

Does secondary chemistry enable lichens to grow on iron-rich substrates?

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Abstract

Lichen substances are shown to increase or to inhibit the adsorption of Fe at cation exchange sites. The influence on the adsorption strongly differs between individual lichen substances and is different for Fe^{2+} and Fe^{3+} . These results add a new biological role to the known functions of lichen secondary metabolites. In an experiment with cellulose filters, which were soaked with acetone solutions of lichen substances and were then incubated with micromolar solutions of FeCl_2 or FeCl_3 , many lichen substances were found to increase Fe^{3+} adsorption, whereas others had no effect. Most lichen substances had no effect on Fe^{2+} adsorption, but two were found to reduce and one to increase the level of adsorption. Lichens of Fe-poor and -rich sites contain lichen substances with different adsorption behavior towards Fe^{2+} and Fe^{3+} . All the studied lichen substances, which only occur in lichens of Fe-poor sites, turned out to be effective Fe^{3+} adsorbents. Lichens of Fe-bearing rock and slag, however, were found to lack lichen substances, or to contain substances that did not adsorb Fe^{3+} and had no effect on Fe^{2+} adsorption, or thirdly, to contain substances that increased Fe^{3+} adsorption, but decreased Fe^{2+} adsorption. These results suggest that lichen substances do play a significant role in Fe adsorption in lichens and determine their tolerance to excess concentrations of Fe. Notwithstanding the strong correlation between the secondary chemistry of lichen species and their preference for Fe-rich or Fe-poor substrates, the postulated mechanism of temporary Fe adsorption by lichen substances has to be subject of future biochemical research.

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Introduction

Iron-bearing rock and slag have long been known to harbor a unique lichen flora (Hilitzer, 1923; Schade, 1933). These lichens form different communities that have been summarized as the *Acarosporion sinopicae* alliance (Purvis and Halls, 1996; Wirth, 1972). Sun and rain-exposed ferrous substrates are inhabited by the

Acarosporium sinopicae, whereas vertical substrates that are sheltered from the rain are preferred by lichens of the *Lecanoreta epanorae* community (Purvis and Halls, 1996; Purvis and James, 1985; Wirth, 1972). While vascular plants, like *Armeria halleri*, *Cardaminopsis halleri* or *Minuartia verna*, are only capable of growing in areas adjacent to such rock outcrops and slag heaps, where soil can accumulate, lichens of the *Acarosporion sinopicae* grow in the chemically most extreme microsites of such habitats, viz. directly on the ore-bearing rock or slag itself. Besides high concentrations of Fe, such

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substrates are characterized by elevated concentrations of other transition metals (e.g., Cu and Pb) and, as most Fe is bound as sulfide, by high concentrations of inorganic S and extremely low pH (Noeske et al., 1970; Wirth, 1972).

Mechanisms which enable lichens of the *Acarosporion sinopicae* to colonize Fe-bearing rock and slag have not been thoroughly investigated. Lichens growing on ferrous substrate belong to various unrelated systematic groups (Eriksson, 2005). Most *Acarosporion sinopicae* lichens have a crustose thallus, but some of them are foliose (*Umbilicaria*) or fruticose (*Stereocaulon*); all have a green photobiont. Lichens growing on Fe-rich rock and slag can accumulate Fe concentrations as high as c. 1 mmol g^{-1} dry weight (Lange and Ziegler, 1963). In many *Acarosporion sinopicae* lichens, most of the total Fe is found in extracellular deposits on the upper thallus surface, giving species such as *Acarospora smaragdula*, *Acarospora sinopica*, *Lecidea silacea*, *Rhizocarpon oederi* or *Tremolecia atrata* a rusty appearance. These encrustations primarily consist of Fe(III) compounds (Lange and Ziegler, 1963). In *A. smaragdula* they were shown to contain S, Si, P, and O as alternative coordinating anionic species (Noeske et al., 1970). Beck (1999, 2002) tried to detect correlations between the Fe and Cu content of the substrate and the occurrence of different species of the most common photobiont genus, i.e., the green alga *Trebouxia*. In contrast to Cu concentrations and tolerance, convincing correlations were not found for Fe. Nevertheless, it is plausible to assume that not only mycobionts, but also photobiont species differ in Fe tolerance. However, since there are no photobiont species unique to *Acarosporion sinopicae* lichens and since photobiont species known from these lichens (e.g. *Trebouxia jamesii* spp. *angustilobata*, *Trebouxia simplex*; Beck, 2002) are also found in lichen species growing on Fe-poor substrates, the photobiont cannot be a primary factor in enabling such lichens to grow on ferrous rock or slag. At present there is no known character shared by all the lichens of the *Acarosporion sinopicae*, which could explain their co-occurrence in this very special habitat.

