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Abstract

In an on-going Microbial Observatory Experiments on the International Space Station (ISS) **molecular phylogeny** and **radiation resistance** of several fungal isolates were characterized. Two strains, ISSF 21 and IF1SW-F4, were isolated from the HEPA filter and the surface of the Cupola of the ISS, respectively. Using primers targeting the internal transcribed spacers ITS1 and 2, both isolates were identified as *A. fumigatus*. The whole genome sequence analysis of ISSF 21 revealed >60,000 single nucleotide polymorphisms (SNPs) when compared to the reference, Af293, which is consistent with the genetic heterogeneity amongst sequenced *A. fumigatus* strains from diverse clinical and environmental source. **Secondary metabolite (SM) profiles** of both ISS isolates were compared to the reference (Af293) but no significant differences were observed. Exposure to various **molecular stresses** did not reveal outstanding differences between ISS and clinical strains however increased UV₂₅₄ resistance of ISS strains was observed. Finally, knowing that *A. fumigatus* is an opportunistic pathogen and microgravity highly influences the antibiotic susceptibility and pathogenicity of microorganisms, we examined **pathogenicity** of both ISS isolates using the zebrafish larval model. Both ISS-isolated strains ISSF 21 and IF1SW-4F were more virulent than two clinical strains (Af293 and CEA10).

