

**Molecular biological characteristics of two  
benomyl-resistant mutants, S-18 and S-19, in an  
entomopathogenic fungus, *Metarhizium anisopliae***

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An entomopathogenic fungus, *Metarhizium anisopliae*, has been used as an important mycopesticide. But the fungus can not be applied with benomyl widely used to control plant pathogens because it is very susceptible to the fungicide. So, two spontaneous mutants, S-18 and S-19 in *M. anisopliae* that had high benomyl resistance were isolated and molecular biological characteristics were investigated. The  $\beta$ -tubulin gene of *M. anisopliae* was cloned and its nucleotide and amino acid sequences were determined since benomyl resistance is known to be involved in structural alteration of  $\beta$ -tubulin. The gene encoded a 448-residue protein and the coding region was interrupted by four introns. The amino acid sequence of *M. anisopliae*  $\beta$ -tubulin was 94~95% homologous to those of other fungal  $\beta$ -tubulins. Homology to the chicken and pig brains was 82%.

Also, the  $\beta$ -tubulin genes in the two benomyl-resistant strains, S-18 and S-19, were cloned and sequenced to determine the mutations responsible for benomyl resistance. The mutant S-18 substituted aspartate for asparagine at position 33 and lysine for glutamate at position 134. And the other mutant S-19 showed alterations at three positions of  $\beta$ -tubulin; arginine for tryptophan at position 21, lysine for asparagine at position 33, and phenylalanine for leucine at position 240.