Calcium oxalate biomineralization by *Piloderma fallax* in response to Ca and P in solid medium

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Introduction

Calcium oxalate on fungal hyphae is formed from soil-derived calcium and biologically synthesized oxalate. Oxalate production may be enhanced by nutrient (e.g., P) deficiency while its precipitation with calcium may be a means of regulating intracellular calcium.

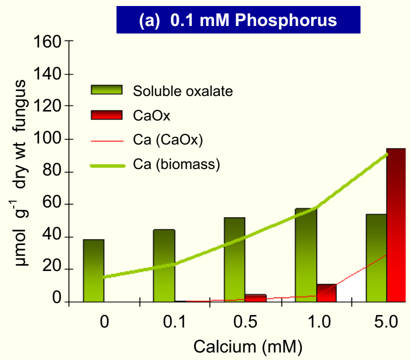
In ectomycorrhizal fungi that also form an extensive network of mycelia throughout the soil, calcium oxalate formation enables the translocation and cycling of calcium. Calcium oxalate can later be oxidized by oxalotrophic bacteria to release carbonate ions that precipitate calcite (calcium carbonate) in soil, representing a potential major carbon sink.

In this study, we quantified and characterized calcium oxalate formed by *Piloderma fallax* in response to varying P and Ca levels in solid medium. This is part of a project to investigate the formation and degradation of calcium oxalate by soil fungi and bacteria.

Materials and Methods

- Test organism - *Piloderma fallax* UAMH9439, a basidiomycete that forms ectomycorrhizal associations with several varieties of conifer and hardwood species
- *P. fallax* was grown in agar media containing 0, 0.1, 0.5, 1, and 5 mM Ca ($CaCl_2$) and 0.1 and 6 mM P (KH_2PO_4).
- Calcium oxalate content was calculated from the concentrations of soluble and total oxalate quantified by HPLC.
- The distribution, morphology and hydration type of calcium oxalate were examined by scanning electron microscopy, energy dispersive X-ray microanalysis, and X-ray diffractometry .

Results



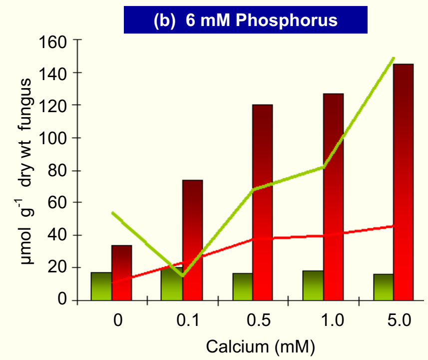


Fig. 1. Comparison of oxalate, calcium oxalate and calcium content of *Piloderma fallax* grown in varying Ca levels at 0.1 and 6 mM P.

Soluble oxalate content was relatively constant across Ca levels at both P concentrations while CaOx content increased as Ca levels increased.

Higher soluble oxalate content was associated with low P concentration. CaOx content and biomass Ca were higher at 6 mM than at 0.1 mM P.

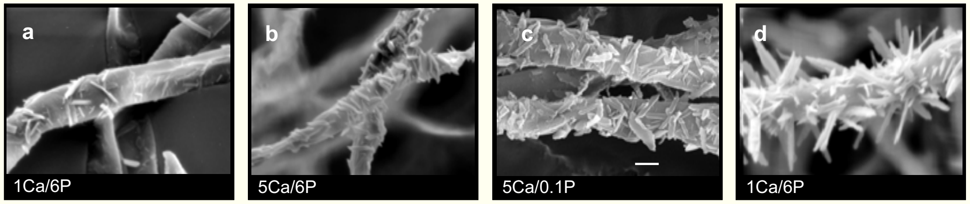
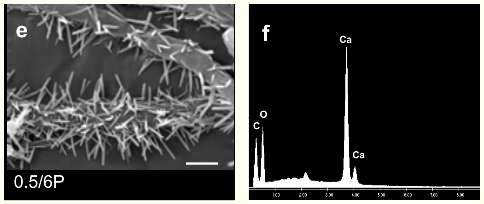


Fig. 2. SEM and SEM-EDS examination of encrusted hyphae of *P. fallax*. Ca and P levels are noted.
(a, b, c) Crystals in various stages of development.
(c, d, e) Three different types of crystals on *P. fallax*
(f) Energy dispersive X-ray analysis of isolated crystals showed that they are composed mainly of calcium
Bar = 1 µm



