Chapter 4

Determining the MPS ability of *Rhizobium* strains containing *A. calcoaceticus pqq* cluster and *P. putida gad* operon

4.1. Introduction

Genetic modification of apoGDH containing *Bradyrhizobium japonicum, Mesorhizobium loti* and *Sinorhizobium fredii* NGR234 with *Acinetobacter calcoaceticus pqq* gene cluster resulted in secretion of ~ 28 mM GA and released P 0.25 mM when grown on 50mM Glucose RP buffered medium. For phosphate solubilization, 2-KGA with pKa 2.6 is known to be much better as compared to GA with pKa 3.86. In addition to acidification, 2KGA can also chelate calcium in soils (Moghimi and Tate, 1978; Moghimi et al., 1978). GA is oxidized to 2-KGA by Gluconate dehydrogenase (GADH) in the periplasm (Yum et al., 1997; Toyama et al., 2007; Saichana et al., 2008). 2GA is secreted by many bacteria and fungi (Webley and Duff 1965; Yum et al., 1997; Walker et al., 2003; Saichana et al., 2008; Vyas and Gulati 2009; Gulati et al., 2010; Park et al., 2010). GADH was isolated and characterized from different species like *Pseudomonas, K. pneumoniae, S. marcescens,* and *E. cypripedii* ATCC 29267 (Matsushita and Ameyama, 1982; Yum et al., 1997; Toyama et al., 2007; Saichana et al., 2008). Three subunit containing FAD-dependent gluconate dehydrogenase (GADH) enzyme, is encoded by *gad* operon comprising of three genes namely FAD dependent gluconate dehydrogenase, cytochrome c and smallest third subunit of unknown function. Overexpression of *E. cypripedii* ATCC 29267 *gad* operon in *E. coli* led to secretion of high amount of 2-KGA (Yum et al., 1997). Tomato and wheat seedlings inoculated with *G. diazotrophicus* PAL5 showed GADH and GDH activity, showed intense acidification and phosphate solubilization (Crespo et al., 2011). Phosphate solubilizing new species *Enterobacter oryziphilus* sp. nov. and *Enterobacter oryzendophyticus* sp. nov. from rice endosphere secretes 2-KGA (Hardoim, et al., 2013). Enhanced P solubilization phenotype due to increased secretion of 2-KGA was observed in *E. asburiae* PSI3 when overexpressed with *Pseudomonas putida* KT 2440 *gad* operon (Kumar et al., 2013). Similarly, genetically modified *H. seropedicae* Z67 with *P. putida gad* operon and *A. calcoaceticus pqq* genes showed efficient P and K solubilisation and increase in Fresh weight, Dry weight N, P, K content upon inoculation to rice plants (Wagh, 2013).

Most of the *Rhizobium* species lack *gadh* operon thereby do not secrete 2-KGA. Thus, the present study investigates the effect of heterologus overexpression of

A. calcoaceticus pqq gene cluster along with *Pseudomonas putida gad* operon in *B. japonicum*, *M. loti* and *S. fredii* NGR234 for 2-KGA and its effect on MPS ability.

4.2: EXPERIMENTAL DESIGN

The experimental plan of work includes the following-

4.2.1: Bacterial strains used in this study

All wild type and genetically modified *E. coli* and *Rhizobium* strains used in this study are listed in **Table 2.1.** The plasmids used in the present study and their restriction maps are given in **Fig. 2.1**. *E. coli* DH10B was used for all the standard molecular biology experiments wherever required.

Table 4.1: Bacterial strains used in this study

Plasmid/Bacterial strains	**Characteristics**	**Source/Reference**
***E. coli* strains**		
E. coli DH10B	Used to maintain plasmids for routine use	Invitrogen, USA
pUCPM18	pUC18 derived Broad-Host-Range vector; Ap^r (100µg/ml)	Hester et al., 2000
pJNK6	pJNK5, Gm^r (20µg/ml) with *gad* operon 3.8 Kb of *P. putida* KT 2440	Wagh, 2013
***Rhizobium* strains**		
Bradyrhizobium japonicum USDA110	Accession number NC_004463.1	NCBI
Mesorhizobium loti MAFF030669	Accession number NC_002678.2	NCBI
Sinorhizobium fredii NGR234	Accession number NC_012587.1	NCBI
***Bj* pUCPM18**	*B. japonicum* with pUCPM18, Gm^r (control vector)	This study
***Bj* pJNK6**	*B. japonicum* with pJNK6Gm^r (20µg/ml)	This study
***Ml* pUCPM18**	*M. loti* with pUCPM18, Gm^r (control vector)	This study
***Ml* pJNK6**	*M. loti* with pJNK6Gm^r (20µg/ml)	This study
***Sf* pUCPM18**	*S. fredii* with pUCPM18, Gm^r (control vector)	This study
***Sf* pJNK6**	*S. fredii* with pJNK6Gm^r (20µg/ml)	This study

Table 4.1: List of bacterial strains used. Detailed characteristics of these strains are given in **Table 2.1** Parent strains and the transformants of *E. coli* and *Rhizobium* were respectively grown at 37°C and 30°C with variations in gentamycin and erythromycin concentrations for rich and minimal media as described in **Section 2.2.**

4.2.2: Development of *B. japonicum, M. loti* and *S. fredii* strains harboring *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6).

