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Article

Which Microbial Growth Model Best Fits to *Fusarium graminearum*?

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Abstract: *Fusarium graminearum* causes head blight in wheat and corn, and produces chemicals harmful for humans and other animals. It is important to understand how it grows in order to prevent outbreaks. There are 3 well-known growth models for microorganisms and they seem applicable to molds: linear, Gompertz and Baranyi. This study aimed to see which could better represent *F. graminearum* growth. Three replicates were grown in yeast extract agar (YEA) for 20 days. The Feret's radius was measured in ImageJ software, and then related to the models. Linear model was the most closely correlated to the actual growth (Pearson's correlation: 0.95). Thus, considering that it was the most representative of the reality and it is easier to use, it seems to be the best logical choice for *F. graminearum* growth studies.

Keywords: *Fusarium graminearum*, mold growth, linear model, Gompertz, Baranyi.

1. Introduction

Fusarium graminearum is a fungal plant parasite responsible for the disease known as FHB (Fusarium head blight) in crops such as wheat and corn, and mycotoxin contamination in humans [1,2]. Toxins include deoxynivalenol (DON) and zearalenone (ZEA) [3-5]. The former is emetic and the second has estrogenic properties. DON inhibits protein synthesis in mammals, causing regurgitation, weight loss, diarrhea and immunosuppression [6]. ZEA has estrogenic activity, potentially causing hyperestrogenism and consequent reproductive problems such as abortion [7].

There is a body of studies on fungal growth [8-11]. Most are conducted for practical purposes such as brewing or drug production rather than mere scientific curiosity [12-15]. Thus, the current knowledge covers the types of substrate or environmental settings, and it is mostly superficial or speculative based on bacterial studies [11]. Thus, there is a need to properly describe how in fact fungi grow, especially molds.

Three major models used to describe fungal growth: one linear and two sigmoidal (Gompertz and Baranyi) [11]. Authors have been arguing about which is the best for practical purposes. While some prefer linear's simplicity, others claim the sigmoidal to be more accurate representing the irregular biological nature of the phenomenon [16]. The choice of an appropriate growth model for *F. graminearum* will allow scientists to more accurately predict the propagation of FHB and prevent outbreaks. This study was undertaken to find out which major model explains growth of *F. graminearum* in a system with limited nutrient supply, in minimal medium and at room temperature.

2. Materials and Methods

This study used a *F. graminearum* isolate from the JCM Catalogue. It is registered as the teleomorph *Giberella zeae* (Schwabe) Petch isolated by Sugiura [3] from rice stubble in Hirosaki, Aomori Prefecture, Japan. It is a known producer of deoxynivalenol, 15-acetyldeoxinivalenol and zearalenone [17].

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Three *F. graminearum* replicates were grown in yeast extract agar (YEA) inside a black box inside a chamber, at room temperature during 20 days. Daily photos were taken using Nikon D3200. The shots were performed vertically at 25 cm above the specimens after opening the Petri dishes. The radii were measured in ImageJ software. After setting the scale by taking the 90 mm of the plate's diameter as reference, the fungal area was isolated through RGB color threshold. Then, the Feret's diameter was determined and converted in radius. Growth models were determined using reference values such as the duration of lag phase (λ), end of the exponential growth (t_{max}), maximum growth rate (μ_{max}) and the maximum radius (y_{max}).

The statistical analysis was performed on Microsoft Excel and Jamovi statistical package. Kinetic graphs of radius x time allowed visual comparisons between the actual growth and the models, and a scatterplot matrix showed how the models were correlated to *F. graminearum* growth.

3. Results and Discussion

3.1. Description of the growth stages

The mold grew in a logistic pattern with its typical sigmoidal curve (**Error! Reference source not found.**). Lag phase took 1 day, followed by 10 days of exponential growth. The maximum growth rate (μ) was 33 mm/d and the maximum radius was 45 mm. The growth rate during the exponential phase was at its peak in the 2nd day, between the 4th and 5th, and also around the 9th and 10th days. It slowed down slightly between the 6th and 7th days.

