

Effect Of Silicon Compounds On Oligotrophic Soil Bacteria

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Abstract. A range of silicon compounds stimulated the population of oligotrophic bacteria when added to soil over the 4 weeks incubation period; with the number increasing with increasing amounts of added silicon compounds. Total counts of soil oligotrophic bacteria were increased after adding all of the silicon compounds. There was a marked stimulation in oligotrophic bacteria population at 3% of silicon compounds. The largest effect of silicon compounds on soil oligotrophic bacteria was recorded in the samples where silicon nitride was added. While in the case of sodium silicate, the observed increase was small and statistically insignificant.

Key words: oligotrophic bacteria; silicon compounds; soil.

Introduction

Most soils can be regarded as being oligotrophic because they are generally considered to contain insufficient carbon to allow the continuous growth of microorganisms (Lynch 1982; Fry 1990). Bacteria which are adapted for growth in extremely low nutrient environments are called oligotrophs (Wainwright 1988). They are found in soils, fresh water and sea water. While data on microbial growth on nutrient-rich media are being rapidly accumulated, the populations of oligotrophic bacteria have been frequently overlooked. Only a few studies have been published about the ecological aspects of the oligotrophic bacteria. Oligotrophic bacteria are considered to play a predominant role in transformations of organic and mineral substances in such environments, since these environments are generally poor in nutrients (Al-Ghamdi 1998).

A recent study began to reveal that a number of bacteria, actinomycetes and fungi appeared to be able to grow on nutrient-free silica gel, fresh air and distilled water (Wainwright *et al.*, 1991). Fungal growth was increased in ultra-pure water containing silicon compounds (Wainwright *et al.*, 1997).

Silicon is the second most common element on earth after oxygen and is abundant in soils (Wainwright *et al.*, 1997). Despite the fact that silicon is generally thought to be biologically inert, it is possible that soil microorganisms will have evolved some means of metabolising this element (Das *et al.*, 1992). Bacteria can solubilize insoluble silicates, a process which may be important in the biological weathering of rocks (Wainwright *et al.*, 1997). It has also been suggested that bacteria use silicon-based autotrophy as a source of energy to support CO₂ fixation (Das *et al.*, 1992).

The purpose of this investigation was to study the effect of silicon compounds on the communities of oligotrophic bacteria in soils collected from different regions of the Kingdom of Saudi Arabia.

Materials and Methods

Collection and analysis of soil samples

Samples of cultivated soils were collected in sterile polyethylene bags from ten different localities at Saudi Arabia (Table 1). For soil characteristics analysis, one kg soil of each locality were taken from the soil surface at a depth of 1-15 cm (five replicates for each site were used).

Table 1. The soil characteristics of different regions in Saudi Arabia (all values are means \pm S.D. of five replicates).

Soil locality	Soil texture	Organic C. %	pH	T.S.S.%	CaCO ₃ %	N%
Abha	Sand	1.80	7.1	0.23	15.00	0.085
Gizan	Sand	0.03	7.8	0.05	24.30	0.041
Hail	Sand	0.07	7.2	0.13	1.87	0.032
Hassa	Sand	1.03	7.3	0.51	1.22	0.050
Jouf	Sandy loam	0.68	7.7	0.14	12.44	0.034
Kharj	Sand	0.62	7.5	0.19	34.62	0.071
Qassium	Sand	0.34	7.9	0.09	24.11	0.045
Riyadh	Sand	0.27	7.7	0.42	30.06	0.061
Tabouk	Sand	0.74	8.1	0.34	7.43	0.042
Taif	Loamy sand	0.12	8.0	0.29	24.92	0.029

Organic C = Organic Carbon N = Total Nitrogen T.S.S = Total Soluble Salts

Mechanical analysis of the soil was determined according to Al-Falih (1996). Soil pH was determined with a glass electrode with a water soil slurry (10:1). The methods described by Jackson (1962) were used for determination of the total soluble salts, calcium carbonates and total nitrogen. The percentage of soil organic carbon was determined colorimetrically using the method described by Walinga *et al.*, (1992). Different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 3%) of each of calcium silicate, silicon nitride, silicic acid and sodium silicate were added to the soil samples. Soils amended with many types of silicon compounds at different rates were set up in triplicate and incubated at 25 °C for four weeks. Soil moisture was adjusted to 70% of maximum water holding capacity (MWHC).

