Weathering of Limestone, Marble, and Calcium Phosphate by Ectomycorrhizal Fungi and Associated Microorganisms

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【Summary】

Ectomycorrhizal fungi and their associated microorganisms were tested for their ability to weather limestone, marble, and calcium phosphate using a plate method. Degradation of these rock materials led to production of clear zones around the microbial colonies. A displacement method also was used to test selected microorganisms for their capacity to release calcium from the insoluble rock materials. Most of the fungi tested were ectomycorrhizal and associated with Douglas-fir (*Pseudotsuga menziesii*) or Scots pine (*Pinus sylvestris*). Most of the associated microorganisms tested were isolated from the mycorrhizal sporocarps, Douglas-fir ectomycorrhizas, or red alder (*Alnus rubra*) nodule surfaces. *Rhizopogon vinicolor, Suillus bovinus, Hysterangium setchellii*, a *Rhizopogon*-associated *Penicillium* sp. and a yeast were able to degrade limestone, marble, and calcium phosphate, as were 4 fluorescent pseudomonads and 3 *Azospirillum* isolates associated with *R. vinicolor* and Douglas-fir ectomycorrhizas. As a result of the rock solubilization, the pseudomonads released significantly more calcium than did other microorganisms. A possible role of a microbially mediated weathering process in nutrient cycling in rhizosphere ecosystems is discussed.

Key words: microbial weathering, biodegradation, microrrhizal fungi.

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外生菌根菌及其相關聯微生物風化石灰岩，大理石及磷酸鈣

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摘 要

本試驗利用洋菜固體平板法測定外生菌根菌株及與外生菌根菌相關聯的微生物包括細菌與真菌之風化溶岩能力。供測定的岩石有石灰岩、大理石及磷酸鈣。將測定之微生物培養於添加上述岩石粉末之培養基，如其菌落周圍形成透明圈表示該微生物具有溶岩能力。使用液體培養法測定可溶性鈣離子濃度以瞭解微生物之溶岩量。供試之外生菌根菌大多分離自花旗松與歐洲赤松的菌根或生長自菌根的子實體。大多數供試之相關聯微生物則分離自菌根菌之子實體，花旗松菌根或赤楊根瘤表面。下列供測定微生物具有風化分解石灰岩、大理石及磷酸鈣：*Rhizopogon vinicolor, Suillus bovinus,*

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Hysterangium setchelli，及分離自*R. vinicolor*子實體之*Penicillium* sp. 和酵母菌；同時，分離自*R. vinicolor*和花旗松菌根之4株螢光性細菌 pseudomonads及3株*Azospirillum*細菌也具有溶岩之能力。液體培養法顯示，以螢光性細菌pseudomonads之菌株具有較強的溶岩能力。本文同時討論微生物在根圈生態系統中溶岩作用對養分循環可能的角色。

關鍵詞：微生物風化作用、生物分解、菌根菌。

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INTRODUCTION

Biological weathering of soil rock minerals by roots and microorganisms plays a crucial role in maintaining supplies of inorganic nutrients for plants (Hinsinger *et al.*, 1991; Hinsinger *et al.*, 1992; Hinsinger and Gilkes, 1993; 1995; Illmer and Schinner, 1995; Illmer *et al.*, 1995; Li *et al.*, 1994; Silverman and Munoz, 1970). Some mycorrhizal fungi, *Laccaria laccata* (Scop: Fr.) Berk. & Br., *Hebeloma crustuliniforme* (Bull. Ex St Amans) Quel., *Pisolithus tinctorius* (Pers.) Coke. & Couch, and *Cenococcum geophillium* Fr., associated with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) also can form ectomycorrhizas with Scots pine (*Pinus sylvestris* L.) (Chakravarty and Unestam, 1987; Unestam and Stenstrom, 1989) which commonly grow on primary rock substrates in Sweden. Significant numbers of bacteria, yeasts, and filamentous fungi are found associated with these mycorrhizal sporocarps or ectomycorrhizas. Thus, these microorganisms are assumed to affect soil by rock-weathering processes and contribute to nutrient availability to and absorption by the mycorrhiza and translocation to the plants. In the present study, we sought to determine the rock weathering activity of these mycorrhizal fungi and their associated microorganisms through biological solubilization. To this end, we used plate and displacement methods on limestone, marble, and rock phosphate substrates to determine the ability of microbes to degrade and release calcium from these rock minerals.

MATERIALS AND METHODS

Specimens of limestone and marble (Cat. # 45E9239 and 45W 9246, respectively, Ward Natural Science Establishment, Inc., Rochester, NY) were each ground to pass through a 90-μm screen. Tricalcium phosphate (J. T. Baker, Inc., Phillipsburg, NJ) also was used. The materials were sterilized by anhydrous ethyl ether and each amended at 0.25% to a medium (Henderson and Duff, 1963) without soil extract: K_2HPO_4, 0.2 g; $(NH_4)_2SO_4$, 0.25 g; $FeCl_4$, 0.005 g; peptone, 0.5 g; yeast extract, 0.5 g; glucose, 5 g; Noble agar, 16 g; and 500 ml distilled water. The test microorganisms (Table 1) were each inoculated onto the center of the medium and incubated at room temperature (21-24℃). Halo production around the microbial colonies indicates the weathering process; the width of the halo zones was taken as a measure of the ability of the microorganisms to solubilize the rocks (Ehrlich, 1990).

