Silicon (2018) 10:267–275
DOI 10.1007/s12633-016-9438-4

ORIGINAL PAPER

Silica Solubilization Potential of Certain Bacterial Species in the Presence of Different Silicate Minerals

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Received: 2 August 2015 / Accepted: 14 April 2016 / Published online: 13 August 2016

Abstract Silicate solubilisng bacteria (SSB) are distributed in soil, water, aquatic sediments and in silicate minerals but their population is smaller than the total bacteria indicating their uniqueness. Pond sediment showed a higher SSB with 40×10^4 g^{-1} while sugarcane field soil recorded 31×10^4 g^{-1} dry weight. SSB population in silicate minerals varied with the highest in phyto-sil followed by muscovite but very low in quartz and illite. The silicate solubilisation potential of the six isolates tested on eight minerals varied with the isolates and mineral. Magnesium trisilicate was more easily solubilised than, quartz, or muscovite. Four of the elite isolates were characterized by 16S r RNA sequencing and were found to be *Bacillus flexus*, *B. mucilaginosus*, *B. megaterium* and *Pseudomonas fluorescens*. The GC/MS analysis of organic acids produced in the medium containing feldspar and quartz by *Bacillus flexus* and *B. muicilaginosus* showed variation with the minerals. The release of silica in solution serves as a nutrient for life forms.

Keywords Silicate solubilising bacteria · Distribution · Dissolution potential · Organic acid production

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1 Introduction

Soil results from weathering of rocks and contains silica, silicates and aluminosilicates. Some microorganisms are geoactive and play a key role in weathering of rocks, mineral formation and in mineral biodegradation [1]. The attack on the siliceous materials in the rocks by microbial metabolites was demonstrated earlier [2]. The role of microorganisms in biologically controlled and biologically induced mineralization resulting in carbonate speleothems, moon milk, silicate speleothems and chalcedony crystals in caves has been reviewed recently [3]. The transformation of polymerised silica to monomeric form by bacteria is important in the biogeochemical cycles of silicates in nature [4]. Bacterial dissolution of silicates in soil gained importance not only because of its involvement in the silica cycle but also because of its role in the release of the plant nutrients like potassium, calcium and magnesium from the silicates. With the increase in cost of potassic fertilizers, silicate minerals and ashes rich in silica and potassium are used as fertilizers. Silicate solubilising bacteria (SSB) are also advocated as a biofertilizer to solubilise silicates. However, studies on silicate solubilising bacteria are limited [5–7]. In the present study the distribution of silicate solubilising bacteria associated with silicate minerals and soils, and their potential for solubilising silicates have been studied.

2 Materials and Methods

Silicate minerals including feldspar (64.59 % SiO_2, Savera minerals, Chennai, India), muscovite (45.21 % SiO_2,), illite (54.01 % SiO_2), calcium silicate (45.17 % SiO_2,) [Astra

chemicals, Chennai, India], quartz (99.71 % SiO_2, Nakoda minerals, Coimbatore, India), talc (54.97 % SiO_2, Fusan orgaincs, Chennai, India) and phyto-sil (78 % SiO_2, Hem Tech, Jodhpur, India) and soil samples from sugarcane field, red soil (Panruti, India), plantation soil (Thadiyankudisai, India) sea sand (Puducherry), pond sediment (Nellikuppam), sea water (Puducherry) were investigated. The moisture content of the samples was determined by oven drying at 105 °C for 24 h. The samples were serially diluted and 1ml aliquots of appropriate dilutions were pour plated on nutrient agar (Glucose 5.0 g; Beef extract 3.0 g; Peptone 5.0 g; NaCl 5.0 g; Agar 15.0 g; Distilled water 1000 ml; pH 7.0–7.2) and soil extract agar (Glucose 1.0 g; K_2HPO_4 0.5 g; Agar 15.0 g; Distilled water 900 ml; soil extract 100 ml prepared by steaming 1 kg of garden land soil in 1l of tap water at 15 lb psi for 30 min, cooled, pinch of $CaCO_3$ added and filtered through double filter paper; pH 7.0-7.2) supplemented with 0.25 % magnesium trisilicate to enumerate the total bacteria and silicate solubilising bacteria (SSB) respectively. After 4 days of incubation at 32 ± 1 °C the colonies were counted. On the soil extract agar, the colonies exhibiting a clearing zone around them due to silicate solubilisation were picked, purified and transferred to nutrient agar slants. About 13 SSB isolates selected were characterised by staining and biochemical tests. All these isolates were screened by plate and liquid assays for their silicate solubilising potential. However, the results of the six most effective cultures are reported hereunder. Four of them were characterised by 16S r RNA sequencing.

3 Determination of Silicate Solubilisation Potential

3.1 Plate Assay

The bacterial isolates were tested on the silicate minerals magnesium trisilicate, feldspar, calcium aluminosilicate, sodium aluminosilicate, talc, muscovite, illite and quartz. The samples were pulverised and sieved through 350 BSS to get particles of less than 45 microns. Each of the minerals was added separately at 0.25 % level in 100 ml of Bunt and Rovira medium taken in a 250 ml flask [8], sterilised and dispensed in sterile petriplates. The culture suspensions containing 6×10^6 ml l^{-1} were streaked by means of an inoculation loop on the respective agar media and incubated at 30±2 °C. After 4 days, the width of the growth was measured at the top, middle and bottom points by a scale and the mean was determined. The clearing zone was measured similarly from the margin of the bacterial growth to the edge of the zone on one side of the growth at three points as above and the mean was expressed in mm.