The recombinant plasmids pUCPM18 Gm (control) and pJNK6 were transformed in *B. japonicum, M. loti* and *S. fredii* by electroporation (Section 2.4.2.2). The transformants were selected on gentamycin selection plates and were confirmed by Restriction endonuclease digestion pattern.

4.2.3: Growth and MPS phenotype of pJNK6 transformant strains of *Rhizobium*

The MPS ability of *B. japonicum, M. loti* and *S. fredii* and its transformants were monitored on Pikovaskya's (PVK) agar and 100 mM Tris buffered RP (TRP) agar as described in **chapter 2**

4.2.4: Effect of heterologous *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6) overexpression on the physiology and glucose metabolism.

B. japonicum, M. loti and *S. fredii* transformants were subjected to physiological experiments involving growth and organic acid production profiles on TRP medium with 50 mM glucose as carbon source.The samples withdrawn at regular interval were analyzed for O.D.600nm, pH, extracellular glucose and organic acid as mentioned in chapter2. The physiological parameters were calculated as mentioned in chapter 2. The enzyme assays were performed as described chapter 2.

4.3: Results

4.3.1: Heterologous overexpression of *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6) in *Rhizobium* strains.

The pJNK6 plasmid was incorporated *in B. japonicum, M. loti and S. fredii* NGR 234 strains and the transformants were confirmed based on restriction endonuclease digestion pattern (**Fig. 4.1).**

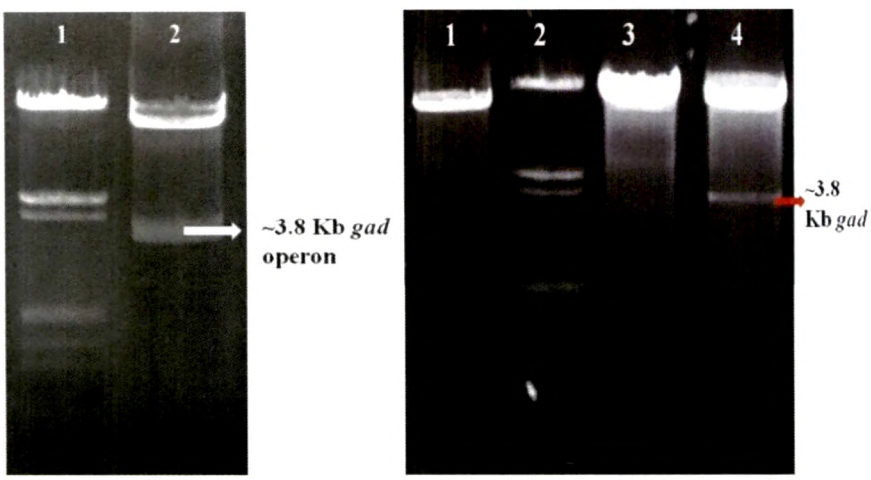


Fig. 4.1: Restriction endonuclease digestion pattern for *Rhizobium* transformants containing pJNK6 (Lane 1- EcoR1/Hind III Marker, Lane- 2, 4 and 6 *Bj* pJNK6, *Ml* pJNK6 and *Sf* pJNK6 BamH1/ XbaI digested (12.1 kb and 3.8 kb).

4.3.2 Effect of heterologous overexpression of *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6) in *Rhizobium* strains on GADH and GDH activity in *B. japonicum, M. loti and S. fredii* NGR 234.

In *Bj* (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) GADH activity was found to be 340.9 U, 438.4 U and 326.3 U, respectively, which is 42, 73 and 36 fold increase compared to native strain. In transformants, GDH activity was found to be 182.8 U, 151.2 U and 219.0 U, respectively, with a fold increase of 14.51,10.10 and 15.24 compared to native strains, which is similar to *Rhizobium* strains transformed with pJNK5 alone

