Modelling the Growth of Lactic acid Bacteria- Starter Culture for Foods

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SUMMARY

The lactic acid bacteria, Lactococcuslactis, is used as a starter culture(s) in food fermentation(s). The specific growth information can be predicted for such starters, that can be practically valuable in exploiting lactic bacteriafor required fermentations. These bacteria are responsible to produce various metabolites. The metabolites of interest are produced from these starters under a set of known growth conditions. Their growth can be modeled using selected mathematical functions. These functions can be used in determining the parameters like specific growth rate and lag time of the organism under defined environmental conditions.

In this study, out of the three functions (Gompertz, Logistic and Richards functions) used, Gompertz function was selected. The model gave out constants that were used to obtain biological parameters for its growth. The main aim was thus to ascertain a particular function and the Goodness of fit (from R^2 values) that was measured from the fitted growth data. Durbin-Watson test was used to test the residuals dependency by autocorrelation. A larger number of fd values was the criteria to suggest if either the data plotted on Logistic or Gompertz function (3 parameters functions), was more helpful. The Gompertz function was found to be superior to Logistic function (both being three parameter function), against Richards function (a four parameter function) that had inherent and variable degree of skewness for testing the Gompertz and Logistic functions. The selected function, Gompertz function, was then derivatized and biological parameters were calculated, from the constant values so obtained. Thus the kinetic data with respect to time was resolved into an easy way to calculate biological parameters in terms of the simple equations. This methodology can be extended to lactic acid bacteria producing various other metabolites of interest.

Keywords: Microbial modelling, Fermentation, Non-linear growth model, Lantum points.

1. INTRODUCTION

The lactic acid bacteria, *Lactococcus lactis*, gram positive homolactic bacteria is used as a starter culture(s) in fermentation. However it can shift from homo to a hetero-lactic fermentation, under metabolic and regulatory conditions. It can primarily produce lactic acid and nisin (anti-microbial metabolite) during fermentation. This starter culture has been used for a neutraceutical lactic drink from black carrots (Singh, 2019). It has been found that the overall metabolism of *L. lactis* comprises an established network of a total of 621 reactions and 509 metabolites (Oliveira *et al.,* 2005). The three main variables that determine cell kinetics are: 1) the intracellular conditions that define the instantaneous state of a cell; 2) the extracellular conditions that are changed by the bacterial metabolism and 3) the extracellular conditions that are independent of the growing culture. Thus in order to use these bacteria as starters, specific information on their growth, like specific growth rate and the lag time (before the exponential phase), needs to be known. This will help know time (lag) to reach exponential phase, in order to exploit its potential growth biomass or any other metabolite of interest in exponential phase.

As bacterial growth curve is asymptotic after a certain period and can be described by 3 different sigmoidal functions:

- Logistic model,
- Gompertz model,
- Richards model.

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It is often very difficult to understand the biological activity/characteristics of an organism in relation to mathematical parameters used in modelling. A possible list of mathematical parameters, associated with growth models have been reported in previous studies (Schnute, 1981; Zwiettering *et al.,* 1990). However in using a model of growth (empirical equation) it becomes more useful, if its constants (mathematical parameters) give information of biological interest. With this in mind, an effort has been made herein to give a step by step methodology used in using above 3 models to predict growth parameters (in biological terms), with more accuracy to help practical use of *L . lactis*, as in starter culture applications.

The best mathematical model for the organism can then be used to calculate biological parameter value(s) (viz., lag time and specific growth rates) for use in batch scale fermentative processes, to allow appropriate information on time to harvest a metabolite (like nisin produced in mid to exponential phase) or biomass of *L. lactis* culture. The asymptotic growth functions include: the three parameter functions: Logistic and Gompertz, as against four parameter function of Richards that was used to compare Logistic vs Gompertz functions. A more appropriate model with 3 parameters was selected (Singh *et al.,* 2015).

