Preliminary selection of phosphate solubilising plant growth promoting microorganisms

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Introduction

- Agriculture is the dominant sector in most Sub-Saharan countries of Africa. It provides employment to the majority of the rural population with a significant contribution to GNP and foreign exchange earnings.
- Agricultural productivity is low and the people depending on agriculture are generally poor. Depletion of soil fertility is a primary cause of low per capita food production (Bremen et al. 2001; Sanchez 2002).
- Fertilizer use is low (8 kg/ha) and inadequate to replace the nutrients removed in harvested crops (Gregory and Bumb 2006).
- A shortage of the essential plant nutrient (P) limits agricultural production on a global scale. Mineral P fertilizers are scarce but are essential to increase and maintain crop yields (George et al. 2006). However, when applied to soils, both P fixation and precipitation occur (Fernandez et al. 2007).
- Increasing problems associated with the use of synthetic chemicals (impacts on health and the environment, resistance development in plant pathogens and pests) has driven an ever-increasing interest in the use of native beneficial microorganisms to improve plant health and productivity (Avis et al. 2008).
- Cameroonian soils are generally low in fertility, particularly phosphorus and nitrogen and need to be fertilized for adequate yield. Research has been undertaken with the aim at maintaining the fertility of Cameroon soils by biological means.
- The objective of this study is to identify and characterize efficient phosphate solubilising microorganisms that allow better mineral nutrition of plants.

Methodology

Soil samples were collected in the five agro ecological zones of Cameroon and assessed for pH and P availability.

- We have generated a culture library of soil isolates using LB media
- Phosphate solubilising ability was tested by growth in P free minimal media supplemented with either $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, or Na-Phytate and with dye (BCG).
- Colony (n) and halo zone (z) diameter and the ratio z/n were evaluated as an indicator for isolate efficiency.

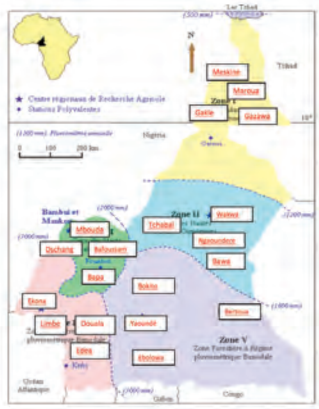

Figure 1: The five agro-ecological zones of Cameroon in different colours and the twenty sampling sites in red colour.

Results

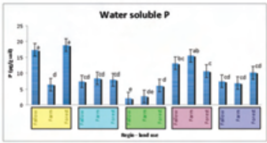

Figure 2: Water P of soils collected in three land use systems of the five agro ecological zones of Cameroon

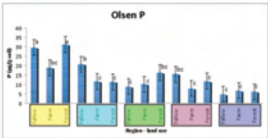

Figure 3: Olsen P of soils collected in three land use systems of the five agro ecological zones of Cameroon

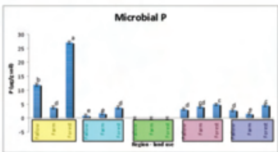

Figure 4: pH of soils collected in three land use systems of the five agro ecological zones of Cameroon

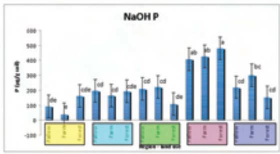

Figure 5: NaOH P of soils collected in three land use systems of the five agro ecological zones of Cameroon

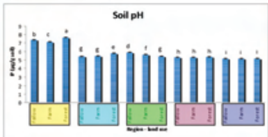

Figure 6: pH of soils collected in three land use systems of the five agro ecological zones of Cameroon

Table 1: Results of ANOVA testing of measures with main assessed factors site was assessed nested within region.

		Water P	Olsen P	Microbial P	NaOH P	pH
Land_Use	F	4.79	19.57	805.63	18.78	8475.59
	P	0.01	<0.001	<0.001	<0.001	<0.001
Region	F	27.83	3.93	395.02	0.06	104.18
	P	<0.001	0.022	<0.001	0.944	<0.001
Interaction	F	6.32	1.94	212.67	1.27	123.21
	P	<0.001	0.061	<0.001	0.268	<0.001

Table 2: Activity of twenty example isolates on agar plates containing minimal medium supplemented with different sparingly soluble phosphate types

