Mathematical modelling of fungal growth *in vitro*

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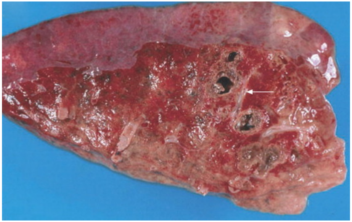
Invasive Aspergillosis

- Invasive Aspergillosis (IA) is a pulmonary disease
- Caused by *Aspergillus* fungi, mainly *A. fumigatus*
- IA has a **high mortality rate** in neutropenic individuals (86%) [1]



Lung tissue of a patient with IA [2]

Current treatments

- **Anti-fungal drugs** (Voriconazole, Itraconazole, etc.)
- High rate of **treatment failure** - about 75-85% [3][4][5]
- Up to 31% of patients have discontinued anti-fungal therapies due to **drug-related adverse effects** such as toxicity ($n = 201$) [6]
- We need to understand fungal growth better to design future treatment strategies!

Background

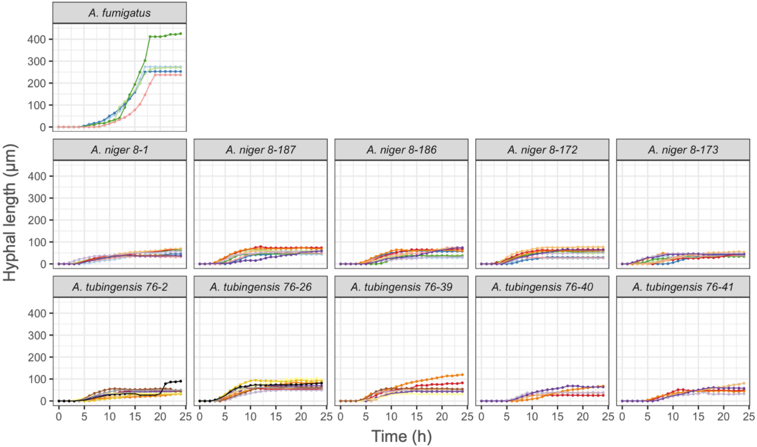
- Araujo et al. (2004): Comparison of **germination rates** for three *Aspergillus* species [7], Paisley et al. (2005): Correlation between virulence and fungal growth rates across nine *A. fumigatus* **isolates** [8]
- No previous studies conducted which compare growth rates based on **hyphal length** across multiple *Aspergillus* strains
- Richard et al. (2018): Epithelial cells (ECs) inhibit *A. fumigatus* **conidia germination**
- Effect of ECs on hyphal growth remains unknown

Aims and Objectives

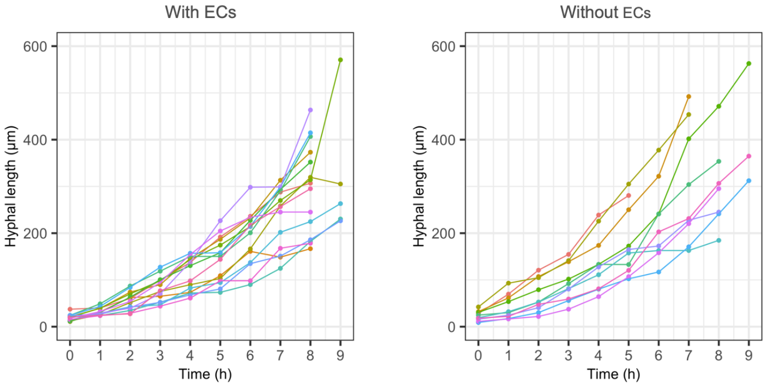
- Aim
  - Investigate why *A. fumigatus* is the most prevalent species in IA by using the hyphal growth rate as a virulence factor

- Objectives
  - Propose fundamental mathematical models of *Aspergillus* hyphal growth and perform model selection
  - Compare the growth rates of *A. fumigatus* growth **with and without epithelial cells**
  - Compare the growth rates of **multiple Aspergillus strains and species**

