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# Gluconic acid production by bacteria to liberate phosphorus from insoluble phosphate complexes

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**Abstract**
Ten indigenous bacterial isolates were identified as efficient mineral phosphate solubilisers namely 7 strains of *Klebsiella pneumoniae*, 2 strains of *Enterobacter aerogenes* and 1 strain of *Pseudomonas aeruginosa*. All the ten strains were able to dissolve calcium phosphate (Ca-P), ferric phosphate (Fe-P) and aluminium phosphate (Al-P) efficiently. STMPSB 8 (*Klebsiella pneumoniae*) could be designated as the best mineral P solubiliser for all the three insoluble mineral phosphates as it exhibited high solubilisation capacity for Ca-P, Fe-P and Al-P whereas STMPSB 6 (*Enterobacter aerogenes*), STMPSB 4 (*Enterobacter aerogenes*) and STMPSB 8 (*Klebsiella pneumonia*) were regarded as good gluconic acid producing bacteria. A strong correlation of 0.795, significant at the level of 0.01 was observed between gluconic acid concentration and calcium phosphate solubilisation. The detection of pyrroloquinoline quinone (*pqq* C) gene in eight of the bacterial isolates indicates that direct oxidation pathway was used during the biosynthesis of gluconic acid with the aid of pyrroloquinoline quinone cofactor.

Keywords: organic acid, phosphate solubilising bacteria, gluconic acid, pyrroloquinoline quinone (*pqq* C) gene

## Introduction

Phosphorus (P) is abundant in soil but mostly not available for plants. High P fixation and low P solubility have led to the unavailability of P for plants (Hoberg et al. 2005). The concentration of soluble P in soil is very low that is around 1 mg/kg (Yanmei et al. 2008; Zixi et al. 2008) as around 95 – 99% of the total soil P comprises insoluble P (Molla et al. 1984). Since the application of chemical P fertilisers to soil is kept increasing from day to day, a large quantity of them become immobilised and unavailable to plants as a consequence of precipitation, adsorption and conversion to organic forms (Zixi et al. 2008). Moreover, it was estimated that the natural P source, rock phosphates may deplete by 2050 (Kai et al. 2006).

Thus, the future use of P in agriculture will be impacted by the declining availability of natural P source and increasing cost and detrimental effects of synthetic P fertilisers. Therefore, there is an urgent need to find a remedy to tackle this devastating phenomenon that occurs all around the world. Malaysian soil is mostly acidic and insoluble phosphates are

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associated with aluminium and ferric ions. Thus, in this study, the capability of mineral phosphate solubilising bacteria to dissolve Ca-P, Al-P and Fe-P, the complex mineral phosphates that commonly found in acidic soils were tested.

Plants and microorganisms may alter the pH of the soil to dissolve the insoluble phosphate especially by exuding low molecular weight organic acids. Many studies have reported that the secretion of organic acid either by plant roots or microorganisms could facilitate phosphate solubilisation (Hoberg et al. 2005; Reyes et al. 2006; Patel et al. 2008; Oburger et al. 2009). However, to date not many reports have been generated on the potential of the indigenous bacterial strains of phosphate solubilisation in Malaysia. The screening of the potential strains in this study shall be useful for the development of phosphate solubilising bacterial inoculants that may help in effective phosphate solubilisation in soil.

A wide range of organic acids were reported to facilitate insoluble phosphate solubilisation (Rodriguez et al. 2004; Hoberg et al. 2005; Chen et al. 2006; Lin et al. 2006; Patel et al. 2008; Vyas and Gulati 2009; Buch et al. 2010; Gulati et al. 2010). They are gluconic acid, 2-ketogluconic acid, oxalic acid, citric acid, acetic acid, malic acid, succinic acid, tartaric acid, propanoic acid, lactic acid, fumaric acid, pyruvic acid etc. (Vyas and Gulati 2009).

