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Research Article

Silica Favours Bacterial Growth Similar to Carbon

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Abstract

Silicate solubilising bacteria were isolated from soil, river water, pond sediment and talc mineral. The isolates were characterised and found to belong to the genera *Bacillus* and *Pseudomonas* sp. Some of the isolates are also identified by 16S r RNA sequencing. Both *Bacillus* and *Pseudomonas* isolates solubilised magnesium trisilicate under in vitro conditions either in the presence or in the absence of glucose. *Bacillus megaterium* isolated from soil solubilised talc, feldspar and magnesium trisilicate by releasing silica in solution. This also exhibited growth exclusively on silicate in the absence of a carbon source and after removal CO_2 in the head space of the flask containing medium. *Bacillus mucilaginosus*, a silicate solubilising species, exhibited growth on acid washed sand (pure quartz) and also in silicic acid in the absence of carbon source revealing the capability of the bacteria to utilise silica or silicate for its growth. The carbon analysis by SEM with EDAX revealed the presence of carbon in cells grown exclusively on silica suggesting the biological transmutation of silica to carbon. The ability of silicate solubilising bacteria to grow exclusively on silica or silicate in the absence of carbon reveals not only their ecological survival in a carbon-free environment in earth but also their likely survival in other celestial bodies..
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1. Introduction

Silicate solubilising bacteria (SSB) are of recent interest as they have been found to solubilise potassium and silica in the soil silicate minerals [1,2]. Silicon is accepted as agronomically a beneficial element for crop plants, which absorbs silicon in the form of *ortho* silicic acid only which is very low (0.1–0.6 mM) in soil solution [3]. It is surprising to see a larger accumulation of silicon in the form of silica in plants when its availability is very low in soil as the entire soil is made up of polymerized silicates that are insoluble in water. Hence the SSB is advocated to solubilise silica in situ in the soil. In an earlier study, SSB was found to grow on sand and in silica in the absence of a carbon source [4]. Therefore, the growth and silicate solubilisation potential of selective isolates were tested on certain silicate minerals

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Table 1. Solubilisation of silica (SiO_2 mg l^{-1}) from magnesium trisilicate by bacterial isolates in the presence and absence of carbon source*.

SSB isolates	Silicate medium without carbon DAI				Silicate medium with carbon DAI			
	0	5	10	15	0	5	10	15
Control	0.8	1.5	1.5	1.5	0.8	1.0	1.0	1.0
Bacillus flexus	0.8	5.1	3.5	0.3	0.8	4.0	1.1	1.4
Bacillus sp	0.8	3.5	7.9	8.2	0.8	4.0	1.6	0.2
Bacillus sp	0.8	6.6	8.7	7.9	0.8	6.9	1.2	1.7
Bacillus mucilaginosus	0.8	5.7	7.2	11.2	0.8	4.0	5.2	8.7
Pseudomonas sp	0.8	6.0	8.2	9.9	0.8	6.0	7.4	8.0

DAI–Days after inoculation; glucose @ 0.1%*.

either in the presence or in the absence of a carbon source. Since the dissolution was observed even in the absence of carbon, perhaps the CO_2 trapped in the head space air in the flask might have favoured growth. A detailed experiment is undertaken after eliminating CO_2, and the results are reported.

2. Materials and Methods

Basal medium (glucose 1.0 g; $(NH_4)_2$ SO_4 1.0 g; KCl 0.2 g; K_2HPO_4 0.1 g; $MgSO_4{\cdot}7H_2O$ 0.2 g; Distilled water 1l; pH 7.0) was prepared and dispensed in 100 ml quantities and insoluble magnesium trisilicate was added at 0.25% concentration. The flasks were sterilized, inoculated with a loopful of silicate solubilising isolates of *Bacillus* and *Pseudomonas*. A similar set of flasks containing basal medium devoid of glucose was similarly inoculated and the flasks were incubated at room temperature (30±2°C) for 2 weeks. At periodical intervals, the culture filtrate was centrifuged to remove the cells and debris and the dissolved silica content was determined in the supernatant as followed in the method of Imaizumi and Yoshida [5]. The ability of *Bacillus megaterium,* another silicate solubilising bacteria to solubilise different silicates is also tested in basal medium supplemented with talc, feldspar and magnesium trisilicate separately at 0.25% level. Since this bacterium solubilised all silicates and dissolution was investigated in magnesium trisilicate with 0.1% glucose and without glucose wherein the CO_2 was excluded by drawing air into the flask aseptically bubbled through alkali. The trace of CO_2 was trapped by suspending vials containing sterile alkali inside the flasks. In order to ascertain the utilization of pure silica by *Bacillus mucilaginosus,* the acid washed sand free from organic matter, were pulverised and sieved through BSS 44 and the precipitated silicic acid (Loba chemicals) were added separately at 0.25% level in the basal medium with and without glucose. The flasks were inoculated with a suspension of 0.1 ml *Bacillus mucilaginosus* (ca 300×10^8 cells/ml). The cell growth was found to be in the carbon containing silicate medium reduced after 7 days due to a low pH level. The cell grown exclusively in silica was subjected to SEM with EDAX (F E I Quanta FEG 200 - High Resolution Scanning Electron Microscope) to detect the carbon content.

