Characterizing genomic and phenotypic traits of the human pathogen *Aspergillus flavus* and its non-pathogenic close relatives

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Purpose

Although fungal diseases affect millions of humans each year, fungal pathogens of humans remain understudied (1). The mold *Aspergillus flavus* is a causative agent of both aspergillosis and fungal keratitis infections (2). Although *A. flavus* is commonly isolated from patients with these infections, species closely related to *A. flavus* are rarely, if ever, isolated from patients and are not considered clinically relevant. To gain insights into why this is the case, we compared genomic and phenotypic traits between *A. flavus* and three closely related non-pathogenic species, namely *A. arachidicola* and *A. parasiticus*, and *A. nomius*.

Methods

We sequenced genomic DNA from seven strains, two each of *A. arachidicola*, *A. parasiticus* and *A. flavus*, and one *A. nomius*. We assembled and annotated draft genomes using SPAdes (3) and predicted biosynthetic gene clusters for each strain using antiSMASH (4). Orthologous proteins were identified and compared using OrthoVenn2 (5). Additionally, we characterized the secondary metabolite production of all seven strains in two clinically relevant conditions: the temperature of the human body and the salt concentration of human tears. We also examined the relative virulence of each strain using the invertebrate model of fungal disease *Galleria mellonella.*

Results

Genomics

A. flavus strains shared seven biosynthetic gene clusters that were absent in strains from the three non-pathogenic species. Furthermore, we identified over 2,000 orthologous protein families unique to *A. flavus*, which were enriched in the gene ontology categories of transmembrane transport and oxidoreductase activity. *A. flavus* had a similar number of predicted biosynthetic gene clusters compared to *A. parasiticus* and *A. arachidicola* and *A. nomius* had the fewest (Fig. 1).

Chemistry

Despite the unique biosynthetic gene clusters and proteins in *A. flavus*, our chemical analyses showed few unique metabolites produced by any species. Temperature changes impacted metabolite production in all species (Fig. 2), but we found a surprising lack of impact of salt on secondary metabolite production. Hierarchical clustering indicated that *A. flavus* and *A. arachidicola* strains are more similar at 37° C than at room temperature.

Virulence

We also found that strains of the same species varied widely in their virulence profiles, and that *A. flavus* strains were not more virulent than strains of the non-pathogenic species (Fig. 3).

Genomics

Figure 1. Orthologous protein families in seven strains of *Aspergillus*. Bars indicate number of protein families in common for all strains with black dots underneath the bar.

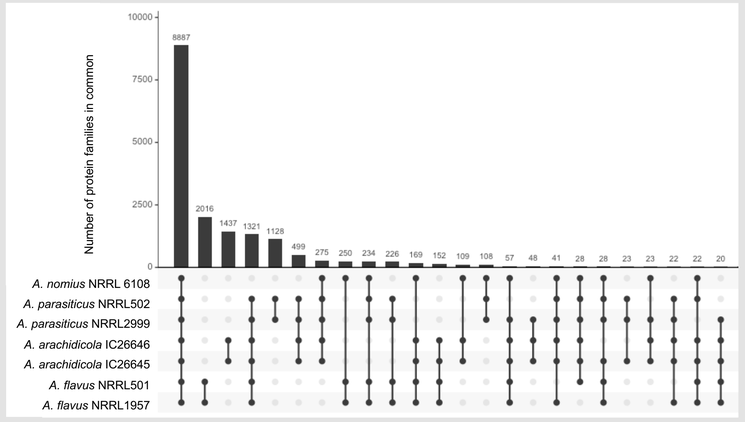
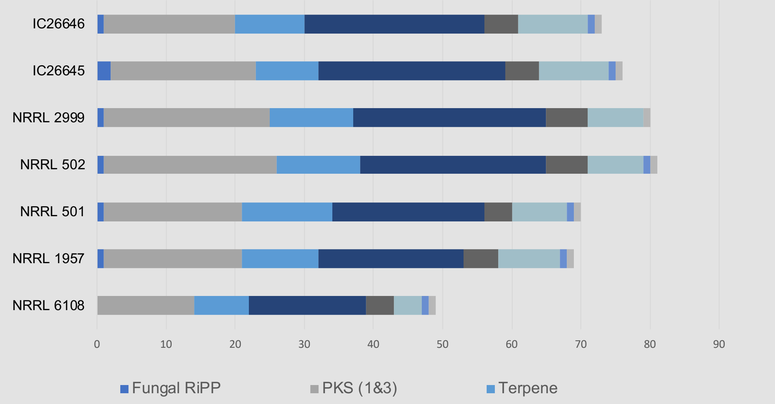
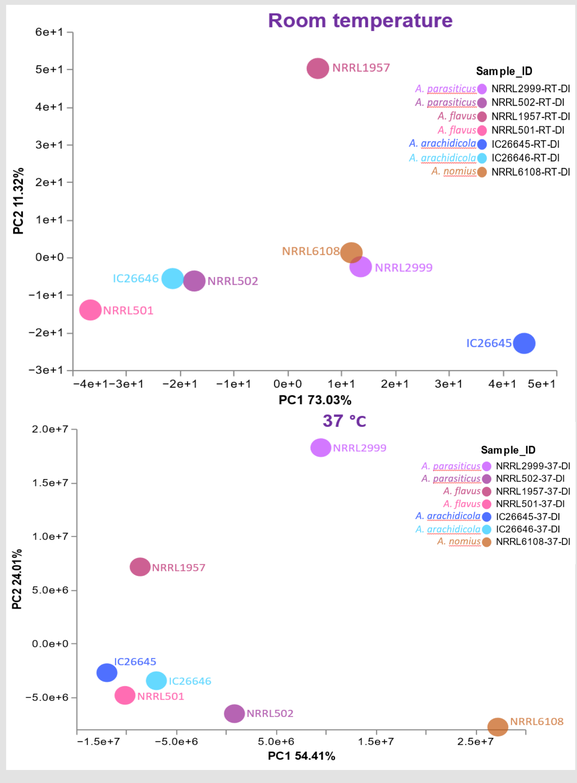