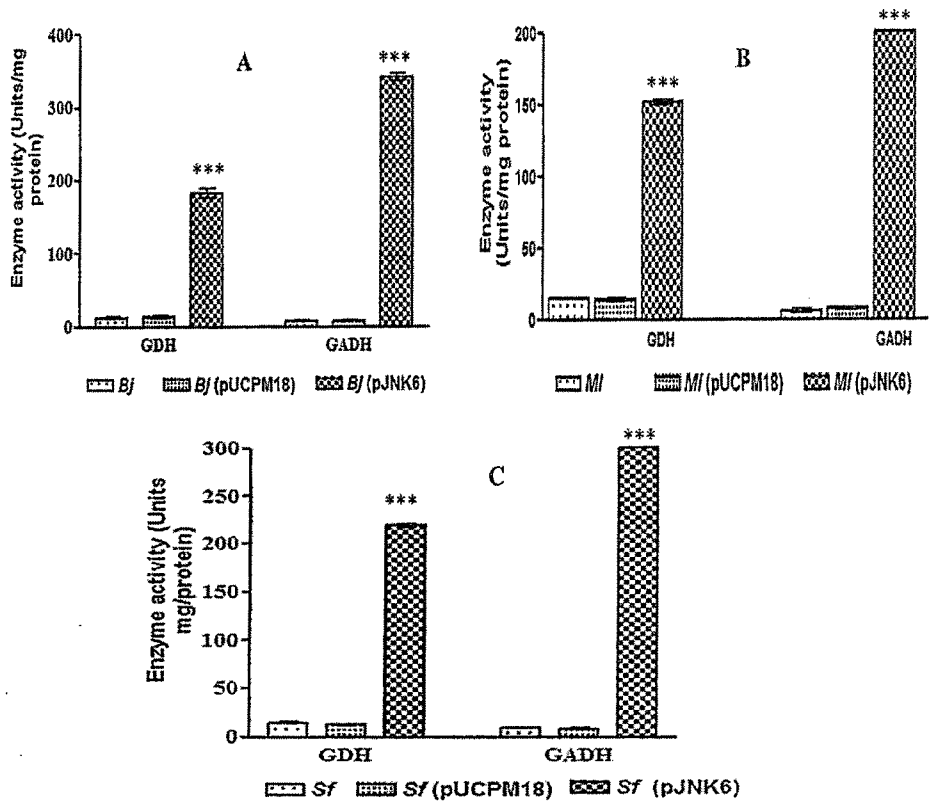


Fig 4.2: GDH and GADH activity of *Rhizobium* transformants containing *pqq* gene cluster (A) *B. japonicum*, (B) *M. loti* (C) *S. fredii* NGR 234. The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

4.3.3: Growth and MPS ability of *Rhizobium* transformants of *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6)

Bj (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) transformants showed good phenotype compared to native and control both on PVK and TRP plates after 3 days of incubation at 30°C. The pJNK6 transformants of *B. japonicum*, *M. loti* and *S. fredii* showed maximum zone of clearance as compared to the control pUCPM18 and pJNK5 transformants (**Table 4.2**).

