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# Rhizine and Upper Thallus Isozymes in Umbilicate Lichens

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## Abstract

Isozymes in rhizines were compared electrophoretically with those in other parts of umbilicate thalli. The distribution of esterase, alkaline phosphatase and superoxide dismutase was investigted in three stands of *Umbilicaria mammulata*, while these three enzymes plus isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, mannitol dehydrogenase and glutamate dehydrogenase were examined in one stand of *U. vellea*. For most enzyme systems in *U. vellea*, the isozymes detected in crude extracts were the same in rhizines as in the main portion of the thallus. However, a few esterase bands in *U. vellea*, and in two stands of *U. mammulata*, were detected only in the main thallus and could have been algal esterases. Alkaline phosphatase bands also unique to the main thallus were found in *U. mammulata*. An unexpected result was that a few electromorphs of alkaline phosphatase and esterase, as well as of glutamate dehydrogenase and pentose phosphate pathway enzymes, were detected only in extracts of rhizines.

Keywords: isoenzymes, rhizines, *Umbilicaria mammulata*, *Umbilicaria vellea*

## 1. Introduction

Many metabolic pathways of free-living fungi and algae are the same. Since glycolytic and respiratory pathways occur in both groups of organisms (Lloyd, 1974; Cochrane, 1976; Watson, 1976), enzymes extracted from lichens cannot

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easily be attributed to one symbiotic partner or the other. Mannitol-1-phosphate dehydrogenase is an enzyme of the mycobiont, because mannitol occurs only in the fungus (Jensen et al., 1991), but the symbiontal source of glucose-6-phosphate dehydrogenase or malate dehydrogenase, for example, is not clear. The respective contributions of individual symbionts is of interest, partly because of the increasing use of enzyme banding patterns for taxonomic purposes and also because many aspects of primary metabolism have been little studied in lichens.

It would seem that electrophoretic comparisons of extracts from isolated symbionts with those from the whole thallus would be the best way to establish which enzymes, and enzyme forms, are generated by each of the two partners. This approach was attempted with *Cladonia cristatella* (Fahselt, 1985), but there are several difficulties. First, normal extraction procedures may not yield comparable subsets of proteins, since the mycobiont in culture differs in a number of respects from that in the thallus. The hyphal mass becomes tough and rubbery, the structure of outer wall surfaces is modified, and the form of cells changes as well, in that some appear swollen and spherical instead of filamentous (Honnegger and Bartnicki-Garcia, 1991).

Cultured photobionts also undergo changes in culture (Bubrick, 1988). Endonuclease-digested DNA, from *Nostoc* symbionts in culture, gives different hybridization patterns with labelled probes than the DNA from *Nostoc* that has recently separated from a lichen (Kardish et al., 1990). The explanation for the difference is not known, but the authors suggest that a different *Nostoc* variant predominates in culture than in the thallus or some kind of genetic rearrangement could be involved.

A further concern regarding cultures is that chemical and physical conditions prevailing *in vivo* may alter gene expression. Moisture status (Vicente, 1990), kinds or proportions of exogenously supplied molecules and light (Vicente, 1991) all may influence lichen enzymes. Any of these factors could complicate comparisons between isolates and intact thalli.

Another consideration is that isolated symbionts may be incapable of producing thallus enzymes. Neither the phycobiont nor mycobiont, when isolated, synthesizes the same form of invertase as the lichen (Martin, 1973a,b). In such a case, electrophoretic comparisons of isolates and whole thalli clearly cannot distinguish which of the partners is producing a lichen enzyme.

An alternative approach to discerning the symbiontal source of lichen enzymes, and one that does not rely on artificial cultures, is to compare freshly separated thallus parts that have differing mycobiont/phycobiont proportions. Dissected thalli were used to study carbohydrate transfer to the mycobiont

(Smith et al., 1969), intrathalline distribution of enzymes (Bernard and Goas, 1979; Yague and Estevez, 1989) and DNA (Kardish et al., 1990).

A convenient way to prepare a reasonable quantity of fresh mycobiont tissue, and one which precludes contamination of mycelial fractions by photobionts, is to remove appendages such as rhizines that are purely fungal, and to compare these with remaining thallus parts that include both symbionts. It seems logical to expect that fungal isozymes would be found in both fractions and algal forms in just one.

The objectives of the present study were (1) to sample the variability within stands of umbilicate lichens in regard to the apportionment of isozymes between thallus parts with differing symbiont composition, and (2) to ascertain electromorph distribution between thallus fractions of enzymes in different metabolic pathways.

