Calcium Oxalate Biomineralization by *Piloderma fallax* in Response to Various Levels of Calcium and Phosphorus

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*Piloderma fallax* is an ectomycorrhizal fungus commonly associated with several conifer and hardwood species. We examined the formation of calcium oxalate crystals by *P. fallax* in response to calcium (0.0, 0.1, 0.5, 1, and 5 mM) and phosphorus (0.1 and 6 mM) additions in modified Melin-Norkrans agar medium. Both calcium and phosphorus supplementation significantly affected the amount of calcium oxalate formed. More calcium oxalate was formed at high P levels. Concentrations of soluble oxalate in the fungus and medium were higher at low P levels. There was a strong positive linear relationship between Ca level and calcium oxalate but only under conditions of phosphorus limitation. Calcium oxalate crystals were identified as the monohydrate form (calcium oxalate monohydrate [COM] whewellite) by X-ray diffraction analysis. Prismatic, styloid, and raphide forms of the crystals, characteristic COM, were observed on the surface of fungal hyphae by scanning electron microscopy. *P. fallax* may be capable of dissolving hyphal calcium oxalate under conditions of limited Ca. The biomineralization of calcium oxalate by fungi may be an important step in the translocation and cycling of Ca and P in soil.

Many fungi from forest litter, including ectomycorrhizal fungi, exhibit calcium oxalate (CaOx) crystals on their hyphae. The ubiquity of CaOx crystals on fungal hyphae suggests that their formation may provide a selective advantage to the organism (4). CaOx formation is hypothesized to regulate intracellular pH and levels of oxalate and Ca and, hence, serves as a major sink for toxic amounts of Ca in soil and other environments (52, 53, 61). In plants, CaOx crystals have also been proposed to serve as a calcium source under conditions of calcium limitation (14, 18, 41), but such a process has yet to be established among fungi.

CaOx on fungal hyphae is formed from soil-derived calcium and biologically synthesized oxalate. Oxalate released by ectomycorrhizae has been correlated with increased phosphorus bioavailability in the rhizosphere (V. Casarin, cited by Hinsinger in reference 25). The ability of oxalate to chelate metal ions makes it important in the solubilization and transport of metals in soil, the weathering and diagenesis of rocks and soil minerals (9, 23, 31, 57), and, consequently, the transport of nutrients. It is generally presumed that CaOx crystals form on the surface of fungal hyphae as a result of precipitation when released oxalic acid interacts with calcium cations (23, 43). However, the regularity of the CaOx crystals suggests that their formation is regulated and that they may be formed within the fungal hyphae at specific sites of origin (3, 5, 7).

CaOx crystals vary in morphology, ranging from plates to raphides, druses, tetragonal bipyramids, and prisms. This variation in morphology can be seen among fungal genera and species (4). The crystals also usually occur either as CaOx monohydrate (COM; whewellite) (29) or CaOx dihydrate (weddellite) (3, 5, 28, 35, 60). Either crystal form or both may be present on fungal hyphae at the same time.

In earlier studies (8, 9), we reported that *Piloderma fallax* is one of the major species of ectomycorrhizal fungi in subboreal forests. In addition, *Piloderma* sp. is found in temperate forest soils in association with conifer and hardwood species (34). *Piloderma* influences nutrient uptake and modifies mineral transformation in rock and soil systems (3, 33). In this study, we chose *P. fallax* because of (i) its ability to produce oxalate and form CaOx crystals (8, 56), (ii) its presence in many types of forest ecosystems, and (iii) its significant role in the breakdown and formation of soil minerals (9).

The objective of this study was to quantify and characterize the formation of CaOx by *P. fallax* in response to various P and Ca levels in agar medium. We tested the hypothesis that P limitation will induce the production of oxalate and that increased concentrations of Ca will result in greater CaOx formation. This study also examined the dissolution of CaOx on *P. fallax* when it is grown on Ca-deficient medium and determined whether CaOx can serve as temporary Ca storage. Our study was conducted to add to knowledge of the ecological significance of CaOx, especially of its influence in biogeochemical cycling of P and Ca in soils.

