Abstract

Split-marker recombination for fast and efficient targeted deletion of *Epichloë* genes

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Endophytic fungi of the genus *Epichloë* exist widely in cool season grasses forming a mutualistic symbiotic relationship with their host. *Epichloë* symbiosis can promote the growth of host plants and improve the hosts resistance to biotic and abiotic stresses. Selected strains of these fungi have therefore been exploited as an important agricultural microbial resource. The establishment of a genetic transformation system in *Epichloë* is important to understand the molecular mechanisms responsible for host stress resistance. However, due to the slow growth of *Epichloë* spp. in culture, this has inhibited research in this space. The rate of false positive colonies in gene deletion experiments is often high, typically because gene replacement fails to occur at the targeted locus. Our work therefore introduced a highly efficient protoplast-based transformation method for targeted gene deletion in *Epichloë*. Cellophane culture was used to disperse mycelium and accelerate its growth, which significantly shortened fungal culture time. High quality protoplasts were subsequently obtained by mixed enzymatic hydrolysis, which reached 4.2×10^8 protoplasts per ml. In addition, using a split-marker recombination method, knockout mutants of *Epichloë* were quickly and efficiently obtained increasing rates by 20% over traditional hygromycin methods. This paper discusses a fast and efficient split marker recombination method for knockout and overexpression of slow-growing *Epichloë* endophytes and its potential use in transforming these fungi for pasture grasses and cereals.

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