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Prion protein degradation by lichens of the genus *Cladonia*

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Abstract: It has recently been discovered that lichens contain a serine protease capable of degrading the pathogenic prion protein, the etiological agent of prion diseases such as sheep scrapie and cervid chronic wasting disease. Limited methods are available to degrade or inactivate prion disease agents, especially in the environment, and lichens or their serine protease could prove important for management of these diseases. Scant information is available regarding the presence or absence of the protease responsible for degrading prion protein (PrP) in lichen species and, in this study, we tested the hypothesis that PrP degradation activity in lichens is phylogenetically-based by testing 44 species of *Cladonia* lichens, a genus for which a significant portion of the phylogeny is well established. We categorized PrP degradation activity among the 44 species (high, moderate, low or none) and found that activity in *Cladonia* species did not correspond with phylogenetic position of the species. Degradation of PrP did correspond, however, with three classical taxonomic characters within the genus: species with brown apothecia, no usnic acid, and the presence of a cortex. Of the 44 species studied, 18 (41%) had either high or moderate PrP degradation activity, suggesting the protease may be frequent in this genus of lichens.

Key words: serine protease, transmissible spongiform encephalopathy

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Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of fatal, infectious, neurodegenerative diseases that afflict a variety of mammalian species (Watts *et al.* 2006). Some TSEs include bovine spongiform encephalopathy, transmissible mink encephalopathy, human Creutzfeldt-Jakob disease, sheep scrapie and cervid chronic wasting disease (CWD). Scrapie and CWD are unique among TSEs in that they are laterally transmitted from infected to uninfected hosts, either through direct animal-to-animal contact or following contact with contaminated environments (Detwiler 2003; Sigurdson 2008). The infectious agent responsible for TSEs is referred to as a 'prion' and is thought to be composed solely of a misfolded form of the prion protein (PrP^{TSE}), which is derived from a normal, cellular form of the protein (Colby & Prusiner 2011). Treatments that inactivate other pathogens are typically ineffective in eliminating TSE infectivity (Taylor 2000). The extreme stability of prions is presumably responsible for allowing scrapie and CWD infectivity to persist in the environment and cause disease in previously healthy animals (Gough & Maddison 2010). Soil is a likely reservoir of scrapie and CWD infectivity, and understanding natural processes or developing procedures to degrade prions in the environment is critical to our limiting the spread of these diseases (Smith *et al.* 2011).

In a previous study, we found that acetone extracts of several lichen species were capable of degrading PrP^{TSE} in brain homogenates, as well as purified PrP^{TSE} (Johnson *et al.* 2011). We found activity in extracts from *Parmelia sulcata*, *Cladonia rangiferina* and *Lobaria pulmonaria*, but surprisingly, not in extracts of the closely related species *P. squarrosa*, *C. stellaris* and *L. oregana* or *L.*

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quercizans. Using specific inhibitors, a serine protease was found to be responsible for PrP^{TSE} degradation and lichen secondary metabolites, and other enzymes were not effective in degrading the protein. Extracts of isolated lichen photobionts were not found to have any anti-prion protein (PrP) activity, suggesting the lichen mycobionts were responsible for the protease activity (Johnson *et al.* 2011).

In further studies, we have found varying levels of anti-PrP activity in a sampling of lichens from more than 10 genera, suggesting activity is widespread, but not evenly distributed in lichen genera (Rodriguez *et al.* 2012). Hundreds of proteases are known in fungi (North 1982), and have been found to have roles in pathogenesis, pathogen resistance, protein digestion, nutrition, morphogenesis, reproduction, germination, and more. The broad diversity of species in our previous studies with proteolytic activity capable of PrP degradation led us to investigate the phylogenetic basis of the character.