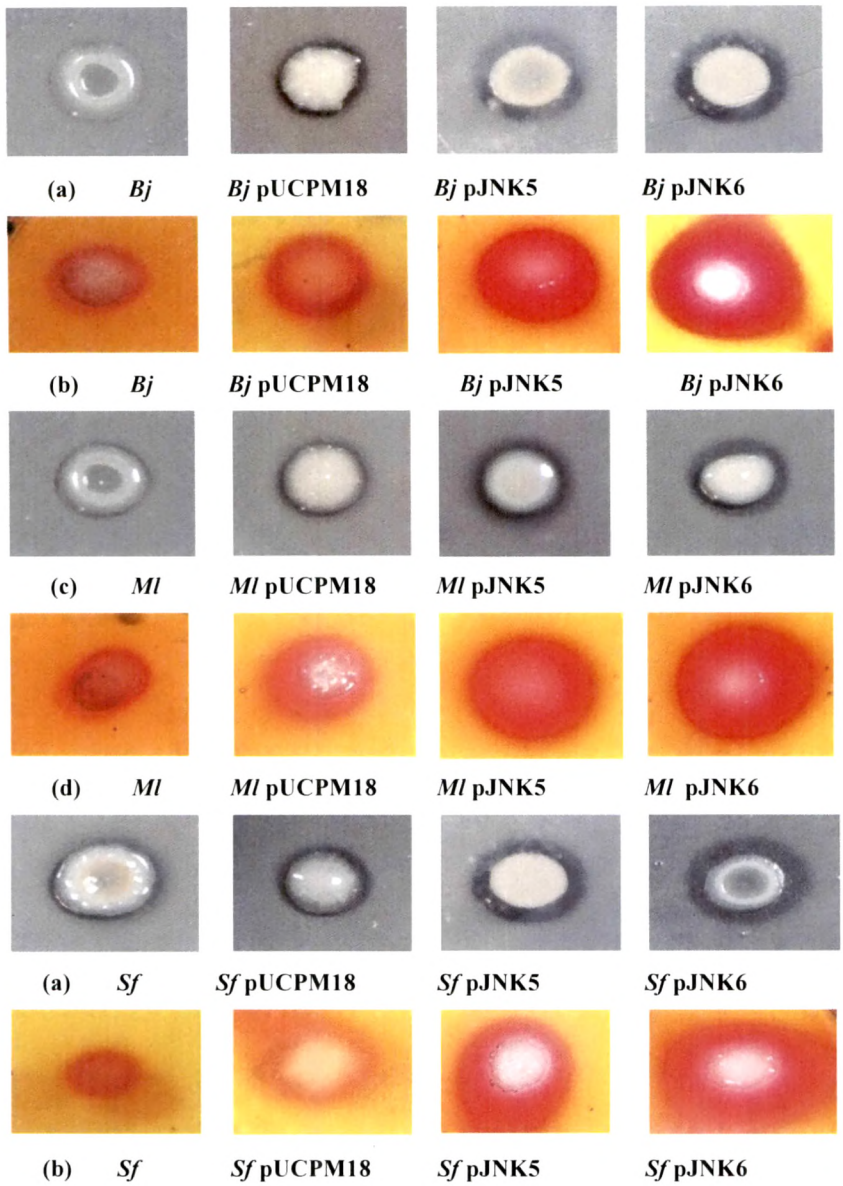


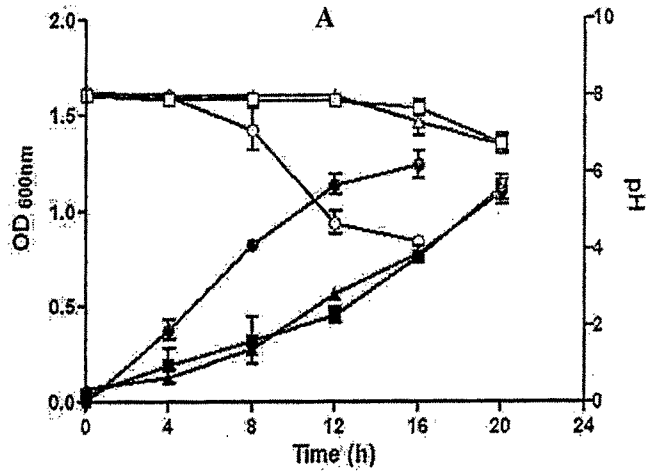
Fig. 4.3: MPS phenotype of *B. japonicum, M. loti* and *S. fredii* strains harboring pJNK6 plasmid. (a), (c) and (e) on Pikovskaya's agar and **(b), (d) and (f)** Tris rock phosphate agar containing 50 mM glucose and 100 mM Tris HCl buffer pH 8.0. The results were noted after an incubation of 3 days at 30 °C. Media composition and other experimental details are as described chapter 2.

Rhizobium Strains	Diameter of zone of clearance (mm)	Diameter of colony (mm)	Phosphate Solubilizing Index
Bj	12.17 ± 0.29	11.17 ± 0.29	1.09
Bj pUCPM18	11.17 ± 0.29	9.50 ± 0.50	1.22
Bj pJNK5	14.50 ± 0.50	10.17 ± 0.29	1.44
Bj pJNK6	14.17± 0.29	9.50 ± 0.50	1.55
Ml	12.83 ± 0.29	11.50 ± 0.50	1.09
Ml pUCPM18	12.17 ± 0.29	10.17 ± 0.29	1.22
Ml pJNK5	12.50 ± 0.50	9.17 ± 0.29	1.36
Ml pJNK6	12.83 ± 0.29	8.83 ± 0.29	1.45
Sf	12.17 ± 0.29	10.50 ± 0.50	1.20
Sf pUCPM18	12.17 ± 0.29	10.17 ± 0.29	1.22
Sf pJNK5	14.50 ± 0.50	11.17 ± 0.29	1.29
Sf pJNK6	14.50 ± 0.50	10.17 ± 0.29	1.42

Table 4.2: P solubilization index on Pikovskyas agar of *B. japonicum*, *M. loti and S. fredii* transformants. *Bj, Ml and Sf*: wild type strain; *Bj* pUCPM18, *Ml* pUCPM18 and *Sf* pUCPM18: *B. japonicum, M. loti and S. fredii* with vector control and *Bj* (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) : *B. japonicum* with *pqq* and *gad* gene cluster, *M. loti* with *pqq* and *gad* gene cluster and *Sf* with *pqq* and *gad* gene cluster. The results were noted after an incubation of 3 days at 30 °C and are given as mean ± S.D. of three independent observations as compared to native *Bj, Ml* and *Sf*. The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

4.3.4: Effect of *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6) overexpression on growth pattern pH profile and physiological effects in presence of 50mM glucose concentration.

pJNK6 transformants of *B. japonicum*, *M. loti* and *S. fredii* showed maximum growth and pH drop of 4.21, 4.21 and 4.23 respectively within 16 h compared to around pH 6.5 to 6.7 of native and control at 20 h **(Fig 4.4).**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0.0
10
8
6
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4
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16
20
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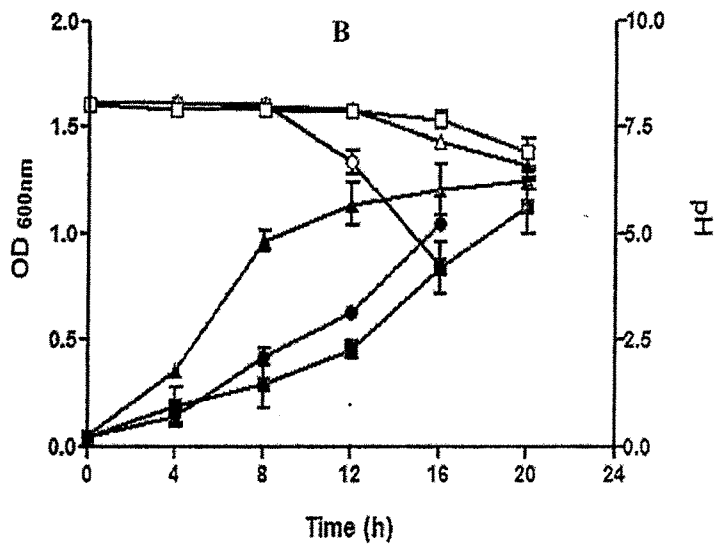
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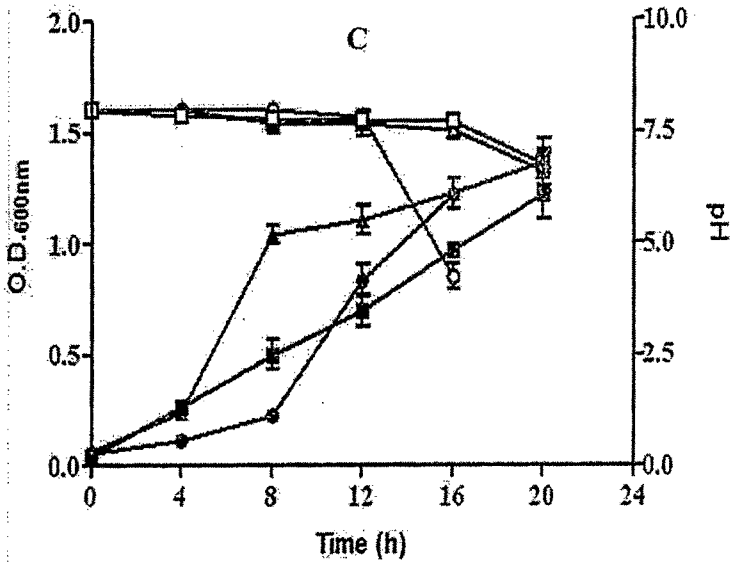