2.   MATERIALS AND METHODS

In predicting growth kinetics, the actual behaviour of a target microorganism, can vary from strain to strain on maximum specific growth rate (μ_{max}) (h^{-1}), (Aryani *et al.,* 2015). Thus we used *L. lactis* NCIM 2114 strain, to model growth with the basic aim to predict growth parameters more precisely in specific fermentation(s), for starter culture applications. In this study, a 24 h growth cycle data of *L. lactis* obtained to model its growth in MRS broth medium (Abs vs time) has been explained in a step wise manner. Elaborate data with different conditions for growth was obtained using specific probes, in a fermenter (Singh *et al.,* 2015).

In a growth curve, 3 main phases of growth can be described by 3 mathematical parameters of interest in the growth function(s) (ie., a, b, c, mathematical parameters with a biological meaning (A, μ_m, and λ) respectively (Zwietering *et al.,* 1990):

1. Tangent (specific growth rate μ) on the inflection point (maximum specific growth rate (μ_m);
2. X-axis intercept of this tangent as Lag period (λ);
3. Asymptote (A) the point of maximum that becomes constant thereafter.

Steps on Modelling:

I.   Fitting growth data using asymptote functions: The growth data of *L. lactis* obtained on a single experimental run (Singh *et al.,* 2015), have been used herein to describe the steps to model growth. For this the nonlinear functions (Logistic, Gompertz and Richards) were used to fit growth data with a nonlinear regression method, using the Marquardt algorithm (Marquardt, 1963) on SPSS software (SPSS 17.1 version). This algorithm uses an iteration method for least square estimation to minimize the sum of squares of the differences between the predicted and measured values. This algorithm also searches for the steepest ascent (slope value) on the curve between four datum points (for estimation of μ_{max}); intersecting this line with the X-axis (for estimation of lag λ), and by taking the final datum point (for estimation of the asymptote A) (Fig 1).
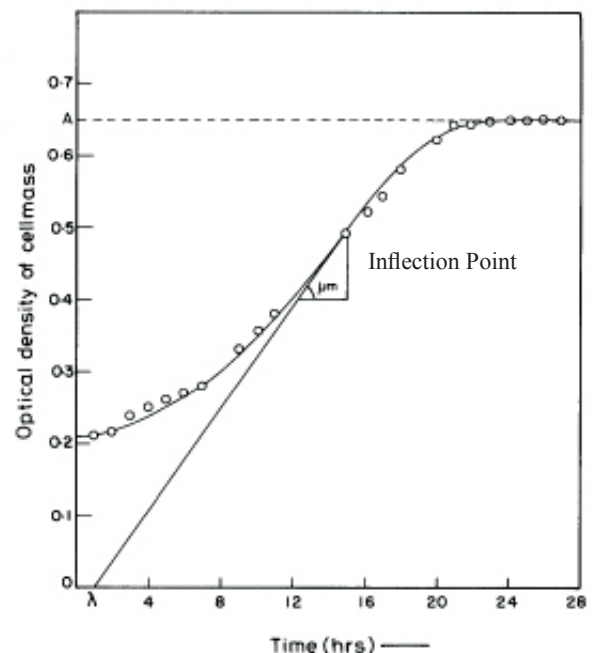

Fig 1. A growth curve (Source: Annadurai *et al.,* 2000).

The values of a, b, c and d were obtained using the SPSS Software (Table 1) with Marquardt algorithm. The growth data of the curve(s) as on a single experimental run data, are fitted to obtain the best fit curve for each of the functions (Logistic, Gompertz and Richards). To measure the Goodness of fit, Root mean square error (RMSE) and Coefficient of Determination (R^2) statistics have been used. To test the presence of autocorrelation (serial correlation) in the data set Box Pierce test was used. Further, to test the residuals dependency Durbin-Watson test has been used (Venugopalan and Prajneshu, 2000).