Phylogenetic characterization

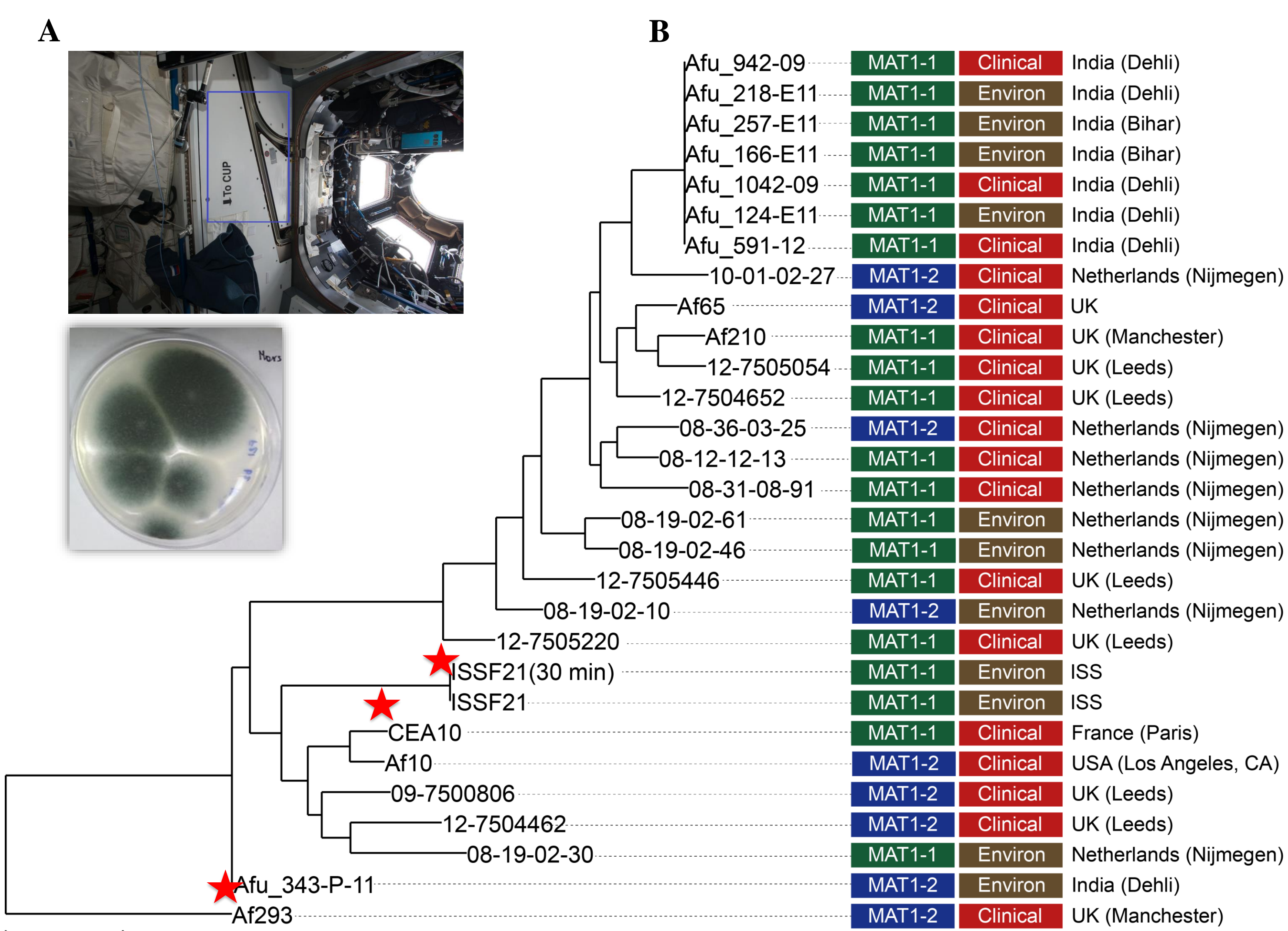


Figure 1. Phylogenetic characterization of ISS isolated strain. (A) ISSF21 was isolated from HEPA filter (not shown) and IF1SW-F4 was isolated from the wall area next to cupola window (blue square). (B) Phylogenetic tree of 28 sequenced strains of *A. fumigatus* also showing mating type (MAT1-1 or MAT1-2), clinical or environmental origin, and sampling location. Red stars designate strains of interest used in this study. At this time, IF1SW-F4 id being sequenced and will be added to this tree.

