Soil phosphate solubilising bacteria of the Okavango floodplains, Botswana

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Introduction

Phosphorus although abundant is among the most limiting macro plant nutrients in some soils. Deficiencies are often seen as purpling of leaves due to unavailability of PO_4 in soil solution. In soil solution PO_4 solubility is pH dependent; at pH<6, it is often bound to Fe or Al, while at pH>7 it forms insoluble complexes with Ca, Mg, K or Na making it unavailable to plants (Brady 2002). Soil organic P requires mineralisation then solubilisation by the enzyme phosphatase before being available. Botswana soils due to the low rainfall (<450 mm/yr) are characterised by high pH and salt pans scattered all over the semi-arid and arid landscape. Cultivation of most of these soils often results in P deficient crops (Leuschner and Manthe 1996). However, in grassland areas of the Okavango, very few grasses show P deficiency. Studies from other areas indicate that some grasses habour PO_4 solubilizing bacteria in their rhizosphere that help them in their P sequencing quest (Duponnois *et al*. 2005). This study was set up to study the PO_4 solubilising bacteria of the Okavango Delta at Seronga. The study also looked at possible mechanisms of solubilisation employed by the bacteria. In the long run, the study will also attempt to use these bacteria on locally grown staple cereal, sorghum (*Sorghum vulgare*).

Methods and Materials

Potential P solubilising bacteria were isolated from the roots of dominant grasses in Seronga floodplains of the Okavango Delta by plating soil dilutions on insoluble phosphate mineral agar medium and incubating at 25^{o}C for 7 d. Clearance of insoluble P on the plates was regarded as ability to solubilise the PO_4. The top ten P solubilising bacteria based on zone of clearance were chosen, further screened and tested in both liquid and solid media for their ability to solubilise P in P complexes *i.e.* Ca, Mg and K phosphate that occur widely in Botswana soils. Modes of P solubilizing studied included production of the enzyme phosphatase, organic acids and ability to lower pH in growth liquid media. Gas chromatography- Mass spectrometry (GC-MS) analysis of the growing liquid cultures was used to assess possible compounds the bacteria excrete during growth.

Results and Discussion

The ten bacterial isolates were selected from the many tested due to their ability to solubilize PO_4 on solid agar The isolates were all Gram negative rods ranging from long to short rods. All except two were motile. The isolates showed ability to solubilize $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$ and K_2HPO_4 on solid agar media at different rates ranging from 9 mm to 2 mm zones of clearance in 27 days (Table 1).

The different isolates were also able to produce the phosphatase in liquid media ranging from 1.19 to 4.23 µgP-nitrophenol/ml supernatant (Table 2). The isolates also showed the ability to lower pH of the growth media as opposed to the uninoculated control medium (Table 2).

Gas chromatography-Mass spectrometry analysis showed that these isolates excrete 34 different acids as extra cellular metabolites in liquid media. These include simple ones such as acetic, formic, phosphoric, propionic acid and more complex and cyclic acids such as 2,2-Biphenylylenephosphoric, 2-Chloroaniline-5-sulfonic , 2-Fluoro-5-trifluromethylbenzoic, 2-trifluromethylbenzoic and 3-Hydroxy-2-methyl-3phenylpropionic acid.

Table 1. Diameter (mm) of zone of clearance for the different isolates with time (days).

Isolate	Grass source	12 d	17 d	19 d	23 d	24 d	27 d
S1	*Eulesine Africana*	4b	4.5b	4.6b	5.4b	5.6b	9c
S2	*Imperata cylindrica*	1a	2a	2a	2a	2a	2a
S3	*Imperata cylindrica*	1a	2a	2a	2a	2a	2a
S4	*Sesbania seban*	1a	2a	2a	2a	2a	2a
S5	*Panicum maximum*	0.6a	2a	2a	2a	2a	2a
S6	Cyperus sp	0.4a	1a	2a	2a	2a	2a
S8	*Cynodan dactylons*	4b	5b	7b	8c	8c	8c
S9	*Urochloa decumbens*	2a	5b	6b	6bc	7c	7bc
S10	*Urochloa trichophus*	2a	4b	5b	5b	6bc	6bc

Table 2. Isolates alkaline phosphatase production andtheir effect on growth media pH.

Isolate	µgP-nitrophenol/ ml supernatant	Media pH on different days			
		Day 1	Day 7	Day 14	Day 21
S1	1.93b	6.54a	5.92ab	5.89ab	5.81a
S2	1.85b	6.54a	6.12b	6.13b	5.91a
S3	1.19b	6.54a	5.97a	5.94b	5.91a
S4	2.28b	6.54a	6.14b	5.52a	6.07ab
S5	1.93b	6.54a	6.00b	5.89ab	5.82a
S6	1.80b	6.54a	5.94ab	5.96b	5.93a
S8	2.13b	6.54a	5.86a	5.85ab	5.82a
S9	4.23c	6.54a	6.02ab	6.01b	5.89a
S10	1.81b	6.54a	5.41a	5.26a	6.14ab
Control	0.01a	6.54a	6.54b	6.54b	6.54a

Conclusions

Seronga grasses are able to flourish in environments where crops show serious P deficiencies because they harbour P solubilising bacteria which they use in acquiring P. The isolates were able to solubilise PO_4 complexes, lower growth media pH, and produce different organic acids in the medium. This study indicates that these isolates are able to solubilise insoluble phosphates in soil by production of organic acids. However, production of alkaline phosphatase may also be a possible mechanism as some of the isolates excreted high amounts in the growth medium. Inoculation of these isolates on cereal crops in the region could increase P availability to crops

References

Brady NC (1990) 'The Nature and Properties of Soils (10th edn)'. (Macmillan Publishing Company: New York).

Duponnois R, Colombet A, Hien V, Thioulouse J (2005) The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. *Soil Biology and Biochemistry* **37,** 1460-1468.

Leuschner K, Manthe CS (1996) Drought-tolerant Crops for Southern Africa: Proc. of the SADC/ ICRISAT Regional Sorghum and Pearl Millet Workshop, 25-29 July, Gaborone, Botswana.