II. Calculate fd and F: The R^2 values were calculated on the SPSS software using the Marquardt algorithm, based on Residual sum of squares (RSS) between observed and predicted values. The RSS of the four parameter Richard's model can exactly predict growth being similar to Schnute's model, a reportedly comprehensive model (Zwietering *et al.*, 1990). In order to choose/describe the data, better with three parameters only and also to choose among either the Logistic or Gompertz function data as sufficient, an F test was used to validate data (Singh *et al.*, 2015; Zwietering *et al.*, 1990). The RSS of the models was used to calculate f_d and F values.

$$f_d = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1}$$ was calculated

and tested against F for DF_2-DF1, DF_1

Tables on critical values for the F distribution at $DF_2 - DF_1$, DF_1 values were used to calculate F values (Timm, 2002). The $DF_2 - DF_1$ (Numerator value) against DF_1 (Denominator value) at F was calculated for DF_1. For P values < 0.05 significance level, the P value calculator (danielsoper.com) was used in the F test. If the f_d value < F (calculated) at P < 0.05 it was considered significant. More the number of runs having significant f_d values, under either Logistic or Gompertz functions, more was the particular function useful in predicting the parameters on that function. In this manner, the data on the 15 experimental runs designed on a Box Behnken design (with 12 factorial points and 3 center points, as replications of control) were tabulated. The results on this aspect have been shown in a previous study (Singh *et al.*, 2015). Since the Gompertz function showed more acceptability to model growth of *L. lactis* NCIM 2114, this function was selected (Singh *et al.*, 2015) to calculate the biological parameters as below.

III. Calculating Biological Parameters From Gompertz Function: The specific growth rate was determined from the slope of exponential growth (phase) after re-parameterizing/re-solving the Gompertz function. For this, derivatives of the function with respect to *t* were calculated, to obtain the point of inflection on tangent across exponential phase on growth curve. This helped to calculate the biological values of specific growth rate and lag period (phase before exponential growth picks up).

Gompertz function: y = a exp [-exp(b-cx)]   (1)

The Gompertz function (1) was derivatized (Zwittering *et al.*, 1990; Derivative-calculator.net).

The function rewritten as:

- The first derivative of the growth equation represented growth with respect to time (rate of change of growth) or growth rate (μ):

$$\frac{dy}{dt} = ac \times [\exp[b - ct]] \times \exp(b - ct)$$ (2)

The second derivative of growth equation

$$\frac{dy^2}{dt^2} = ac^2 \exp[-\exp(b - ct) \times \exp(b - ct)]$$

- The second derivative of Gompertz function with respect 't' equals zero, at inflection point on the curve, where the rate of change was maximum, to obtain maximal growth rate μ_m:

$$d^2y/dt^2 = 0 \longrightarrow t_i = b/c$$ (3)

Now the first derivative at the inflection point gives maximal specific growth rate (μ_m)

$$\mu_m = (\frac{dy}{dt})t_i = \frac{ac}{e}$$ (4)

The parameter 'c' in the Gompertz function can thus be obtained as

c = μ_m e/a (where e = 2.71828)

Using equations 3 and 4,

The lag time (l) is defined as the t-axis, intercept of the tangent through the inflection point, where

$$0 = \mu_m \times \lambda + \frac{a}{e} - \mu_m t_i \qquad (5)$$

From equations 3, 4 and 5

Lag $(\lambda) = (b-1)/c$

The parameter 'b' can be substituted by

$$b = \frac{\mu_m e}{a} \lambda + 1 \qquad (6)$$

3. RESULTS AND DISCUSSION:

The growth curves were modeled by curve fits (Singh *et al.,* 2015) and the R^2 values showed the extent of goodness of fit. Table 1 indicates that Logistic, Gompertz and Richards models provide satisfactory results. R^2 values being almost equal in all the three models, very low RSME values in case of Gompertz and Richards models. Durbin-Watson test statistic values computed under all the models suggest that residuals of all the models are uncorrelated. Hence the fitted models are well justified for explaining the bacterial growth.

Table 1. Summary statistics of the models fitted for growth of *L. lactis*.

