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Degradation of Serpentine and Muscovite Rock Minerals and Immobilization of Cations by Soil *Penicillium* Spp.

By

R. H. CRAWFORD*), M. FLOYD**) & C.Y. LI***)

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Summary

CRAWFORD R. H., FLOYD M. & LI C. Y. 2000. Degradation of serpentine and muscovite rock minerals and immobilization of cations by soil *Penicillium* spp. – Phyton (Horn, Austria) 40 (2): 315–322. – English with German summary.

Two *Penicillium* species were isolated from the soil of two seed orchards on the Siskiyou National Forest in southwest Oregon, where magnesium-rich serpentine rock mineral is abundant. One species was isolated from soil of Douglas-fir rhizosphere, the other species from the nonrhizosphere soil. Both isolates were tested for their ability to degrade serpentine and muscovite. Both isolates degraded serpentine, releasing silicon and magnesium. The two fungal isolates also degraded muscovite, releasing aluminum, potassium, and silicon. Limited quantities of the cations released from mineral degradation were immobilized in mycelial tissue.

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*) Dr. R. H. CRAWFORD, USDA Forest Service, Delaware State University, Department of Agriculture, 1200 North DuPont Highway, Dover, DE 19901, USA; fax 302-857-6402; e-mail: rcrawford01@fs.fed.us

**) M. FLOYD, Department of Plant and Soil Science, Alabama A&M University, Normal, AL 35762, USA.; fax 256-851-5906; e-mail: aammaf01@aamu.edu

***) C. Y. LI, USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR, 97331, USA; fax 541-758-7760; e-mail: cli@fs.fed.us

Address correspondence to Dr. Ralph H. CRAWFORD, USDA Liaison Officer, Delaware State University, Department of Agriculture, DuPont Highway, Dover, DE 19901, USA.

Zusammenfassung

CRAWFORD R.H., FLOYD M. & LI C.Y. 2000. Der Abbau von Serpentin- und Muscovit-Felsmineralien und die Immobilisierung von Kationen durch Boden-*Penicillium*-Arten. – Phyton (Horn, Austria) 40 (2): 315–322. – Englisch mit deutscher Zusammenfassung.

Zwei *Penicillium*-Arten wurden aus der Erde von zwei Pflanzgärten beim Siskiyou National Forest im Südwesten Oregons isoliert, wo magnesiumreiche Serpentinfelsminerale vorkommen. Eine Art wurde aus dem Boden der Douglas-Föhren-Rhizosphaere isoliert, die andere Art von der nicht durchwurzelten Erde. Beide Isolate wurden darauf geprüft, inwieweit sie Serpetin und Muscovit abbauen können. Beide Isolate bauten Serpentin ab, wobei sie Silizium und Magnesium freisetzten. Beide Pilzisolate bauten auch Muscovit ab und setzten dabei Aluminium, Kalzium und Silizium frei. Eine begrenzte Menge an Kationen, welche durch den Mineralabbau freigesetzt wurde, wurde im Mycel festgelegt.

Introduction

Primary weathering of soil rock minerals by biochemical activities of rhizosphere microbes could accelerate mobilization of inorganic nutrients for uptake by plants (WEED & al. 1969, BERTHELIN 1983, LEYVAL & al. 1990, BERTHELIN & al. 1991, LEYVAL & BERTHELIN 1991, ADAMS & al. 1992, PARIS & al. 1995a, 1995b, 1996). Such biochemical activities include production of organic acids that are cation complexing agents (DUFF & al. 1963, ECKHARDT 1980, LYALIKOVA & PETUSHKOVA 1991, PALMER & al. 1991) or siderophores that mobilize ferric iron from otherwise insoluble ferric oxides and oxyhydroxides.

Plant roots can also induce weathering processes of minerals and contribute significantly to the supply of cations to the plants (HINSINGER & al. 1991, 1992, 1993, HINSINGER & JAILLARD 1993), which is probably due to the severe root-induced acidification in the rhizosphere. Combined induced weathering processes by plants and soil microbes may thus be responsible for transformation of soil mineral structure, thereby removing mineral cations for rapid uptake by plants, especially in ecosystems developing on primary mineral substrates. *Penicillium* was the most abundant fungal genus isolated from the rhizosphere of two seed orchard sites on the Siskiyou National Forest in southwest Oregon where magnesium-rich serpentine rock mineral is abundant. This study was designed to determine the ability of these two *Penicillium* spp. to solubilize this rock mineral. A silicon-rich muscovite mineral also was included in this study.