Among all the organic acids, gluconic acid is the most effective in solubilising mineral phosphates (Perez et al. 2007; Patel et al. 2008; Werra et al. 2009; Gulati et al. 2010; Ogut et al. 2010). Gluconic acid is further oxidised to 2-ketogluconic acid, an effective chelating agent for calcium ions and the strongest monobasic carboxylic acids for the dissolution of hydroxyapatite (Moghimi and Tate 1978; Werra et al. 2009). In general, mineral phosphate solubilising bacteria use the direct oxidation pathway to produce gluconic acid (GA) and further oxidise it to 2-ketogluconic acid (2-KGA) (Krishnaraj and Goldstein 2001).

The usage of P-based bio-inoculant not only functions as plant growth promoter but also recycles the abundant phosphorus accumulated in soil. This can pave way for a sustainable agriculture system and also prevent the phosphorus loss to the environment via leaching or surface run-off.

**Materials and methods**

The ideal growth medium for phosphorus solubilisers was obtained from Nautiyal (1999). National Botanical Research Institute's phosphate growth medium (NBRIP) contained glucose, 10 g/litre; calcium phosphate, 5 g/litre; ammonium sulphate, 0.5 g/litre; potassium chloride 0.2 g/litre; sodium chloride, 0.2 g/litre; magnesium sulphate, 0.1 g/litre; manganous sulphate, 0.002 g/litre and ferum sulphate, 0.002 g/litre. The bacterium was grown in NBRIP medium at room temperature (28 – 30 ºC) in orbital shaker at 180 rpm. The NBRIP medium was modified by substituting calcium phosphate with ferric phosphate and aluminium phosphate.

Bacteria isolated from soil were screened for their ability to solubilise insoluble P using agar plate assay. About 420 bacteria were tested on NBRIP agar medium to produce clear zone consistently even after five subcultures. Only 10 bacteria were selected for further experiments based on their consistency of producing clear zone even after five subculturing. They were designated as STMPSB 1 until STMPSB 10.

Later the bacterial isolates were identified by PCR amplification of the 16S rDNA gene using universal primers. Sequence data were compared with available sequences of bacteria in the National Centre for Biotechnology Information GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST Search. Biochemical tests such as catalase test, oxidase test, motility test, citrate test, triple sugar iron agar test, indole test, nitrification test methyl red test, voges-

praskaeur test and carbon utilisation and gas production test were done to characterise the bacterial isolates.

Each bacterium was grown in nutrient agar at 35 °C overnight. One loopful of bacteria was inoculated in NBRIP broth and incubated for 5 days in a rotary shaker at a speed of 180 rpm at room temperature. The bacterial cultures were centrifuged at 9,000 rpm for 10 min. The supernatant was filtered using Whatman filter paper No. 42. Soluble orthophosphate concentration was determined using the molybdenum blue method as described Olsen and Sommers (1982). The absorbance was read at 882 nm using a spectrophotometer. A calibration curve was prepared using potassium anhydrous dihydrogen phosphate.

***High performance liquid chromatography (HPLC) analysis***

Each bacteria was grown overnight in nutrient agar at 35 ºC. The pure colony of each isolate was transferred into 5 ml of respective NBRIP medium which is considered as the inoculum. About 10% of the inoculum was transferred into 50 ml of fresh NBRIP medium and grown in rotary shaker for 4 days. The sample was centrifuged at a speed of 10,000 x g for 15 min. The supernatant was filtered through 0.22 µm nylon membranes. The filtrate was subjected to the HPLC analysis. Each sample of bacteria had two replicates. About 1 µl of the filtrate from each sample was injected into the system. The retention time of the standard gluconic acid was compared with the peak at the same retention time in the samples. The concentration of gluconic acid secreted by bacteria was calculated based on the area of peak and standard calibration graph.

The detection of gluconic acid was carried out by HPLC (Agilent Technologies 1200 series, model 1311 A). Secreted organic acids were quantified using a ZORBAX SB-Aq column (4.6 x 150 mm, 5 µm). The column was operated at room temperature with a mobile phase of 20 mM aqueous phosphate buffer (pH 2.0) and acetonitrile, 99/1 (v/v) at a flow rate of 0.7 ml/min. Elute was monitored using an UV-DAD detector. The gluconic acids were identified and quantified by comparing the retention time and peak areas with the standard organic acids. Pure gluconic acid was purchased from Sigma-Aldrich (Germany). Stock standard solutions were dissolved in Milli-Q water, diluted to different concentration using mobile phase and filtered using 0.45 µm nylone membranes. They were stored at 4 ºC in the dark. A linear calibration curve was plotted for each organic acid on the basis of peak area against concentration.

