JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1409-1414

Evaluation of Suitability of Primary Growth Models to Fit Bacterial Growth Under Metal Stress Conditions

C. Shiny Matilda and C. Shanthi*

School of Bioscience and Technology, VIT University, Vellore - 632 014, India.

(Received: 11 February 2016; accepted: 19 March 2016)

The goal of this study was to evaluate the suitability of growth models to fit the growth data of metal resistant bacteria. The growth data obtained for chromium resistant isolates namely *Bacillus cereus* VITSH1 and *Alcaligenes faecalis* VITSIM2 cultured on nutrient agar amended with increasing concentrations Cr^{3+} Cu^{2+} and Cd^{2+} were fit into growth models. The traditional methods for enumerating bacteria are not instantaneous, therefore necessitates mathematical models to study the behavior of microbes in a specific experimental condition. The logistic and Gompertz models were applied to obtain the predicted microbial numbers and to evaluate the suitability of the models to study the growth pattern in metal stress conditions. The logistic model fit the experimental data and was found to be superior to the Gompertz model. The results suggested that the logistic model could be successfully applied to study the growth pattern of bacteria in metal stress conditions.

Keywords: *Alcaligenes faecalis*, *Bacillus cereus*, Growth, logistic model.

Study of microbial growth curve is a fundamental aspect of predictive microbiology and is indispensable in diverse fields of biotechnology, genetics and ecology (Kovarove at al., 1998; Malakar *et al*., 2003). Predictive microbiology is a combination of statistical, mathematical and microbiological principles to quantify the behavior of particular microorganism (Schultze, 2006). Microbial response has been expressed in terms of concentration of colony forming units or optical density as an indirect measurement (McMeekin *et al*., 1993). Modeling of bacterial growth kinetics enables one to describe the behavior of a particular microorganism under different environmental conditions and hence appropriate models are needed to extract parameters from such growth curves (Lopez, 2004). Microbial growth curves are characterized by a sigmoidal shape and various mathematical models are proposed to fit the curves (Baranyi *et al*., 1993; Mckellar *et al*., 1997). Among the various models two popular mathematical models reported in literature are the Logistic and Gompertz model (Pearl, 1927; Peleg *et al*., 2011). These models serve as primary models which describe the microbial response over time with a characteristic set of parameter values (Whiting, 1995; McMeekin and Ross, 2002). The inability of the logistic model to generate a sigmoidal curve on a semi logarithmic plot led to the development of a modified logistic model (Fujikawa *et al*., 2011). Gibson *et al* modified the Gompertz model to better fit the bacterial growth (Gibson *et al*., 1988). Some bacteria have developed resistance strategies to cope up with metals which exert toxicity at high concentration. A metal resistant isolate namely *Bacillus cereus* VITSH1 and *Alcaligenes faecalis* VITSIM2 isolated from soil have been employed in the current study to analyze the effect of Cr^{3+}, Cu^{2+} and Cd^{2+} on the growth of the isolates. On comparison of the models prediction and

* To whom all correspondence should be addressed.
Tel.: +91 416 2202549; Fax: +91 416 2243092;
E-mail: cshanthi@vit.ac.in

observations of the experimental data a good compatibility between the model and the measurement is necessary for the model to serve as an explanatory tool explicitly elucidating the behavior of the organisms upon exposure to a particular experimental condition. The present study was intended to investigate the suitability of primary mathematical models to fit the bacterial growth data.

MATERIALS AND METHODS

Nutrient broth and the salts used in the present study namely basic $Cr_2(SO_4)_3$, $CdSO_4.H_2O$ and $CuSO_4.5H_2O$ were purchased from (HiMedia, India).

