Aspergillus fumigatus persistence in Cystic Fibrosis: adaptation to hypoxia and osmotic stress through in-host HOG pathway mutation

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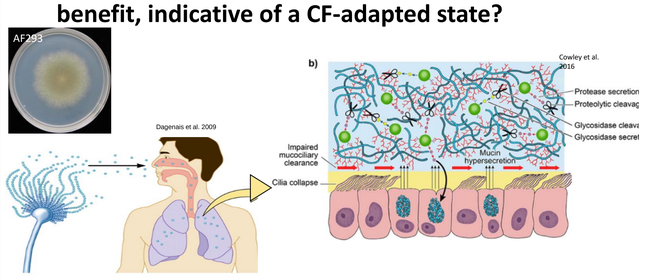
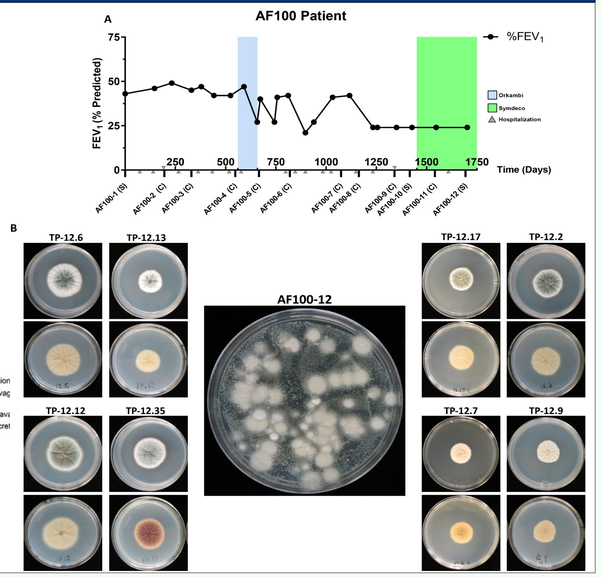
Aspergillus fumigatus is a ubiquitous fungus that can cause chronic infections in patients with Cystic Fibrosis (CF)

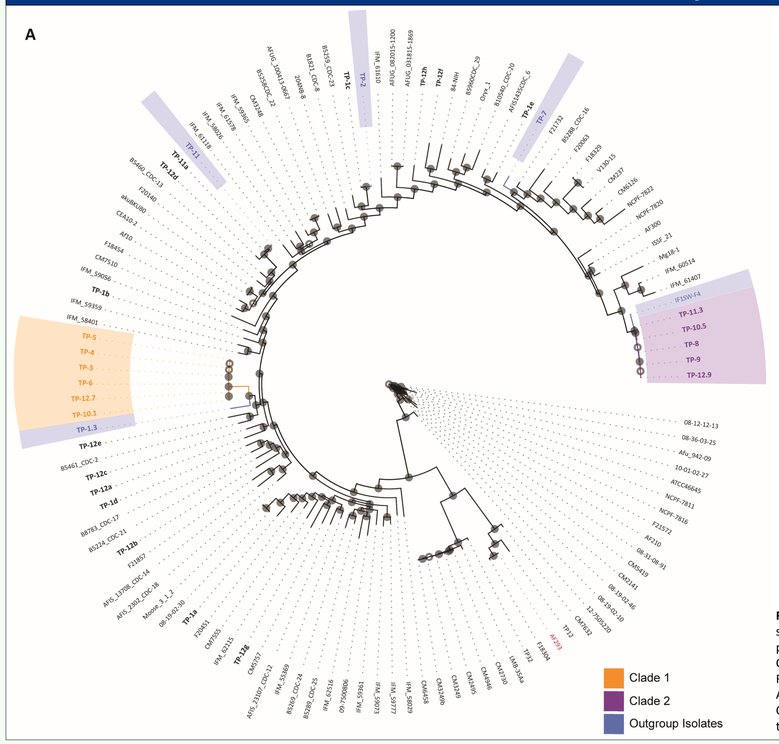
Despite increasing prevalence, it is not well understood if persistent *A. fumigatus* colonization is important to treat in CF. We are using the AF100 series of isolates collected from one patient over >6 years to address the following:

- **Can we identify persistent isolates with relevant phenotypic profiles using CF environmental stresses?**
- **Can we identify specific genetic changes in CF isolates associated with a relevant phenotypic benefit, indicative of a CF-adapted state?**





Illumina MiSeq whole-genome sequencing (WGS) generated genome data for 30 AF100 isolates. We compared these to ~60 non-CF clinical and environmental isolate genomes to generate this phylogenetic tree. AF100 isolates occupy ~15 distinct positions, indicating genetic diversity. Analysis also identified two clades of closely related isolates from multiple timepoints