***Pqq C gene detection***

Pure colony of bacteria was grown overnight in nutrient broth at 35 ºC in orbital shaker. Bacterial DNA was extracted using UltraClean$^{TM}$ Microbial DNA Isolation Kit. The purified DNA was used as a template for PCR amplification. Two oligonucleotides, namely, OIMBF 3 (5'-CCC GCG AGC AGA TCC AGG GCT GGG T-3') and OIMBF 4 (5'-TAG GCC ATG CTC ATG GCG TC-3') were used for the amplification of a fragment internal to the *pqq* C genes according to published sequence by Farhat et al. (2009). The amplification was carried out in 50 µl reaction mixture containing 1.0 U of 5 U/µl Taq polymerase (Fermentas Life Sciences), 5 µl of 10 x PCR buffer (200 mM Tris-HCI, pH 4.4, 500 mM KCl), 1 µl of 10 mM of dNTP mix, 1.5 µl of 50 mM $MgCl_2$, 1 µl of 10 mM of each primer and 1 µl of genomic DNA. The thermal programme include denaturation at 95 ºC for 3 min, annealing at 95 ºC for 1 min, 59 ºC for 1 min, 72 ºC for 2 min (35 cycles) and final extension at 72 ºC for 3 min. The PCR products were separated with a 1% (w/v) agarose gel and purified by the Nucleospin Extract 11, PCR clean up kit (Germany) and sequenced by 1$^{st}$ Base laboratory (Serdang, Malaysia). The nucleotide sequences were compared with the BLASTN programme. The partial

sequences which showed similarity with the published *pqq* C gene in the database were deposited in GenBank.

***Data analysis***

Data analysis was done using SPSS 16.0. The values were given as means of at least two replications. The differences with $p < 0.05$ were considered significant.

**Results and discussion**

The mineral phosphate solubilising bacteria were selected based on the consistency of clear zone production in NBRIP agar medium after a five series of subculturing. These bacteria were identified to 99 – 100% similarity using molecular method as *Klebsiella pneumoniae* (STMPSB 1, STMPSB 3, STMPSB 5, STMPSB 7, STMPSB 8, STMPSB 9 and STMPSB 10), *Enterobacter aerogenes* (STMPSB 4 and STMPSB 6) and *Pseudomonas aeruginosa* (STMPSB 2). The biochemical characteristics of the bacteria are shown in *Table 1*.

The consistency of the clear zone formation which indicates the mineral phosphate solubilising trait was vital to rule out the possibility of selecting bacterial strains which show instability in phosphate solubilisation after a few inoculations (Igual et al. 2001). It is believed that the production of clear zone is due to the secretion of organic acids into the surrounding medium. Goldstein et al. (1999), Chen et al. (2006), Jha et al. (2009) and Gulati et al. (2010) have suggested the acidification of the surrounding medium as the major mechanism of mineral phosphate solubilisation by bacteria due to the production of organic acids.

A total of 10 isolates of phosphate solubilising bacteria were tested for their capability to dissolve Ca-P (calcium phosphate), Fe-P (ferric phosphate) and Al-P (aluminium phosphate) in NBRIP broth.

