Isolation, Culture, and Degradative Behavior of the Lichen Parasite *Hobsonia Santessonii*

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Abstract

The fact that lichens are generally well-defended chemically against pathogens suggests that mycoparasites known to attack lichens (parasitic lichenicolous fungi) have been able to overcome lichen chemical defenses. Recently, an isolate of the relatively specialized lichen mycoparasite *Hobsonia santessonii* was obtained from *Peltigera scabrosa* collected in northern Sweden. In laboratory experiments, the ability of this lichen parasite to degrade lichen tissues was compared with that of a related nonlichenicolous species, *Hobsonia mirabilis*. The lichen pathogen *H. santessonii* was able to degrade a variety of lichens, including a *Peltigera* species; interestingly, the degradation was greater for tissues with lichen compounds present than it was when compounds were removed using acetone. The nonlichenicolous *H. mirabilis* also exhibited degradative ability, in some cases greater than that of *H. santessonii*; however, acetone-soluble lichen compounds were far more inhibitory for *H. mirabilis* than for *H. santessonii*. These results indicate a similarity in the general types of degradative polysaccharidases produced by these apparently related fungi; however the more specialized *H. santessonii* appears to produce enzymes that are more tolerant of lichen compounds.

1. Introduction

Lichenicolous fungi colonize lichens to form a variety of associations ranging from parasitic/necrophytic to commensalistic, and even mutualistic in some cases (Clauzade et al., 1989; Hawksworth, 1982). The relative infrequency of lichen attack by parasitic fungi has been attributed to the production by lichens of a variety of secondary metabolites (Hawksworth, 1979), many of which exhibit antibiotic activities (Vartia, 1973; Rundel, 1978; Lawrey, 1984, 1986, 1987). Recent experimental studies have demonstrated that lichen secondary metabolites limit distributions of selected lichenicolous members of the ascomycetous order Hypocreales (Lawrey et al., 1994). Generally, lichen compounds have been found to elicit negative responses from lichen parasites; however, there is also some evidence (Lawrey, 1993) for positive effects of lichen secondary metabolites on the growth of highly specialized fungi.

This paper describes a study of a lichenicolous hyphomycete, *Hobsonia santessonii* Lowen & D. Hawksw., which is known from the literature (Lowen et al., 1986) to be restricted to thalli of the lichen *Peltigera scabrosa* in Scandinavia. Field observations indicate it is a mild pathogen which does little damage to its host, but it has not been the subject of experimental study. In 1995, a fresh specimen of *H. santessonii* was sent to me by Professor Rolf Santesson, and this was subsequently isolated and brought into culture. This culture was used in a series of laboratory experiments designed to investigate the following questions: 1) To what extent does the degradative ability of this pathogen under laboratory conditions help to explain its extreme host specialization in natural habitats? 2) How do lichen secondary compounds influence its degradative behavior? 3) How do the laboratory responses of this lichen parasite compare with those of a related nonlichenicolous hyphomycete, *Hobsonia mirabilis*?

2. Materials and Methods

Hobsonia isolates

Cultures of the lichen parasite *Hobsonia santessonii* Lowen & D. Hawksw. were derived from a single-conidium isolate (labelled JL105-95) obtained from part of a specimen collected by Starri Heidmarsson (n. 550) at Abisko in Torne Lappmark in northern Sweden; the original specimen is housed in the Botanical Museum, Uppsala University. It was found growing on the lichen *Peltigera scabrosa* Th. Fr.

Conidia were removed using a sterile needle and deposited on petri plates containing the following media: Sabouraud's agar supplemented with

dextrose, Sabouraud's agar supplemented with maltose, corn meal agar, potato dextrose agar, and plain agar. Germination of conidia was observed between 10 and 15 days after being deposited on the surface of Sabouraud's with maltose; no other medium elicited germination. Maintained in a growth chamber at 18°C, colonies grew slowly, producing tufts of conidia on irregularly-shaped sporodochia as they enlarged and coalesced with each other. The isolate has been deposited in the American Type Culture Collection (ATCC 200311).

A culture of *Hobsonia mirabilis*, which is not known to infect lichens, was obtained from the American Type Culture Collection (ATCC 18961) and used in degradative experiments to provide data for comparison with *H. santessonii*. This nonlichenicolous fungus was initially isolated by Roger D. Goos (RDG272) from decaying palm leaves collected in Hawaii; it was maintained on Sabouraud's supplemented with dextrose at the same culture conditions as the culture of *H. santessonii*.