The genus *Cladonia* is one of the largest genera of lichens, with *c.* 450 species worldwide, and about 175 in North America (Ahti & Hammer 2002; Esslinger 2011). Species of *Cladonia* typically grow on soil and rotting wood, are found in many different habitats, and are known to produce secondary metabolites with bioactive properties (Thomson 1967). Members of the genus *Cladonia* grow naturally in areas where scrapie or CWD are endemic, and the soil habitat is of interest due to the potential for scrapie and CWD to persist in soils. Fresh *Cladonia* material of many species is easy to collect in a small number of localities, facilitating sampling of a large number. In addition, recent phylogenetic studies of the genus based on 73 species (Guo & Kashiwadani 2004) and 168 species (Stenroos *et al.* 2002) have provided data for grouping species based on molecular, morphological and chemical data.

The objectives of our present study were to determine the PrP degradation activities of *Cladonia* species and then determine the correlations of the activities to known phylogenies. In addition, PrP degradation activity correlations with classical taxonomic characters would also be established. We tested the hypotheses that species with the activity were closely, rather than distantly related, and that activity would be related to classical taxonomic characters which are genetically based. We therefore screened the anti-PrP protease activity in 44 species of one genus to look for the emergence of hereditary patterns.

Materials and Methods

Lichens and extracts

During 2010 we collected specimens of 44 species of *Cladonia* at localities in Wisconsin, Michigan, Louisiana and New Brunswick, Canada (see Appendix). We were also provided with a fresh collection of *C. jaliscana* from Mexico by T. Nash, and we included three specimens recently collected from Alaska, deposited in WIS. Specimens were chosen to represent all major phylogenetic groups of the genus. Specimens were cleaned of extraneous material under a dissecting microscope and small pieces of both squamules and podetia were selected for extraction. Not knowing *a priori* if the agent of activity was localized in the thallus, we sampled the entire lichen thallus. All specimens had been dried after collection and identified before extraction. Species identifications were based on Ahti & Hammer (2002), Hinds & Hinds (2007), Brodo *et al.* (2001) and Thomson (1967) keys. Original specimens are deposited in WIS.

We selected three classical taxonomic characters for tabulating and comparing with anti-prion activity: apothecial colour, presence/absence of a cortex, and presence/absence of usnic acid. These were selected because they are traditionally used in dividing major groups of *Cladonia* species, and are easily determined both from specimens and the literature.

Dried lichens were then powdered by bead-beating with 2·8 mm ceramic beads (MoBio, Carlsbad, CA). Extracts were produced by suspending lichen powders in acetone at 10% (w/v) and incubating at 37°C for 24 h with vigorous shaking. Following incubation, solid particles were removed by filtration through Whatman filter paper (Grade #1, Piscataway, NJ), and the acetone in the filtrate was evaporated. The remaining residue was resuspended in dimethyl sulfoxide (DMSO) (ThermoFisher Scientific, Rockford, IL) so that each g of lichen powder starting material would yield 1 ml of product. Our testing was based on the weight of the lichen samples, not the residues, so that the results would be relevant to the activities of the individual specimens.

Lichen extract-prion protein reactions

All experiments used brain homogenate derived from clinically-affected CD-1 mice, experimentally inoculated with mouse-passaged RML scrapie agent. Animal work was conducted with the approval of the National Wildlife Health Center Institutional Animal Care and Use Committee (Protocol #EP080716). Brain homogenate was

generated by adding brain tissue to a final concentration of 10% (w/v) in deionized H_2O and disrupting with a Dounce homogenizer. Degradation of PrP by lichen extracts was assessed by incubating brain homogenate (10 µl; 1 mg brain equivalent) with 10 µl of lichen extract in DMSO (10 mg of lichen equivalent), or DMSO vehicle as a control in a solution of 10 mM sodium acetate pH 5·0 in a total volume of 30 µl. Reactions proceeded for 1 h at 37°C and were halted by the addition of 4× lithium dodecyl sulphate (LDS) sample buffer and 10× NuPAGE reducing agent (Invitrogen, Carlsbad, CA), each to final concentrations of 1×. Samples were then heated at 95°C for 5 min.

