following ways:

#### 5.2.1.1 Plate method

0.1 ml of the infection mix was dispensed in 2.5 ml of LB soft agar and poured on freshly prepared Z-agar plates and incubated at 37°C for overnight. Next day, 2 ml of Z-broth was added to each plate and incubation continued for 2 more hours. The Z-broth and soft agar layers were transferred into a 15 ml falcon tube and 1 ml of chloroform was added followed by vortexing vigorously for 30 seconds. After centrifuging down the debris, the clear supernatant was removed into a fresh sterile 15 ml falcon tube, treated again with chloroform and stored at 4°C.

#### 5.2.1.2 Broth method

To 0.225 ml of infection mixture, 10 ml of Z-broth containing 0.2% glucose was added and incubated at 37°C with slow shaking until growth followed by the visible lysis of the culture occurred (approximately 4 hours). The lysate was treated with chloroform, centrifuged and the clear supernatant was stored at 4°C.

### 5.2.2 P1 Transduction

2 ml of freshly overnight culture of the recipient strain grown in Z-broth was mixed with $10^8$ pfu of P1 (0.2 ml) and incubated at 37°C for 20 minutes to facilitate phage adsorption. The mixture

was centrifuged, and unadsorbed phage particles in the pellet were removed by washing twice with citrate buffer and finally cells were suspended in 0.2 ml of N-saline. 0.1 ml of infected cells were plated on selective medium. In case of antibiotic selection, where phenotypic expression is required, either of two procedures followed: (a) The bacterial pellet after the initial centrifugation was resuspended in 10 ml of LB medium supplemented with 25 mM Na citrate followed by centrifuged and repeat the same procedure. After 1 hr incubation at 37°C, the cells were recovered by centrifugation and plated directly onto appropriate antibiotic containing LB plates. (b) The mixture, after washing, was plated in soft agar on medium not containing selective antibiotic and incubated for 1-2 hrs at 37°C. Afterwards the appropriate antibiotic was added in a soft agar and it was overlaid in sufficient quantities enough to achieve the desired final concentration after diffusion throughout the medium.

### 5.2.3 Assaying of *relA* phenotype

**5.2.3.a 3-amino, 1, 2, 4-triazole (3-AT)** induces histidine starvation by acting as a competitive inhibitor of the imidazoleglycerol-phosphate dehydratase. This enzyme catalyses the sixth step of histidine production (Hilton, 1965). The sensitivity was determined by using M9 minimal medium supplemented with glucose (0.2%), all amino acids except histidine (4 µg/ml), adenine (1 mM), thiamine (1 mM), and AT (15 mM) (Silva & Benitez, 2006).

**5.2.3.b SMG (serine, methionine and glycine)** induces isoleucine starvation as serine is competitive inhibitor of threonine deaminase which catalyzed the 1$^{st}$ step of isoleucine biosynthesis. Serine, glycine, methionine (SMG) are one carbon amino acids and primary effect of SMG is to increase the level of THF (tetrahydrofolate) in cell which leads to inhibition of growth due to isoleucine starvation (Uzan & Danchin, 1976). The sensitivity to inhibition by serine, methionine, glycine, (SMG) was tested with M9 glucose medium containing SMG (100 µg/ml each), adenine (50 µg/ml), thymine (50 µg/ml), and calcium pantothenate (1 µg/ml) (Silva & Benitez, 2006).

**5.2.3.c Serine hydroxamate** inhibits charging of seryl t-RNA resulting in auxotrophy for serine (Tosa & Pizer, 1971). Amino acid starvation was induced by addition of 0.5-1mg/ml serine hydroxamate in minimal medium containing 0.2% glucose.

### 5.2.4 Determining growth curve

Growth curve experiment was performed in either LB and/or Minimal glucose broth. Overnight grown cultures were inoculated at 1% density in 50ml LB flasks and/or 50ml Minimal glucose broth. The inoculated flasks were kept on rotary shaker at 37°C and 25°C temperature. O.D.600 was measured at different time intervals. This experiment was repeated twice.