Numeration of oligotrophic bacteria

Concentration of organic carbon required by oligotrophic bacteria was suggested by some researchers to be 1 to 15 mg C/L (Kuznetsov *et al.*, 1979; Martin and Macleod 1984), and by others to be less than 1 mg C/L (Ishida and Kadota 1981). Based on the above mentioned criteria, the 10,000 fold dilutions of nutrient broth medium (composed of 5 g peptone, 5 g beef extract, 5 g NaCl, and one

liter distilled water) was used as oligotrophic media (Al-Ghamdi 1998). The diluted nutrient broth medium assumed to contain approximately 0.4 mg C/L. This medium was solidified by 1.5% (w/v) of purified agar (Oxoid) and sterilized by autoclaving at 121°C; 15 lb squ. inch for 20 minutes.

For the quantitative estimation of bacteria six replicates for each site were used. Plate count was made by spreading 0.1 ml of the appropriate dilutions of soil samples using a sterile glass spreader onto the surface of agar plates. The inoculated plates were incubated aerobically at 25 °C for one week and the colonies were enumerated. Counts were expressed as number of colony-forming units (CFU) per gram dry weight of soil samples.

Statistical analysis

Analysis of variance was carried out and significance between groups was evaluated with the Duncan test ($P = 0.05$).

Results

All soil samples tested, with exception of Abha soil, contained low percentages of organic carbon and total nitrogen (Table 1). The soils were slightly

Table 2. The number of oligotrophic bacteria in soil samples treated with different concentrations of calcium silicate during incubation at 25 °C for 4 weeks (all values are means \pm S.D. of six replicates).

Soil locality	Number of oligotrophic bacteria $\times 10^6$ /g soil					
	0.0 %	0.5 %	1.0 %	1.5 %	2.0 %	3.0 %
Abha	4.1 \pm 0.1	4.4 \pm 1.3	9.0 \pm 0.2	9.4 \pm 0.5	15.0 \pm 0.3	20.9 \pm 2.0*
Gizan	3.2 \pm 0.3	5.3 \pm 1.7	8.5 \pm 0.8	9.3 \pm 1.6	14.0 \pm 1.7	25.1 \pm 0.4*
Hail	3.0 \pm 1.0	6.4 \pm 1.7	8.6 \pm 0.6	8.3 \pm 1.0	15.6 \pm 1.2	21.9 \pm 0.6*
Hassa	3.5 \pm 1.0	3.9 \pm 0.0	4.5 \pm 0.1	7.1 \pm 1.0	16.2 \pm 2.2	23.6 \pm 0.1*
Jouf	2.0 \pm 0.4	4.5 \pm 1.0	5.7 \pm 0.7	6.5 \pm 0.9	14.1 \pm 1.2	20.0 \pm 1.4*
Kharj	2.8 \pm 1.0	5.8 \pm 0.7	7.2 \pm 0.0	8.4 \pm 2.4	15.7 \pm 1.2	21.1 \pm 0.3*
Qassium	3.0 \pm 0.7	2.4 \pm 0.0	8.9 \pm 1.2	9.1 \pm 1.0	14.5 \pm 1.8	25.5 \pm 0.1*
Riyadh	3.4 \pm 1.6	6.1 \pm 1.1	8.0 \pm 0.1	8.5 \pm 1.6	19.0 \pm 1.0*	28.2 \pm 2.2*
Tabouk	2.4 \pm 0.4	2.7 \pm 1.0	6.4 \pm 0.8	7.0 \pm 0.5	14.9 \pm 0.9	27.0 \pm 1.2*
Taif	3.2 \pm 0.7	7.5 \pm 0.4	8.3 \pm 1.1	8.5 \pm 0.0	17.6 \pm 1.3*	19.2 \pm 0.2*

*: Significant at 0.05.

Table 3. The number of oligotrophic bacteria in soil samples treated with different concentrations of silicon nitride during incubation at 25 °C for 4 weeks (all values are means \pm S.D. of six replicates).

