

Breaking the mould: rethinking 'wild type' in fungal pathogens

Should we really be using one strain to generalize an entire species? Having spent my PhD studying bacterial pathogens, I often observed that isolates of the same species varied greatly in their virulence strategies, the diseases they caused and the genes encoded in their genomes. Therefore, my answer was no. However, at the time I entered the world of fungal pathogenesis as a postdoctoral researcher, the concept of intraspecies heterogeneity had still not gained significant traction. Most researchers used a single 'wild type' reference strain to study their favourite pathogen. As a result, I was excited when Robert Cramer's team clearly demonstrated the relevance of the intraspecies heterogeneity in *Aspergillus fumigatus* for its virulence. This work sparked my own research, which currently involves the characterization of *A. fumigatus* genomic diversity, revealing a tremendous amount of genomic heterogeneity underpinning its phenotypic heterogeneity.

A. fumigatus is a ubiquitous filamentous fungus that lives naturally in the soil. As an opportunistic pathogen, it can cause invasive pneumonia in immunocompromised individuals, contributing to an estimated 1.8 million deaths each year. Most researchers studying this pathogen use one of two wild-type reference strains called AF293 and CEA10. Kowalski, Beattie et al. demonstrated that these two strains have significant phenotypic differences in traits relevant to infection. Despite both strains being wild type, CEA10 was more fit under hypoxic growth in vitro. It was also significantly more lethal in a mouse model of infection, in part due to its ability to better invade lung tissue relative to AF293, which remained in the large airways. The authors extended their in vitro and in vivo analyses to a panel of environmental and clinical isolates of *A. fumigatus*, and they observed

that the isolates presented a wide range of fitness under hypoxic stress, as well as variable lethality in a mouse model, mirroring what they observed with AF293 and CEA10 strains. Moreover, the authors observed that higher fitness in hypoxia was associated with shorter median survival times in the mouse. As a final demonstration of the role of hypoxic fitness in *A. fumigatus* virulence, the authors took the less virulent AF293 strain and experimentally evolved it under hypoxia. Strikingly, after 20 generations the strain exhibited significantly increased fitness under hypoxic conditions and virulence in the mouse model. The evolved strain now behaved more like CEA10, exhibiting more tissue invasion and higher mortality than the ancestor.

While previous studies had described phenotypic heterogeneity between *A. fumigatus* strains or among fungal strains of other species, this study stands out because it also delineated the specific consequences of phenotypic variation during infection and pinpointed a key contributing factor – fitness in hypoxia. Another unique aspect is that the study included environmental isolates. Even though soil is the primary habitat for this organism, most of the previous research focused almost exclusively on strains obtained from patients. This study has inspired me and other research groups to expand our knowledge of intraspecies heterogeneity in fungal pathogens, often discovering similar divergence at both the genomic and phenotypic levels. In *A. fumigatus*, I and other groups have revealed that underneath the phenotypic, there is a dazzling level of genomic diversity and only about two-thirds of the genomic content is conserved across the species.

The work by Kowalski, Beattie et al. underscores the limitations of using 'reference' strains when studying microorganisms. The microbial kingdom possesses enormous

genomic and phenotypic diversity, even within what we define as the same species. This is particularly true for opportunistic bacterial and fungal pathogens, which commonly occupy many habitats and have consequently evolved myriad strategies to succeed in these diverse and often rapidly changing conditions. By conducting experiments in a single strain, we miss the full spectrum of virulence strategies used by a pathogen. This also raises the question of what 'wild type' refers to in the case of virulence-associated traits, such as resistance to specific stressors, which often exist along a continuum but vary widely between isolates.

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The extraordinary diversity of the microorganisms that fascinate us so much should be embraced rather than ignored. Fortunately, advances in high-throughput experimental approaches and next-generation sequencing are making this goal increasingly possible.

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Competing interests

The author declares no competing interests.

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Related article: Barber, A. E. et al. *Aspergillus fumigatus* pan-genome analysis identifies genetic variants associated with human infection. *Nat. Microbiol.* **6**, 1526–1536 (2021)