Determination of growth parameters

The first three phases of the growth curve are described by the growth parameters such as the maximum specific growth rate defined as the tangent in the inflection point µmax, λ, the x-axis intercept of the tangent and the asymptote, maximum value reached. The growth parameters (µmax, λ and A) were determined from the experimental growth data of *B.cereus* VITSH1 and *A.faecalis* VITSIM2 upon exposure to maximum tolerable concentrations of Cr^{3+}, Cu^{2+} and Cd^{2+} in nutrient broth.

Fitting of the Models

Primary model

The mathematically modified forms of the logistic and Gompertz equations (Eq. (1) and Eq. (2)) given by (Zwietering *et al*., 1990) were used to fit the growth curves of metal resistant isolates under metal stress conditions.

$y = A / \{1 + \exp[4\mu m/A(\lambda - t) + 2]\}$ Eq. (1)

$y = A\exp\{- \exp[\mu m .e/A(\lambda - t) + 1]\}$ Eq. (2)

where A- asymptote**,** µm- tangent in the inflection point, λ- x-intercept of the tangent, e =exp(1)

Model Comparison and Evaluation

From the two primary models employed in this study, the one giving the best fit was determined by comparison of Mean square error (MSE), which evaluates the difference between the growth data estimated by the model and measured experimentally. The model with the lower value of MSE was satisfied to describe the data well (Sutherland and Bayliss, 1994).

$$MSE = RSS/n = \frac{\Sigma(\mu observed - \mu predicted)^2}{n}$$

where RSS is the residual sum of squares, n is the number of data points. The goodness of fit was evaluated by chi-square test for 24 degrees of freedom with 99% confidence limits for the primary models and for 3 and 4 degrees of freedom for secondary model.

The bias factor developed as an index of model performance (Ross, 1996) in terms of average deviation between the predicted and observed values was calculated as BF = $\exp[\Sigma \ln(P/O)/n]$ and the average accuracy of the estimates was assessed using the 'accuracy factor' (Baranyi *et al*., 1999) where AF = $\exp[\sqrt{\Sigma}(LnP-LnO)\ 2 / n]$, where 'P' is the predicted, 'O' is the observed values and 'n' is the number of data points.

RESULTS AND DISCUSSION

The growth data obtained under metal stress condition was fit into primary models namely the logistic and the Gompertz model. The logistic model served as a good primary model for the isolates whereas the Gompertz model failed to fit the growth data of both the isolates (data not shown). Graphically the logistic model fit the growth data better (Fig. 1 and 2). The statistical evaluation is given in table 1 and 2.

On plotting the predicted values of the logistic model against time, (Fig. 1) and (Fig. 2) the shape of the curve was steep and the lag phase region was extended. The lag phase as described by Monod is one of the poorly understood growth phases controlled by unknown regulatory mechanisms (Monod, 1949). In order to ascertain the effect of metals on lag phases of both the isolates the growth rates were measured at maximum tolerable concentration of metals. The prolonged lag phase at maximum concentration of metal shows a remarkable increase of delay time which is an indication of the stress on the isolates. On comparison of the models by MSE values, chi square validation, bias factor and accuracy factor assessment (Table 1 and 2) it was found that the logistic model gave the best fit than Gompertz model in all the data sets of both the isolates. The chi-square values of logistic model fit the growth data at 99% confidence limits, whereas the chi

Table 1. MSE, Chi-square values, Bias factor and Accuracy Factor determination from logistic model of *Bacillus cereus* VITSH1

Organism Metal	*Bacillus cereus* VITSH1			
concentration(ppm)	MSE	Chi-Square	BF	AF
Cr^{3+} (1500)	0.34	32.10	0.96	1.26
Cu^{2+} (350)	0.28	7.25	0.99	1.04
Cd^{2+} (80)	0.22	13.60	0.96	1.23

Table 2. MSE, Chi-square values, Bias factor and Accuracy Factor determination from logistic model of *Alcaligenes faecalis* VITSIM2