3.2 Liquid Assay

Basal medium (Glucose 10 g; NH_4SO_4 1.0 g; KCl_2 0.2 g; $MgSO_4$ 0.2 g; K_2HPO_4 0.1 g; Dis.H_2O 1000 ml; pH 7.0-7.2) was dispensed in polyacrylate bottles in 100 ml quantities. Each of the test minerals was then added in 0.25 g quantities to separate sets of these bottles, plugged with cotton, sterilised and inoculated with a loopful of 24 h old culture suspension (cells 6×10^6 ml l^{-1}). At 5 and 10 days of incubation, the culture from a given medium was collected and centrifuged at 10000 rpm for 5 min to remove the debris. The silica concentration in the clear supernatant was determined by the method of [9]. The pH of the culture filtrate was determined with a Elico digital pH meter model LI 120. Triplicates were maintained for each of the minerals. The data were subjected to statistical analysis by the Duncan Multiple Range Test (DMRT). The Analysis of Variance tool was applied for calculating means with significant difference and compared by using Multiple Regression Analysis in Microsoft Excel 2007, where the analyses were done in triplicate.

3.3 Identification by 16S r RNA sequencing

Four of the six isolates were identified by their 16S r RNA sequence. A fragment of 16S r RNA gene from the total genomic DNA was amplified by PCR using universal primer. A single band was observed when resolved on agarose gel. The PCR product was purified by using an EZ-10 spin column PCR product purification kit, BIOBASIC Inc. The 16S r RNA gene sequence was used to carry out BLAST with the nucleotide database of the NCBI gene bank.

3.4 Characterisation of organic acids

The filtrates from the cultures inoculated with *Bacillus mucilaginosus* (sugarcane field soil isolate) and *B. flexus* (talc isolate) grown in the presence of feldspar and quartz were analysed by a GC/MS Joel GC mate II series for identification of major organic acids. One μ l of sample was injected into the GC/MS system with HP 5 MS column and 0.25 mm in thickness and the carrier gas was helium with average velocity of 35 cm sec^{-1} and flow rate of 1 ml min^{-1}. The GC oven temperature was from 50 − 250 °C and the injector and detector temperature were set at 250 °C. The GC/MS data were analyzed qualitatively with the NIST software, which performed a completely computerized analysis of the mass-spectral data. The results obtained with the NIST software, as well as the identity of compounds not contained in the libraries, were checked by consecutively

Table 1 Distribution of total bacteria and silicate solubilising bacteria in soil, sediments and minerals

Samples	Moisture Content (%)	Total Bacteria* $\times 10^5$	SSB* $\times 10^4$	Proportion of SSB to total bacteria (%)
Sugarcane field soil	14.0	12.3^{e}	31.1^{c}	25.3
Plantation soil	25.0	68.9^{b}	10.9^{e}	1.6
Red soil	2.1	33.3^{c}	2.0^{j}	0.6
Pond sediment	24.0	5.5^{h}	40.7^{b}	74.0
Sea sediment	17.2	4.1^{i}	4.6^{i}	11.2
River sand	1.1	19.2^{d}	6.1^{g}	3.2
Sea water	–	0.8^{k}	1.9^{j}	23.7
Talc	5.0	89.2^{a}	5.1^{h}	0.6
Phyto-sil	8.0	10.5^{f}	57.0^{a}	54.3
Feldspar	5.0	0.3^{l}	0.3^{m}	10.0
Muscovite	3.2	2.0^{j}	16.9^{d}	84.5
Illite	4.3	0.8^{k}	1.0^{k}	12.5
Calcium silicate	5.0	9.0^{g}	10.0^{f}	11.1
Quartz	5.0	0.7^{k}	0.5^{l}	7.1

* On dry weight basis; Mean of three replicates

Values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$)

examining narrow regions of the chromatogram on the data system display.

4 Results

4.1 Silicate Solubilising Bacterial Population Density

The soil samples, silicate minerals, sediments and water harboured a higher number of total bacteria than SSB. The population of SSB was much smaller in numbers in these samples than the total bacteria. The total bacteria were higher in plantation soil followed by red soil, river sand and sugarcane soil while sea water and sea sediment and pond sediment contained far fewer. The highest population of total bacteria was recorded in plantation soil which is rich in organic matter. However the proportion of SSB was just 1.6 %. Red soil, although containing a large total bacterial population contained a small number of SSB, amounting to only 0.6 % of the total population. The sugarcane field soil from the same area showed a relatively smaller total bacterial population than red soil but with a higher concentration of SSB, amounting to 25.3 % of the total population. This might be due to successive cultivation of sugarcane which upon harvest adds a large quantity of sugarcane trash rich in silica season after season. The pond sediment showed more SSB than the sea sediment (Table 1). Among the silicate minerals, talc exhibited the highest total bacterial load of