Soil locality	Number of oligotrophic bacteria $\times 10^6$ /g soil					
	0.0 %	0.5 %	1.0 %	1.5 %	2.0 %	3.0 %
Abha	4.0 \pm 0.1	14 \pm 0.3	34 \pm 9.2*	54 \pm 0.7**	52 \pm 3.9**	79 \pm 7.3**
Gizan	3.3 \pm 0.4	23 \pm 1.7*	25 \pm 0.8*	53 \pm 1.0**	60 \pm 4.2**	61 \pm 8.4**
Hail	3.1 \pm 1.1	13 \pm 1.2	20 \pm 5.2*	83 \pm 1.5**	56 \pm 12**	89 \pm 7.6**
Hassa	3.5 \pm 1.3	11 \pm 4.0	45 \pm 8.1**	51 \pm 8.0**	67 \pm 9.2**	96 \pm 9.1**
Jouf	2.0 \pm 0.4	25 \pm 1.3*	11 \pm 9.7	65 \pm 0.8**	71 \pm 5.7**	85 \pm 4.4**
Kharj	2.5 \pm 1.0	18 \pm 2.8*	22 \pm 5.0*	84 \pm 2.4**	57 \pm 9.2**	91 \pm 9.3**
Qassium	3.1 \pm 0.4	24 \pm 1.0*	39 \pm 6.2**	41 \pm 1.0**	45 \pm 7.8**	75 \pm 7.1**
Riyadh	3.4 \pm 1.2	11 \pm 1.3	30 \pm 9.1*	35 \pm 1.6*	50 \pm 3.7**	42 \pm 5.2**
Tabouk	2.6 \pm 0.7	17 \pm 1.5*	24 \pm 9.1*	30 \pm 7.5*	47 \pm 6.9**	60 \pm 5.2**
Taif	3.1 \pm 0.3	15 \pm 2.9	33 \pm 9.1*	55 \pm 3.0**	76 \pm 3.3**	72 \pm 5.2**

*: Significant at 0.05.

**: Highly significant.

alkaline and sandy in texture in all cases, with exception of sandy loam and loamy sand soils in Jouf and Taif respectively. There was a high content of total soluble salts in all soils studied. The highest content of total soluble salts was observed in Hassa soil (0.51%), while the lowest content was recorded in Gizan soil (0.05%). The Kharj and Riyadh soils exhibited the highest percentage of CaCO₃ which were 34.62 and 30.06 respectively. The population of oligotrophic bacteria in all soils before receiving any amount of silicon compounds ranged between 2.0 $\times 10^6$ CFU/g of soil to 4.1 $\times 10^6$ CFU/g of soil (Tables 2-5).

Changes in population of soil oligotrophic bacteria of different regions in Saudi Arabia after amendment of silicon compounds are shown in Tables 2-5. It was found after 4 weeks of incubation that the addition of silicon compounds caused a marked stimulation in population of soil oligotrophic bacteria; with the number increasing with increasing amounts of added silicon compounds. The population of oligotrophic bacteria in soil amended with calcium silicate ranged between 1.9 to 2.8 $\times 10^7$ CFU/g of soil at 3% concentration (Table 2).

The highest population of oligotrophic bacteria was observed at 3% of silicon nitride (Table 3).

Table 4. The number of oligotrophic bacteria in soil samples treated with different concentrations of silicic acid during incubation at 25 °C for 4 weeks (all values are means \pm S.D. of six replicates).

