

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 can be data variabilities, confounding factors, inaccurate assumptions or models being too-specific. This data-mining project is aimed at comparing and contrasting published phenological models on microbial population size data, highlighting which is a better model under what conditions. The hypotheses are:

- published phenological models are significantly better fits than polynomials for describing microbial growths;
- appropriate phenological model(s) is/are identifiable through distinguishable microbial population size parameter values; and
- parameter values of each phenological model from successful fits are clusters with well-defined boundaries between models.

Methods

Microbial population sizes data were given, sourced from ten different publications (Table1). The collection contained different microbial clades growing at various conditions for varying times. Also, these data were recorded in multiple time and population units. Some of the population data were direct counts while some were not.

Experimental microbial population growth data library were divided into individual data subsets through six filters (“Temperature (in °C)”, “Microbial clade”, “growth substrate mate-

rials”, “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) method was used only on the four phenological models and linear approach was taken 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) and relative time lag (t.lag) were obtained through recursive linear modelling with shrinking independent range (from maximum and minimum). In this project 5% was chosen as the shrinking threshold assuming this resolution was sufficient for initiating NLLS fits. The final slope value (i.e. r.max) would be a positive finite number with the highest R² value (i.e. best-fit slope on considered data). The x-intercept for that slope was the t.lag. Time which this r.max 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: $g1 \leq t.lag < g2 < t.K \leq g3$. Inputs for phenological models were listed below (popn & time were the dependent and independent variables respectively):

Verhulst (classical): $popn = f(N0, K, r.max, time)$

modified Gompertz: $popn = f(N0, K, r.max, time, t.lag)$

Baranyi: $popn = f(N0, K, r.max, time, t.lag)$

Buchanan: $popn = f(N0, K, r.max, time, t.lag, gx)$

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. Hence a small sd was precise assuming this extraction was an accurate estimate. r.max was a guesstimated value. So a large sd would be sufficient for getting the “true” value with fair accuracy. 100 trials were done as a optimal value under a trade-off between efficiency and accuracy.

AIC⁵⁻⁷ was used to select optimal parameter values (i.e. meeting the “minimal AIC +2”⁸ criteria) within each phenological model and best candidate model(s) for a data subset. All available parameter sets were included in principal component analysis (PCA). AIC tolerance threshold was expanded to $\min(\text{AIC})+2^8$ 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 test was significant.

Parameter weights were assessed across phenological models by PCA R-way analysis method on natural-logged parameter data. Datasets would be expected clustering together if parameter(s) were representing the observed data. Then with Kruskal-Wallis test, each parameter was tested for statistical differences across phenological models. Post-hoc Tukey pairwise comparisons would be carried out upon significance.

Main Assumptions

- there was no negative population growth throughout experiments from source publications. Data not fitted this assumption were set to zeros;
- all parameter estimates converged to global optimal using NLLS method.

Computing tools

R (ver 3.6.0)⁹ was used with packages “minpack.lm”¹⁰ (NLLS), “stats”⁹ (Kruskal test and PCA) and “PMCMR”¹¹ (post-hoc Nemenyi pairwise comparisons). Python (ver 3.7.3)¹² was used with package “subprocess”¹² (streamline project workflow).

