Mineral phosphate solubilizing activity of *Acetobacter diazotrophicus*: A bacterium associated with sugarcane

Mineral phosphate solubilization (MPS) is a characteristic trait of many rhizosphere[1] and endorhizosphere bacteria[2], that release Pi in the readily available form as H_2PO_4 and HPO_4 from insoluble fixed forms. Such organisms gain importance in low-capital, eco-friendly agriculture, as nearly 90 per cent of applied phosphate fertilizer is fixed in the soils, rendering these fertilizers unavailable for plant consumption[3,4]. *Acetobacter diazotrophicus* is a nitrogen-fixing bacterium isolated from sugarcane roots and stems[5]. Low-capital agriculture, aiming simultaneously at sustainability, must rely on increased efficiency of mineral uptake or partial replacement of chemical fertilizers. Hence, we looked for the property of mineral phosphate solubilization in these strains.

The strains of *A. diazotrophicus* used in the study are listed in Table 1. The strains were maintained on LGI medium[6]. Quick analysis of the MPS trait was made on modified Sperber's medium (MSM) by measuring the zone of solubilization around the colony growth. For assessing the release of Pi from tricalcium phosphate (TCP), 100 µl of 24 h cultures in 10 ml LGI broth were inoculated into 100 ml tricalcium phosphate broth[7] and incubated at 30°C for 10 days. The P released and the profile of organic acids produced were estimated by methods described by Gaur[8]. The drop in pH in the external medium was measured by a pH meter.

On MSM agar, the strains showed large zones of solubilization indicating the ability of these strains to solubilize insoluble and fixed phosphates (Table 1, Figure 1). Estimates of P released in the medium revealed that all the strains released Pi from tricalcium phosphate. Strain PaP5 solubilized 50.9 per cent TCP while, Pal5 and PPe4 solubilized 21.78 and 23.96 per cent P respectively, indicating a tremendous potential of these strains to release P from fixed phosphates for plant uptake.

The descending paper chromatography revealed that *A. diazotrophicus* releases succinate, tartarate, citrate, and gluconate. Release of organic acids has been attributed to be one of the mechanisms by which microbes bring about phosphate solubilization[9,10]. It has been reported earlier that *A. diazotrophicus* forms 2-ketogluconic acid and 2,5-diketogluconic acid from glucose[5]. Additionally, this bacterium is known to oxidize glucose extracellularly to gluconic acid via pyrroloquinoline-quinone-linked glucose dehydrogenase (PQQ-GDH)[11]. The mode of mineral P solubilization by *A. diazotrophicus* thus, is probably through the PQQ-dependent production of gluconic acid. Cloning of the gene that codes for PQQ enabled transgenic *E. coli* HB 101 to produce gluconic acid and solubilize hydroxy apatite[12].

However, it is also known that MPS bacteria solubilize mineral phosphate through proton extrusion[13]. It is still to be analysed whether a coordinated mechanism involving organic acid production and proton extrusion, as hypothesized for *Pseudomonas* sp. Psd 201 (ref. 14), exists in these strains.

A. diazotrophicus, apart from stimulating sugarcane growth through nitrogen fixation and production of plant growth promoting substances[15], can also improve the availability of P in its rhizosphere. Agronomic experiments are required to analyse the capacity of these strains to partially replace phosphate fertilizers and use low grade rock phosphate by co-

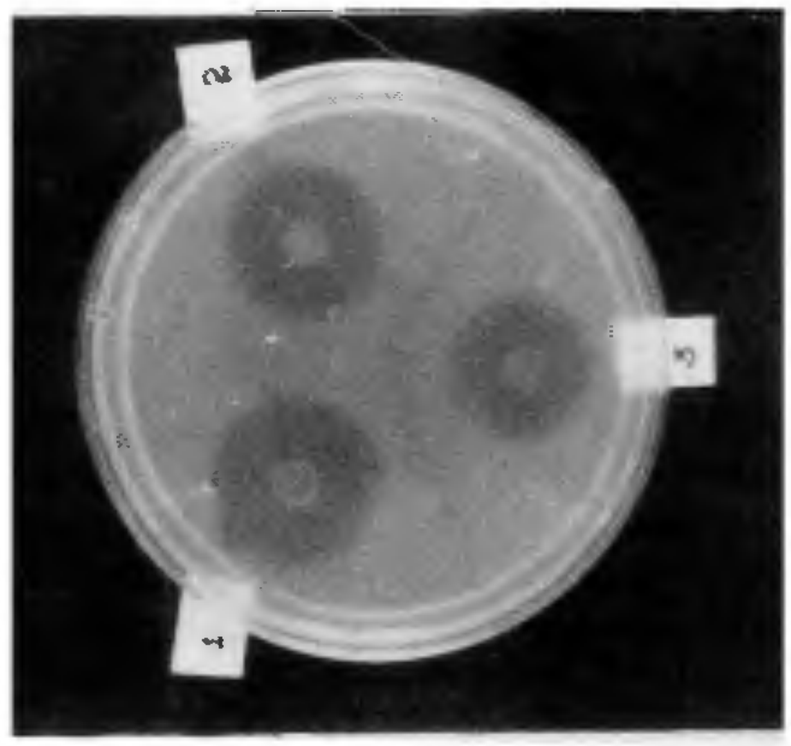

Figure 1. *In vitro* solubilization of dicalcium phosphate by *Acetobacter diazotrophicus*. 1, PaP5; 2, PPe4; 3, PaL5.

Table 1. Mineral phosphate solubilization by *Acetobacter diazotrophicus*

Strain	Zone of solubilization (on MSM), mm	%P solubilized Pikovskaya's broth	pH drop* (units)
Pal5	17	21.78	1.5
Ppe4	21	23.96	1.5
PaP5	24	50.09	1.5

*The pH of Pikovskaya's broth was brought down from 6.5 to 5.0, 10 days after inoculation.

inoculating these strains. The *in vitro* analysis of this study, reflects the possibility in sugarcane.

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K. S. MAHESHKUMAR*
P. U. KRISHNARAJ**
A. R. ALAGAWADI*

**Department of Agricultural Microbiology and*
***Department of Biotechnology, University of Agricultural Sciences, Dharwad 580 005, India*