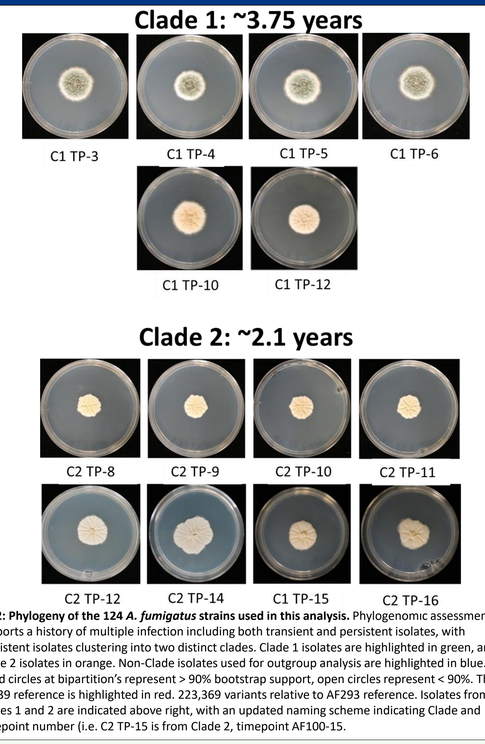
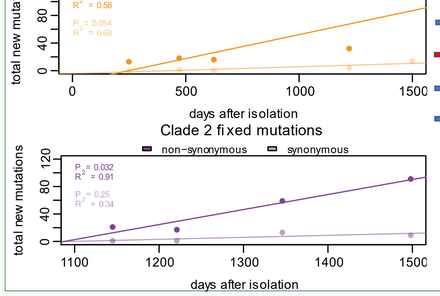


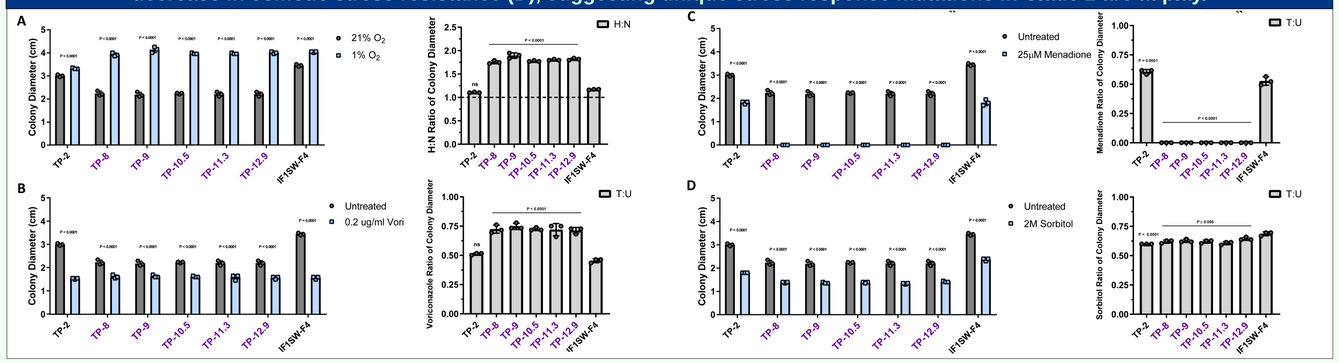
Fig 2: Phylogeny of the 124 *A. fumigatus* strains used in this analysis. Phylogenomic assessment supports a history of multiple infection including both transient and persistent isolates, with persistent isolates clustering into two distinct clades. Clade 1 isolates are highlighted in green, and Clade 2 isolates in orange. Non-Clade isolates used for outgroup analysis are highlighted in blue. Filled circles at bipartition's represent > 90% bootstrap support, open circles represent < 90%. The AF239 reference is highlighted in red. 223,369 variants relative to AF293 reference. Isolates from Clades 1 and 2 are indicated above right, with an updated naming scheme indicating Clade and timepoint number (i.e. C2 TP-15 is from Clade 2, timepoint AF100-15.

We used the WGS data to identify mutations found in Clades 1 and 2 and performed positive selection analysis to determine which clade to study further. Our analysis suggests Clade 2 may be under positive selection; here we show a short list of mutations of interest unique to Clade 2

