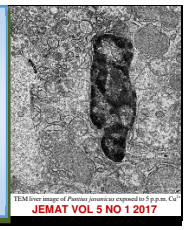
HIBISCUS PUBLISHER

JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEMAT



Comparison Between the Modified Gompertz and Churchill Death Models in Modelling the Growth of Cell Culture from Leaf-Derived Cell Suspension of *Barringtonia racemose*

Hussein, S.[1] and Shukor, M.Y.[2]*

[1]Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia.
[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Mohd Yunus Shukor
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 Serdang,
Selangor,
Malaysia.
Email: mohdyunus@upm.edu.my / yunus.upm@gmail.com

HISTORY

Received: 12th April 2017
Received in revised form: 27th of May 2017
Accepted: 19th of June 2017

KEYWORDS

Barringtonia racemose
cell suspension
growth curve
Churchill
modified Gompertz

ABSTRACT

The growth of plant cell suspension culture often is observed to show a declining phase. Commonly used growth models such as can be used to model sigmoidal growth curves but do not fit curves showing a declining or death phase. In this study, the growth curves of *Barringtonia racemose* cell suspension under light and dark conditions were modelled according to the Churchill model, which incorporates growth and decline phases, and was compared to the popular modified Gompertz model. For both growth conditions, the Churchill model gave better results for the error functions Root Mean Square Error, the coefficient of determination, adjusted coefficient of determination, bias factor and accuracy factor with the exception of the corrected Akaike Information Criterion (AICc). The regressed parameters or constants obtained from the Churchill model shows growth and decline rates with higher values for the growth rate compared to the decline rates for both growth conditions. The decline and growth rate parameters signify as λ_1 and λ_2 for growth under light conditions were 0.367 (95% confidence; 0.103 to 0.632) and 0.796 (95% confidence; 0.458 to 1.134), respectively, while the decline and growth rate parameters λ_1 and λ_2 for growth under dark conditions were 0.158 (95% confidence; 0.314 to 0.629) and 1.491 (95% confidence; -0.809 to 3.720), respectively. The 95% confidence interval values were overlapped for both growth rate parameters under light, and dark conditions are indicating that the differences observed were not significant.

INTRODUCTION

The tropical region is rich in plants having medicinal properties [1–4]. In Malaysia, the shoots of *B. racemosa* are commonly found, and its leaves are eaten as a salad. *Barringtonia racemosa* (L.) Spreng leaves are used to reduce high blood pressure. It belongs to the Lecythidaceae family. The roots, leaves, and barks can be pounded as a remedy for itchiness as a result of chicken pox [5–8]. The plant has high phenolic, and antioxidant content with the majority of the active compounds composition include compounds such as steroids, saponins, diterpenes and triterpenoids [8,9]. The leaves of this plant contained compounds such as ferulic acid, naringin, gallic acid, luteolin, rutin, kaempferol, ellagic acid, protocatechuic acid, and quercetin [6]. One of the methods to engineered active compounds enhancement by this plant is through metabolic engineering of the callus culture.

Many callus growth curves including *B. racemosa* exhibited death phases [10–14] of which normal growth models such the sigmoidal curve can be fitted by various mathematical functions such as Richards, logistic, Schnute, Gompertz, [15], Buchanan three-phase [16] Baranyi-Roberts [17],von Bertalanffy [18,19] and more recently the Huang model [20] will have problem to model them. A one-step growth and decline model is available in predictive microbiology and has shown great promise in further secondary modelling such as to study the effect of environmental parameters on growth rate. Such an approach is not available for

plant callus or cell suspension growth. This paper aims to do this by comparing the applicability of the Churchill death model in comparison to the modified Gompertz model often used in modelling callus and cell suspension growth of *B. racemosa* [21–23].

MATERIALS AND METHODS

Data acquisition

Graphical data of published work [24] from Figure 3a were electronically processed using WebPlotDigitizer 2.5 [25] which helps to digitize scanned plots into a table of data with good precision and reliability [26,27].

Mathematical modelling

The modified Gompertz model [15] (**Eqn. 1**) is as follows';

$$y=A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda-t)+1\right]\right\} \qquad \textbf{(Eqn. 1)}$$

where A=growth at lower asymptote; μ_m= maximum specific growth rate, λ=lag time, e = exponent (2.718281828) and t = sampling time.

