Comparing *in vitro* growth rates of fungal species using multi-level mixed effects

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Introduction

Critical fungal infections are currently managed by antifungals, such as azoles, that reduce the rate of fungal proliferation. However, due to their high cost and less than desirable efficacy in vulnerable patients[1], alternative treatments are actively being sought. The antifungal efficacy is often evaluated by estimating fungal growth rates under different conditions[2]. But they are usually estimated from the snapshot data that pooled all biological replicates together. Here we propose a method to estimate fungal growth rates in an experimental population by accounting for the rates of each biological replicate over time. We applied the method to estimate growth rates of different fungal strains and *Aspergillus fumigatus* with or without epithelial cells (ECs).

Methods

We proposed a mixed effects method to estimate growth rates of the main fungal hyphal branch at the population level (fixed effects) and the individual biological replicate level (random effects) using exponential and logistic models, as well as more bespoke previously published growth models[3,4]. We employed a multilevel mixed effects method if there was additional grouping to the data such as strains and species.

We applied the mixed effects method to the dynamic data of hyphal lengths (μm) of 11 *Aspergillus* strains, which are subtypes of 3 *Aspergillus* species, namely *A. fumigatus*, *A. niger* and *A. tubingensis,* as well as the dynamic data of hyphal lengths of *A. fumigatus* grown with or without epithelial cells (ECs). The growth rates obtained by all of the models were compared using student t-tests and the statistical significance and power were recorded.

Results

Information criterion-based model selection favoured using our mixed effects method over the non-mixed effect methods. Our method allows for successful derivation of the growth rate for each dataset. The growth rates of the 11 *Aspergillus* strains were significantly different at the Bonferroni corrected 0.05 significance level. The population growth rates of the 3 *Aspergillus* species were significantly different from each other with that of *A. fumigatus* being the largest (43.39 ± 2.92 for *A. fumigatus,* 8.09 ± 1.05 for *A. tubingensis* and 6.95 ± 0.91 for *A. niger)*. No significant difference was observed in the growth rates with or without ECs.

Discussion and Conclusion

We proposed a new method of estimating and comparing different fungal growth rates of *Aspergillus* fungi. We demonstrated the usefulness of the method by applying it to derive the growth rates. The results highlighted differences in growth rates of multiple *Aspergillus* species and strains, and the lack thereof for *A. fumigatus* with or without ECs. This study sets the groundwork for analyses investigating any *in vitro* fungal growth in varying experimental conditions, such as with or without antifungal drugs.

References

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