NuPAGE and immunoblotting

Samples were subjected to 12% NuPAGE gel electrophoresis using MOPS running buffer and electroblotted to polyvinyl difluoride membranes. Membranes were then immunoblotted using monoclonal antibody SAF83 (1:5000) (Chemicon, Billerica, MA). Immunoreactivity was visualized using an anti-mouse secondary antibody conjugated with horseradish peroxidase (Santa Cruz Biotech, Santa Cruz, CA), Pierce SuperSignal West Pico Chemiluminescent Substrate System (Rockford, IL) and an EC3 imaging system (UVP, Upland, CA). Densitometric measurements were made using UVP VisionWorks®LS software. All immunoblot results were confirmed by at least three independent experimental replicates done in separate reaction tubes.

Data analyses

Species frequencies (i.e. counts) were cross-tabulated by PrP degradation activity and character states and analyzed by log-likelihood significance tests. The resulting Chi-Square probabilities were calculated using JMP version 8 software to test null hypotheses about the frequency distributions in the tables (SAS Institute 2009).

Results

PrP degradation conditions

We tested extracts prepared from 44 *Cladonia* species for their ability to reduce PrP immunoreactivity in samples of TSE-infected brain homogenate as compared to a control treatment with DMSO alone. Conditions for reactions were previously optimized and were conducted at pH 5·0 (Johnson *et al.* 2011). When reactions were buffered to pH 7·0 with either HEPES or Tris, no degradation was observed for any lichen tested (data not presented). The current pH findings are consistent with our previous work indicating that low pH favours PrP degradation by an extract of the lichen *Parmelia sulcata*.

Categorizing *Cladonia* lichens by activity levels

We have previously assigned lichen species to three PrP degradation levels: 1) below the limit of immunoblot detection, 2) some ability to reduce PrP levels, and 3) no activity (Johnson *et al.* 2011; Rodriguez *et al.* 2012). For our experiments of PrP^{TSE} with *Cladonia* extracts, we used slightly modified categories for the amount of remaining immunoreactivity present: 1) none/low, indicating a range of no reduction in immunoreactivity to $<50\%$ reduction; 2) moderate, indicating $>50\%$ reduction in immunoreactivity but some remaining signal; and 3) high, indicating no detectable PrP immunoreactivity remaining. In Fig. 1, we present a representative immunoblot showing 10 species at the various levels of activity used for categorizing the *Cladonia* lichens in our study. Using the detection method shown in Fig. 1, we grouped the 44 *Cladonia* species into the three activity categories (Table 1). We found 15 species with high anti-PrP proteolytic activity, 13 with moderate, and 16 with none/low activity.

Comparison of degradation activity with phenotypic characters and ecological factors

A little over half of our specimens were from Wisconsin, and they were almost evenly divided among the three PrP degradation categories. The remaining specimens from North America were too few to draw conclusions about the effect of specimen origin on PrP degradation activity. Almost 40% of the species are considered boreal or montane species, and these were almost evenly divided between having and not having PrP degradation activity. Our sampled *Cladonia* species grow mostly on soil and rotting wood, and there was no obvious relationship between substratum and PrP degradation activity. Half of the 44 species grow on soil or rock, while the other half occur mainly on rotting wood. About half of the species studied prefer sunny exposures, while about one fifth prefer shade, and the remainder can tolerate sun or shade. Over half of the sun species have PrP degrada-