We have now investigated the role that secondary lichen metabolites may play in enabling a particular species to grow on Fe-bearing substrata. Lichens possess more than 800 aliphatic, cycloaliphatic, aromatic and terpenoid compounds, the so-called lichen substances, which are formed by the mycobiont and are deposited as crystals on the cell wall surfaces of both the fungal and the algal partners. In nature, the distribution of the majority of these compounds is limited to lichens (Huneck, 1999, 2001; Huneck and Yoshimura, 1996). Several interesting functions have been ascribed to these secondary metabolites. Lichen substances may, for example, protect the photobiont from UV light and from excessive amounts of photosynthetically active

radiation (Gauslaa and Ustvedt, 2003; Solhaug et al., 2003). Many lichen substances exert anti-herbivore activities, primarily against invertebrates (Nimis and Skert, 2006). Some lichen secondary metabolites, such as usnic acid, are known to have antibiotic properties (Yilmaz et al., 2004), while this compound and other substances may inhibit potential fungal, bryophyte and vascular plant competitors (Lawrey, 1986).

Purvis et al. (1987) found that the depsidone norstictic acid immobilized Cu in the lichens *A. smaragdula* and *Lecidea lactea* and detected the presence of a Cu^{2+} –norstictic acid complex. Furthermore, the chemically related psoromic acid was suggested to sequester Cu^{2+} by chelation in *Lecidea bullata* and *Tephromela testaceoatra* (Purvis et al., 1990). These lichen species belong to the *Lecideion inops* alliance, which includes several species which can survive on Cu-bearing rock and slag, and show a markedly green color in parts of the thallus or in apothecia due to Cu deposits (Purvis and Halls, 1996). Takani et al. (2002) reported the formation of a Cu^{2+} –(+)-usnic acid complex *in vitro*, but there is no evidence that usnic acid improves the performance of species of the *Lecideion inops* alliance in the field, although there are several cuprophytic lichens (e.g. *Lecanora cascadiensis*) which do contain this compound (Purvis and Halls, 1996).

It has been suggested that the anthraquinone parietin may form a complex with Fe^{3+} (Engstrom et al., 1980), but this study has been largely overlooked by lichenologists as it dealt with ethanol extracts of the mold *Aspergillus ruber*. Although parietin occurs in non-lichenized fungi, it is a common cortical pigment in the lichen family Teloschistaceae (Huneck and Yoshimura, 1996). From their results Engstrom et al. (1980) proposed that parietin could facilitate intracellular Fe uptake by temporary chelation of Fe^{3+} ions. Although Engstrom et al. (1980) had no experimental evidence for their hypothesis, their assumption seems plausible as many parietin-containing lichens are calciphilous (Purvis et al., 1992; Wirth, 1995) and can, thus, be assumed to grow at sites with low Fe availability (Zohlen and Tyler, 1997). In the Poaceae, phytosiderophores perform a similar function to that assumed for parietin, i.e. they mobilize Fe from the soil by temporary chelation and facilitate its intracellular uptake (Curie et al., 2001).

Since lichen substances which promote Fe acquisition from the substrate would be a disadvantage for lichen species growing on Fe-bearing rock and slag, we suspected that lichens of the *Acarosporion sinopicae* alliance would not contain such secondary metabolites. Furthermore, we tested the hypothesis that lichen substances of species which are restricted to non-ferrophytic substrata would actually adsorb Fe. In order to test these hypotheses we studied whether isolated lichen substances would alter the affinity of Fe^{2+} and Fe^{3+} to organic surfaces with cation binding

sites. Lichen cell walls harbor numerous cation exchange sites, consisting primarily of carboxyl and hydroxyl groups (Sarret et al., 1998).

