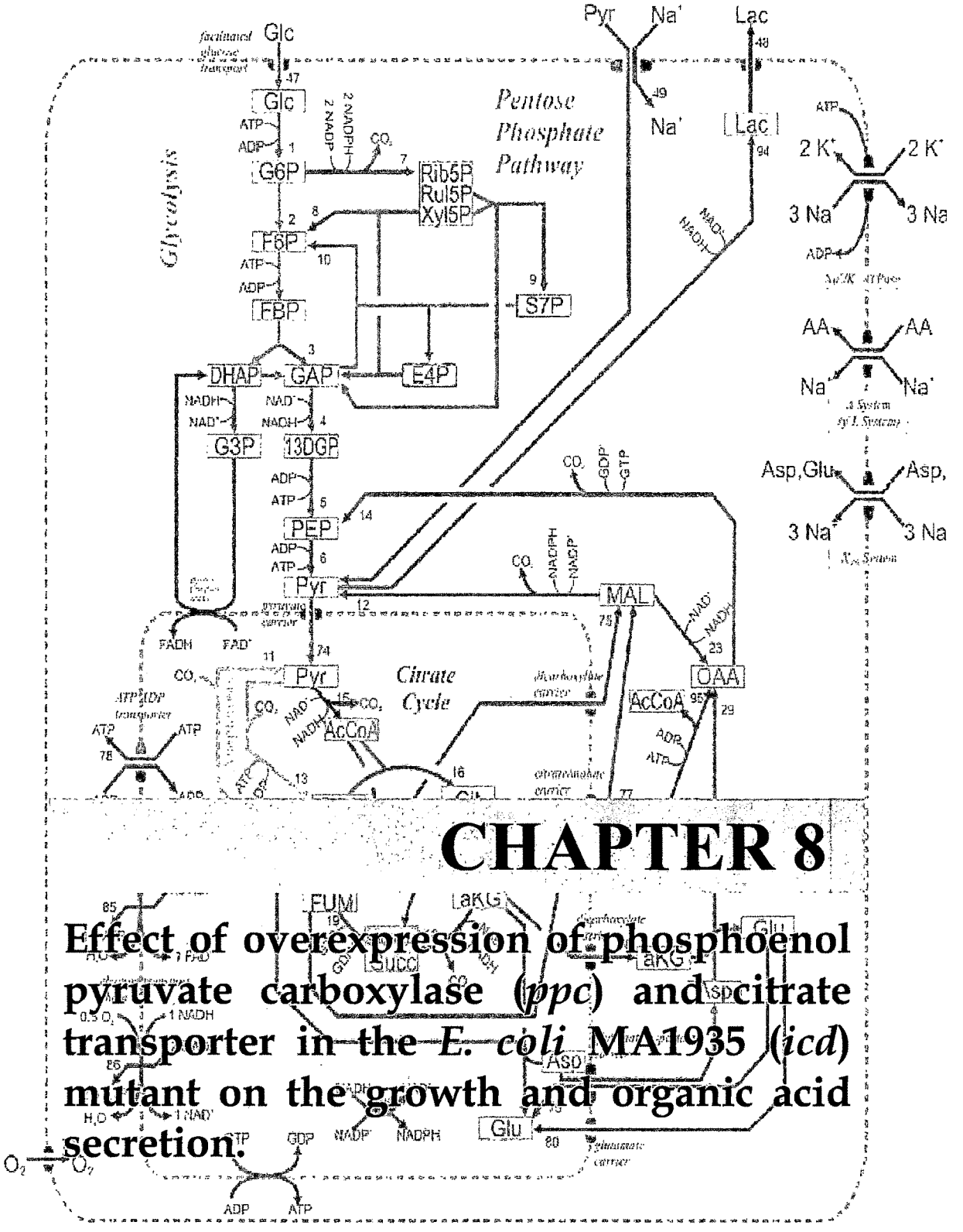

CHAPTER 8

Effect of overexpression of phosphoenol pyruvate carboxylase (*ppc*) and citrate transporter in the *E. coli* MA1935 (*icd*) mutant on the growth and organic acid secretion.

8.1: INTRODUCTION

In *E. coli*, glycolysis and TCA cycle constitute the central carbon metabolism. The former constitutes carbon breakdown leading to the formation of PEP, pyruvate and acetyl CoA. TCA cycle plays a dual role in the catabolism and anabolism. Complete oxidation of acetyl CoA yields energy and also generates various metabolic intermediates important for biosynthesis. *E. coli* when grown on TCA cycle intermediates or on substrates that enter TCA cycle though acetyl CoA (acetate, fatty acids, ethanol) operates the gluconeogenic pathway wherein the TCA cycle intermediates malate and OAA needs to be converted to PEP and pyruvate which are further converted to sugar phosphate by a set of reactions. This metabolic link between the glycolysis/gluconeogenesis and TCA is called the *PEP-pyruvate-OAA node* or the *anaplerotic node*. Regulatory role of the node is well explained in **Chapter 1**. This node plays a critical role in directing the carbon flux by involving a series of reactions into appropriate directions in response to growth conditions and its regulation varies in different bacteria.

This node has been well demonstrated for *E. coli*, *Bacillus subtilus*, *Corynebacterium* and *Pseudomonas citronellolis* (Sauer and Eikmanns, 2005). In *E. coli*, this node has been exploited for succinate overproduction both under aerobic and anaerobic conditions (**Chapter 1**).

8.1.1: Effect of *ppc* overexpression in *E. coli* and other bacteria.

Altering the metabolic flux between the PEP and OAA branch point by various genetic manipulations including *ppc* overexpression under glycolytic conditions has been well-demonstrated in *E. coli* (Farmer and Liao, 1999; Lin et al., 2005a). PEP and OAA are the key metabolites at the junction of biosynthesis and catabolism. In *E. coli*, *ppc* overexpression under anaerobic conditions directed the carbon flux towards fermentation products with succinic acid as the major fermentative product on glucose which otherwise is a minor product (Millard et al., 1996). Studies on the overexpressing *ppc* or *pyc* genes in *E. coli* demonstrated that the metabolic network adapts to these genetic alterations by

adjusting the flux to lactate, ethanol and acetate (Gokarn et al., 2000). In addition, *pyc* overexpression improved the glucose consumption rate, ATP formation rate and the specific growth rate.

ppc overexpression under aerobic condition in *E. coli* resulted in lower glucose consumption rates and organic acid excretion without altering the growth and respiration rates; thereby resulting into improved growth yield on glucose (Chao and Liao, 1993). Acetate overflow under aerobic conditions in presence of excess glucose reduced recombinant protein production. Flux analysis also supported that directing the flux through PPC could help in lowering the acetate outflow. This hypothesis was successfully supported by *ppc* overexpression in *E. coli* which reduced the acetate excretion by 60% without affecting the growth and the glucose consumption rate (Farmer and Liao, 1997; Abdel-Hamid et al., 2001). Simultaneous overexpression of *ppk* and *pck*, or *pps* alone in the presence of glucose led to futile cycling, which did not affect the growth rate significantly (Liao et al., 1994).