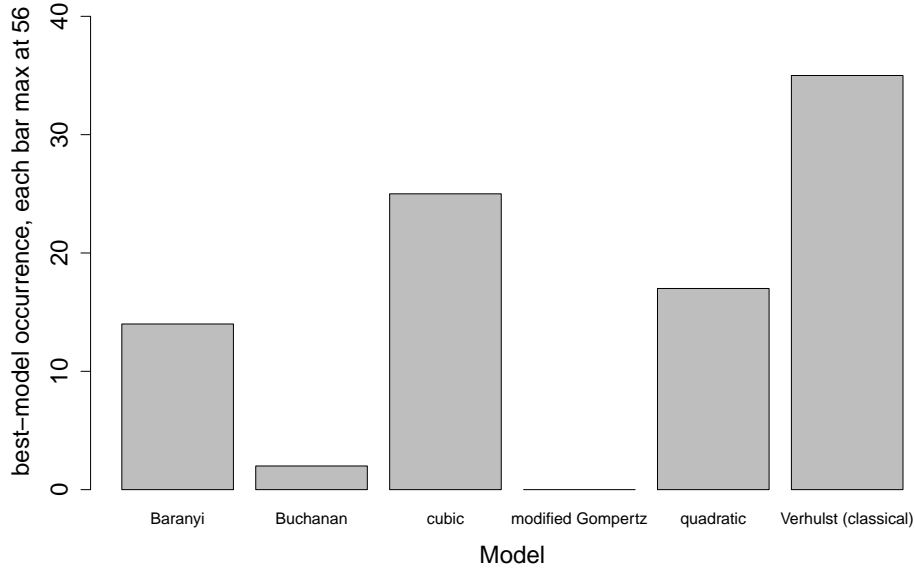


Figure 1: Barplot showing the number of “best model” identification under AIC model-selection methods with “ Kruskal-Wallis rank sum test ” statistic $X^2 = 5$, $df = 5$, $p = 0.42$

84 From Fig.1, large fluctuations between each model to be described as “best-fit” were observed.
 85 However the occurrence difference was not statistical significant. Among the counts, there were
 86 37 datasets with more than one “best-fit” models. Verhulst (classical) and cubic were the top
 87 two models selected as “best-fit” for the 56 datasets (35 for Verhulst (classical) and 25 for
 88 cubic). There are 9 datasets calling both “best-fit” at the same trial. Between Baranyi and
 89 quadratic, the counts were 14 and 17 respectively with 5 datasets calling both models “best-fit”.
 90 The only outstanding performance was from modified Gompertz, which 0 datasets were called
 91 it as “best-fit”.

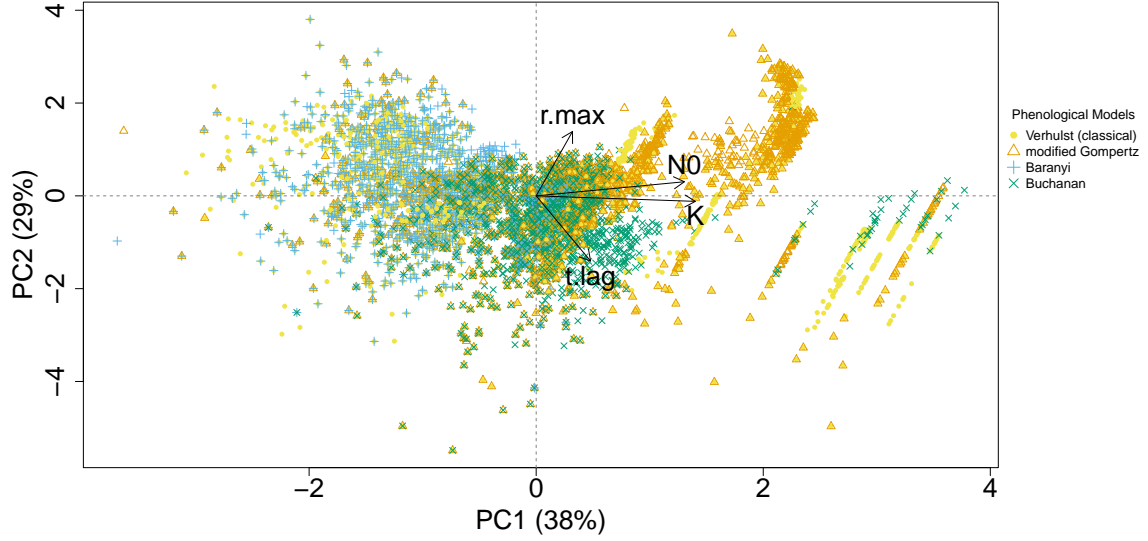


Figure 2: Biplot of Principal Component Analysis (PCA) comparing phenological models using estimated parameter values with “minimal AIC +2”⁸ evaluations.

