

Evolution of host resistance in a toxin-producing bacterial-fungal alliance.

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Abstract

The rice seedling blight fungus *Rhizopus microsporus* harbors endosymbiotic *Burkholderia* sp. for the production of the virulence factor, the antimetabolic agent rhizoxin. Since the toxin highly efficiently blocks mitosis in most eukaryotes, it remained elusive how self-resistance emerged in the fungal host. In this study, rhizoxin sensitivity was systematically correlated with the nature of beta-tubulin sequences in the kingdom Fungi. A total of 49 new beta-tubulin sequences were generated for representative species of Ascomycota, Basidiomycota and Zygomycota. Rhizoxin sensitivity assays revealed two further amino acids at position 100 (Ser-100 and Ala-100), in addition to the known Ile-100 and Val-100, which convey rhizoxin resistance. All sensitive strains feature Asn-100. This hot spot was verified by modeling studies, which support the finding that rhizoxin preferentially interacts with the tubulin molecule in a cavity near position 100. Ancestral character state reconstructions conducted in a Bayesian framework suggest that rhizoxin sensitivity represents the ancestral character state in fungi, and that evolution of rhizoxin resistance took place in the ancestor of extant resistant Zygomycota. These findings support a

model according to which endosymbiosis became possible through a parasitism--mutualism shift in insensitive fungi.

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