Modelling growth of *Lactobacillus plantarum* as a function of temperature: Effects of media

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Summary

The growth response of *Lactobacillus plantarum* as affected by incubation temperature was studied in various media. Parameters resulting from growth curves were analysed in secondary phase using the Ratkowsky model and cardinal temperature model with inflection (CTMI) in a temperature range from 8 °C to 40 °C. Using Ratkowsky model, minimal and maximal temperatures (T_{min} and T_{max}) were calculated for de Man, Rogosa and Sharpe (MRS) broth, milk and lactose-free milk, respectively (T_{min} = 0.9 °C, T_{max} = 41.6 °C; T_{min} = 7.1 °C, T_{max} = 41.2 °C; T_{min} = 4.7 °C, T_{max} = 41.6 °C) and compared with CTMI model that provided also optimal growth temperatures (T_{opt}) in MRS broth (T_{opt} = 36.6 °C, T_{min} = 2.0 °C, T_{max} = 41.0 °C), milk (T_{opt} = 34.7 °C, T_{min} = 7.8 °C, T_{max} = 41.0 °C) and lactose-free milk (T_{opt} = 34.2 °C, T_{min} = 5.4 °C, T_{max} = 41.4 °C). Optimal specific growth rates of 0.81 h^{-1}, 0.52 h^{-1} and 0.40 h^{-1} were estimated from the experiments performed in MRS broth, milk and lactose-free milk, respectively. Results of the modelling can be taken into account to optimize fermentation processes in which *Lb. plantarum* strains are used.

Keywords

Lactobacillus plantarum; mathematical modelling; cardinal parameters; lactose-free milk

Several mathematical equations and models describing the behaviour of microorganisms under different environmental factors were developed in predictive microbiology in the last few decades. Primary and secondary models are used to characterize the growth of bacterial cultures in relation to time and food environmental conditions. These have immediate practical applications to improve microbiological food safety, quality and are leading to the developement of quantitative understanding of microbial ecology of food products. They also serve as a basis for tertiary models used for prediction of microbial behaviour in food [1].

In predictive microbiology, mathematical modelling has been mainly applied to evaluate the potential outgrowth of spoilage bacteria or foodborne pathogens [2, 3]. However, in the last ten years, there has been an increasing interest in modelling the kinetics of beneficial food-grade microorganisms, such as lactic acid bacteria. In food industry, lactic acid bacteria are intentionally added as starter cultures to food products with a purpose to develop a new kind of foods, achieve their stability and safety with unique organoleptic characteristics [3, 4]. Moreover, in this effort as well as in safety assessment of naturally fermented food products, the newly developed competition models, in which LAB play relevant role, can be taken into consideration [5, 6].

Regarding fermentation of hexoses, *Lactobacillus plantarum* is a member of facultative heterofermentative group of lactobacilli, which ferment sugars via Embden-Meyerhof-Parnas pathway or the pentose-phosphate pathway [7]. Free sugars can be transported through either permease systems or by an inducible specific phosphotrans-

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ferase system. The transport of glucose, fructose, mannose, mannitol, galactose and lactose can occur through the phosphotransferase system to end up as glucose-6-phosphate. In total, twenty-five complete phosphotransferase sugar transport systems were identified in *Lb. plantarum* WCFS1, reflecting its efficient adaptive capacity [8].

Lb. plantarum is commonly found in the human gastrointestinal tract and is encountered in a range of environmental niches [9]. It is also naturally and frequently presented in human breast milk [10–12]. *Lb. plantarum* has several applications in the food industry and has been used as a starter culture in various food fermentation processes contributing to the organoleptic properties of food products. In a study of Matejčeková et al. [13, 14], *Lb. plantarum* HM1 preserved viability in buckwheat and soya fermented products during cold storage period within 14–21 days. Pakbin et al. [15] showed that a combination of *Lb. casei*, *Lb. delbrueckii* and *Lb. plantarum* was able to grow in peach juice with counts higher than 10^9 CFU·ml^{-1}. In another study by Nagpal et al. [16], fermented orange, apple, grape and tomato juices were incubated with *Lb. plantarum* and *Lb. acidophilus*, which reached counts of 10^8 CFU·ml^{-1} after 72 h of fermentation. *Lb. plantarum* is also one of the most frequent species related with cheese production, playing an important role in ripening [17]. Furthermore, *Lb. plantarum* is often the dominating *Lactobacillus* spp. in traditional lactic acid-fermented foods based on plant material such as sauerkraut, green olives or cucumbers. The fact that *Lb. plantarum* frequently dominates in spontaneously lactic acid-fermented foods with pH < 4 and is able to survive the passage through the acid conditions of the human stomach, naturally points to its high resistance to acid conditions [18].