## 2. Materials and Methods

*Umbilicaria mammulata* (Ach.) Tuck. and *U. vellea* (L.)Ach., both relatively robust lichens with copious rhizines consisting of purely fungal tissue on their lower surfaces and both with previously determined isozyme banding patterns of whole thalli (Hageman and Fahselt, 1990, 1992), were chosen as experimental material. Eight to 10 thalli of *Umbilicaria mammulata* were removed from granite outcrops (44° 55′N, 79° 20′W) near Kilworthy, Ontario, Canada on June 7, 1990. These were collected from each of three stands, located on separate granite faces within 100 m of one another. Three thalli of *U. vellea* were taken May 27, 1992 from a stand west of Huntsville, Ontario at 45° 16′N, 79° 38′W. In each case, the collected thalli were non-apotheciate and approximately 10–15 mm in the longest dimension. Consistency of intrathalline isozyme distribution within stands was examined in *U. mammulata*, and a wider range of enzymes was tested in *U. vellea.*

After three cold acetone extractions to remove phenolics, rhizines were scraped off the lower surface with a scalpel. Associated bits of darkly pigmented lower cortex were detached with the rhizines, but little or no medullary tissue was taken, and the rhizine preparations did not include algal cells. The thallus that remained after removal of rhizines included the medulla, intact algal layer and entire upper cortex. Rhizines and upper thallus parts were stored separately at −17° C within 3 days of collection.

Extraction, isoelectric focussing, staining and scoring methods were those described in Hageman and Fahselt (1984). The assumption was made that electromorphs with the same staining reaction and isoelectric point were the same form of a given enzyme. Enzyme systems that were studied in *U. mammulata*

included the two that contributed most to group structure when umbilicate species were ordinated using principal componens analysis (Hageman, 1989), esterase (EST, EC 3.1.1.1) and alkaline phosphatase (ALP, EC 3.1.3.1). Since these enzymes are both highly polymorphic, they are especially useful at the population level. Superoxide dismutase (SOD, EC 1.15.1.1) was selected for intensive study as one of the enzymes which is least variable, both within and between conspecific umbilicte stands, and is more diagnostic at the level of species. In dissected thalli of *U. vellea*, the same three enzymes and five others, isocitrate dehydrogenase (IDH, EC 1.1.1.41), 6-phosphogluconate dehydrogenase (6PG, EC 1.1.1.44), glucose-6-phosphate dehydrogenase (G6P, EC 1.1.1.49), mannitol dehydrogenase (MAN, EC 1.1.1.138) and glutamate dehydrogenase (GDH, EC 1.4.1.4), were analyzed.

## 3. Results

Isozyme distribution between rhizines and other parts of the thallus was similar among individual thalli in the three stands of *U. mammulata* (Table 1). A few EST electromorphs were confined either to the main part of the thallus or to rhizines, but many bands occurred in both thallus fractins. In all stands the common EST bands generally stained more heavily in extracts of rhizines than in extracts of other tissues. In the two stands with five ALP enzyme forms each, only one of hte electromorphs was common to both thallus fractions. In the stand with 10 isozymes, 4 occurred in both parts of the thallus. A number of ALP forms were detected exclusively in rhizines in each of the stands, and the unique rhizine bands appeared in every thallus that was dissected. In all three stands of *U. mammulata*, some thalli had one or two infrequent ALP electromorphs that were found only in the main thallus and not in rhizines. The most evident band of *U. mammulata* SOD was clearly present in both rhizines and upper thallus parts, but one other isozyme was found only in the rhizines of some individuals at each sampling site.

In one thallus of *U. vellea* (Table 2) three electromorphs of EST were exclusively associated with the upper part of the thallus and one with rhizines, but most isozymes were found in both thallus fractions. Also, most ALP bands were detected in both rhizines and the main thallus. Only one ALP band was detectable in rhizines alone. Although two isozymes each of G6P, 6PG and GDH were found only in rhizines, most of the isozymes of *U. vellea* were the same in both upper parts of the thallus and rhizines.

Table 1. Number of enzyme electromorphs of *U. mammulata* in rhizines only (R), in upper thallus layers only (T) and common to both parts of the thallus. Fractions denote the proportion of samples of one thallus part which contained at least one enzyme band not found in the other. In each stand 8–10 thalli were analyzed

| | Stands | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 2 | | | | 3 | | | |
| Enzyme | Common | T | R | Total | Common | T | R | Total | Common | T | R | Total |
| EST (carboxylic ester hydrolase) | 17 | 3 (8/8) | 3 (6/8) | 23 | 21 | 0 | 0 | 21 | 24 | 3 (10/10) | 1 (3/10) | 28 |
| ALP (phosphoric monoester hydrolase) | 1 | 1 (1/8) | 3 (8/8) | 5 | 4 | 1 (1/10) | 5 (10/10) | 10 | 1 | 2 (3/10) | 2 (10/10) | 5 |
| SOD (oxido-reductase) | 1 | 0 | 1 (5/8) | 2 | 2 | 0 | 1 (6/10) | 3 | 1 | 0 | 1 (2/10) | 2 |

