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Concurrent Release of Secondary and Micronutrient by a *Bacillus* sp.

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Abstract: Soil is the resource and reservoir of plant nutrients but it cannot sustain crop requirement continuously unless replenished by manures or fertilizers. To supplement chemical fertilizer inputs, biofertilizers for Nitrogen fixation, Phosphate solubilisation and Potash mobilisation have been identified and widely used. But organisms solubilising secondary nutrients like Mg and Ca micronutrient like Zn are yet to be identified. A Silicate solubilising bacterium *Bacillus* sp isolated from sugarcane field soil was found to release Magnesium and Calcium from Dolomite, Talc and Magnesium trisilicate under *in vitro* condition and in soil amended with these minerals. The Magnesium content in the soil solution from Talc amended soil increased from 9 to 24 ppm and 32 to 43 ppm in Magnesium trisilicate amended soil. During the same period Ca increased from 32 to 64 ppm in Talc amended soil, 40 to 72 ppm in Dolomite and 32 to 72 ppm in Magnesium trisilicate. The study revealed that this Silicate solubilising bacterium could solubilise concurrently Magnesium (Mg), Calcium (Ca), Silicon (Si) and also had the Zinc (Zn) solubilisation potential.

Key words: Silicate solubilisation · *Bacillus* · Secondary · Micronutrient

INTRODUCTION

Soil is the reservoir of nutrients required by plants. But it cannot sustain the demand by crops continuously, season after season and hence farmers have resorted to application of fertilizers. Even here farmers apply major nutrients like Nitrogen, Phosphorus and Potassium often and seldom the secondary and micronutrients. Magnesium and Calcium are secondary nutrients required in substantial quantities by crops but applied rarely. Similarly Zinc is a micronutrient usually applied after observing its deficiency in crops. The crustal abundance of Mg, Ca and Zn are 2.90%, 5.00% and 79 ppm, respectively. But the availability in soil and uptake by plants is limited by several factors like low pH, cationic competition and low temperature of the cropping season. Considering the plant content ranging from 0.05 to 1.0% of Mg, 0.1-6.0% of Ca and 10-250 ppm of Zn one can visualise their demand for the biomass and grain yield of cultivated crops and their continuous removal from soil. A crop yielding a biomass of 10 t/ha inclusive of grain and straw (assuming an average moisture content of 30% may yield 7 t/ha dry matter) may remove 3.5-70 kg Mg, 7.0-420 kg Ca and 0.07-1.75 kg Zn from a hectare soil. Excepting for the residues and compost that supply very small quantities no addition of these nutrients is resorted to. But only in plantation crops dolomite is applied once in 2 or 3 years to correct acidity that supplies Magnesium as well. Soil is rich in Calcium but poor in Magnesium content. Therefore in order to sustain the supply, Magnesium of soil has to be released to cope up to the crop demand. The present study reports the role of a Silicate solubilising bacterium *Bacillus* sp isolated from a sugarcane field soil in releasing these nutrients in soil.

MATERIALS AND METHODS

The solubilisation of Mg and Ca was investigated in liquid culture and in soil incubation studies. Basal medium (Glucose-10g; $(NH_4)_2SO_4$-1.0g; KCl-0.2g; K_2HPO_4-0.1g; $MgSO_4$-0.2g; Water-1000ml; pH7.0) was prepared and dispensed in 100ml quantities in 500ml Erlenmeyer flasks to which Talc, Dolomite and Magnesium trisilicate were added separately @ 0.25% level. The flasks were sterilised at 15lb psi for 20 min, cooled and inoculated with 0.1ml of actively growing culture of Silicate solubilising *Bacillus* sp isolated from sugarcane field (clayey loam) soil collected from Panruti, Tamilnadu, India. The bacterium

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was isolated in Modified Bunt and Rovira medium containing 0.25 % magnesium trisilicate. This isolate exhibited clearing zone around its growth indicating the dissolution of insoluble silicate. The isolate was identified by Gram staining and biochemical tests.The flasks were incubated at room temperature (30±2°C). At 0, 2, 4, 8 days interval, flasks were withdrawn, the culture was centrifuged at 10,000 rpm for 15 min to remove the cells and debris and the supernatant was analysed for the Mg, Ca and Silica.