Several studies showed that *Lb. plantarum* exerts inhibitory activity against pathogenic and food spoilage microorganisms such as *Listeria monocytogenes* [19], *Enterococcus faecalis* [19, 20], *Escherichia coli* [21, 22], *Pseudomonas aeruginosa, Staphylococcus aureus* [20], *Helicobacter pylori* [23], *Clostridium difficile* [24] or *Yersinia enterocolitica* [21]. Because of this antagonistic feature, *Lb. plantarum* strains are used in food preservation, providing extension of shelf life and reducing or even replacing chemical additives. Also, they can be used as a supporting therapeutic agents in the treatment of certain infections [25, 26].

Despite the broad interest in the application of *Lb. plantarum* in the food industry, data on growth of specific isolates are not generally available. Studying the growth kinetics of *Lb. plantarum* in food and in artificial media, considering temperature changes, can make it possible to control proliferation of undesirable microorganisms in food. Further, obtained data may contribute to a better control of fermentation processes and may help to clarify in which manner and to which degree will the food environment interfere with the functionality of the strains. This study deals with quantification of the temperature effects on the growth of *Lb. plantarum* in real and model growth media, and further with application of several models.

MATERIALS AND METHODS

Microorganism

Lb. plantarum HM1 isolated from breast milk and identified by Liptáková et al. [11] was used as a model microorganism in this study. The isolate was first sub-cultured three times for 24 h at (37 ± 0.5) °C, 5% CO_2 in de Man, Rogosa and Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France) from the frozen stock containing MRS broth and 25 % glycerol before using it as an inoculum (stored at –30 °C).

Substrate inoculation and culture conditions

The standard suspension of the strain was prepared by overnight incubation at (37 ± 0.5) °C, 5% CO_2 in MRS broth and used in the individual experiments for inoculation of pre-tempered MRS broth, commercially available ultra-pasteurized (UHT) milk or lactose-free milk (glucose and galactose present after enzymatic hydrolysis, concentration of lactose < 0.2 g·l^{-1}) with 1.5 g·l^{-1} fat content (both Meggle, Bratislava, Slovakia) at an initial level of 10^3 CFU·ml^{-1}. Sterility of milks was regularly confirmed by plating prior to inoculation. Three parallel static cultures of samples were prepared at ranked temperatures from (8 ± 0.5) °C to (40 ± 0.5) °C under aerobic conditions.

Enumeration of bacteria and determination of active acidity

At time intervals, serial ten-fold dilutions of samples were prepared in a solution of 8.5 g·l^{-1} NaCl and 0.1 g·l^{-1} peptone (Biolife, Milan, Italy). Presumptive numbers of *Lb. plantarum* were estimated using MRS agar (Biokar Diagnostics) according to STN ISO 15214 [27]. Inoculated Petri dishes with *Lb. plantarum* were cultured in anaerobic conditions (37 ± 0.5) °C, 5% CO_2 for 48 h. The pH values were measured at the same time as the microbiological analysis using the pH meter WTW 720 (Inolab, Weilheim, Germany).

Growth data modelling

The growth parameters were fitted and calculated using the mechanistic D-model by BARANYI and ROBERTS [28] that is incorporated in DMFit tools kindly provided by Dr. J. Baranyi (University of Debrecen, Debrecen, Hungary). The growth function of Baranyi and Roberts expressed in the explicit form was applied as follows:

$$y(t) = y_0 + \mu_{max} A(t) - c$$

$$c = \frac{1}{m} \ln\left(1 + \frac{e^{m\mu_{max}A(t)} - 1}{e^{m(y_{max} - y_0)}}\right) \quad (1)$$

