

Bacterial symbionts of *Epichloë* fungal endophytes of grasses

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Bacteria often form symbiotic associations with plant beneficial fungi, and they can increase the fitness of either their fungal and/or plant hosts. We characterised the bacterial microbiota associated with *Epichloë* fungal endophytes of perennial ryegrass (*Lolium perenne*) and investigated the effects of the bacterial symbionts on the performance of *Epichloë* or *L. perenne* (e.g., growth, *Epichloë*-derived alkaloid concentrations). We hypothesised that the bacterial microbiota associated with *Epichloë* would not be detrimental towards the fungal or plant host and instead promote plant growth without affecting either fungal growth or production of *Epichloë*-derived alkaloids. The bacterial microbiota associated with *Epichloë* was dominated by members of the genus *Paenibacillus*, although bacteria within genera *Delftia*, *Micrococcus* and *Bradyrhizobium* were also present. Two bacterial strains, *Paenibacillus* E222

and *Micrococcus* E226, were isolated from *Epichloë* mycelia and characterised further. Both E222 and E226 formed ectosymbiotic relationships with the fungal endophyte (i.e., bacterial cells were located on the hyphal surface) and possessed plant-growth promoting traits identified by whole genome analysis. Further experiments, enriching the abundance of E222 and E226 by inoculating these bacteria onto perennial ryegrass seed colonised by *Epichloë*, confirmed their plant growth promoting abilities *in planta*. E226 did not affect the growth of *Epichloë* or the concentration of *Epichloë*-derived alkaloids while E222 did the opposite. The mechanism of plant growth promotion by E222 and E226 is likely related to the production of growth promoting hormones/volatiles, abilities to solubilise phosphorus, and/or synthesise vitamins/metabolic cofactors.