PHOSPHORUS MOBILISATION MECHANISMS OF *Klebsiella pneumoniae*

Since the 1980s, the plant growth-promoting rhizobacteria (PGPR) have been increasingly recognised as capable of promoting the nutrient-efficient growth of cereal crops. A cooperative set of factors associated with PGPR including stimulated root growth, biological nitrogen fixation by the PGPR organisms, mobilisation of soil N and P, facilitated uptake of nutrients and biocontrol of antagonists and pathogens have been postulated to improve plant growth and maximise the yield of grain (Kennedy and Roughley, 2002). Strong demand pressure for biofertiliser or probiotic products existing in Vietnam these days is also due to a need to protect the environment from chemical pollution

The BioGro inoculant comprises three different microbes *Pseudomonas fluorescens/putida* (1N), *Citrobacter freundii* (3C) and *Klebsiella pneumoniae* (4P). Preliminary research funded by ACIAR and AusAID has verified in field trials near Hanoi that the BioGro effect can increase the average yield of rice by 10-20% and reduce input costs for chemical fertilisers (Nguyen Thanh Hien et al., 2002). In order to establish inoculant biofertilisers for rice as a reliable technology, it is necessary to understand the mechanisms in which PGPR improve rice growth and yield.

Effect of substrate (Glucose) on microbial population of *Klebsiella pneumoniae*.

The number of *Klebsiella pneumoniae* was rapidly increased after inoculation on the Pikovskaia culture medium and was significantly ($P<0.01$) increased with the increase in the amount of glucose added three days after inoculation (Figure 1). However, at day 9 after inoculation, *Klebsiella pneumoniae* population in all treatments dropped and continued to drop in treatments received glucose while in the control, it was gradually increased. Results obtained from the effect of substrate to the proliferation of *Klebsiella pneumoniae* are important for management practices in the field condition to improve the effectiveness of this bacterium in solubilising soil phosphates.

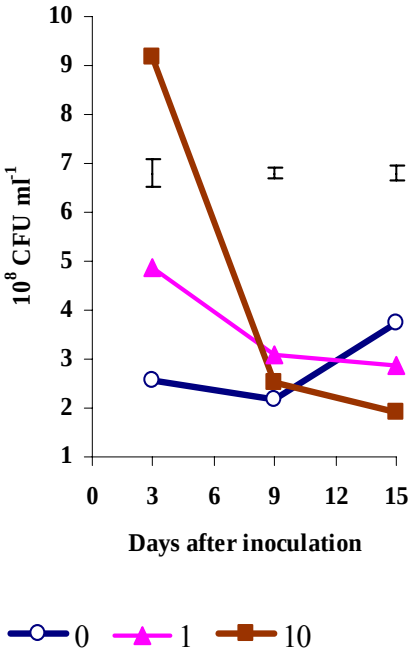

Figure 1: Effect of glucose addition on *Klebsiella pneumoniae* proliferation on Pikovskaia culture medium. Addition rates are 0, 1 and 10 mg glucose L^{-1}. Error bars are LSD (0.05) at each sampling day.

Effect of substrate (Glucose) on pH of Pikovskaia culture medium

Culture medium pH dramatically dropped (1 and 2 units at 1g and 10 g glucose L^{-1}, respectively) after inoculation three days (Figure 2) and did not change very much up to day 15. This coincided with the increase in *Klebsiella pneumoniae* population at 3 days after inoculation.

Phosphate solubilising capacity of *Klebsiella pneumoniae* on Pikovskaia culture medium

Calcium phosphate is considered as a slow-release phosphate source and is one of the components of culture medium used to test P solubilising capacity of

microbes. In the Pikovskaia culture, its soluble P was 1.96 mg P L^{-1}, accounting for 2.3 % of added P.

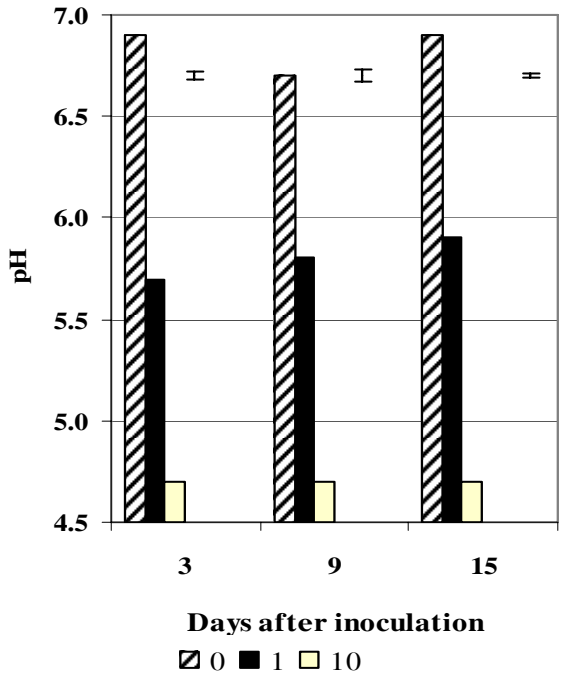

Figure 2. Effect of inoculation with *Klebsiella pneumoniae* on pH of Pikovskaia culture medium. Addition rates are 0, 1 and 10 mg glucose L^{-1}. Error bars are LSD (0.05) at each sampling day.