where $y(t)$ is the natural logarithm of the cell concentration, y_0 is the natural logarithm of the cell concentration at $t = t_0$, t is time, t_0 is initial time of the growth, μ_{max} is the maximum specific growth rate, m is the curvature parameter to characterize the transition from the exponential phase (suggested values m ranging from 1 to 10), y_{max} is the natural logarithm of the maximum cell concentration, $A(t)$ is the function that plays the role of a gradual delay in time:

$$A(t) = t + \frac{\ln(e^{-m\mu_{max}t} + e^{-h_0} - e^{-\nu t - h_0})}{\mu_{max}} \quad (2)$$

where t is time, h_0 is the dimensionless parameter quantifying the initial physiological state of the cells, ν is the rate determining the rate of the transition from lag to the exponential phase. The lag time λ can be calculated as:

$$\lambda = \frac{h_0}{\mu_{max}} \quad (3)$$

For comparison purposes, another primary growth model modified by HUANG [29, 30] was used:

$$\ln Y = \ln Y_0 + \ln Y_{max} - \ln f$$

$$\ln f = e^{Y_0} + (e^{Y_{max}} - e^{Y_0}) e^{-\mu_{max} B(t)} \quad (4)$$

$$B(t) = t + \frac{1}{\alpha} \ln \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}} \quad (5)$$

where Y is the bacterial count, Y_{max} and Y_0 are the maximum and initial bacterial counts, μ_{max} is the maximum specific growth rate, λ is the lag phase duration and α in $B(t)$ is the lag phase transition coefficient (LPTC).

Using decimal logarithm of bacterial counts, the growth rates (Gr) were estimated from the primary models. For secondary modelling, they were recalculated to the specific growth rates (μ) according to the equation:

$$\mu = Gr \cdot \ln 10 \quad (6)$$

Effect of temperature on growth rates

The specific growth rates (μ) calculated by curve fitting according to BARANYI and ROBERTS [28] were modelled as a function of temperature with the following models as applied by RATKOWSKY et al. [31]:

$$\sqrt{\mu_{max}} = [b(T - T_{min})]^2 \cdot g$$

$$g = 1 - \exp[c(T - T_{max})] \quad (7)$$

where T is temperature, T_{min} and T_{max} are the theoretical minimum and maximum temperatures, b is a Ratkowsky parameter to be calculated, and c is the parameter enabling the model to fit the data above the optimal temperature.

Then, a cardinal temperature model with inflection (CTMI) was introduced to empirically describe the influence of selected environmental factors on the data, which is described by the equation:

$$\mu_{max} = \mu_{opt} \frac{h}{i \times j}$$

$$h = (T - T_{max})(T - T_{min})^2$$

$$i = (T_{opt} - T_{min})$$

$$j = h \times (T - T_{opt}) - \\ -(T_{opt} - T_{max})(T_{opt} + T_{min} - 2T) \quad (8)$$

where T is actual incubation temperature, T_{min} is the theoretical temperature below which no growth is observed, T_{max} is the temperature above which no growth occurs, and T_{opt} is the temperature at which the maximum specific growth rate equals its optimal value (μ_{opt}) [32].

After the parameters were calculated, regression coefficient (R^2) was determined for each model using the following equation:

$$R^2 = 1 - \frac{\sum_1^n (Y_{obs} - Y_{est})^2}{\sum_1^n (Y_{obs} - \bar{Y})^2} \quad (9)$$

where Y_{obs} are the natural logarithms (base e or 10) of bacterial counts, Y_{est} are the logarithms of bacterial counts estimated by a model and $\bar{Y}$ is the average of the logarithms of bacterial counts observed experimentally.

Another parameter, root mean square error ($RMSE$), an estimate of the standard error of a model, was calculated using the following formula:

$$RMSE = \sqrt{\frac{\sum (Y_{obs} - Y_{est})^2}{n}} \quad (10)$$

Standard error of prediction (SEP) was calcu-

lated according to Zurerra-Cosano et al. [33] and expressed as percentage:

$$SEP = \frac{100}{\mu_{mean\ obs}} \sqrt{\frac{\sum(\mu_{obs} - \mu_{pred})^2}{n}} \quad (11)$$

The ordinary least-squares criterion was used to fit the models to the data. The sum of the squared residuals (*RSS*) was defined as follows:

$$RSS = \sum_{k=1}^{n} (\mu_{obs} - \mu_{cal})_k^2 \quad (12)$$

where n is the number of data points. The smaller the *RSS*, the better fit.