Materials and methods

Affinity of selected lichen substances to Fe^{2+} and Fe^{3+}

The experiment was carried out with 18 isolated lichen substances from the collections of S. Huneck and J.A. Elix. The lichen substances were selected by compiling a list of the secondary metabolites occurring in ferrophytic lichen species from the literature (Huneck and Yoshimura, 1996; Purvis et al., 1992; Wirth, 1995). The selection of lichen species considered was based on floristic literature (Hafellner and Türk, 2001; Purvis and Halls, 1996; Wirth, 1972, 1995) and from our own field experience (Hauck, 1996). Nomenclature of lichen species mentioned in the text refers to Santesson (1993) and Wirth (1994). The set of lichen substances was extended to include seven additional secondary metabolites, which have not been found in ferrophytic lichens. These included the depsides divaricatic and perlatolic acid, the depsidones fumarprotocetraric, physodalic, physodic, and protocetraric acid as well as pulvinic acid. Acaranoic acid, acarenoic acid, barbatic acid, epanorin, and psoromic acid, which occur in some ferrophytic lichen species, were not studied as isolates were not available.

The individual lichen substances were dissolved in acetone (250 μM) and applied to ash-free cellulose filter paper (Blue Ribbon Filters, Schleicher & Schuell, Dassel, Germany) by shaking filter stripes (c. 20 cm^2 , 160 mg) in the lichen substance solution for 1 h. The filter strips served as standardized surfaces. Cellulose filters consist of randomly interlaced fibers which are littered with exchange sites that bind to metal ions, similar to the cell wall surfaces of lichens (Klemm et al., 1998). Even adsorption of lichen substances could be monitored visually in the case of colored compounds, such as usnic acid and the pulvinic acid derivatives. Untreated filter strips were used as controls. Two strips of impregnated filters per replicate were exposed for 1 h to 25 ml of 85 μM FeCl_2 or FeCl_3 ; the concentration of 85 μM referred to the optimum measuring range of the AAS used (Vario 6, Analytik Jena, Germany). After incubation, the filter paper was removed with forceps and the Fe concentration in the solution was analyzed.

Statistics

Data are given as arithmetic means $\pm$ standard error of five replicate samples. The statistical significance of

differences between means was calculated with Duncan's multiple range test ($P \leq 0.05$). The effects of two independent variables on Fe adsorption from the incubation medium was studied with a two-way analysis of variance (ANOVA). The first independent parameter included the affiliation of the secondary metabolites to the class of lichen substances (depsides, depsidones, dibenzofurans, pulvinic acid derivatives, terpenoids). The second parameter was the frequency an individual lichen substance in the ferrophytic lichen species was listed in Table 1 (0–6 species). The statistical significance was tested by calculating F values. The statistical analyses were computed with SAS 6.04 software (SAS Institute Inc., Cary, North Carolina, USA).

Results

Secondary chemistry of ferrophytic lichen species

Table 1 presents a compilation of lichen species that occur on Fe-bearing substrata in Europe. Many of the species are confined to ferrous rock and slag and are thereby obligate members of the Acarosporion sinopicae alliance. Examples of such species include *A. sinopica*, *Lecanora epanora*, *Lecanora subaurea*, *L. silacea*, and *R. oederi*. Other species, such as *A. smaragdula*, *Rhizocarpon lecanorinum*, *Stereocaulon nanodes*, *T. atrata*, and *Umbilicaria torrefacta*, are members of Acarosporion sinopicae communities, but are also found on siliceous rock that is poor in Fe. A few species included in Table 1 do not occur in the Acarosporion sinopicae alliance. However, the normally saxicolous *Lecania erysibe*, is regularly found on rusty steel (Hauck, 1996). *Micarea bauschiana*, *Micarea lutulata* and *Micarea sylvicola* are pioneer species in rock overhangs with varying Fe content (Purvis et al., 1992; Wirth, 1995). *Staurothele frustulenta*, on the other hand, is locally abundant along the shores of rivers (Hauck, 1996), where it apparently accumulates Fe from industrial effluents (T. Feuerer, unpublished). *Vezdaea leprosa* is found in the dripzone of corroded wires and crash barriers (Ernst, 1995; Purvis et al., 1992).