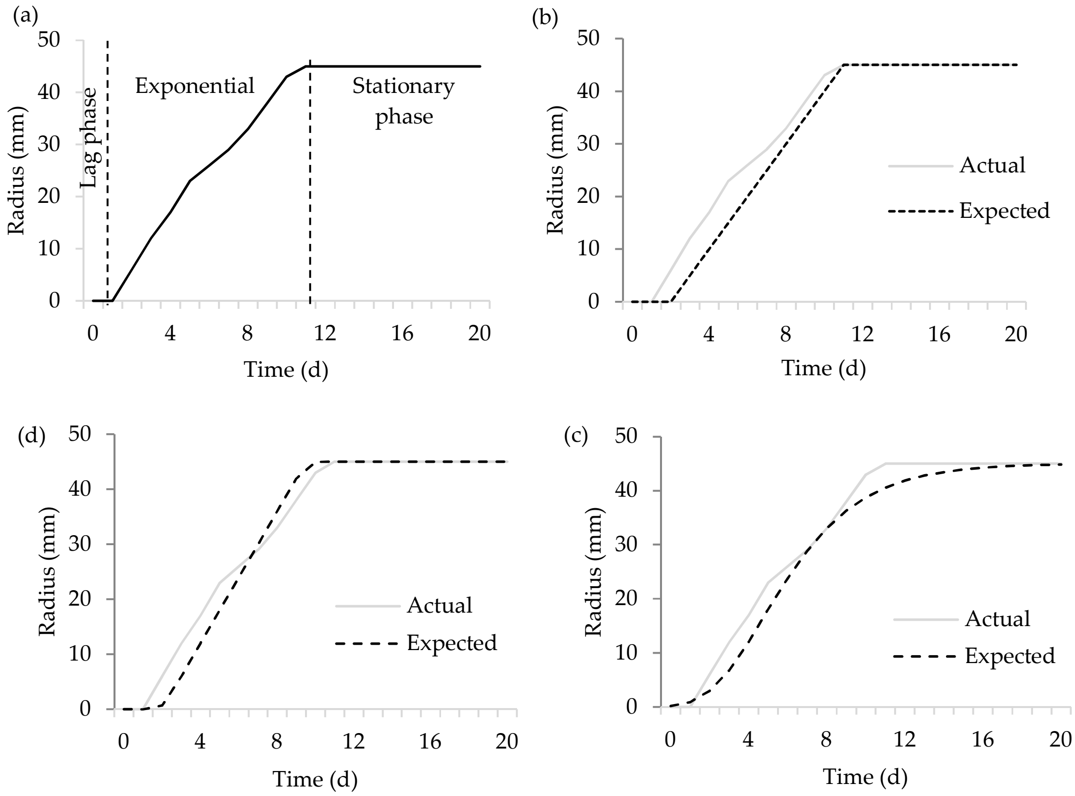

Figure 1. *F. graminearum* growth during 20 days: (a) distinct stages; (b) actual growth x linear model; (c) actual growth x Gompertz model; (d) actual growth x Baranyi model.

First, the linear model was compared to actual growth. The differences are noticeable, especially during the log phase. The lag phase also shows some discrepancy, as the actual growth started at least one day before what the linear model shows. On the other hand, the curves seem to get closer as the time passes and finally connect in the beginning of the stationary phase. The

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Gompertz model appeared very smooth, almost without a lag phase and showing very harmonious transitions between the phases. The stationary phase's onset also takes days longer than expected. Thus, this model is not the most adequate to represent *F. graminearum* growth. They have noticeable discrepancies, especially in the slope variations in the log phase. Baranyi's model also seems smooth but not as much as Gompertz. Its shape is somewhere between the two previously analyzed. Yet, besides the considerably irregular shape of the actual growth curve during the log phase, Baranyi's model seems fit enough to represent *F. graminearum* growth.

3.2. Correlations

All models seemed fit to represent the growth of *F. graminearum* over time (Figure 2). Yet, they present different degrees of correlation: the actual growth was more correlated to the linear model, followed by Gompertz and then Baranyi.

