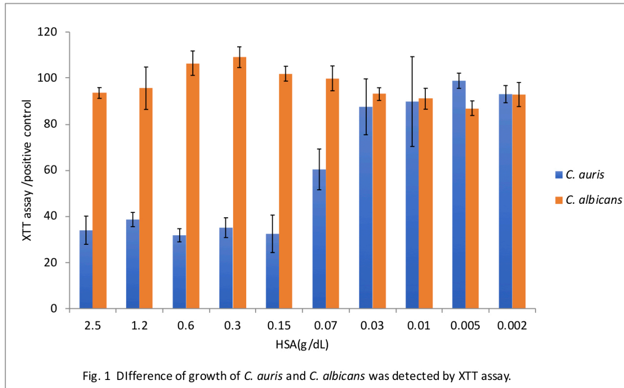

Fig. 1 Difference of growth of *C. auris* and *C. albicans* was detected by XTT assay.

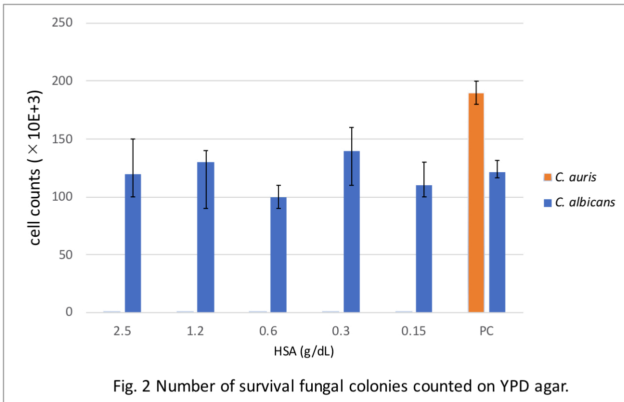

Fig. 2 Number of survival fungal colonies counted on YPD agar.

***Disclosures.* All authors:** No reported disclosures.

1724. Plasmid-free CRISPR-Cas9 System for Genetic Engineering of *Rhizopus delemar*

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Background. Mucormycosis is a serious infection caused by fungi of the order Mucorales. *Rhizopus delemar* is the most common etiologic agent of mucormycosis. Pathogenesis studies of mucormycosis have been hampered by poor genetic trackability of the organism, owing to rare chromosomal integration events and multinucleated nature of the cells. The clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease 9 (Cas9) system has been widely used in genetic manipulation through efficient homologous and non-homologous break points in a variety of organisms including *R. delemar*. However, plasmid-free CRISPR/Cas9 system has not been previously described in the fungus. Here, we introduce a rapid plasmid-free system for inducing orotidine 5'-phosphate decarboxylase (pyrF) gene mutation in *R. delemar*.

Methods. Protoplasts of *R. delemar* 99–880 strain were transformed with 20 nucleotide gRNA targeting the N-terminus of pyrF gene and the Cas9 enzyme. Screening for pyrF auxotrophy was carried out by plating transformed protoplasts on potato dextrose agar (PDA) plates containing 1 mg/mL 5-fluoroorotic acid (5-FOA) and 100 µg/mL uracil. Putative mutant strains were selected for uracil auxotrophy by plating simultaneously on media with or without uracil. pyrF disruption was verified by using PCR and qRT–PCR.

Results. Approximately100 transformants were generated through plating on 5-FOA plates. Only three transformants did not grow on minimal medium lacking uracil, indicating that they were true pyrF null mutants. PCR analysis showed that these three transformants have undergone nucleotide deletion events within the pyrF gene. The lack of pyrF gene expression was further verified by using qRT–PCR relative to wild-type *R. delemar* 99–880.

Conclusion. Similar to the plasmid-based genome manipulation strategy, the plasmid-free CRISPR/Cas9 system can induce gene editing in *R. delemar.* This rapid and simple approach adds an additional tool in our conquest to understand pathogenesis of mucormycosis.