Table 2 Growth and clearing zone by the SSB isolates in different minerals

Organism	A		B		C		D		E		F		G		H	
	G	Z	G	Z	G	Z	G	Z	G	Z	G	Z	G	Z	G	Z
B. flexus	15	18	6	-	16	1	4	2	8	-	28	-	4	1	4	1
Pseudomonas sp	10	5	4	11	19	1	6	8	2	12	8	-	2	-	4	1
Bacillus sp	18	6	5	-	12	-	6	1	4	1	6	-	5	1	10	1
B. megaterium	4	15	3	-	4	-	4	1	6	-	3	-	3	-	4	-
B. mucilaginosus	17	5	4	11	22	1	6	9	6	15	8	-	3	1	5	-
P. fluorescens	5	10	4	6	1.0	1	4	7	5	12	2	-	3	1	4	-

G- Growth; Z- Zone

A-Magnesium trisilicate; B- Potassium aluminosilicate, C-Talc; D- Calcium aluminosilicate; E-Sodium aluminosilicate; F- Quartz; G-Muscovite; H- Illite

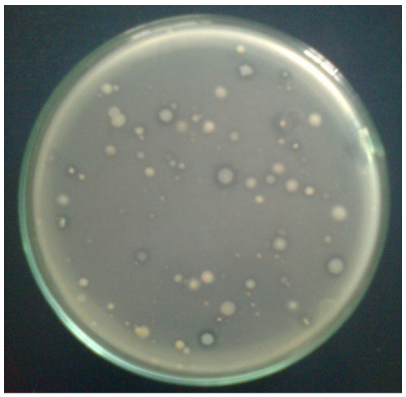
89.2×10^5 g^{-1} followed by phyto-sil 10.5×10^5 g^{-1}. On the other hand the SSB in these samples were 5.1×10^4 g^{-1} dry weight and 57×10^4 g^{-1} respectively. Feldspar, illite and quartz had a very low population of SSB.

Most of the SSB isolates belong to *Bacillus* and *Pseudomonas* genera. The isolate from sugarcane field soil was *Bacillus mucilaginosus*, the talc isolate was *B. flexus*, the paddy soil isolate was *Pseudomonas fluorescens,* the other paddy soil was *Pseudomonas* sp, that from river water was *Bacillus* sp and that from casuarina soil was *B. megaterium*.

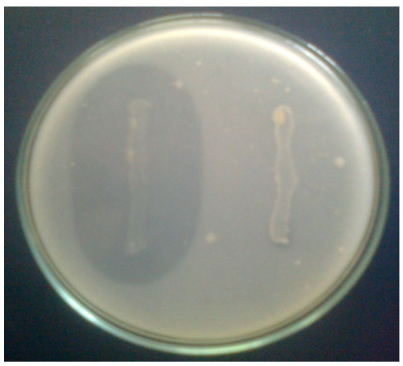
5 Solubilisation of Silicates

5.1 Plate Assay

All the six isolates showed a good growth and solubilisation of magnesium trisilicate. *B. flexus*, *B. megaterium* and *P. fluorescens* showed a larger clearing zone than others (Table 2). However, all isolates grew in agar medium containing feldspar (Potassium aluminosilicate) but only *B. mucilaginosus*,*P. fluorescens* and *Pseudomonas* sp exhibited a clearing zone around their growth. All organisms grew in the presence of the silicate but only *Bacillus* sp and *B. megaterium* formed very small clearing zones. On calcium aluminosilicate containing agar all isolates grew poorly and exhibited a low solubilising activity. Here again *B. mucilaginosus* and the two *Pseudomonas* strains exhibited greater solubilising activity. Similar results were observed in sodium aluminosilicate. On muscovite agar medium all organisms grew relatively poorly and only *B. flexus*, *Bacillus* sp, *B. mucilaginosus* and *P. fluorescens* formed small clearing zones. On illite agar all the organisms showed growth but only *B. flexus*, *Pseudomonas* sp and *Bacillus* sp showed a small zone indicating a poor solubilisation. On quartz, containing agar medium, none of the organisms formed zones although all of them showed growth. The appearance of growth and clearing zones around streaks of *B. mucilaginosus* on some minerals in agar media are shown in Fig. 1a and b. The results showed that all the isolates had silicate solubilising activity, but this activity varied on different minerals.



a Colonies exhibiting a halo of clearing zone in the silicate medium



b Solubilisation zone in plate assay

Fig. 1 **a** Colonies exhibiting a halo of clearing zone in the silicate medium. **b** Solubilisation zone in plate assay

5.2 Liquid Assay

Extensive silicate solubilisation was observed with magnesium trisilicate and calcium aluminosilicate whereas moderate solubilization was observed with talc and sodium aluminosilicate and low solubilisation with quartz and feldspar (Table 3). There was a difference in the levels of silica released at both the intervals sampled. Certain of the isolates that did not exhibit any clearing zone with the minerals in the plate assay also showed dissolution in the corresponding liquid media. None of the isolates exhibited any clearing zone in quartz in plate assay but all of them showed a very slight dissolution in liquid medium (Fig. 2).