Secondary metabolite production

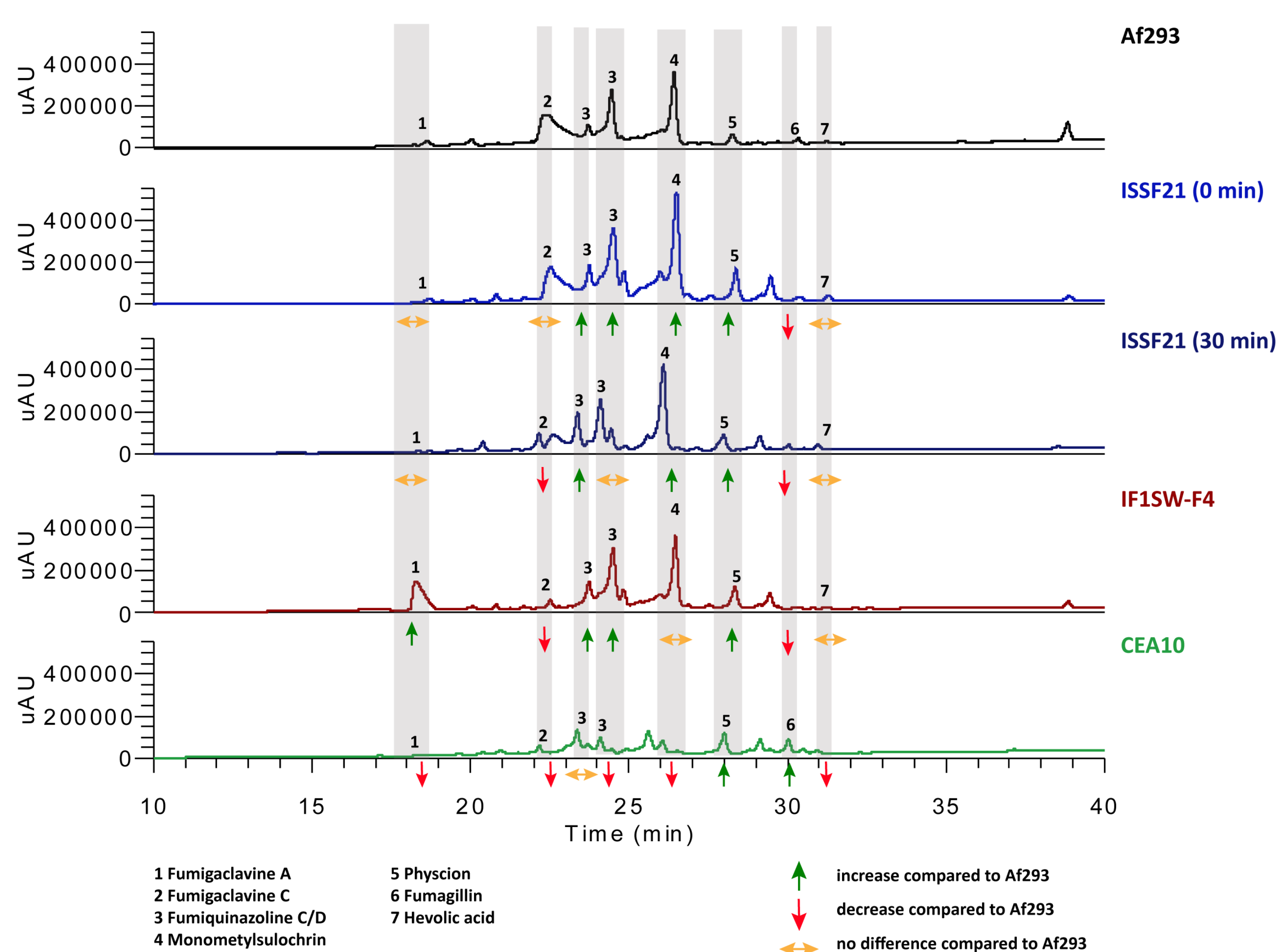


Figure 1. Secondary metabolite production of ISS strains. LC/MS traces compare SM profiles of ISSF21, ISSF21 (30 min), IF1SW-F4 and CEA10 to Af293 when grown on glucose minimal media. Individual metabolite production reported as either increased, decreased, or no difference compared to Af293.

