Targeted Disruption of Nonribosomal Peptide Synthetase pes3 Augments the Virulence of Aspergillus fumigatus

Nonribosomal peptide synthesis (NRPS) is a documented virulence factor for the opportunistic pathogen Aspergillus fumigatus and other fungi. Secreted or intracellularly located NRP products include the toxic molecule gliotoxin and the iron-chelating siderophores triacetylfusarinine C and ferricrocin. No structural or immunologically relevant NRP products have been identified in the organism. We investigated the function of the largest gene in A. fumigatus, which encodes the NRP synthetase Pes3 (AFUA_5G12730), by targeted gene deletion and extensive phenotypic analysis. It was observed that in contrast to other NRP synthetases, deletion of pes3 significantly increases the virulence of A. fumigatus, whereby the pes3 deletion strain (A. fumigatus Δpes3) exhibited heightened virulence (increased killing) in invertebrate (P <0.001) and increased fungal burden (P = 0.008) in a corticosteroid model of murine pulmonary aspergillosis. Complementation restored the wild-type phenotype in the invertebrate model. Deletion of pes3 also resulted in increased susceptibility to the antifungal, voriconazole (P <0.01), shorter germlings, and significantly reduced surface β-glucan (P = 0.0325). Extensive metabolite profiling revealed that Pes3 does not produce a secreted or intracellularly stored NRP in A. fumigatus. Macrophage infections and histological analysis of infected murine tissue indicate that Δpes3 heightened virulence appears to be mediated by aberrant innate immune recognition of the fungus. Proteome alterations in A. fumigatus Δpes3 strongly suggest impaired germination capacity. Uniquely, our data strongly indicate a structural role for the Pes3-encoded NRP, a finding that appears to be novel for an NRP synthetase.