5.3 Acidification and Alkalination by Cultures

The pH of the culture filtrates varied with the bacterial species, mineral and period of incubation. The pH of the culture was shifted to acidity in some isolates while still others to alkalinity. Further certain of the isolates exhibited acidity in one mineral and alkalinity in the other. The pH of *B. flexus* cultures rose only slightly during growth in the presence of all minerals except quartz, muscovite, illite and feldspar. In the presence of the latter minerals the pH dropped slightly, indicating marginal acidification. The other five isolates caused a drop in pH when growing in the presence of quartz, feldspar, muscovite and illite. Growth of *Bacillus* sp. caused a similar acidification in quartz, feldspar, muscovite and illite but in others showed initial acidification and a subsequent increase in pH. Growth of *B. megaterium* in the presence of calcium aluminosilicate,

Table 3 Dissolution of silica in liquid medium in different silicate minerals

Organism	A		B		C		D		E		F		G		H		
	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10	(DAI)
SiO_2 (mg/l)																	
Bacillus flexus	24.3^{c}	14.80^{b}	3.4^{e}	1.2^{e}	1.69^{d}	2.89^{c}	1.00^{f}	0.82^{f}	4.69^{d}	4.65^{f}	2.76^{b}	4.96^{a}	2.07^{d}	2.20^{d}	1.38^{d}	3.31^{b}	
Pseudomonas sp	27.0^{a}	13.40^{c}	4.0^{d}	2.4^{d}	1.51^{e}	3.45^{b}	4.14^{b}	8.28^{d}	3.82^{e}	4.84^{e}	2.48^{c}	3.45^{b}	2.48^{c}	1.93e	1.10e	3.03^{c}	
Bacillus sp	4.10^{f}	12.80^{d}	3.3^{e}	1.3^{e}	4.41^{a}	3.58^{b}	5.52^{a}	12.0^{a}	6.69^{b}	6.62^{b}	2.76^{b}	2.90^{c}	2.89^{b}	2.55^{c}	1.65^{c}	2.20^{e}	
B.megaterium	7.00^{e}	10.30^{e}	8.1^{a}	6.5	1.51^{e}	4.69^{a}	3.50^{c}	10.3^{c}	6.07^{c}	5.55^{d}	3.58^{a}	3.58	1.93^{d}	3.17^{ab}	2.07^{b}	3.58^{a}	
B.mucilaginosus	24.0^{d}	15.50^{a}	6.6^{b}	4.5^{b}	2.20^{c}	2.93^{c}	1.38^{e}	1.93^{e}	7.03^{a}	7.92^{a}	1.38^{d}	1.82^{d}	3.17^{a}	3.03^{b}	1.51^{cd}	1.51^{f}	
P.fluorescens	26.4^{b}	13.50^{c}	5.5^{c}	2.7^{c}	2.62^{b}	2.34^{d}	1.93^{d}	10.7^{b}	7.17^{a}	6.07^{c}	3.45^{a}	2.86^{c}	2.89^{b}	3.31^{a}	2.76^{a}	2.70^{d}	
Initial	2.20		1.2		0.82		0.55		4.83		2.0		2.07		1.50		
pH																	
Bacillus flexus	6.93	7.43	6.82	6.12	7.67	7.48	7.40	7.34	7.79\	7.23	6.34	6.39	6.74	6.36	6.12	6.02	
Pseudomonas sp	5.97	8.62	5.75	7.12	8.06	8.45	7.77	7.14	7.55	7.28	6.74	6.46	6.65	6.53	6.45	6.15	
Bacillus sp	6.45	8.46	5.73	6.15	7.67	8.53	6.49	7.08	6.74	7.14	5.12	4.84	4.57	4.72	4.46	4.84	
B.megaterium	8.34	7.29	3.96	6.10	7.38	8.54	6.46	6.20	7.62	7.17	5.16	4.73	4.58	4.63	6.15	6.40	
B.mucilaginosus	6.92	6.06	5.14	5.83	8.27	8.61	5.42	5.13	5.59	5.33	4.06	3.80	3.72	3.55	6.03	4.16	
P.fluorescens	8.56	8.86	5.57	5.67	8.01	8.26	5.70	5.40	7.17	6.07	4.19	3.49	4.12	4.50	4.72	4.08	
Initial	7.24		7.09		7.29		7.36		7.05		7.10		7.02		7.09		

DAI-Days After Inoculation; A-Magnesium trisilicate; B-Potassium aluminosilicate, C-Talc; D-Calcium aluminosilicate; E-Sodium aluminosilicate; F-Quartz; G-Muscovite; H-Illite

Values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$)

quartz, illite and muscovite caused acidification of the medium. However, in the presence of magnesium trisilicate, talc, and sodium aluminosilicate, its growth caused a rise in pH initially but then caused a decline. Growth of *B. mucilaginosus* in the presence of all the minerals except talc caused a drop in pH indicating acid production whereas in talc it raised the pH to alkalinity. Growth of *Pseudomonas* sp from causarina field soil in the presence of magnesium trisilicate and of Feldspar caused a sharp drop in pH initially followed a rise in pH into the alkaline range on the 10th day. In the presence of talc, calcium- and sodium aluminosilicate, growth of *Pseudomonas* sp. shifted the pH towards alkalinity but to acidity in quartz, muscovite and illite. Growth of *P. fluorescens* caused a rise in pH of the culture in the presence of magnesium trisilicate and talc and a drop in pH to acidity in the presence of the other minerals. The bacterial species differed in their reaction with the minerals.

