Attempts to Liberate Protoplasts Enzymatically from Virulent Cultures of Endothia parasitica

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A method that may be used to study the transmission of hypovirulence in Endothia *parasitica is to* remove the fungal cell wall enzymatically and infect protoplasts with double-strand RNA (deRNA) obtained from hypoviruleut strains. The five enzymes that have been used, singly and in combination, to liberate protoplasts are: B-glucuronidase, chitinase, laininarinase, helicase, and a *Trichoderma* viride culture filtrate. Of these, only B-glucuronidase has been effective in releasing protoplasts from either 2-day-old mycelial plugs after a 5- to 6-hour incubation period or germinating spores after a 12- to 24-hour period. During incubation, mycelia or spores are shaken rapidly (150 rpm) at room temperature (.24° C) in the enzyme solutions to which 0.5 m MgSO4 and 0.05 m Na-maleate are added to maintain protoplast stability.