



A NEW METHOD FOR PRODUCING HYBRID STRAINS OF THE ENTOMOPATHOGENIC FUNGUS *VERTICILLIUM LECANII* (*LECANICILLIUM* SPP.) THROUGH PROTOPLAST FUSION BY USING NITRATE NON-UTILIZING (*NIT*) MUTANTS

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ABSTRACT

Mycotal and Vertalec are mass-produced fungal strains for insect control. Strain B-2, which was isolated in Japan, has high epiphytic ability on cucumber leaves. Protoplast fusion was performed using these strains of *Verticillium lecanii* to obtain new strains possessing useful characteristics as biological control agents (BCAs). We used *nit* mutants for visually selecting the protoplasts. Hybrid strains were subjected to molecular analysis using the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs) and arbitrarily primed-PCR (AP-PCR) in order to determine protoplast fusion and/or genetic recombination. We detected 126, 44, and 4 hybrid strains from the combinations of Vertalec × Mycotal, B-2 × Mycotal, and B-2 × Vertalec, respectively. Morphological characteristics of hybrid strains differed from

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those of their parental *nit* mutants. Protoplast fusion of hybrid strains was confirmed in genomic DNA, but not in mitochondrial DNA (mtDNA). A uniform biased tendency of the DNA banding pattern was observed depending on the combination of parental strains. The molecular analysis also revealed genetic recombination. These results showed a novel method for producing hybrid strains of the entomopathogenic fungus *V. lecanii*.

Key words: AP-PCR, breeding of biological control agents, *Lecanicillium longisporum*, *Lecanicillium muscarium*, mtDNA, *nit* mutants, protoplast fusion, *Verticillium lecanii*.
