The University Of Sheffield.

BioMedEng21

6 - 7 September 2021 | Sheffield, UK

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

Joshua G. Sia[1], Tara Hameed[1], Reiko J. Tanaka[1]
joshuasia2000@gmail.com

[1]Department of Bioengineering, Imperial College London

Introduction

- Novel treatments for invasive aspergillosis (IA) are urgently needed to combat anti-fungal resistance.
- Antifungal efficacy is often evaluated by estimating fungal growth rates under different conditions.
- However, growth rates are commonly estimated using data pooled from different biological replicates.

Aim

To propose a mixed effects method to estimate fungal growth rates in an experimental population by accounting for the rates of each biological replicate, strain or species.

Methods

A multilevel mixed effects method was used to estimate growth rates of the main fungal hyphal branch at the population level (fixed effects) and the individual biological replicate, strain and species levels (random effects) for growth of different *Aspergillus* fungal strains.

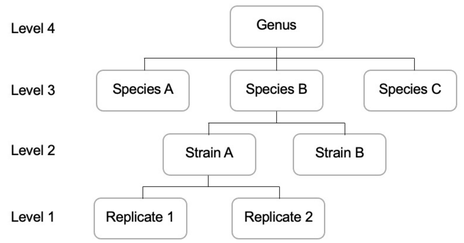
The growth rates obtained by all of the models were compared using student t-tests and the statistical significance was recorded.

$$\text{Level 1}: \hat{y}_{ijkl}(x) = \frac{A_{ijk}}{1 + \mathrm{e}^{\frac{B_{ijk} - x}{C_{ijk}}}} + \epsilon_{ijkl}$$

$$\begin{aligned}\text{Level 2}: A_{ijk} &= A_{ij} + u_{ijk}\,, \quad u_{ijk} \sim \mathcal{N}(0, \sigma_u^2)\\ B_{ijk} &= B_{ij} + v_{ijk}\,, \quad v_{ijk} \sim \mathcal{N}(0, \sigma_v^2)\\ C_{ijk} &= C_{ij} + w_{ijk}\,, \quad w_{ijk} \sim \mathcal{N}(0, \sigma_w^2)\end{aligned}$$

$$\begin{aligned}\text{Level 3}: A_{ij} &= A_i + \bar{u}_{ij}\,, \quad \bar{u}_{ij} \sim \mathcal{N}(0, \sigma_{\bar{u}}^2)\\ B_{ij} &= B_i + \bar{v}_{ij}\,, \quad \bar{v}_{ij} \sim \mathcal{N}(0, \sigma_{\bar{v}}^2)\\ C_{ij} &= C_i + \bar{w}_{ij}\,, \quad \bar{w}_{ij} \sim \mathcal{N}(0, \sigma_{\bar{w}}^2)\end{aligned}$$

$$\begin{aligned}\text{Level 4}: A_i &= A_0 + \tilde{u}_i\,, \quad \tilde{u}_i \sim \mathcal{N}(0, \sigma_{\tilde{u}}^2)\\ B_i &= B_0 + \tilde{v}_i\,, \quad \tilde{v}_i \sim \mathcal{N}(0, \sigma_{\tilde{v}}^2)\\ C_i &= C_0 + \tilde{w}_i\,, \quad \tilde{w}_i \sim \mathcal{N}(0, \sigma_{\tilde{w}}^2)\end{aligned}$$



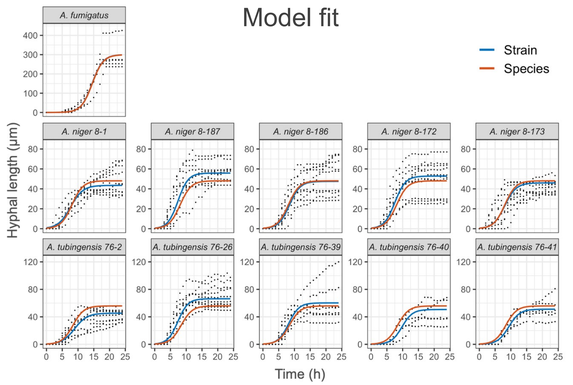
Multilevel mixed effects method applied to the data of 11 Aspergillus strains, which are subtypes of 3 Aspergillus species, namely A. fumigatus, A. niger and A. tubingensis.

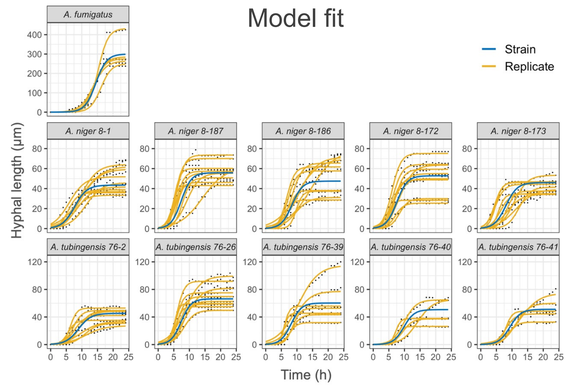
Results

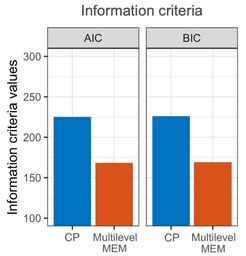
The multilevel mixed effects model (MEM) was able to account for variations in growth from different groupings in the data which led to better generalisation.

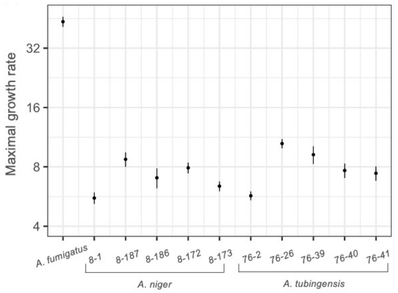
Information criteria based model selection favoured the multilevel mixed effects model over the complete pooling model.

Growth rates of the 11 *Aspergillus* strains were significantly different at the Bonferroni corrected 0.05 significance level. The population level growth rates of the 3 *Aspergillus* species were significantly different from each other with that of *A. fumigatus* being the largest (43.39 $\pm$ 2.92 for *A. fumigatus*, 8.09 $\pm$ 1.05 for *A. tubingensis* and 6.95 $\pm$ 0.91 for *A. niger*).









Conclusion

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

Acknowledgements

The data for this study was collected by Professor Elaine Bignell (University of Exeter) and Ms Natasha Motsi (University of Manchester).

MANCHESTER 1824 The University of Manchester

UNIVERSITY OF EXETER

Imperial College London