Growth of 11 *Aspergillus* strains



A. fumigatus growth with and without ECs



Workflow

Data

- Multiple species
- Epithelial cells

Model

- Exponential and logistic models
- Complete and partial pooling models

Evaluation

- AIC
- BIC
- Cross validation

Growth models

ECs dataset

Exponential model

$$\hat{y}(x) = A\,\mathrm{e}^{Bx} \quad (1)$$

A: Intercept
B: Growth rate

MS dataset

Logistic model

$$\hat{y}(x) = \frac{A}{1 + \mathrm{e}^{\frac{B-x}{C}}} \quad (2)$$

A: Asymptote
B: Inflection point
C: Inverse of slope

Logistic model growth rate

Derivative of the logistic model

$$\frac{dy}{dx} = \frac{A}{C}\frac{z}{(1+z)^2}\,, \quad z = \mathrm{e}^{\frac{B-x}{C}} \tag{3}$$

Derivative evaluated at $x = B$ to obtain maximal growth rate (4) with standard deviation (5)

$$\left.\frac{dy}{dx}\right|_{x=B} = \frac{A}{4C} \tag{4}$$

$$\sigma_d = \frac{d}{4}\sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_C}{C}\right)^2} \tag{5}$$

Models - Dealing with noise

- Additive noise

$$y \sim \mathcal{N}\left(\hat{y}, \sigma^2\right) \tag{6}$$

- Multiplicative noise

$$\log(y) \sim \mathcal{N}\left(\log(\hat{y}), \sigma^2\right) \tag{7}$$

Models - Dealing with groupings

- Complete Pooling

$$\hat{y}_i = \frac{A}{1 + \mathrm{e}^{\frac{B-x}{C}}} \tag{8}$$

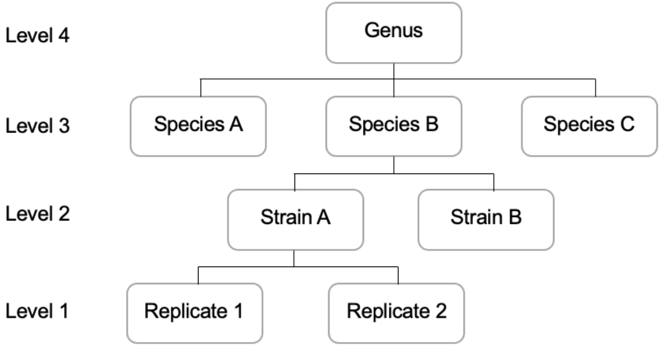
- Mixed Effects Model, MEM (Partial Pooling)

$$\hat{y}_{ij} = \frac{\left(A + a_j\right)}{1 + \mathrm{e}^{\frac{\left(B+b_j\right)-x}{\left(C+c_j\right)}}} \tag{9}$$

$$a_j \sim \mathcal{N}\left(0,\, \sigma_a^2\right) \quad b_j \sim \mathcal{N}\left(0,\, \sigma_b^2\right)$$
$$c_j \sim \mathcal{N}\left(0,\, \sigma_c^2\right)$$