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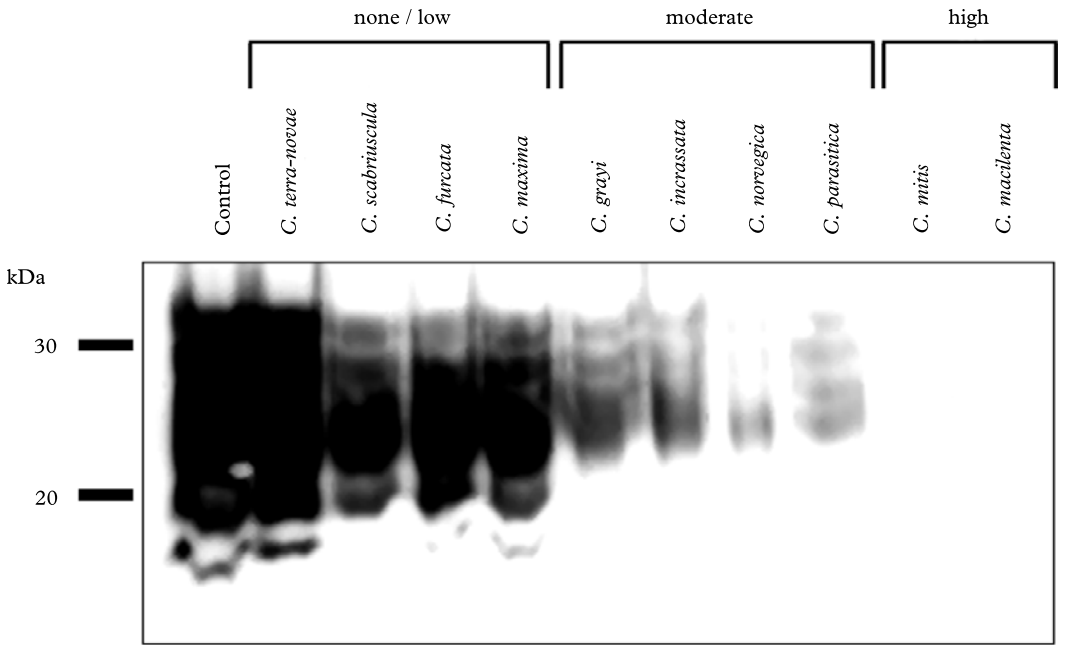

FIG. 1. Immunoblot indicating varying degrees of PrP degradation by different *Cladonia* species.

tion activity, while the shade species were evenly split between having PrP degradation activity and not having it.

Of the 34 species we studied that have brown apothecia, 22 have PrP degradation activity, while 5 of the 10 species with red apothecia have PrP degradation activity. Of the 15 species with high PrP degradation activity, brown fruited species outnumber the red fruited species by 4 to 1. The log-likelihood ratio for this pattern was not statistically significant, probably due to the small sample sizes.

Half of the species studied (22) with PrP degradation activity do not produce usnic acid. Of the 14 species that do produce usnic acid, 6 have PrP degradation activity and 3 have high PrP degradation activity. Species with high PrP degradation activity without usnic acid outnumber those with usnic acid by 4 to 1. The log-likelihood ratio for this pattern of 1·9 resulted in a Chi-square value of 3·8, which had a probability of occurring due to chance of 0·15.

Twenty-two of the 37 species with a cortex on the thallus (based on the literature) have PrP degradation activity, compared with five of the seven without a cortex. Even though the sample sizes of species with and without a cortex are uneven, the ones with PrP degradation activity outnumber those without it by 4 to 1. However, this pattern was not statistically significant due to the small sample sizes.

A tabulation of the frequencies of 44 *Cladonia* species categorized by three taxonomic characters and PrP degradation activity is shown in Table 2. When the latter three characters were combined into one analysis, 17 of the 44 species (39%) with either high or moderate PrP degradation activity have brown apothecia, no usnic acid, and a cortex present, the largest frequency of any combined group of characters. The log-likelihood ratio Chi-square of 16·5 had a probability of 0·087 of this pattern being due to chance.

Thirty-seven of the 44 species studied were included in the phylogeny of Stenroos *et al.*

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TABLE 1. *Forty four* Cladonia *species categorized by PrP degradation activity*