As a measure of goodness of fit of the models, the percent variance for (V) was also calculated, using the formula [34]:

$$V = \left[1 - \frac{(1 - r^2)(n - 1)}{(n - n_T - 1)}\right] \times 100 \quad (13)$$

where n is number of observations, n_T number of terms and r^2 is the multiple regression coefficient.

Statistical analysis

Each experiment was performed in three separate trials. Results were presented as mean with a standard deviation. Statistical analyses were carried out using Microsoft Excel 2013 (Microsoft, Redmond, Washington, USA). Data were treated by Student's *t*-test with a least significant difference of 95 %.

RESULTS AND DISCUSSION

Some of *Lb. plantarum* growth parameters covering broad temperature range in UHT milk (1.5 g·l^{-1} fat content) were presented in our previous study [35]. In order to provide growth description of the strain over the whole temperature range in different media, additional growth experiments were carried out at optimal and beyond optimal growth temperatures.

Generally, *Lb. plantarum* showed better growth in MRS broth compared to lactose-free milk as expected, due to the presence of easily fermentable glucose, peptones, mixture of oleic esters, manganase and magnesium salts, supplying the nutritive elements required for the growth of lactobacilli. *Lb. plantarum* growth in MRS broth was characterized by specific growth rates ranging from 0.15 h^{-1} to 1.89 h^{-1} (Tab. 1), which were by about 20–73 % higher compared to those in lactose-free milk (from 0.04 h^{-1} to 0.39 h^{-1}) (Tab. 2).

Different substrates affected also the maximal population densities (MPD) that ranged within the average values of (9.60 ± 0.38) log CFU·ml^{-1} (V = 3.9 %); (7.58 ± 0.33) log CFU·ml^{-1} (V = 4.3 %) and (7.72 ± 0.20) log CFU·ml^{-1} (V = 2.6 %) in broth, milk and lactose-free milk, respectively. *Lb. plantarum* could not grow well at 8 °C neither in milk nor in broth, and showed 21 days adaptation phase without any increase of its numbers. This was the reason why these growth experiments were excluded from the secondary modelling. On the contrary, a slight decrease in cell numbers was observed by Valík et al. [36] for *Lb. rhamnosus* GG (μ = 0.069 h^{-1}), which was by about 93 % higher in comparison to our results. Increasing the incubation temperature by 4 °C resulted in proliferation of the studied strain in both media. The growth was still slow (μ = 0.040 h^{-1} and μ = 0.065 h^{-1} in lactose-free milk and broth, respectively) represented by the doubling time of 17.3 h and 10.6 h in milk and in broth, respectively. Further increase in temperature led to a more intensive growth in almost whole studied temperature range, except for marginal 40 °C. Aerobic incubation of *Lb. plantarum* at 15 °C decreased the lag phase duration 4.4-fold in comparison with that at 12 °C, while the specific growth rate was by about 69 % faster. At 15 °C, specific growth rate of *Lb. plantarum* in lactose-free milk was shown to be the same as the specific growth rate of *Lb. rhamnosus* VT1 in milk [37]. At this temperature, growth of *Lb. plantarum* in MRS broth was by about 48 % faster in comparison to lactose-free milk. In lactose-free milk, maximal specific growth rates were observed at temperatures of 30 °C and 34 °C (0.386 h^{-1} and 0.388 h^{-1}, respectively), which was consistent with the optimal temperature of 34.7 °C estimated using CTMI model for the studied strain in UHT milk [35]. The specific growth rate of *Lb. plantarum* at 37 °C (μ = 0.369 h^{-1}) was a half of the specific growth rate of *Lb. acidophilus* in UHT milk at the same temperature (μ = 0.77 h^{-1}) [38]. In MRS broth, the growth of *Lb. plantarum* in the exponential phase at 34 °C was faster by about 37 % and by about 51 % in milk and lactose-free milk, respectively. The fastest growth, as expressed by the specific growth rate (μ = 0.829 h^{-1}), was noticed in MRS broth at 37 °C. Further increasing the incubation temperature had a negative effect on the growth dynamics and led to deceleration of growth. In the last selected temperature (40 °C), specific growth rate decreased by about 50 % in comparison to 37 °C in lactose-free milk and by about 49.8 % in broth.

