

# Investigation of adaptation pattern in hypoxia condition of clinical *Aspergillus fumigatus*



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*Aspergillus fumigatus* (Af) is a major cause of invasive fungal infection in human being. Previous study confirmed the hypoxia condition as an important factor in the host environment and the positive correlation between Af's hypoxia resistance and its virulence. However, the phenotypic and genomic changes during adaptation to hypoxia remain unknown. Here, we aim to investigate how clinical Af adapts to hypoxia condition.

Using two Af type strains (Af293 and KU) and two clinical strains which have different hypoxia resistance, we generated hypoxia-adapted strains (G20) by passaging conidia for 20 generations for a total of 80 days of hypoxic growth in lab. Hypoxia-adapted and original strains are compared with the application of phenotypic assay and genomic analysis. Developed hypoxia resistance has been observed among G20 strains, compared to the original ones. According to the genomic analysis, we identified an insertion on gene Afu4g10200 of Af293, and a SNP on gene Afu5g04170, both of which are in CDS region of the gene, and may be responsible for the hypoxia regulation. However, these two genes exclusively exist on Af293 and IFM 58401, respectively, indicating the heterogeneity of hypoxia adaptation of different Af clinical strains.

We managed to illustrate the underlying genomic mechanism of hypoxia adaptation of Af, and confirmed the correlations between hypoxic fitness and other properties during adaptation in the host environment.

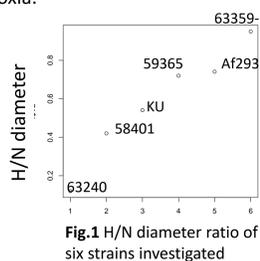
## MATERIAL & METHODS

### Generation of hypoxia adapted strains

#### Six Af strains are selected

Six Af strains, including two laboratory used strains, and four clinical ones are investigated. According to hypoxia/normoxia diameter ratio, they have different tolerance to hypoxia.

Af293	IFM 58401
KU	IFM 59365
	IFM 63240
	IFM 63359-1



#### lab passaging

5d old conidia AMM 37°C  
 1% oxygen, AMM w/o Fe<sup>2+</sup>, 37°C  
 Passage every four days  
 Bring 1~5x10<sup>7</sup> conidia to new AMM  
 totally 20 generations

### Afterwards evaluation

**hypoxia resistance checked by colony diameter and dry weight**  
 dry weight: preculture: 5d, AMM, 37°C; liquid culture: 5x10<sup>7</sup> conidia, 200rpm, 100ml, liquid AMM, 48h; dry.

**Whole genome analysis is performed for all hypoxia resistance developed strains.**

**SNPs identification and SNPs -based phylogenetic analysis**  
 Based on Af293 type strain

**GO analysis**  
 FDR corrected values are calculated

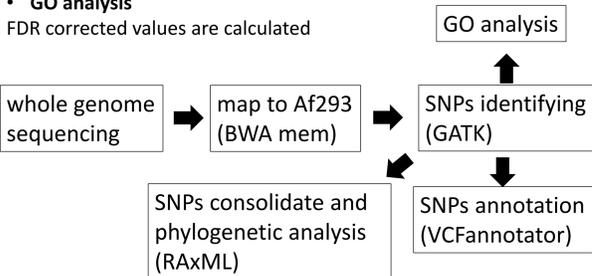


Fig.2 whole genome analysis pipeline

## CONCLUSIONS & PLANS

We generated four hypoxia adapted clinical Af strains in the lab, including two lab used strains, and two clinical ones. They initially had different hypoxia resistance.

Four strains with different genetic backgrounds have different adaptation patterns to hypoxia, but generally they all become more resistant to hypoxia.

As for IFM 63559-1, G20 on normoxia has larger colony diameter, but less dry weight, which may indicate that after adaptation to hypoxia, fungal hyphae tissue may become more scarce, although it needs to be checked on other strains.

Until now, we managed to get two mutations which may be responsible for Af's adaptation to hypoxia, i.e., Afu4g10200 and Afu5g04170. No genetic mutations overlap are found among these four strains, indicating different hypoxia regulation mechanisms.