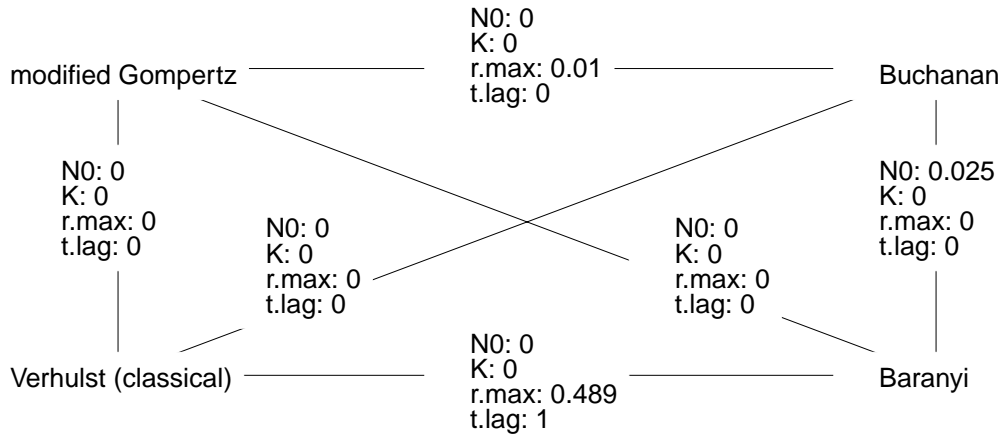


Figure 3: P-value summary between models on the four parameters under post-hoc Tukey-Dist pairwise comparison from Kruskal-Wallis Test. Kruskal tests for all four factors were significant ($N0 : X^2 = 427.58$, $df = 3$, $p\text{-value} = 0$; $K : X^2 = 1545.51$, $df = 3$, $p\text{-value} = 0$; $r.\text{max} : X^2 = 133.58$, $df = 3$, $p\text{-value} = 0$; $t.\text{lag} : X^2 = 1378.71$, $df = 3$, $p\text{-value} = 0$).

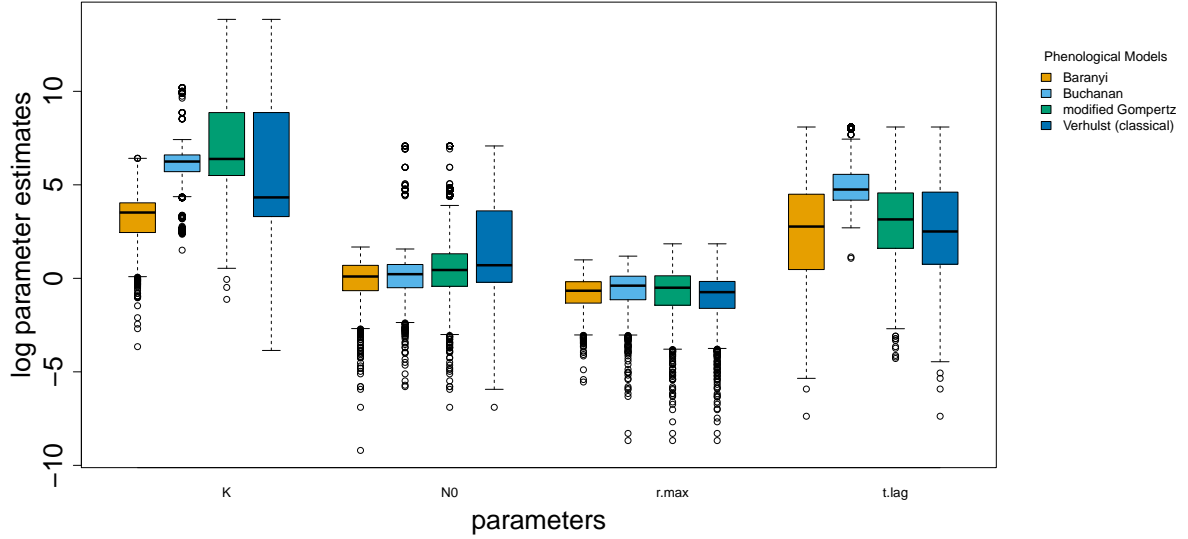


Figure 4: Boxplot of log parameter values grouped by phenological models. Statistical results were summarized in Fig.3

In Fig.2, principal component 1 (PC1) was capturing 38 % variability. It was composed approximately by 0.65 N0, 0.7 K, 0.16 r.max and 0.24 t.lag. PC2 was capturing 29 % variability. It was composed approximately by 0.15 N0, -0.06 K, 0.69 r.max and -0.7 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). Verhulst (classical) was having the widest neutral coverage across parameter space (Fig.2,4). All other three models (modified Gompertz, Baranyi and Buchanan) were generally modelling within the Verhulst (classical) coverage (Fig.4). modified Gompertz was the second widest coverage model but Verhulst (classical) was evaluated better if both equations fitted the same dataset (Fig.1). More successful trials were towards positive responses for N0, K and r.max. Baranyi was a specific model more specified in describing datasets with negative responses towards most parameter factors (all except r.max). Baranyi had the strictest r.max acceptance for successful NLLS modelling (Fig.4). Buchanan had the narrowest parameter ranges in most parameters (all except r.max, Fig.4). Datasets describable by this model were generally neutral responses towards all four parameters (Fig.2). In the analysis for individual parameters, the parameter value ranges overlapping between phenological models (Fig.4). Hence the differences were not observable although most “differences” were statistically significant (Fig.3).