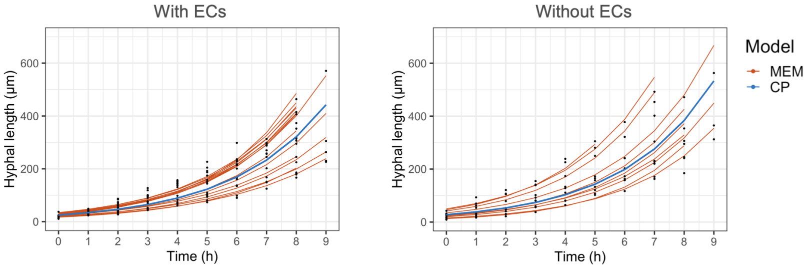
Models - Dealing with groupings

- Multilevel mixed effects model



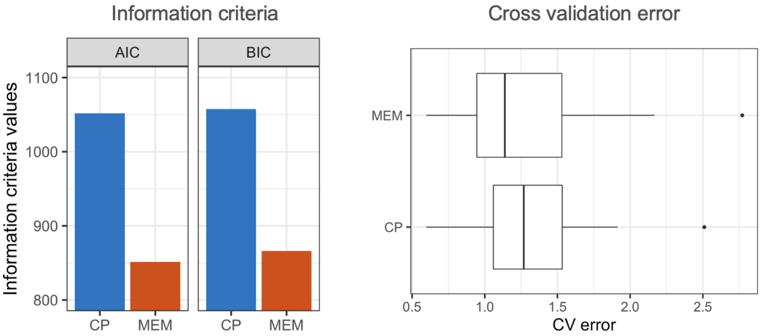
ECs dataset: Model fits

The MEM allows for **variability between replicates**



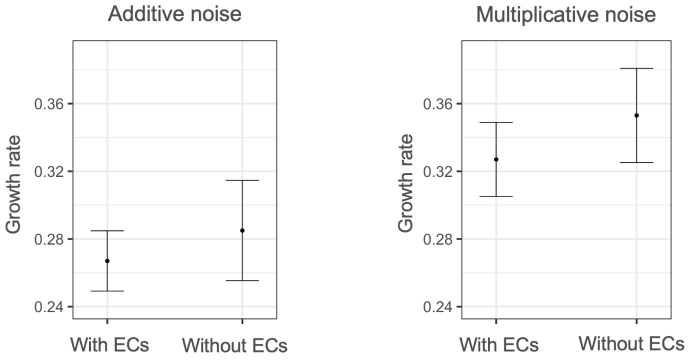
ECs dataset: Model selection

The MEM has **lower** information criteria values than the CP model

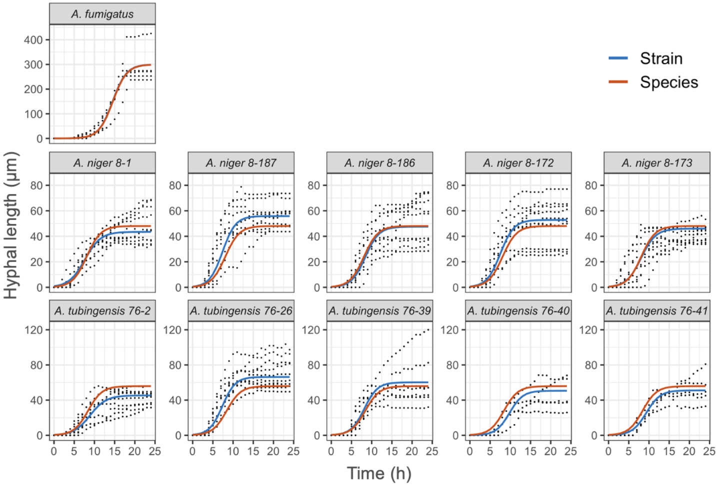


ECs dataset: Growth with ECs

No significant difference found at the 0.05 significance level

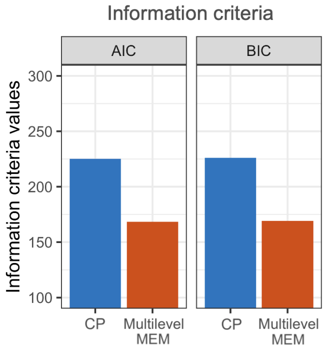


MS dataset: Multilevel MEM fit



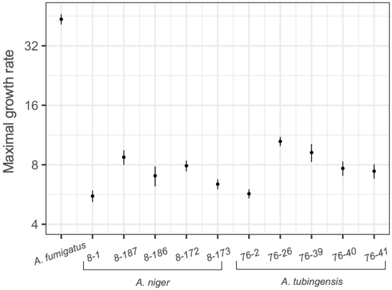
MS dataset: Model selection

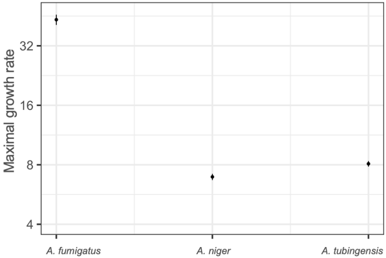
The multilevel MEM allows for relatively **good generalisation** and has **lower information criteria values**