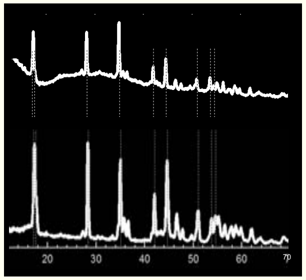


Fig. 3. X-ray diffraction of crystals from *P. fallax*.
Crystals isolated from *P. fallax* were identified as calcium oxalate monohydrate. Other crystal types were not detected by XRD and SEM examination.

Conclusions

- Calcium oxalate concentration increased with increasing calcium. Higher phosphorus level resulted in greater production of calcium oxalate.
- Soluble oxalate content did not vary with calcium level. Accumulation is favoured by low phosphorus level.
- Calcium oxalate formed as raphide and elongated flat crystals on *P. fallax* hyphae. Isolated calcium oxalate was identified as whewellite (monohydrate type).

Acknowledgments

Canada Research Chairs

NSERC CRSNG Natural Sciences and Engineering Research Council of Canada

Author's current research projects

The influence of microbial interactions and processes on the weathering and formation of soil minerals

Soil microorganisms play vital roles in both the physical and chemical processes involved in mineral weathering and formation in soil. One way by which soil organisms, in general, can influence weathering is by the production of acids, ligands, and extracellular polymers that interact with mineral surfaces. Our research focuses on low molecular weight organic acids produced by ectomycorrhizae and ectomycorrhizosphere bacteria. These organisms have received much attention because of their role in the diagenesis of minerals and soil formation as well as for their potential in reforestation, soil bioremediation, and plant nutrition management. Some of our studies are carried out using individual organisms while some look at the effect of interactions among different organisms on mineral weathering and formation. The multitude and diversity of microorganisms in soil are expected to result in diverse and complex manners of microbe-microbe and microbe-mineral interactions. For this reason, understanding rhizosphere processes that lead to mineral weathering/formation necessitates looking at microbial interactions.

- **Influence of ectomycorrhizal organic acids and ectomycorrhizosphere bacteria on soil mineral weathering**

  Pinus contorta and several species of ectomycorrhizal fungi (*Piloderma fallax, Paxillus* sp*., Cenococcum geophilum)* are used in *in vitro* experiments to determine differences in organic acid production and mineral dissolution by ectomycorrhizae, non-colonized roots, and free-living fungi. These *in vitro* experiments also look at the effect of ectomycorrhizosphere bacteria on organic acid production and mineral weathering by ectomycorrhizae. Mineral dissolution is analyzed by monitoring released cations, plant/fungal nutrient uptake, physical changes in minerals, and changes in the composition and distribution of elements in minerals.

- **Formation and degradation of calcium oxalate**

  This study focuses on one of the most commonly produced organic acid by soil microorganisms – oxalic acid – and the associated production of calcium oxalate crystals by these organisms. Many fungi, including ectomycorrhizal fungi, exhibit calcium oxalate crystals on their hyphae. The ubiquity of these crystals on fungal hyphae suggests that they present a selective advantage to the organism. Several hypotheses on the functions that these crystals play have been proposed including regulation of pH and Ca levels as well as serving as Ca reservoir under Ca-limited conditions.

  Our study focuses on some factors that may affect calcium oxalate crystal formation by *Piloderma fallax*. We are interested in finding out how variations in the morphology, distribution, amount, and type of calcium oxalate on fungal hyphae are affected by calcium and phosphorus levels in the medium. Part of the study is devoted to finding out if fungi can utilize the calcium oxalate crystals on their hyphae for growth under conditions of calcium deficiency. We are also looking at the degradation of oxalate and calcium oxalate by oxalotrophic bacteria and how the presence of these bacteria will affect the formation of calcium oxalate crystals by the fungus.

<u>**About the author**</u>

Lizette Tuason is a Ph. D. student (Natural Resources & Environmental Studies) at the University of Northern British Columbia. Prior to her move to Canada, she worked as Research Associate and, later, Assistant Professor at the University of the Philippines Los Baños where she also obtained her B. S. Biology (major in microbiology) and M.S. Microbiology degrees.

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