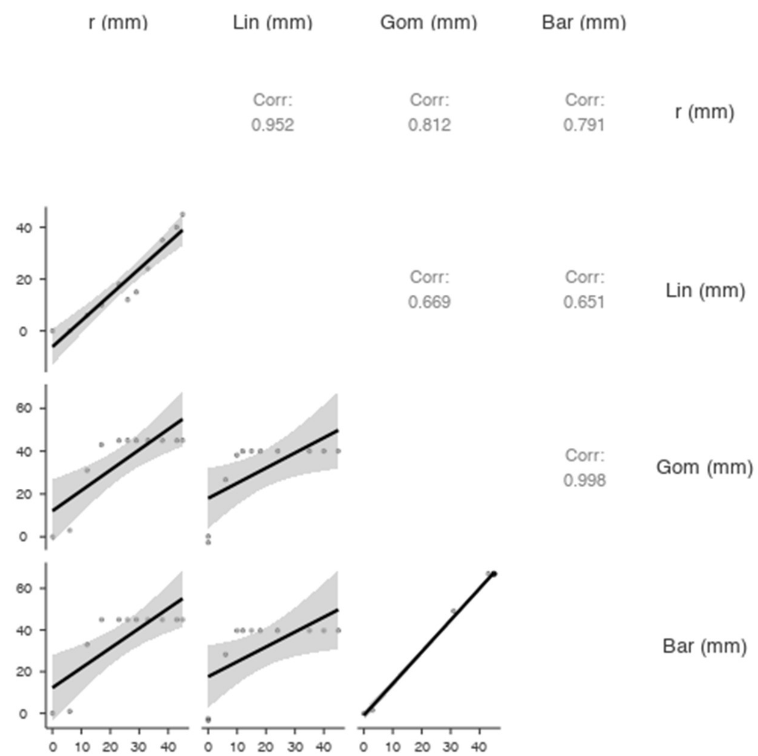

Figure 2. Correlation between *F. graminearum* growth and the major microbial growth models. r = radius (actual growth); Lin = linear; Gom = Gompertz; Bar = Baranyi; corr = Pearson's correlation.

The scatter plots also show more consistency between the actual growth and the linear model, with a narrower "shade" of standard error. The highest consistency was between Gompertz and Baranyi models but both showed the weakest correlations and fitness with actual growth.

4. Discussion

The logistic pattern was expected, as it is common for biological systems, especially if subjected to closed systems rich in nutrients. In this case, the mold stopped growing in size due to the surface area available, rather than nutrient shortage. Once it happens, a color change is noticeable and it is probably a result of nutrient shortage. Neagu and Borda [12] also studied *F. graminearum* growth. Their maximum growth rate was higher (13.5 mm/d) and the fungus attained its maximum size on the 8th day. The difference might have been due to their media, enriched with barley and wheat extract, while here the medium is minimal, with yeast extract. Unfortunately, they did not us the current models and ignored details such as phase distinction, probably because they just wanted to see how much time the mold takes to occupy the plate's surface.

The growth rate might be explained by some biological interactions [18,19]. The lag phase consists of adaptation, followed by rapid growth. The following reduction is likely due to signals sent by the first hyphae reaching the plate's borders and facing the first signs of nutrient exhaustion. But it is not a major problem because there are more nutrients underneath the surface. After a re-adaptation to the new situation, they grew some millimeters and finally stopped growing as most reached the borders.

The linear model is certainly the easier to work with and it is the most widely used [11], and it seems to be the best for *F. graminearum* among the ones analyzed in this study. Gompertz can be used but might not be as representative of the actual growth. Baranyi was the less fit. This result disagrees with Garcia's opinion on fungal growth [11] based on Buchanan's meta-analysis on bacterial growth [16]. The relatively simple radial expansion of the mold in comparison with bacteria might have been due to the fact that the spores ultimately produce a single multinuclear organism [19], with single protoplasm, more efficiently spreading nutrients across the mycelium in a more coordinated fashion. Gompertz and Baranyi developed their models to reflect the smoother transitions of the bacterial growth stages [16], but they might not be as necessary for *F. graminearum*.

In summary, *F. graminearum* growth exhibited a sigmoidal shape. A 4^{th} degree polynomial regression was fit to predict its growth rate. Further studies may provide more insight at the current observations but this experiment indicated linear model as the best to represent the growth of *F. graminearum* and certainly most closely related fungi.

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