## 5.3 Results and Discussion

### 5.3.1 Construction of *relA*$^+$ derivative of MC4100

*spoT1* mutation in MC4100 is substitution (H255Y) and a two-amino acid insertion between residues 82 and 83(+QD) of the SpoT protein. The compromised ppGpp hydrolytic function is responsible for slow (20 fold) decay in first order kinetics of ppGpp during stringent response as well as severe impairment in ppGpp degradation during exponential growth, resulting in high intracellular concentration (Spira *et al.*, 2008, Laffler & Gallant, 1974, Fiil *et al.*, 1977). It is not unexpected then that the intracellular ppGpp pool is even higher in MC4100 *relA*$^+$ (KP4) than in MC4100 (*relA1*) with phenotypic consequences. Indeed growth characteristics of KP4 (MC4100 *relA*$^+$) and its *relA1* parents are striking (Figure 5.1).

The strains used for this comparison were constructed by P1 transduction. *relA*$^+$ allele was transduced into different strains by cotransduction or *relA* with each of the three markers, viz., *cysI3152::*Tn*10kan* (CAG12182), *ΔbarA784*::*KAN* (JW2757-1), *ΔgudD785*::*KAN* (JW2758-5). For markerless strain construction, *CysI*$^-$ transductants were converted into *CysI*$^+$ prototrophs again by P1 transduction, screening for loss of Kan$^r$ gene.

MG1655 (*spoT*$^+$) *relA*$^+$/*relA*$^-$ pair is no different from each other for growth on any medium and at any temperature as against MC4100 (*spoT1) relA*$^+$/*relA1* pair. The growth of MC4100 *relA*$^+$ is markedly slow on minimal medium in comparison to *relA1* counterpart. (Table 5.2).

If ppGpp pool directly impinges on growth with negative correlation, the slow growth of MC4100 *relA*$^+$ possibly means its high intracellular concentration resulting from RelA dependent nutritional shiftdown-induced increase in ppGpp synthesis (intracellular ppGpp measurements are being done). The pool amounts are large enough to cause slowing down of growth. Interestingly the pool amounts can be increased further in response to starvation condition.

Under these conditions, the *relA*$^+$ strain grows better on minimal medium containing 3-AT than on minimal medium (Figure 5.1, B).