For the manufacture of lactose-free base milk, mainly free enzymes are used [39]. In the literature, only a few studies refer to the influence of

Tab. 1. Specific growth rates of *Lb. plantarum* in relation to temperature in de Man, Rogosa and Sharpe broth calculated using Baranyi and Huang models.

T [°C]	Baranyi model				Huang model			
	μ [h^{-1}]	R^2	*RMSE*	λ [h]	μ [h^{-1}]	R^2	*RMSE*	λ [h]
12	0.07^{x}	0.997	0.284	35.6	0.07^{x}	0.951	0.411	40.9
15	0.13^{x}	0.997	0.079	26.0	0.13^{x}	0.993	0.193	25.5
18	0.19^{x}	0.991	0.189	9.4	0.19^{x}	0.952	0.500	9.1
21	0.39^{x}	0.994	0.179	4.9	0.39^{x}	0.966	0.419	4.9
25	0.48^{x}	0.996	0.187	3.9	0.47^{x}	0.963	0.503	3.7
30	0.62^{x}	0.999	0.225	2.6	0.60^{y}	0.955	0.527	2.4
34	0.78^{x}	0.996	0.166	2.2	0.77^{x}	0.967	0.364	1.8
37	0.83^{x}	0.997	0.200	2.2	0.83^{x}	0.992	0.215	2.3
40	0.53^{x}	0.974	0.406	–	0.63^{y}	0.930	0.662	3.1

Means within a line with a different superscript letters are significantly different ($p < 0.05$).
T – incubation temperature, μ – specific growth rate, R^2 – correlation coefficient, *RMSE* – root mean square deviation, λ – lag phase duration.

Tab. 2. Specific growth rates of *Lb. plantarum* in relation to temperature in lactose-free milk calculated using Baranyi and Huang models.

T [°C]	Baranyi model				Huang model			
	μ [h^{-1}]	R^2	*RMSE*	λ [h]	μ [h^{-1}]	R^2	*RMSE*	λ [h]
12	0.04^{x}	0.996	0.100	78.0	0.04^{x}	0.971	0.265	55.6
15	0.07^{x}	0.997	0.061	17.5	0.07^{x}	0.992	0.107	17.2
18	0.12^{x}	0.994	0.116	13.5	0.11^{x}	0.987	0.180	12.8
21	0.17^{x}	0.994	0.115	–	0.18^{x}	0.970	0.304	2.7
25	0.26^{x}	0.996	0.117	2.0	0.28^{y}	0.991	0.170	5.1
30	0.36^{x}	0.997	0.090	1.5	0.37^{x}	0.994	0.131	1.4
34	0.39^{x}	0.996	0.114	–	0.40^{x}	0.986	0.201	1.6
37	0.37^{x}	0.995	0.103	–	0.37^{x}	0.999	0.208	0.0
40	0.19^{x}	0.996	0.103	–	0.19^{x}	0.989	0.158	1.6

Means within a line with different superscript letters are significantly different ($p < 0.05$).
T – incubation temperature, μ – specific growth rate, R^2 – correlation coefficient, *RMSE* – root mean square deviation, λ – lag phase duration.

lactose hydrolysis on the characteristics of fermented milk. Some studies reported on a reduction of fermentation time in case of hydrolysed base milk that supposed higher growth rates of the lactic acid bacterial culture used [40, 41], whereas others observed an increase in fermentation time or rather no effects [42, 43]. Similar results were reported in our study, when specific growth rates of *Lb. plantarum* close to the optimal growth temperature range where lower compared to milk despite the content of easily fermentable glucose and galactose in the former substrate.

