Microbial growth models – which is better and why?

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Abstract

Introduction

Phenological models are expected to fit data trends within its biological field. Yet due to different reasons, models developed and published from one sample may not fit the others. These reasons may be due to data variabilities, confounding factors, inaccurate assumptions or models being too-specific. This project is aimed at compare and contrast published phenological models on microbial population size data, highlighting which is a better model under what conditions. The hypotheses are:

- published phenological models are better than polynomials in describing microbial population size;
- appropriate phenological model(s) is/are identifiable through distinguishable shapes of microbial population size; and
- parameters of data under each phenological model is clustered, similar with dataset best-described by the same model but different from those described by other models.

Methods

Experimental microbial population growth data library were divided into individual data subsets through six filters ("Temperature (in ^{o}C)", "Microbial clade", "growth substrate materials", "experimental replicate number", "population data recording unit" and "data source"). Records with data unit "OD_595" were scaled into optical density percentages (i.e. data*100) to facilitate general analyses workflow. Independent (or explanatory) variable was "Time (hr)" and dependent (or response) variable was "population size".

Some raw data were recorded in minutes (instead of hour). This record artifact was not corrected because of two reasons: 1. shape of curves were the main concern instead of independent variable's scale; and 2. the unit was consistent within each data subset.

Model assessment

Six candidate models were assessed, four phenological and two polynomial equations. They were "Verhulst (classical)"[1], "modified Gompertz"[2], "Baranyi"[3], "Buchanan"[4], "quadratic" and "cubic". Non-linear least square (NLLS) approach was used only on the four phenological models and linear model-fitting was done on the two polynomials. Starting values selection (for phenological models only) was described below:

Initial (N0) and final (K) population sizes were selected to be the minimum and maximum values of each data subset respectively. Maximum growth rate (r.max) was selected by linear model through a recursive manner. For every iteration, population size data from the top 5% independent variable values were excluded from the linear model calculation. The data and slope would only be recorded if it was positive, higher adjusted R^2 value and larger slope than the recorded "best slope" value. After scanning from the maximum side, the best slope and its respective data were taken out and screened from the minimum side. Final best slope and x-intercept were regarded as the r.max and relative time lag (t.lag) of the population (in the source experiment) respectively. Time which this linear model intersected with K was regarded as the time achieving carrying capacity (t.K). Population data was then classified into three groups (gx) according to the time: $\text{g1} \leq \text{t.lag} < \text{g2} < \text{t.K} \leq \text{g3}$. 5% was chosen as the scanning threshold because the author assumed this resolution was fine enough for achieving good starting values for NLLS fitting. Inputs for phenological modelswere listed below (popn & time were the dependent and independent variables respectively):

$$
\begin{aligned}
\text{Verhulst (classical):}\quad & \text{popn} = f(\text{N0, K, r.max, time}) \\
\text{modified Gompertz:}\quad & \text{popn} = f(\text{N0, K, r.max, time, t.lag}) \\
\text{Baranyi:}\quad & \text{popn} = f(\text{N0, K, r.max, time, t.lag}) \\
\text{Buchanan:}\quad & \text{popn} = f(\text{N0, K, r.max, time, t.lag, gx})
\end{aligned}
$$

All test starting values were than sampled from normal distribution with mean as the estimated value and standard deviation (sd) of 1. The sd value was chosen because of different reasons for each parameters. N0 and K were directly extracted from the raw experimental data, which could be assumed being an accurate estimate for that data subset (hence a small sd was logical). r.max was a guesstimated value from fitting linear models. This process could potentially be affected by extreme values in the data and hence a large sd should be preferred. 100 trials were done as a optimal value under a trade-off between efficiency and accuracy.

Only AIC[5–7] was used to select for optimal parameter values within each phenological model and best model between the six candidates for a data subset. Reasons would be listed in Discussion section. For models with more than one parameter sets as sharing the lowest AIC value, the first set of values from the random sampling trials were used for Kruskal-Wallis analysis. All the available parameter sets were used to principal component analysis (PCA). AIC tolerance threshold was expanded to min(AIC)+2[8] to incorporate more accepted models for analyses.