Materials and Methods

Rock minerals

Serpentine rock was collected from the two seed orchard sites in the Siskiyou National Forest in southwest Oregon. The sites were planted with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) following intensive disturbance, which includes clearcutting, burning, scarification, ripping, stumping, brushing raking, and grading. The trees were 5 years old at the time of isolation of *Penicillium*. Muscovite was obtained from Ward's Natural Science Est., Inc. (Rochester, NY). The compositions of these two rock minerals were analyzed using the CAMECA SX50 electron microprobe at Oregon State University, Corvallis (Table 1). The rocks were ground to pass through a 120-µm screen. *Penicillium* species were isolated by a serial dilution method from the rhizosphere and nonrhizosphere soils of Douglas-fir (CRAWFORD 1995). The two fungal species isolated were plated with 0.25% serpentine or muscovite on the agar medium (HENDERSON & DUFF 1963). Halo zones produced around fungal colonies indicated solubilization of rock minerals.

Table 1. Chemical composition of rock minerals (% weight).

	O_2	F	Na	Mg	Al	Si	P	Cl	K	Ca
Muscovite[a]	43.55	0.24	0.38	0.17	18.73	21.46	0.01	0.01	8.16	0.07
Serpentine[b]	39.28	0.13	0.01	23.65	0.34	19.12	0.02	0.05	0.004	0.02

[a] means of 24 samples, [b] means of 18 samples.

Methods

Determination of elements released

To determine the release of elements by weathering action of these two soil fungi, a displaced method developed by HENDERSON & DUFF 1963 was used. The isolated fungi were each grown on the above liquid medium. The resulting fungal mass was harvested and washed three times in distilled water. The washed fungal mass was suspended in 25 ml of a 8 % glucose solution in a flask containing 0.01 g muscovite or serpentine, previously sterilized by anhydrous diethyl ether. After 3 weeks incubation at room temperatures on a shaker, the cultural solutions were centrifuged to remove the fungal mats and minerals. The clear supernatant were decanted and filtered through 0.2 µm filters to remove any other microbes. Solutions with *Penicillium* but without minerals served as controls. The concentration of aluminum, magnesium, potassium, and silicon released into solutions was determined by a flame atomic absorption spectroscopy at the Cooperative Chemical Analytic Laboratory, USDA Forest Service, Pacific Northwest Research Station, and Oregon State University, Corvallis, Oregon. The amount released was expressed as mg cations released into 1 L solution per 0.5 mg fungal dried weight. The contents of aluminum, potassium, and magnesium in fungal mats were determined by acid digestion followed by a Perkin-Elmer 500 atomic absorption spectrophotometer, according to the methods of ALLEN & al. 1976.

Results and Discussion

The two *Penicillium* spp., isolated from rhizosphere and nonrhizosphere soils of Douglas-fir, degraded both serpentine and muscovite rocks, releasing aluminum, potassium, magnesium, and silicon ions. The amounts released significantly differed between the *Penicillium*-inoculated and the *Penicillium* alone (Tables 2, 3); no release was noted from either of the rocks alone. The amounts of silicon and magnesium released from serpentine were not significantly different between the rhizospheric and nonrhizospheric *Penicillium* isolates (Table 2). The two isolates, however, significantly differed in the release of aluminum, potassium, and silicon from muscovite (Table 3).

Table 2. Release of aluminum (Al), potassium (K), silicon (Si), and magnesium (Mg) after incubation of rhizospheric or nonrhizospheric *Penicillium* sp. with serpentine for 3 weeks.

Treatment	Al (mg/l)	K (mg/l)	Si (mg/l)	Mg (mg/l)
Serpentine+Pr	–	–	11.86+0.74d	8.23+0.49f
Serpentine+Pn	–	–	12.42+3.24d	11.13+4.36f
Control (Pr without rock)	0.23+0.05a	9.50+2.45b	0.87+0.25e	0.79+0.31g
Control (Pn without rock)	0.25+0.10a	0.21+0.12c	0.73+0.36e	0.11+0.06h

Means of 4.

–: Not determined due to minor components; means with the same letter along the same column did not significantly differ at p=0.05, according to Duncan's multiple range test; Pr: rhizospheric *Penicillium* sp.; Pn: nonrhizospheric *Penicillium* sp.

Table 3. Release of aluminum (Al), potassium (K), silicon (Si), and magnesium (Mg) after incubation of rhizospheric or nonrhizospheric *Penicillium* sp. with muscovite for 3 weeks.

Treatment	Al (mg/l)	K (mg/l)	Si (mg/l)	Mg (mg/l)
Muscovite+Pr	1.13+0.13a	9.84+1.08d	3.90+0.36h	–
Muscovite+Pn	5.50+0.68b	1.09+0.30e	6.87+0.68i	–
Control (Pr without rock)	0.23+0.05c	9.50+2.45f	0.87+0.25j	0.79+0.31m
Control (Pn without rock)	0.25+0.10c	0.21+0.12g	0.73+0.36j	0.11+0.06n

Means of 5.

–: Not determined due to minor components; means with the same letter along the same column did not significantly differ at p=0.05, according to Duncan's multiple range test; Pr: rhizospheric *Penicillium* sp.; Pn: nonrhizospheric *Penicillium* sp.