In soil incubation studies earthen pots of 9" dia were filled with rice field clayey loam soil collected from Kanniakoil village, Puducherry during off season. The soil was air dried, straw bits and roots were removed, powdered and sieved through 60 mesh BSS. One Kg of sterilized soil was filled in earthen pots. Intermittent sterilisation was done for 3 days by steaming for 1h. Each of the silicate mineral was added at 0.5% level and mixed aseptically. Five ml of an actively growing culture (Ca. 20 x 10^6 cell ml^{-1}) was added to each pot. Control pots without addition of Silicate mineral and without inoculation of the culture were also maintained. The pots were filled with distilled water to submerge the soil. About 600ml of water was added; the level marked and was maintained throughout the experiment by addition of water to compensate the quantity of sample withdrawn from each pot at every interval. Samples were withdrawn using a pipette with least disturbance to soil, centrifuged at 10,000 rpm for 15 min and supernatant was analysed for Mg, Ca and Silica. Calcium and Magnesium were estimated by Versanate method [1] and Silica was analysed by the method described by Yoshida [2].

Growth on Silica: The ability of the organism to grow entirely on Silica without any added carbon source was tested on quartz (acid washed sand passing through BSS 64 mesh) and also in precipitated and dried silica (Loba Chem, Mumbai). Basal medium was prepared and aliquots of 100 ml quantities were dispensed in 500ml Erlenmeyer flasks. To one set of flasks 0.25% quartz and to another set of flasks 0.25 % silica without any carbon source were added. The flasks were sterilised at 15 lb psi for 20 min and cooled. An actively growing culture was inoculated @ 1 ml (26x 10^6 cells ml^{-1}) per 100 ml of the medium. The flasks were incubated at room temperature. The growth of bacteria in each of the flask was enumerated by employing serial dilution technique.

Zinc Solubilization: The zinc solubilization potential of the isolate was investigated in Bunt and Rovira medium (glucose-20.0g; peptone-1.0g; yeastextract-1.0g; $(NH_4)_2SO_4$-0.50g; K_2HPO_4-0.40g; $MgCl_2$-0.10g; $FeCl_3$-0.01g; soil extract-250ml; Water-750ml;pH 6.6-7.0) containing 0.1% insoluble zinc oxide [3]. The medium was sterilized poured into petri dishes and allowed to cool. On this solid medium a loopful of the culture was streaked and incubated for 3 days at room temperature (30±2°C). A clear zone around the bacteria indicated the solubilisation of insoluble zinc oxide. The bacterial growth and clear zone were measured (Table 4).

RESULTS AND DISCUSSION

In soil incubation studies it was observed that mere addition of the water was found to render Mg and Ca soluble in water besides silica increasing their content in water. Silicate minerals like Talc, Dolomite and Magnesium trisilicate were easily solubilised by the bacterium. Inoculation of the *Bacillus* sp solubilising silica to soil without any added silicate mineral also increased the solubility of Mg and Ca suggesting the ability of the bacterium to act upon the native soil silicate releasing Mg and Ca. However, the increase was relatively lesser than that observed in added silicates. The calcium release was higher than Mg. Application of Talc, Dolomite, Magnesium trisilicate generally augmented the Mg and Ca level in water. The release of Mg was higher due to inoculation and this was especially higher in Talc and magnesium trisilicate owing to a higher content of magnesium in this salt than dolomite. The Magnesium content in Talc and Magnesium trisilicate was 24% and 18% respectively when compared to 13% in dolomite. The higher level of Ca was observed in *in vitro* studies in Magnesium silicate might arise from the impurities contained in it as soluble salts to the tune of 1.5% (Table 1 and 2).