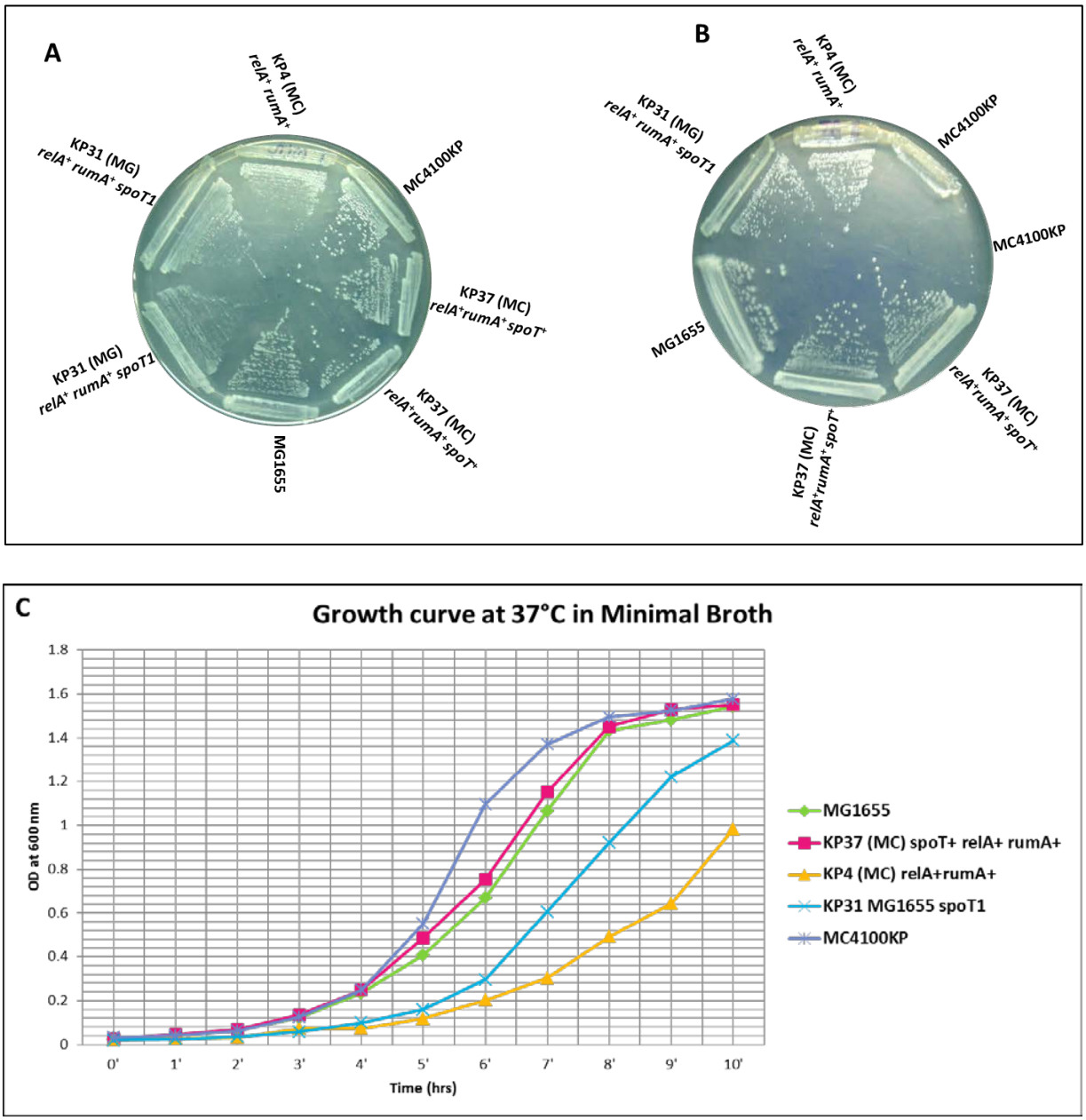


**Figure 5. 1: Comparing the effect of exchange of *spoT* allele between *relA*$^+$ derivative of MC4100 and MG1655.** **(A)** Growth on minimal medium; **(B)** assaying *relA* phenotype on minimal agar plate supplemented with 3-AT and **(C)** Growth rate measurement in minimal broth at 37°C.

### 5.3.2 Interchanging *spoT* alleles between KP4 (MC4100 *relA*$^+$) and MG1655

Interchanging *spoT* allele was done by P1 transduction using linked *ΔpyrE748*::*KAN*. *spoT*$^+$ allele of JW3617-1 was mobilized into KP4 generating KP35 (*spoT*$^+$ *ΔpyrE748*::*KAN*). *spoT1* allele of MC4100KP was first linked to *ΔpyrE748*::*KAN* marker (KP11) (67% cotransduction) followed by its transfer into MG1655 producing KP29 (*spoT1 ΔpyrE748*::*KAN*). Selection for growth on minimal plate lacking uracil was carried out for construction of *pyrE*$^+$ derivatives KP37 (MC4100 *relA*$^+$ *spoT*$^+$) and KP31 (MG1655 *spoT1*).

Phenotypes resulting from interchanging *spoT* alleles between KP4 and MG1655 once again revealed undefined genetic differences between the two strains. As described earlier (Sarubbi *et al.*, 1988, Fiil *et al.*, 1977), KP31 (MG1655 *spoT1*) is marginally affected for growth (Table 5.2), in contrast to KP4 which is significantly affected for growth in minimal medium. Importantly *spoT*$^+$ enabled growth of KP37 (MC4100 *relA*$^+$ *spoT*$^+$) to the same level as MG1655 (Figure 5.1A), indicating convincingly that the undefined genetic difference(s) between MG1655 and KP4 is(are) relevant to ppGpp metabolism.

**Table 5. 2: Doubling time and growth rate of different mutants**

| Strains | Doubling time in MB at 37°C | Growth rate* |
|---|---|---|
| MG1655 | 84.04 | 0.492 |
| KP4 | 108 | 0.384 |
| KP31 | 98.3 | 0.426 |
| KP37 | 78.5 | 0.528 |

*Values represent the growth rates ($\mu$, $h^{-1}$) of exponentially growing cells in MB at 37°C.