**MATERIALS AND METHODS**

Fungal culture. *P. fallax* UAMH9439 was maintained in the dark at 25°C on modified Melin-Norkrans (MMN) medium (39). Stock cultures were grown on MMN medium with a soluble P concentration of 0.1 mM for 4 weeks prior to inoculation to the test medium, MMNb. Base MMNb medium contained 3 g liter⁻¹ malt extract, 10 g liter⁻¹ glucose, 15 g liter⁻¹ agar, 0.1 μg liter⁻¹ thiamine HCl, 0.025 g liter⁻¹ NaCl, 0.202 g liter⁻¹ NH₄Cl, 0.274 g liter⁻¹ KCl, 0.15 g liter⁻¹ MgSO₄·7H₂O, and 0.012 g liter⁻¹ FeCl₃·6H₂O; CaCl₂ and KH₂PO₄ were added to the base MMNb medium to obtain 10 test media (M1 to M10) containing different concentrations of calcium and phosphate (Table 1). Some medium components were filter sterilized/autoclaved separately and combined after autoclaving to prevent the thermal degradation of glucose/thiamine-HCl and the precipitation of insoluble phosphates. The pH of the test medium was adjusted to 5.5 after autoclaving.
Aliquots of 25 ml of the medium were dispensed into 90-mm petri dishes. A sterile dialysis membrane was placed on top of each solidified medium to facilitate easy removal of the fungal colony (49). Five-mm fungal plugs were cut from the edges of 1-month-old fungal colonies and deposited at the center of each test plate. The plates were incubated in the dark at 25°C for 3 months. Fungal colonies were then harvested and dried at 70°C for 48 h. The agar medium underneath each fungal colony and extending outward by 3 mm was also collected and dried. There were five replicates for each test medium.

In order to test if CaOx crystals on hyphae are dissolved when the Ca supply is limiting, P. fallax was grown on a dialysis membrane on MMNb medium with 5 mM CaCl2 and 6 mM H3PO4. After 2 months, five fungal colonies were harvested for analysis of the “initial” content of calcium, oxalate, and CaOx. The rest of the fungal colonies were immediately transferred to fresh MMNb medium supplemented with 5, 0.5, and 0.0 mM CaCl2 and incubated for 4 months at room temperature. The experiment was replicated five times.

Chemical analyses. Soluble and total oxalate in mycelia and agar were extracted using water (Millipore Milli-Q water was used throughout the study) and 60% HNO3 (OmniTrace). Each sample was centrifuged, and the supernatant was filtered through a 0.20-m-pore-size nylon (for soluble oxalate) or 1.5 M HCl (for total oxalate) and heating for 10 min in a boiling water bath. Both H2SO4 and H3PO4 failed to extract CaOx, and H2SO4 as the mobile phase, a column temperature of 45°C, and a UV detector at a 200-nm wavelength. Oxalate peaks were identified based on the retention time. A solution of 2 M HCl, on the other hand, rapidly dissolved CaOx. The extraction method involved homogenizing the sample in either water (for soluble oxalate) or 1.5 M HCl (for total oxalate) and heating for 10 min in a boiling water bath. HCl-treated samples were further diluted to 0.5 M and centrifuged, and the centrifugate was filtered through a 0.20-μm-pore-size nylon filter prior to high-performance liquid chromatography (HPLC) analysis.

An HPLC Bio-Rad Aminex HPX-87H column (300 by 7.8 mm) was used for the identification and quantification of oxalate. Samples were diluted to a final concentration of 0.025 M HCl before injection into the HPLC column. Separation and detection were carried out at a flow rate of 0.7 ml min−1 using 0.008 N H2SO4 as the mobile phase, a column temperature of 45°C, and a UV detector set at a 200-nm wavelength. Oxalate peaks were identified based on the retention time of a standard solution of oxalate alone and oxalate spiked into unknown samples. Preliminary runs of standard solutions revealed that retention times and peak areas differed between standard solutions in water and dilute acid. For this reason, separate standard curves were used for water and acid extracts. Additionally, standard and sample solutions were prepared following similar methods and conditions.

Oxalates occur as free acids; as soluble salts of K, Na, and Mg; and as insoluble salts of Ca (19). CaOx concentration was calculated based on the concentrations of total and soluble oxalate (27) as follows: the g of insoluble oxalate = g of total oxalate − g of soluble oxalate and g of CaOx = g of insoluble oxalate × 1.45, where 1.45 is the factor of conversion of oxalate (CaC2O4) to CaOx (Ca(C2O4)), Total Ca and P amounts in fungal biomass were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion with 70% HNO3 (Omnitrace).