5.4 Qualitative Analysis of Organic Acids

A variety of acids were detected by GC/MS in culture filtrates from *B. flexus* and *B. mucilaginosus* that had grown in the presence of feldspar and quartz (Table 4). *B. flexus* elaborated malic, citric, tartaric, gluconic and oxalic acids in quartz whereas in feldspar malic, citric, tartaric and hydroxyl propionic acids alone were produced in varying quantities. On the other hand *B. mucilaginosus* showed more than seven organic acids in feldspar whereas it produced only four acids in quartz. The predominant acids produced were acetic, hydroxypropionic, phthalic, gluconic, oleic, tartaric and citric acids in feldspar whereas acetic, gluconic, hydroxypropionic and tartaric acids were produced in quartz (Fig. 3).

6 Discussion

Silicates are broken down readily in the biosphere. Microorganisms play an important role in the dissolution of silicates and in the formation of clay minerals in soils and sediments [10]. Physico-chemical and biochemical mechanisms are involved in the silicate dissolution [11] but biochemical action by microbial activity is considered more important than others. Weathering of silicates has a major impact on the environment including formation of soil and sediments, release of nutrients for the microflora and plants, release of cations like Ca^{2+} and Mg^{2+} that sequester atmospheric CO_2 and in the geochemical cycling of silicon in nature. Soil is the habitat for a variety of bacteria that are either adsorbed or carried on soil silicates and where microbial metabolites have direct access to soil particles. Although soil supports

