Dissection of the epoxyjanthitrem pathway in *Epichloë* sp. LpTG-3 strain AR37 by CRISPR gene editing to deliver improved SDN-1 type endophytes for grasses

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Epichloë festucae var. *lolii* and *Epichloë* sp. LpTG-3 are filamentous fungal endophytes of perennial ryegrass (*Lolium perenne*) that have a substantial impact on New Zealand's agricultural economy by conferring biotic advantages to the host grass. *Epichloë* endophyte strain AR37 provides ryegrass with improved agronomic performance, insect protection and plant persistence. Indole diterpenes are a class of well characterised secondary metabolites produced by *Epichloë* endophytes, with the AR37 strain expressing epoxyjanthitrems, a class of decorated indole diterpenes. These have been associated with the observed effects of AR37 on livestock and insect pests. Here we used gene inactivation by CRISPR-Cas9 to deconvolute the genetic basis for epoxyjanthitrem biosynthesis and manipulate this secondary metabolite pathway to reduce or remove endophyte-induced mammalian toxicity whilst retaining activity against some important agricultural insect pests. We also show that gene editing of *Epichloë* can be achieved without off-target events or introduction of foreign DNA (footprint-less) and that these gene edits can be classified as site-directed nuclease 1 (SDN-1), which are not regulated as genetically modified organisms in selected jurisdictions, including Australia. These SDN-1 technology edits have been successfully grown out of containment in the field in Australia for seed multiplication, followed by agronomy trials, which are now being evaluated. This has the potential to provide a step change in the future use of animal safe *Epichloë* strains in New Zealand pastures which will significantly reduce chemical inputs and increase animal welfare.

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