High	Moderate	None or low
C. bellidiflora (Ach.) Schaer.	*C. caespiticia* (Pers.) Flörke	*C. apodocarpa* Robbins
C. chlorophaea (Flörke ex Sommerf.) Spreng.	*C. cenotea* (Ach.) Schaer.	*C. borealis* S. Stenroos
C. didyma (Fée) Vain.	*C. cervicornis* (Ach.) Flot.	*C. cariosa* (Ach.) Spreng.
C. evansii Abbayes	*C. fimbriata* (L.) Fr.	*C. cristatella* Tuck.
C. macilenta Hoffm.	*C. grayi* G. Merr. Ex Sandst.	*C. cylindrica* (A. Evans) A. Evans
C. mitis Sandst.	*C. incrassata* Flörke	*C. deformis* (L.) Hoffm.
C. multiformis G. Merr.	*C. jaliscana* Ahti & Guzm.	*C. furcata* (Huds.) Schrad.
C. peziziformis (With.) J. R. Laundon	*C. magyarica* Vain.	*C. leporina* Fr.
C. phyllophora Hoffm.	*C. norvegica* Tons. & Holien	*C. maxima* (Asahina) Ahti
C. pocillum (Ach.) Grognot	*C. ochrochlora* Flörke	*C. scabriuscula* (Delise) Nyl.
C. pyxidata (L.) Hoffm.	*C. parasitica* (Hoffm.) Hoffm.	*C. squamosa* Hoffm.
C. rangiferina (L.) F. H. Wigg.	*C. sobolescens* Nyl. ex Vain.	*C. stellaris* (Opiz) Pouzar & Vězda
C. robbinsii A. Evans	*C. subtenuis* (Abbayes) Mattick	*C. subulata* (L.) F. H. Wigg.
C. subradiata (Vain.) Sandst.		*C. sulphurina* (Michx.) Fr.
C. verticillata (Hoffm.) Schaer.		*C. terrae-novae* Ahti
		C. uncialis (L.) F. H. Wigg.

TABLE 2. *Frequencies of forty four* Cladonia *species grouped by taxonomic characters and PrP degradation activity (PDA)*

Colour of apothecia	Presence of usnic acid	Presence of cortex	High PDA	Moderate PDA	No PDA	Total
Brown	−	−	3	0	0	3
Brown	−	+	7	10	8	25
Brown	+	−	1	1	2	4
Brown	+	+	1	0	1	2
Red	−	+	2	0	0	2
Red	+	+	1	2	5	8
Total			15	13	16	44

(2002), and 30 were included in Guo & Kashiwadani's (2004) phylogeny. Of the high PrP degradation activity species, 13 were included in Stenroos *et al.* and 11 in Guo & Kashiwadani. Of the moderate PDA species, 8 were included in Stenroos *et al.* and 6 in Guo & Kashiwadani. Of those that had no PDA, 16 were in Stenroos *et al.* and 13 in Guo & Kashiwadani. In both phylogenies, there was a complete overlap of the three PrP degradation activity categories across the entire tree, and no clustering of the character in any one clade. In some instances, all three PrP degradation activity categories occurred next to one another in the tree, for example *C. cylindrica* (none), *C. grayi* (moderate), and *C. phyllophora* (high) in Stenroos *et al* (2002). No phylogenetic association of the PrP degradation activity character could be detected in either study, nor was any association found when compared with the phylogeny of Lendemer & Hodkinson (2009) for apodetiate *Cladonia* species.

Selected algal partners of 17 of our 44 species studied were examined phylogenetically by Piercey-Normore & DePriest (2001). No patterns of association among the three PrP degradation activity categories and the algal clades were evident. As above, pairs of algal species with opposing PrP degradation activities were sometimes paired in the same clade, for example algae from *C. grayi* and algae from *C. robbinsii*.

Discussion

The frequencies of the three PrP degradation activity categories are about evenly divided among the 44 species, indicating that the protease involved is either potent in a species, completely absent, or has some degree of potency in between the two. This could mean that there is more than one protease involved and they have different potencies. It could also be interpreted that other factors regulate the protease activity or modulate PrP^{TSE} structure to promote PrP degradation. Nevertheless, a species of *Cladonia* has about a one third chance of having the protease in a potent form, not at all, or a moderate expression of the activity. However, if the high and moderate categories are merged, then 18/44 *Cladonia* species surveyed (41%) have some form of PrP degradation activity. If this result were extrapolated to the entire genus, it might suggest that a species of *Cladonia* has a 2·5:1 chance of having PrP degradation activity of some potency.