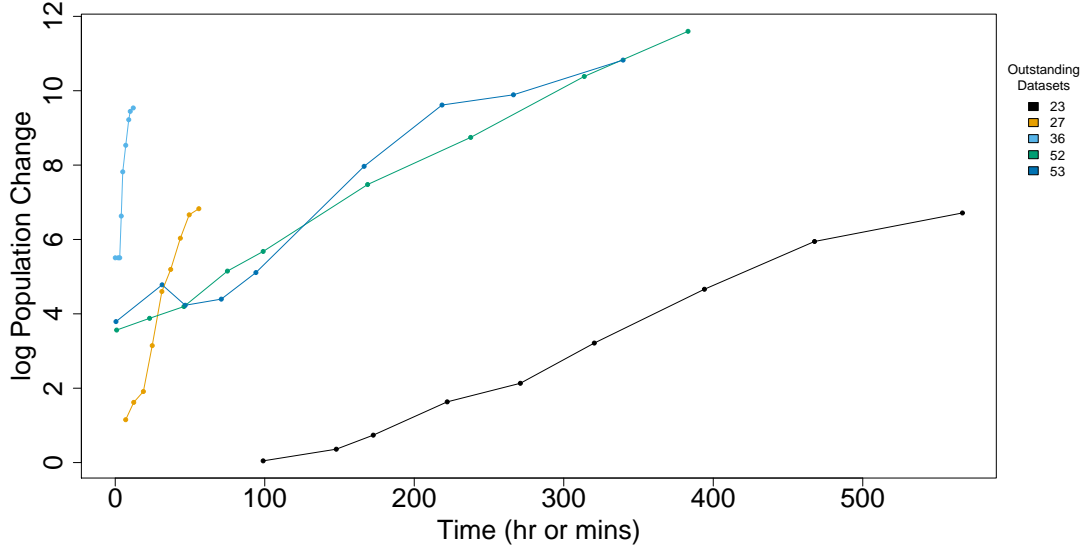


Figure 5: Line plot of datasets restricted to polynomial fits. Dataset details could be found in Table 1

Discussion

AIC is the most suitable approach (compare with BIC and R^2) for model evaluation within and between models in this project because AIC 1. is accurate with small sample size^{13,14} and sparse data¹⁴; 2. does not assume a “true model” was under examination^{15–17}; and 3. take number of parameters into evaluation consideration⁵. Also none of the phenological models used are “nested”¹⁸, leaving AIC as the only appropriate model-selection method.

Phenological models were not always better than polynomials. Since experimental data is highly variable, some may not fit assumptions of these models (Fig.5). Even among the fitted ones, phenological models are not statistically better than polynomials (Fig.1).

Between phenological models, some of them may be highly specific (e.g. Baranyi and Buchanan) or general (e.g. Verhulst (classical) and modified Gompertz). Although PCA result shows significant separation of model properties (Fig.2,4), these differences are not observable in the data (Fig.4). The clustering of NLLS parameter estimates is also not having defined boundaries (Fig.2), increasing the difficulty of choosing the most appropriate phenological model for a newly-generated microbial population sizes dataset. Due to the different phenological model properties towards their own parameters (Fig.2), the PCA graph can be a reference for determining the best phenological model on those new data in the future. However Fig.2 can

only be taken in literal sense when the experimental temperature, substrate and population unit of the microbial clades fall within the categories or ranges listed in Table 1. If not, the same methodology can be used with suitable published data to generate the PCA result for reference. Generally speaking, Verhulst (classical) will be sufficient to explain most behaviours of the population. modified Gompertz can be used when authors want to incorporate t.lag into description, although this factor may be redundant. Using Fig.2 as reference, one can pinpoint the data response to the parameters and refine their model-of-choice to a more specific one if necessary.

