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The Dissolution of K and P-bearing Minerals by Silicate Dissolving Bacteria and Their Effect on Sorghum Growth

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Abstract: Potassium and phosphorus are vital components of plant nutrition package limiting crop yield and quality. In particular, the crop production in Egypt relies quietly on the world potash fertilizers. It thus becomes urgent to exploit the indigenous mineral resources to alleviate the importation of fertilizers. Therefore, dissolution of feldspar (orthoclase) and rock phosphate by silicate dissolving bacteria was monitored in pure culture and soil pot experiments to determine the effect of these bacteria on releasing K and P as well as their effect on plant growth. Six strains of bacteria capable of dissolving silicate minerals were isolated from feldspar samples. The release of K and P from feldspar and rock phosphate was substantially enhanced in pure culture inoculated with bacterial strains. The different strains however, had obvious abilities to release K and P from the minerals. The strain SBS had a higher capacity to release K and P from the minerals than others did. The released K by this strain (490 mg/l) increased by 21-112 % while the released of P (758 mg/l) increased by 25-75 % compared with that by any of the five strains. The release of K from the feldspar was obviously affected by pH and aerobic condition. The strain SBS had a much higher potential to release K in the pH 6.5-8.0 than other pHs. More aerobic condition produced more K and P than a less aerobic one. Soil pot experiments with sorghum plants indicated that the silicate dissolving bacteria could grow in the tested soils and have a potential of minerals dissolution. The mean effect of silicate bacteria on feldspar dissolution was appreciable greater under the tested soils (62 % of total K) rather than under bacterial culture (53 % of total K). Bacterial inoculation combined with K and P bearing minerals gave 48, 65 and 58 % increase in dry matter yield of sorghum plants in clay, sandy and calcareous soil respectively compared to non-inoculated soils. The uptake of K by sorghum plants also increased by 71, 110 and 116 % while the uptake of P increased by 41, 93 and 79 % in the same soils respectively. Residual soil fertility estimated by K and P concentration after harvest also underwent an increase through inoculation of silicate dissolving bacteria especially with mineral additions in the tested soils.

key words: dissolution, feldspar, rock phosphate, bacillus, sorghum

INTRODUCTION

Most of the Egyptian desert soils are poor or marginal in nutrient status and require adequate fertilization to sustain high productivity. However, due to economic consideration, the cost of applying imported or locally produced water-soluble fertilizers is becoming more expensive. Thus, the use of alternative indigenous resources such as feldspar (orthoclase) and rock phosphate are gaining importance to alleviate the dependence of imported or costly commercial fertilizers.

Several laboratory studies have shown that microbes can increase the dissolution rate of silicate and aluminum silicate minerals in laboratory patches experiments, primarily by generating organic and inorganic acids[1]. Although some of these organisms are free-living (plank tonic) in solution, most of these bacteria are attached to mineral surfaces[2,3], where they can impact water-rock interaction, mineral surface chemistry, dissolution and precipitation of minerals, the evolution of ground water geochemistry and soil formation[4-9]. The interaction between microbes and mineral substrate can involve the oxidation or reduction of mineral constituents from which the organism derives energy for growth and reproduction[10-12]. However, other types of interaction are also possible and these may have impact on the diagnosis of wider range of mineral including abundant classes of silicate and aluminum-silicate minerals[13,14].

Microbes can enhance mineral dissolution rate by producing and excreting metabolic by-products that...
interact with the mineral surface. Complete microbial respiration and degradation of particulate and dissolved organic carbon can elevate carbonic acid concentration at mineral surfaces, in soils and in ground water\textsuperscript{[14,15]}, which can lead to an increase in the rates of mineral weathering by a proton-promoted dissolution mechanism. In addition to carbonic acid, microbes can produce and excrete organic ligands by a variety of processes such as fermentation and degradation of organic macromolecules, or as a response to nutrient stress\textsuperscript{[13,16-20]}.

It is well known that many organic compounds produced by microorganisms, such as acetate, citrate and oxalate can increase mineral dissolution rate\textsuperscript{[21]}. Carboxylic acid groups which shown to promote dissolution of silicates are also common in extracellular organic materials. Moreover, some microorganisms in soil environment contain enzymes that function in ways analogous to chitinase and celluloses, i.e. they specifically break down mineral structure and extract elements required for metabolism or structure purposes (e.g., mineralizes)\textsuperscript{[11]}. This may be especially important for ions such as Fe\textsuperscript{3+} and Al\textsuperscript{3+}, which are expected to be rather insoluble.