Recently, a suitable primary model was developed by HUANG [29] with a transition coefficient $\alpha = 4$ (or $\alpha = 25$). All 18 growth curves were re-analysed using $\alpha = 4$ (the maximum specific growth rates were not significantly different ($p < 0.05$) from either with $\alpha = 25$) by non-linear regression to obtain the kinetic parameters (μ_{max} and λ) (Eq. 4) for each growth curve. The only parameters to estimate by non-linear regression (at minimal residual sum of square) were just lag phase duration and growth rate. The estimated specific growth rates of the studied strain varied from 0.07 h^{-1} to 0.83 h^{-1} (Tab. 1) and from 0.04 h^{-1} to 0.40 h^{-1} (Tab. 2) in MRS broth and lactose-free milk, respectively. In most cases, these values were not significantly different ($p < 0.05$) from those estimated using Baranyi model (Fig. 1). According to the results, both models were equally suitable options for fitting the growth curves and calculating specific growth rates. However, the quality of data in a growth curve also affected the determination of lag phase duration (10 data points

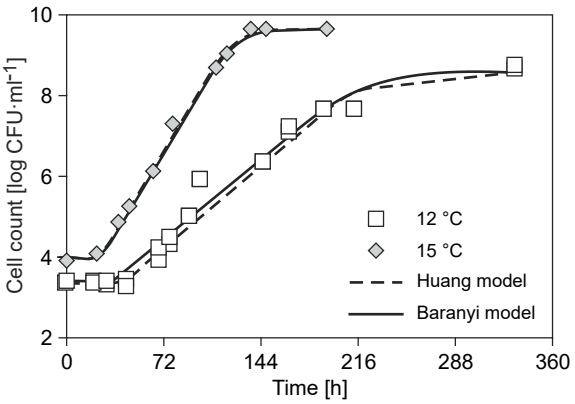

Fig. 1. Curve fitting of *Lb. plantarum* HM1 using the Huang model (α = 4) and the Baranyi model for the growth of cultures in MRS broth at 12 °C and 15 °C.

of the lag phases among the 18 data points were substantially different). The Huang model takes into account adaptation of bacteria and transition from lag phase to exponential phase after they are exposed to the new environment. Using Huang model, lag phase of each growth curve is clearly defined and is visibly distinguishable from the exponential phase of growth. If the prior history and accumulation of critical substances affects the bacterial growth, the Baranyi model is probably a better choice [29].

During the growth of culture, a decrease in pH values by about 0.17–1.16 and 2.05–2.54 was observed in lactose-free milk and MRS broth, respectively (Tab. 3). In our previous study in UHT milk (1.5 g·l^{-1} fat content), no significant changes of pH values (0.00–0.24) during the growth of *Lb. plantarum* were reported [35]. In another study done by Østlie et al. [44], *Lb. rhamnosus* GG inoculated into UHT milk supplemented in advance with 7.5 g·l^{-1} fructose, reached pH of 3.9–4.1 after 24 h of incubation at 37 °C. In our study, the fastest decrease in pH was reported at 37 °C (–0.1535 h^{-1} and –0.2699 h^{-1}) in lactose-free milk and broth, respectively. Smetánková et al. [45] reported pH values below 4.3 in MRS broth at temperatures of 30 °C, 37 °C and 45 °C during the growth of *Lb. plantarum*, with a faster decrease in pH under aerobic conditions than under anaerobic conditions in most cases.

Secondary modelling

The temperature effect on the growth rate was described by Ratkowsky square root model [31] in the whole temperature range. Individual points in Fig. 2A illustrate the observed values of the specific growth rate and the line is displayed for a model course of the influence of temperature on the growth dynamics. Comparison of predicted versus observed values of the specific growth rates is presented in Fig. 2B. Until the temperature reached the optimum level, the growth rate was increasing almost linearly with the rising incubation temperature.

By using Eq. 7, minimal and maximal temperatures as the model parameters for *Lb. plantarum* growth in different media, were calculated as follows: T_{min} = 0.9 °C, T_{max} = 41.6 °C; T_{min} = 7.1 °C, T_{max} = 41.2 °C; T_{min} = 4.7 °C, T_{max} = 41.6 °C for MRS broth, milk and lactose-free milk, respectively. To confirm these temperatures, also the CTMI model as described by Eq. 8 was used. Its graphical representation is shown in Fig. 3A and comparison of predicted versus observed values of the specific growth rates is presented in Fig. 3B. From the graphical representation it is visible that the model fitted