Displacement methods as described by Henderson and Duff (1963) were used to detect the release of calcium after incubation of the rock minerals with microbes. Microbes were individually grown in liquid media, with fungi in modified Melin-Norkrans' (MMN; Marx, 1969), *Azospirillum* in Dobereriner's (Zuberer, 1987), and fluorescent *Pseudomonas* in King's (King *et al.*, 1954). The resulting microbial mass was harvested and washed 3 times with sterile distilled water. The total washed microbial mass from a flask was suspended in 25 ml of 8% glucose solution in a flask, to which 25 ml of 0.5% rock minerals in sterile distilled water was later added. Five flasks containing rock minerals were used for each microbe as replicates. Rock mineral solutions without microbes served as controls. After 4 wk of incubation on a shaker at room temperature (21-24 ℃), the cultured solutions were centrifuged (9000 ×g, 20 min) to remove microbes and unsolubilized

Table 1. Mycorrhizal fungi and associated microorganisms tested for rock weathering

Species and isolate number	Collected / isoloted from
Cenococcum geophilium, A145	*Pseudotsuga menziesii*
Hebeloma crutuliniforme, 8166	〃
Hysterangium setchellii, HS 206	〃
Laccaria laccata, 10440	*Abies lasiocarpa*
Piloderma croceum, 85.009	*Pinus syvestris*
Rhizopogon vinicolor, 11970	*P. menziesii*
Suillus bovinus, SB 24.6	*P. syvestris*
Azospirillum sp., Az-1	Sporocarp of *L. laccata*
Azospirillum sp., Az-2	Sporocarp of *R. vinicolor*
Azospirillum sp., Az-4	Douglas-fir ectomycorrhizae formed with *R. vinicolor*
Penicillium sp.	Sporocarp of *R. vinicolor*
Pseudomonas fluorescens, Ps-1[1)]	Douglas-fir ectomycorrhizae
P. fluorescens, Ps-2 [1)]	*Alnus rubra* nodule surface
P. fluorescens, Ps-3 [1)]	Sporocarp of *Chanterella tubaeiformis*
P. pudita, R20 [2)]	*Beta vulgaris*
Yeast	Sporocarp of *R. vinicolor*

[1)]Identified by the 2nd author.
[2)]Obtained from R. Osburn, University of California, Berkley.

rock minerals; the solutions were decanted and then filtered through 0.2-μm filters. The cultured solutions were analyzed for release of calcium from rock minerals by microbes with a flame atomic absorption spectrophotometer (Perk-in-Elmer 5000, Perkin-Elmer Corp., Norwalk, CT) (Li *et al.*, 1994). The amount of calcium released was expressed as milligrams of calcium released into 1 L of solution.

RESULTS AND DISCUSSION

Of the 7 ectomycorrhizal fungi tested, *Rhizopogon vinicolor, Suillus bovinus*, and *Hysterangium setchellii* degraded limestone, marble, and calcium phosphate, as indicated by halo formation on the plates (Table 2, Fig. 1). Other mycorrhizal fungi showed no detectable weathering activity. *Penicillium* sp. and a yeast, isolated from the sporocarp of *R. vinicolor*, were also able to solubilize these rock minerals. All 4 fluorescent *Pseudomonas* isolates and 2 nitrogen-fixing *Azospirillum* isolates showed positive degradation activity (Table 2).

Selected microbes were tested with the displacement method for their capacity to release calcium from these calcium-containing rock minerals. *Pseudomonas putida* (Trevisan) Migula and 3 *P. fluorescens* (Trevisan) Migula isolates released significantly more calcium than did the other microorganisms (Table 3, Fig. 2). *Penicillium* sp. and the yeast released moderate amounts of calcium while *R. vinicolor, S. bovinus*, and the 2 *Azospirillum* isolates released small amounts. *Laccaria laccata*, as in the results with the plate method, released a nondetectable level of calcium from these rocks.

