

Similarities and differences of metabolic potential among three *Epichloë* species by LC-MS

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Many *Epichloë* endophytes are characterized by their mutualistic relationship with Poaceae grasses, which not only enhances the host's resilience to a range of biotic and abiotic stresses but also increases its ecological competitiveness. Alkaloids produced by *Epichloë* spp. play crucial roles in these mutualistic interactions. Numerous studies have systematically characterized four primary classes of *Epichloë*-derived alkaloids: ergot alkaloids, pyrrolopyrazines (including peramine), indole-diterpenes, and 1-aminopyrrolizidines (including lolines). However, emerging empirical evidence challenges the traditional alkaloid-centric paradigm by demonstrating that the defensive traits mediated by *Epichloë* in host plants are not exclusively governed by alkaloids. The defensive potential of other metabolites derived from *Epichloë* remains inadequately understood. Further metabolite profiling could enhance our understanding of the utilization value of *Epichloë* strains for agricultural benefit. In this study, we employed Liquid Chromatography-Mass Spectrometry (LC-MS) to analyse the metabolites of five *Epichloë* strains (IB8, QG6, ED11, AD16 and LE7) across three *Epichloë* species (*Epichloë sibirica*,

E. sinensis, *E. bromicola*) isolated from five grass species (*Achnatherum sibiricum*, *Psathyrostachys lanuginosa*, *Festuca sinensis*, *Elymus cylindricus*, and *E. dahuricus*). Our analysis sought to identify both common and strain-specific metabolites. The findings revealed that 1752 compounds, encompassing 15 distinct classes, were detected across five endophytic fungal strains. The predominant components comprised lipids and lipid-like substances (25.63%), organic acids and their derivatives (24.49%), and organic heterocyclic compounds (12.90%). Although strains with varying alkaloid-producing potential generated similar categories of metabolites, they exhibited quantitative differences. Unique metabolites from strains IB8, AD16, LE7, and ED11 were associated with at least one specific functional pathway each. These pathways corresponded to penicillin and cephalosporin biosynthesis (IB8), folate biosynthesis (AD16), sesquiterpene and triterpenoid biosynthesis (LE7), and the sulphur relay system (ED11). The next phase of this research will focus on elucidating functional linkages between these characterized metabolites and defensive phenotypes in endophyte-symbiotic grasses.