Our data do not provide evidence to support the hypothesis that phylogeny determines PrP degradation activity. No patterns of association of the 44 species were found with recently published *Cladonia* phylogenies. Although our sample of 44 species is about one tenth of the 450 *Cladonia* species described, they represent important phylogroups in the genus, including four type species at the group or supergroup levels according to a recent phylogenetic study by Stenroos *et al.* (2002), and each of them were categorized in different PrP degradation activities. *Cladonia rangiferina* (type species for the *Cladinae* group) has high PrP degradation activity, *C. cenotea* (type species for the supergroup *Perviae*) has moderate activity, and *C. uncialis* and *C. subulata* (type species for the group *Unciales* and supergroup *Cladonia*, respectively) have no activity. No phylogenetic relationships were found with selected photobionts of *Cladonia* species, suggesting that the protease may be a component of the mycobiont instead. This remains to be determined, however, because some enzymes in lichens are from the mycobiont or photobiont exclusively, and some are found in both bionts (Legaz *et al.* 2004).The concept that the protease is derived from the mycobiont is consistent with our previous data, in which we failed to identify anti-PrP protease activity in extracts of a small group of isolated photobionts (Johnson *et al.* 2011). However, since no relationships were found with the *Cladonia* species, which are taxonomically the mycobionts, the location of the protease or PrP degradation activity in either symbiont is still an open question.

We attempted to relate PrP degradation activity to three characters that are used in classical *Cladonia* taxonomy: apothecial colour, presence/absence of usnic acid, and presence/absence of a cortex. The strength of the association of PrP degradation activity with these characters increased in the order apothecial colour, cortex presence, and finally usnic acid presence, but none of these were statistically significantly associated below the 0·10 probability level. When combined into one multivariate cross-tabulation, however, the association had a 0·09 probability of occurring by chance, suggesting that PrP degradation activity is associated with many characters. *Cladonia* species with brown apothecia, no usnic acid, and a cortex are most likely to have some PrP degradation activity based on this data set. Curiously, *C. subulata*, which is the type species of the genus (Ahti & Hammer 2002), has these characters but does not have PrP degradation activity.

No associations were found between PrP degradation activity and the geographic origin or habitat of the specimens examined. This suggests that PrP degradation activity is not determined by environmental or geographical factors, at least in this sample of 44 species. We still believe that this should be studied further, however, as some enzymes in lichens, such as urease, are present in some species only in the winter and disappear in the summer (Legaz *et al.* 2004). All of our specimens were collected during warm season months.

Although neither environment nor phylogeny explain the presence of the PrP degradation protease in these 44 species, we remain convinced that there is a genetic basis for it because the property was demonstrated

repeatedly with different samples of a given species. We screened many isolates of several species and the activity was always present or absent depending on the species (Rodriguez *et al.* 2012). For example, over ten *C. rangiferina* isolates all behaved the same in our tests. Although we do not know the chemical nature of the activity, we believe it is an inherent and species-specific character of certain lichens, not related to secondary compound production.

In conclusion, our hypothesis that closely related species of *Cladonia* would have PrP degradation activity, and distantly related species would not, was not supported by the results of this study. However, they did indicate that this property is widespread among *Cladonia* species, probably inherent and probably a characteristic of the mycobiont. As more lichens are surveyed for PrP degrading ability, it may be possible to determine the chemical basis for this property and its mode of action.

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References

Ahti, T. & Hammer, S. (2002) *Cladonia*. In *Lichen Flora of the Greater Sonoran Desert Region. Vol. 1* (T. H. Nash III, B. D. Ryan, C. Gries & F. Bungartz, eds): 131–158. Tempe, Arizona: Lichens Unlimited.

Brodo, I. M., Sharnoff, S. D. & Sharnoff, S. (2001) *Lichens of North America*. New Haven and London: Yale University Press.