Table 2. In vitro dissolution of silica (SiO_2 mg l^{-1}) from different silicate minerals by *Bacillus megatherium* in the presence and absence of carbon source.

Treatment	Silicate medium without carbon DAI			Silicate medium with carbon DAI		
	0	8	16	0	8	16
Control	1.3	1.3	1.3	1.2	1.2	1.2
Talc	2.4	1.9	3.0	3.0	1.3	3.6
Feldspar	5.1	1.9	6.3	4.1	3.8	5.8
Magnesium trisilicate	5.9	5.1	0.4	6.6	6.2	7.5

DAI–Days after inoculation; glucose @ 0.1%.

Table 3. Growth of *Bacillus megaterium* in silica medium in the presence and absence of CO_2.

Treatments	0 DAI		8 DAI		16 DAI	
	SiO_2 (mg l^{-1})	Bacterial count $\times 10^6$	SiO_2 (mg l^{-1})	Bacterial count $\times 10^6$	SiO_2 (mg l^{-1})	Bacterial count $\times 10^6$
Silicic acid alone	2.3	14	3.2	5	3.4	49
Silicic acid + glucose 0.1%	2.3	14	2.8	4	3.7	67
Silicic acid-air devoid of CO_2	2.3	15	1.8	4	0.8	21
Silicic acid + glucose 0.1% +air	2.3	14	2.1	14	1.5	29

DAI–Days after inoculation; carbon as glucose @ 0.1%.

3. Results and Discussion

Dissolution of silica was observed in silicate containing medium and also in silicate containing glucose medium. The silicate solubilising bacterial isolates from different sources from talc, pond sediment, sugarcane field soil and river water belonging to the genera *Bacillus* and *Pseudomonas* solubilised magnesium trisilicate in vitro. Some of the isolates like talc, sugarcane field soil were characterised by 16S r RNA sequencing are found to be *B.flexus*, *B. megaterium*, *B. mucilaginosus*. *Bacillus* sp isolated from river water and pond sediment of solubilised more silica in carbon-free silicate medium than in glucose containing medium. It is likely that these organisms can thrive in river water and pond sediment with very low nutrient content which might have an inherent higher solubilisation potential than those isolated from other sources. The *Bacillus* sp isolated from talc and the field soil effectively solubilised silicate in the presence of glucose. The solubilisation by *Pseudomonas* sp was nearly the same with regard to the absence and presence of glucose (Table 1).

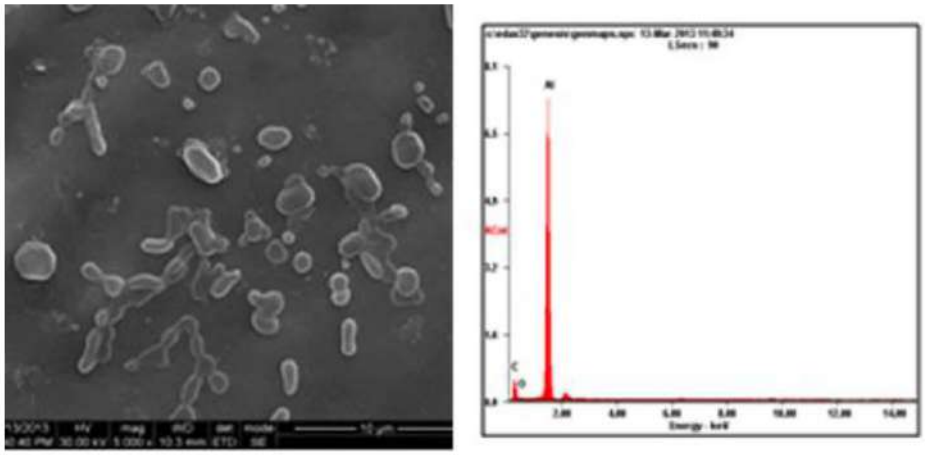
The *Bacillus megaterium* solubilised different silicate minerals like talc and feldspar (Table 2). However, solubilisation was relatively higher in magnesium trisilicate. A reduction in dissolved silica was observed in the initial stages of incubation. It is likely that this might be due to utilization of silica by the bacterium for its growth.

Bacillus mucilaginous was found to grow exclusively on silicate and in the absence of carbon source. Bacterial multiplication was observed in flasks, where the CO_2 was excluded (Table 3). This bacterium grows well in acid washed sand and silicic acid in the presence and absence of glucose. It is of interest to note that multiplication continued in silica was unabated, whereas in combination with glucose, the growth was arrested after initial multiplication (Table 4). The growth of bacteria on silicic acid and acid washed sand indicates their capability to utilize silica for its cellular build up (Table 5). All of these have conclusively shown that silica can serve as a substrate for the growth of bacteria. This has an environmental significance as the bacteria can survive on soil silicates even when utilizable carbon is absent. The retardation of growth in media containing both silica and glucose on prolonged incubation suggests that the acids formed might inhibit the growth of bacteria. Since organic acids were implicated in silicate dissolution [6] their accumulation might have limited its growth. Carbon and silicon are similar in structure and function and silicon is considered as an analogue of carbon. Both share several properties in common and the dioxides of both carbon and

