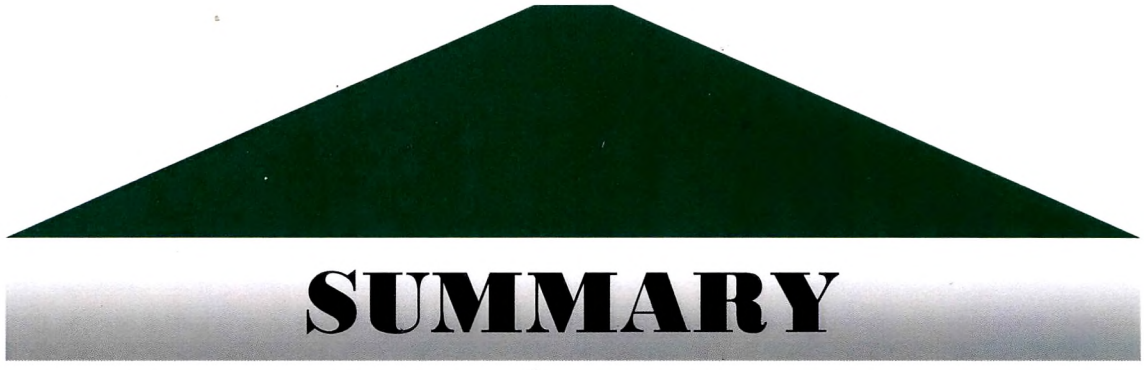

SUMMARY

Summary

Phosphate solubilizing bacteria release P from inorganic complexes by the secretion of wide range of low molecular weight organic acids. P solubilization potential of these bacteria varies based on the amount and nature of organic acid produced. Citric, oxalic, and gluconic acids are the product of central carbon metabolism. Fluorescent pseudomonads show myriads of catabolic diversity in their carbon metabolism. The amount of the organic acid secretion may differ between members of the same genus and sometimes between strains of the same species (Vyas and Gulati 2009; Buch et al. 2010; Archana et al. 2012). Moreover, organic acid secretion in the rhizosphere depends not only on the metabolic potential of the organism but also on the plant physiology as complex set of interactions mediated many compounds including the root exudates as the major C source. The nature of root exudates varies from plant to plant and composed of a complex mixture of several nutrients in low amount. Therefore, it is difficult to predict the organic acid secretion by rhizobacteria which may also vary with plant physiology. Hence, to develop an efficient P biofertilizer with the potential plant growth promoting properties for multiple host systems and diverse eco habitats, the present study was an effort to understand the following aspects: (i) to develop a potential genetic modification strategy to increase the MPS ability of plant growth promoting fluorescent pseudomonads; (ii) to understand *in vitro* metabolic effects of genetic modification on different strains of *P. fluorescens* including rhizospheric isolates; and (iii) to determine the consistency and performance of genetically modified strains in alkaline vertisol soils.

Chapter 3-4 demonstrates the construction of *P. fluorescens* PfO-1 strain harbouring *E. coli* NADH insensitive citrate synthase and *S. tphimurium* sodium citrate transporter (*yc*) operon under *lac* promoter in broad host range plasmid pUCPM18 containing gentamycin resistance. The recombinant plasmid complemented the *cs* mutant phenotype of *E. coli* W620 and citrate transporter mutant phenotype of *E. coli* DH5α when grown on M9 minimal medium without glutamate and in Koser citrate broth respectively. This indicates the expression of respective genes. The expression of yc operon resulted in increased CS activity with a simultaneous increase in citric acid accumulation as well as secretion as compared to the wild type, vector control and strain harbouring only wild type citrate

synthase. The high amount of intracellular citric acid accumulation (~50mM) had no deleterious effect on cell growth given in

Table 1 : Comparative effects of overexpression of *yc* operon in *P. fluorescens* PfO-1 grown under M9 minimal medium containing 100 mM glucose as a carbon source

Parameters	WT	pGm	pAB7	pYF	pYC
CS activity	-	No change	2.97 fold increase	5.6 fold increase	4.7 fold increase
Intracellular citric acid	-	No change	2.3 fold increase	5.7 fold increase	5.1 fold increase
Extracellular citric acid	-	10 fold increase	170 fold increase	320 fold increase	533 fold increase
Gluconic acid	-	1.7 fold increase	2.7 fold increase	6.6 fold increase	6.7 fold increase
Acetic acid	-	1.2 fold increase	1.97 fold increase	2.9 fold increase	4.8 fold increase
Pyruvic acid	-	1.26 fold decrease	1.87 fold decrease	2.8 fold decrease	1.8 fold decrease
Growth rate	-	No change	No change	No change	No change
Glucose consumed	-	1.35 fold increase	No change	No change	1.24 fold decrease
Biomass yield	-	1.33 fold increase	1.58 fold increase	3.16 fold increase	2.58 fold increase
G6PDH	-	1.7 fold increase	1.8 fold increase	1.4 fold increase	1.46 fold increase
PYC	-	1.1 fold increase	1.96 fold increase	2.4 fold increase	4.9 fold increase
ICDH	-	No change	No change	No change	No change
ICL	-	No change	No change	No change	No change
GDH	-	1.44 fold increase	1.65 fold increase	2.1 fold increase	1.6 fold increase

