Supplementary Data Set S5. Bioinformatics search for a gene encoding an enzyme catalyzing the first step of phosphorus-carbon (P-C) compound biosynthesis

One highly expressed *Epichloë* ORF (m.299598) encoded a predicted protein highly similar to multiple fungal phosphonopyruvate decarboxylases. These enzymes catalyze the most common second step in phosphonate biosynthesis (Peck and van der Donk, 2013). The first step can be catalyzed by phosphonopyruvate mutase, also referred to as phosphoenol pyruvate (PEP) mutase (Yu et al., 2013), or by carboxyphosphonoenolpyruvate phosphomutase (Hidaka et al., 1990).

We searched the Trinity-generated ORFs with protein sequences representing these enzymes. We found no Trinity ORF assigned to *Epichloë* that was predicted to encode proteins with significant similarity to a fungal PEP mutase (AHL24479; *Glycomyces*). Proteins predicted to be encoded by *L. perenne* ORFs m.49255, m.49254 and m.225236 had limited similarity to this enzyme (E-values 2×10^{-8} - 4×10^{-7}).

Two *L. perenne* ORFs, m.90678 and m.40418, are predicted to encode proteins with significant similarity (E-values of 4×10^{-42} and 2×10^{-27}, respectively) to a phosphoenolpyruvate mutase (accession number AAA02862.1) from *Dianthus caryophyllus* (Wang et al., 1993) The former was among the 2% most highly expressed ryegrass ORFs. Its expression was uniform in all tissues (and not significantly affected by endophyte infection).

No other Trinity-generated ORFs are predicted to encode proteins with significant similarity to this enzyme.

Literature Cited

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