MS dataset: Comparing growth rates

Maximal growth rate of *A. fumigatus* is **significantly larger** than all other species and strains





Discussion

- Maximal growth rate of *A. fumigatus* is higher than *A. tubingensis* and *A. niger*
  - Supports the hypothesis that the maximal growth rate is a virulence factor indicative of prevalence in IA
- Richard et al. (2018): ECs inhibit *A. fumigatus* conidia germination
  - Lack of significance obtained suggests that ECs only inhibit conidia germination but **not hyphal growth**
- Criticism of the logistic growth model - unable to sufficiently model the lag phase
  - Baranyi et al. [9]
  - Mavridou et al. [10]

Thank you!

Q&A

Appendix A - ECs dataset: Model fits

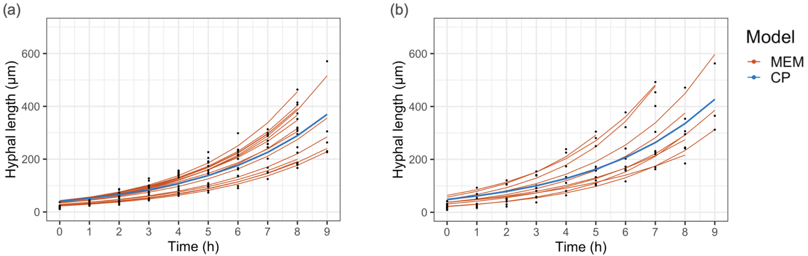


Figure 1: Fits of the additive noise CP and MEM models for *A. fumigatus* growth (a) with ECs and (b) without ECs

Appendix B - ECs dataset: Model selection

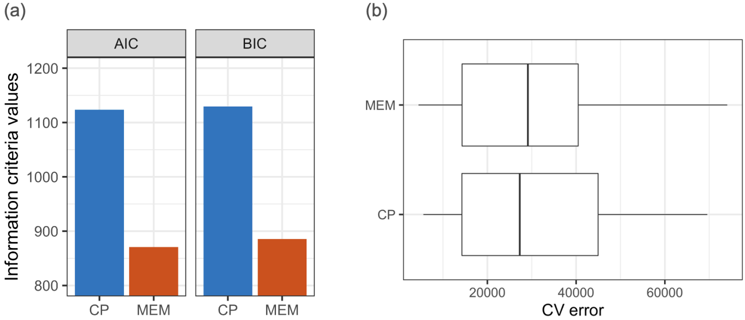


Figure 2: (a) Information criteria values and (b) Cross validation errors for the CP and MEM additive noise models

Appendix C - Multilevel mixed effects model

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

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

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

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

Appendix D - Model proposed by Baranyi et al. [9]

$$y(t) = y_0 + g\,A_n(t) - \frac{1}{m}\ln\left(1 + \frac{\mathrm{e}^{m\,g\,A_n(t)} - 1}{\mathrm{e}^{m(y_{max} - y_0)}}\right) \tag{10}$$

$$A_n(t) = \int_0^t \frac{s^n}{\lambda^n + s^n} ds$$

y: hyphal length

y_0: hyphal length at time $t = 0$

y_{max}: maximum hyphal length

m: Richard's curvature parameter after the exponential phase

n: curvature parameter after the lag phase

g: maximum hyphal growth rate

λ: duration of lag phase

s: integral variable

Appendix E - Model proposed by Mavridou et al. [10]

$$y(t) = \begin{cases} y_0 & 0 \leq t < \tau \\ (y_0 + v(t-\tau))\mathrm{e}^{-\lambda(t-\tau)} & t \geq \tau \end{cases} \tag{11}$$

y: hyphal length
y_0: hyphal length at the lag phase
τ: duration of lag phase
v: growth rate
λ: growth decay constant

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