According to our analysis, GATK pipeline is not so accurate for the mutation calling of serial passage strains which raised from the same ancestor (we failed to find any SNPs or INDELS of KU; the results are all false positive, according to the IGV data). Methodology part should be revised to find more subtle differences among the serial passage strains.

We also plan to investigate how hypoxia adaptation of Af affects other survival related properties like antifungal resistance, virulence, etc.

## RESULTS

### Adaptation to hypoxia measured by colony diameter

After 20 generation hypoxia passaging, six strains exhibited different hypoxia resistance pattern. Generally the growth under hypoxia was increased for all four strains; while for the growth under normoxia, growth of strains which initially have larger colony diameter (i.e. IFM 58401) was decreased.

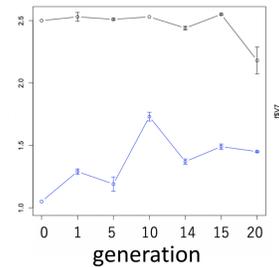
### Adaptation to hypoxia measured by dry weight

We measured the dry weight of IFM 63559-1 and Af293's G0 and G20, under both hypoxia and normoxia conditions.

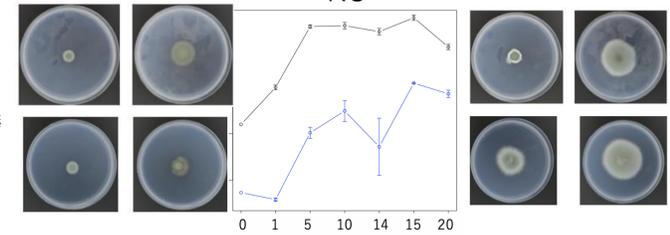
As for IFM 63559-1 (fig.4 left), under hypoxia condition, dry weight of G20 is significant higher than that of G0, while the opposite situation is observed under normoxia condition.

Generally hypoxia tolerance increased, according to the H/N ratio. However, we didn't notice a significant difference of either hypoxia or normoxia dry weight on G20 and G0 for another strain Af293.

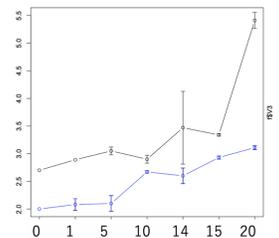
IFM 58401



KU



Af293



IFM 63559-1

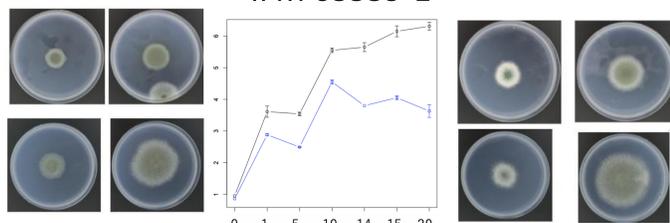


Fig.3 colony diameter (generation 0, 5, 10, 15, 20) and morphology of generation 0 and 20 under hypoxia and normoxia

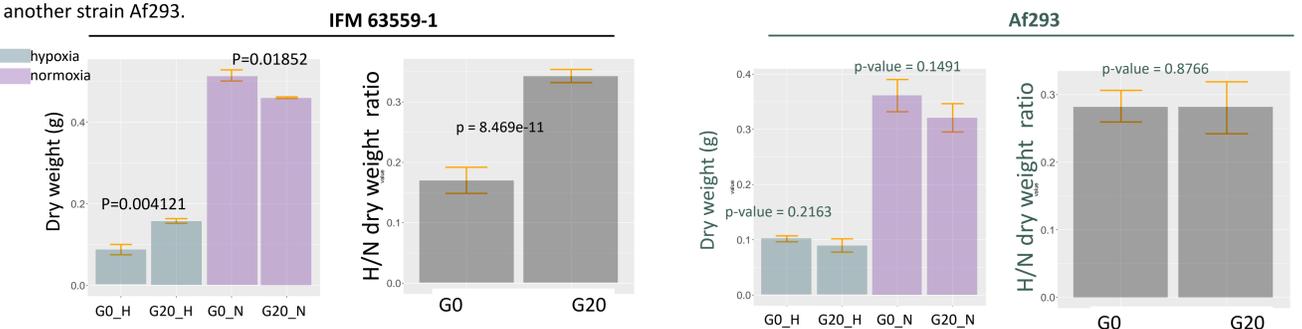


Fig.4 dry weight and H/N ratio of strain Af293 (left) and IFM 63559-1 (right)

### Genetic mutations analysis

Af293 type strain based phylogeny is shown in fig.5.