Analysis of lichen chemistry

Standard thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques (Culberson, 1972; Culberson and Johnson, 1976; Culberson et al., 1981; Culberson and Elix, 1989) were used to analyze acetone extracts of six lichen species used in degradation experiments (listed below). HPLC was also used to evaluate the extent to which lichen tissues subjected to acetone washing for degradation experiments were free of phenolic compounds.

Preparation of inoculum

Mycelium of *Hobsonia santessonii* or *H. mirabilis* was aseptically scraped from the surface of six 2-week-old agar plate cultures (incubated at 18°C) into a sterilized Waring blender with enough sterile distilled water to cover the blades. This was blended for 30 sec, and the homogenized inoculum was added to experimental dishes at a level of 5 mg dry wt/12.5 ml culture medium.

Laboratory experiments

Laboratory experiments were carried out to determine (1) if the two *Hobsonia* species could grow on lichens, (2) whether *H. santessonii* could use lichens other than *Peltigera scabrosa*, its normal host, and (3) if lichen secondary metabolites influence degradation of lichen tissues by either fungus. Six lichens were chosen to be used as substrates for growth of the fungi. Five of these – *Flavoparmelia baltimorensis* (Gyel. & Fór.) Hale, *Xanthoparmelia*

conspersa (Ehrh. ex Ach.) Hale, *Punctelia rudecta* (Ach.) Krog, *Myelochroa aurulenta* (Tuck.) Elix & Hale, and *Lasallia papulosa* (Ach.) Llano – have been used in previous experiments involving other lichenicolous fungi (Lawrey, 1993; Lawrey et al., 1994) and one – *Peltigera canina* – is presumably similar to the preferred host species, *Peltigera scabrosa*, sufficient material of which could not be obtained from the field.   All lichen material was collected in Rappahannock County, VA, and used while still fresh. Thalli were cleaned of debris, washed briefly in distilled water, dried, and ground in a Wiley mill to pass a 20-mesh screen. A subsample of material for each species was washed several times in absolute acetone to remove all lichen phenolic compounds. Both washed and unwashed samples were then autoclaved and oven-dried (100°C) for 4 hours and stored in a desiccator. These materials were used as growth media for inocula of *H. santessonii* or *H. mirabilis*.

To separate the effects of lichen secondary metabolites from those of other possible factors on fungal degradative ability, washed tissues of *Lasallia papulosa* were treated with acetone extracts of each of the test lichens to create growth media that were identical in every way except secondary chemistry. Extracts were added to acetone-washed tissues of *Lasallia papulosa* (chosen because each *Hobsonia* species was known to be able to degrade this lichen after it is washed with acetone) in the same concentration found in natural lichens. The tissue was then dried in a hood and stored in a desiccator for 1 week prior to use in growth/degradation experiments.

Fresh suspensions of either *Hobsonia santessonii* or *H. mirabilis* were used to inoculate lichen tissues in each growth experiment. Approximately 100 mg of lichen tissue was weighed to the nearest 0.01 mg, poured into 5-cm glass petri plates, and inoculated with 1 ml of *Hobsonia* suspension. These were then sealed in plastic film and placed in a growth chamber (12–12 hour light-dark cycle, 18°C) for 30 days. At the end of the experiment, the growth of the parasite was estimated by determining the net weight loss of the lichen tissue upon which it had been growing. Weights obtained at the end of each experiment included any fungal biomass that accumulated during the experiment since this could not be separated from the growth medium.

3. Results

Analysis of lichen chemistry

The acetone extracts of the test lichens contained the lichen compounds expected for these species (Table 1), and the chemical profiles were the same as those reported for previous studies involving these lichens (Lawrey, 1993;

Table 1. Phenolic compounds identified in acetone extracts of lichens used in fungal growth experiments.

Lichen species	Compounds
Flavoparmelia baltimorensis	Protocetraric, Usnic, Gyrophoric and Caperatic acids, Atranorin
Xanthoparmelia conspersa	Stictic, Norstictic, Constictic, Connorstictic and Usnic acids
Lasallia papulosa	Gyrophoric, Lecanoric, Papulosin, unknown pigments
Punctelia rudecta	Lecanoric acid, Atranorin
Peltigera canina	No polyketide compounds
Myelochroa aurulenta	Secalonic acid A, Atranorin, Zeorin, Leucotylic acid

Lawrey et al., 1994). Repeated tests of washed substrates revealed no detectable compounds.