Colby, D. W. & Prusiner, S. B. (2011) Prions. *Cold Spring Harbor Perspectives in Biology* **3**: a006833.

Detwiler, L. A. (2003) The epidemiology of scrapie. *Scientific and Technical Review* **22:** 121–143.

Esslinger, T. L. (2011) A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. North Dakota State University: http://www.ndsu.edu/pubweb/~esslinge/chcklst/chcklst7.htm (First Posted 1 December 1997, Most Recent Version (#17) accessed 16 May 2011), Fargo, North Dakota.

Gough, K. C. & Maddison, B. C. (2010) Prion transmission: prion excretion and occurrence in the environment. *Prion* **4:** 275–282.

Guo, S. & Kashiwadani, H. (2004) Recent study on the phylogeny of the genus *Cladonia* (s. lat.) with the emphasis on the integrative biology. *National Science Museum Monographs* **24:** 207–225.

Hinds, J. W. & Hinds, P. L. (2007) *The Macrolichens of New England*. New York: New York Botanical Garden Press.

Johnson, C. J., Bennett, J. P., Biro, S. M., Duque-Velaszuez, J. C., Rodriguez, C. M., Bessen, R. A. & Rocke, T. E. (2011) Degradation of the disease-associated prion protein by a serine protease from lichens. *PLoS ONE* **6:** e19836.

Legaz, M-E., Fontaniella, B., Millanes, A-M. & Vicente, C. (2004) Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. *European Journal of Cell Biology* **83:** 435–446.

Lendemer, J. C. & Hodkinson, B. P. (2009) The wisdom of fools: new molecular and morphological insights into the North American apodetiate species of *Cladonia*. *Opuscula Philolichenum* **7:** 79–100.

North, M. J. (1982) Comparative biochemistry of the proteinases of eukaryotic microorganisms. *Microbiological Reviews* **46:** 308–340.

Piercey-Normore, M. D. & DePriest, P. T. (2001) Algal switching among lichen symbioses. *American Journal of Botany* **88:** 1490–1498.

Rodriguez, C. M., Bennett, J. P. & Johnson, C. J. (2012) Lichens: unexpected anti-prion agents? *Prion* **6:** 1–6.

SAS Institute. (2009) *JMP Statistical Discovery Software*. Cary, North Carolina: SAS Institute, Inc..

Sigurdson, C. J. (2008) A prion disease of cervids: chronic wasting disease. *Veterinary Research* **39:** 41.

Smith, C. B., Booth, C. J. & Pedersen, J. A. (2011) Fate of prions in soil: a review. *Journal of Environmental Quality* **40:** 449–461.

Stenroos, S., Hyvönen, J., Myllys, L., Thell, A. & Ahti, T. (2002) Phylogeny of the genus *Cladonia* s. lat. (Cladoniaceae, Ascomycetes) inferred from molecular, morphological and chemical data. *Cladistics* **18:** 237–278.

Taylor, D. M. (2000) Inactivation of transmissible degenerative encephalopathy agents: a review. *The Veterinary Journal* **159:** 10–17.

Thomson, J. W. (1967) *The Lichen Genus* Cladonia *in North America*. Toronto: University of Toronto Press.

Watts, J. C., Balachandran, A. & Westaway, D. (2006) The expanding universe of prion diseases. *PLoS Pathogens* **2:** e26.

Appendix. Details of origin and character data for the 44 *Cladonia* species used in PrP degradation experiments