***Disclosures.* All authors:** No reported disclosures.

1725. Shear Forces Induce a Transient, Calcineurin-Dependent Hyper-Virulent Phenotype in Mucorales via Soluble Factors

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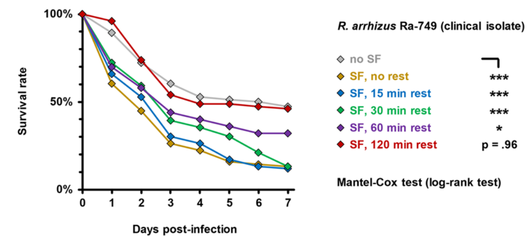
Background. Myocutaneous mucormycosis is encountered in settings of extreme mechanical forces such as combat-related blast injuries or natural catastrophes. It is unclear whether the virulence of Mucorales is affected by mechano-biological factors such as shear forces (SF).

Methods. Spores of clinical strains of *Rhizopus arrhizus*, *Rhizomucor pusillus*, and *Mucor circinelloides* (10^7/mL in PBS) were either kept in static culture (control) or exposed to shear forces (SF) by magnetic stirring for 30 minutes. Mycelial expansion was monitored in the IncuCyte time-lapse microscopy system. For *in vivo* studies, the dorsal thorax of wild-type *Drosophila melanogaster* flies (n = 66–76 per condition) was pricked with a needle dipped in control or SF-exposed spore solutions. Flies were also infected with non-exposed spores suspended in cell-free supernatant taken from SF-exposed spores. Survival was monitored for 7 days post-infection.

Results. Growth rates and morphogenesis of all isolates were not altered by SF. However, SF-exposed spores of all tested Mucorales isolates exhibited increased pathogenicity in the fly model (7-day survival: SF 8–14%, control 36–44%, $P < 0.001$). Introducing different resting periods after SF resulted in gradual attenuation of the hyper-virulent phenotype, with survival rates of infected flies returning to the level seen with non-SF-exposed spores after 120 min post-SF resting (Panel A). To gain a mechanistic insight, we added cyclosporine A (CsA, 100 µg/mL) during shear challenge. Compared with SF-exposed spores, CsA addition improved 7-day survival of *R. arrhizus*-infected flies from 1% to 29% ($P < 0.001$), whereas the pathogenicity of non-SF-exposed spores was not influenced by CsA. Interestingly, supernatants from SF-exposed *R. arrhizus* rendered non-exposed spores hyper-virulent (Panel B, $P = 0.003$).

Conclusion. SF induces a transient hypervirulent phenotype of Mucorales. Our findings suggest that soluble mediators contribute to increased pathogenicity. Largely attenuated hyper-virulence in the presence of CsA corroborated the previously described relevance of the calcineurin pathway in fungal mechano-biology. RNA sequencing studies are in progress to identify epigenetic alterations in Mucorales following SF.

A



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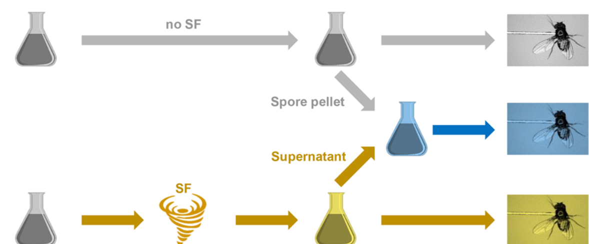

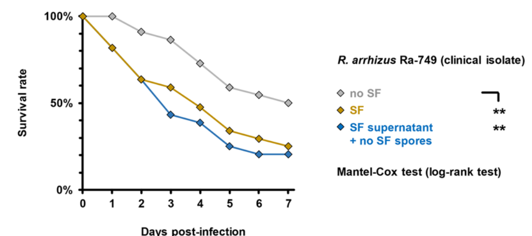

* p < 0.05 ** p < 0.01 *** p < 0.001

***Disclosures.* All authors:** No reported disclosures.