Since lichen compounds are known to be relatively unstable in excessively hot environments (Culberson et al., 1977), chemical changes to the substrates were expected to result from sterilization. Indeed, a few changes in TLC and HPLC pattern were observed following autoclaving of lichen material. This was especially evident in *Xanthoparmelia conspersa*, extracts of which appeared to contain several breakdown products of norstictic acid. However, inasmuch as the extracts of both autoclaved and untreated lichens always contained the same compounds (with the addition of small quantities of breakdown products for some), and autoclaving was deemed the most reliable way to sterilize the experimental substrates, these substrates are as similar chemically to the cell walls of natural lichens as is possible in a laboratory simulation.

Degradative ability of lichenicolous and nonlichenicolous Hobsonias

In general, each of the two *Hobsonia* species exhibit some ability to utilize a variety of lichen tissues when lichen compounds are present (Fig. 1). It is interesting that each species exhibited the lowest degradative activity on normal, unwashed material of *Myelochroa aurulenta*, although some enhancement of growth was observed if acetone-soluble compounds were removed. The net weight gain observed for *H. mirabilis* on this lichen is caused

by the addition of approximately 5 mg of inoculum to the substrate and then having no degradative activity take place during 30 days. Both species also exhibited maximum degradation rates on *Peltigera canina*, which produces no lichen compounds. In addition, the nonlichenicolous *H. mirabilis* appeared to prefer *Xanthoparmelia conspersa* and the lichenicolous *H. santessonii* appeared to prefer *Lasallia papulosa*.
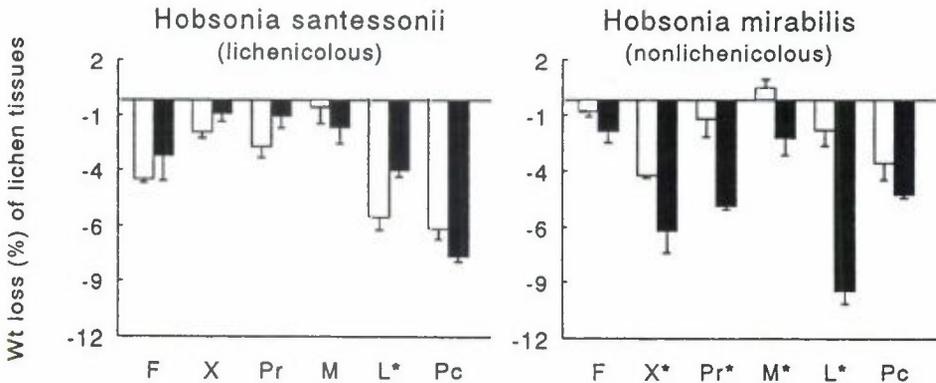

Figure 1.   Degradative ability (measured as mean percent net wt. loss of substrate after 30 days ± standard error of the mean) of lichenicolous *Hobsonia santessonii* and nonlichenicolous *Hobsonia mirabilis* inoculated onto six lichen substrates (F = *Flavoparmelia baltimorensis*; X = *Xanthoparmelia conspersa*; Pr = *Punctelia rudecta*; M = *Myelochroa aurulenta*; L = *Lasallia papulosa*; Pc = *Peltigera canina*. Lichen material was either washed with acetone to remove secondary metabolites (filled bars) or left unwashed (open bars). Sample size for each treatment was six. An asterisk (*) indicates that a significant (Mann-Whitney test, p≤0.05) difference was found between the washed and unwashed samples of the lichen tissue.