Soil locality	Number of oligotrophic bacteria $\times 10^6$ /g soil					
	0.0 %	0.5 %	1.0 %	1.5 %	2.0 %	3.0 %
Abha	4.1 \pm 0.4	2.0 \pm 1.1	4.2 \pm 0.2	9.4 \pm 0.6	13.0 \pm 0.1	11.9 \pm 1.0
Gizan	3.0 \pm 0.1	3.3 \pm 1.0	5.5 \pm 0.5	8.3 \pm 1.0	13.0 \pm 1.2	14.1 \pm 0.6
Hail	2.9 \pm 0.3	2.6 \pm 0.7	6.9 \pm 0.4	7.3 \pm 0.0	13.6 \pm 0.2	15.9 \pm 0.6
Hassa	3.4 \pm 1.0	3.0 \pm 0.3	5.5 \pm 0.1	9.1 \pm 1.1	14.2 \pm 0.2	17.6 \pm 0.8*
Jouf	2.0 \pm 0.2	2.5 \pm 0.7	4.0 \pm 0.2	8.5 \pm 0.3	13.1 \pm 1.0	16.0 \pm 0.4
Kharj	2.7 \pm 1.0	2.1 \pm 0.3	3.3 \pm 0.4	7.4 \pm 0.4	12.7 \pm 0.2	17.1 \pm 0.9*
Qassium	3.0 \pm 0.6	3.4 \pm 0.4	6.1 \pm 0.2	8.1 \pm 0.1	13.5 \pm 1.0	16.6 \pm 0.8
Riyadh	3.4 \pm 1.0	3.1 \pm 1.0	7.0 \pm 0.3	9.5 \pm 1.0	13.0 \pm 0.0	15.3 \pm 1.2
Tabouk	2.2 \pm 0.5	2.2 \pm 0.3	5.5 \pm 0.0	6.0 \pm 0.5	13.9 \pm 0.9	14.6 \pm 1.0
Taif	3.2 \pm 0.1	3.5 \pm 0.4	4.3 \pm 0.4	7.5 \pm 0.3	12.6 \pm 0.6	15.3 \pm 0.6

*: Significant at 0.05.

Table 5. The number of oligotrophic bacteria in soil samples treated with different concentrations of sodium silicate during incubation at 25 °C for 4 weeks (all values are means \pm S.D. of six replicates).

Soil locality	Number of oligotrophic bacteria $\times 10^6$ /g soil					
	0.0 %	0.5 %	1.0 %	1.5 %	2.0 %	3.0 %
Abha	4.1 \pm 0.1	3.0 \pm 0.8	3.4 \pm 3.2	4.4 \pm 0.7	5.2 \pm 3.0	6.9 \pm 7.0
Gizan	3.2 \pm 0.6	3.3 \pm 1.9	3.5 \pm 0.2	3.3 \pm 1.9	4.0 \pm 4.2	5.1 \pm 4.4
Hail	3.0 \pm 1.4	3.5 \pm 2.2	3.3 \pm 5.2	4.3 \pm 1.4	4.6 \pm 1.4	4.7 \pm 2.6
Hassa	3.5 \pm 1.0	3.0 \pm 2.3	3.5 \pm 8.0	3.1 \pm 3.0	5.7 \pm 5.2	4.6 \pm 9.4
Jouf	2.0 \pm 0.4	2.1 \pm 4.0	2.0 \pm 9.0	2.5 \pm 2.8	4.1 \pm 5.0	4.5 \pm 3.4
Kharj	2.8 \pm 1.2	2.4 \pm 2.3	3.2 \pm 5.5	4.4 \pm 2.3	3.7 \pm 9.6	4.1 \pm 3.3
Qassium	2.9 \pm 0.7	3.1 \pm 4.2	3.1 \pm 0.4	3.5 \pm 4.4	3.1 \pm 4.0	3.9 \pm 2.0
Riyadh	3.3 \pm 1.1	3.3 \pm 1.3	3.3 \pm 2.0	3.3 \pm 2.6	4.3 \pm 1.6	5.8 \pm 1.5
Tabouk	2.0 \pm 0.4	2.4 \pm 5.0	3.9 \pm 3.2	4.1 \pm 1.2	5.2 \pm 4.8	5.5 \pm 5.1
Taif	3.0 \pm 0.4	3.2 \pm 1.2	3.0 \pm 9.0	3.5 \pm 7.1	5.0 \pm 7.7	5.2 \pm 5.7

Addition of silicon nitride caused a highly significant ($P=0.05$) increase in the population of oligotrophic bacteria. The Hassa soil showed the largest bacterial count (9.6×10^7 CFU/g of soil) at 3% of silicon nitride. The lowest bacterial population was exhibited by Riyadh soil (4.2×10^7 CFU/g of soil) when the soil was supplemented with 3% of silicon nitride. Generally, all concentrations of silicon nitride caused a significant increase of the population of oligotrophic bacteria. But only for the maximum concentration used in this study (3%) of calcium silicate the increase was significant on the population of oligotrophic bacteria.