The death model of Churchill [28] (**Eqn.** 2) is as follows;

$$\hat{Y} = \left[\frac{1}{K_1}.exp(\lambda_1.t) + \frac{1}{K_2}.exp(\lambda_2.t)\right]^{-1} \qquad \textbf{(Eqn. 2)}$$

Since log N=log N_0 at time t=0, there occurs a relationship between K_1 and K_2 as follows (**Eqn. 3**);

$$\frac{1}{K_1} = (\log N_0)^{-1} - \frac{1}{K_2} \qquad \textbf{(Eqn. 3)}$$

Statistical analysis

Statistical discriminatory methods utilized in this work takes into account the penalty for the number of parameters and include corrected AICc (Akaike Information Criterion), Root-Mean-Square-Error (RMSE), bias factor (BF), accuracy factor (AF) and adjusted coefficient of determination (R^2) [29].

The RMSE was calculated according to **Eqn. 4** [30], and smaller number of parameters is expected to give a smaller RMSE value. n is the number of experimental data, Ob_i and Pd_i are the experimental and predicted data while p is the number of parameters.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Pd_i - Ob_i)^2}{n-p}} \qquad \textbf{(Eqn. 4)}$$

A modified form of the coefficient of determination, $adjR^2$, which is an adjusted coefficient of determination was utilized to indicate the closeness of data to the experimental model. The formulas are as follows (**Eqns 5 and 6**), where the Residual Mean Square is RMS and s^2_y is the total variance of the y-variable [30].

$$Adjusted\left(R^2\right) = 1 - \frac{RMS}{s_Y^2} \qquad \textbf{(Eqn. 5)}$$

$$Adjusted\left(R^2\right) = 1 - \frac{\left(1-R^2\right)(n-1)}{(n-p-1)} \qquad \textbf{(Eqn. 6)}$$

The Akaike Information Criterion (AIC) evaluates the trade-off between the complexity of a model and the goodness of fit. It is based on information theory [31]. To handle data with a smaller number of values the Akaike information criterion (AIC) with correction or AICc is employed. It is a corrected version of the AIC and also has a penalized factor for models with a larger number of parameters [32]. The AICc is calculated as follows (**Eqn. 7**);

$$AICc = 2p + n\ln\left(\frac{RSS}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \qquad \textbf{(Eqn. 7)}$$

Accuracy Factor (AF) and Bias Factor (BF) originates from the work of Ross [33] to test for the goodness-of-fit of the models and were calculated (**Eqns. 8 and 9**) as follows;

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{(Pd_i/Ob_i)}{n}\right)} \qquad \textbf{(Eqn. 8)}$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|(Pd_i/Ob_i)|}{n}\right)} \qquad \textbf{(Eqn. 9)}$$

The growth data needs to be first transformed to logarithmic values. The Levenberg–Marquardt algorithm (LMA) nonlinear regression embedded in the CurveExpert Professional software (Version 1.6) was utilized to fit the data. The algorithm minimizes the sum of the squares of the differences between the measured and predicted values [30].

RESULTS AND DISCUSSION

Data from the cell suspension was first transformed in log values (**Fig. 1**). The data appears to show that growth measured as the dry weight of cell culture under light conditions was lower than under dark conditions.

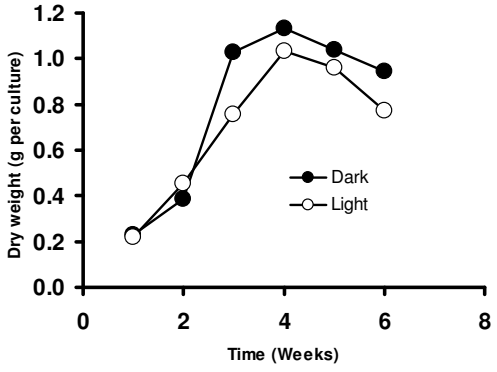


Fig. 1. Profile of growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension *of Barringtonia racemosa* cultured at 25ºC in light and dark conditions. Data on the y-axis represent log (+2).