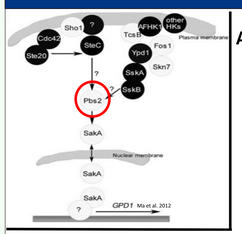


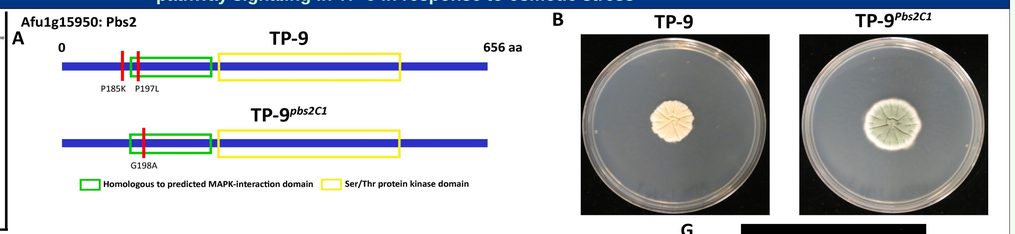
Gene ID	Product Description (FungiDB)	Type of Mutation	Nucleotide Change
Afu1g07160 (*ubp3*)	Ortholog(s) have ubiquitin-specific protease activity, role in protein deubiquitination and cytosol, nucleus localization	Splice donor variant	522+2T>G
Afu1g15950 (*pbs2*)	**Putative mitogen-activated protein kinase (MAPKK)**	**Missense variant**	**590C>T**
Afu2g10620	Ortholog(s) have protein serine/threonine kinase activity, protein serine/threonine kinase inhibitor activity	Missense variant	199G>A
Afu3g05900 (*ste7*)	MAPK kinase (MAPKK)	Start site lost	2T>C
Afu3g08010 (*ace1*)	C2H2 transcription factor	Missense variant	2175G>C
Afu3g10080	Ortholog(s) have cytosol localization	Missense variant	531C>G
Afu3g11940	Has domain(s) with predicted zinc ion binding activity	Stop codon gained	1711C>T
Afu3g11970 (*pacC*)	C2H2 finger domain transcription factor	Frameshift variant	1628dupC

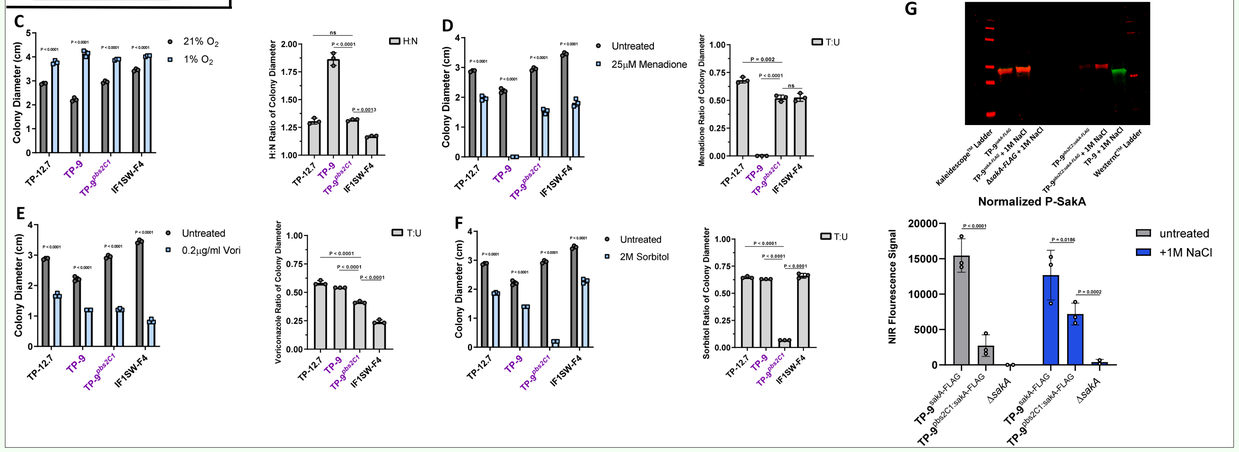
Using IF1SW-F4, a closely-related non-CF outgroup for Clade 2, and TP-2, a non-persistent AF100 isolate, we performed phenotypic assays with the AF100 isolates using CF-relevant stresses. Clade 2 isolates have increased growth in hypoxia (A) and are more tolerant to voriconazole treatment (B). Clade 2 isolates are also severely sensitive to oxidative stress via menadione (C), and show a slight decrease in osmotic stress resistance (D), suggesting unique stress response mutations in Clade 2 are at play.



We identified a unique mutation in Clade 2 in the HOG pathway MAPKK *pbs2* (A). We asked if the unique allele contributes to Clade 2 phenotypes. Using *pbs2^{C1}*, we generated TP-9^{pbs2C1} which shows improved normoxia growth (C) and rescued oxidative stress defect (D) at the cost of a severe osmotic stress defect (F). Using Near-Infrared Fluorescence Western Blotting (G), we show that *pbs2^{C2}* is necessary for HOG pathway signaling in TP-9 in response to osmotic stress







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We are investigating a spontaneous fludioxonil resistance phenomenon observed in the Clade 2 isolates. Using hyphal plugs grown on AMM + 0.1ug/ml fludioxonil, we identified and isolated colony sectors from TP-9 that showed increased growth on fludioxonil at the cost of severe osmotic impairment (A). These strains also show a reduced H:N ratio compared to TP-9; Unlike TP-9^{Pbs2C1} this is due mostly to reduced growth in hypoxia, further pointing to the importance of the HOG pathway (B). Efforts are focused on characterizing the SakA activity of these strains, which appear capable of SakA phosphorylation in response to NaCl stress despite their osmotic defect (C). We are also looking into similar sectors from the IF1SW-F4 outgroup (D).