Table 1. Biochemical test results for the three main genera of mineral phosphate solubilising bacteria

| Biochemical tests | *Klebsiella* sp. | *Enterobacter* sp. | *Pseudomonas* sp. |
|---|---|---|---|
| Catalase | + | + | + |
| Oxidase | – | – | + |
| Motility | +/– | + | – |
| Citrate | + | + | + |
| Glucose (triple sugar iron agar) | + | + | – |
| Gas production | +/– | +/– | – |
| Indole | +/– | – | + |
| Nitrification | + | + | +/– |
| Methyl red | – | – | – |
| Voges proskaeur | + | + | + |
| Maltose | + | + | – |
| Lactose | +/– | – | – |
| Sorbitol | + | +/– | – |
| Sorbose | + | + | – |
| Fructose | + | + | + |
| Mannitol | +/– | + | + |
| Cellobiose | + | + | + |
| Sucrose | +/– | + | – |
| Xylitol | + | + | + |
| Glucose | + | + | + |

All the 10 MPSB bacteria were able to solubilise Ca-P, Fe-P and Al-P according to various levels of efficiency. The correlation between time of incubation and soluble P released from Ca-P, Fe-P ad Al-P was 0.779, significant at $p \leq 0.05$.

The best Ca-P solubiliser was STMPSB 8, the best Fe-P solubiliser was STMPSB 9 and the best Al-P solubiliser was STMPSB 8, which could release 1,772.5, 1,679.1 and 1,198.6 mg/litre of orthophosphate respectively after 96 h of incubation (*Figure 1*). STMPSB 8 could be designated as the best mineral P solubiliser for all the three insoluble mineral phosphates as it exhibited high solubilisation capacity for Fe-P, Al-P and Ca-P.

The average concentrations of orthophosphate released throughout the experiment were 1,250, 1,088 and 786 mg/litre for Ca-P, Fe-P and Al-P respectively. The highest solubility of mineral phosphate was achieved at 96 h where ferric phosphate, calcium phosphate and aluminium phosphate released 1,547.26, 1,541.28 and 914.02 mg/litre of orthophosphate respectively. The solubility of all the three mineral phosphates were dropped after 24 h but started to increase after 48 h (*Figure 1*).

The concentration of solubilised phosphate declined at 48 h and consequently increased after 48 h. With regard to this observation, Delvasto et al. (2006) have stated that the formation of an intermediate phosphate known as brushite caused the occurrence of oscillating levels of soluble P or drop in the concentration of soluble P. Illmer and Schinner (1995) also stated that Al-P and Ca-P follow two different solubilisation mechanisms. The later increase of soluble P after 48 h could have been caused by the re-dissolution of brushite crystals formed earlier as reported by Delvasto et al. (2006) and Illmer and Schinner (1995).

Furthermore, Tripura et al. (2007) have speculated that the decrease in concentration of the soluble P after an initial increase could have been caused by the decrease in the production of organic acid once soluble P is released into the medium. They also indicated that the decrease in the concentration of soluble P at the end of the incubation could be due to the utilisation of soluble P and organic acid for the metabolic activity of bacteria. However, an increase in orthophosphate concentration for all three the insoluble phosphates at 96 h also shows that the efficiency of phosphorus solubilisation increases over time.

In general, Ca-P could release more soluble P followed by Fe-P and Al-P. This result is in agreement with Vyas et al. (2007) and Zaidi et al. (2009) who have reported that the aluminium phosphate solubilisation rates were lower than the Ca-P solubilisation. Other authors also found that soluble P with Ca-P was significantly higher compared to Fe-P and Al-P (Heekyung et al. 2005; Hong et al. 2006; Sulbaran et al. 2009; Xuan et al. 2011). Furthermore, Perez et al. (2007) also have observed that the mobilisation of P from $FePO_4$ happens at much lesser level than $Ca_3(PO_4)_2$.

Organic acids react with metal elements to form organic chelates which aids in the mobilisation of soluble phosphates (Jones and Darrah 1994; Wang and Zhou 2006). A number of research papers have indicated the ability of the organic acids in facilitating mineral P solubilisation (Chen et al. 2006; Frankem et al. 2006; Vassilev et al. 2006; Patel et al. 2008; Vyas and Gulati 2009). It was postulated that the organic acids may release ions from the surface of minerals via anion exchange or chelation of cations attached to phosphates (Whitelaw et al. 1999; Hoberg et al. 2005; Reyes et al. 2006; El-Tarabily et al. 2008). Among many organic acids, gluconic acid is considered to be the strongest organic acids (Goldstein et al. 1999; Chul et al. 2003). Lin et al. (2006) have reported that the process of acidification and chelation by gluconic acid had liberated insoluble phosphates from the culture medium.