Expression of *E. coli ppc* gene in *Synechococcus* PCC 7942 *ppc* mutant had lower PPC activity with reduced growth, chlorophyll-a content and photosynthetic activity (Luinenburg and Coleman, 1993). Overexpression of *ppc* gene in combination with ornithine carbamoyltransferase and carbamoylphosphate synthetase genes triggered the biosynthesis of cyanophycin in *Acinetobacter sp.* strain ADP1 (Elbahloul and Steinbüchel, 2006). Overexpression of *ppc* gene in *C. glutamicum* resulted in lysine overproduction containing feedback-resistant aspartate kinase while it did not contribute much in glutamate overproduction (Cremer et al., 1991; Shirai et al., 2007).

8.1.2: Why heterologous *ppc* gene?

PPC of *E. coli* is activated by acetyl CoA (Canovas and Kornberg 1966), Fructose 1,6- bisphosphate (Sanwal and Maeba, 1966), GTP and long chain fatty acids, and inhibited by L-aspartate, L-malate and other C-4 dicarboxyclic acids (Gold and Smith, 1974; Morikawa, 1980). Majority of the PPC enzymes of non-photosynthetic bacteria including

E. coli and *P. citronellolis* belong to class I which get allosterically activated by acetyl-CoA and inhibited by L-aspartate (Newaz and Hersh, 1975; O'Brien *et al.*, 1977). On the contrary as a rare case, PPC in *Pseudomonas* AM-1 and *Pseudomonas* MA grown on methylamine as sole carbon source belonged to Class III as they were independent of acetyl-CoA and aspartate mediated allosteric regulations (Large et al., 1962; Newaz and Hersh, 1975). PPC in *Pseudomonas* MA was also activated by NADH and inhibited by ADP (Millay et al., 1978).

To overcome the allosteric regulations exerted at the anaplerotic node by *E. coli* metabolism, *ppc* gene from a heterologous host *Synechococcus elongatus* PCC 6301 (*Anacystis nidulans*, cyanobacteria) was selected for the present study. This PPC is known to be non-allosteric and has been demonstrated to be insensitive to the allosteric effectors including dioxane (non-physiological activator) and L-aspartate (Ishijima et al., 1985; Kodaki et al., 1985). Cyanobacetrial PPC is not activated by acetyl-CoA (Luinenburg and Coleman, 1993). *S. elongatus ppc* gene codes for a 1053 amino acid residue polypeptide with the codon usage not so markedly different from that of the *E. coli ppc* (Katagiri et al., 1985). Like most of the known PPCs, this cyanobacterial PPC functions as a homotetramer of ~95–110-kDa subunits, and is more closely related to bacterial PPCs due to presence of conserved bacterial type (including *E. coli*) catalytic domain and lack of N-terminal phosphorylation domain typical of plant PPC (Kai et al., 1999; Sanchez and Cejudo, 2003; Xu et al., 2006; Sugita et al., 2007).

The present study involves manipulation at this node in *E. coli* BL21(λDE3) *icd* mutant (MA1935) to enhance citrate production through overexpression of phosphoenol pyruvate carboxylase (*ppc*) in the mutant as OAA levels might be limiting for citrate production on glucose. Hence the present study investigates the effect of overexpression of cyanobacterial *ppc* gene in *E. coli* BL21(λDE3) *icd* mutant and accumulation of citrate.

8.2: WORK PLAN

8.2.1: Bacterial strains used in the present study.

E. coli strains	Genotype	References
BL21(λDE3)	F⁻, *ompT,hsdS$_B$* (r_{B-}, m^{B+}), *dcm, gal,* (DE3).	Sambrook and Russell, 2001
MA1935	*icd* gene mutant	Aoshima et al., 2003
MA1935 pVSD1	*icd* mutant carrying citrate transporter pVSD1, Chl^r	Present study
MA1935 pJE3:pVSD1	P_{tac} promoter, citrate transporter. Chl^r, Tc^r, Ap^r	Present study
MA1935 pVSppc:pVSD1	*ppc* gene under P_{tac} promoter, citrate transporter. Chl^r, Tc^r, Ap^r	Present study
DH5α pJE3	P_{tac} promoter, Tc^r, Ap^r	Present study
DH5α AB5	tet resistance gene in pUCpM18 plasmid Tc^r, Ap^r	Buch, 2008
DH5α pVSppc	*ppc* gene under P_{tac} promoter. Chl^r, Tc^r, Ap^r	Sharma, 2008

Table 8.1: *E. coli* strains used for the present study. The details of the plasmids and the concentrations of the antibiotics used are given in the table 2.2 and 2.3.

8.2.2: Construction of control vector.

To construct the control vector, pTTQ18 and pAB5 plasmids were digested with *Hind*III, pTTQ18 was linearised (4,563bp) while pAB5 gave two fragments 5,349bp (vector backbone) and 2,817bp fragment corresponding to Tc^r fragment respectively. The vector and insert were gel purified and ligated to yield a plasmid designated as pJE3 (7,367bp).