When 4P was inoculated, soluble P increased by 5.7 fold (9.66 mg P L^{-1}). Apatite and Gafsa are phosphate rocks from Vietnam and Tunessie, respectively which have extremely low solubility. However, the solubility of Gafsa increased by 133 fold with the presence of *Klebsiella pneumoniae*.

Fused-magnesium phosphate, a heat-treated phosphate which is relatively soluble, was dissolved two fold higher in the medium when *Klebsiella pneumoniae* was inoculated. An acid-treated high soluble P, SSP had a highest percentage of soluble P, did not affected by *Klebsiella pneumoniae* inoculation. Medium pH's were reduced by 4P inoculation in the Apatite, Gasfa and SSP containing flasks, whilst pH values increased in flasks containing $Ca_3(PO_4)_2$ and FMP when 4P was inoculated. Flasks with SSP resulted in the lowest medium pH (pH = 3,5) (Figure 3).

Two days after inoculation and at each phosphate source, flasks inoculated with 4P showed a high level of turbidity compared to the control flasks. The exception was due to flasks with SSP. Specific reaction with α -methyl D-glucoside resulted in a colour change from red to yellow in all inoculated flasks, except for those treated with SSP. This implies there was very few or none *Klebsiella pneumoniae* in inoculated flasks containing SSP leading to a conclusion that *Klebsiella pneumoniae* could not grow in the culture media due to a high soluble P (>50 mg P L^{-1}) and a low pH (pH<3.5) when SSP was added. These key findings gave a good example for the establishing of a solubility capacity benchmark of *Klebsiella pneumoniae*.

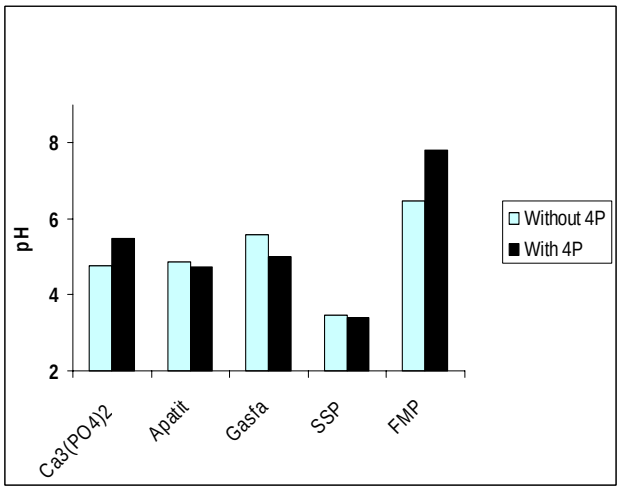

Figure 3. Effect of inoculation with *Klebsiella pneumoniae* on medium pH containing different phosphate sources

Effect of glucose concentration and *Klebsiella pneumoniae* inoculation on rice growth grown on an Acrisol soil of Tay Ninh province

In the pot trial where glucose was added to Tay Ninh soil, rice seeds did not emerge three days after sowing while the control pots did. Population of P solubilising bacteria significantly increased (P<0.05) with glucose doses.

Effect of *Klebsiella pneumoniae inoculation and* phosphate (source and rates) application on P solubilising population and Olsen P of an Acrisol from Tay Ninh province

Inoculation with *Klebsiella pneumoniae* at 10^6 CFU g^{-1} soil significantly (P<0.05) increased number of P solubilising bacteria across P rates and sources.