Table 2. Number of enzyme electromorphs of *U. vellea* in rhizines only (R), in upper thallus layers only (T) and in both parts of the thallus. Fractions denote the proportions of samples of one thallus part which contained at least one enzyme band not found in the other. Three replicate thalli were examined from the same stand.

| Enzyme | Pathway* | Number of common bands | Number of exclusive bands | | Total number of bands |
|---|---|---|---|---|---|
| | | | T | R | |
| EST | X | 12 | 3 (1/3) | 1 (1/3) | 16 |
| ALP | X | 4 | 0 | 1 (1/3) | 5 |
| SOD | OD | 2 | 0 | 0 | 2 |
| G6P | PP | 3 | 0 | 2 (3/3) | 5 |
| 6PG | PP | 2 | 0 | 2 (2/3) | 4 |
| MAN | MN | 2 | 0 | 0 | 2 |
| IDH | TCA | 1 | 0 | 0 | 1 |
| GDH | AA | 7 | 0 | 2 (2/3) | 9 |

* AA = amino acid metabolism, MN = mannitol pathway, OD = oxygen detoxification, PP = pentose phosphate pathway, TCA = tricarboxylic acid cycle, X = various

## 4. Discussion

Electromorphs that were extractable only from the main thallus and lacking in rhizines were not unexpected since such an intrathalline distribution would be consistent with isozymes produced only by the photobiont. In both *U. mammulata* and *U. vellea*, some forms of esterase displayed this type of distribution and may be algal enzymes. Although relatively few isozymes were restricted to the main thallus, it does not mean that the algal partner is inactive metabolically. For example, although no forms of pentose phosphate pathway enzymes were detected exclusively in the upper thallus of *U. vellea*, the pathway is not necessarily inoperative in the photobiont. Instead, this result is probably a consequence of the low representation of photobiont cells in the biomass of the lichen thallus.

Since mycobiont hyphae extend throughout a lichen, it was also anticipated that some enzyme forms would be common to both the rhizine fractionand the main portion of the thallus. Banding patterns, in fact, were similar between the two thallus parts, particularly in *U. vellea*. Based on intrathalline distribution, therefore, it appears that many electromorphs in lichen extracts are primarily attributable to the mycobiont. This finding supports conclusions reached by comparison of enzyme forms of the thallus and isolated symbionts of *Cladonia cristatella* (Fahselt, 1985).

The occurrence, however, of some electromorphs only in rhizines, and not in other parts of the thallus, is more surprising. One explanation for distinctive

rhizine patterns might be that genetically different fungal strains are incorporated into upper and lower thallus regions. Larson and Carey (1986) did not investigate separated layers, but their results indicteed differing isozyme patterns with a patch-work type of distribution over large umbilicates, or a mosaic or fungal strains arranged side by side in the thallus.

Another possible explanation is that fungal metabolism is under regulatory control and gene expression varies in different thallus layers or structures. Indications of biosynthetic disparency within one thallus include distinctive pigmentation of the prothallus as compared to areoles and lower levels of phenolics, in the prothallus than in the thallus proper (Fahselt, 1976). Extracellular deposits of crystals also may differ; for example, in *Rhizocarpon geographicum*, they are much heavier on hyphae within an areole than in the prothallus. An example of a localized or structure-specific lichen phenolic is the anthraquinone, skyrin, which is mainly concentrated in the umbilicus of *Lasallia pustulata* (Posner, Feige, and Huneck, 1990). Different secondary metabolites in the layers of stratified lichens (Hale, 1983), suggests metabolic differences between the medulla and cortex. As well, there are differences in cell wall chemistry from one thallus layer to another. In some phycolichens, chitin may be limited to mycobiont cells within the algal layer or, depending on the species, to hyphae in the algal layer and those in either the upper cortex or proximal medulla (Schlarmann et al., 1990).

Regulation within a lichen seems a probable explanation for those enzyme forms that are found specifically in rhizines. Microenvironment probably differs between the upper and lower thallus surfaces, and the chemical environment of a thallus also could vary internally. For example, the photobiont must affect hyphal metabolism in the algal layer and perhaps some distance beyond, depending upon the possibility of transport via apoplastic space in the hyphal wall (Honegger, 1991). Therefore, both physical and biochemical factors could alter gene expression in lichens and enable hyphal aggregations in different parts of the thallus to produce distinctive enzyme forms, or even different enzymes.

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