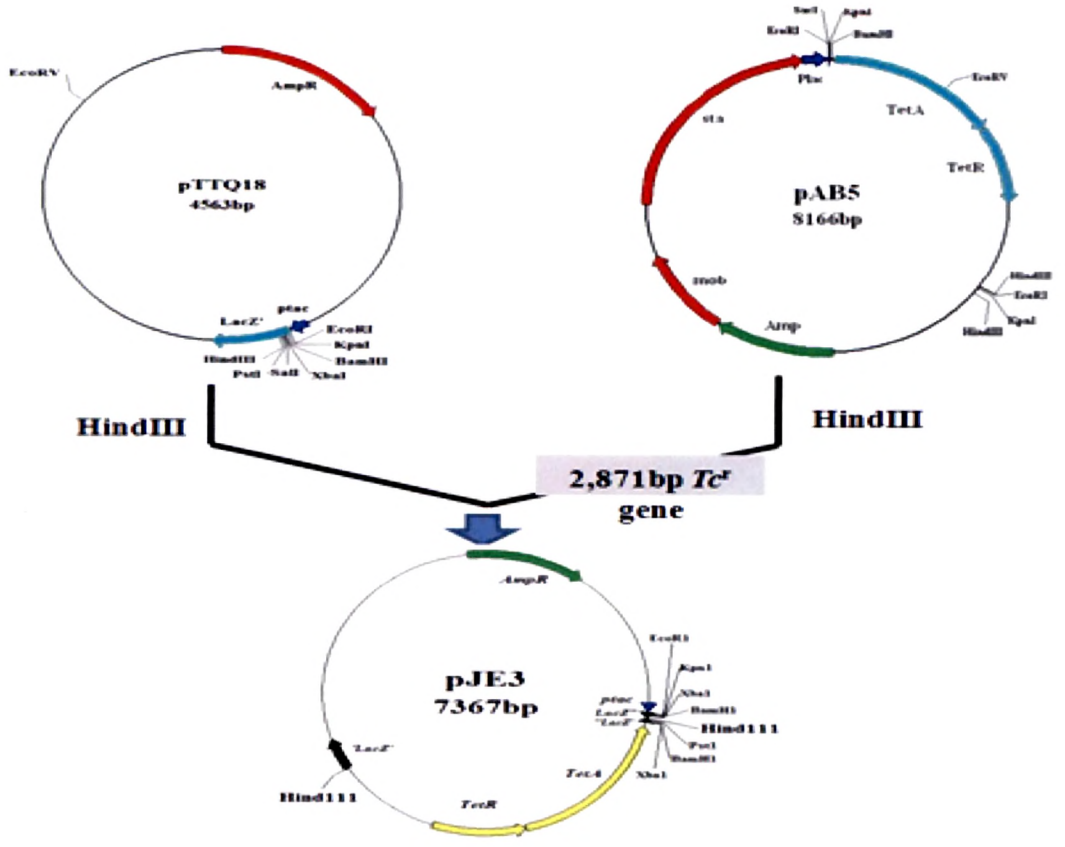

Fig. 8.1: Schematic representation of construction of vector carrying tetracycline resistance (*Tc*^{r}) gene (pJE3).

All the molecular biology techniques like plasmid preparation, transformation, gel elution and purification, restriction digestion, DNA ligation and gel electrophoresis were performed based on the protocol described in section 2.3.

8.2.3: Developing *E. coli* BL21(λDE3) *icd* mutant overexpressing *ppc* and citrate transporter.

E. coli MA1935 *icd* gene mutant was transformed with *ppc* gene harboring plasmid and co-transformed with the pVSD1 plasmid. Control *E. coli* was obtained by transformation of the mutant with pJE3 and pVSD1 plasmids.

8.2.4: Monitoring growth and physiological parameters of *E. coli* BL21(λDE3) *icd* mutant.

The *E. coli* MA1935 transformants were grown on M9 minimal media with micronutrients and 50mM glucose and 100mM glycerol as carbon source. IPTG (0.1 mM) was used for induction. Samples were collected at regular interval for O.D. $_{600nm}$, glucose utilized and organic acid in the medium. The physiological parameters were calculated as described in (section 2.5). Enzyme assays were performed as per protocol mentioned in (section 2.6); ICDH, PPC, G-6-PDH, CS and ICL at stationary phase.

8.3: RESULTS

8.3.1: Developing *E. coli* BL21(λDE3) *icd* mutant overexpressing *ppc* and citrate transporter

The control vector was constructed using single site cloning yielding pJE3. Plasmids were confirmed by restriction digestion analysis (**Fig.8.2**). *E. coli* MA1935 was transformed with pVSD1, these transformants could utilize citrate as sole carbon source as compared to the *E. coli* BL21(λDE3) wild type parent and *E. coli* MA1935 (**Fig. 8.3**). *E. coli* MA1935 pVSD1 was co transformed with pVSppc and pJE3 giving rise to *E. coli* MA1935 pVSppc: pVSD1 and *E. coli* MA1935pJE3:pVSD1. Plasmids were isolated from these strains and restriction digestion was carried out to confirm the plasmid. pVSD1 on HindIII digestion yielded two bands one corresponding to 7,148 bp and 700bp and the pJE3:pVSD1on HindIII digestion gave three bands 7,148bp 5,349bp and 2,817bp and pVSppc :pVSD1 with EcoRI digestion gave four bands 7,323bp, 7,148bp, 3,951bp and 700bp (**Fig. 8.2**).

8.3.2: Monitoring the effect of *icd* gene mutation and overexpression of citrate transporter on the physiological parameters in *E. coli* MA1935 on M9 minimal media with glucose and glycerol as carbon sources.

E. coli MA1935 (*icd* mutant) required glutamate supplementation to grow on glucose or glycerol as carbon source (**Fig. 8.3**). On glucose, *E. coli* MA1935 (*icd* mutant) showed a poor growth rate, reduced glucose consumption rate and also overall reduction in the amount

of glucose consumed as compared to wild type (**Table 8.2, Fig. 8.5 (i) and (ii)**). There was no change in the biomass as a result of mutation. Incorporation of citrate transporter in the mutant did not affect the growth but significantly decreased the glucose consumption rate and the glucose consumed.

