Abstract

Improved methodology for the isolation of *Epichloë* endophytes from *Achnatherum inebrians*

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Achnatherum inebrians, a toxic perennial bunchgrass native to northwestern China, establishes a mutualistic symbiotic relationship with two distinct endophyte species, *Epichloë gansuensis* and *Epichloë inebrians*. These endophytes produce ergot alkaloids that accumulate in plant tissues and are responsible for frequent poisoning incidents in grazing livestock. The bioaccumulation of these compounds poses significant agricultural risks, necessitating the development of targeted control strategies. Research investigations require the isolation of these fungi from plant material. The current isolation method is internationally accepted but results in a low endophyte isolation frequency.

The conventional protocol involved sequentially treating seeds with 75% ethanol (30 s) followed by 10% sodium hypochlorite (1 min), with 5 consecutive sterile-water rinses performed after each sterilisation step. The sterilised seeds were dried on autoclaved filter paper and transferred to potato dextrose agar (PDA) supplemented with antibiotics (50 mg/L benzylpenicillin potassium and 50 mg/L streptomycin sulphate). Following a certain period of time, actively growing mycelia were aseptically selected from the colony periphery for sequential subculturing. Any microbiological contamination was removed as soon as it was detected throughout the experimental process. The methodological enhancements involved two modifications: (1) implementation of a 10 min 50% sulphuric acid pretreatment for seed coat prior to sterilisation, and (2) formulation optimisation of PDA through supplementation with 0.1% (w/v) yeast extract and 5% (v/v) aqueous foliage extract from *A. inebrians*. These improvements significantly increased the isolation success rate from 4% to 18% while accelerating fungal growth by 10-20%.

The optimised methodology discussed in this study significantly enhanced the isolation frequency of endophytes from *A. inebrians*, thereby allowing more effective research studies to be conducted. This method establishes a referenceable technical pathway for the isolation of endophytes from grasses, enhances the practical application potential and advances the technological innovation process in endophyte symbiosis research.

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