Morphology and identification of crystals. CaOx crystals were identified by powder X-ray diffraction (XRD) analysis using a Bruker D8 diffractometer with a general area detector diffraction system. Isolation of CaOx was done by dissolving the fungus in NaOCl and repeated washings in water and methanol (21, 40). Sodium hypochlorite was chosen to dissolve organic matter because other chemical reagents, specifically H2O2, have been reported to transform organic carbon into oxalate (24, 38). It was confirmed that organic matter dissolution by NaOCl did not yield CaOx by trial extractions on CaOx-free fungal mycelia collected by pretreatment with acid to remove any existing CaOx. XRD analysis of these test samples, after NaOCl dissolution, did not detect any CaOx crystals.

Freeze-dried fungal samples and NaOCl-isolated crystals used in XRD analysis were examined using a Phillips XL30 scanning electron microscope (SEM) with an EDAX energy-dispersive X-ray spectrometer (EDS). The fungal samples were mounted on Al stubs and sputter-coated with Au for examination of crystal morphology and distribution. The elemental composition of NaOCl-isolated crystals was determined by SEM-EDS analysis. We were unsuccessful in doing SEM-EDS analysis of individual crystals on the hyphae because of drifting at high magnifications, making it impossible to keep the electron beam focused on the same spot for the entire length of the scan. Additionally, the hyphae were subject to beam damage at the larger electron beam spot size required for SEM-EDS analysis.

Statistical analyses. All data were analyzed using SPSS 16. The normality of distribution and homogeneity of variances were assessed using the Shapiro-Wilk and Levene’s tests, respectively. Logarithmic transformation of data was done when necessary. The transformed data were analyzed by two-way analysis of variance with calcium and phosphorus levels as the independent variables. Differences between treatments were determined by Tukey’s honestly significant differences test. Data that did not fit a normal distribution or whose variances were not homogeneous after transformation were analyzed using Kruskal-Wallis and Mann-Whitney U tests. Correlations between variables were determined using Spearman’s coefficient. Differences were considered statistically significant at a P value of <0.05.

### RESULTS

CaOx production. At 0.1 mM P, the CaOx concentration was significantly higher at 5 mM Ca than at the lower Ca concentrations (P = 0.008) (Fig. 1). There were no significant differences in CaOx concentrations across all five calcium levels at 6 mM P while total oxalate concentration at 5 mM Ca was significantly higher than the total oxalate concentration only at 0 mM Ca. CaOx amounts were always higher at 6 mM P than at 0.1 mM P. At lower Ca levels, CaOx concentrations were, at most, over than 100-fold higher at 6 mM P than at 0.1 mM P (0.44/50.93 [0.1/6 mM P] μmol g−1 CaOx at 0.1 mM Ca).

In contrast to CaOx concentrations, the concentrations of soluble oxalate content in P. fallax and in the medium were both higher at 0.1 mM P than at 6 mM P at all Ca levels. The concentrations of both soluble oxalates in the fungus and in the medium were >40% higher at 0.1 mM than at 6 mM P. There were no significant differences among soluble oxalate concentrations across Ca levels at 0.1 and 6 mM P.

Both Ca and P levels had a significant effect on the total calcium content of P. fallax (P = 0.000) while only P had a significant effect on fungal biomass P content (P = 0.0000) (Fig. 2). The interaction between Ca and P levels was not statistically significant (P = 0.137). Total calcium content was higher at 6 mM than at 0.1 mM P and increased with increasing Ca levels at both P concentrations (Fig. 2A). Conversely, P content was higher at 0.1 mM than at 6 mM P with no significant differences across Ca levels (Fig. 2B). Fungal biomass was higher at 6 mM than at 0.1 mM P. There was little difference in the fungal biomass across Ca levels at 0.1 mM P and no significant difference at 6 mM P (Fig. 2C).

The CaOx content of P. fallax was negatively correlated to the concentrations of soluble oxalate in the fungus (Spearman’s r = −0.415; P = 0.01), soluble oxalate in the medium (r = −0.390; P = 0.01), and fungal biomass P (r = −0.364; P = 0.05) but positively correlated to total Ca in the fungus (r = 0.665; P = 0.01). Fungal biomass P was positively correlated to both soluble oxalate in the fungus (r = 0.694; P = 0.01) and soluble oxalate in the medium (r = 0.816; P = 0.01). There was no correlation between fungal biomass and P, CaOx, and soluble oxalate.