The two isolates also assimilated aluminum, potassium, and magnesium released from these two rocks into their tissues. The rhizospheric *Penicillium* isolate showed 0.1 % increase of aluminum, 0.05 % increase of potassium, and no decrease of magnesium in tissue for muscovite (Table 4). With serpentine, the same isolate showed 0.01 % increase of aluminum, 0.03 % increase of potassium, and 0.45 % increase of magnesium. The nonrhizospheric *Penicillium* showed 0.38 % increase of aluminum, 0.43 % increase of potassium, but no increase of magnesium in tissue for muscovite. With serpentine, the same isolate showed 3.22 % increase of magnesium; no absorption was observed for aluminum and potassium (Table 4).

Table 4. Presence of aluminum (Al), potassium (K), and magnesium (Mg) in fungal mycelium after incubation of fungus with muscovite or serpentine for 3 weeks.

Rock	Fungus	Al	K	Mg
		(% of fungal mycelial wt.)		
Serpentine	Pr	0.01	0.03	0.45
Serpentine	Pn	0.00	0.00	3.22
Muscovite	Pr	0.10	0.05	0.00
Muscovite	Pn	0.38	0.43	0.00

Means are the average of two replicates over control. Fungal mats in 8 % sucrose solution without rock served as control. Pr: rhizospheric *Penicillium* sp.; Pn = nonrhizospheric *Penicillium* sp.

The experiment reported here shows that the two *Penicillium* isolates growing in vitro in the presence of muscovite or serpentine resulted in degradation of the rocks and release of aluminum, potassium, magnesium, and silicon. The fungal isolates also incorporated the released cations into their tissues. This information suggests that the cations released as results of attack could add nutritional availability to plants and *Penicillium*. The biochemical mechanisms by which *Penicillium* mediates metal ion solubilization – by high concentration of organic acid, formed by *Penicillium* in metabolism of root exudates at root surfaces, by changing the redox state of the cations, or by chelation – are not known and need to be explored. Rock-inhabiting fungi produce organic acids, many of which can be found in rocks. In pure culture with rock powder these fungi can extract many cations from the rocks (SIVERMAN & MUNOZ 1970, HIRSCH & al. 1991). Other fungi, including mycorrhizal, produce low-molecular-weight organic acids for solubilzation of rocks (PARIS & al. 1996, VASSILEV & al. 1997, ILLMER & al. 1995). In pure culture, the two *Penicillium* isolates in this study did not lead to the same degradation and immobilization capacities and so could differentially affect cation ion exchange capacity between rhizosphere and nonrhizosphere soil of Douglas-fir. Additionally, immobilization of excess minerals by these two fungi could reduce metal toxicity toward plants and ecosystems. The plant uptake of released minerals through analysis of plant mineral content warrants continued research efforts to better understand the microbially mediated micronutrient availability in the rhizosphere (LEYVAL & al. 1990, HINSINGER & al. 1992). This study suggests that the role of *Penicillium* in soil may be broader than just its saprophytic activity on organic matters.

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Recensio

WAGNER Hildebert (Ed.) 1999. Immunomodulatory Agents from Plants. – In: PARNHAM M. J. (Ed.), Progress in Inflammation Research. – Gr. 8°, X + 365 Seiten, zahlreiche Abb. (Diagramme, Formelbilder); geb. – Birkhäuser Verlag Basel, Boston, Berlin. – DM 258,–. – ISBN 3-7643-5848-3.

Der Band enthält 13 Beiträge zum Thema Immunsystem beeinflussender, vor allem stimulierender, Reinsubstanzen und Extrakte aus Pflanzen i.w.S. (inkl. Pilze). Im ersten Beitrag (p. 1–39) geben WAGNER, KRAUS & JURCIC eine allgemeine Übersicht zu „search for potent immunostimulating agents from plants and other natural sources“, in dem auch obsolete Behandlungsmethoden und Screening-Methoden erwähnt sind. Er enthält eine Liste (Tabelle 1) von 125 Arten (unter denen so bekannte wie *Lentinula edodes* und *Schizophyllum commune* fehlen), für deren Extrakte oder daraus isolierte Reinsubstanzen stimulierende Wirkung beschrieben worden ist; einige weitere sind in Tabelle 2 und 5 enthalten. Einige Beispiele davon sind diskutiert (z.B. Vincristin, *Tabebuia*-Chinone, ribosome inactivating proteins aus *Trichosanthes, Bryonia, Ecballium* und *Cucurbita,* Lentinan, Polysaccharide aus *Achyrocline satureioides* u.a.).

Auf den Seiten 41–135 beschäftigen sich gleich vier Beiträge mit den verschiedenen Gesichtspunkten (inkl. klinischen Studien) von *Echinacea*-Inhaltsstoffen, die zur Zeit die meist verwendeten Immunstimulantien sind (alleine über 800 verschie-

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