Solubilization of Iron-Containing Minerals by Soil Microorganisms

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Eighty-eight strains of microorganisms were isolated from soils collected in northern and southern Chile, and 10 fungi which showed the highest solubilizing action upon the iron in granodiorite were then selected. These fungi were incubated with the following iron-containing minerals: augite, hornblende, biotite, magnetite, hematite, and the igneous rock granodiorite. The solubility of iron in these minerals depended on their nature, crystalline structure, the concentration of metabolic products, or all three. Complex formation could be the mechanism involved, as a strong cation-exchange resin was not able to extract Fe from culture solutions. This conclusion is also confirmed by the $R_f$ values obtained by thin-layer chromatography of iron-containing culture solutions.

Certain groups of microorganisms can directly or indirectly transform rocks and minerals in quantities large enough to influence their geological distribution (11, 14). These transformations include enzymatic oxidations and reductions and the formation of chelates and complexes with proteins, amino acids, organic acids, etc. (4, 11). The action of some organic products of microbial metabolism on minerals has been reported (4, 5). It was demonstrated that microorganisms transform crystalline biotite (2), mica to vermiculite (15), and certain rocks to an amorphous state (12). The present work examines the solubilization of iron minerals of different composition and crystalline forms by soil microorganisms. Complexing of iron by metabolic products of Aspergillus niger was also studied.

MATERIALS AND METHODS

Eighty-eight strains of microorganisms were isolated from soils collected in northern and southern Chile. The soils were diluted in water and cultivated on glucose agar and Sabouraud glucose agar. Ten fungi were selected which, when incubated in the presence of 100 mg of granodiorite, solubilized the highest amounts of iron. For incubation, a sterile basal medium was used which had the following composition, per liter: (NH₄)₂SO₄, 0.5 g; KH₂PO₄, 0.5 g; and sucrose, 50 g; it was dissolved in distilled water and adjusted to pH 7.0 with a diluted NaOH solution. The selected fungi were numbered from 1 to 10, corresponding to the following genera: Penicillium (1 and 10), Mucor (2), Aspergillus (3, 4, and 5), Cephalosporium (6, 7, and 8) and Fusarium (9). They were maintained in Sabouraud agar medium.

Minerals whose composition and crystallographic characteristics are described in Table 1 were utilized (3). The minerals were supplied by the Department of Geology, Universidad de Chile, Santiago, Chile. Total iron in the minerals was determined by using the technique of Bandemer and Shaible (1). Determination of iron in solution was carried out with a Perkin Elmer atomic absorption spectrophotometer (model 303), and pH was determined with a pH meter (model 360 WTW).

The minerals were ground in an agate mortar, and the particles between 160 and 180 mesh were used. They were leached with 0.002 N HCl to remove exchangeable bases, washed with distilled water until a negative chloride reaction occurred, and then dried at 105°C (9). A 100-mg sample of the powdered mineral was placed in a test tube and sterilized with hot air. After sterilization, 5 ml of the sterile basal growth medium and five drops of the microorganism suspension (from a colony suspended in 2 ml of the basal medium) were added. The tubes were incubated at 30°C for 21 days with daily stirring. The dissolving action of HCl alone was also studied during the same period of time. At the end of the incubation period, the content of the tubes was filtered through Whatman no. 1 filter paper to remove the mixture of mycelium and residual rock, and pH values and iron concentration were determined in the clear filtrate. To study the iron-complexing ability of metabolic products, granodiorite and A. niger strain 3 were selected and incubated for 30 days in the basal growth medium. Variable volumes (10, 20, and 30 ml) of the solution to be tested were placed in 125-ml plastic flasks, and 5 ml of an iron stock solution (metallic iron dissolved in diluted HNO₃) was added in a concentration sufficient to reach 20 to 25 μg/ml in a 50-ml final volume. A 5-ml amount of a 2 N KNO₃ solution was also added to reach a uniform ionic
Table 1. Characteristics of minerals

| Mineral      | Formula*          | Crystallographic system | Total iron |
|--------------|-------------------|-------------------------|------------|
| Augite       | (Al, Si),O₆Ca(Mg, Fe, Al) | Monoclinic              | 5.2        |
| Hornblende   | Si₆O₉₂(Al, Fe, Ti)₃Ca₃Na₃(Mg, Fe)₆(O, OH)₂ | Monoclinic              | 5,200      |
| Biotite      | Si₆O₉K(Mg, Fe, Al)(OH)₂ | Monoclinic              |           |
| Magnetite    | Fe₆O₄             | Cubic                   | 14.2       |
| Hematite     | Fe₂O₃             | Trigonal                | 65.8       |
| Granodiorite | Igneous rock      |                         | 3.2        |

* See reference 3.

Experimental error: in this determination, hornblende was insufficient for the total iron analysis.