Tab. 3. Effect of temperature on pH values during the growth of *Lb. plantarum.*

T [°C]	Lactose-free milk			de Man Rogosa, Sharpe broth		
	Δ pH	Final pH	k_{pH}	Δ pH	Final pH	k_{pH}
12	0.17	6.28	–0.0005	2.20	4.30	–0.0152
15	0.05	6.59	0.0002	2.11	4.24	–0.0338
18	0.41	6.14	–0.0040	2.24	4.02	–0.0423
21	0.18	6.36	–0.0025	2.05	4.25	–0.0199
25	0.52	5.97	–0.0055	2.31	4.00	–0.1353
30	0.30	6.14	–0.0081	2.34	3.99	–0.1607
34	0.47	5.97	–0.0113	2.38	3.94	–0.1645
37	0.52	5.99	–0.1535	2.51	3.99	–0.2699
40	1.16	5.33	–0.0247	2.54	3.97	–0.1883

T – incubation temperature, ΔpH – difference in pH value after the incubation period, k_{pH} – rate constant for decrease of pH.

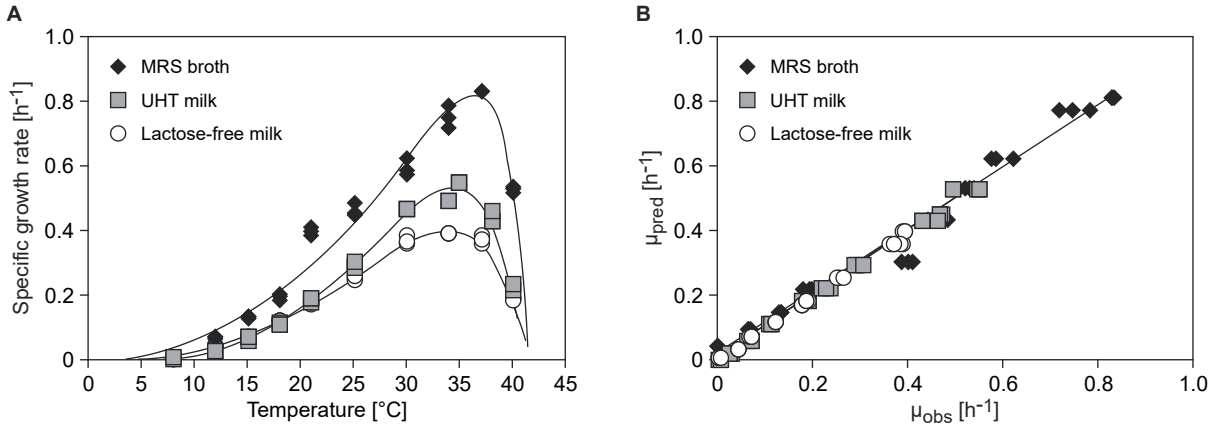

Fig. 2. Application of Ratkowsky model to specific growth rate of *Lb. plantarum*.

A – experimental data fitting, B – comparison of predicted and observed specific growth rates.
μ_{pred} – predicted specific growth rate, μ_{obs} – observed specific growth rate.