Fig. 4.4 : Effect of *pqq* gene cluster and *gad* operon overexpression on extracellular pH (□, Δ, ○) and growth profile (■, ▲,●) of (A) *B. japonicum* and (B) *M. loti*, (C) *S. fredii* on TRP medium with 50 mM glucose .(□, ■, *Bj, Ml, Sf* wild type); {Δ, ▲, *Bj* (pUCPM18Gm^r), *Ml* (pUCPM18Gm^r), *Sf* (pUCPM18Gm^r)}; {○, ●, *Bj* (pJNK6), *Ml* (pJNK6), *Sf* (pJNK6)};. OD_{600} and pH values at each time point are represented as the mean ± SD of six independent observations.

In presence of 50 mM glucose, increase in GDH and GADH activity significantly affected growth profile. The total glucose utilization rate at the time of pH drop remained unaffected but there was ~ 2 fold decrease in glucose consumed due to increase in GA and 2-KGA acid secretion. However, the Specific Glucose Utilization Rate Q_{Glc} (g.g dcw^{-1}.h^{-1}) decreased by ~1.2 to ~1.9 fold in pJNK6 transformants. Additionally, the increase in GDH and GADH activity increased the specific growth rate by ~1.8 to ~2.0 fold, and improved the biomass yield by ~1.3 to ~ 1.6 fold in pJNK6 transformants compared to control (**Table 4.3**).

Rhizobium Strains	Specific Growth Rate $K(h^{-1})^a$	Total Glucose Utilized (mM) ^{b}	Glucose Consumed (mM) ^{b}	Biomass Yield $Y_{dcw/Glc}$ (g/g) ^{a}	Specific Glucose Utilization Rate Q_{Glc} $(g.g\ dcw^{-1}.h^{-1})^a$
Bj	0.186 ± 0.026	46.20 ± 0.20	38.23 ±1.33	1.78 ± 0.14	0.14 ± 0.01
Bj pUCPM18	0.280 ± 0.051	48.30 ± 0.46	40.65 ± 0.30	1.21 ± 0.16	0.21 ± 0.02
Bj pJNK6	0.363 ± 0.07***	49.10 ± 0.26	14.20 ± 1.57	2.39 ± 0.48**	0.11 ± 0.02
Ml	0.221 ± 0.03	45.91 ± 0.64	37.07 ± 0.55	1.36 ±0.26	0.19 ± 0.04
Ml pUCPM18	0.265 ± 0.02	48.40 ± 0.36	40.38 ± 0.02	1.0 ±0.04	0.26 ± 0.01
Ml pJNK6	0.438 ± .05***	49.67 ± 0.12	15.89 ± 0.22	1.96 ± 0.18*	0.13 ± 0.01
Sf	0.260 ± 0.02	46.10 ± 0.42	37.17 ± 0.55	1.69 ± 0.22	0.15 ± 0.02
Sf pUCPM18	0.311 ± 0.02	48.10 ± 0.10	40.67 ± 0.40	1.85 ± 0.10	0.14 ± 0.01
Sf pJNK6	0.468 ± .03***	49.03 ± 0.06	16.10 ± 0.21	2.68 ± 0.25**	0.09 ± 0.01

Table 4.3: Physiological variables and metabolic data from of *B. japonicum* , *M. loti* and *S. fredii pqq* transformants grown on TRP medium 100mM Tris-Cl buffer pH 8 and 50mM Glucose containing Rock Phosphate 1mg/ml. The results are expressed as Mean ± S.E.M of six independent observations. ^{a} Biomass yield $Y_{dcw/Glc}$, specific growth rate (k) and specific glucose utilization rate (Q_{Glc}) were determined from mid log phase of each experiment. ^{b} Total glucose utilized and glucose consumed were determined at the time of pH drop. The difference between total glucose utilized and glucose consumed is as explained. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

4.3.6 : P solubilization and organic acid secretion in 100mM Tris-Cl Buffer pH 8 and 50mM Glucose containing Rock Phosphate 1mg/ml.

