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Natural efficiency of *Bacillus mucilaginosus* on the solubilization of silicates

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Abstract
Silicon a vital compound in the plant life which support the plants to have rigid structure and helps the plant to overcome stress, in addition silicon induce systemic resistance which helps the plants to overcome variety of diseases. In present research about 20 isolates of silicon solubilizing organisms were isolated from 20 different locations of Cuddalore district, they were purified and studied for silicon solubilization. Among the isolates, the isolate SSB-17 was found to be a superior isolate and recorded much significant values in the solubilization of silicon compared with other isolates. The best isolate showed much significant efficiency in the degradation of organic siliceous materials in the form of recording clear zone. Further research needed to exploit the potentiality of *Bacillus mucilaginosus* on solubilization silicon compound and degradation of silicon materials in the open environment.

Keywords: Silicon, *Bacillus mucilaginosus,* (SSB), Rice, *Pyricularia oryzae*

Introduction

Silicon a known vital mineral present in abundant amount to the extent of 28% in the biosphere of earth crust. In majority of cases silicon associated with lignin and carbohydrate compounds of plant cell wall. Soil consists of sand particles, silt and clay particles in soil all these soil portions known to contains primary and secondary silicate minerals. Weathering, disintegration and biological breakdown of silicate compounds can liberate silicon from soluble form that can be absorbed by the plants. Rice a well studied siliceous plant that can accumulate more silicon. So that silicon fertilization is highly essential for rice crop. Silicon nutrition promotes the plant growth and yield by increasing the tillerings, leaf area index, increased photosynthetic activity, phosphorous, calcium and magnesium utilization, water economy of the plants (Vandervorm, 1980) [12], disease resistance (Winslow, 1992) [13] and reducing the mineral toxicity.

Bacillus mucilaginosus, a silicate solubilizing bacterium, and also common soil bacteria and a model microorganism in research on silicate mineral weathering (Basak and Biswas, 2009; Malinovskaya *et al*., 1990) [2, 8] and the extensive studies on the bacterium were mainly focused on the silicon releasing from soil minerals and the application of the same as plant growth promoting rhizo bacteria (PGPR) and seems to be a promising approach in increasing the rice productivity growing under lowland condition. *B. mucilaginosus* cells produce biologically active compounds that can stimulate plant growth. Bio-inoculation of *B. mucilaginosus* and yield increase of wheat, corn, barley, potato, sugar beet, tomato, cabbage, grape, pea and sunflower has been frequently reported and demonstrated considerable increases in their crop yields.

1. *Bacillus mucilaginosus* is the predominant microbial genera that are frequently encounted in the rhizosphere of lowland rice crop and involved in microbial dissolution of silicon.
2. Based on the above view the present research was undertaken to exploit the potentiality of *Bacillus mucilaginosus* on the release of silicon from its compounds.

Materials and Methods

Isolation of SSB Isolates from the Rhizosphere Soil of Rice

Ten gram of air dried rhizosphere soil sample of rice collected from each location, was transferred to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on rotary shaker (100 rpm) for 30 min. at ambient temperature. The well mixed suspension of each soil sample was subjected to serial dilution ranging from 10^{-1} to 10^{-7} dilution. One ml of the suspension from dilution 10^{-6} to 10^{-7}, was aseptically transferred to sterile petriplates and 15-20 ml of SSB medium was poured, rotated in clockwise and anticlockwise direction for uniform distribution and incubated at 28 ± 2°C for 48 hr. At the end of incubation period, SSB colonies which appeared with clear zone were transferred to slants of SSB medium and maintained at 4°C for further study.

i) Medium for silicate solubilizing bacteria (Bunt and Rovira, 1955) [4]
Peptone - 1.00 g
Yeast extract - 1.00 g
Glucose - 20.00 g
Ammonium sulphate - 0.50 g
Dipotassium hydrogen phosphate - 0.40 g
Magnesium chloride - 0.l 0 g
Soil extract - 250 ml
Magnesium trisilicate - 2.5 g
Distilled water - 750 ml
Agar - 20.0 g
pH - 6.6-7.0

Purification of SSB isolates
All the SSB isolates of were purified by streak plate method by using tryptic soy agar medium, frequently.

Enumeration of SSB Population from the Rhizosphere of Rice
The adhered soil of rice roots, collected from five rice plants of particular location, were pooled and one g of soil sample was transferred to 9 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min. at ambient temperature. The well mixed suspension was then diluted appropriately upto 10^{-6} dilution. One ml of suspension from 10^{-5} and 10^{-6} dilution was aseptically transferred to sterile petriplate and 15-20 ml of selective silicate solubilizing bacterial medium was added. The plate was rotated in a clockwise and anticlockwise direction for uniform distribution and incubated at 28±2°C for 48 hr. Three replications were maintained for each dilution. After the incubation period, the colonies which appeared with clear zones on SSB medium were counted. The total number of colonies in the original samples was expressed as CFU g^{-1} of oven dry soil.

