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# Predicting growth of *Weissella viridescens* in culture medium under dynamic temperature conditions

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**Abstract**

The lactic acid bacteria (LAB) are among the main spoilage microorganisms of foods, and the *Weissella viridescens* (formerly *Lactobacillus viridescens*) is well known to cause deterioration on the meat surface in vacuum packed meat products, even under refrigerated conditions. Therefore, this study evaluated the predictive ability of Baranyi and Roberts dynamic model to describe *W. viridescens* growth in culture medium (which simulates a food rich in nutrients), subjected to dynamic temperature conditions. Baranyi and Roberts primary model was fitted to the growth curves of *W. viridescens* in culture medium under six different isothermal temperatures (4, 8, 12, 16, 20 and 30 °C) previously obtained in our laboratory. Four secondary models (linear, square root, exponential and Arrhenius type) were assessed to describe the influence of temperature on the growth parameters. The square root was the best model to describe temperature influence on $\mu_{max}$ parameter. For $Y_{max}$ parameter, the secondary model was considered the mean values obtained experimentally in the studied temperature range. Experimental data were used to evaluate the model predictions under dynamic conditions for two different temperature profiles, NIP-1 (12-16-20-25 °C) and NIP-2 (16-12-8-4 °C). According to the statistical indexes, the model showed better predictive ability for NIP-1, with RMSE of 0.3341, $R^2$ of 0.9939, bias factor of 1.0046 and accuracy factor of 1.0197; the growth of *W. viridescens* under NIP-2 conditions was underestimated, indicating a fail dangerous prediction. The results showed that the predictive model can be used to predict the shelf life of meat products spoiled by *W. viridescens*.



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*Keywords:* mathematical modeling; dynamic temperature; microbial growth.

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## 1. Introduction

Meat and meat products are highly perishable, with a shelf life strictly dependent on the number and type of microorganism present initially, and on storage conditions, particularly pH, temperature and gas atmosphere[2]. Among the main spoilage microorganisms in meat products are lactic acid bacteria (LAB) which are a group of gram-positive bacteria[3]. Some species such as *Weissella viridescens* (formerly *Lactobacillus viridescens*) can produce peroxide which reacts with meat pigment, causing greenish appearance[4,5,6,7].

The temperature of refrigerated foods usually varies greatly during transport, retail and especially at home, and it has great influence on the kinetics of microbial growth in food[8]. The temperature increase causes the decrease of the adaptation time and the increase of the maximum specific growth rate of the spoilage bacteria, which can lead to negative effects, such as reduction of food shelf-life and impact on food safety[9].

The objective of this study was evaluate the predictive ability of Baranyi and Roberts dynamic model to describe *W. viridescens* growth in rich culture medium, subjected to dynamic temperature conditions.

Material and methods

### *1.1. Microorganism and experimental procedures*

*Weissella viridescens* (CCT 5843 ATCC 12706) used in this study was purchased in lyophilized form from the *André Tosello* Foundation of Tropical Cultures (Campinas, Brazil). The strains were rehydrated in MRS – *Lactobacillus* medium (Acumedia Manufactures, Michigan, USA), and stored in Eppendorf tubes with MRS medium containing 20% glycerol at -24 °C until use. The reactivation of the culture for preparing the inocula was carried out in MRS medium at 30 °C for 18 hours. The experiments were performed in 250 mL Erlenmeyer flasks with 160 mL of MRS medium and approximately $10^3$ CFU/mL. The flasks were inserted in incubators (Dist, Florianópolis, Brazil) at different temperature conditions.

The growth under non-isothermal conditions were performed in two experiments, NIP-1 (12-16-20-25 °C) and NIP-2 (16-12-8-4 °C). All experiments were conducted until the stationary growth phase, and the temperature was recorded every 5 minutes by mini data loggers (Testo 174, Lenzkirch, Germany).

### *1.2. Mathematical models*

The Baranyi and Roberts primary model[10] was fitted to *W. viridescens* growth curves in culture medium under six different isothermal temperatures (4, 8, 12, 16, 20 and 30 °C) previously obtained in our laboratory. Four secondary models (linear, square root, exponential and Arrhenius type) were assessed to describe the influence of temperature on the growth parameters. Square root model[11], shown in Eq (1), was the best model to describe temperature influence on $\mu_{max}$ parameter. For $Y_{max}$ parameter, the secondary model was considered the mean values obtained experimentally in the studied temperature range. The experimental data were used to evaluate the model predictions, shown in Eq (2), (3) and (4) under dynamic conditions for two different temperature profiles, NIP-1 (12-16-20-25 °C) and NIP-2 (16-12-8-4 °C). In Equations (1), (2), (3) and (4), $y$ is the natural logarithm of the cell concentration $N$ ($y = \ln(N)$) at time $t$; $Q$ is the physiological state of the cells at time $t$, $\mu_{max}$ is the maximum specific growth rate, $y_{max}$ is the natural logarithm of the maximum cell concentration; $T$ is the temperature; $T_{min}$ is the theoretical temperature for minimal microbial growth; and $b$ is an empirical parameter. The initial conditions to solve the differential equations (2), (3) and (4) are $y(0) = y_0$ and $Q(0) = Q_0$, in which $y_0$ is the value of the natural logarithm of initial cell concentration, and $Q_0$ is the value of the initial physiological state of cells, wherein $Q_0$ is associated with the parameter $h_0$. The parameters $b$ and $T_{min}$ were estimated from the experimental data.