Chemical stresses resistance

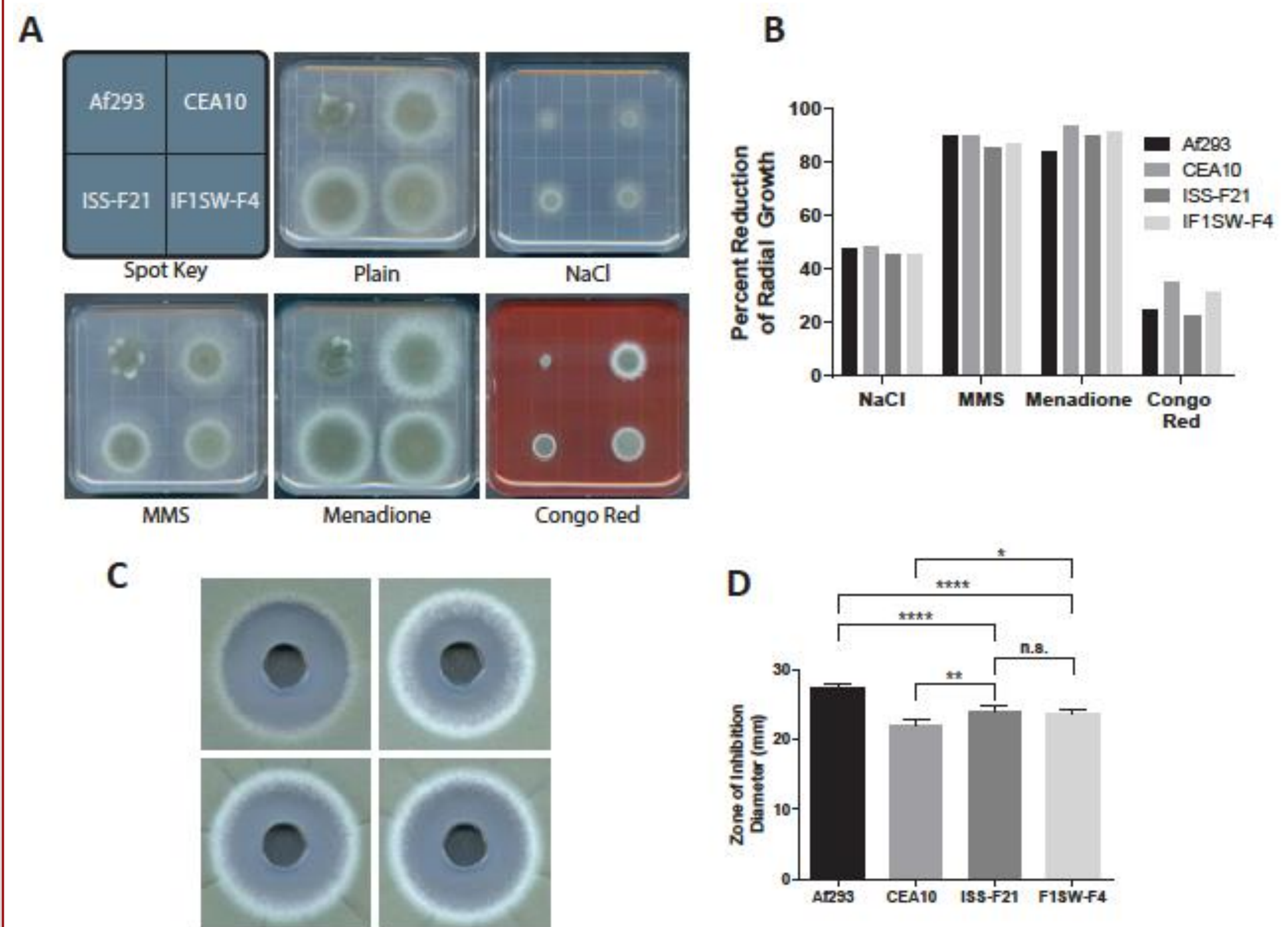


Figure 3. Virulence assessment in a larval zebrafish model of invasive aspergillosis. A) Cartoon representation of 48 hour post fertilization zebrafish larvae undergoing microinjection of conidia into the hindbrain ventricle (shaded red). B) Survival outcome through 7 days post infection (dpi) of neutrophil-deficient *mpx:mCherry-2A-Rac2D57N* larvae, where neutrophils specifically are unable to reach the site of infection. Shown are data pooled from three independent experimental replicates. Statistical analyses were performed using Cox Proportional-Hazard regression analysis. IF1SW-F4 was also shown to be statistically more virulent compared to CEA10 ($p=0.041$) but no different from ISS-F21 ($p=0.15$) (data not shown).