Several lichen species which occur on Fe-bearing substrate, including the characteristic Acarosporion sinopicae species *A. sinopica* and *R. oederi*, are devoid of lichen substances (Table 1). Other obligate or facultative ferrophytic lichens, namely *A. smaragdula*, *Adelolecia pilati*, *L. silacea*, *Rhizocarpon sorediosum*, and *U. torrefacta*, contain varying amounts of lichen substances and these are often low. The most frequent substance present in the ferrophytic species are the pulvinic acid derivative, rhizocarpic acid, the depsidones stictic and norstictic acids, the triterpene zeorin, the depside atranorin, and the dibenzofuran usnic acid (Table 1). More rarely present are the depsides barbatic,

Table 1. Selected lichen species occurring on Fe-rich substrata with their ecological preferences and secondary chemistry

Lichen species	Ecology ^a	Secondary metabolites
<i>Acarospora impressula</i>	Fe +/Fe –	–
<i>Acarospora sinopica</i>	Fe +	–
<i>Acarospora smaragdula</i>	Fe +/Fe –	± Norstictic acid
<i>Adelolecia pilati</i>	Fe +/Fe –	± Atranorin
<i>Lecania erysibe</i>	Fe +/Fe – ^b	–
<i>Lecanora epanora</i>	Fe +	Epanorin* rhizocarpic acid, zeorin
<i>Lecanora gisleriana</i>	Fe +	Usnic acid
<i>Lecanora handelii</i>	Fe +	Usnic acid, zeorin
<i>Lecanora rubida</i>	Fe +	Norstictic acid
<i>Lecanora soralifera</i>	Fe +	Usnic acid, zeorin
<i>Lecanora subaurea</i>	Fe +	Pannarin, rhizocarpic acid, zeorin
<i>Lecidea silacea</i>	Fe +	± Porphyrilic acid
<i>Lecidea ullrichii</i>	Fe +	–
<i>Micarea bauschiana</i>	Fe +/Fe –	–
<i>Micarea lutulata</i>	Fe +/Fe –	–
<i>Micarea sylvicola</i>	Fe +/Fe –	–
<i>Miriquidica atrofulva</i>	Fe +	Stictic acid, ± norstictic acid
<i>Placopsis lambii</i>	Fe +/Fe –	Gyrophoric acid, ± lecanoric acid (trace)
<i>Pleopsidium chlorophanum</i>	Fe +/Fe –	Rhizocarpic acid, acaranoic acid* acarenoic acid*
<i>Rhizocarpon furfurosum</i>	Fe +	Stictic acid
<i>Rhizocarpon lecanorinum</i>	Fe +/Fe –	Rhizocarpic acid, ± stictic acid
<i>Rhizocarpon norvegicum</i>	Fe +/Fe –	Rhizocarpic acid, ± stictic acid
<i>Rhizocarpon oederi</i>	Fe +	–
<i>Rhizocarpon ridescens</i>	Fe +	Psoromic acid*
<i>Rhizocarpon sorediosum</i>	Fe +	± Barbatic acid* ± psoromic acid*
<i>Scoliciosporum umbrinum</i>	Fe +/Fe – ^c	–
<i>Staurothele frustulenta</i>	Fe +/Fe – ^d	–
<i>Stereocaulon leucophaeopsis</i>	Fe +/Fe – ^e	Atranorin, lobaric acid
<i>Stereocaulon nanodes</i>	Fe +/Fe –	Atranorin, lobaric acid
<i>Stereocaulon vesuvianum</i>	Fe +/Fe –	Atranorin, stictic acid, ± norstictic acid
<i>Tremolecia atrata</i>	Fe +/Fe –	–
<i>Umbilicaria torrefacta</i>	Fe +/Fe –	± Gyrophoric acid, ± stictic acid
<i>Vezdaea leprosa</i>	Fe+ ^f	–

*Lichen substance not considered in present study.

^aFe +, iron-bearing siliceous rock or slag; Fe – iron-poor siliceous rock.

^bOn rusty steel, limestone, sandstone.

^cFe-bearing or Fe-poor siliceous rock, bark, wood.

^dFe-bearing or Fe-poor limestone and nutrient-rich siliceous rock, terrestrial or aquatic sites near (polluted) rivers.

^eFe-bearing siliceous or calcareous rock.

^fFe-rich soil and plant debris.

gyrophoric and lecanoric acid, the depsidones pannarin, lobaric acid and psoromic acid, the dibenzofuran porphyritic acid, the pulvinic acid derivative epanorin, and the cycloaliphatic acids acaranoic and acarenoic acids (Table 1).