Table 4. shows the number of oligotrophic bacteria in soil samples treated with different concentrations of silicic acid during incubated at 25

°C for 4 weeks. The total count of oligotrophic bacteria in soils treated with 3% concentrations of silicic acid ranged between 1.2 to 1.8×10^7 CFU/g of soil. While the lowest population of oligotrophic bacteria was recorded in soil treated with sodium silicate and ranged from 3.9 to 6.9×10^6 CFU/g of soil at 3% concentration (Table 5). The silicic acid and sodium silicate caused insignificant increase in the numbers of oligotrophic bacteria in all soil samples, with exception of Hassa and Kharj soils for silicic acid.

Discussion

Most of the investigated soil samples tested had a high percentage of total soluble salts and low percentage of organic carbon. The Gizan soil had the lowest percentage of T.S.S and organic carbon

(0.05% and 0.03% respectively), and there was a high number of oligotrophic bacteria in this soil compared to other control soils. On the other hand, Hassa soil that were having the highest level of T.S.S and organic carbon exhibited the lowest number of oligotrophic bacteria. The results show that these oligotrophic bacteria are affected by salinity and carbon; a similar relationship between salt, carbon content and number of oligotrophic bacteria in the soil were reported earlier by other workers (Ishida and Kadota 1981; Kaszubiak and Muszynska 1992).

Wainwright *et al.* (1997) reported that the presence of silicon in clays and other soils minerals may help explain why soil microorganisms, including bacteria and fungi, can grow in such an apparently nutrient-poor environment by: (i) using nutrients adsorbed by silicon compounds from the soil atmosphere, or (ii) by using energy derived from silicon metabolism to fix CO₂ chemoautotrophically.

There was a significant stimulation in population of oligotrophic bacteria at 3% of silicon compounds. The same effect of silicon compounds on fungal growth was observed by other workers (Wainwright *et al.* 1997).

It is interesting that in case of sodium silicate, the observed increase was small and statistically insignificant. Why sodium silicate should be exceptional in this respect is not clear. However, like the other compounds it was not completely soluble, so the lack of growth stimulation was not due to the absence of insoluble particulates.

Silicon compounds acted as nutrient sources for bacterial growth, because they are efficient at adsorbing combined carbon and nitrogen from the atmosphere. The possibility that bacteria can use silicon compounds as an energy source was suggested by Das *et al.*, (1992). Bigger and Nelson (1943) also suggested that silicon compounds might

adsorb ammonia and CO₂ from the atmosphere, thereby allowing bacteria to fix CO₂, using energy obtained from the oxidation of ammonium. Although not conclusively proved but Chakrabarty *et al.*, (1988) suggested that certain bacteria can grow as silicon autotrophs. It is generally thought that silicon compounds are biologically unreactive. But Alison (1968) stated that there is no theoretical reason why the reaction of Si-Si-Si with oxygen or oxygen compounds could not act as an energy-yielding reaction.

Conclusion

Whatever the mechanism involved, it is clear that silicon compounds promote bacterial population. Overall, the population changes of soil oligotrophic bacteria were correlated with amounts of silicon compounds added (Tables 2-5). The counts of oligotrophic bacteria increased as the concentration of silicon compounds increased. Addition of silicon nitride and calcium silicate caused a highly significant stimulation on the population of oligotrophic bacteria.

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تأثير مركبات السيليكون على أعداد بكتيريا التربة الأوليجوتروفية

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ملخص البحث: إضافة عدد من مركبات السيليكون الى عينات التربة خلال أربعة أسابيع من فترة التحضين حفزت

تكاثر أعداد البكتيريا الأوليجوتروفية. تزداد أعداد البكتيريا الأوليجوتروفية بزيادة تركيز مركبات السيليكون.

وجد في هذه الدراسة أن الزيادة الملحوظة في أعداد البكتيريا الأوليجوتروفية ظهرت عند إضافة ٣ % من مركبات

السيليكون. نيتريتات السيليكون سجلت أعلى تأثير على أعداد البكتيريا الأوليجوتروفية في عينات التربة. بينما لوحظ

ان سيليكات الصوديوم أعطت أقل تأثير على أعداد البكتيريا الأوليجوتروفية.