### 5.3.3 Qualitative differences of *relA1* and *relA2009* phenotype in MG1655 and MC4100 background:

In the work described in the thesis, we have used two types of *relA*$^-$ mutant alleles, namely *relA1* and *relA2009*. *relA1* mutation is an IS*2* insertion in *relA* gene that retains 1% residual RelA activity. *relA2009* mutation constructed in this work (Chapter 6; Pg. 82 & 83, on the other hand is apparently null mutant with no RelA activity remaining. Behaviour of *relA* mutant alleles in MC4100 and MG1655 background in terms of their growth on SMG/3-AT seemingly correlated well with intracellular ppGpp levels. Since MC4100 is also *spoT1* there is high basal level of ppGpp even when there is *relA1* mutation. MG1655, on the other hand has low ppGpp levels.

pTE4 plasmid contains *relA*$^+$ DNA cloned downstream of arabinose inducible promoter in pBAD18Kan vector. Following introduction of *relA*$^+$ plasmid (pTE4) in each of the strains viz., MC4100KP (*relA1*), KP7 (MC4100 *relA2009* Δ*rumA*), KP22 (MG1655 *relA1*) and KP23 (MG1655 *relA2009* Δ*rumA*) strains the *relA* mutation in each was tested for complementation of RelA phenotype as assayed by sensitivity-resistance to 3-AT/SMG. The phenotype of growth on starvation plate was possible to be correlated with the intracellular levels of ppGpp (are being monitored). MC4100KP (*relA1)*, KP7 (MC4100 *relA2009* Δ*rumA*) strains grow in the presence of 3-AT plate without any added arabinose, whereas KP22 (MG1655 *relA1*) and KP23 (MG1655 *relA2009* Δ*rumA*) grew only when arabinose was present at 6.65 mM; there was no growth on minimal plate and minimal plate with 0.665 mM and 3.33 mM of arabinose.

Furthermore there is correlation also between the severity of *relA* allele, expression of *relA* gene on the plasmid represented in terms of arabinose concentration and growth on minimal plate supplemented with 3-AT or SMG (Table 5.3).

**Table 5. 3: Comparison of *relA1* and *relA2009* on 3-AT/SMG supplemented media in MC4100 and MG1655 background.**

| | | Growth | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strains/Plasmids (Derivatives of MC4100 or MG1655) | *relA rumA* genotype | **Min+ glucose** | **Min+ SMG** | **Min+SMG+Arabinose** | | | **Min+3-AT** | **Min+3-AT+Arabinose** | | |
| | | | | **0.665 mM** | **3.33 mM** | **6.65 mM** | | **0.665 mM** | **3.33 mM** | **6.65 mM** |
| KP22/pBAD18Kan (MG1655) | *relA1 rumA*$^+$ | + + + | − | − | − | − | − | − | − | − |
| KP23/pBAD18Kan (MG1655) | *relA2009*Δ*rumA1* | + + + | − | − | − | − | − | − | − | − |
| KP22/pTE4 (MG1655) | *relA1 rumA*$^+$ | + + + | − | − | ± | +++ | − | + | ++ | +++ |
| KP23/pTE4 (MG1655) | *relA2009*Δ*rumA1* | + + + | − | − | ± | +++ | − | + | ++ | +++ |
| MC4100KP/pBAD18Kan | *relA1 rumA*$^+$ | + + + | − | − | − | − | − | − | − | − |
| KP7/pBAD18Kan (MC4100) | *relA2009*Δ*rumA1* | + + + | − | − | − | − | − | − | − | − |
| MC4100KP×pTE4 | *relA1 rumA*$^+$ | + + + | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| KP7/pTE4 (MC4100) | *relA2009*Δ*rumA1* | + + + | − | + | ++ | +++ | +++ | +++ | +++ | +++ |

MC4100 *relA1* mutant grows on Minimal plate containing SMG forming small size colonies; growth after arabinose supplementation (3.33mM and more) is faster. MC4100 *relA2009* mutant does not grow on Min+SMG in the absence of arabinose; growth is seen only when supplemented with arabinose at 3.33mM and more. *relA1* and *relA2009* mutants of MG1655 do not grow on Min+SMG in the absence of arabinose; growth is possible only when supplemented with arabinose at 3.33mM and more. Growth on SMG seemingly required more ppGpp i.e., more levels of *relA* expression than growth on 3-AT plate is also apparent from Table 5.3.

### 5.3.4 Construction of MC4100 *relA*$^+$ strain by other groups

Curiously this effect of introduction of *relA*$^+$ gene in MC4100 on growth has not been described adequately.