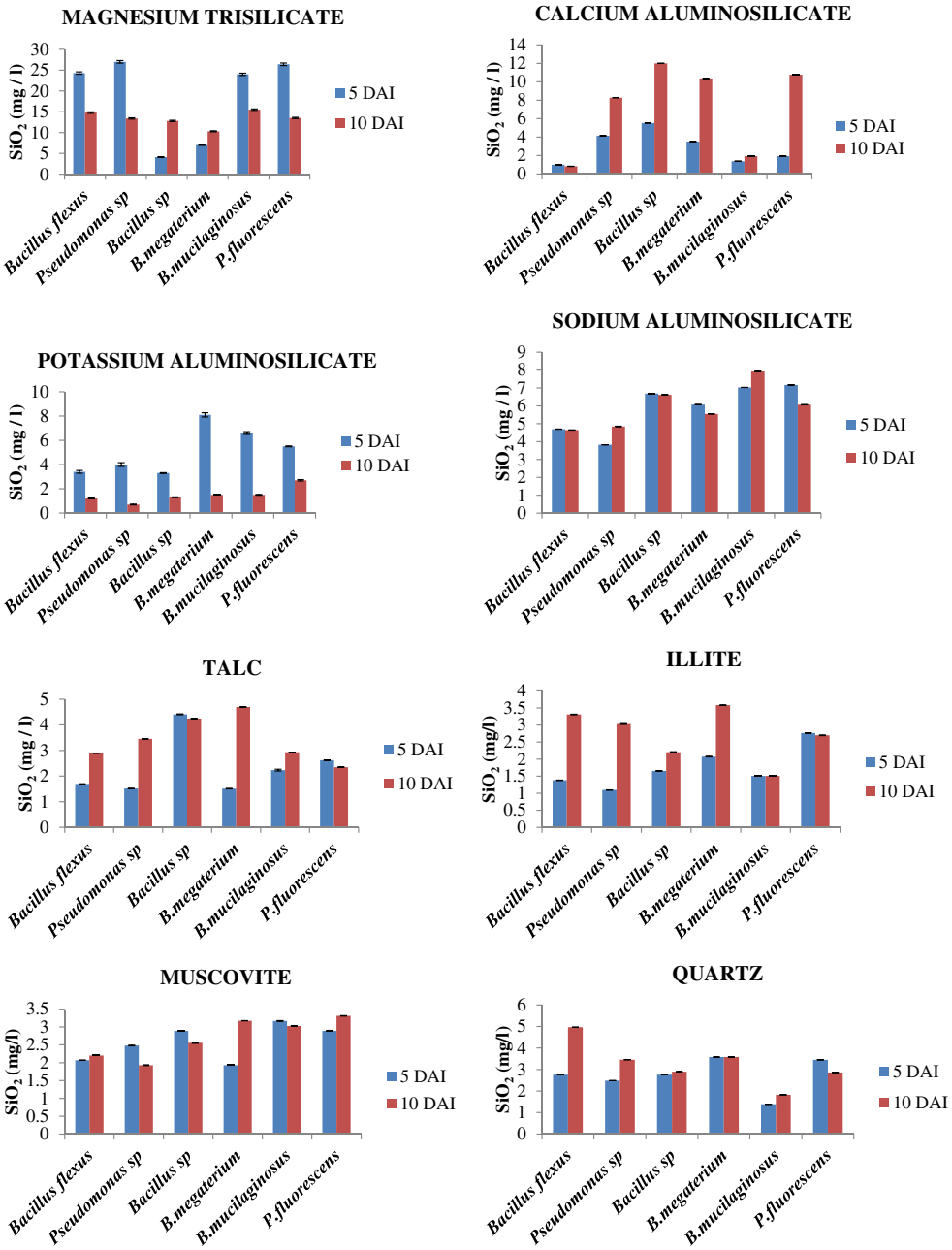


Fig. 2 Silicate solubilisation by different species in various silicate minerals

different kinds of bacteria, not all of them can dissolve silicates. If every dwelling organism is endowed with this ability the soil substratum may be liquefied over the years and lost to the sea. The observation that the total number of bacteria is greater in soil than the silicate dissolving bacteria clearly shows that not all bacteria have the ability to dissolve silicates but only a few indicating their uniqueness. A relatively large number of SSB found associated with phyto-sil, muscovite and calcium aluminosilicate suggests that these minerals harbour them from the natural deposits from which they were excavated. The proportion of SSB associated with a mineral is not related to its silica content as muscovite with 21 % silica carried a higher proportion of SSB followed by phyto-sil with 78 % silica compared to quartz with 98 % silica, talc with 54 %, and feldspar with 45 % silica. The results clearly show a wide difference between the total bacteria present in soil or silicate minerals and the SSB.

All the six bacterial species *viz Bacillus flexus*, *B. muicilaginosus*, *B. megaterium*, *Bacillus* sp, *Pseudomonas fluorescens* and *Pseudomonas* sp effectively solubilised magnesium trisilicate while their solubilisation potential was much less in the presence of illite, quartz and muscovite. These SSB also differed in the solubilisation

Table 4 Organic acids produced by *B. flexus* and *B. mucilaginosus* in the presence of feldspar and quartz

Organism	Silicate mineral	Organic acids produced
B. flexus	Feldspar	citric, malic, tartaric, hydroxypropionic acid
	Quartz	citric, malic, tartaric, gluconic, oxalic acid
B. mucilaginosus	Feldspar	citric, tartaric, hydroxypropionic, gluconic, acetic, phthalic, oleic acid
	Quartz	tartaric, hydroxypropionic, gluconic, acetic, hexadecanoic, heptadecanoic methyl ester

potential which varied with the type of mineral irrespective of the sources from which they were isolated. The potential of a single species also differed with different silicate minerals. In the natural environment a variety of silicate minerals coexist and the different SSB species may preferentially attack certain of them rapidly and others slowly but when all of them are acting in concert the solubilisation will be much more than that observed individually under *in vitro*.

Several mechanisms have been found to be involved in silicate dissolution. They include i) Hydration of respiratory CO_2 produced by soil microbes forming carbonic acid which acts on silicates as observed in orthoclase degradation to kaolinite [12]. It was also reported that CO_2 sequestration in basaltic acquifers and associated carbonate mineralization might maintain an environment suitable for silicate mineral dissolution [13]. ii) Microbially excreted metabolites like amino acids, phenolic compounds, organic and inorganic acids have metal complexing properties that may bind with Al and Fe of silicates rendering silicates soluble. In the soil environment oxidation of sulphur and reduced sulphide to sulphuric acid by *Thiobacillus* and oxidation of ammonia to nitrates by *Nitrosomonas* and conversion of