$$\sqrt{\mu_{\max}} = b\left(T - T_{\min}\right) \quad (1)$$

$$\frac{dy(t)}{dt} = \mu_{\mathbf{max}}\left[\frac{1}{1+e^{-Q(t)}}\right]\left\{1-\mathbf{exp}\left[y(t)-y_{\mathbf{max}}\right]\right\} \quad (2)$$

$$\frac{dQ(t)}{dt} = \mu_{\mathbf{max}} \quad (3)$$

$$Q_0 = -\ln\left[\exp(h_0)-1\right] \quad (4)$$

The prediction of *W. viridescens* growth curves in different temperature profiles was obtained using *ode23* function in computational routines programmed and executed in Matlab 7 (MathWorks®, Natick, USA), which is based on the Runge-Kutta method, applying the initial conditions. The statistical index $R^2$, mean square error (RMSE), factor bias and factor accuracy were used to assess the ability of the mathematical models in representing the growth data.

## 2. Results and discussion

In this study, the secondary models linear, square root, exponential and Arrhenius type were fitted to the experimental data of *W. viridescens* maximum specific growth rate ($\mu_{máx}$) in the temperature range from 4 to 30 ° C. The selection of the secondary model was based on correlation coefficients ($R^2$) obtained fitting secondary models, as shown in Table 1.

Table 1. Secondary models which describe the influence of temperature on the parameter maximum specific growth rate of *W. viridescens*.

| Model | Equation | $R^2$ |
|---|---|---|
| Linear | $\mu_{máx} = 0.03*(T) - 0.1668$ | 0.9706 |
| Arrhenius type | $\ln(\mu_{máx}) = -15.518*(1/T) - 0.259$ | 0.8983 |
| Exponential | $\mu_{máx} = 0.0217*\exp^{0.134*T}$ | 0.9069 |
| Square root | $\sqrt{\mu_{máx}} = 0.029*(T+1.32)$ | 0.9930 |

Square Root model represented well the dependence of the maximum specific growth rate with temperature, with $R^2$ value above 0.99 (Figure 1). The parameters $b$ and $T_{min}$ were $b$ = 0.0290 (± 0.0020) $h^{-0.5}$ $°C^{-1}$ and $T_{min}$ = -1.33 (± 1.26) °C, with $R^2$ = 0.993.

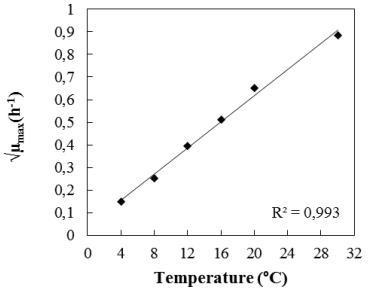


Fig. 1. (a) Fitting of Square Root model (solid line) to the maximum specific growth rate experimental data (symbols)

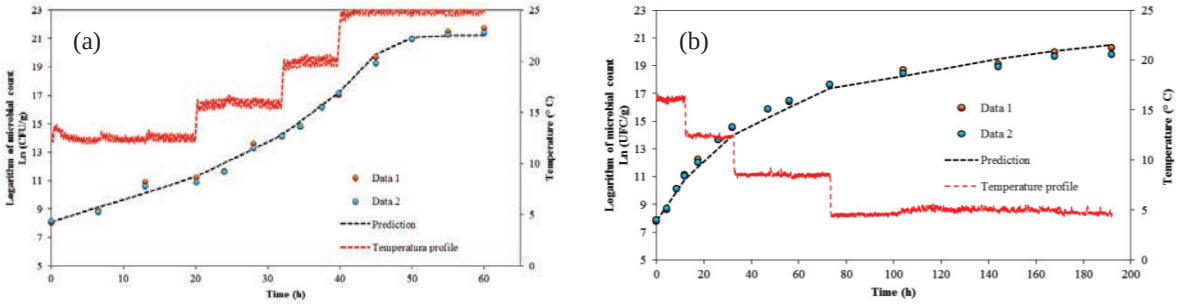


Fig. 2. Mathematical model prediction (dashed line) and experimental data (symbols) of *W. viridescens* growth for the (a) profile NIP-1 (12-16-20-25 °C) and (b) profile NIP-2 (16-12-8-4 °C) and xperimental temperature profile (red dashed line).

According to the statistical indexes, the model showed better predictive ability in the NIP-1, with RMSE of 0.3341, $R^2$ of 0.9939, bias factor of 1.0046 and accuracy factor of 1.0197. *W. viridescens* growth under NIP-2 profile was underestimated, indicating a fail dangerous prediction by the model. Thus, the predictive ability of the Baranyi and Roberts model was greater when the temperature was closer to the optimal growth temperature for this bacterium (30 °C). The moderate temperatures of this profile (12-16-20-25 ° C) contributed to a good prediction model.

## 3. Conclusion

The results showed that the predictive model can be used to predict the shelf life of meat products spoiled by *W. viridescens*, but additional studies are needed to improve the prediction under refrigeration temperatures.

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