Gluconic acid is synthesised by direct oxidation pathway mediated by glucose

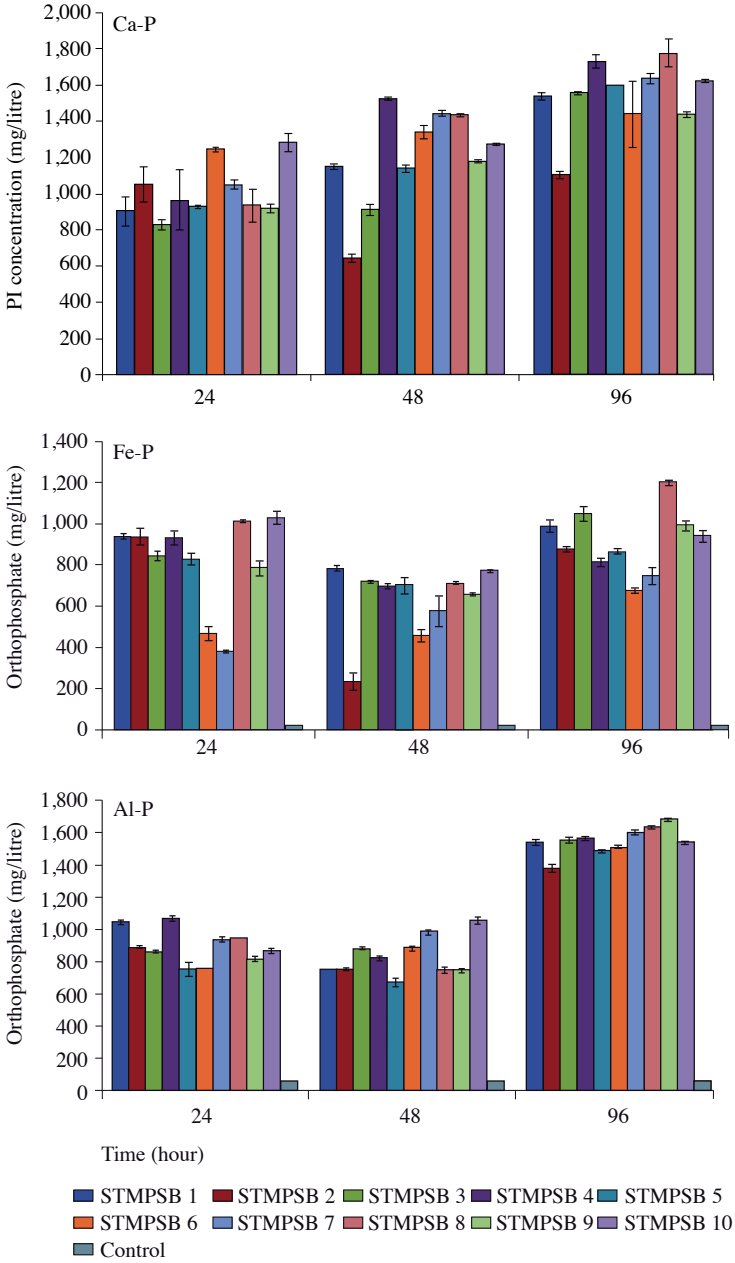


*Figure 1. Orthophosphates released from Ca-P, Fe-P and Al-P by bacterial isolates at different intervals. Values are means of two independent readings. Bars indicate standard error*

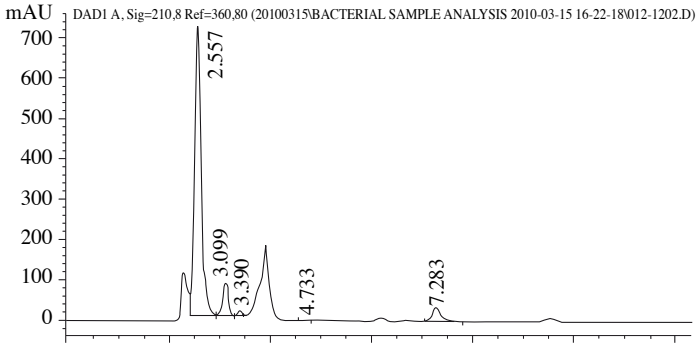


*Figure 2. Chromatogram of HPLC with gluconic acid peak detected at RT 2.557 in bacterial culture*