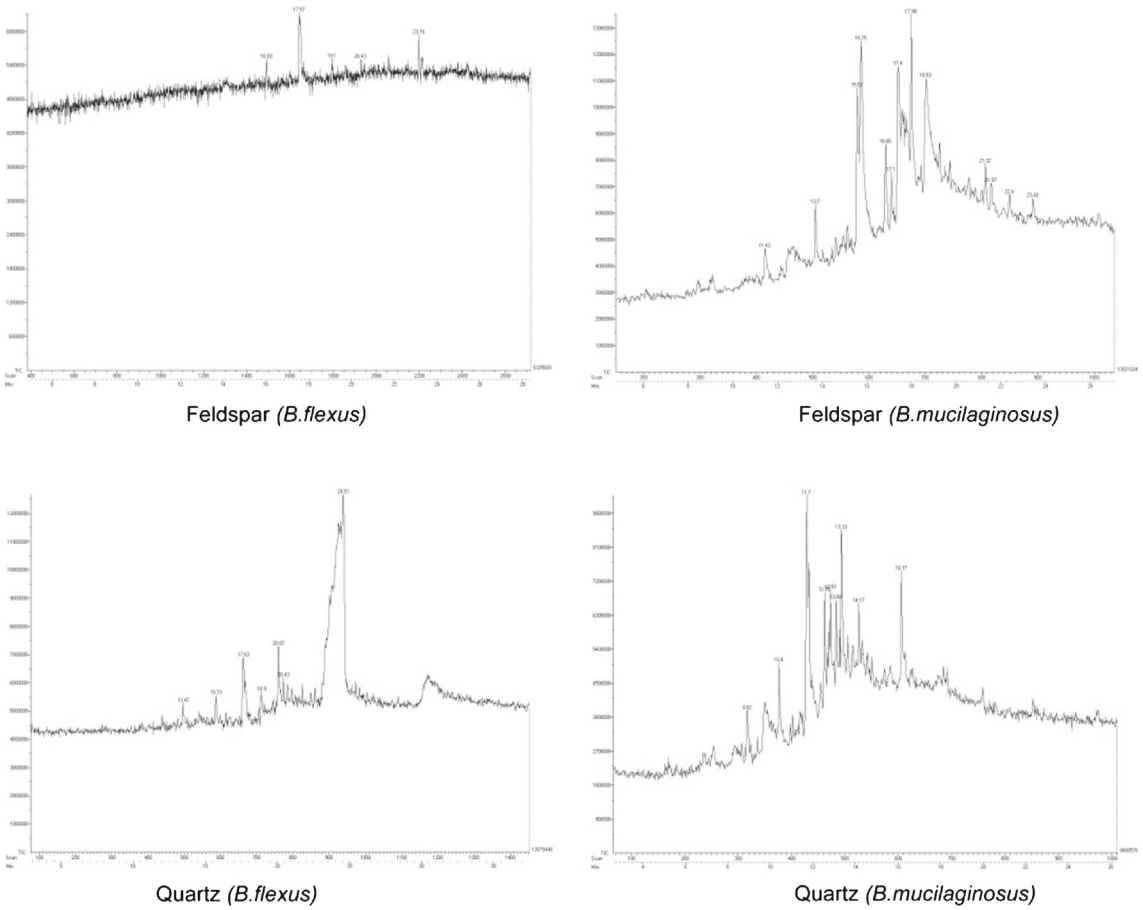


Fig. 3 GC MS chromatograms of the organic acids produced by SSB on feldspar and quartz

nitrate to nitric acid by *Nitrobacter* can generate inorganic acids that can act on silicates. The organic and inorganic acids cannot only acidify promoting silicate dissolution but also provide protons (H^+) for protonation for hydrolysis of silicates on one hand and on the other hand complexing with cationic components of silicates (chelation) as they are potential chelating agents [14]. Oxalic acid elaborated by bacteria can react with Al and Fe to form oxalate complex from minerals [15].The dissolution of silicates due to *keto*-gluconic acid production by bacteria which complexes and chelates with metals was also reported [16] iii) The formation of a surface complex by organic molecules and ligand promoted dissolution of silicate can also occur. iv) Alkali production was suggested as a possible mechanism [17]. In the present study also the pH of the culture was shifted to alkalinity by SSB. However this phenomenon differed with the organisms and minerals tested. It is also of interest to note that the pH of the culture was shifted to alkalinity by all the six species except *B. mucilaginosus* in magnesium trisilicate while by all of them to alkalinity in talc. At the same time all these organisms acidified the medium in quartz, muscovite and illite. Bacteria are known to elaborate ammonia and amines shifting the pH of the environment. In the soil ecosystem decomposition of organic matter and nitrogen fixation can form ammonia and amines creating alkalinity. These organic metabolites can solubilise silicates. v) The exopolysaccharides produced by microbes are implicated in weathering of rocks and breakdown of silicates due to their wetting and drying properties [18]. The biofilm formation in sewers and pipes can corrode not only pipes but also solubilise silicates in their microenvironment. Polysaccharides can bind silicates and render them soluble. *Bacillus* in general are good polysaccharide producers and in the present study many of the isolates belonged to *Bacillus* which made the culture thick and sticky by the production of polysaccharides. Bacterial surfaces, because of their high reactivity due to ionisable carboxylates and phosphates of lipopolysaccharides in Gram negative bacteria, due to peptidoglycan, teichuronic acids and teichoic acids in Gram positive bacteria can absorb and bind the inorganic silicate ions rendering dissolution [19]. In the present study silicate solubilisation was observed both in Gram negative *Pseudomonas* and Gram positive *Bacillus* indicating that both Gram positive and Gram negative organisms are involved in silicate solubilisation in soil and other natural environments. vi) The bacterial sulphate reduction by bacteria can generate H_2S and this reduced sulphur could react with cations like Ca and Fe of silicate minerals forming their sulphides [20]. In a flooded rice ecosystem H_2S formation is common and this can react with the cations thus rendering silicate solubilisation. vii) The siderophores formed by bacteria bind, transport and shuttle iron and this process can play a role in silicate solubilisation by scavenging iron from silicate minerals as observed in hornblende degradation [21]. *Pseudomonas* are known to produce siderophores and in the present investigation two different *Pseudomonas* were found to solubilise silicates.

The results indicate that not only acidolysis but also other mechanisms may also operate in dissolution of silicates. The culture filtrates of all the SSB were not acidic but some were alkaline in reaction. Even the same species showed a different reaction according to the silicate mineral. The acid or alkali production also depended on the type of mineral and the species of organism interacting with it. In the natural environment a host of organisms colonize soil and rhizosphere producing varied types of metabolites that are likely to have a bearing on soil reaction, resulting in silicate dissolution. The silica soluble in water is assimilated by several microbes, plants etc. to fabricate cell support structures. Although the dissolution may be low in some cultures, depending upon the mineral in the laboratory study, their role in the natural environment is likely to be much more as these organisms may coexist and act on a variety of soil silicates releasing silica in solution.