(i) The study compares much used laboratory strains MC4100 *relA1*and MG1655 with no mention of MC4100 *relA*$^+$ (Spira *et al.*, 2008).

(ii) Hanna Engelberg–Kulka et al. described construction of MC4100 *relA*$^+$ in the paper (Engelberg–Kulka *et al.*, 1998). This is referred to extensively in their papers on genetics of two component of addiction module in *E. coli* (Sat *et al.*, 2003, Hazan *et al.*, 2004, Amitai *et al.*, 2009, Vesper *et al.*, 2011, Erental *et al.*, 2012). However, Tsilibaris *et al.*, (2007) showed that MC4100 *relA*$^+$ strain is in fact *relA*$^-$ by sequencing the *relA* region in the strain obtained from Kulka *et al* lab. It harbored a frameshift mutation at the 5' end of the *relA* gene that rendered it *relA*$^-$ (deletion of cytosine 623 modifies the amino acid sequence from codon 115 and generates a stop at codon 118) (Tsilibaris *et al.*, 2007). The authors constructed MC4100 *relA*$^+$ by selection for growth on SMG plate. Again there is no phenotype of slow growth on minimal medium described for this strain.

(iii) Other publications which elaborate on the construction of MC4100 *relA*$^+$ derivative used cotransducible selectable markers like *fuc-3072*::Tn*10* (Lange *et al.*, 1995, Majumder *et al.*, 2001). However, the description of growth phenotype of the strain is clearly lacking in these papers.

Results described above in the chapter for the *relA* phenotype of the two strains MC4100 and MG1655 has direct bearing on the work explained in the following chapter. We report our

finding that insertion of chloramphenicol acetyltransferase (*CAT*) cassette upstream of *relA* gene causes overproduction of RelA protein without affecting its transcript levels. The genetic characterization of the mutant strain forms the scope of the work.

## 5.4 References


Amitai, S., I. Kolodkin-Gal, M. Hananya-Meltabashi, A. Sacher & H. Engelberg-Kulka, (2009) Escherichia coli MazF leads to the simultaneous selective synthesis of both "death proteins" and "survival proteins". *PLoS genetics* **5**: e1000390.

Engelberg–Kulka, H., M. Reches, S. Narasimhan, R. Schoulaker-Schwarz, Y. Klemes, E. Aizenman & G. Glaser, (1998) rexB of bacteriophage λ is an anti-cell death gene. *Proceedings of the National Academy of Sciences* **95**: 15481-15486.

Erental, A., I. Sharon & H. Engelberg-Kulka, (2012) Two programmed cell death systems in Escherichia coli: an apoptotic-like death is inhibited by the mazEF-mediated death pathway. *PLoS biology* **10**: e1001281.

Fabich, A.J., M.P. Leatham, J.E. Grissom, G. Wiley, H. Lai, F. Najar, B.A. Roe, P.S. Cohen & T. Conway, (2011) Genotype and phenotypes of an intestine-adapted Escherichia coli K-12 mutant selected by animal passage for superior colonization. *Infection and immunity* **79**: 2430-2439.

Ferenci, T., Z. Zhou, T. Betteridge, Y. Ren, Y. Liu, L. Feng, P.R. Reeves & L. Wang, (2009) Genomic sequencing reveals regulatory mutations and recombinational events in the widely used MC4100 lineage of Escherichia coli K-12. *Journal of bacteriology* **191**: 4025-4029.

Fiil, N.P., B.M. Willumsen, J. Friesen & K. von Meyenburg, (1977) Interaction of alleles of therelA, relC andspoT genes inEscherichia coli: Analysis of the interconversion of GTP, ppGpp and pppGpp. *Molecular and General Genetics MGG* **150**: 87-101.

Freddolino, P.L., S. Amini & S. Tavazoie, (2012) Newly identified genetic variations in common Escherichia coli MG1655 stock cultures. *Journal of bacteriology* **194**: 303-306.

Hazan, R., B. Sat & H. Engelberg-Kulka, (2004) Escherichia coli mazEF-mediated cell death is triggered by various stressful conditions. *Journal of bacteriology* **186**: 3663-3669.