Table 2. Iron solubilized* and pH changes produced by the interaction of different minerals and soil fungi after 21 days of incubation at 30°C

| Fungus       | pH | Augite | pH | Hornblende | pH | Biotite | pH | Magnetite | pH | Hematite | pH | Granodiorite | pH |
|--------------|----|--------|----|------------|----|---------|----|-----------|----|----------|----|-------------|----|
| Control      |    | 6.8    |    | 6.7        | 10.0 | 6.5     |    | 6.7       | 0.0 | 6.7      | 0.0 | 6.5         | 0.0 |
| Penicillium  |    | 4.0    |    | 10.0      | 2.7  | 139.5   |    | 3.1       | 151.0 | 2.7     | 81.0 | 2.7         | 106.0 |
| Muscor (2)   |    | 4.5    |    | 19.5      | 2.2  | 136.0   |    | 3.2       | 156.5 | 2.7     | 19.5 | 3.1         | 59.5 |
| Aspergillus  (3) |    | 3.1    |    | 24.0      | 2.4  | 217.0   |    | 3.1       | 315.0 | 2.8     | 305.0 | 3.1         | 46.0 |
| Aspergillus  (4) |    | 5.2    |    | 7.0       | 2.8  | 204.0   |    | 3.2       | 202.5 | 3.2     | 220.5 | 3.8         | 9.0  |
| Aspergillus  (5) |    | 3.5    |    | 38.5      | 2.6  | 193.0   |    | 3.2       | 256.0 | 2.6     | 91.0  | 3.0         | 27.5 |
| Cephalosporium (6) |    | 3.6    |    | 29.5      | 2.7  | 164.0   |    | 3.0       | 246.5 | 2.5     | 49.5  | 3.6         | 13.5 |
| Cephalosporium (7) |    | 5.2    |    | 19.0      | 3.2  | 92.5    |    | 3.5       | 193.0 | 3.3     | 20.5  | 2.5         | 169.5 |
| Cephalosporium (8) |    | 5.5    |    | 15.5      | 3.2  | 51.0    |    | 3.5       | 135.0 | 3.5     | 37.0  | 3.0         | 229.5 |
| Fusarium     |    | 4.4    |    | 33.5      | 2.9  | 186.0   |    | 3.2       | 132.5 | 2.9     | 82.5  | 3.1         | 27.0 |
| Penicillium  (10) |    | 5.3    |    | 3.5       | 2.9  | 116.5   |    | 3.3       | 118.0 | 2.7     | 24.5  | 2.9         | 30.5 |

* Micrograms of Fe solubilized per 100 mg of mineral in 5 ml of medium.

RESULTS AND DISCUSSION

All microorganisms studied grew well in the medium used. Table 2 shows that all microorganisms dissolved iron from minerals with a simultaneous pH decrease. No relation between solubilized iron and final pH was found (cf. Tables 2 and 3). Initial solubilization may result from H ions interacting with organic metabolites and then complexing iron to prevent precipitation. The iron-dissolving capacity of each strain of microorganism is different. This specificity can be attributed to the different nature or concentration of metabolic products of each microorganism, or both of these factors. The action of the same microorganism on different minerals is dependent on the chemical and crystallographic characteristics of the mineral. For example, microorganisms dissolved more iron from biotite...
than from augite, both of which are monoclinic minerals. This would mean that for the same crystalline structure, iron extraction depends on its abundance in the mineral. Both hematite, which has a trigonal structure, and magnetite, which has a cubic structure, consist of only Fe and O combinations, and they liberated less iron than biotite and augite, although they contain a greater relative amount of it. This could indicate that iron solubilization from a mineral depends less on its abundance than on its crystalline structure and purity (13). These facts have importance in soil formation, because if a crystalline structure loses or exchanges some of its components, internal forces would be unbalanced, and subsequently disintegration would occur.

Table 4 shows the action of *A. niger* metabolites and basal medium upon Fe$^{3+}$. The basal medium complexed with iron. When the basal medium increased, the iron removed by the cation-exchange resin decreased, which resulted in more iron remaining in solution (8).

When the solubilizing abilities of *A. niger* filtrate grown in basal medium were compared with the uninoculated control, it was found that much more iron goes into solution when *A. niger* is present, which confirms that the iron-complexing action is due to the metabolic products of the fungus. The filtrate of *A. niger* with granodiorite has still higher iron-complexing ability. Since the cation-exchange resin does not extract iron solubilized by the microorganism, this iron must be present as an anionic or charge zero complex. The complexing action of the *A. niger*
metabolites was confirmed by the $R_F$ values obtained from TLC: iron aqueous solution = 0.06; basal growth medium = 0.08; filtrate from incubation without mineral = 0.14; and filtrate from incubation with mineral = 0.24. This proves the existence of one or more iron-complexing-compounds.

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