Both of the growth curves were subjected to the Churchill and modified Gompertz model (**Figs. 2 and 3**). Based on visual observation, the Churchill model gave very good fits to both growth curves under light and dark conditions, whilst the Gompertz model gave poor fitting. Very few calli and cell suspension profile have been modelled using the modified Gompertz model [21,22,34] while another study claims that the modified Gompertz model is not adequate to model callus growth in some situation [35].

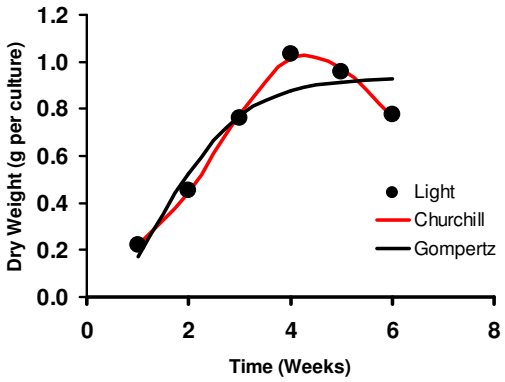


Fig. 2. The growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25ºC under light conditions modelled according to the modified Gompertz and Churchill models.

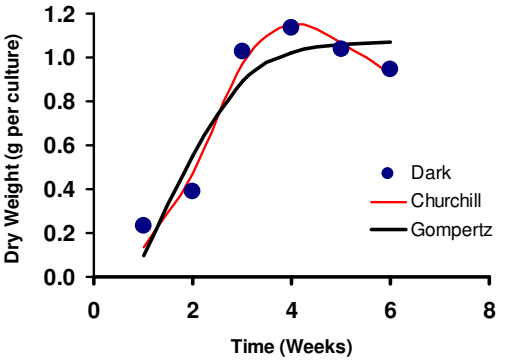


Fig. 3. The growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25 ºC under dark conditions modelled according to the modified Gompertz and Churchill models.

For both conditions, the Churchill model gave better results for the error functions Root Mean Square Error, coefficient of determination, adjusted coefficient of determination, bias factor and accuracy factor with the exception of the corrected Akaike Information Criterion (AICc), where the latter values were better for the modified Gompertz models under both light (**Table 1**) and dark (**Table 2**) conditions. Based on the majority of the statistical tests or error functions, the Churchill model should be selected as the best model based on the visual and statistical analysis. The AICc test does not work well with very few sample points [36,37], and future studies should be carried out with a large number of sample points.

Table 1. Error functions for the Churchill and modified Gompertz models utilized for the regression of the growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25ºC under light conditions.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Churchill	4	0.02	1.00	1.00	1.01	1.00	16.48
modified Gompertz	3	0.12	0.91	0.82	1.13	0.98	0.53

Note:
p no of parameters
RMSE Root Mean Square Error
R^2 Coefficient of determination
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor

Table 2. Error functions for the Churchill and modified Gompertz models utilized for the regression of the growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25ºC under dark conditions.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Churchill	4	0.08	0.98	0.94	1.13	0.95	36.91
modified Gompertz	3	0.15	0.91	0.82	1.26	0.91	3.68

Note:
p no of parameters
RMSE Root Mean Square Error
R^2 Coefficient of determination
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor

The regressed parameters or constants obtained from the Churchill model shows growth and decline rates with higher values for the growth rate compared to the decline rates (**Tables 3 and 4**) for both growth conditions. The decline and growth rate parameters signify as λ_1 and λ_2 for growth under light conditions were 0.367 (95% confidence; 0.103 to 0.632) and 0.796 (95% confidence; 0.458 to 1.134), respectively, while the decline and growth rate parameters λ_1 and λ_2 for growth under dark conditions were 0.158 (95% confidence; 0.314 to 0.629) and 1.491 (95% confidence; -0.809 to 3.720), respectively.

The 95% confidence interval values were overlapped for both growth rate parameters under light, and dark conditions are indicating that the differences observed were not significant despite the visual observation which appears to show that there is a difference. The statistical analysis indicates that more sample data are needed for the confidence interval to be minimized in order for the two curves to be significantly different if it is indeed they are

Table 3. Churchill regressed values for growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25ºC under light conditions.