Some recent reports showed that silicate dissolving bacteria could activate soil P, K, Si reserves and promote plant growth\textsuperscript{[22,23]}. Styriakova et al\textsuperscript{[24]}, reported that the activity of silicate dissolving bacteria played a pronounced role in the release of Si, Fe and K from feldspar and Fe oxyhydroxides. At present, little information about the K and P releasing condition by the silicate bacteria are available. The aim in this study was to investigate K and P releasing condition by silicate bacteria and their effects on plant growth.

**MATERIAL AND METHODS**

**Mineral perpetration:** Feldspar (orthoclase) was obtained from three locations represent the sediments of potash feldspar in Eastern Desert of Egypt between Ras Benas and Quseir. Rock phosphate was obtained from New Valley Project. All samples were ground and sieved and the 250-µm-size fraction was collected for these experiments. The mineral powders were rinsed with distilled water to remove the fine particles. The element contents of the minerals are presented in (Table 1).

**Bacteria and media:** Six strains of silicate dissolving bacteria were isolated from different feldspar samples according to the dilution-plate methods. Prior to experimental use, these strains were initially grown in Nutrient broth No. 2 at 28 °C for 2 days and then harvested by centrifugation at 4000 rpm. The cultures were then resuspended and rinsed several times with deionized water to remove any remaining culture medium and added in concentration of (10\textsuperscript{10} CFU/ml) to modified Bromfield\textsuperscript{[25]} liquid medium (1954); (NaH\textsubscript{2}PO\textsubscript{4} - 0.5 g; MgSO\textsubscript{4}. 7H\textsubscript{2}O - 0.5 g; (NH\textsubscript{4})\textsubscript{2} SO\textsubscript{4} - 1.0; NaCl - 0.2; molasses - 5.0 g; 1000 ml distilled water; pH 7.5).

**Pure culture experiments:** Two parallel dissolution experiments were performed under laboratory conditions in 500 ml-Erlenmeyer flasks containing 10 g of either feldspar or rock phosphate in 200 ml of modified Bromfield medium inoculated with either active or dead cells of silicate bacteria (NaH\textsubscript{2}PO\textsubscript{4} was not added to the culture in the rock phosphate experiment). The medium pH was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 with diluted HCl or NaOH solutions in the case of feldspar experiment or to 7.5 in the case of rock phosphate to avoid any effect of pH medium on rock phosphate dissolution. A control without inoculation was also made.

All cultures were sterilized at 80°C with shaking to stop the microbial metabolism after 30 days incubation. The cultures were further treated with 6% H\textsubscript{2}O\textsubscript{2} and filtered to separate mineral powder residues from the cells. The filtered culture solutions were digested on a hot plate with 30% H\textsubscript{2}O\textsubscript{2} until all organic matter was oxidized. The concentrations of K and P in the digested solutions were determined with the flame and spectrophotometer, respectively.

**Cultivation experiment:** A pot culture experiment was conducted in clay, sandy and calcareous soil. The basic properties of the soils are presented in (Table 2). Five Kg of each soil was transferred into plastic pots. Prior to planting, the minerals of feldspar and rock phosphate were added to the soils at 0 and 10 g each per pot. A constant dose of nitrogen in the form of urea was applied to all treatments at the rate of 1.5 g per pot in two equal splits. The treatments were replicated four times in a complete randomized block design.

The seeds of sorghum plants sterilized with 6% H\textsubscript{2}O\textsubscript{2} and dipped in distilled water for two days at 30 °C and then air-dried. The tested bacterium SBS was identified as a strain of \textit{(Bacillus cereus)}. The seeds were soaked in either fermented liquid containing SBS strain or distilled water for control. It was measured that there were about (2x10\textsuperscript{7} CFU) SBS strain per seed of sorghum. The treated seeds were planted with 5 plants per pot after thinning. Soil moisture was maintained at field capacity throughout the course of the experiment. The plants were cut after 30 days incubation and the dry matter of each treatment was recorded. Sorghum dry matter samples were digested with triacid mixture and the concentration of K and P of the above-ground parts were determined using standard
Chemical analysis of the minerals (%).