Isolate codes		MM with Ca-P			MM with Al-P			MM with Fe-P			MM with Na-phytate			
		z (mm)	n (mm)	z/n (a)	z (mm)	n (mm)	z/n (b)	z (mm)	n (mm)	z/n (c)	z (mm)	n (mm)	z/n (d)	Rating
1	Mar-fm B1	35	5.5	6.36	46	5	9.20	27	6	4.50	21	6	3.50	a,b,c,d
2	Gaz-ft B7	18	8	2.25	22	7	3.14	13	9	1.44	17	10.5	1.62	a,b,c,d
3	Gak-ft F4	19	11	1.73	35	8	4.38	0	15	0.00	22	17.5	1.26	a,b,d
4	Mes-ft F4	11	6.5	1.69	16	6	2.67	0	5.5	0.00	15	6	2.50	a,b,d
5	Baw-fm B8	18	5.5	3.27	18	5.5	3.27	0	0	0.00	14	7	2.00	a,b,d
6	Wak-ft B15	39	5.5	7.09	51	5	10.02	23	6	3.83	28	7	4.00	a,b,c,d
7	Nga-ft F4	38	29	1.31	50	19.5	2.56	42	31.5	1.33	41	32.5	1.26	a,b,c,d
8	Tch-ft F8	21	12	1.75	39	5.5	7.09	22	12	1.83	22	11	2.00	a,b,c,d
9	Dsc-ft B15	27	5.5	4.90	46	6	7.66	22	7	3.14	16	6.5	2.46	a,b,c,d
10	Mbo-ft B2	13	6	2.17	25	5.5	4.55	15	6.5	2.31	14	5	2.80	a,b,c,d
11	Bap-ft F5	18	5	3.60	25	6	4.17	13	6.5	2.00	13	7.5	1.73	a,b,c,d
12	Baf-ft F6	18	10	1.80	37	6	6.17	21	13	1.62	20	11	1.82	a,b,c,d
13	Ede-ft B4	15	6.5	2.31	0	5	0.00	27	8	3.38	23	7.5	3.07	a,c,d
14	Eko-fm B5	27	6	4.50	40	5.5	7.27	17	5	3.40	18	6	3.00	a,b,c,d
15	Dou-fm F6	13	9	1.44	36	7.5	4.80	14	10	1.40	19	9	2.11	a,b,c,d
16	Lim-ft F1	47	41	1.15	25	13.5	1.85	47	40	1.18	49	36	1.36	a,b,c,d
17	Yao-ft B5	15	6.5	2.31	14	6	2.33	22	6	3.67	20	7	2.86	a,b,c,d
18	Bok-fm B6	14	5.5	2.55	44	6	7.33	19	8	2.37	15	7	2.14	a,b,c,d
19	Ebo-ft F2	14	9	1.56	13	5	2.60	0	7	0.00	15	8	1.88	a,b,d
20	Ber-fm F5	20	8	2.50	43	11	3.91	0	9	0.00	17	13	1.31	a,b,d

Note: MM, minimal medium; z, diameter of halo zone; n, diameter of the colony; a, solubilisation of calcium-phosphate; b, solubilisation of aluminium-phosphate; c, solubilisation of iron-phosphate; d, solubilisation of sodium-phytate; B, bacteria; F, fungus; ft isolate from forest soil; fm, isolate from farm soil.

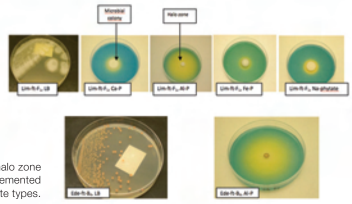

Figure 7: Microbial colonies on LB plates and halo zone (yellow zone in this case) on agar plates supplemented with the different phosphate types.

Conclusion

- P availability increases with soil alkalinity.
- Significant differences in P availability between sites within a region and between land uses, showing depletion of P availability in farmers' field.
- The acidifying activity of the microorganism is demonstrated by the pH decrease observed as a halo (yellow) zone surrounding the colony.
- The solubility of the different phosphate types decreases in the order: Ca-P > Al-P > Na-phytate > Fe-P.
- The ratio z/n serves as an indicator for the isolate efficiency; the higher the ratio, the greater the activity of the microorganism.
- Ongoing research is focused on: -Analysis of the bacterial community structures of soils by T-RLFP. -Quantitative assay in liquid media containing sparingly soluble phosphates and determination of the organic acids involved in the process of phosphate solubilisation. -Identification of isolates by sequencing of the SSU ribosomal gene combined with phylogenetics. -Green house trials using the most efficient strains to assess effect on plant performance.

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