Significant increase was seen in P release by pJNK6 transformants of *B. japonicum, M. loti* and *S. fredii* compared to control and native, 13, 12 and 12 fold, respectively, in TRP medium in presence of 50mM glucose and 100mM Tris Cl Buffer pH 8.0 (**Fig. 4.5**).

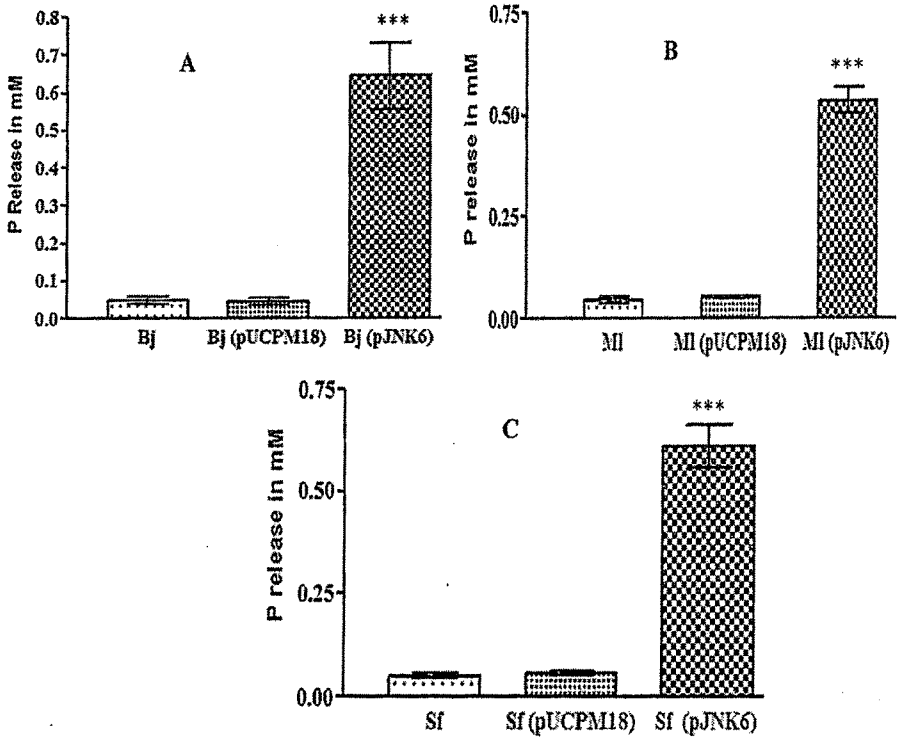

Fig.4.5: Phosphate released by *Rhizobium* transformants containing *pqq* gene cluster and *gad* operon (A) B. *japonicum* (B) *M. loti* and (C) *S. fredii.* The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

Extracellular medium of pJNK6 transformants of *B. japonicum, M. loti* and *S. fredii* had 6, 5.27 and 6.83 folds higher amounts of 2-ketogluconic acid, respectively, with its specific yield ($Y_{C/G}$) increased by 4.06, 3.07 and 2.74 fold whereas levels of gluconic acid were 3.31, 3.07 and 2.64 folds higher with specific yield ($Y_{C/G}$) increased by 2.02, 1.82 and 1.13 fold compared to native and controls (**Table 4.4**)

Rhizobium Strain	GA mM	2-KGA mM	Specific Yield GA $Y_{C/G}$	Specific Yield 2-KGA $Y_{C/G}$
Bj	6.26 ± 0.23	2.37 ± 0.47	1.39 ± 0.12	0.49 ± 0.09
Bj pUCPM18	5.72 ± 0.06	1.93 ± 0.07	1.39 ± 0.08	0.46 ± 0.05
Bj pJNK6	20.73± .82***	14.17 ± 0.23***	3.11 ±0.15***	1.99 ±0.12***
Ml	6.53 ± 0.18	2.60 ± 0.20	1.01 ± 0.21	0.40 ± 0.1
Ml pUCPM18	5.80 ± 0.10	2.21 ± 0.13	0.98 ± 0.03	0.36 ± 0.04
Ml pJNK6	20.06± .24***	13.72 ± .22***	1.84 ± 0.03**	1.23 ±0.04***
Sf	6.760 ± 0.10	2.20 ± 0.15	1.59 ± 0.05	0.54 ± 0.10
Sf pUCPM18	5.50 ± 0.30	1.92 ± 0.03	1.10 ± 0.11	0.37 ± 0.01
Sf pJNK6	17.90±0.17***	15.03 ± .05***	1.81 ± 0.12**	1.48 ±0.07***

Table 4.4: Organic acid Secretion and Organic acid yield from of *B. japonicum* , *M. loti* and *S. fredii pqq* transformants grown on TRP medium 100mM Tris-Cl buffer pH 8 and 50mM Glucose containing Rock Phosphate 1mg/ml. The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

Bj (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) also secreted ~7.30 µM, ~7.00 µM and ~8.75 µM PQQ, which is similar to *Rhizobium* strains transformed with pJNK5 alone (**Fig. 4.6**).

