First in Situ Imaging of Usnic Acid in Cladonia (Lichen) by Vibrational Spectromicroscopy

C. R. Liao\textsuperscript{a}, J. Sorensen\textsuperscript{a}, M. D. Piercey-Normore\textsuperscript{b}, and K. M. Gough\textsuperscript{a}

\textsuperscript{a} Department of Chemistry, University of Manitoba, \textsuperscript{b} Department of Biological Sciences, University of Manitoba

A lichen is composed of a fungal partner, and an algal and/or a cyanobacterial partner, living in a symbiotic relationship characterized by physiological integration between these organisms. Lichens present a variety of defensive mechanisms, one of which is the production of usnic acid (UA) (Fig. 1). This polyketide and secondary metabolite produced by certain lichenized fungi has a protective function for the lichen and valuable biomedical properties. These include an anti-proliferative effect on tumor cells, an antibiotic function as part of lichen’s disruptive effect on herbivore gut microflora, and a strong absorption in the ultraviolet range, allowing photoprotective functionality.

We demonstrate the first \textit{in situ} detection of usnic acid (UA) in selected species of the lichen \textit{Cladonia}, using FPA-FTIR imaging and Raman microscopy. Fruticose lichens show a defensive mechanism in production of UA. Upon confirming the distinct spectral signature of UA in lichen tissue, we mapped its distribution in \textit{Cladonia arbuscula}, \textit{C. uncialis} and \textit{C. sulphurina} tissues. Spectroscopic images were obtained from cryosectioned lichen fragments embedded in media and from hand-sectioned fragments that were media-free. UA was present in the pycnidia, and younger walls of \textit{C. arbuscula} and \textit{C. uncialis}, the spore-producing region of a \textit{C. uncialis} apothecium, and in both the younger and older soredia of \textit{C. sulphurina}. The localization of UA in lichens is an important precursor to future work that includes the identification of the gene cluster responsible for its biosynthesis. In addition, we have found that UA production (associated with the mycobiont) is concentrated in areas that contain the photobiont, in our samples. A possible explanation is that fungal hyphae surrounding the algae may be producing higher concentrations of usnic acid than hyphae that are physically farther away from the algae. Precursors for the biosynthetic polyketide pathway, including nutrients and acetyl and malonyl CoA, could be more available in this region, since they are produced by the mycobiont. Thus, the localization of UA may thus be an evolutionary response to the requirement for the UV protection that it provides. Our results show that FTIR and Raman imaging can be an effective way to study the distribution of natural products in lichens with micron-scale precision.