Statistical analysis

Kruskal test was used for identify the best-fit model among all included model because the count was categorical and not assumed being normally-distributed. Pairwise Nemenyi comparisons would be carried out to identify the best test if p-value of the above test was significant.

Using PCA, parameter weights could be observed across phenological models. All parameter values met the "minimal AIC +2"[8] criteria were extracted. The t.lag values for datasets calling Verhulst (classical) as "best-fit" were set zero (as this model do not need this parameter). With datasets as rows, R-way analysis was done after all parameter data was natural-logged. Phenological models would be positively-correlating with a parameter if dataset observations were concentrated towards the positive side of the factor and vice versa. It was expected that datasets would cluster together (or being on similar positions) if parameter(s) were representing the observed data.

After that, all data used in the PCA analysis were analysed by individual factor (i.e. N0, K, r.max and t.lag). This was done by Kruskal-Wallis test. The data would be also analysed using post-hoc Tukey pairwise comparison if the Kruskal test showed significance.

Main Assumptions

- there was no negative population growth (i.e. starting population was always lower than carrying capacity), so negative population growth data were set to zeros;
- estimated parameter estimates would always result in a global optimal status in parameter space through the NLLS method.

Computing tools

R (ver 3.6.0)[9] was used with "minpack.lm"[10] for computing non-linear least square statistics for model comparisons. "stats"[9] was used for Kruskal test and PCA analysis. "PMCMR"[11] was used for carrying out Nemenyi post-hoc pairwise comparisons for Kruskal test when needed.

Results

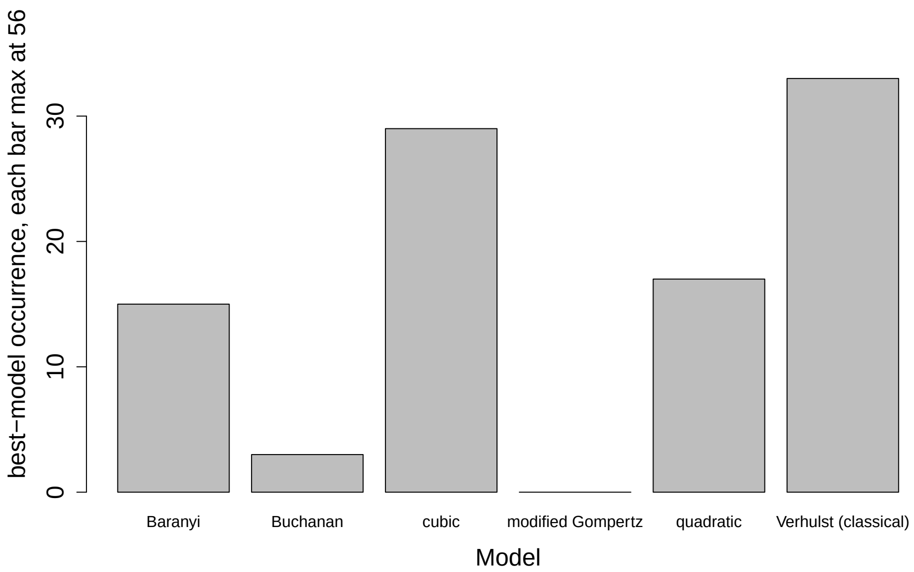

Figure 1: Barplot showing the number of "best model" identification under AIC model-selection methods with " Kruskal-Wallis rank sum test " statistic $X^2 = 5$, df = 5 , p = 0.42

From Fig.1, large fluctuations between each model to be described as "best-fit" were observed. However the occurrence difference was not statistical significant. Among the counts, there were 41 datasets with more than one "best-fit" models. Verhulst (classical) and cubic were the top two models selected as "best-fit" for the 56 datasets (33 for Verhulst (classical) and 29 for cubic). There are 12 datasets calling both "best-fit" at the same trial. Between Baranyi and quadratic, the counts were 15 and 17 respectively with 6 datasets calling both models "best-fit". The only outstanding performance was from modified Gompertz, which 0 datasets were called it as "best-fit".