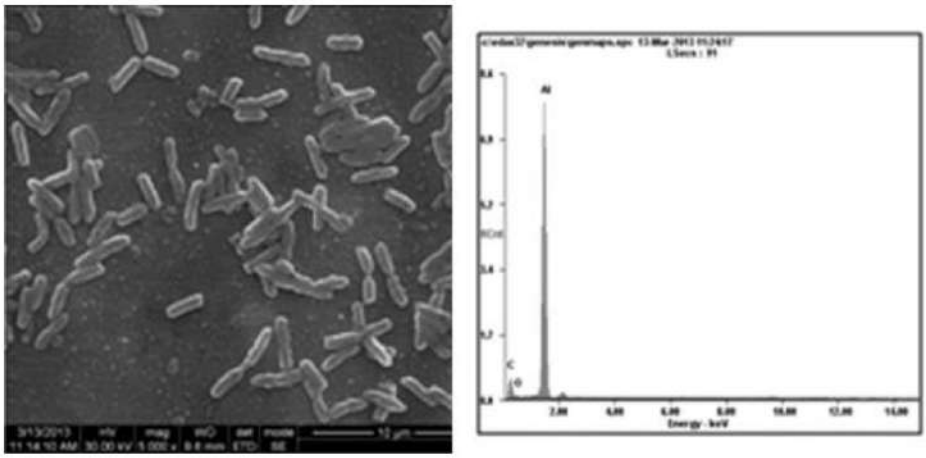
Table 4. Growth of *Bacillus mucilaginosus* on pure sand and silicic acid with and without carbon source.

0 DAI		8 DAI		16 DAI	
	SiO_2 (mg l^{-1})	Bacterial count $\times 10^5$	SiO_2 (mg l^{-1})	Bacterial count $\times 10^7$	SiO_2 (mg l^{-1})	Bacterial count $\times 10^7$
Silicic acid alone	2.4	296	9.1	350	9.9	350
Silicic acid + glucose 0.1%	2.4	294	10.2	*	9.6	*
Sand alone	0.9	295	4.4	438	2.8	438
Sand+ glucose 0.1%	0.9	295	4.4	*	2.8	*

DAI–Days after inoculation.
*No viable cells.



(a) Sand alone



(b) Glucose alone

Figure 1. Bacteria grow under the presence and absence of carbon source the image shown with SEM with EDAX analysis (sand grown cells are slightly different in their morphology).

silicon are available in large quantities on this planet. But carbon alone forms the backbone of all organic forms. The utilisation of silicon for growth is observed in this study even though a slight morphological variation was observed in bacterial cells. The SEM with EDAX analysis also revealed the presence of carbon in bacterial cells which were grown in silica containing medium (Fig. 1). Although carbon compounds are abundant in living organisms and it is the basis for life, silicon compounds might have played a role in the development of primitive forms of life when earth was inhospitable for the development of carbon based life, as carbon compounds appeared very late.

Many living organisms are known to accumulate silicon since bacteria contain about 180 mg Si/kg of dry matter [7]. Silicon was found to exert a growth accerlating effect on bacteria [8]. Das and Chattopadyay [9]reported that

Table 5. Growth of *Bacillus mucilaginosus* on pure pulverized quartz in the presence and absence of any carbon source.

Treatments	0 DAI			3 DAI			7 DAI			10 DAI		
	pH	SiO_2	Bacterial count $\times 10^5$	pH	SiO_2	Bacterial count $\times 10^{16}$	pH	SiO_2	Bacterial count $\times 10^{20}$	pH	SiO_2	Bacterial count $\times 10^{20}$
Quartz	7.2	0.9	228	7.0	1.2	278	7.0	1.3	1256	7.0	0.69	206
Quartz+glucose 0.1%	7.2	0.8	227	3.0	0.6	572	3.0	0.6	43	3.0	0.41	*
Quartz+glucose 0.2%	7.2	0.8	227	3.0	1.9	203	3.0	0.6	30	3.0	0.60	*

DAI–Days after inoculation; SiO_2 expressed in mg l^{-1}.
* No viable cells.

Mycobacterium tuberculosis is capable of utilizing silicate facultatively in the absence of carbon. The formation of silicic acid in esterified cell wall of bacteria was also reported [9]. It is also likely that silicon might have been converted to carbon by biological transmutation or by nucleido – biological reactions which is common in life forms. The transmutation of potassium in chickens to meet their calcium requirement for egg shells by pecking up micas containing potassium is well known. The existence of biological transmutations is also well established [10,11]. The present study shows that gram positive *Bacillus* and gram negative *Pseudomonas* utilize silicate exclusively which is an immense significance in their ecological survival, where carbon compounds are lacking in the natural environment. In the search for extraterrestrial life on other planets also the phenomenon of utilising silicon *in lieu* of carbon becomes significant as the bacteria can occur in a carbon-free environment.

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