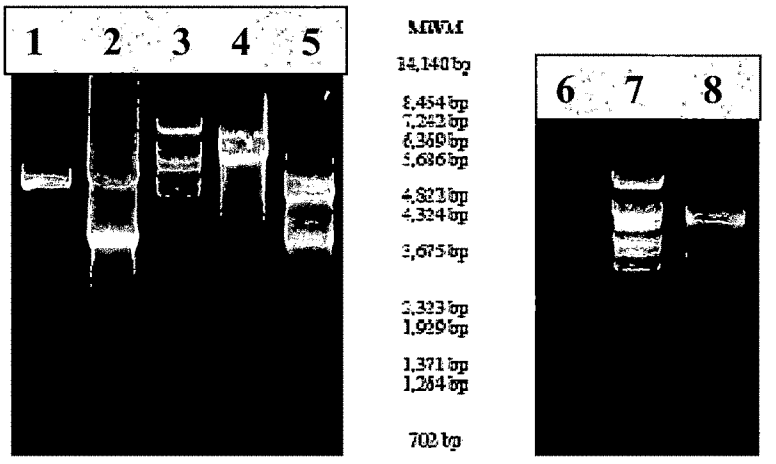

Fig. 8.2: Restriction Digestion pattern for pJE3, pJE3:pVSD1 and pVSppc:pVSD1

Lane 1: pTTQ18 HindIII digest, Lane 2: pAB5 HindIII digest (5,349bp, 2817bp), Lane 3,7 : BstEII molecular marker, Lane 4: pJE3 EcoRI digest (7367bp), Lane 5: pJE3 HindIII digest (4563bp, 2817bp) Lane 6: pJE3:pVSD1 HindIII (7148bp 5,349bp and 2817bp), Lane 7: pJE3:pVSD1 HindIII (7323bp, 7148bp, 3951bp and 700bp).

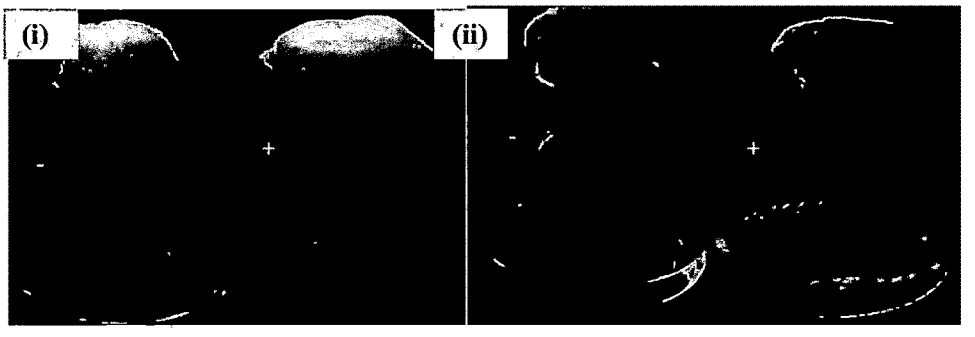

Fig 8.3: *E. coli* BL21 (λDE3) and *E. coli* MA1935 in presence and absence of citrate transporter. (i) *E. coli* BL21 (λDE3) in presence of citrate transporter (ii) *E. coli* MA1935 in presence of citrate transporter -/+ indicate growth.

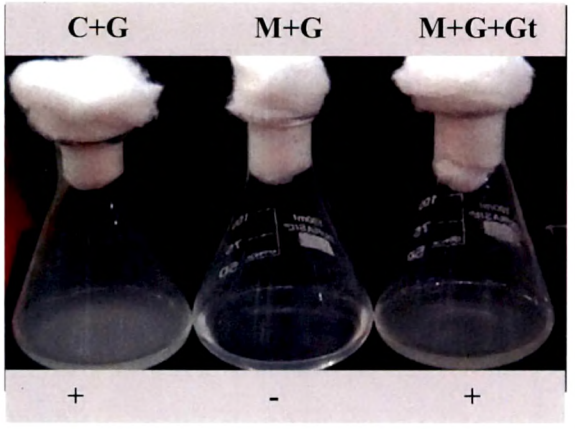

Fig. 8.4: *E. coli* MA1935 icd mutant showing glutamate auxotrophy. C- *E. coli* BL21(λDE3), M- *E. coli* MA1935 *icd* mutant , G-glucose, Gt-Glutamate. -/+ indicate growth

***E. coli* strains**	**Growth rate μ (h^{-1})**	**Specific glucose consumption rate (Q_{glc}) ($gglc.g^{-1}dcw.h^{-1}$)**	**Total glucose utilized (mM)**	**Biomass $Y_{dcw/Glc}$ ($g\ dcw.\ g^{-1}.h^{-1}$)**
BL21(λDE3)	0.74 ± 0.1	7.0 ± 0.72	45.5 ± 4.0	0.15 ± 0.05
MA1935	**0.34 ± 0.03** ** [a]	**4.42 ± 0.36** * [a]	**20.5 ± 2** *** [a]	**0.13 ± 0.1** ns
MA1935 pVSD1	0.53 ±0.04	2.18 ± 0.58 **[b]	6.7 ± 1.9 ***[b]	0.15 ± 0.02
MA1935 pJE3:pVSD1	0.20 ± 0.07	4.18 ± 0.17	27.01 ± 4.14	0.08± 0.01
MA1935 pVSppc:pVSD1	**0.45 ± 0.11** *[c]	**3.50 ± 1.00** [ns] c	**24.75 ± 4.0** ns c	**0.13 ± 0.01** ** [c]

Table 8.2: Physiological variables and metabolic data from *E. coli* MA1935 *ppc* transformant grown on M9 minimal medium. The results are expressed as Mean ±SD of 8-10 independent observations. Biomass yield $Y_{dcw/Glc}$, specific growth rate ($\mu(h^{-1})$) and specific glucose consumption rate (Q_{Glc}) were determined from mid log phase of each experiment. Total glucose utilized was determined at the end of growth curve. [a] comparison between *E. coli* MA1935 and parent strain [b] comparison between *E. coli* MA1935 in absence and presence of citrate transporter [c] comparison between *E. coli* MA1935 overexpressing *ppc* gene and respective control.. * $p<0.05$, ** $p<0.01$, ns=non-significant.

On glycerol, *E. coli* MA1935 (*icd* mutant) demonstrated lower growth rate and improved glycerol uptake compared to that of the control. Incorporation of citrate transporter in the mutant further reduced the growth and amount of glycerol consumed **(Table 8.3, Fig. 8.6 (i) and (ii)).**

E. coli strains	Growth rate μ (h^{-1})	Total glycerol consumed (mM)
BL21(λDE3)	0.34 ± 0.02	45.8 ± 3.6
MA1935	**0.21 ± 0.02 **[a]**	**64 ± 4.0**
MA1935 pVSD1	0.16 ± 0.03	29.7 ± 8.0 **[b]
MA1935 pJE3:pVSD1	0.13 ± 0.01	67 ± 6.8
MA1935 pVSppc:pVSD1	**0.13 ± 0.02 [ns c]**	**58 ± 4.0 [ns c]**

Table 8.3: Physiological variables and metabolic data from *E. coli* MA1935 *ppc* transformant grown on M9 minimal medium with glycerol as carbon source. The results are expressed as Mean ±SD of 4-6 independent observations. Specific growth rate ($\mu(h^{-1})$) was determined from mid log phase of each experiment. Total glycerol utilized was determined at the end of growth curve. [a] comparison between *E. coli* MA1935 and parent strain [b] comparison between *E. coli* MA1935 in absence and presence of citrate transporter [c] comparison between *E. coli* MA1935 overexpressing *ppc* gene and respective control. * $p<0.05$, ** $p<0.01$, ns=non-significant.