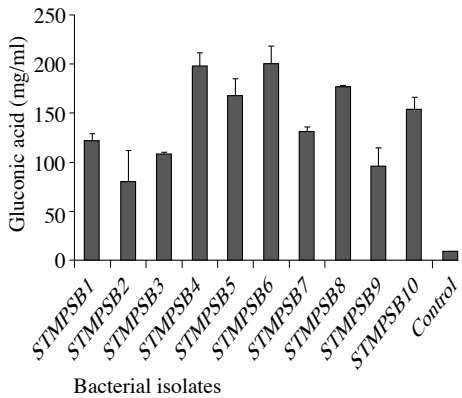


*Figure 3. Concentration of gluconic acid secreted by mineral phosphate solubilising bacteria. Values are means of two independent readings. Bars indicate standard error*

dehydrogenase enzyme (GDH) and a co-factor, pyrroloquinoline quinone (PQQ) in the outer face of cytoplasmic membrane. Gluconic acid is produced in the periplasm (outer face of the cytoplasmic membrane) of Gram negative bacteria and released outside the cells (Goldstein et al. 1999; Perez et al. 2007). Therefore, the analysis of culture supernatant allows the detection of gluconic acid present in bacterial culture. Gluconic acid peak in HPLC chromatogram was detected at 2.557 retention time as shown in *Figure 2*.

All the ten bacterial isolates were able to produce gluconic acid ranging from 80.13 – 199.51 mg/ml (*Figure 3*). STMPSB 6, STMPSB 4 and STMPSB 8 are good source of gluconic acid where they have produced 199.51, 197.04 and 175.91 mg/ml of gluconic acid respectively. The lowest amount of gluconic acid was produced by STMPSB 2, *Pseudomonas aeruginosa* (80.13 mg/ml).

Besides the quantification of low molecular gluconic acids, the relationship between gluconic acid production and mineral phosphate solubilisation was also observed in this study. For some bacterial isolates, gluconic acid production was in parallel with the amount of orthophosphates released from the complex insoluble P. A strong correlation of 0.795 (*Table 2.*) was observed between gluconic acid concentration and mineral P solubilised by bacterial cells.

*Figure 4* shows the comparison between the production of gluconic acid and the amount of P solubilised. It was obvious that certain bacteria which produced high concentration of gluconic acid were also able to release high amount of orthophosphate through acidification of the medium. Since gluconic acid is considered as the strongest acid to dissolve insoluble phosphate, the increase of its concentration also amplifies the amount of orthophosphate released from Ca-P.

Similarly, Ogut et al. (2010) have reported a linear regression between soluble P and gluconic acid concentration in bacterial cultures ($R^2 = 0.59$). Likewise,

Table 2. Correlation of gluconic acid and soluble P

| | | Gluconic acid | Soluble P |
|---|---|---|---|
| Gluconic acid | Pearson Correlation | 1.000 | .795** |
| | Sig. (2-tailed) | | .006 |
| | N | 10.000 | 10 |
| Soluble P | Pearson Correlation | .795** | 1.000 |
| | Sig. (2-tailed) | .006 | |
| | N | 10 | 10.000 |