The results of this study suggest that some mycorrhizal fungi and their associated microorganisms can break down or weather limestone, marble, and rock phosphate and release calcium from the rocks. Because the ectomycorrhizal fungi are major components of the rhizosphere

Table 2. Solublization of limestone, marble, and calcium phosphate as indicated by halo formation around microbial colonies

Species and isolate number	Lime-stone	Marble	Calcium phosphate
Cenococcum geophilium, A145	-[1)]	-	-
Hebeloma crutuliniforme, 8166	-	-	-
Hysterangium setchellii, HS 206	+	+	+
Laccaria laccata, 10440	-	-	-
Piloderma croceum, 85.009	-	-	-
Rhizopogon vinicolor, 11970	+	+	+
Suillus bovinus, SB 24.6	+	+	+
Azospirillum sp., Az-1	-	-	-
Azospirillum sp., Az-2	+	+	+
Azospirillum sp., Az-4	+	+	+
Penicillium sp.	+	+	+
Pseudomonas fluorescens, Ps-1	+	+	+
P. fluorescens, Ps-2	+	+	+
P. fluorescens, Ps-3	+	+	+
P. pudita, R20	+	+	+
Yeast	+	+	+

[1)]+: positive solubilization; -: solubilization not detected.

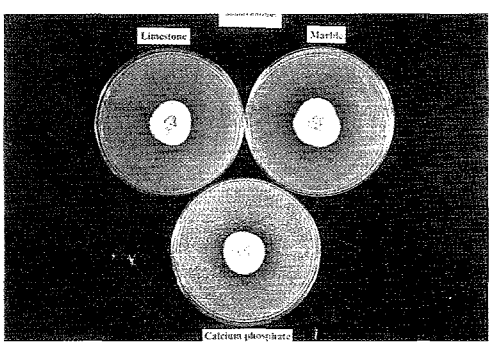

Fig. 1. Decomposition of limestone, marble, and calcium phosphate by *Suillus bovinus* as indicated by halo formation around the fungal growth.

and form a symbiotic relationship with Douglas-fir and Scots pine, they may play a crucial role in maintaining the supply of calcium and possibly other elements such as phosphorus from relatively insoluble minerals in the soil. Leyval *et al.* (1990)

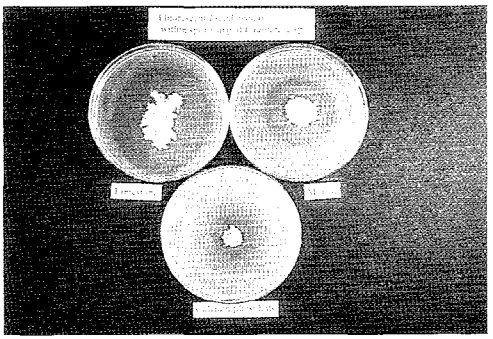

Fig. 2. Decomposition of limestone, marble, and calcium phosphate by fluorescenet Pseudomonas as indicated by halo formation around the colony.

reported that inoculation with mycorrhizal fungi or with rock-dissolving bacteria promotes release of cations from micas in pine and beech rhizospheres; inoculation with both microorganisms enhances mobilization of elements in these 2 treerhizospheres. Thus, synergistic effects of mycorrhizal fungi plus associated microorganisms may potentially increase nutrient availability for Douglas-fir and Scots pine.

Organic acids produced by soil microor-ganisms, including mycorrhizal fungi, enhance the weathering of primary rock minerals (Hiebert and Bennett, 1992; Leyval *et al.*, 1990; Watteau and Berthelin, 1994) by chelating cations from minerals. Production of siderophores by soil microorganisms mobilizes iron from otherwise insoluble ferric oxides and oxyhydroxides (Watteau and Berthelin, 1990; 1994). The production of organic acids and/or siderophores by these rhizosphere microorganisms, however, needs to be explored. Plant roots under axenic conditions also can induce weathering of minerals and contribute significantly to the supply of cations to plants (Hinsinger *et al.*, 1991; Hinsinger *et al.*, 1992; Hinsinger and Gilkes, 1993; 1995) probably due to rhizosphere acidification by proton excretion from plant roots. Combined induced weathering processes by plant roots and soil microbes may thus be responsible for transformation of mineral structure, removing cations from the minerals for rapid uptake by plants.

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Table 3. Calcium (mg-l^{-1}) released into cultural solutions after incubating limestone, marble, or calcium phosphate with selected mycorrhizal fungi and associated microorganisms[1)]

Species and isolate number	Limestone	Marble	Calcium phosphate
Laccaria laccata, 10440	30 a[2)]	39 a	7 a
Rhizopogon vinicolor, 11970	54 b	73 b	14 b
Suillus bovinus, SB 24.6	50 b	60 b	11 b
Azospirillum sp., Az-2	51 b	72 b	12 b
Azospirillum sp., Az-4	54 b	66 b	10 b
Penicillum sp.	154 c	210 c	45 d
Pseudomonas fluorescens, Ps-1	420 d	624 e	91 e
P. fluorescens, Ps-2	508 e	548 d	106 f
P. fluorescens, Ps-3	516 e	679 ef	107 f
P. pudita, R20	544 ef	749 f	108 f
Yeast	124 c	193 c	40 d
Control	34 a	38 a	7 a

[1)] Incubated for 4 wk at 21-24℃.

[2)] Means in each column followed by the same letter are not significantly different at $P = 0.05$, according to Duncan's multiple range test. Data are averages of 5 replicates.

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