Affinity of selected lichen substances to Fe²⁺ and Fe³⁺

The affinity of Fe for the exchange sites present in the cellulose filters was considerably higher for Fe³⁺ than for Fe²⁺. The filters, which were not, pretreated with lichen substances, removed 70% of the Fe³⁺ ions, but

only 20% of the Fe²⁺ ions from the incubation medium. Eight lichen substances increased the adsorption of Fe³⁺ from the FeCl₃ solution (Table 2). Among them, the depsidones norstictic, physodalic, and fumarprotocetraric acid exerted the strongest effect, with the latter removing Fe³⁺ more or less quantitatively from the solution. The depsidones protocetraric and physodic acid, the depside divaricatic acid as well as pulvinic acid and its derivative rhizocarpic acid increased the adsorption of Fe³⁺ by around 40–50% (Table 2).

Most lichen substances did not affect the adsorption of Fe²⁺ (Table 3). Only pulvinic acid increased the adsorption of Fe²⁺ significantly, whereas rhizocarpic and norstictic acid reduced the adsorption of Fe²⁺ by

Table 2. Adsorption of Fe from 85 μM FeCl_3 by filter paper soaked with 250 μM solutions of different lichen substances

Lichen substance	Conc. (μM) ^a		Relative change of conc. (%) ^b
(+)-Usnic acid	27.4 $\pm$ 3.0	a	9
Atranorin	27.3 $\pm$ 3.6	a	8
Control	25.2 $\pm$ 3.1	ab	0
Porphyrilic acid	25.2 $\pm$ 3.2	ab	0
Pannarin	24.5 $\pm$ 4.3	ab	−3
Stictic acid	21.7 $\pm$ 3.3	abc	−14
Zeorin	20.0 $\pm$ 2.3	abcd	−21
Lobaric acid	19.3 $\pm$ 2.6	abcd	−24
Perlatolic acid	19.1 $\pm$ 3.0	abcd	−24
Gyrophoric acid	16.8 $\pm$ 0.5	bcd	−33
Lecanoric acid	16.6 $\pm$ 3.6	bcd	−34
Protocetraric acid	14.4 $\pm$ 2.1	cd	−43
Physodic acid	14.0 $\pm$ 3.1	cd	−44
Pulvinic acid	13.5 $\pm$ 3.7	cd	−47
Rhizocarpic acid	12.7 $\pm$ 2.0	cd	−50
Divaricatic acid	11.9 $\pm$ 2.4	d	−53
Norstictic acid	2.5 $\pm$ 0.5	e	−90
Physodalic acid	1.2 $\pm$ 1.1	e	−95
Fumarprotocetraric acid	0.2 $\pm$ 0.2	e	−99

Arithmetic mean $\pm$ standard error of five replicate samples. Statistics: Duncan's multiple range test, $P \leq 0.05$, $df = 76$. Means sharing a common letter do not differ significantly.

^aRemaining Fe concentration after shaking two filter strips impregnated with different lichen substances or not (control) for 1 h in 85 μM FeCl_3 .

^bRelative change of Fe concentration after shaking two filter strips impregnated with different lichen substances or not (control) for 1 h in 85 μM FeCl_3 . Control is set to 100%. Positive values indicate higher and negative values indicate lower Fe concentration in the solution.

the filter paper. The effect of these lichen substances on Fe adsorption was much lower than with Fe^{3+} , as they increased or decreased Fe^{2+} adsorption by only 10% compared to the control (Table 3).

Discussion

The lichen substances investigated showed marked differences in their affinity to Fe. Moreover, most lichen substances exhibited different affinities to Fe^{2+} and Fe^{3+} . While some secondary metabolites removed most of the Fe^{3+} from solution, others had no effect on Fe^{3+} adsorption. In contrast to the adsorption of Fe^{3+} , that of Fe^{2+} was only influenced by three lichen substances. Except for norstictic and rhizocarpic acid, the ferrophytic lichens considered in this study contain only lichen substances which do not have a significant effect on Fe^{3+} adsorption. However, rhizocarpic acid occurs in several species of *Rhizocarpon* and in *Pleopsidium*