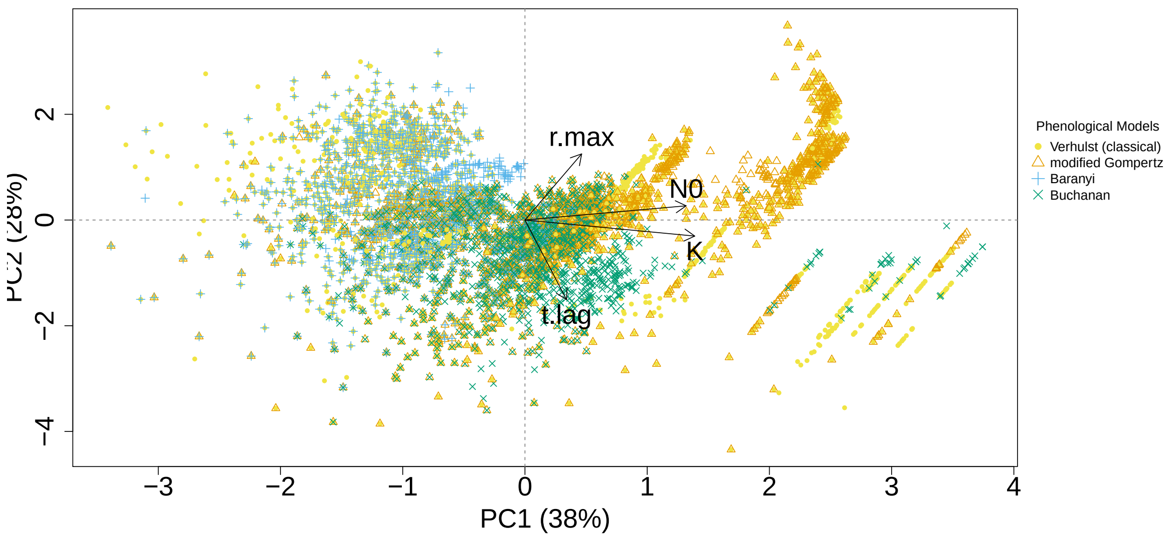

Figure 2: Biplot of Principal Component Analysis (PCA) comparing phenological models using estimated parameter values with "minimal AIC +2"[8] evaluations.

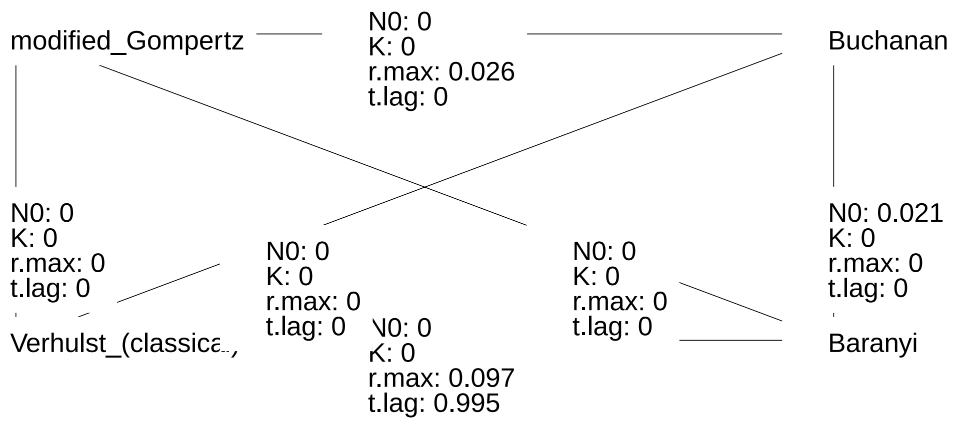

Figure 3: P-value summary between models on the four parameters under post-hoc Tukey-Dist pairwise comparison from Kruskal-Wallis Test. Kruskal tests for all four factors were significant (N0 : X^2 = 409 , df = 3 , p-value = 0 ; K : X^2 = 1568.91 , df = 3 , p-value = 0 ; r.max : X^2 = 131.62 , df = 3 , p-value = 0 ; t.lag : X^2 = 1431.68 , df = 3 , p-value = 0).