**Correlation is significant at the 0.01 level (2-tailed)

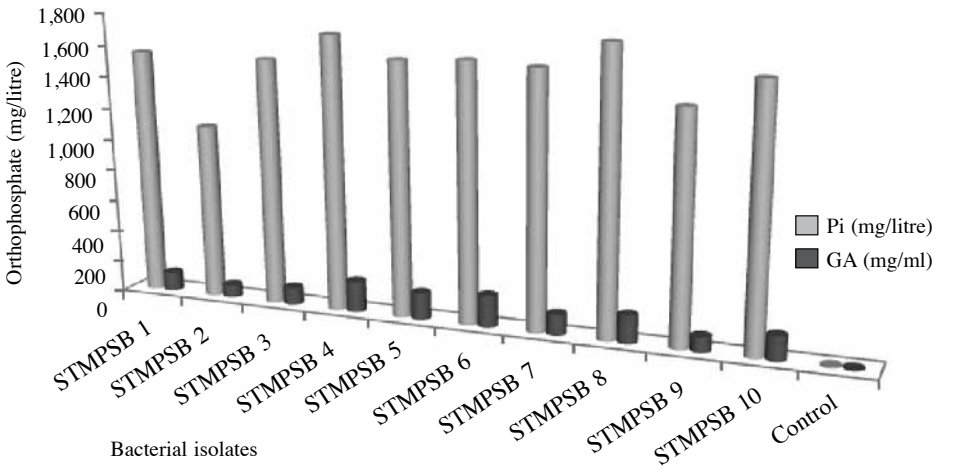


*Figure 4. Comparison between gluconic acid (GA) production (mg/ml) and orthophosphates (Pi) released (mg/litre) by mineral phosphate solubilising bacteria*

Whitelaw et al. (1999) and Vassilev et al. (2001) have observed a strong relationship between the concentration of gluconic acid and soluble phosphate. However, Yanmei et al. (2008) have proved that there was no significant correlation between quantities of organic acids produced by phosphate solubilising bacteria and orthophosphates released from tri-calcium phosphate. Similarly, Gulati et al. (2010) found that there was no relationship discovered between the quantity of organic acids produced and the solubilisation of rock phosphates. According to them, the higher solubilisation of phosphate in the presence of low quantity of organic acids could be due to the higher reactivity and greater variation of organic acids.

In the present study, the *pqq* C gene was detected in all strains except STMPSB 2 and STMPSB 9. The partial sequences of *pqq* C genes were deposited in GenBank. The genetic data is now available at EMBL Europe and the DNA Data Bank of Japan (DDBJ). The list of the bacteria and accession numbers were described in *Table 3*. The detection of *pqq* C genes show that the biosynthesis pathway involved in the production of gluconic acid in 8 out of 10 mineral phosphate solubilising strains is via direct oxidation pathway with the presence of cofactor PQQ.

The absence of *pqq* C in STMPSB 2 and STMPSB 9 indicates that an alternative pathway may have involved in the production of gluconic acid in these 2 strains (Kil et al. 1998; Krishnaraj and

Table 3. The accession numbers of the *pqq* C gene sequence deposited in NCBI Gene Bank

| Code of MPSB | Detection of *pqq* C gene | Length of gene | Accession number of *pqq* C gene sequence deposited in Gene Bank (Locus) |
|---|---|---|---|
| STMPSB 1 | YES | 551 bp | JF683614 |
| STMPSB 2 | NO | – | – |
| STMPSB 3 | YES | 563 bp | HQ727983 |
| STMPSB 4 | YES | 565 bp | JF683615 |
| STMPSB 5 | YES | 550 bp | JF683616 |
| STMPSB 6 | YES | 600 bp | JF683617 |
| STMPSB 7 | YES | 570 bp | HQ727985 |
| STMPSB 8 | YES | 597 bp | JF683613 |
| STMPSB 9 | NO | – | – |
| STMPSB 10 | YES | 529 bp | JF683618 |

Goldstein 2001; Goldstein et al. 2003; Buch et al. 2008). It can be speculated that the absence of *pqq* C gene confirms that other genes apart from PQQ, might have catalysed the production of PQQ via an alternative biosynthetic pathway. Krishnaraj and Goldstein (2001) mentioned that several clones from different Gram negative bacteria that do not synthesize *pqq* gene are able to induce gluconic acid production in *E. coli* via an alternative pathway for PQQ biosynthesis which is not expressed under normal metabolic conditions. From these reports, it was quite obvious that other genes apart from PQQ genes may code for the biosynthesis of gluconic acid. In that manner, Babu Khan et al. (1995) have speculated that *gab Y* plays some unknown role in the regulation of the direct oxidation pathway in *P. cepacia* in order to produce gluconic acid. Besides *gab Y* gene, Rodriguez et al. (2006) suggested the synthesis of gcd co-factor that can be related to the biosynthesis of PQQ using an alternative pathway.