Conclusion

Published phenological models were data-specific, which none of them were found significantly performing better than the others. Although most of the parameter values are significantly different between models, their ranges are superimposing with one another. Phenological models correlate with parameters differently, but the correlations are unobservable through plotting a log-linear logistic growth curve. 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 publicly available at GitHub.

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190 university press, 2003).

191 Appendix

Table 1: Table showing dataset id details in this project.

id	T°C	clade	substrate	replicate	Source	Pop unit
1	5	Chryseobacterium balustinum	TSB	1	1	OD_595
2	5	Enterobacter sp	TSB	1	1	OD_595
3	5	Pantoea agglomerans	TSB	1	1	OD_595
4	5	Bacillus pumilus	TSB	1	1	OD_595
5	5	Clavibacter michiganensis	TSB	1	1	OD_595
6	5	Pseudomonas fluorescens	TSB	1	1	OD_595
7	5	Acinetobacter clacoaceticus	TSB	1	1	OD_595
8	5	Stenotrophomonas maltophilia	TSB	1	1	OD_595
9	5	Klebsiella pneumonia	TSB	1	1	OD_595
10	5	Dickeya zeae	TSB	1	1	OD_595
11	5	Pectobacterium carotovorum	TSB	1	1	OD_595
12	15	Chryseobacterium balustinum	TSB	1	1	OD_595
13	25	Chryseobacterium balustinum	TSB	1	1	OD_595
14	35	Chryseobacterium balustinum	TSB	1	1	OD_595
15	5	Tetraselmis tetrahele	ESAW	1	2	N
16	5	Tetraselmis tetrahele	ESAW	2	2	N
17	5	Tetraselmis tetrahele	ESAW	3	2	N
18	5	Tetraselmis tetrahele	ESAW	4	2	N
19	5	Tetraselmis tetrahele	ESAW	5	2	N

20	8	Tetraselmis tetrahele	ESAW	1	2	N
21	16	Tetraselmis tetrahele	ESAW	1	2	N
22	32	Tetraselmis tetrahele	ESAW	1	2	N
23	2	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
24	4	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
25	7	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
26	10	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
27	20	Staphylococcus sp	Raw Chicken Breast	1	3	CFU
28	2	Staphylococcus sp	Salted Chicken Breast	1	3	CFU
29	2	Staphylococcus sp	Cooked Chicken Breast	1	3	CFU
30	2	Pseudomonas sp	Raw Chicken Breast	1	3	CFU
31	2	Aerobic Psychotropic	Raw Chicken Breast	1	3	CFU
32	2	Aerobic Mesophilic	Raw Chicken Breast	1	3	CFU
33	8	Spoilage	Vacuum Beef Striploins	1	4	N
34	8	Escherichia coli	Vacuum Beef Striploins	1	4	N
35	8	Salmonella Typhimurium	Vacuum Beef Striploins	1	4	N
36	10	Spoilage	C02 Beef Striploins	1	4	N
37	12	Spoilage	Vacuum Beef Striploins	1	4	N
38	30	Spoilage	Vacuum Beef Striploins	1	4	N
39	6	Serratia marcescens	Pasteurised Skim Milk	1	5	N
40	6	Serratia marcescens	UHT Skim Milk	1	5	N
41	6	Serratia marcescens	Pasteurised Full-fat Milk	1	5	N
42	6	Serratia marcescens	UHT Full-fat Milk	1	5	N
43	6	Serratia marcescens	Pasteurised Double Cream	1	5	N
44	6	Serratia marcescens	UHT Double Cream	1	5	N
45	0	Arthrobacter sp	TGE agar	1	6	CFU
46	37	Arthrobacter sp	TGE agar	1	6	CFU
47	0	Arthrobacter aureus	TGE agar	1	6	CFU
48	0	Arthrobacter citreus	TGE agar	1	6	CFU
49	0	Arthrobacter globiformis	TGE agar	1	6	CFU
50	0	Arthrobacter simplex	TGE agar	1	6	CFU
51	8	Lactobacillus plantarum	MRS broth	1	7	N
52	4	Weissella viridescens	MRS broth	1	7	N
53	4	Lactobacillus sakei	MRS broth	1	7	N
54	15	Oscillatoria agardhii	Z8	1	8	DryWeight
55	15	Pseudomonas sp	APT Broth	1	9	CFU

192 “Source” column publication key:

193

- 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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