Parameter	Logistic function	Gompertz function	Richards function
a	1.463	1.481	1.482
b	3.037	1.677	0.027
c	0.599	0.415	0.419
d			4.079
R^2	0.993	0.997	0.977
RMSE	0.041	0.027	0.027
Durbin-Watson test statistic value #	2.01 (p=0.49)	2.541 (p=0.89)	2.293 (p=0.92)
# Values > 2 show no autocorrelation			

Further to describe the entire set of data with the main aim to simplify the growth kinetics, from a selected 3 parameter function, the Gompertz function, it was easy to precisely calculate the biological values μ_m, l and A from the mathematical values a, b and c of Gompertz function (Table 1). The simple derived equations (Table 2) were then used to calculate the biological parameters obtained from derived equations of Gompertz function.

Table 2. Simplified calculation(s) of biological parameters on equations derived from the Gompertz function.

Estimate of Parameters (See ANOVA Table 2)			m_{max} (h^{-1})	l (lag time) (h)	Inflection Point (h)
a	b	c	ac/e	(b-1)/c	b/c
1.481	1.677	0.415	0.23	1.62	4
where e = 2.71828					

The results (Table 2) showed that in each case of the bacteria, L lactis, the specific growth rate which has started at a value of zero has accelerated to a maximum value 0.23/h (m_{max}) in 1.62 h (l) and has maintained the same specific growth rate till inflection point 4.04 h (A). After 4.04 h there was no enhancement of specific growth rate.

It was shown earlier that the slope from Gompertz function is always positive for finite values of x and differentiating it, led to the ordinate at the point of inflection when 37 per cent of the final growth was reached (Winsor, 1932). This inflection point in the early part of the growth cycle, was shown to give a good approximation of the data as compared to Logistic function where the point of inflection was mid-way (50 per cent) between the asymptotes. That a bias in selecting Gompertz function cannot take place (Winsor, 1932) since the mathematical properties of the Gompertz and the Logistic, functions had same degree of "skewness" (Winsor, 1932). The Richards function on the other hand has four constants. Thus this function with four constants was a help to introduce a variable degree of skewness into a growth curve and be useful in comparing Logistic and Gompertz functions. A selected model thus becomes useful when models are microbiologically relevant to understand the mathematical expressions, in terms of biological values.

The algorithm used herein to calculate the set of parameters with the lowest residual sum of squares (RSS) and their 95% confidence intervals, also had several advantages. This algorithm has been shown to close in on the converged values rapidly, after the vicinity of the converged values was reached. Also it is an optimum interpolation between the Taylor series method and the Gradient method, to give adequate representation of the nonlinear model (Marquardt, 1963). The Gompertz model was thus a suitable function to model growth of *L. lactis* NCIM 2114.

The modelling information on derived biological parameters (Table 2), that practically helped to deduce, maximal specific growth rate in exponential phase, at which the maximal metabolite (of interest) as produced, like nisin, can be harnessed. *L. lactis* is of particular interest, due to its potential to produce 'nisin' an antimicrobial metabolite (natural bio-preservative). Such microbial products produced can be further purified and used as high value product, in preserving cheeses and other foods. A microbial metabolite obtained after a lowest possible lag time, before it enters exponential phase to produce nisin, in its exponential phase, can be determined precisely by modelling growth. We can exploit these parameters on potential time period of exponential growth with high concentrations of nisin produced, for further downstream processing in practical applications of a modelled growth.

4. CONCLUSIONS

The large amount of data obtained can be easily analyzed for growth kinetics by an automated method to model growth of *L. lactis* NCIM 2114. Especially where starter culture strains require biomass production, the growth kinetics can be a useful method to determine its specific growth rate and lag period. This can also be of consequence in precisely determining lag times and maximal specific growth of the organism to harvest metabolites produced during its exponential growth. The procedure to model hence turned out as a stochastic model with the use of a statistical package software along, and a very precise tool to study the exponential growth of *L. lactis*.

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