Parameter	Value	Std Err	Range (95% confidence)
K_1	7.413	2.761	-4.469 to 19.296
λ_1	0.367	0.061	0.103 to 0.632
K_2	0.104	0.015	0.039 to 0.168
λ_2	0.796	0.078	0.458 to 1.134

Table 4. Churchill regressed values for growth of *Barringtonia racemosa* cell suspension in leaf derived cell suspension of *Barringtonia racemosa* cultured at 25ºC under dark conditions.

Parameter	Value	Std Err	Range (95% confidence)
K_1	2.384	1.418	-3.718 to 8.486
λ_1	0.158	0.110	-0.314 to 0.629
K_2	0.032	0.034	-0.114 to 0.179
λ_2	1.491	0.535	-0.809 to 3.720

CONCLUSION

The growth of cell suspension under light and dark condition was successfully modelled using primary growth models Churchill and the modified Gompertz with the Churchill model coming out as the better model based on the overwhelming majority of the statistical test. An important result obtained is that the 95% confidence interval for the two growth rates were wide and overlapped indicating that the growth rates under light and dark conditions were not significantly different from one another. More data points are needed.

REFERENCES

1. Mahyuni S, Radzali M, Marziah M, Johari R, Mohd Aspollah S. Studies on the production of flavonoids (quercetin and hesperitin) from callus culture of *Citrus aurantifolia* (Christm and Panzer) swingle. In: Proceedings of the Seminar Medicinal Plants: Quality Herbal Products for Healthy Living. Forest Research Institute Malaysia (FRIM); 2000.
2. Wazir D, Ahmad S, Muse R, Mahmood M, Shukor M. Antioxidant activities of different parts of *Gnetum gnemon* L. J Plant Biochem Biotechnol. 2011;20(2):234–240.
3. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. BioMed Res Int. 2014;Article ID 480258:11 pages.
4. Salvamani S, Gunasekaran B, Shukor MY, Shaharuddin NA, Sabullah MK, Ahmad SA. Anti-HMG-CoA Reductase, Antioxidant, and Anti-Inflammatory Activities of *Amaranthus viridis* Leaf Extract as a Potential Treatment for Hypercholesterolemia. Evid Based Complement Alternat Med. 2016;2016:1–10.
5. Rahman MM, Polfreman D, MacGeachan J, Gray AI. Antimicrobial activities of *Barringtonia acutangula*. Phytother Res. 2005;19(6):543–545.
6. Hussin NM, Muse R, Ahmad S, Ramli J, Mahmood M, Sulaiman MR, et al. Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L.(Lecythidaceae). Afr J Biotechnol. 2009;8(12).
7. Mmushi T, Masoko P, Mde L, Mokgotho M, Mampuru LJ, Howard R. Antimycobacterial evaluation of fifteen medicinal plants in South Africa. Afr J Tradit Complement Altern Med. 2010;7(1).
8. Kong KW, Mat-Junit S, Ismail A, Aminudin N, Abdul-Aziz A. Polyphenols in *Barringtonia racemosa* and their protection against oxidation of LDL, serum and haemoglobin. Food Chem. 2014 Mar 1;146:85–93.
9. Kong KW, Mat-Junit S, Aminudin N, Ismail A, Abdul-Aziz A. Antioxidant activities and polyphenolics from the shoots of *Barringtonia racemosa* (L.) Spreng in a polar to apolar medium system. Food Chem. 2012 Sep 1;134(1):324–32.
10. Miachir JI, Romani VLM, Amaral AF de C, Mello MO, Crocomo OJ, Melo M. Micropropagation and callogenesis of *Curcuma zedoaria* Roscoe. Sci Agric. 2004;61(4):427–32.
11. Al-Khayri JM. Determination of the date palm cell suspension growth curve, optimum plating efficiency, and influence of liquid medium on somatic embryogenesis. Emir J Food Agric. 2012;24(5):444–55.
12. Hussein S, Ling APK, Ng TH, Ibrahim R, Paek KY. Adventitious roots induction of recalcitrant tropical woody plant, *Eurycoma longifolia*. Romanian Biotechnol Lett. 2012;17(1):7026–35.
13. Huang B, Han L, Li S, Yan C. Optimization of induction, subculture conditions, and growth kinetics of *Angelica sinensis* (Oliv.) Diels callus. Pharmacogn Mag. 2015 Jul 1;11(43):574.