8.3.3: Monitoring the effect of *ppc* overexpression on the physiological parameters in *E. coli* MA1935 carrying citrate transporter.

On glucose *E. coli* MA1935 expressing *ppc* gene improved growth and increased biomass as compared to the control. No alteration was seen with respect to glucose consumption rate and the amount of glucose consumed **(Table 8.2, Fig. 8.7 (i) and (ii)).** On glycerol *ppc* overexpression in *E. coli* MA1935 did not show any alteration with respect to growth and glycerol uptake **(Table 8.3, Fig. 8.8 (i) and (ii)).**

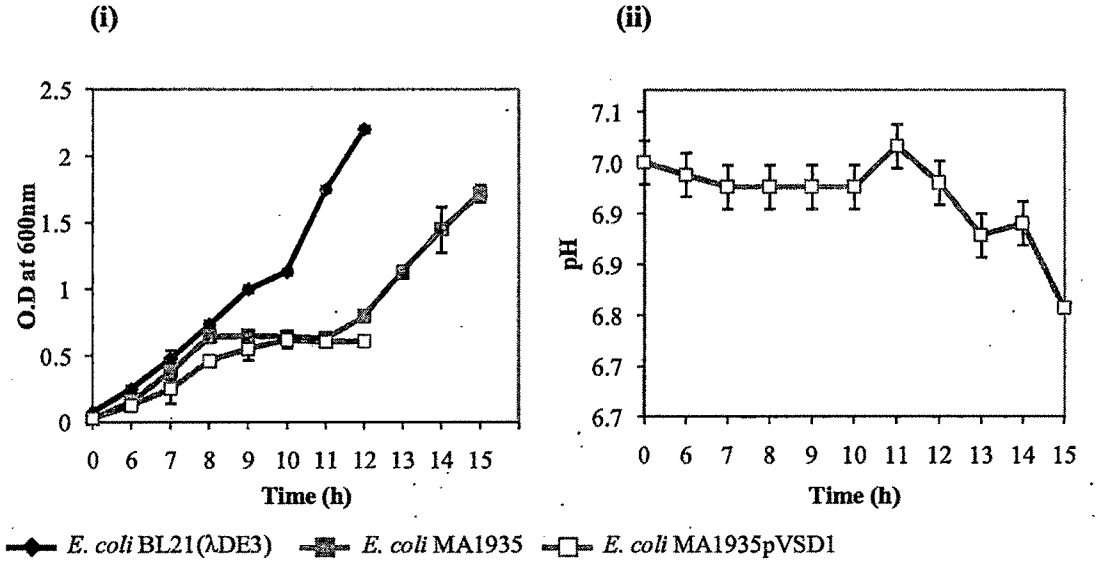

Fig. 8.5: Growth characteristics of *E. coli* MA1935 on M9 minimal medium with 50 mM glucose. (i) & (ii) $O.D_{600nm}$ and pH on glucose.. All values plotted are represented as Mean ± SD for n= 4 to 7 observations.

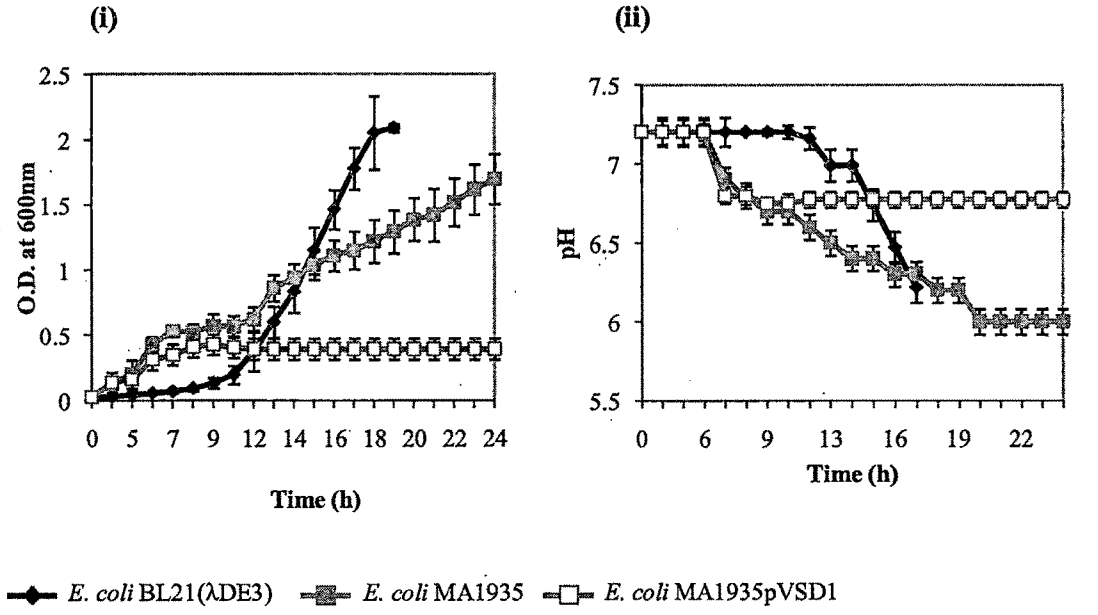

Fig. 8.6: Growth characteristics of *E. coli* MA1935 on M9 minimal medium with 50 mM glucose and 100mM glycerol. (i) & (ii) $O.D_{600nm}$ and pH on glycerol. All values plotted are represented as Mean ± SD for n= 4 to 7 observations.