Organism Metal	*Alcaligenes faecalis* VITSH1			
concentration(ppm)	MSE	Chi-Square	BF	AF
Cr^{3+} (2000)	0.21	5.09	0.99	1.06
Cu^{2+} (300)	0.28	5.52	0.98	1.05
Cd^{2+} (80)	0.8	2.30	0.99	1.02

The chi-square values for 23 degrees of freedom at 99% confidence limits is 44.18

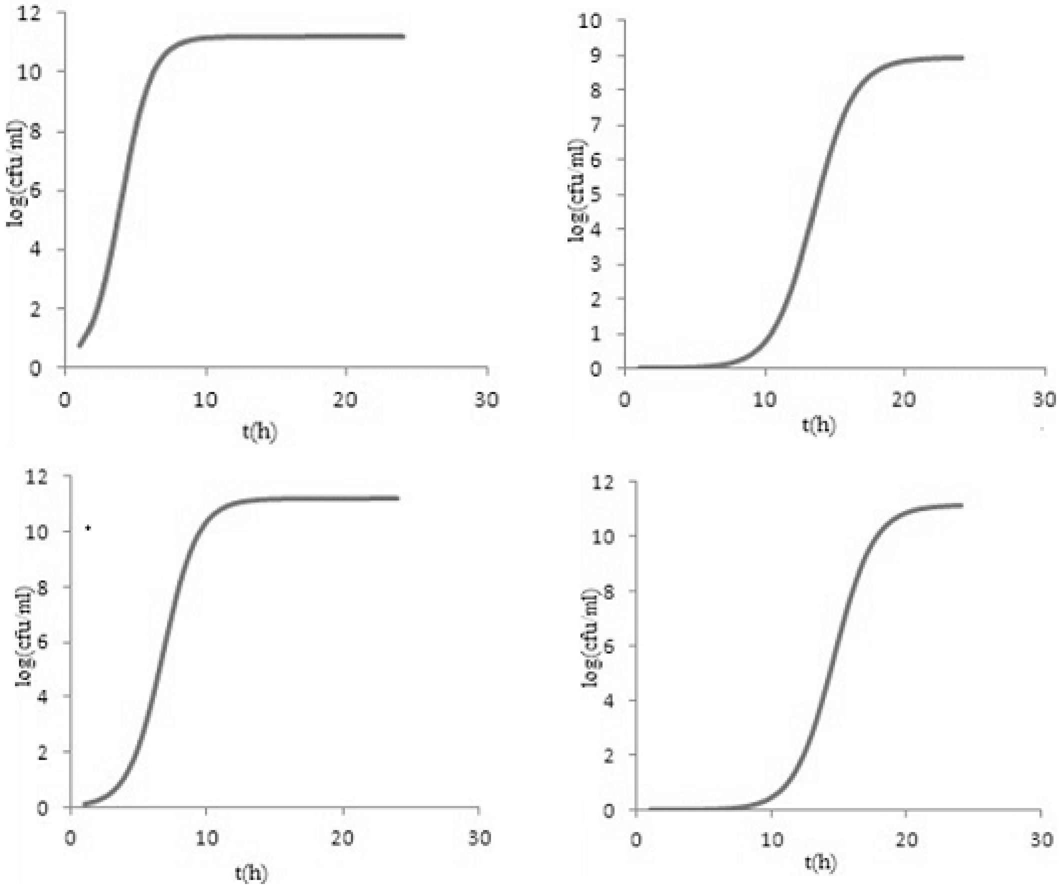

Fig.1. Predicted microbial numbers of Bacillus cereusVITSH1 (a) control (b) Cr^{3+} 2000ppm (c) Cu^{2+} 300ppm (d) Cd^{2+} 60ppm with time from logistic model