In vitro studies showed that this *Bacillus* can solubilize Mg and Ca from the silicate minerals. The lowest silica content was observed in the culture filtrate compared to Mg and Ca might be due to its utilization by the bacterium. In the present study utilization of silica by the *Bacillus* sp under *in vitro* condition was clearly established. This bacterium grew well on quartz (Sand) and Silica under *in vitro* conditions (Table 3). Bacteria were known to utilize silica in the absence of carbon [4]. The utilization of silica by diatoms and certain bacteria

Table 1: *In vitro* solubilisation of Mg, Ca and Si by a silicate solubilising *Bacillus* sp

		Magnesium (ppm)				Calcium (ppm)				Silica (ppm)			
		0	2	4	8	0	2	4	8	0	2	4	8
S.no	Treatments	DAI				DAI				DAI			
1.	Talc + *Bacillus* sp	28	28	28	28	56	408	480	488	0.5	3.1	3.3	2.4
2.	Dolomite + *Bacillus* sp	38	254	177	220	64	408	520	177	1.2	2.0	0.6	1.1
3.	Magnesium trisilicate + *Bacillus* sp	22	360	273	418	32	120	184	380	5.5	8.1	0.4	3.5

DAI: Days after Incubation

Table 2: Solubilisation of Mg, Ca and Si by a Silicate solubilising *Bacillus* sp in soil incubation studies:

		Magnesium (ppm)				Calcium (ppm)				Silica (ppm)			
		0	2	4	8	0	2	4	8	0	2	4	8
S.no	Treatments	DAI				DAI				DAI			
1.	Talc + *Bacillus* sp	9	33	24	48	32	64	64	88	2.8	2.0	9.5	11.5
2.	Dolomite + *Bacillus* sp	48	33	9	43	40	88	72	88	2.0	2.9	6.2	9.7
3.	Magnesium trisilicate + *Bacillus* sp	32	24	43	52	32	120	72	100	1.0	2.6	10.3	12.8

DAI: Days after Incubation

Table 3: Growth of *Bacillus sp* on sand (quartz) and pure silica in the absence of carbon source

	Population			
	0 DAI	3 DAI	7 DAI	14 DAI
Particulars	$x10^6$	$x10^6$	$x10^9$	$x10^9$
Sand (quartz) 0.25%	0.22	258	365	344
Silica 0.25%	0.20	631	351	523

DAI: Days after Incubation

Table 4: *In Vitro* solubilisation of Zinc by a Silicate solubilising *Bacillus sp*

Medium	Growth (mm)	Clearing Zone (mm)
Medium containing 0.25% Magnesium trisilicate	5	16
Medium containing 0.1% Zinc oxide	2	3

were also reported earlier [5, 6]. An enhanced growth acclerating effect of silicon on *Staphylococcus aureus* [7] and utilization of silicon by certain Nocardioform organisms was also observed [8]. Although, multiplication of bacteria purely on silica without any carbon was demonstrated it is claimed that there is a minor role of silicon compounds in the development of primitive forms of life when earth was inhospitable for the development of carbon based life [9].

The ability to solubilise Talc, Dolomite and Magnesium trisilicate *in vitro* and when added to the soil indicate that this bacterium can attack other silicate mineral in soil and liberate Mg and Ca. Although solubilisation was observed due to the mere water addition, inoculation of the bacterium enhanced the solubilisation. This bacterium species solubilised insoluble Zinc oxide *in vitro* indicating its capability to solubilise Zinc. This showed that it can solubilize zinc from the native total zinc of the soil that may be absorbed by plants. Senthilkumar [10] isolated a *Bacillus* sp from spheralite zinc ore that solubilised Zinc and studied its beneficial effect on rice. This showed that the *Bacillus* sp can concurrently release Mg, Ca, Si and Zn from soil silicates containing these ions. It is imperative that silicate solubilising bacteria can liberate Potassium from Potassium bearing silicate minerals. Although no specific test was made for mobilisation of K by this silicate solubilizer, it is likely it is capable of mobilising K. Soil is

the abode for a variety of primary and secondary minerals containing silicon which binds the elements like Mg, Ca, Zn and K [11]. When once silica is solubilised it is likely that the nutrients contained in them might be available for plant absorption.

There is already a band of biofertilizer for each of the nutrient like Nitrogen, Phosphorus, Potash etc. but so far there is no specific biofertilizer for releasing Mg in soil. This is perhaps the beginning for identifying effective bacterial isolate capable of solubilising the secondary nutrient Magnesium with concurrent release of Ca, Zn and Silica.

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