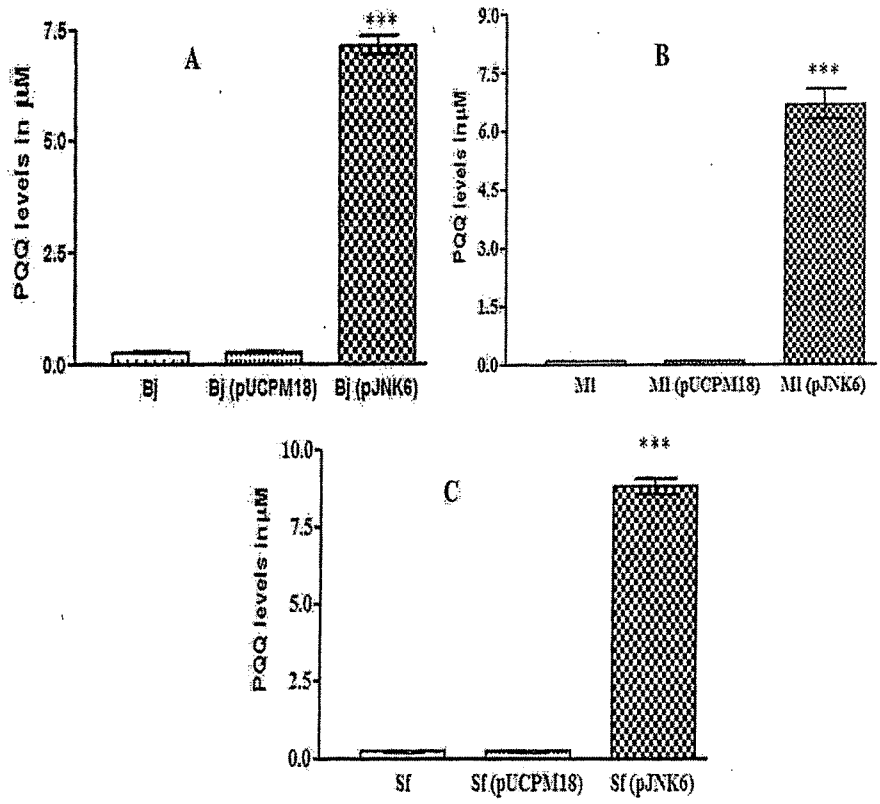

Fig. 4.6: PQQ secreted by *Rhizobium* transformants containing *pqq* gene cluster and *gad* operon (A) *B. japonicum* (B) *M. loti* and (C) *S. fredii.* The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

4.3.8: Effect of *A. calcoaceticus pqq* gene cluster and *P. putida gad* operon (pJNK6) overexpression on Biofilm, EPS and IAA secretion in *Rhizobium* transformants.

Biofilm and EPS synthesis on an average showed significant increase by ~2.5 fold, while ~ 1.8 fold increase in IAA secretion in *Bj* (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) transformants in 100 mM Tris-Cl buffer pH 8.0, 50 mM glucose and rock phosphate 1 mg/ml in comparison to native and control (**Table 4.5**).

Rhizobium **Strains**	**Biofilm O.D.at 550nm**	**EPS (g/100ml)**	**IAA (µg/ml)**
Bj	1.39 ± 0.02	11.48 ± 0.1	20.14 ± 1.33
***Bj* pUCPM18**	1.45 ± 0.03	12.56 ± 0.2	22.32 ± 1.65
***Bj* pJNK6**	4.67 ± 0.03***	28.45 ± 0.2***	42.69 ± 1.34***
Ml	1.51 ± 0.06	11.34 ± 0.05	28.17 ± 1.35
***Ml* pUCPM18**	1.81 ± 0.05	11.89 ± 0.1	29.67 ± 1.81
***Ml* pJNK6**	5.64 ± 0.04***	33.33 ± 0.5***	47.22 ± 2.12***
Sf	1.61 ± 0.10	12.53 ± 1.54	32.79 ± 1.87
***Sf* pUCPM18**	2.10 ± 0.16	13.43 ± 1.11	34.10 ± 1.32
***Sf* pJNK6**	6.12 ± 0.2***	38.11 ± 1.88***	52.12 ± 1.22***

Table 4.5: Biofilm, EPS and IAA production by *Bj* (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) transformants in TRP medium. The results are expressed as Mean ± S.E.M of six independent observations. * P<0.05, ** P<0.01 and *** P<0.001.

Discussion

Rhizobium species lack GADH enzyme which converts GA to 2-KGA. Transformation of *B. japonicum, M. loti* and *S. fredii* NGR 234 strains with pUCPM18 Gm^r containing *A. calcoaceticus pqq* gene cluster under constitutive P*lac* promoter along with *P. putida* KT2440 *gad* operon with its own promoter showed GADH activity ~390 U, while GDH activity was ~190 U with 50 mM glucose in M9 medium. Incorporation of *E. cypripedii* ATCC 29267 *gad* operon in *E. coli* increased GADH activity from ~410 U to ~2150 U in presence of gluconate (Yum et al., 1997). Similar increase in GAD activity (1738 U) was found in *E. asburiae* PSI3 *gad* transformant in presence of gluconate (Kumar et al., 2013). GADH activity of *H. seropedicae* Z67 when overexpressed with *A. calcoaceticus pqq* gene cluster along with *P. putida* KT2440 *gad* operon (*Hs* pJNK6) was ~414 U, in HRP minimal medium which is similar to *E. asburiae* PSI3 *gad* transformant (438 U) in TRP medium (Wagh, 2013). *Bj* (pJNK6), *Ml* (pJNK6) and *Sf* (pJNK6) showed similar expression of *P. putida* KT2440 *gad* operon as with *E. asburiae* PSI3 and *Hs* (pJNK6), this shows that *P. putida* KT2440 *gad* operon is functional in *Rhizobium* strains.

