

Endophytic fungal flora in the living leaves of *Fraxinus excelsior* healthily growing in Hokkaido, Japan

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Purpose: Ash dieback caused by *Hymenoscyphus fraxineus* (Helotiales) is threatening *Fraxinus* species in Europe. Kato (2018) showed that *H. fraxineus* endophytically inhabits several ashes growing in Hokkaido Pref., Japan by detection of fungal DNA. Among them, *F. excelsior* (European ash) that is known as a susceptible species, but healthily growing in the Sapporo campus of Hokkaido Univ. was included. Other fungi coexisting in the same tissues may affect to *H. fraxineus* and function as one of the biological factors to control the disease. In this study, assemblages of endophytes in healthy leaves of the *F. excelsior* were investigated toward exploration of biological agents for ash dieback control.

Methods: Assemblages of endophytes in leaflets and rachises of the healthily growing *F. excelsior* were explored by a culture-based method and a metagenome analysis. *Fraxinus pennsylvanica* (Green ash) and *F. mandshurica* (Manchurian ash) in the site were examined by a culture-based method as well.

Results and Conclusion: 22 species of 15 genera in 14 families were isolated from the surface-sterilized leaves of *F. excelsior*. *Aureobasidium pullulans*, *Diaporthe* spp. and *Colletotrichum* spp. were isolated in high frequency. *Talaromyces marneffe* was isolated only from *F. excelsior*. In the metagenome analysis, 186 species were recognized in the leaves that were processed by ultrasonic-washing with dioctyl sodium sulfosuccinate (Aerosol OT). The most frequently detected species was *A. pullulans*, followed by *Papiliotrema flavescens*. Such fungi detected in high frequency may dominate the endophytic flora. Further studies are required to explore the antagonisms of those fungi against *H. fraxineus*.