Table 2 shows the fungal biomass and CaOx formed when P. fallax colonies grown in 5 mM Ca–6 mM P for 2 months were then transferred to fresh medium containing equal or less Ca to investigate the possible utilization of CaOx during Ca limitation. Biomass (dry weight) increased at all Ca concentra-

<table>
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<th>Supplement</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
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<td>1</td>
<td>5</td>
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<tr>
<td>H2SO4</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>1</td>
<td>5</td>
<td>0</td>
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### Table 1. Concentrations of CaCl2 and H2SO4 included in MMNb test media

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tions, with no significant differences between means. CaOx and total Ca increased at 5 mM Ca but decreased in media containing 0.5 and 0 mM Ca, with the lowest concentrations observed at 0 mM Ca. While transfer to 0.5 mM Ca showed a decrease in CaOx per unit of biomass (35.00 ± 6.17 μmol per g), there was, in fact, an increase in CaOx from 4.17 ± 0.63 to 5.48 ± 1.02 μmol (Table 2). However, CaOx did not increase to the same extent as the biomass (175% increase to 155.5 ± 5.6 mg), resulting in a lower reported value of CaOx per unit of biomass. The Ca concentration per unit of biomass was positively correlated to the CaOx per unit of biomass (Spearman’s ρ = 0.812; P = 0.01).

**Morphology and identification of hyphal encrustations.** Hyphal encrustations were abundant on *P. fallax* grown in 6 mM P at all levels of Ca (Fig. 3a to g). In contrast, hyphal encrustations at 1 mM Ca–0.1 mM P were also abundant but appeared to be in an early stage of development (Fig. 3i). At Ca concentrations below 1 mM at 0.1 mM P, observed encrustations (also at early stage of development) ranged from none to very few.

Prismatic and elongated crystals were observed on hyphae (Fig. 3g). Elongated crystals may be flat and broad and arranged parallel to the hyphal surface (Fig. 3a, b, d, h, and i) or may be raphides tangentially protruding from the hyphae (Fig. 3e). The first type of elongated crystals ranged from 1.0 to 2.2 μm long although thinner and shorter forms could be seen, which appeared to be crystals in early stages of development (Fig. 3a and d). These early forms can be seen even on hyphae that were already heavily encrusted with long crystals (Fig. 3b and h). Some elongated crystals were found on hyphae that were also covered with prismatic crystals (Fig. 3e, f, and g). SEM-EDS analysis of crystals (isolated by the NaOCl method) produced spectra high in calcium (Fig. 3j). XRD analysis confirmed the presence of CaOx (Fig. 4). However, only the monohydrate form (whewellite) was found. Spot-scanning of individual crystals for SEM-EDS analysis was tried but was not successful because of the tendency of the sample to drift at very high magnifications. Area scanning was considered as an alternative, but it, like spot scanning, yielded combined scans of both crystal and hyphae because it was not possible to find areas where there would be no hyphae around or underneath the crystals.

**DISCUSSION**

In soils, phosphorus availability is low because of the higher amount of P bound to soil minerals (46). The exudation of organic anions is one of the strategies that plants may adapt to deal with a limited P supply (47). Our results appear to indicate a similar strategy in *P. fallax* as fungal biomass P and the production and release of soluble oxalate by the organism increased under limited P availability. Previous studies in plants have shown that P deficiency is associated with enhanced exudation of organic acids that are involved in the solubilization and desorption of P from minerals (26, 32, 47, 58). Increased exudation of organic anions from P-deficient plants has been associated with enhanced activities of enzymes involved in organic acid synthesis and decreased activities of enzymes involved in organic acid catabolism (30, 42). The association of organic acid exudation and P deficiency suggests that P-deficient conditions activate both organic anion and P transporters. The associated influx of P i may be a means to restore internal balance and membrane potential stability during the release of organic anions (44, 45).

The kind and amount of organic acids secreted seem to be dependent on the fungal and plant species. Pure cultures of the
ectomycorrhizal fungus *Paxillus involutus* produced 22 µmol of malonate g⁻¹ of mycelium but very little to no oxalate under conditions of limited P (10, 56). *Pinus sylvestris* colonized by *Hebeloma longicaudum* secreted more malonate (226 µM) than oxalate (44 µM) while seedlings colonized by *P. involutus* secreted more oxalate (310 µM) than malonate (38 µM) under limited P conditions (56). The legume pigeon pea [*Cajanus cajan* (L.) Millsp.] is normally intercropped with cereals to increase total P availability. It can utilize Fe-bound phosphorus, and this ability has been attributed to the exudation of piscidic acid and its *p*-O-methyl derivative which release P by chelating Fe³⁺ (1).