UV₂₅₄ resistance

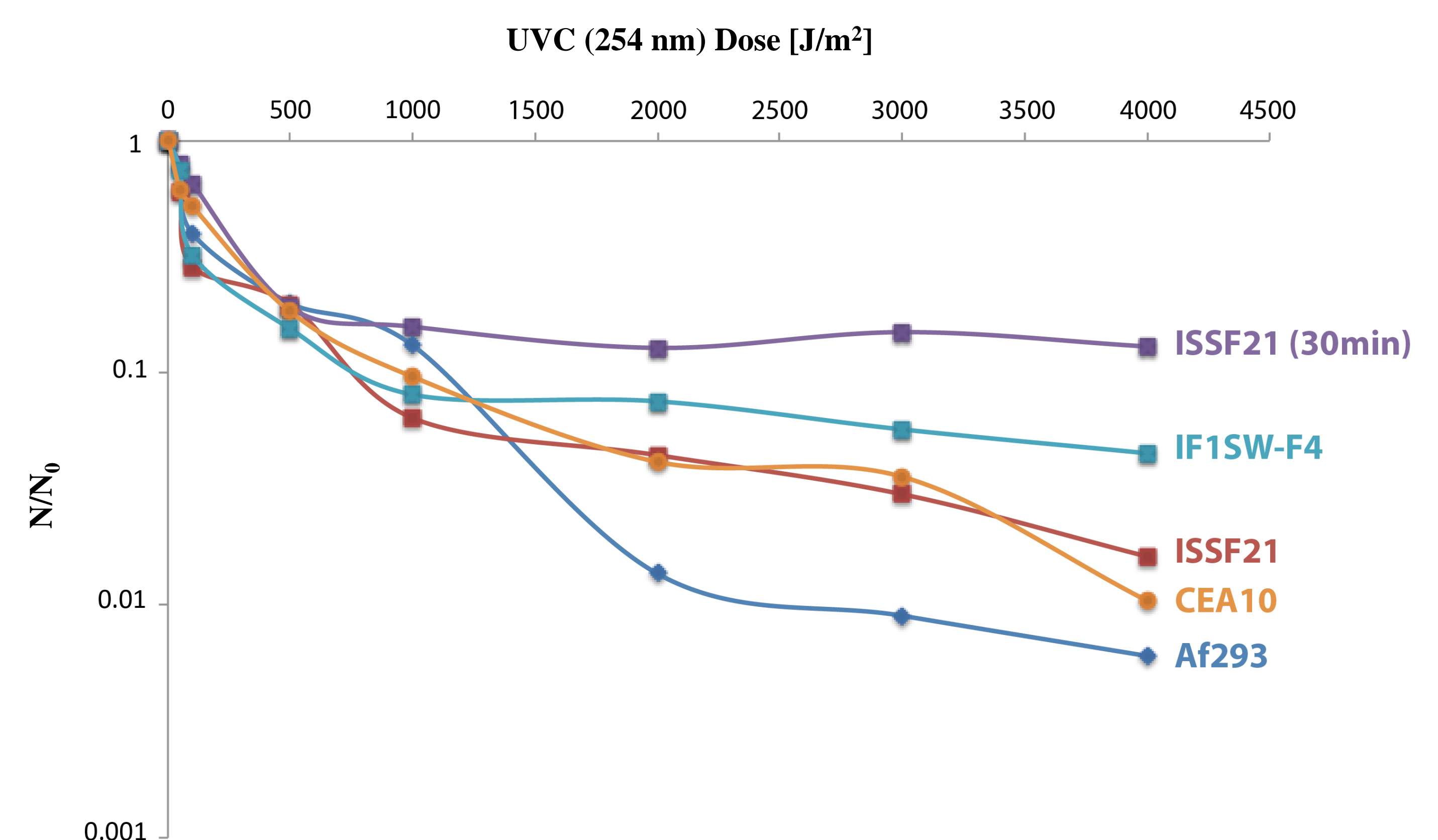


Figure 4. UV₂₅₄ resistance of *A. fumigatus* ISS-isolated and clinical strains. Purified spores of ISS-isolated [ISS-F21, ISS-F21 (30 min), and IF1SW-F4] and clinical [CEA10 and Af293] strains were exposed to various UV₂₅₄ doses. The three *A. fumigatus* strains isolated from ISS showed higher UV₂₅₄ survival rates (N/N_0 ; # of spores survived/# of spores exposed at Time 0) when compared to the two clinical isolates. The average fungal spore survival rates from three different experiments are plotted above. ISSF21 strain was exposed to the simulated space conditions (Mars UV 200 to 400 nm; and Mars atmosphere) for 30 minutes (105.6 kJ/m²).

Virulence

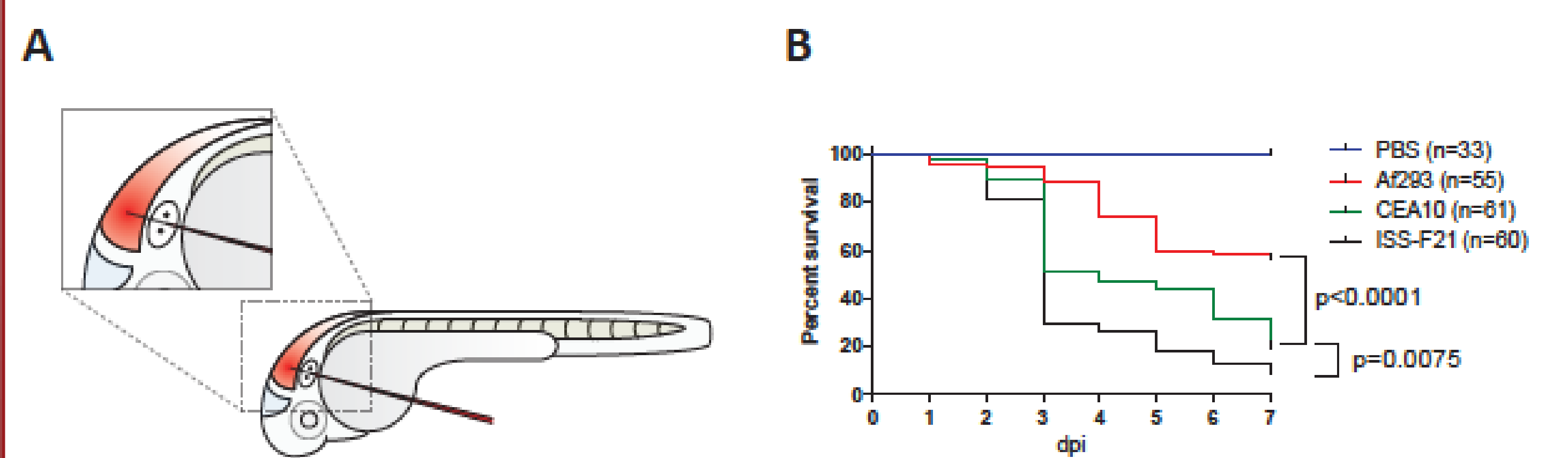


Figure 5. Virulence assessment in a larval zebrafish model of invasive aspergillosis. A) Cartoon representation of 48 hour post fertilization zebrafish larvae undergoing microinjection of conidia into the hindbrain ventricle (shaded red). B) Survival outcome through 7 days post infection (dpi) of neutrophil-deficient *mpx:mCherry-2A-Rac2D57N* larvae, where neutrophils specifically are unable to reach the site of infection. Shown are data pooled from three independent experimental replicates. Statistical analyses were performed using Cox Proportional-Hazard regression analysis. IF1SW-F4 was also shown to be statistically more virulent compared to CEA10 ($p=0.041$) but no different from ISS-F21 ($p=0.15$) (data not shown).

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