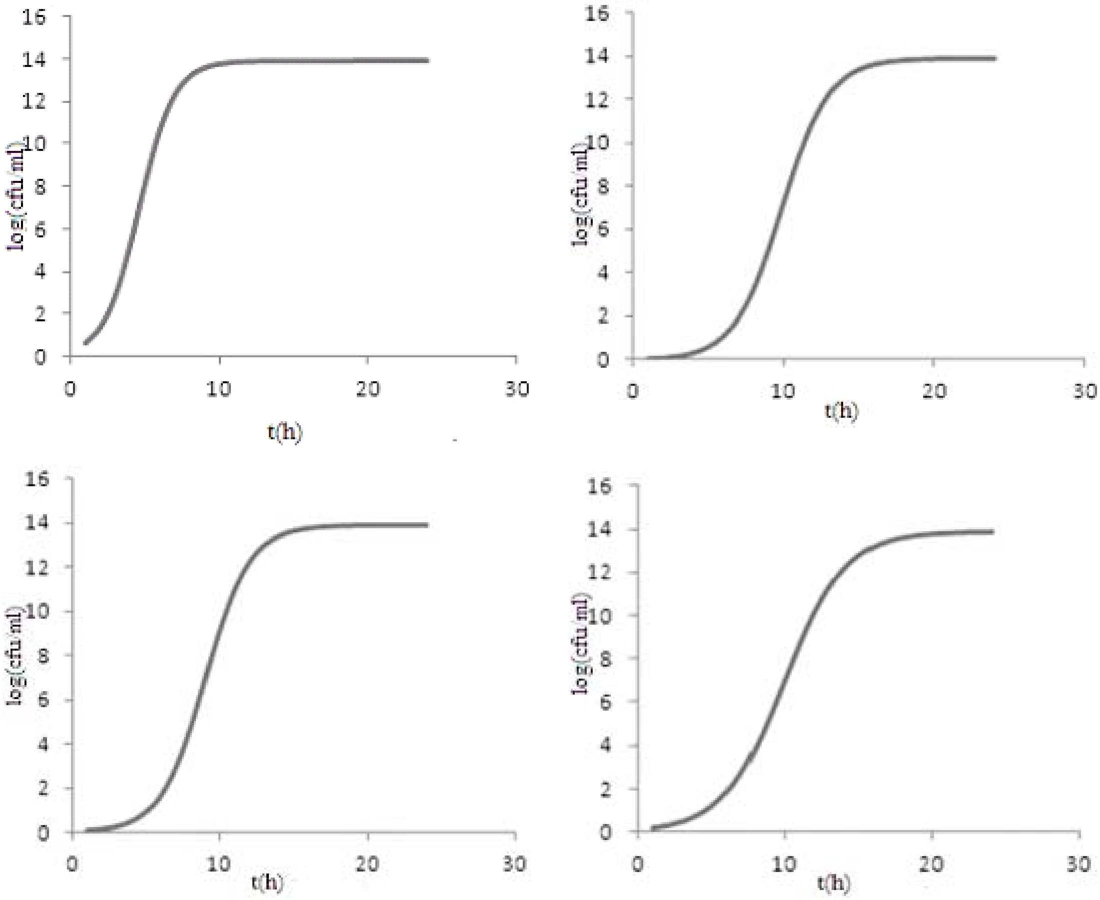

Fig. 2. Predicted microbial numbers of *Alcaligenes faecalis* VITSIM2 (a) control (b) Cr^{3+} 1000ppm (c) Cu^{2+} 300ppm (d) Cd^{2+} 80ppm with time from logistic model