7 Conclusion

Although the total bacteria present in soil and silicate minerals were numerically high, the proportion of silicate solubilising bacteria (SSB) was low indicating that they are unique. The SSB was lower in proportion in soil than aquatic sediments suggesting more active silicate solubilization in aquatic environments. Both Gram positive *Bacillus* and Gram negative *Pseudomonas* were found to solubilize silicates and they were identified as *Bacillus mucilaginosus*, *B. megaterium*, *B. flexus* and *P. fluorescens* by biochemical characterisation and 16S r RNA sequencing.

Even though a few of isolates did not exhibit a dissolution zone in the plate assay, all of them solubilised silicates in liquid medium but the extent of dissolution varied with the species, silicate mineral and the incubation period. The reaction of the medium varied with the organism, the mineral and period of incubation. However in quartz, muscovite and illite all these bacteria exhibited acidification. This indicates that the mechanism of silicate solubilisation may vary between species and between silicates. A variety of organic acids was produced by *B. mucilaginosus* and *B. flexus* in feldspar and quartz. This has significance in the natural environment where a host of different species coexist and interact with the variety of silicate minerals when different species can preferentially solubilise a particular mineral over the others. The combined action of these organisms on different minerals in soil may release the nutrients like phosphorus, potassium, iron, calcium and magnesium bound in the silicate minerals. This has a bearing not only on the

geochemical cycling of silicon but also on nutrient release for microbial and plant assimilation suggesting the feasibility of using SSB as a bioinoculant either alone or in conjunction with silicates as shown by Sheng and Coworkers [5]. The silicate solubilisation can be exploited profitably to enhance crop production either alone or in conjunction with silicate materials as silica itself is considered as agronomically beneficial and its mobilization is always accompanied by the release of other macro and macro and micronutrients that are bound in silicate minerals.

References

1. Gadd GM (2010) Metals, minerals and microbes: Geomicrobiology and bioremediation. Microbiology 156:609–643
2. Webley DM, Henderson MEK, Taylor IF (1963) The microbiology of rocks and weathered stones. J Soil Sci 14:102–112
3. Northup DE, Lavoie KH (2001) Geomicrobiology of caves: A Review. Geomicrobiol J 18:199–222
4. Lauwers AM (1974) Biodegradation and utilization of silica and quartz. Arch Microbiol 95:67–78
5. Sheng XF, Zhao F, He LY, Qiu G, Chen L (2008) Isolation and characterization of silicate mineral – solubilising Bacillus globisporus Q12 from the surfaces of weathered feldspar. Can J Microbiol 54:1064–1068
6. Uroz S, Calvaruso C, Turpault MP, Frey-Klett PP (2009) Mineral weathering by bacteria: Ecology, actors and mechanisms. Trends Microbiol 17:378–387
7. Vasanthi N., Saleena LM, Anthoni Raj S (2012) Concurrent release of secondary and micronutrients by a Bacillus sp. American – Eurasian. J Agric Sci 12:1051–1064
8. Bunt JS, Rovira AD (1955) Microbiological studies of some sustainable soils. J Soil Sci 6:119–128
9. Imaizumi K, Yoshida S (1958) Edaphological studies on silicon supplying power of paddy fields. Bull Natl Inst Agric Sci Jpn Ser 8:261–304
10. Barker WW, Banfield JF (1996) Biologically versus inorganically mediated weathering reactions: Relationships between minerals and extracellular microbial polymers in lithobiontic communities. Chem Geol 132:55–69
11. Lian B, Chen Y, Zhu L (2008) Effect of microbial weathering on carbonate rocks. Earth Sci Front 15:90–99
12. Waksman SA, Starkey RL (1924) Microbiological analysis of soil as an index of soil fertility. 7. Carbon dioxide evolution. Soil Sci 17:141–161
13. Pokrovsky O, Shirokova L, Stockman G, Zabelina S, Binizeth P, Gerard E, Menez B, Alfredsson H (2011) Quantifying the role of microorganisms in silicate mineral dissolution at the condition of CO_2 storage in basalts. Geophys Res Abstr 13:13904
14. Avakayan ZA, Pivovarova TA, Karavaiko GI (1986) Properties of new species - Bacillus mucilaginosus. Mikrobiologiya 55:477–482
15. Drever JI, Stillings LL (1977) The role of organic acids in mineral weathering. Colloids Surf A Physicochem Eng Asp 120:167–181
16. Duff RB, Webley DM (1959) 2-ketogluconic acid as a natural chelator produced by soil bacteria Chemistry and industry 1376–1377
17. Kutuzova RS (1969) Release of silica from minerals as a result of microbial activity. Mikrobiologiya 38:596–602
18. Malinovskaya IM, Kosenko LV, Votselko SK, Podgorskii VS (1990) Role of Bacillus mucilaginosus polysaccharide in degradation of silicate minerals. Mikrobiologiya 59:70–78
19. Urrutia MM, Beveridge TJ (1994) Formation of fine grained silicate minerals and metal properties by a bacterial cell surface (Bacillus subtilis) and implications on global cycling of silicon. Chem Geol 116:261–280
20. Ehrlich HL (1981) Geomicrobiology. Marcel Dekker Inc., New York, p 393
21. Kalinowski BE, Liermann LJ, Brantley SL, Barnes A, Pantano CG (2000) X- ray photoelectron evidence for bacteria – enhanced dissolution of hornblende. Geochim Cosmochim Acta 64:1331–1343