Table 3. Adsorption of Fe from 85 μM FeCl_2 by filter paper soaked with 250 μM solutions of different lichen substances

Lichen substance	Conc. (μM) ^a		Relative change of conc. (%) ^b
Rhizocarpic acid	74.4 $\pm$ 0.8	a	10
Norstictic acid	73.8 $\pm$ 0.6	ab	9
Pannarin	71.5 $\pm$ 1.6	abc	6
Lobaric acid	70.2 $\pm$ 1.3	abc	4
Stictic acid	70.0 $\pm$ 2.0	abc	3
Protocetraric acid	69.8 $\pm$ 1.4	abc	3
Lecanoric acid	69.4 $\pm$ 1.9	abcd	3
Zeorin	68.9 $\pm$ 1.9	abcd	2
Divaricatic acid	68.6 $\pm$ 1.5	abcd	1
Physodic acid	68.6 $\pm$ 2.2	abcd	1
Perlatolic acid	68.2 $\pm$ 1.8	bcd	1
Fumarprotocetraric acid	68.0 $\pm$ 1.5	bcd	0
Porphyrilic acid	67.7 $\pm$ 0.9	cd	0
Control	67.7 $\pm$ 1.2	cd	0
Gyrophoric acid	66.7 $\pm$ 2.3	cd	−2
(+)-Usnic acid	66.5 $\pm$ 2.4	cd	−2
Physodalic acid	65.6 $\pm$ 2.6	cd	−3
Atranorin	63.7 $\pm$ 2.1	de	−6
Pulvinic acid	60.3 $\pm$ 1.5	e	−11

Arithmetic mean $\pm$ standard error of five replicate samples. Statistics: Duncan's multiple range test, $P \leq 0.05$, $df = 76$. Means sharing a common letter do not differ significantly.

^aRemaining Fe concentration after shaking two filter strips impregnated with different lichen substances or not (control) for 1 h in 85 μM FeCl_2 .

^bRelative change of Fe concentration after shaking two filter strips impregnated with different lichen substances or not (control) for 1 h in 85 μM FeCl_2 . Control is set to 100%. Positive values indicate higher and negative values indicate lower Fe concentration in the solution.

chlorophanum, which colonize Fe-bearing rock or slag and norstictic acid occurs in varying amounts in the ferrophytic lichens *A. smaragdula*, *Miriquidica atrofulva*, and *Stereocaulon vesuvianum* and is always found in *Lecanora rubida*. The occurrence of the Fe^{3+} -adsorbing secondary metabolites norstictic and rhizocarpic acid contradicts our initial hypothesis that lichens growing on Fe-bearing substrates should lack Fe-adsorbing lichen substances. However, norstictic and rhizocarpic acid are the only substances investigated that significantly reduce the adsorption of Fe^{2+} . This implies that the inhibition of Fe^{2+} adsorption compensates for the increased Fe^{3+} adsorption. Such an assumption is justified as Fe^{2+} is generally more mobile than Fe^{3+} and is the prevailing ionic species under the acidic conditions typically found on Fe-rich rock and slag (Purvis, 1996; Wirth, 1972).

All lichen substances studied which do not occur in ferrophytic lichen species are effective in Fe^{3+} adsorption. This supports the hypothesis that lichen substances do not

adsorb Fe for permanent immobilization, as proposed by Purvis et al. (1987, 1990) for the Cu–norstictic acid and Cu–psoromic acid complexes, but function as chelators that facilitate intracellular uptake (Engstrom et al., 1980). The subsequent uptake of cations temporarily bound to lichen substances is quite conceivable, as delayed intracellular uptake is also known from extracellular exchange sites in the cell wall (Brown and Beckett, 1985; Hauck et al., 2006). In many lichen habitats, the occurrence of Fe-adsorbing substances would be advantageous, because the availability of Fe is low. This certainly applies to the microhabitats of most epiphytes (Hauck and Spribille, 2005; Schmull and Hauck, 2003), to lichens on calcareous rock and soil, as well as to some acidic, nutrient poor rocks and soils (Wirth, 1972).