square values of Gompertz model fit the growth data for control of both the isolates but failed to fit the data of both the isolates in the presence of metals (data not shown). Therefore further validation was done for logistic model alone. Upon data fitting, though the logistic and the Gompertz curves were not congruent in the lag phase region as well as in the transition region of the log phase to stationary phase, the chi square validation results show that the overflow of error occurred in the lag phase compared to the transition region of log to stationary phase. A good fit was observed with the Gompertz model without the lag phase data which distorted the model fit. Some authors reported in the past that the Gompertz function shows some disadvantages. It does not give exactly N=N0 at t=0, the lack of this information may have significant effects on predicted growth (Van and Zwietering, 1998) in the case of metal treated condition. The modified Gompertz equation overestimates the growth rate by 15% in comparison with other models (Membre *et al*., 2004). Gompertz equation overestimated the maximum population density, particularly when the number of data points during the stationary phase was limited (Buchanan, 1997). In contrast to the present study, Gompertz model fails in data fitting (Corbo *et al*., 2009) if there is no lag phase or the lag phase is too short (paradox of negative lag phase). Modeling the lag time is technically elusive and also necessitates quantitative data. Cells with lengthy lag phase may remain inscrutable by viable count procedures. As the lag phase is influenced by so many factors, accurate predictions of the lag phase are difficult to track. Moreover lag phase depends not only on current conditions but also on the physiological history of the cells. In spite of the issues encountered with modeling the lag phase of the isolates by Gompertz model, the logistic model successfully described the growth curves of both the isolates under metal stress conditions. The logistic model was found to be compatible with the experimental data including lag phase data. The model with the lower value of MSE was satisfied to describe the data (Sutherland and Bayliss, 1994). In the present study the MSE values were found to be low. Perfect agreement between the observed and the predicted values will have a bias factor equal to 1.Values > or < 1 will indicate over or under Prediction. Accuracy factor will always be equal to greater than 1. The larger the value, the less accurate is the average estimate. An accuracy factor of 2 indicates that the prediction is on average different from the observed value by a factor of 2 (Ross, 1996). The accuracy factor of both the isolates falls between 1 and 1.3. Models are categorized as good, acceptable and unacceptable (Ross, 1999). Good model - BF= 0.9 - 1.05, acceptable model – BF = 0.7 – 0.9, use with caution – BF = 1.06 – 1.15 and the unacceptable model BF<0.7 and BF>1.15. A bias factor >1 indicates a failsafe model (Zhou *et al*., 2008). The bias factor values for food spoilage micro-organism should be between 0.75 and 1.25 (Dalgaard, 2002) for a microbial spoilage model to be successfully validated. In the present study the bias factor of the logistic model was found to be a good and acceptable model. In spite of the time delay encountered by the cells to cope up with stress, the logistic model which depends upon the lag time delay probably was found to be a suitable model to predict the growth of the organisms in stress conditions.

CONCLUSIONS

The findings of the present study suggest that the logistic model would be a suitable primary model to study the growth pattern of resistant bacteria under stress conditions, especially metal stress conditions. Therefore the model could also be used to develop secondary models as a useful tool to predict the microbial behavior in various other stress conditions. The model could be used to evaluate microbial safety in food under temperature stress, and to evaluate the crop yield, soil fertility and plant growth by analyzing the growth pattern of halotolerant bacteria in salt resistant bacteria. As both the isolates also showed resistance to antibiotics (data not shown) and are of clinical importance, the growth behavior of these microbes could be applied in therapeutic areas for pharmacodynamic and pharmacokinetic modeling.

ACKNOWLEDGEMENTs

The authors thank VIT University for providing the facilities to carry out this work and also gratefully acknowledge Mr. Madhusudan HV and Mr.Gaurav Isola, VIT University for their help in carrying out this study.