<table>
<thead>
<tr>
<th>Minerals</th>
<th>K, %</th>
<th>P, %</th>
<th>SiO₂, %</th>
<th>Fe₂O₃, %</th>
<th>Na₂O, %</th>
<th>CaO, %</th>
<th>Al₂O₃, %</th>
<th>SO₂, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthocalse</td>
<td>11.25</td>
<td>0.12</td>
<td>65.23</td>
<td>0.16</td>
<td>3.68</td>
<td>0.26</td>
<td>18.72</td>
<td>0.41</td>
</tr>
<tr>
<td>Rock phosphate</td>
<td>0.33</td>
<td>30.0</td>
<td>8.5</td>
<td>4.2</td>
<td>1.0</td>
<td>45.0</td>
<td>0.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table 2: Some basic properties of soils used in the experiment.

<table>
<thead>
<tr>
<th>Soil</th>
<th>EC (dS/m)</th>
<th>pH</th>
<th>CaCO₃ (mg/kg)</th>
<th>Avail. N (mg/kg)</th>
<th>Avail. P (mg/kg)</th>
<th>Avail. K (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>0.35</td>
<td>7.6</td>
<td>2.7</td>
<td>35</td>
<td>14</td>
<td>115</td>
</tr>
<tr>
<td>Sandy</td>
<td>0.78</td>
<td>8.2</td>
<td>1.5</td>
<td>12</td>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>Calcareous</td>
<td>1.24</td>
<td>8.5</td>
<td>28.3</td>
<td>17</td>
<td>5</td>
<td>47</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS

Releasing capacity in pure culture: The releases of K and P from feldspar and rock phosphate were used as an overall indicator of mineral dissolution during these experiments (Fig. 1&2). Six strains of silicate dissolving bacteria were obtained from the samples of feldspar. Higher K and P concentration was observed in the culture inoculated with bacterial strains compared to controls due to lower resistance of the tested minerals to activity of silicate bacteria. However, the different strains showed various abilities on dissolution minerals and their effect were more pronounced with rock phosphate.

The SBS strain had a higher capacity to release K (53 % of total K) and P (58 % of total P) from the minerals than others did. The release of K by the SBS strain (490 mg/l) increased by 21-112 % compared with that by any of the five strains, while the release of P (758 mg/l) increased by 25-75 %. All dead cells also released a significant amount of K and P from the minerals.

Previous studies however, confirmed that these bacterial strains produced several organic acids such as acetic, butyric, byruvic and formic acid which can specifically break down mineral structure and extract elements required for metabolism or structure purpose1,2,4.

The organic acids produced by microbial colonization on the mineral surfaces greatly accelerated the release of mineral elements to solution from feldspar sample27. In the present dissolution experiments with silicate dissolving bacteria, K and P released to solution were greatly increased due to the production of organic acids, which decreased solution pH to about 3.5. The dead cells also had a small but significant enhancement of dissolution rates, as microbial cell surfaces contain sites that can complex with metal ions released from mineral cultures28,30.

Effect of pH on K releasing from feldspar: Acidity of culture significantly influenced the bacterial activity (SBS strain) in releasing K from feldspar (Fig. 3).
This strain could dissolve much more K under the pH from 6.5-8.0 while the maximum release was obtained at neutral pH. In this respect, Vandeviver et al.[29], reported that several strain of bacteria enhanced dissolution at neutral pH in a buffered glucose media by producing organic ligends, primarily gluconate. The releasing by the dead cells was not significantly affected by the culture acidity.

**Effect of culture volume:** The SBS bacterium was a facultative microorganism. It can survive in both aerobic and anaerobic conditions. However, the releasing capacity of the isolate was different under these conditions (Fig. 4). A small culture volume, i.e. a more aerobic condition, had a much higher K releasing capacity than a large culture volume. The K releasing by the dead cells was not significantly affected by the culture volumes.

**Response of sorghum plants:** The dry matter of sorghum plants inoculated with silicate dissolving bacteria (SBS strain) and supplied with minerals (feldspar and rock phosphate) increased by 48, 65 and 58 % for clay, sandy and calcareous soil respectively compared to the plants supplied with minerals alone (Table 3). Furthermore, the dry weight of sorghum inoculated with bacteria alone increased by 34, 28 and 26 % respectively compared to non-inoculated treatment. Potassium and phosphorus uptake improved markedly in both cases with inoculation of bacteria in the tested soils compared to corresponding controls.