Hs pJNK6 secreted 15.83 mM 2-KGA along with 3.79 mM GA, when grown on 100 mM HEPES containing 50 mM Glucose (Wagh, 2013). Overexpression of *P. putida* KT2440 *gad* operon alone in *E. asburiae* PSI3 possessing high level of PQQ biosynthesis secreted 11.63 mM 2-KGA along with 21.65 mM GA (Kumar et al., 2013). *E. cypripedii* ATCC 29267 *gad* operon in *E. coli* could secrete ~ 13mM 2KGA in LB containing 100mM glucose (Yum et al., 1997).Our study shows similar results as seen in different species, *Rhizobium* strains secreted up to 15 mM 2-KGA along ~21 mM GA, respectively, when grown on 50 mM glucose on TRP medium. *Rhizobium* strain oxidizes glucose chiefly using ED pathway, overexpression of *pqq* cluster and *gad* operon channelized glucose towards direct oxidation pathway, resulting in to secreting higher amounts of GA and 2-KGA, this change in glucose flux in no way affected growth rate and biomass yield .

Hs pJNK6 released 0.457 mM P and ~1.1 µM PQQ (Wagh, 2013). *E. asburiae* PSI3 *gad* transformant could solubilize 0.84 mM rock phosphate in presence of 45 mM glucose while native stain requires 75mM glucose to solubilize rock phosphate in

TRP medium (Kumar et al., 2013). Our study showed similar results as *Hs* pJNK6, but P release was less compared to *E. asburiae* PSI3 *gad* transformant. The reason for this variation in P release in different species is not clear.

Increase in EPS, Biofilm and IAA secretion of *Rhizobium pqq-gad* transformants was similar to that of *pqq* transformants which could improve the nodulation, nitrogen fixation and plant growth promotion by *Rhizobium* strains.

Studies show that, genetic modification of *Rhizobium* species were predominantly done to increase nitrogen fixing ability or nodule formation (Vieira, et al., 2010). Using biotechnology tools, soil inhabiting indigenous species or Non PSMs can be converted to PSM. Most of the genetic modifications carried out till date is to enhance gluconic acid secretion, very few studies are there for 2-KGA secretion (**Table 4.6**) (Kumar et al., 2013). *Rhizobium* strains expressing *A. calcoaceticus pqq* cluster and *P. putida gad* operon togather for 2-KGA secretion would make *Rhizobium* strains very efficient P solubilzer. For stable genetic modification, genomic integration of *pqq-gad* operon is desirable in *Rhizobium* strains.

Table 4.6 Genetic modifications for enhanced MPS by bacteria

Gene/Function	Source	Host	Mineral P solubilized	Organic acid	References
PQQ biosynthesis	*S. marcescens*	*E. coli*	TCP	GA	Krishnaraj and Goldstein (2001)
pqqE	*E. herbicola*	*Azospirillum sp.*	TCP	GA ?	Vikram et al. (2007)
pqqED genes	*R. aquatilis*	*E. coli*	HAP	GA	Kim et al. (1998)
Unknown	*E. agglomerans*	*E. coli*		GA ?	Kim et al. (1997)
pqqABCDEF genes	*E. intermedium*	*E. coli* DH5α	HAP	GA	Kim et al. (2003)
Ppts-gcd, P gnlA-gcd	*E. coli*	*A. vinelandii*	TCP	GA	Sashidhara and Podille (2009)
gabY Putative PQQ transporter	*P. cepacia*	*E. coli* HB101		GA	Babu-Khan et al. (1995)
Unknown	*Erwinia herbicola*	*E. coli* HB101	TCP	GA	Goldstein and Liu (1987)
gltA/ citrate synthase	*E. coli* K12	*P. fluorescens* ATCC 13525	DCP	Citric acid	Buch et al (2009)
Unknown	*Synechocystis* PCC 6803	*E. coli* DH5α	RP	Unknown	Gyaneshwar et al 1998

NADH insensitive cs /Na^+ dependent citrate transporter operon	*E. coli* /*S.typhimurium*	Genomic integration in *P. fluorescens* PfO-1	RP	Citric acid	Adhikary, 2012
gad / Gluconate dehydrogenase	*P. putida* KT2440	*E. asburiae* PSI3	RP	GA and 2KGA	Kumar et al 2013
pqq/gad PQQ/ GADH	*A. calcoaceticus* /*P. putida* KT2440	*H. seropedicae* Z67	RP	GA and 2KGA	Wagh, 2013
pqq/gad PQQ/ GADH	*A. calcoaceticus* /*P. putida* KT2440	*B.japonicum. M. loti* and *S. fredii* NGR234	RP	GA and 2KGA	This Study

GA- Gluconic acid, 2KG- 2 ketogluconic acid, DCP- Dicalcium phosphate, TCP- Tricalcium phosphate, RP- Senegal rock phosphate, HAP- Hydroxyapatite.