The *Hobsonia* species differed markedly in their response to the removal of lichen compounds, however. The nonlichenicolous *H. mirabilis* degrades significantly (Mann-Whitney test, p≤0.05) larger fractions of most lichens (*X. conspera, P. rudecta, M. aurulenta* and *L. papulosa*) when they were washed with acetone to remove lichen compounds, indicating that they are generally inhibitory to this fungus. On the other hand, the lichenicolous *H. santessonii* generally degrades the same or a smaller fraction of lichen tissues when these compounds are removed, indicating that their presence is not inhibitory to this fungus, and can even be stimulatory. In the case of *Lasallia papulosa* tissue, for example, significant (Mann-Whitney test, p≤0.05) enhancement of tissue degradation was observed when compounds were present.
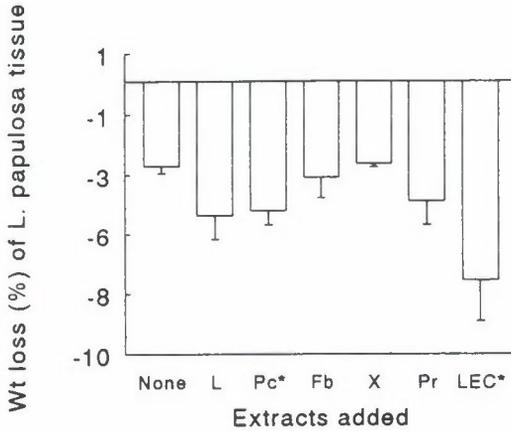
Figure 2.   Degradative ability (measured as mean percent net wt. loss of substrate after 30 days ± standard error of the mean) of *Hobsonia santessonii* inoculated onto sterilized tissues of *Lasallia papulosa* that had been washed with acetone to remove secondary metabolites and then treated with acetone extracts of several lichen species (None = acetone only, no extract added; L = *Lasallia papulosa*; Pc = *Peltigera canina*; Fb = *Flavoparmelia baltimorensis*; X = *Xanthoparmelia conspersa*; Pr = *Punctelia rudecta*; LEC = pure lecanoric acid at 5% (wt/wt) thallus dry wt). Sample size for each treatment was four. An asterisk (*) indicates a significantly greater degradation for a treated substrate (Mann-Whitney test, p≤0.05) than for the control (NONE).

Since each species is able to utilize washed tissues of *Lasallia papulosa*, acetone extracts of the test lichens were added to washed tissues of this lichen to create substrates that differed only with regard to secondary chemistry. These were inoculated with either *Hobsonia santessonii* or *H. mirabilis* and weighed after 30 days. Results (Fig. 2) indicated that the presence of lichen extracts either had no significant effect on degradation when compared to untreated tissues or significantly enhanced the degradation of washed *Lasallia papulosa* tissues. Interestingly, the acetone extract of *Peltigera canina* had a significant (Mann-Whitney test, p≤0.05) stimulatory effect on degradation; also, addition of 5% lecanoric acid to the medium stimulated degradation. None of the lichen extracts elicited an inhibition of degradative ability by *H. santessonii*.

4. Discussion

Results of these laboratory experiments indicate: (1) that both lichenicolous

Hobsonia santessonii and the related nonlichenicolous *H. mirabilis* are able to degrade lichens; but (2) that *H. santessonii* is less sensitive to the presence of lichen secondary metabolites than *H. mirabilis*, and may even exhibit enhanced degradative activities in the presence of these compounds.

An enhancement of degradative ability by a lichen mycoparasite in the presence of lichen compounds has been observed once before (Lawrey, 1993) for a fungus originally called *Hobsonia christiansenii*. This organism has since been identified by Paul Diederich as *Marchandiomyces corallinus*, which has a similar appearance. [Although it has been suggested by Lowen et al. (1986) that *H. christiansenii* and *M. corallinus* are actually syanamorphs inasmuch as they appear to have identical chemistries, the elucidation of this will require further experimental work and it would seem prudent to keep the two names distinct at this point.] The fungus attacked thalli of *Flavoparmelia baltimorensis* almost exclusively, and was found to degrade this lichen best in laboratory experiments. Acetone extracts of this preferred lichen host also elicited enhanced degradative behavior. However, the parasite was generally quite sensitive to the secondary metabolites of other nonhost lichens. In a similar study (Lawrey et al., 1994) of lichenicolous hypocrealean fungi tested with the same lichen tissues, the presence of lichen compounds was also frequently found to be inhibitory.

The chemical constituents of the most inhibitory extracts have not been investigated completely; however, pure samples of a few lichen compounds have been used in a number of unpublished degradation experiments; of these, lecanoric acid is among the most inhibitory. This result is especially interesting in light of the present results indicating a stimulatory effect on tissue degradation by *H. santessonii* of this compound.

Lawrey et al. (1994) suggested that the ability of a fungus to parasitize lichens is strongly coupled to the production of enzymes that are resistant to the chemical defenses of lichen hosts. There is some evidence for this. Torzilli and Lawrey (1995) documented the enzymatic basis for the degradation of lichen tissues by the lichen mycoparasite *Nectria parmeliae*, and showed also that lichen compounds have the ability to alter enzymatic activity. It is likely that a narrowly-distributed and ecologically-specialized lichen parasite such as *Hobsonia santessonii* makes a limited number of degradative enzymes which are most effective in degrading tissues of the lichen host on which it specializes. However, the fact that its behavior is enhanced in the presence of many lichen compounds, including those not produced by its normal host, may indicate an earlier use of lichens as hosts by members of this particular hyphomycete lineage.