The MPS ability in terms of P solubilization index in PVK agar medium was in the order of WT≤Gm<pAB7<pYF<pYC clearly indicating the enhanced effect of both NADH insensitive citrate synthase and citrate transporter over wild type citrate synthase in terms of citric acid secretion. The distribution of glucose between two catabolic pathways: GDH mediated extracellular direct oxidation pathway and intracellular phosphorylative pathway involving active glucose uptake followed by the action of glucokinase and G-6-PDH are responsible for MPS ability of pseudomonads. Higher amount of total glucose utilized and less glucose consumption in *P. fluorescens* PfO-1 (pYC) strain compared to the control correlated with enhanced gluconic acid production and MPS ability. The strain when tested under P limiting condition in buffered TRP medium also showed significant enhancement in MPS phenotype.

The main objective of the present study was to design a stable and broad host range expression system for enhancing citric acid secretion which could provide MPS ability in fluorescent pseudomonads and other PGPR. Considering the stability of the established genetic modification and to test the efficacy in multiple host system the 5th chapter describes the effect of *yc* operon integration into the genome of 6 different *P. fluorescens* strains PfO-1, Pf-5, CHAO-1, ATCC13525 including wheat rhizospheric isolates P109 and Fp315. All these strains are known for bioremediation and biocontrol activities. *P. fluorescens* P109 and Fp315 are reported to possess poor P solubilizing ability. A systematic study was carried out to determine the effects of genomic integration in TRP broth medium and compare with the wild type and plasmid transformants of *yc* operon. MPS phenotype and Pi release of the genomic integrants of all strains were better than the wild type and plasmid bearing strains (**Table 2**). Overexpression of genes using multicopy plasmids can severely burden the host cell by alteration of the energy status of the cell as the resources are diverted towards recombinant protein synthesis and maintenance of the multicopy plasmid and hence overall productivity could be compromised. Therefore, genomic integration results in better effects.

Table 2: Comparative effects of overexpression of *yc* operon plasmid transformants and genomic integrants in *P. fluorescens* PfO-1 and Fp315 grown under TRP minimal medium containing 75 mM glucose as a carbon source.

Parameters	PfO-1		Fp315	
	pYC	pYCInt	pYC	pYCInt
CS activity	2.64 fold increase	3.17fold increase	3.4 fold increase	2.65 fold increase
Intracellular citric acid	5.6 fold increase	4.4 fold increase	5.2 fold increase	4.7 fold increase
Extracellular citric acid	60 fold increase	53 fold increase	77.2 fold increase	80.5 fold increase
Gluconic acid	1.4 fold increase	1.57 fold increase	1.3 fold increase	1.99 fold increase
Acetic acid	UD	UD	UD	UD
Pyruvic acid	UD	UD	UD	UD
Pi release	2 fold increase	2.2 fold increase	2.1 fold increase	2.85 fold increase
Growth rate	No change	No change	No change	1.9 fold increase
Glucose consumed	1.2 fold decrease	1.6 fold decrease	2 fold decrease	1.7 fold decrease
Biomass yield	2.3 fold increase	3.4 fold increase	4.1 fold increase	4.8 fold increase
G6PDH	2 fold decrease	2.1 fold decrease	1.8 fold decrease	1.6 fold decrease
PYC	5.1 fold increase	4.9 fold increase	1.45 fold increase	1.6 fold increase
ICDH	No change	No change	No change	No change
ICL	No change	No change	No change	No change
GDH	1.6 fold increase	2.2 fold increase	1.8 fold decrease	1.6 fold decrease

All parameters are compared with the respective wild type strain

Chapter 6 also describes the effect of genomic integration on growth promotion ability of mung bean plants in alkaline vertisol soil without supplememntation of rock phosphate in green house conditions. All treatments on an average showed a consistent increase in growth to similar extent as the SSP control or in some cases even better. Further, genomic integrants performed better in some parameters than the strain bearing the operon in the plasmid.

In conclusion, the present study illustrates a novel genetic engineering approach of enhancing citric acid secretion and MPS ability by genomic integration of NADH insensitive *E. coli* cs and *S. tphimurium* sodium citrate transporter operon constructed under *lac* promoter in metabolically distinct fluorescent pseudomonads. Genomic integration appears to be a better strategy than plasmid based expression which creates a milestone for getting stable expression system in metabolic engineering studies. Further, genomic integration of yc operon led to enhanced plant growth promotion under P limitation and without supplementation of external rock phosphate by fluorescent pseudomonads. Out of all *P. fluorescens* strain tested, the overall performance of PfO-1 and Fp315 genomic integrants was found to be the best in terms of MPS ability in laboratory condition as well as growth promotion of mung bean in green house. Future prospective will be to test the efficacy of these strains in field condition with multiple host plant under different environmental condition to determine their consistency of P supplementation and other plant growth promotion abilities in agricultural field conditions.