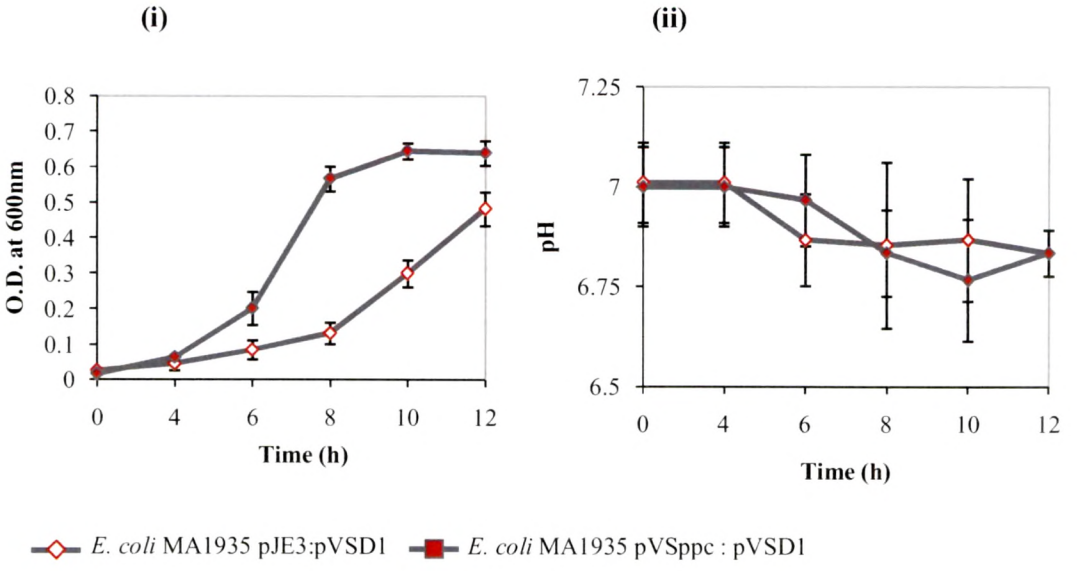

Fig. 8.7: Growth characteristics of *E. coli* MA1935 overexpressing *ppc* on M9 minimal medium with 50 mM glucose. (i) & (ii) $O.D_{600nm}$ and pH on glucose. All values plotted are represented as Mean ± SD for n= 4 to 7 observations.

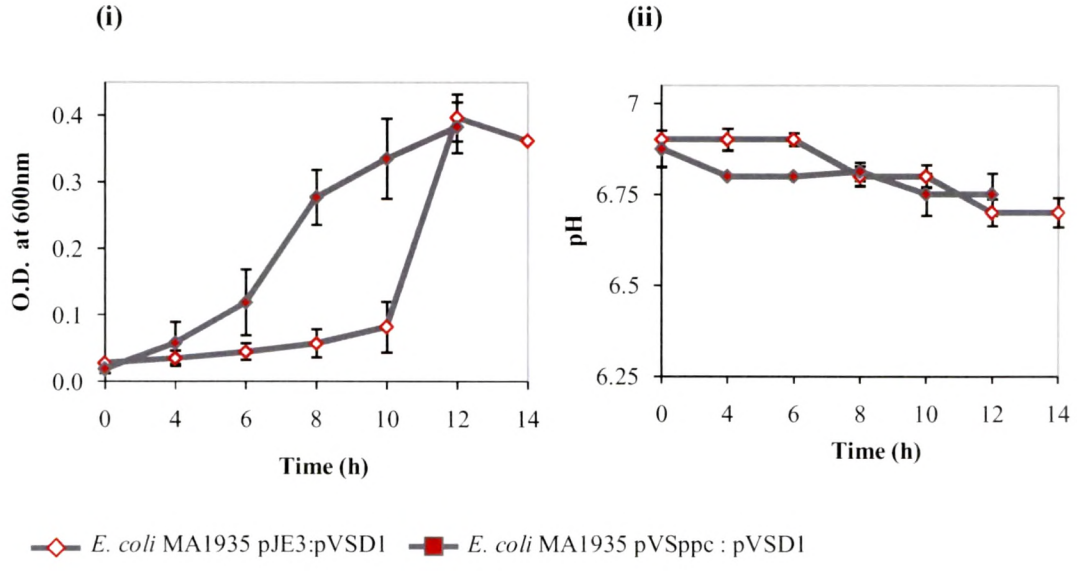

Fig. 8.8: Growth characteristics of *E. coli* MA1935 overexpressing *ppc* on M9 minimal medium with 100mM glycerol. (i) & (ii) $O.D_{600nm}$ and pH on glycerol. All values plotted are represented as Mean ± SD for n= 4 to 7 observations.

8.3.4: Effect of *icd* mutation and *ppc* gene overxpression on the PPC, CS, G-6-PDH and ICL activities in *E. coli* MA1935.

On glucose, *E. coli* MA1935 and the plasmid transformants showed no ICDH activity. Mutation in *icd* gene resulted in decrease in G-6-PDH activity but no alteration in PPC, CS and ICL activities were seen with respect to the control. Incorporation of citrate transporter also demonstrated similar results (**Fig. 8.9**). On glycerol, *E. coli* MA1935 showed similar results as obtained on glucose with respect to PPC, CS and G-6-PDH activity but ICL activity was low compared to that on glucose (**Fig. 8.10**).

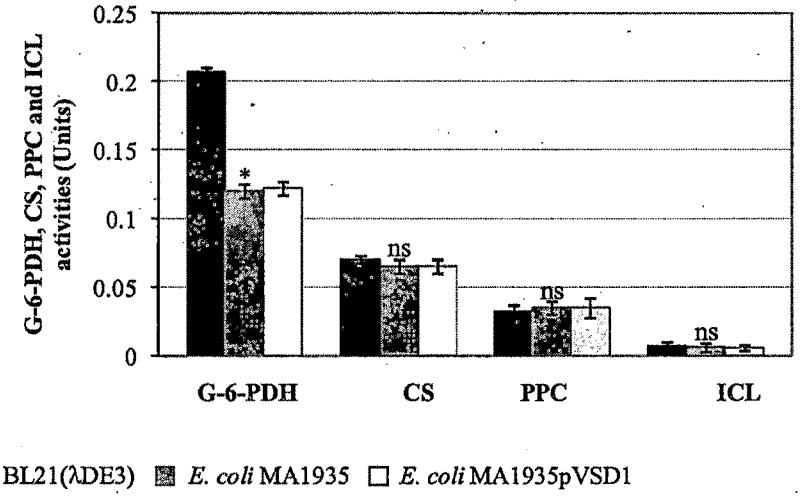

Fig. 8.9: Effect of *icd* gene mutation in *E. coli* BL21(λDE3) on enzyme activities in presence and absence of citrate transporter on M9 minimal medium with 50 mM glucose. Units for the specific activity are given as μmoles/mg. protein/min. All the values are represented as Mean ± SD for n= 5-8 observations. Units- μmoles/mg. protein/min. *$p<0.05$, ns-non significant.