In addition to this, some other genes were also found to involve in mineral phosphate solubilisation in different species namely, pKKY in *Enterobacter agglomerans* (Kim et al. 1997), pK1M10 in *Rahnella aquatilis* (Kil et al. 1998) and pKG3791 in *Serratia marcescens* (Krishnaraj and Goldstein 2001). All these findings demonstrate the complexity of mineral phosphate solubilisation in different bacterial strains.

Production of organic acid, mainly gluconic acid is considered as the major mechanism of mineral phosphate solubilisation. Apart from its role in solubilising mineral phosphate, gluconic acid is also well known for its bio-control activity. According to Kaur et al. (2006), D-gluconic acid is the most significant antifungal agent in bio-control of take-all disease on wheat root. Furthermore, Kaur et al. (2006), Song et al. (2008) and Werra et al. (2009) also have similar observations on gluconic acid produced by *Pseudomonas* sp. and *Enterobacter* sp. In fact, PQQ was also found to be involved in the production of antifungal metabolites and induction of systemic resistance (Song et al. 2008). The 10 strains of mineral phosphate solubilising bacteria have the potential of bio-control activity as they were able to produce gluconic acid which has dual role, both in mineral phosphate solubilisation and bio-control activity.

## Conclusion

All the 10 mineral phosphate solubilising bacteria strains were able to produce gluconic acid which could have contributed to mineral phosphate solubilisation. It was confirmed that the major mechanism of mineral phosphate solubilisation by these 10 bacteria was through organic acid production, mainly gluconic acid. The production of gluconic acid and the presence of *pqq C* gene also have confirmed that these strains possess both the phosphate solubilising trait and bio-control activity trait. Besides facilitating mineral phosphate solubilisation, gluconic acid is a significant antifungal agent too. PQQ is also involved in the production of antifungal metabolites and induction of systemic resistance. The ability of the bacterial isolates to dissolve insoluble phosphate and to produce phytohormones reveals the potential of the bacterial strains to be utilised in the bio-processing of gluconic acid and production of bio-fertiliser. However, a proper field test should be done to verify the efficiency of the mineral phosphate solubilising bacteria to increase the uptake of soluble P by crops.

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**Abstrak**

Sepuluh isolat bakteria asli telah dikenal pasti sebagai pelarut mineral fosfat yang efisien iaitu 7 strain *Klebsiella pneumoniae*, 2 strain *Enterobacter aerogenes* dan 1 strain *Pseudomonas aeruginosa*. Kesemua sepuluh strain dapat melarutkan kalsium fosfat (Ca-P), ferik fosfat (Fe-P) dan aluminium fosfat (Al-P) dengan efisien. STMPSB 8 (*Klebsiella pneumoniae*) merupakan pelarut fosfat mineral yang terbaik bagi ketiga-tiga fosfat mineral tak larut kerana ia telah menunjukkan keupayaan keterlarutan yang tinggi untuk Ca-P, Fe-P dan Al-P manakala STMPSB 6 (*Enterobacter aerogene*s), STMPSB 4 (*Enterobacter aerogene*) dan STMPSB 8 (*Klebsiella pneumonia* ) telah dianggap sebagai bakteria penghasil asid glukonik yang baik. Satu korelasi kukuh sebanyak 0.795, signifikan pada aras 0.01 diperhatikan antara kepekatan asid glukonik dengan keterlarutan kalsium fosfat. Pengesanan gen pirrolokuinolin kuinon (*pqq* C) dalam lapan isolat bakteria menunjukkan bahawa tapak jalan pengoksidaan langsung digunakan dalam biosintesis asid glukonik dengan bantuan kofaktor pirrolokuinolin kuinon.