It was expected that *Hobsonia santessonii* would degrade tissues of *Peltigera canina*, a close relative of its normal host, *Peltigera scabrosa*. Although these

two *Peltigera* spp. differ chemically, they are likely to produce similar cell wall compounds to which the enzymes of *H. santessonii* are optimally adapted. Cell wall constituents of lichen mycobionts are known to vary considerably (Ahmadjian, 1993), so variation in the cell wall-degrading enzymes of lichen parasites is a good source of information to explain the host ecology of these organisms.

Members of the Peltigeraceae are known to harbor numerous lichenicolous fungi. Hawksworth (1980, 1982) has argued that the unusually high number of fungal parasites observed on members of the Peltigeraceae may be due to the ancient age of this group as a distinct lineage of lichen-forming fungi; other possible explanations he mentioned include the low diversity of secondary chemicals, and the moist habitat and generally high nitrogen content of these lichens. The present study indicates that although *P. canina* is relatively depauperate chemically, *H. santessonii* is unusually tolerant of lichen secondary compounds. For this particular mycoparasite, then, the presence or absence of secondary metabolites in lichens seems to be unimportant.

Acknowledgments

REFERENCES

Ahmadjian, V. 1993. *The Lichen Symbiosis*. Wiley, NY.

Clauzade, G., Diederich, P., and Roux, C. 1989. Nelikenigintaj fungoj likenlogaj. Illustrita determinlibro. *Bulletin de la Société linnénne de Provence* 1: 1–142.

Culberson, C.F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* 72: 113–125.

Culberson, C.F. and Elix, J.A. 1989. Lichen substances. In: *Methods in Plant Biochemistry*. Academic Press, London, pp. 509–535.

Culberson, C.F. and Johnson, A. 1976. A standardized two-dimensional thin-layer chromatographic method for lichen products. *Journal of Chromatography* 128: 253–259.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1977. Thermally induced chemical artifacts in lichens. *Phytochemistry* 16: 127–130.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1981. A standardized TLC analysis of β-orcinol depsidones. *Bryologist* 84: 16–29.

Hawksworth, D.L. 1979. The lichenicolous Hyphomycetes. *Bulletin of the British Museum of Natural History* (Botany) 6: 183–300.

Hawksworth, D.L   1980.   Notes on some fungi occurring on *Peltigera*, with a key to accepted species. *Transactions of the British Mycological Society* **74**: 363–386.

Hawksworth, D.L. 1982. Secondary fungi in lichen symbioses: parasites, saprophytes and parasymbionts. *Journal of the Hattori Botanical Laboratory* **52**: 357–366.

Lawrey, J.D. 1984. *Biology of Lichenized Fungi.* Praeger, NY.

Lawrey, J.D. 1986. Biological role of lichen substances. *Bryologist* **89**: 111–122.

Lawrey, J.D. 1987. Nutritional ecology of lichen/moss arthropods. In: *Nutritional Ecology of Insects, Mites, and Spiders.* F. Slansky, Jr. and J.G. Rodriguez, eds. Wiley, NY. pp. 209–233.

Lawrey, J.D. 1993. Chemical ecology of *Hobsonia christiansenii*, a lichenicolous hyphomycete. *American Journal of Botany* **80**: 1109–1113.

Lawrey, J.D., Rossman, A.Y., and Lowen, R. 1994. Inhibition of selected hypocrealean fungi by lichen secondary metabolites. *Mycologia* **86**: 502–506.

Lowen, R., Brady, B.L., Hawksworth, D.L., and Paterson, R.R.M. 1986. Two new lichenicolous species of *Hobsonia*. *Mycologia* **78**: 842–846.

Rundel, P.W. 1978. The ecological role of secondary lichen substances. *Biochemical Systematics and Ecology* **6**: 157–170.

Torzilli, A.P. and Lawrey, J.D. 1995. Lichen metabolites inhibit cell wall-degrading enzymes produced by the lichen parasite *Nectria parmeliae*. *Mycologia* **87**: 841–845.

Vartia, K.O. 1973. Antibiotics in lichens. In: *The Lichens.* V. Ahmadjian and M. E. Hale, Jr., eds. Academic Press, NY. pp. 547–561.