Overexpression of *ppc* gene in *E. coli* MA1935 decreased CS and increased ICL activity with respect to control. No alteration was seen with respect to G-6-PDH activity. 3 fold increase in PPC activity was observed in *E. coli* MA1935 carrying pVSppc (**Fig. 8.11**). On glycerol, *E. coli* MA1935 expressing *ppc* gene showed similar results as obtained on glucose with respect to PPC, CS and G-6-PDH activity but ICL activity was low compared to that on glucose (**Fig. 8.12**).

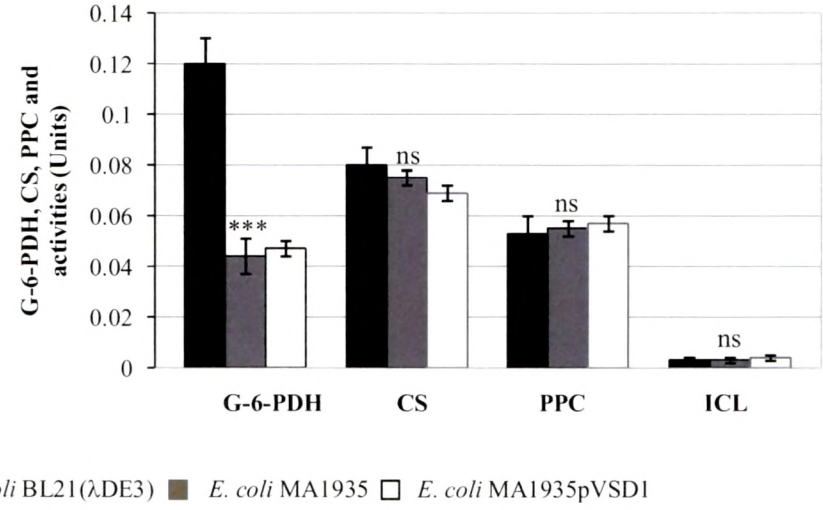

Fig. 8.10: Effect of *icd* gene mutation in *E. coli* BL21(λDE3) on enzyme activities in presence and absence of citrate transporter on M9 minimal medium with 100 mM glycerol. Units for the specific activity are given as μmoles/mg. protein/min. All the values are represented as Mean ± SD for n= 5-8 observations. Units- μmoles/mg. protein/min, ***p<0.001, ns-non significant..

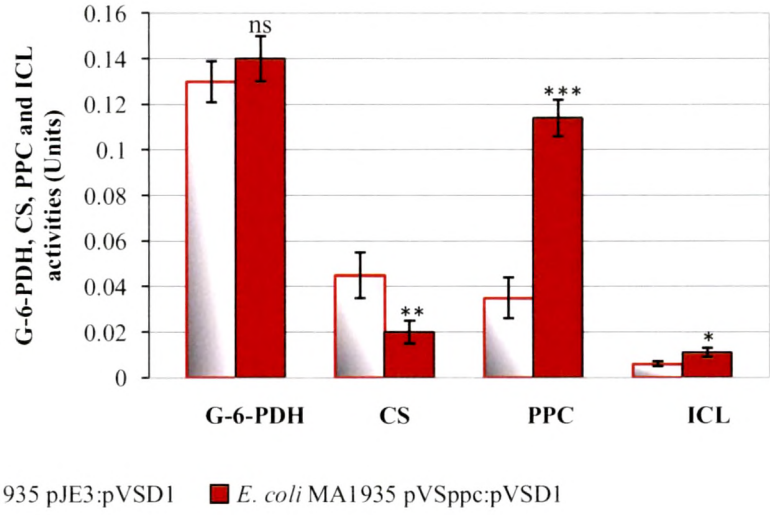

Fig. 8.11: Effect of *ppc* overexpression in *E. coli* BL21(λDE3) *icd* mutant on enzyme activities in presence and absence of citrate transporter on M9 minimal medium with 50 mM glucose. Units for the specific activity are given as μmoles/mg. protein/min. All the values are represented as Mean ± SD for n= 5-8 observations. Units- μmoles/mg. protein/min. *p<0.05, **p<0.01, ***p<0.001, ns-non significant.

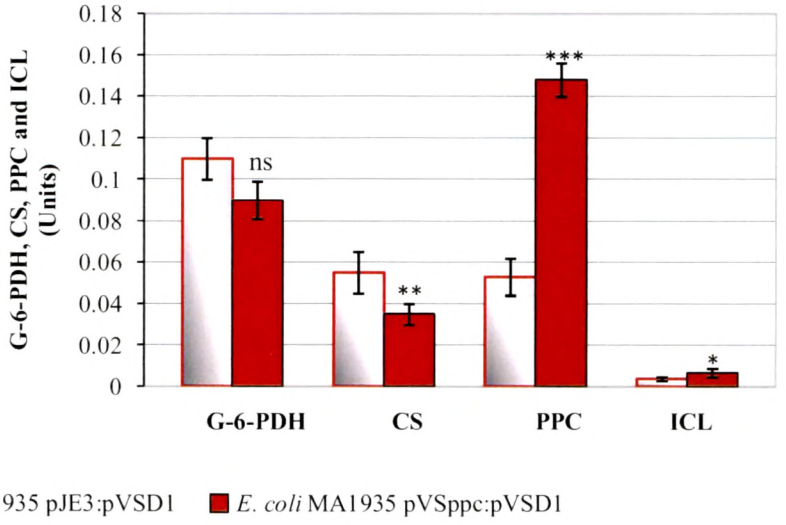

Fig. 8.10: Effect of *ppc* overexpression in *E. coli* BL21(λDE3) *icd* mutant on enzyme activities in presence and absence of citrate transporter on M9 minimal medium with 100 mM glycerol. Units for the specific activity are given as μmoles/mg. protein/min. All the values are represented as Mean ± SD for n= 5-8 observations. Units- μmoles/mg. protein/min. *p<0.05, **p<0.01, ***p<0.001, ns-non significant.