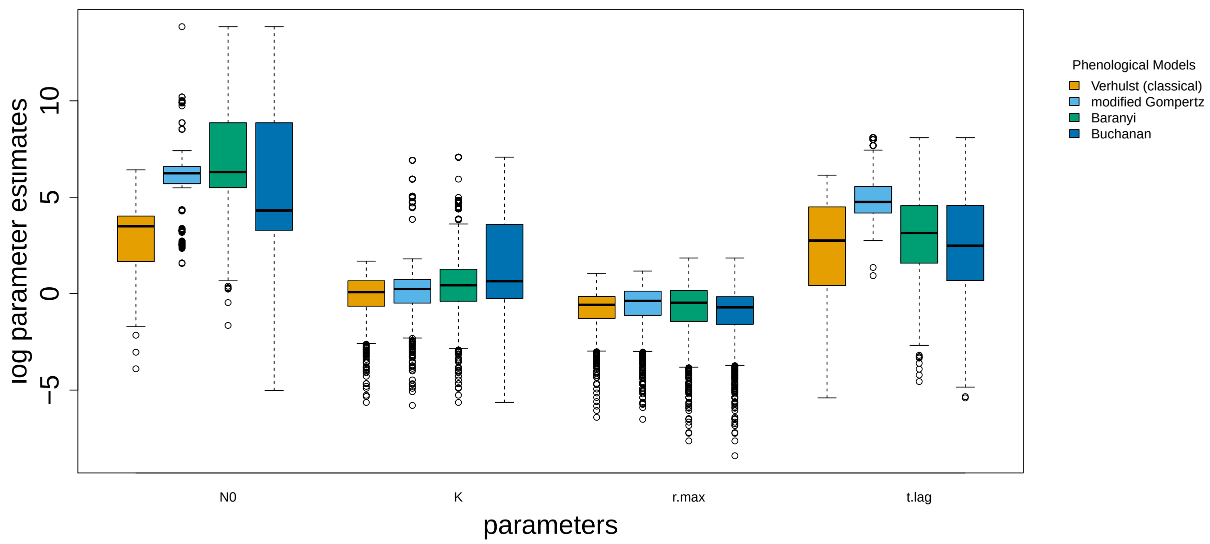

Figure 4: Boxplot of log parameter values grouped by phenological models. Statistical results were summarized in Fig.3

In Fig.2, principal component 1 (PC1) was capturing 38 % variability. It was composed approximately by 0.66 N0, 0.69 K, 0.23 r.max and 0.17 t.lag. PC2 was capturing 28 % variability.

It was composed approximately by 0.13 N0, -0.15 K, 0.63 r.max and -0.75 t.lag.

There were 51 datasets with phenological models fitting, although they may not be the "best-fit" ones. Datasets 23, 27, 36, 52, 53 were strictly limited to polynomial-fitting (Fig.5). Among the phenological modelsfitting datasets, Verhulst (classical) was the umbrella function, which can be fitted to most phenological model-friendly datasets.