However, these bacteria were found to develop in the rhizosphere of the sorghum plants. The release of dissolved K and P-bearing minerals indicates that the silicate dissolving bacteria could grow under the soil conditions and have a potential of mineral dissolution. After 30 days inoculation, the cells in rhizosphere of sorghum plants treated with minerals increased to 38.6 x 10^4, 30.5 x 10^4 and 26.8 x 10^4 for clay, sandy and calcareous soil respectively. In comparison to non-inoculated treatments, the release of K and P from the added minerals was substantially enhanced with bacterial treatments. Soil available K increased to 135 (clay), 82 (sandy) and 73 (calcareous) mg/kg soil respectively.

Soil available P also increased to 37 (clay), 32 (sandy) and 12 (calcareous) mg/kg soil respectively. The release of K was about 129, 242 and 306 % much higher than that of non-inoculated treatment while the release of P was 106, 146 and 71 % in the clay, sandy and calcareous soil respectively. On the other hand, inoculation of silicate dissolving bacteria without mineral additions appreciably activates the initial K and P content in all soils. The available K content in the rhizosphere soil of sorghum was 69, 43 and 50 % much higher than that of non-inoculated treatments for clay, sandy and calcareous soil respectively. The most possible reason was that the inoculation of silicate dissolving bacteria accelerated the transformation of non-available forms of K and P into an available one.

Microbial K-releasing power was appreciably raised in the tested soils upon bacterial inoculation (Table 4). The mean value of released K from the feldspar reached to (62 % of total K) in the soil culture experiments compared to (53 % of total K) in bacterial culture experiment. This discrepancy can be attributed to the limited extent of microbial attachment to mineral surfaces, the limited contact time between microbes and minerals, or to the accelerated mineral dissolution rates found under soil conditions. Similar result has been reported by[27].
The release of potassium from feldspar added to different soils inoculated by silicate dissolving bacteria. Table 4:

<table>
<thead>
<tr>
<th>Soil</th>
<th>Feldspar</th>
<th>Initial mg/pot</th>
<th>Uptake mg/pot</th>
<th>Residual mg/pot</th>
<th>Released mg/pot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>+</td>
<td>575</td>
<td>878</td>
<td>675</td>
<td>611(65%)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>575</td>
<td>562</td>
<td>380</td>
<td>-</td>
</tr>
<tr>
<td>Sandy</td>
<td>+</td>
<td>340</td>
<td>740</td>
<td>410</td>
<td>578(62%)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>340</td>
<td>422</td>
<td>150</td>
<td>-</td>
</tr>
<tr>
<td>Calcareous</td>
<td>+</td>
<td>235</td>
<td>657</td>
<td>365</td>
<td>542(58%)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>235</td>
<td>345</td>
<td>135</td>
<td>-</td>
</tr>
</tbody>
</table>

- Figures in parenthesis indicate percent of total K.
- Feldspar containing 9.34 % total K applied at the rate of 10 g per pot.

In soils and aquifers, most bacteria are attached to surfaces, therefore any dissolution enhancing metabolites (protons, ligands, oxidants) that are produced are most concentrated at or near the mineral surfaces where dissolution occurs. In laboratory experiments, the bacteria are primarily in suspension and any metabolites produced by the bacteria are diluted by the bulk solution. Under soil conditions, the length of contact time between the minerals, microbe and solution is also much greater than which can be maintained in the laboratory with bacterial culture. However, silicate dissolving bacteria greatly accelerated the release of mineral elements to soil solution from feldspar and rock phosphate.

The dissolution of these minerals by silicate bacteria under soil condition suggested that this bacteria specie, ubiquitous in the soil, plays an important role in destruction of K and P-bearing minerals as well as in the cycle and transformation of rocks. Mounting evidence, however, suggested that bacteria attached to feldspar surfaces could greatly accelerate the rate of feldspar desolation to gain access to trace or limiting nutrients for metabolic process, primarily by the production of organic ligands. The result of these ligands excretion is the accelerated weathering of feldspar. The activity of these bacteria was restricted to soil condition, K containing minerals, pH and incubation temperature. Potassium concentration in the soil also influenced the dissolving process. Sheng, *et al.* reported that silicate bacteria had the maximum efficiency of releasing potassium in the K
concentration 26.5-57.8 mg/kg. In addition, other factors like plants, soil fertility, moisture, temperature and soil organisms, particularly in the field, may largely affect the activity of microorganisms.

Finally, from the foregoing discussion, it is possible to use the locally available feldspar mineral in combination with silicate dissolving bacteria as a bio-fertilizer to replace chemical fertilizer and reducing the cost of crop production.

REFERENCES

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