14. Santos MRA dos, Souza CA de, Paz ES, Santos MRA dos, Souza CA de, Paz ES. Growth pattern of friable calluses from leaves of *Capsicum annuum* var. annuum cv. Iberaba Jalapeño. Rev Ciênc Agronômica. 2017 Jul;48(3):523–30.
15. Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990;56(6):1875–81.
16. Buchanan RL, Whiting RC, Damert WC. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 1997;14(4):313–26.
17. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23(3–4):277–94.
18. Cloern JE, Nichols FH. A von Bertalanffy growth model with a seasonally varying coefficient. J Fish Res Board Can. 1978;35(11):1479–82.
19. Darmani Kuhi H, Kebreab E, Lopez S, France J. A derivation and evaluation of the von Bertalanffy equation for describing growth in broilers over time. J Anim Feed Sci. 2002;11(1):109–25.
20. Huang L. Growth kinetics of *Escherichia coli* O157:H7 in mechanically-tenderized beef. Int J Food Microbiol. 2010;140(1):40–8.
21. Hussein S, Halmi MIE, Kiong ALP. Modelling the growth kinetics of callus cultures from the seedling of *Jatropha curcas* L. according to the modified Gompertz model. J Biochem Microbiol Biotechnol. 2016 Jul 31;4(1):20–3.
22. Hussein S, Halmi MIE, Ling APK. The modified Gompertz model demonstrates a variable growth rate between two *Centella asiatica* phenotypes. J Biochem Microbiol Biotechnol. 2017 Jul 31;5(1):18–20.
23. Kiong ALP. Addendum: Hussein et al. The modified Gompertz model demonstrates a variable growth rate between two *Centella asiatica* phenotypes. Journal of Biochemistry, Microbiology and Biotechnology, 2017, Vol 5, No 1, 18-20. J Biochem Microbiol Biotechnol. 2017 Dec 31;5(2):1–1.
24. Behbahani M, Shanehsazzadeh M, Hessami MJ. Optimization of callus and cell suspension cultures of *Barringtonia racemosa* (Lecythidaceae family) for lycopene production. Sci Agric. 2011;68(1):69–76.
25. Rohatgi,A.WebPlotDigitizer. http://arohatgi.info/WebPlotDigitizer/app/ Accessed June 2 2014.; 2015.
26. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.
27. Drevon D, Fursa SR, Malcolm AL. Intercoder reliability and validity of WebPlotDigitizer in extracting graphed data. Behav Modif. 2017;41(2):323–39.
28. Membré JM, Thurette J, Catteau M. Modelling the growth, survival and death of *Listeria monocytogenes*. J Appl Microbiol. 1997;82(3):345–350.
29. Yakasai MH, Ibrahim KK, Yasid NA, Halmi MIE, Rahman MFA, Shukor MY. Mathematical modelling of molybdenum reduction to mo-blue by a cyanide-degrading bacterium. Bioremediation Sci Technol Res. 2016 Dec 31;4(2):1–5.
30. Motulsky HJ, Ransnas LA. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J Off Publ Fed Am Soc Exp Biol. 1987;1(5):365–74.
31. Akaike H. New look at the statistical model identification. IEEE Trans Autom Control. 1974;AC-19(6):716–23.
32. Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media; 2002. 528 p.
33. Ross T, McMeekin TA. Predictive microbiology. Int J Food Microbiol. 1994;23(3–4):241–64.
34. Shukor MS, Masdor N., Halmi MIE, Ahmad SA, Shukor MY. Modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):20–5.
35. Omar R, Abdullah MA, Hasan MA, Rosfarizan M, Marziah M. Kinetics and modelling of cell growth and substrate uptake in *Centella asiatica* cell culture. Biotechnol Bioprocess Eng. 2006;11(3):223–9.
36. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modeling of the growth kinetics of *Bacillus* sp. on tannery effluent containing chromate. J Environ Bioremediation Toxicol. 2014;2(1):6–10.
37. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Modeling the growth kinetics of *Chlorella vulgaris* cultivated in microfluidic devices. Asian J Plant Biol. 2014;2(1):7–10.