Determination of the SSB Isolates Silicate Solubilizing ability in Vitro
Plate assay
All the twenty (SSB) bacterial isolates were inoculated on silicate solubilizing bacterial agar medium containing 0.25% insoluble $MgSiO_3$. After the incubation period (36-48 hr) at room temperature, the isolates exhibiting prominent clearing zone around the colonies were selected and the area of clearing zone was measured using slide and calculated and recorded.

Broth assay
The bacterial isolates were screened for the silicate solubilizing efficiency further in basal medium containing 0.25% $MgSiO_3$ as described elsewhere in the text. One ml of respective isolate was inoculated into 50ml of the medium in a 100 ml Erlenmeyer flask and incubated at room temperature (30±1˚C) at 5, 10 and 15 days interval, the content were filtered and centrifuged to remove the cell and debris. The clear supernatant was analyzed for silica content as per the procedure described by Saxena (1989) [11].

Composition of Basal medium
Glucose - 10 g
Ammonium sulphate - 1 g
Potassium chloride - 0.2 g
Di-potassium hydrogen phosphate - 0.1g
Magnesium sulphate - 0.2 g
Distilled water - 1000 ml
pH - 7.0

Result and Discussion
Occurrence and Community Population of Silicate Solubilizing Bacteria from the Rhizosphere of Lowland Rice, Grown at Cuddalore District, Tamil Nadu
The occurrence of silicate solubilizing bacterial (*Bacillus mucilaginosus*) population from the rhizosphere IR 50 rice grown twenty different locations were estimated during the Kharif season (June-September, 2010) and the results were presented in Table 1.
Among the twenty different locations, soil samples were collected from Perampattu recorded a maximum of 1.20 per cent as community population of silicate solubilizing bacteria from the rhizosphere soil sample of IR 50 whereas the location, namely, Muttam recorded the least population 0.41% as community population of SSB from the rhizosphere soil sample of IR 50 and the other places recorded intermediate values in the community population of silicate solubilizing bacteria.

Table 1: Physio-chemical Properties of soil sample collected from twenty locations of Cuddalore District, Tamil Nadu, India

Location	Soil Type	Organic matter Content (g/kg)	pH	EC (dsm^{-1})
Annamalainagar	Loamy	S	6.8	0.8
Sivapuri	Clay loamy	0.61	6.9	0.4
Varagurpettai	Loamy	0.74	7.1	0.1
Jayankondapattinam	Clay loamy	0.68	7.2	0.9
Perampattu	Loamy	0.64	6.9	0.5
Kanakarapattu	Loamy	0.34	6.5	0.7
Uthamasolamangalam	Clay loamy	0.74	7.2	0.3
Kodipallam	Loamy	0.46	7.4	0.5
Killai	Clay loamy	0.72	7.5	1.1
Pichavaram	Loamy	0.35	6.8	0.9
Neyvasal	Loamy	0.73	6.2	0.8
Nedumbur	Loamy	0.69	7.5	0.5
Melavanniur	Clay loamy	0.64	7.7	0.9
Parivilagam	Loamy	0.58	7.3	1.2
Themmur	Loamy	0.34	7.9	0.8
Meiyathur	Loamy	0.68	7.8	0.7
Neduncheri	Clay loamy	0.52	7.2	0.8
Vilagam	Loamy	0.78	7.5	0.3
Muttam	Loamy	0.64	6.2	0.6
Pannapattu	Clay loamy	0.43	6.9	0.5

EC- Electrical Conductivity **dSm^{-1}** – deci siemens per meter

The survey for the occurrence of silicate solubilizing bacteria from rhizosphere of rice during Kharif season revealed the ubiquitous occurrence of silicate solubilizing bacteria in the rhizosphere of IR 50 rice grown at all the twenty different locations of Cuddalore district, Tamil Nadu, India.
In the present study on twenty isolates of silicate solubilizing bacteria (SSB-1 to SSB-20) were isolated from the

rhizosphere of lowland rice grown at 20 different locations of Cuddalore district, Tamil Nadu, India and the isolates were identified based upon the morphological and physiological characteristics as reported by Gerherdt (1981) and Awad Galal Osman (2009) [6, 1]. On the basis of the above parameters, it was found that all the SSB isolates were belonging to *Bacillus mucilaginosus*. The silicate solubilizing ability of *Bacillus mucilaginosus* has already has been reported (Berthelin and Belgy, 1979; Malinovskaya *et al*., 1990; Welch *et al*., 1999 [3, 8].

The occurrence of efficient silicate solubilizing bacteria that might solubilize silicate could pave the way for the release of other nutrients in soil (Murali kannan, 1996; Archana, 2007).

In the present investigation, the occurrence of silicate solubilizing bacteria, from the rice rhizosphere of lowland rice grown at Cuddalore district, Tamilnadu, India, was surveyed at 20 different locations where rice is grown as monocrop and under lowland condition. Twenty SSB cultures were isolated from all the selected locations, their silicate solubilizing ability was studied under *in vitro* condition and all the isolates were characterized. Based on the performance, the most efficient SSB isolate was selected and use the same for silicon nutrition.