Soluble oxalate concentration was lower at 6 mM P than at 0.1 mM P, probably because most of the oxalate was present in bound form (as CaOx). The higher amount of CaOx may account for the higher fungal biomass observed. The lower soluble oxalate concentration in the medium and P in fungal biomass indicate that enhanced transport of organic acid and P was not triggered under conditions of high P levels. Less CaOx at 0.1 mM P coupled with a higher soluble oxalate (fungus) concentration may indicate that CaOx formation in fungi is not simply a passive precipitation process of calcium and oxalate. The fungus can control how soluble oxalate is utilized. At low P concentrations, release of oxalate is favored over CaOx formation, perhaps to aid the fungus to sequester any available P in the growth medium.

The high CaOx concentration at 5 mM Ca even at low P levels supports the hypothesis that CaOx formation is involved in regulating Ca levels. Changing properties of CaOx crystals in response to Ca levels have been reported in previous studies in plants (20), but the process has yet to be studied in the case of fungi. Oxalic and other carboxylic acids can serve as ligands that complex with metal cations. These complexation reactions may be affected by pH. Citrate and oxalate, for example, can complex with Fe³⁺ at a pH of <4. At pH 7, oxalate does not form any complex while citrate still exhibits some complexation activity (32). In general, the number of charged carboxylate groups determines the stability constant of the metal complexes. Acetate, a monocarboxylate common or dominant in some soils (11, 50, 55), forms a complex with Ca²⁺ with a stability constant of only 0.93 while the dicarboxylic oxalate has a higher stability constant at 3.27 (16). The tricarboxylic citrate forms a complex with Ca²⁺ with a stability constant of 4.85, but Ca-oxalate, unlike Ca-citrate, has a tendency to precipitate (32).

The ease of forming CaOx presents an advantage for plants in nutrient (e.g., P) acquisition. Apatite, Ca₅(PO₄)₃(F, Cl, OH), is a common source of inorganic phosphate in soil (37). Oxalate complexes with Ca²⁺ on apatite, weakening the metal-oxygen bonds and releasing P into the soil solution (32, 54). Apatite dissolution experiments with oxalate show small amounts of Ca released despite large amounts of released P, suggesting CaOx formation. SEM analysis confirmed CaOx crystals on the apatite surface (22, 59).

The final total CaOx content of *P. fallax* in 5 mM Ca medium was higher than the initial concentration (Table 2), evidently because the final agar medium contained the same optimum levels of Ca and P for CaOx production. At 0.5 mM Ca, there was no significant difference between the initial and final CaOx per total weight of fungal biomass. The calcium level in the medium may have been sufficient to maintain cell function but did not enhance CaOx production. Final CaOx (per total weight of fungal biomass) decreased at 0.0 mM Ca from 6.1 to 2.8 µmol, suggesting the dissolution of previously formed CaOx. CaOx formation had previously been demonstrated to

![FIG. 2. Calcium content (A), phosphorus content (B), and fungal biomass (C) of *P. fallax* grown with various Ca levels at 0.1 and 6 mm P. Bars are standard errors of the means (n = 4 or 5). For each analyte, bars with the same letter are not significantly different (P > 0.05). In panel A, the letters above the bars compare the values for the sum of calcium in fungus and CaOx.](http://aem.asm.org/)

secreted more oxalate (310 µM) than malonate (38 µM) under limited P conditions (56). The legume pigeon pea [*Cajanus cajan* (L.) Millsp.] is normally intercropped with cereals to increase total P availability. It can utilize Fe-bound phosphorus, and this ability has been attributed to the exudation of piscidic acid and its *p*-O-methyl derivative which release P by chelating Fe³⁺ (1).
be a reversible process in duckweed *Lemna minor* L. when polarizing microscope examination showed the formation and disappearance of CaOx crystals in medium with high and 0.0 concentrations of Ca, respectively (18). It has been suggested earlier that CaOx in plants may provide a reserve of Ca for conditions when Ca is limiting for growth (6, 23). We believe that our results are the first to document the possible utilization of Ca in CaOx crystals by a fungal species. Future studies (e.g., radiolabeled C and Ca) can investigate the mechanism of dissolution of hypha-bound CaOx and the fate of Ca.