8.3.5: Effect of *icd* mutation and *ppc* gene overxpression on the organic acid profile in *E. coli* MA1935.

On glucose, *E. coli* MA1935 showed a significant increase in citrate accumulation compared to wild type. Incorporation of citrate transporter demonstrated low citrate accumulation compared to the mutant (**Table 8.4**). No acetate was observed in either of the mutant cultures except wild type (20 ± 3 mM). No extracellular citrate levels were observed in presence of citrate transporter. Overexpression of *ppc* in *E. coli* MA1935 reduced intracellular citrate levels as compared to control. Similar results with respect to growth rate and enzyme activities were observed for all *E. coli* MA1935 strains on glycerol. Intracellular citrate levels on glycerol were comparatively lower compared to that on glucose (**Table 8.5**).

E. coli strains	Intracellular citrate on glucose	
	(mM)	Yield ($g.g^{-1}$)
BL21(λDE3)	2.57 ± 0.58	0.06 ± 0.004
MA1935	**3.98 ± 0.51 **[a]**	**0.31 ± 0.03*** [a]**
MA1935 pVSD1	2.13 ± 0.55 [ns]	0.38 ± 0.01 [ns]
MA1935 pJE3:pVSD1	3.12 ± 0.43	0.10 ± 0.01
MA1935 pVSppc:pVSD1	**0.81 ± 0.05 ***[b]**	**0.05 ± 0.006** [b]**

Table 8.4: Intracellular citrate accumulation in *E. coli* MA1935 expressing *ppc* gene under P_{tac} promoter. The table depicts the citrate (intracellular) late stationary phase cultures of *E. coli* MA1935 transformants (plasmid control and test) grown on M9 minimal media with 50 mM glucose as carbon source. All the values are represented as Mean ± SD of n=4-8 observations. * $p<0.05$, *** $p<0.001$, ns=non-significant.

E. coli strains	Intracellular citrate on glycerol	
	(mM)	Yield ($g.g^{-1}$)
BL21(λDE3)	0.25 ± 0.07	0.14 ± 0.03
MA1935	**1.2 ± 0.02 *****	**1.3 ± 0.06 *****
MA1935 pVSD1	1.4 ± 0.06	1.6 ± 0.04
MA1935 pJE3:pVSD1	1.3 ± 0.03	0.5 ± 0.07
MA1935 pVSppc:pVSD1	**0.6 ± 0.02 *****	**0.3 ± 0.02 *****

Table 8.5: Intracellular citrate accumulation in *E. coli* MA1935 expressing *ppc* gene under P_{tac} promoter. The table depicts the citrate (intracellular) late stationary phase cultures of *E. coli* MA1935 transformants (plasmid control and test) grown on M9 minimal media with 100 mM glycerol as carbon source. All the values are represented as Mean ± SD of n=4-8 observations. * $p<0.05$, *** $p<0.001$, ns=non-significant.

8.4: DISCUSSION

E. coli MA1935 *icd* mutant showed poor growth rate and reduced glucose consumption rate compared to wild type; and required glutamate supplementation for growth on glucose and glycerol as carbon source. These results were in agreement with earlier reports on *icd* mutants (Lakshmi and Helling, 1976; Aoshima et al., 2003). 3 fold increase in *ppc* gene expression in *E. coli* MA1935 *icd* mutant improved growth and increased biomass. But no alterations were found with respect to glucose consumption rate or the amount of glucose consumed. Earlier reports on *ppc* overexpression (as low as ~ 7 fold and as high as ~ 70 fold) under aerobic condition demonstrated low glucose consumption rate with improved biomass production, no alteration in growth was observed (Chao and Liao, 1993). In contrast plasmid borne overexpression of *ppc* under constitutive promoter demonstrated 20% reduction in growth rate (De Mey, 2007). Reduction in glucose consumption was attributed to decreased PEP/pyruvate ratio which could decrease PEP-PTS dependent glucose uptake. Contradictorily, *E. coli ppc* mutant also reported reduction in glucose consumption rate (Peng et al., 2004). Hence *ppc* overexpression in an *icd* mutant background in the present study demonstrated to be beneficial to growth and suggested that glucose consumption is independent of glucose transport.

E. coli MA1935 had lower G-6-PDH activity and unaltered CS and ICL activity on minimal medium with glucose. On rich medium, *E. coli* MA1935 showed 2 fold increase in CS activity with a very low ICL activity compared to wild type in stationary phase (Aoshima et al., 2003). This suggests that change in media condition could influence the overall cellular metabolism which is also in agreement with the functional genomics profile of *E. coli* on rich and minimal media where minimal media reported an increase in the number of gene expression regulating the central carbon metabolism (Tao et al., 1999). Overexpression of *ppc* gene in *E. coli* MA1935 showed unaltered G-6-PDH with increased ICL activities compared to mutant. Reduced CS activity demonstrated by this strain suggested that the due to the block in ICDH the TCA cycle is underplayed and PPC overexpression directs the flux to anaplerotic pathway for biomass production thus resulting

in low CS acitivity. Increased ICL activity in response to *ppc* overexpression remained unexplained.

High intracellular levels of citrate in *E. coli* MA1935, were in agreement with earlier reports (Aoshima et al., 2003) but was comparatively lower compared to the levels obtained on rich media. Overexpression of *ppc* gene in *E. coli* MA1935 reduced intracellular citrate levels supporting low CS activity resulting in low flux through TCA. Acetate was absent in the extracellular medium which is in agreement with earlier reports on *icd* mutation (Aoshima et al., 2003). Incorporation of citrate transporter did not result in citrate efflux but showed an overall reduction in glucose consumed and consumption rate.

E. coli BL21 showed poor growth rate on glycerol compared to glucose media. This is in agreement with the earlier reports on glycerol (Holms, 2001). Mutations in *icd* gene resulted in poor growth rate on glycerol compared to wild which supported the poor growth rate on glucose. Intracellular citrate levels in *E. coli* BL21 were lower compared to that on glucose. Although the flux through TCA was reported to be higher (Holms, 2001) the low intracellular citrate levels were unexplainable.

The present study demonstrates that down regulation of *icd* gene plays a crucial role in citrate production but overexpression of *ppc* to meet the requirement of oxaloacetate directs the flux to anaplerotic pathway hence enhancing CS activity in *E. coli* MA1935 coupled with *ppc* overexpression could result in improved citrate production.