Grading the SSB isolates based on silicate solubilization

All the twenty isolates recorded appreciable value in silicate solubilization. Among the twenty isolates, about three isolates namely SSB-8, SSB-11 and SSB-17 were more efficient in silicate solubilization and recorded clear zone about 15 mm and thirteen SSB isolates were showed medium silicate solubilizing efficiency are recorded 10 to 14.99 mm clear zones and remaining four isolates are recorded below 10 mm clear zones the values were presented in Table 2.

Table 2: Interstrain difference of SSB isolates for the degradation of organic siliceous rice residues under *in vitro* condition (plate assay)

Isolate	Clearing zone**			Statistics*
	Straw	Husk	Black ash	
SSB-8	6.9	5.3	4.9	A
SSB-11	6.3	4.9	4.6	C
SSB-17	6.5	5.0	4.5	B

* Values followed by different letters are significantly differed at 5% level according to student 't' test.
** in mm dia

In the present investigation, all the twenty isolates were screened for their silicate solubilizing ability and antagonistic activity against *Pyricularia oryzae* under *in vitro* condition. The present results revealed that a marked variation existed among SSB isolates on silicate solubilization and antagonistic acitivty against *Pyricularia oryzae*. Exopolysaccharides (EPS) productions of the SSB isolates and EPS mediated silicate solubilization through organic acid have been proposed by Malinovskaya *et al*. (1990) [8] and Welch *et al*. (1999). Epstein (1999), the results of the present study clearly revealed the silicate solubilizing ability of the isolates and the microbially solubilized silicon might elicit the biochemical defense reaction against *Pyricularia oryzae* under *in vitro* condition.

Breakdown of Organic Siliceous Materials by Silicate Solubilizing Bacteria (*Bacillus mucilaginosus*) under *in vitro* condition

Plate assay

The efficiency of three different best isolates of silicate solubilizing bacteria to degrade organic siliceous materials was determined under *in vitro* condition by the area of clearing zone in agar media containing silica source. The results are summarized. The results of present study clearly revealed the efficiency of the isolates to degrade the organic siliceous material but with a variation in their efficiency. Larger clearing zones were observed by SSB-11 and the lowest clearing zone were observed by SSB-8 respectively.

Broth assay

The results on the solubilization of silica from the organic siliceous materials are presented in Table 3. The results of the present study clearly revealed the efficiency of the isolates to degrade the organic material with a slight variation in their efficiency. Among the best three isolates, the isolate SSB-17 was found to be a best one in the degradation of organic siliceous residues.

Table 3: Interstrain difference of SSB isolates for the degradation of organic siliceous rice residues under *in vitro* condition (broth assay)

Isolate	Silica content*								
	Straw			Husk			Black ash		
	5 DAI	10 DAI	15 DAI	5 DAI	10 DAI	15 DAI	5 DAI	10 DAI	15 DAI
SSB-17	30.41	35.83	45.90	26.91	28.70	30.80	23.25	25.51	26.71
SSB-11	26.00	29.80	35.90	23.15	26.29	27.10	23.00	23.70	24.90
SSB-8	28.29	30.80	43.20	26.80	27.79	29.91	23.20	25.12	25.00

DAI –Days after inoculation
***** - mg/L

The recycling of crop residues to replenish the soil with nutrients that has been removed by the biomass and to preserve soil health is of great importance in sustainable agriculture. Rice straw, rice husk and black ash are the important rice residues available in Cuddalore district, Tamil Nadu where rice is grown under monoculture and in lowland condition. These rice residues contain 11 per cent, 14.5 per cent and 65 per cent of silicon, respectively, in the above mentioned crop residues. The recycling of these organic siliceous, rice residues, as a source of silica, will be a low cost technology for effective silicon nutrition and blast disease management in lowland rice.

Rice straw, rice husk and black ash are the important organic siliceous rice residues abundantly available in Cuddalore district where rice is known as mono crop and under lowland condition. Microorganisms play a vital role in solubilization of silicates in nature mainly through the production of different types of organic acids. The primary and secondary silicate minerals in rocks can be attacked through products of microbial metabolism (Muntz, 1980) [9]. Silicate solubilization by micro organisms in rice ecosystem become an important disease resistant as well as for the resistance against insects attacks. Silica also influencing the nutrition of rice. The positive role of *Bacillus*, as silicate solubilizing bacterium, in rice ecosystem has been reported by many workers (Norkina and Pumpyanskaya, 1956; Duff and Webley, 1959; Webly *et al.*, 1960) [10, 5]. *Bacillus mucilaginosus*, as a member of *Bacillus* play a significant role on enhancing the silicon nutrition, plant growth promotion and biocontrol of *Pyricularia oryzae* in rice crop (Datnoff *et al*., 1991). Silicate solubilizing organisms secrete plant growth promoting substances like IAA and GA in the rhizosphere and promote the growth and yield of rice. Hence, the development of this organism, as agricultural bio-inoculant needs to be exploited in detail for the maximization of growth and yield in rice (Lian Bin *et al*., 2002) [7].

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