REFERENCES

1. Kovarove-Kovar, K., Egli, T. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. *Microbiol. Mol. Biol. Rev.* 1998; **62**: 646–666.
2. Malakar, P.K., Barker, G.C., Zwietering, M.H., Van't Riet, K. Relevance of microbial interactions to predictive microbiology. *Int. J. Food. Microbiol.* 2003; **84**: 263–272.
3. Schultze, K.K., Linton, R.H., Cousin, M.A., Luchansky, J.B., Tamplin, M.L. A predictive model to describe the effects of temperature, sodium lactate, and sodium diacetate on the inactivation of a serotype 4b strain of *Listeria monocytogenes* in a frankfurter slurry. *J. Food. Prot.*, 2006; **69**:1552–1560.
4. McMeekin, T.A, Olley, J.N., Ross, T., Ratkowsky, D.A. Predictive Microbiology: Theory and Application Wiley, New York 1993; 1-9.
5. Lopez, S., Prieto, M., Dijkstra, J., Dhanoa, M.S., France, J. Statistical evaluation of mathematical models for microbial growth. *Int. J. Food. Microbiol*., 2004; **96**:289–300.
6. Baranyi, J., Roberts, T.A., McClure, P.J. A non-autonomous differential equation to model bacterial growth. *Food. Microbiol.,* 1993;**10**:43-59.
7. McKellar, R.C. A heterogeneous population model for the analysis of bacterial growth kinetics. *Int. J. Food. Microbiol.* 1997; **36**:179-186.
8. Pearl, R. The growth of populations. *Q. Rev. Biol.*, 1927; **2**(4):532–548.

9. Peleg, M., Corradini, M.G. Microbial growth curves: what the models tell us and what they cannot. *Crit Rev Food Sci Nutr.*, 2011; **51**: 917–945.
10. Whiting, R.C. Microbial modeling in foods. *Crit. Rev. Food. Sci. Nutr.*, 1995; **35**: 464-494.
11. McMeekin, T.A., Ross, T. Predictive microbiology: providing a knowledge-based framework for change management. *Int. J. Food. Microbiol.*, 2002; **78**: 133-153.
12. Fujikawa, H., Kai, A., Morozumi, S. A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures. *Food. Microbiol.*, 2004; **21**(5): 501-509.
13. Gibson, A.M., Bratchell, N., Roberts, T.A. Predicting microbial growth: growth responses of *salmonella* in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food. Microbiol.* 1988; **6**: 155-178.
14. Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.*, 1990; **56**: 1875–1881.
15. Sutherland, J.P., Bayliss, A.J. Predictive modeling of *Yersinia enterocolitica*: the effects of temperature, pH and sodium chloride. *Int. J. Food. Microbiol.*, 1994; **21**: 197–215.
16. Ross, T. Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol.*, 1996; **81**(5): 501-508.
17. Baranyi, J., Pin, C., & Ross, T. Validating and comparing predictive models. *Int. J. Food. Microbiol.*, 1999; **48**(3): 159-166.
18. Monod, J. The growth of bacterial cultures. *Annu. Rev. Microbiol.*, 1949; **3**: 371–394.
19. Van Gerwen, S.J., Zwietering, M.H. Growth and inactivation models to be used in quantitative risk assessments. *J. Food. Protect.*, 1998; **61**(11): 1541-1549.
20. Membre, J.M., Kubaczka, M., Dubois, J., Chene, C. Temperature effect on *Listeria monocytogenes* growth in the event of contamination of cooked pork products. *J. Food. Prot.* 2004; **67**(3): 463-9.
21. Buchanan, R.L., Whiting, R.C, Damert, W.C. When simple good is enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.*, 1997; **14**(4): 313-326.
22. Corbo, M.R., Bevilacqua, A., Campaniello, D., Amato, D., Speranza, B., Sinigaglia, M. Prolonging microbial shelf life of foods through the use of natural compounds and non thermal approaches–a review. *Int. J. Food. Sci. Technol.*, 2009; **44**: 223-41.
23. Ross, T. Predictive food microbiology models in the meat industry. Meat and Livestock Australia, North Sydney, Australia. 1999; 196.
24. Zhou, K., Cui, T.T., Li, P.L., Liang, N.J., Liu, S.C., Ma, C.W., Peng, Z.H. Modeling and predicting the effect of temperature, water activity and pH on growth of *Streptococcus iniae* in Tilapia. *J. Appl. Microbiol.*, 2008; **105**: 1956–1965.
25. Dalgaard, P., Bremner, H.A. Modelling and predicting the shelf-life of seafood. *Safety and quality issues in fish processing.*, 2002; 191-219.