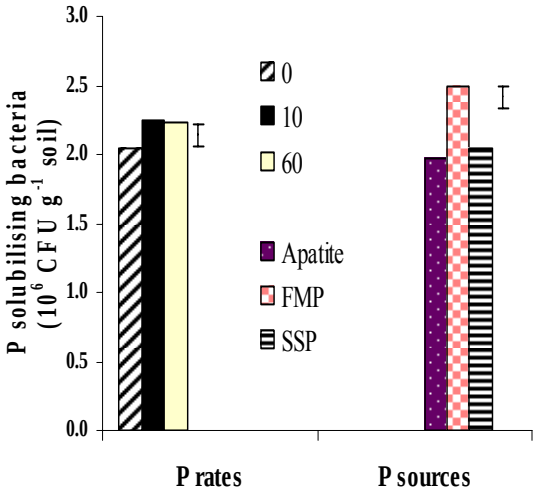

Figure 4: Effect of inoculation with *Klebsiella pneumoniae*, P (rates & sources) application on P solubilising bacteria of an Acrisol from Tay Ninh province. P rates were 0, 10 and 60 mg P kg^{-1}soil. Error bars are $LSD_{(0.05)}$ for P rates or P sources.

Number of P solubilising bacteria was significantly (P<0.05) increased compared with the control when P was added (Figure 4) either at 10 or 60 mg P kg^{-1} soil. There was no significant difference in number of P solubilising bacteria between P addition rates. Among three P sources, treatments received FMP had highest number of P solubilising bacteria on this soil type, significantly (P<0.05) higher than those received apatite and SSP.

Alkaline extractable soil P (Olsen method) was significantly (P<0.05) increased (11%) across P rates and sources as affected by *Klebsiella pneumoniae* inoculation to the Acrisol in Tay Ninh province. Treatments received P had significantly higher Olsen P (Figure 5) concentration than the control. Treatments received SSP had highest Olsen P, followed by FMP then apatite. The addition of 4P bacteria (10^6 CFU g^{-1}) to Tay Ninh soil significantly increased (P<0.05) dry weight of rice seedlings and number of tillers across P rates and P sources.

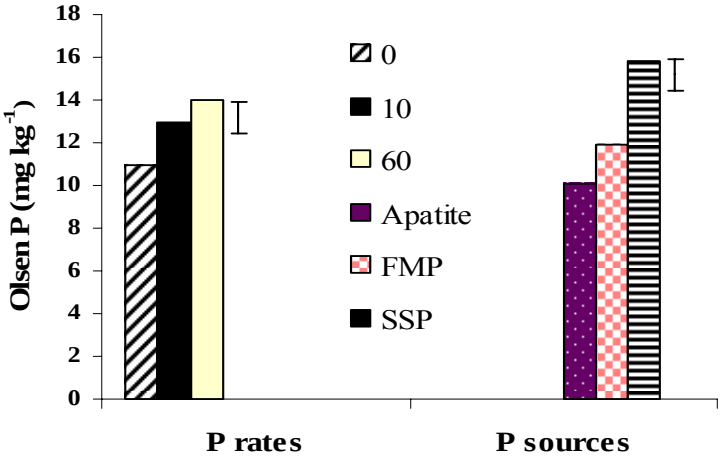

Figure 5: Effect of inoculation with *Klebsiella pneumoniae*, P (rates and sources) application on available P of an Acrisol from Tay Ninh province. P rates were 0, 10 and 60 mg P kg^{-1}soil. Error bars are $LSD_{(0.05)}$ for P rates or P sources.

Substrate greatly affected proliferation of Klebsiella pneumoniae. In modified Picovskaia medium, the population of 4P rapidly significantly ($P<0.05$) increased with glucose doses added after three days but it declined afterwards while in the control without sugar, the population of 4P gradually increased. Culture medium pH dropped one or two pH units in response to a higher glucose application dose of 1 and 10g glucose L^{-1}, respectively.

Klebsiella pneumoniae inoculation increased the phosphate solubilising capacity of slow-release phosphates as followed: Gafsa> apatite > $Ca_3(PO_4)_2$> FMP. Culture medium pH declined as followed: FMP>Gafsa>$Ca_3(PO_4)_2$>apatite>SSP. Proliferation of 4P bacteria was inhibited when culture medium P was high (>50 mg P kg^{-1}) and culture medium pH was low (pH<3.5), as was the case of single super phosphate addition.

In the pot trial with an Acrisol from Tay Ninh province, inoculation of Klebsiella pneumoniae (across glucose doses) tended to increase the P solubilising bacterial population and Olsen P but the differences were not significant at $P<0.05$. The population of 4P increased with the application doses of glucose. Olsen P significantly ($P<0.05$) increased at the highest glucose dose. Inoculation of Klebsiella pneumoniae (across P rates and P sources) significantly increased dry weight of straw and number of tillers ($P<0.05$).

Although *Klebsiella pneumoniae* has high P solubilising capacity, it is been recently assessed that *Klebsiella pneumoniae* is an important opportunistic pathogen and a frequent cause of nosocomial infections (Struve, C. and K.A. Krogfelt, 2004). It is ubiquitous in nature. The pathogenic potential of environmental *Klebsiella pneumoniae* isolates is unknown.

References

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