Some very effective Fe^{3+} -adsorbing lichen substances, including fumarprotocetraric, divaricatic and protocetraric acid, are very widespread in lichens. Furthermore, lichens that are capable of growing on Fe-bearing substrates (Table 1) belong to different orders and 10 families (Acarosporaceae, Hymeneliaceae, Lecanoraceae, Lecideaceae, Pilocarpaceae, Ramalinaeae, Rhizocarpaceae, Stereocaulaceae, Umbilicariaceae, Vezdaeaceae) of the Lecanoromycetes (Eriksson, 2005; Wedin et al., 2005). *S. frustulenta*, which is not, however, a member of the Acarosporion sinopiceae alliance, is affiliated to the Eurotiomycetes (Eriksson, 2005). If Fe^{3+} adsorption by lichen substances serves primarily for detoxification, some lichen species containing fumarprotocetraric, divaricatic and protocetraric acid or parietin would have been expected to colonize ferrous rock during the course of evolution. Rather, lichen species with these substances only grow in the surroundings of Fe-bearing substrata, like for example, some terricolous *Cladonia* species (Hauck, 1996).

At present, we have no convincing explanation for the physico-chemical causes of the different affinities to Fe of the secondary metabolites investigated. A simple dependence on varying hydrophobic and hydrophilic properties of the substances studied can be ruled out, as such correlation was not observed (Huneck, 2003). Chemically related substances often behaved very differently in their adsorption of Fe. Pulvinic acid, for instance, had the highest affinity to Fe^{2+} , whereas its derivative, rhizocarpic acid, reduced the adsorption of Fe^{2+} most effectively. The affinity for Fe^{3+} was nearly identical for both pulvinic and rhizocarpic acid. The depsides and depsidones, the two major classes of lichen substances, did not show any clear trend for similar behavior with either Fe^{2+} nor to Fe^{3+} . However, two-way ANOVA revealed that 27% of the total variance in Fe^{3+} adsorption data could be attributed to the affiliation of a secondary metabolite to a particular class of lichen substances ($F = 11.22$, $P \leq 0.001$, $df = 76$). For Fe^{2+} , this affiliation had no significant

effect on the adsorption rate. The same two-way ANOVA showed that the frequency of lichen substances in ferrophytic lichens was dependent upon their affinity to both Fe^{2+} and Fe^{3+} . In the case of Fe^{2+} , 27% of the total variance in the adsorption data could be explained by the frequency of the lichen substances studied in ferrophytic lichens ($F = 6.00$, $P \leq 0.001$, $df = 76$), whereas this value was 13% in the case of Fe^{3+} ($F = 3.60$, $P \leq 0.01$, $df = 76$).

Further work on the potential of lichen substances to modify cation adsorption is necessary in order to substantiate our hypothesis that secondary chemistry plays a decisive role in the Fe budget of lichens. Though the surface structure of the cellulose filters employed is superficially similar to that of the cortex of heteromorous lichen thalli, cation adsorption rates may be different for cellulose fibers and fungal hyphae saturated with these compounds. Furthermore, the delayed uptake of ions that are bound to lichen substances has still to be proven. However, we doubt that these limitations are crucial to the outcome of our experiments because of the close correlation of the observed Fe adsorption rates with the known ecological preferences of lichen species with and without Fe-adsorbing secondary metabolites.

Although we assume that lichen secondary chemistry is a key factor in enabling lichens to grow on Fe-rich substrata, it is probably not the only factor that is essential for high Fe tolerance. There are many lichen species that lack Fe-adsorbing lichen substances, but do not grow on ferrous rock and slag. Further, prerequisites are probably the tolerance to high acidity (Wirth, 1972) as well as the ability for effective extra- and intracellular Fe immobilization (Lange and Ziegler, 1963; Noeske et al., 1970; Paul et al., 2003).

Conclusions

Lichens that are capable of colonizing Fe-rich habitats have special characteristics in terms of their secondary chemistry. Such species

- either lack lichen substances,
- or contain lichen substances which have low affinity to both Fe^{2+} and Fe^{3+} ,
- or contain lichen substances which inhibit Fe^{2+} adsorption, but increase Fe^{3+} adsorption.

Lichens that avoid Fe-rich habitats often contain lichen substances, which increase Fe^{3+} adsorption.

These observations support the hypothesis forwarded by Engstrom et al. (1980) that lichen substances adsorb Fe to facilitate its intracellular uptake. However, the physiological significance of Fe adsorption by lichen

substances for intracellular uptake has still to be substantiated.

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