Heath, J., J.D. Perkins, B. Sharma & G.M. Weinstock, (1992) NotI genomic cleavage map of Escherichia coli K-12 strain MG1655. *Journal of bacteriology* **174**: 558-567.

Hilton, J., (1965) Modes of action of 3-amino-1, 2, 4-triazole: current status. In: Isotope Weed Research Proc. Symp. Vienna. pp. 71-83.

Jishage, M. & A. Ishihama, (1997) Variation in RNA polymerase sigma subunit composition within different stocks of Escherichia coli W3110. *Journal of bacteriology* **179**: 959-963.

King, T., A. Ishihama, A. Kori & T. Ferenci, (2004) A regulatory trade-off as a source of strain variation in the species Escherichia coli. *J Bacteriol* **186**: 5614-5620.

Laffler, T. & J. Gallant, (1974) spoT, a new genetic locus involved in the stringent response in E. coli. *Cell* **1**: 27-30.

Lange, R., D. Fischer & R. Hengge-Aronis, (1995) Identification of transcriptional start sites and the role of ppGpp in the expression of rpoS, the structural gene for the sigma S subunit of RNA polymerase in Escherichia coli. *Journal of bacteriology* **177**: 4676-4680.

Maharjan, R.P., P.-L. Yu, S. Seeto & T. Ferenci, (2005) The role of isocitrate lyase and the glyoxylate cycle in Escherichia coli growing under glucose limitation. *Research in microbiology* **156**: 178-183.

Majumder, A., M. Fang, K.-J. Tsai, C. Ueguchi, T. Mizuno & H.-Y. Wu, (2001) LeuO expression in response to starvation for branched-chain amino acids. *Journal of Biological Chemistry* **276**: 19046-19051.

Peters, J.E., T.E. Thate & N.L. Craig, (2003) Definition of the Escherichia coli MC4100 genome by use of a DNA array. *J Bacteriol* **185**: 2017-2021.

Sarubbi, E., K.E. Rudd & M. Cashel, (1988) Basal ppGpp level adjustment shown by new spoT mutants affect steady state growth rates and rrnA ribosomal promoter regulation in Escherichia coli. *Mol Gen Genet* **213**: 214-222.

Sat, B., M. Reches & H. Engelberg-Kulka, (2003) The Escherichia coli mazEF suicide module mediates thymineless death. *Journal of bacteriology* **185**: 1803-1807.

Sawers, R.G., (2005) Expression of fnr is constrained by an upstream IS5 insertion in certain Escherichia coli K-12 strains. *Journal of bacteriology* **187**: 2609-2617.

Silva, A.J. & J.A. Benitez, (2006) A Vibrio cholerae relaxed (relA) mutant expresses major virulence factors, exhibits biofilm formation and motility, and colonizes the suckling mouse intestine. *J Bacteriol* **188**: 794-800.

Soupene, E., W.C. van Heeswijk, J. Plumbridge, V. Stewart, D. Bertenthal, H. Lee, G. Prasad, O. Paliy, P. Charernnoppakul & S. Kustu, (2003) Physiological studies of Escherichia coli strain MG1655: growth defects and apparent cross-regulation of gene expression. *Journal of bacteriology* **185**: 5611-5626.

Spira, B., X. Hu & T. Ferenci, (2008) Strain variation in ppGpp concentration and RpoS levels in laboratory strains of Escherichia coli K-12. *Microbiology* **154**: 2887-2895.

Tosa, T. & L.I. Pizer, (1971) Biochemical bases for the antimetabolite action of L-serine hydroxamate. *J Bacteriol* **106**: 972-982.

Tsilibaris, V., G. Maenhaut-Michel, N. Mine & L. Van Melderen, (2007) What is the benefit to Escherichia coli of having multiple toxin-antitoxin systems in its genome? *J Bacteriol* **189**: 6101-6108.

Uzan, M. & A. Danchin, (1976) A rapid test for the rel A mutation in E. coli. *Biochem Biophys Res Commun* **69**: 751-758.

Vesper, O., S. Amitai, M. Belitsky, K. Byrgazov, A.C. Kaberdina, H. Engelberg-Kulka & I. Moll, (2011) Selective translation of leaderless mRNAs by specialized ribosomes generated by MazF in Escherichia coli. *Cell* **147**: 147-157.