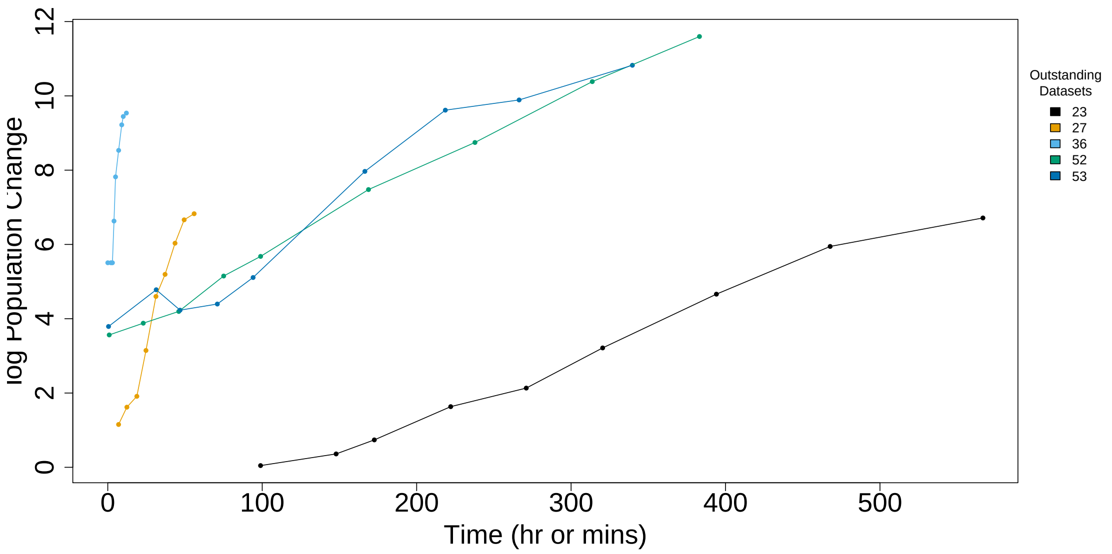

Figure 5: Line plot of datasets restricted to polynomial fits.

From Fig.2, Verhulst (classical) was having the widest coverage across parameter space. However it did not have specific inclination towards any factors. All other three models (modified Gompertz, Baranyi and Buchanan) were generally modelling subsets of the Verhulst (classical) coverage. modified Gompertz was able to model most of the data covered by the Verhulst (classical). Yet a larger successful trials were towards positive responses for N0, K and r.max. Baranyi was a more specific model better in describing datasets with negative responses towards all parameter factors except r.max. Buchanan was having similar specificity comparing with Baranyi. Datasets describable by this model were generally neutral responses towards all four parameters. No models were found well-describing datasets positively correlating with t.lag while negative correlating with r.max.

Discussion

AIC is considered the most suitable model-selection approach within model and between models in this project. Unlike BIC, AIC are more accurate with small sample sizes[12,13] and sparse data[13]. AIC did not assume a "true model" was under examination[14–16]. Since candidate models were not "nested model", BIC is not a better choice than AIC[17]. Hence the use of only AIC as model-selection criterion should be justified.

Although Baranyi and Buchanan were observed occupying different parameter space (Fig.2), these differences were not statistical significant (Fig.1).

Conclusion

Published phenological models were data-specific, which none of them were found significantly performing better than the others in general. Parameters defined by these phenological models were appropriate, which none of them were having observable domination nor negligible weights on the function calculations. There were assumptions embedded within the phenological models which have limited its ability to describe data without a distinct sigmoid shape.

Code and Data Availability

All scripts and data used for this report were publicity available at GitHub.

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Appendix

Table 1: Table showing dataset id details for Fig.5

id	Temp (^{o}C)	clade	substrate	replicate	Source	Pop unit
23	2	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
27	20	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
36	10	Spoilage	C02 Beef Striploins	1	4	N
52	4	Weissella viridescens	MRS broth	1	7	N
53	4	Lactobacillus sakei	MRS broth	1	7	N

"Source" column publication key:

1 Bae, Y.M., Zheng, L., Hyun, J.E., Jung, K.S., Heu, S. and Lee, S.Y., 2014. Growth characteristics and biofilm formation of various spoilage bacteria isolated from fresh produce. Journal of food science, 79(10), pp.M2072-M2080.

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