The pyrrolodiazipic acids: a new class of alkaloids produced during the virulent sexual cycle of *Epichloë* fungi

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Fungi from the genus *Epichloë* form systemic and seed-transmissible endophytic infections of grasses in a symbiosis that is considered predominately mutualistic. During asymptomatic growth these *Epichloë* fungi produce a variety of secondary metabolites that benefit their host by providing protection against herbivory and disease. However, the *Epichloë* sexual cycle is antagonistic to its host, characterised by the proliferation of hyphae on reproductive tillers to form fungal stromata that encase and sterilise developing host inflorescences. Production of known host-protective *Epichloë*-derived secondary metabolites is suppressed in these stromata; however, we describe here the pyrrolodiazipic acids, a novel class of fungal alkaloids that are specifically produced in *Epichloë* stromata. Transcriptomic analysis identified the five-gene *PZA* cluster that, unlike other *Epichloë* biosynthetic gene clusters, is specifically expressed in stromata. Reconstruction of this *PZA* cluster in a heterologous host enabled the function of the encoded enzymes to be defined, key biosynthetic intermediates to be identified, and the final product characterised – stromatine, a pyrrolodiazepine with carboxylic acid functionality. Analysis of material from many different *Epichloë*-host associations revealed that stromatine was universally and exclusively found in stroma tissues. Stromatine was also produced in exceptionally large amounts, contributing almost 1% of the total stroma dry mass in some grass-endophyte associations. *PZA* gene deletion analyses across three different *Epichloë* species yielded interesting results; stromatine appears to be required for host infection by some *Epichloë* species, while the accumulation of PZA pathway intermediates appears to be detrimental to symbiosis. Genomic analyses reveal the *PZA* cluster is conserved among sexual *Epichloë* species but is dispensable for asexual strains. Combined with the tissue specificity and high levels of production, this suggests that stromatine plays a critical role in the *Epichloë* sexual cycle, with potential bioactivities and functions to be dissected further.

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