Figure 2. Stacked bar plot of predicted biosynthetic gene clusters for each *Aspergillus* strain.



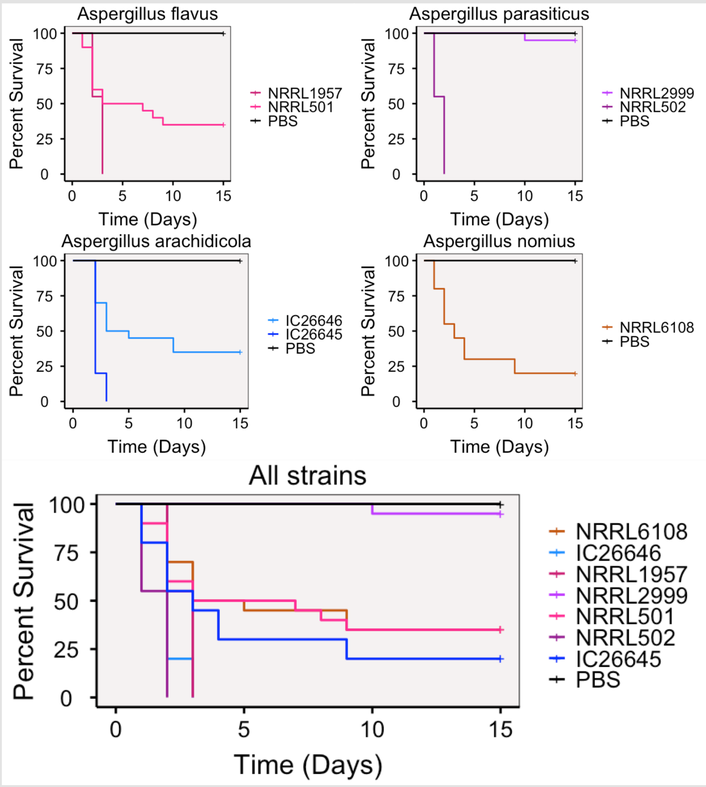
Chemistry

Figure 2. Principal component analysis of *Aspergillus* strains at room temperature and at 37º C. Each dot represents the total metabolite production for one strain. Dots closer together indicate more similar metabolite production.



Virulence

Figure 4. *Aspergillus flavus* is not significantly more virulent than related species in an invertebrate model of fungal disease. Cumulative survival of *Galleria mellonella* larvae inoculated.



Conclusion

Unexpectedly, strains of the same species varied in chemistry and virulence, but not genetics, and *A. flavus* strains were not the most virulent.

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