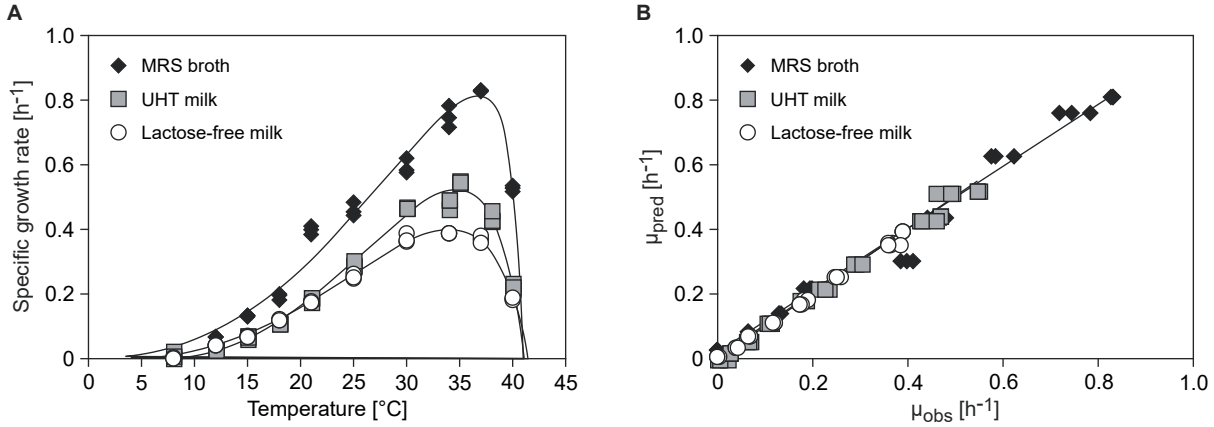

Fig. 3. Application of Cardinal temperature model with inflection to specific growth rate of *Lb. plantarum*.

A – experimental data fitting, B - representation of predicted against observed specific growth rates.
μ_{pred} – predicted specific growth rate, μ_{obs} – observed specific growth rate.

the experimental data very well for all substrates (R^2 = 0.989). A good prediction of this model was also reported in a study of MEDVEĎOVÁ et al. [38, 46] for *Lb. acidophilus* NCFM and *E. coli* BR, respectively. Since the parameters of CTMI model are based on their biological interpretation and due to the lack of structural correlation between parameters, the simple and accurate estimation of cardinal temperature values is facilitated [32]. According to CTMI model, the optimal conditions in MRS broth for *Lb. plantarum* growth were expected at 36.6 °C, in milk at 34.7 °C and in lactose-free milk at 34.2 °C. Under the optimal temperature conditions, the last parameter of the CTMI model provided the maximal specific growth rates of 0.81 h^{-1}, 0.52 h^{-1} and 0.40 h^{-1} for MRS broth, milk and lactose-free milk, respectively. This approach can be used in dairy practice after recalculation to a generation time of 51 min, 80 min and 104 min in broth, milk and lactose-free milk, respectively. A closer look at both models revealed satisfactory results with respect to different modelling techniques. The narrow range of each cardinal temperature for *Lb. plantarum* could be expected even in such different media as milk and MRS broth. Moreover, the studied strain grows under different conditions, affected by temperature and media composition, in a food processing plant, with defined errors in expectation, taking into account the calculated discrepancies.

As shown in Tab. 4, a good fit between the predictions with a discrepancy factor (*Df*) of 0.8–4.2 % according to BARANYI et al. [47] was achieved, which is in agreement with our previous works [48–50]. Taking the *Df* (0.8–3.9 %) and *SEP* (4.2–8.0 %) values into account, cardinal

Tab. 4. Statistical indices for the growth of *Lb. plantarum* fitted by various models.

	Df [%]	*SEP* [%]	*RSS*	*RMSE*
Ratkowsky model				
Broth	4.2	11.0	0.050	0.044
Milk	1.4	5.7	0.006	0.015
Lactose-free milk	0.8	4.6	0.002	0.009
Cardinal temperature model with inflection				
Broth	3.9	7.2	0.044	0.038
Milk	1.9	8.0	0.010	0.019
Lactose-free milk	0.8	4.2	0.002	0.008

D_f – discrepancy factor, *SEP* – standard error of prediction, *RSS* – the sum of the squared residuals, *RMSE* – root mean square error.

temperature model with inflection exhibited the best data fit. As experienced by VALÍK et al. [51], CTMI model provided the best statistical indices. However, the predicted growth rates of *Lb. plantarum* using Ratkowsky model can be still considered as acceptable (*Df* varied from 0.8 % to 4.2 %).

CONCLUSIONS

Lb. plantarum is widely used in various kinds of food fermentations. In order to provide information on growth of certain relevant strains introduced to fermentation of fruit juices and plant-based or dairy-based products, performance of two secondary models was evaluated against a set of experimentally determined data. In a detailed comparison of predictive models, both applied models were suitable for estimation of *Lb. plantarum* growth, while CTMI was a little more accurate in prediction with percent discrepancies within 0.8 % to 3.9 %. Prediction of growth parameters has practical application in shelf life estimation, whereas food safety and quality still provide relevant challenges for predictive microbiology, one of which is the growth description of lactic acid bacteria during food fermentation of food matrices.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0006 and Vega No. 1/0363/19.

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Received 3 October 2018; 1st revised 26 October 2018; accepted 31 October 2018; published online 20 March 2019.