Examination of fungal hyphae by SEM showed that the abundance of CaOx crystals was dependent on the Ca level but only under conditions of limited P. At high P concentrations, CaOx was abundant at all levels of Ca except at 0.0 Ca (Fig. 3). A similar dependency on Ca supply was observed in bird’s nest fungi by Shinners and Tewari (51). Calcium oxalate crystals on

<table>
<thead>
<tr>
<th>Condition and CaCl₂ concn in medium (mM)</th>
<th>Fungal biomass (mg)</th>
<th>CaOx (µmol g⁻¹ of fungus)</th>
<th>Total CaOx (µmol)ᵇ</th>
<th>Ca (µmol g⁻¹ of fungus)ᶜ</th>
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<tbody>
<tr>
<td>Before transferᵈ</td>
<td>56.6 ± 6.5</td>
<td>73.23 ± 5.33</td>
<td>4.17 ± 0.63</td>
<td>95.95 ± 15.41</td>
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<tr>
<td>After transfer</td>
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</tr>
<tr>
<td>5</td>
<td>163.1 ± 10.3 A</td>
<td>259.00 ± 5.45 A</td>
<td>42.22 ± 2.71 A</td>
<td>239.73 ± 6.59 A</td>
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<tr>
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<td>35.00 ± 6.17 B</td>
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<td>94.30 ± 7.28 B</td>
</tr>
<tr>
<td>0</td>
<td>169.0 ± 7.7 A</td>
<td>11.30 ± 3.46 B</td>
<td>1.93 ± 0.64 B</td>
<td>29.07 ± 1.07 C</td>
</tr>
</tbody>
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ᵃ Data are the averages of five replicates. Means with different letters are significantly different (P < 0.05).
ᵇ Calculated from data in the second and third columns.
ᶜ Total Ca was determined by ICP-MS after digestion of fungi with HNO₃. Thus, total Ca is the amount of Ca in the biomass plus any CaOx present in the biomass.
ᵈ Cultures were grown for 2 months on 5 mM CaCl₂ before transfer to medium containing various concentrations of CaCl₂.

FIG. 3. SEM and SEM-EDS examination of encrusted hyphae of *P. fallax*. Ca and P levels (mM) are noted. (a to g) Crystals are seen in various stages of development with 6 mM P. Both verrucose and elongated structures were observed. At 0.1 mM P (h and i) abundant encrustations are seen only at 5 mM Ca. Hyphae at other Ca concentrations had encrustation at an early stage of development similar to panel i. (j) EDS of isolated crystals showed that they are composed mainly of calcium.

TABLE 2. Fungal biomass, calcium, and CaOx content of *P. fallax* grown in media with various CaCl₂ concentrations*
P. fallax were prismatic or elongated, with the elongated crystals varying in thickness, shape, and length. The elongated crystals at their early development stage are similar to those found on Armillariella tabescens (4). However, the shape of the elongated crystals on P. fallax is different from shapes observed in other Piloderma species (8). The different shapes of elongated crystals may represent different stages of crystal development, with crystals starting out as small globular particles on the hyphae, growing into the wider elongated forms, and becoming dehydrated into raphide crystals (57).

The significance of CaOx formation in ectomycorrhizal fungi extends beyond its physiological role in fungi to its contribution to plant host nutrition and geochemical processes. Ectomycorrhizal colonization presents a nutrient acquisition advantage to the tree host as colonized seedlings can produce as much as seven times more oxalic acid than nonmycorrhizal seedlings (2). As shown in our results, the production of large amounts of soluble oxalate in low-P treatments may improve the availability of P through chelation, thus favoring high fungal growth. Fungal hyphae form an extensive network of mycelia throughout the soil and facilitate transport of limited resources to the roots. Nutrients (e.g., P) can be translocated to other host plants that are connected to the mycelial network (17), and reciprocal transfer of nutrients can occur between the interacting hyphae of ectomycorrhizal and saprophytic fungi (36). Our study also shows that CaOx on fungal hyphae is an active Ca reserve for fungal use under conditions of low Ca availability. The important role of oxalate in P mobilization and the storage of Ca in CaOx ensure a steady supply of these two essential nutrients to maintain vigorous growth of fungi that are active in the breakdown of primary soil minerals (33, 9). In addition, CaOx is a C reservoir that can be oxidized by oxalotrophic soil bacteria, forming CaCO3 in the process and providing a C sink for atmospheric CO2 (12, 13).

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