Species	Anti-PrP activity*	Origin of specimen†	Substratum	Exposure	Distribution‡	Apothecial colour	Usnic acid	Cortex present
C. apodocarpa	N	WI	soil or rock	sun	E US	brown	−	+
C. bellidiflora	H	E Can	rotting wood, stumps, moss, soil	shade	Arctic	red	+	+
C. borealis	N	AK	soil or rock	sun	Boreal, montane NA	red	+	+
C. caespiticia	M	WI	moss, rock, soil	shade	E US	brown	−	+
C. cariosa	N	WI	soil	sun	Boreal, montane NA	brown	−	+
C. cenotea	M	WI	wood, soil	shade	Boreal, montane NA	brown	−	+
C. cervicornis	M	MI	soil	sun		brown	−	+
C. chlorophaea	H	WI	wood, bark, rock, soil	sun or shade	NA	brown	−	+
C. cristatella	N	WI	wood, soil	sun or shade	E US	red	+	+
C. cylindrica	N	WI	rotting wood	sun or shade	E US	brown	−	+
C. deformis	N	AK	rotting wood, soil	sun	Boreal, montane NA	red	+	+
C. didyma	H	WI	rotting wood, soil	sun	SE US	red	−	+
C. evansii	H	LA	soil	sun or shade	SE US	brown	−	−
C. fimbriata	M	WI	rotting wood, soil	sun or shade	Boreal, montane NA	brown	−	+
C. furcata	N	E Can	soil, moss	shade	E US	brown	−	+
C. grayi	M	WI	wood, bark, rock, soil	sun or shade	NA	brown	−	+
C. incrassata	M	E Can	rotting wood	sun	E US	red	+	+
C. jaliscana	M	Mex	soil	shade	Cen America	red	+	+
C. leporina	N	LA	soil	sun	SE US	red	+	+
C. macilenta	H	E Can	soil, old wood	sun or shade	NA	red	−	+
C. magyarica	M	WI	soil	sun	E US	brown	−	+
C. maxima	N	E Can	moss	shade	E Can	brown	−	+
C. mitis	H	E Can	soil or rock	sun	Boreal, montane NA	brown	+	−
C. multiformis	H	WI	soil	sun or shade	Boreal, montane NA	brown	−	+
C. norvegica	M	E Can	rotting wood, soil	sun or shade	E US	brown	−	+
C. ochrochlora	M	WI	rotting wood	shade	E & W US	brown	−	+
C. parasitica	M	E Can	old wood	sun or shade	E US	brown	−	+
C. peziziformis	H	LA	soil	sun	E US	brown	−	+
C. phyllophora	H	WI	soil	sun or shade	Boreal, montane NA	brown	−	+
C. pocillum	H	WI	soil	sun	Boreal, montane NA	brown	−	+
C. pyxidata	H	WI	soil	sun	Boreal, montane NA	brown	−	+
C. rangiferina	H	WI	soil	sun or shade	Boreal, montane NA	brown	−	−

Appendix. ***Continued***

Species	Anti-PrP activity*	Origin of specimen†	Substratum	Exposure	Distribution‡	Apothecial colour	Usnic acid	Cortex present
C. robbinsii	H	WI	soil	sun or shade	E US	brown	+	+
C. scabriuscula	N	E Can	soil, moss	shade	E & W US	brown	−	+
C. sobolescens	M	WI	soil	sun	E US	brown	−	+
C. squamosa	N	WI	soil, old wood	shade	Boreal, montane NA	brown	−	+
C. stellaris	N	WI	soil	sun or shade	Boreal, montane NA	brown	+	−
C. subradiata	H	LA	soil, wood	sun	SE US	brown	−	−
C. subtenuis	M	LA	soil	sun	SE US	brown	+	−
C. subulata	N	WI	rotting wood, soil	sun	Boreal, montane NA	brown	−	+
C. sulphurina	N	AK	rotting wood, soil	sun	Boreal, montane NA	red	+	+
C. terrae-novae	N	E Can	soil	sun	E Can	brown	+	−
C. uncialis	N	WI	soil or rock	sun	Boreal, montane NA	brown	+	+
C. verticillata	H	WI	soil or rock	sun	Boreal, montane NA	brown	−	+

* N = none/low, M = moderate, H = high; † AK = Alaska, E Can = Eastern Canada, LA = Louisiana, Mex = Mexico, MI = Michigan, WI = Wisconsin;
‡ NA = North America, E, SE are cardinal directions, US = United States, Can = Canada, Cen = Central