Comparing G20 to G0, Af293 has 3 indels (one deletion, two insertion), one of which resides in CDS region, other two are in intron and p5UTR, respectively (CDS is shown in fig.6). According to the mapping result data shown in IGV (upper in fig. 6), this insertion appears between G10 and G15. This insertion exclusively exists in Af293, not in other three strains.

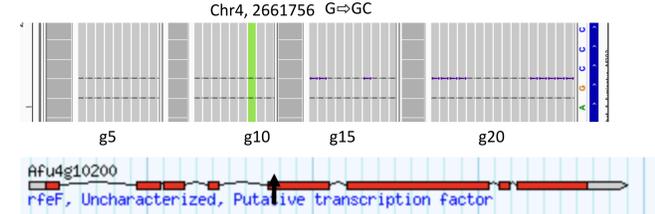


Fig.6 An Insertion at chromosome no. 4 of Af293 G20 to G0

As for SNPs, none is found for Af293 and KU (G20 to G0). One non-synonymous SNP, T to C, is found in IFM 58401, which caused a amino acid transition from Leu to Pro at 92nd CDS region. This mutation appears generally, according to IGV data, from G5 to G10. Also this mutation only exists in IFM 58401.

### Hypoxia tolerance related genetic mechanism differs among these four strains

We analyzed the two mutation point on gene Afu4g10200 and Afu5g04170, respectively, of four strains, and didn't find common mutations (SNPs or INDELS) among them (fig. 9), indicating various adaptation mechanisms of *A. fumigatus* to hypoxia.

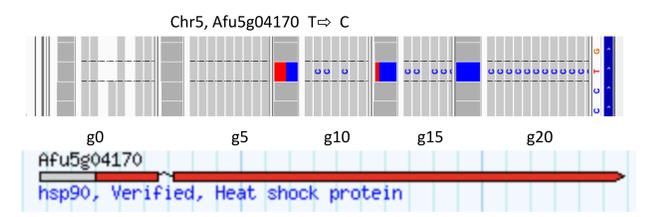


Fig.7 An non-synonymous SNP at chromosome no. 5 of IFM 58401 G20 to G5

Previous research indicated Afu5g14900 controls the hypoxia tolerance of Af293 (CH Kowalski, nature microbiology, 2019). But in our research, we didn't find a mutation in this gene for Af293. Moreover, we found that other three strains even don't have this gene (fig. 8), indicating other possible genetic mechanisms for their hypoxia tolerance controlling.

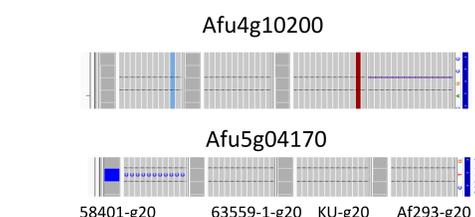


Fig.9 One Insertion on Afu4g10200 on Af293, and one SNP on Afu5g04170 on 58401

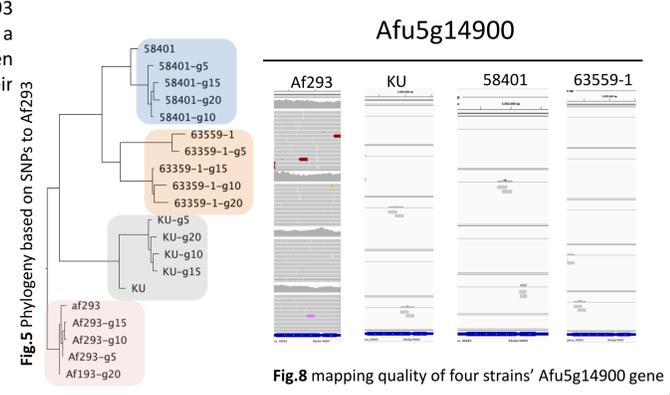


Fig.8 mapping quality of four strains' Afu5g14900 gene

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