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Characterization of the growth of *Lactobacillus plantarum* in milk in dependence on temperature

Zuzana Matejčeková, Denisa Liptáková, Sabína Spodniaková, Ľubomír Valík

Department of Nutrition and Food Quality Assessment, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK 812 37, Bratislava
zuzana.matejcekova@stuba.sk

Abstract: The effect of temperature on the growth dynamics of *Lactobacillus plantarum* in the model environment of UHT milk was investigated. Based on the experiments between temperatures ranged from 8 to 40 °C, growth dynamics of the studied isolate was positively determined by the cultivation temperature that led to increasing intensity of growth in the exponential phase (except for temperatures 38 and 40 °C). Final counts in stationary phase have reached concentrations about 10^7 CFU · ml^{-1} from initial 10^3 CFU · ml^{-1}, except for marginal 8 and 40 °C. Experimentally, it was found, that optimal temperature was close to 37 °C, where the fastest specific growth rate in an exponential phase was recorded (μ = 0.7683 h^{-1}, t_d = 54 min) and the stationary phase was reached after 24 h of incubation. During the growth and multiplication of *Lb. plantarum*, no significant decrease of pH values in comparison to initial ones in dependence on temperature were determined (0.00—0.24 units).

Keywords: lactic acid bacteria, *Lactobacillus plantarum*, predictive microbiology

Introduction

Genus *Lactobacillus* is by far the largest of the genera included in a group of lactic acid bacteria (LAB) (Tannock, 2004) and is recognized as being phylogenetically very heterogeneous (broad interval of % G-C content). Lactobacilli are characterized as gram-positive, microaerophilic, non-sporeforming, non-flagellated rods or coccobacilli found in diverse environments, including nutrient-rich dairy products, microbial-heavy host habitats (human mucosal surfaces), as well as natural ecological niches (Barrangou et al., 2011). In humans and animals, lactobacilli are considered as essential components, playing a large variety of health promoting functions, such as immunomodulation, intestinal integrity and pathogen resistance (Vaughan et al., 2005). For those reasons some strains have traditionally been used as probiotics in various food commodities (Smetánková et al., 2012). In many cases, lactobacilli occupy a central role in fermentation processes as a starter cultures with a long and safe history since they contribute to the conservation, flavour and texture of the final products. While the fermentative conversion of carbohydrates present in the raw materials mainly into lactic acid is the main function, production of anti-microbial peptides, exopolysaccharides and a variety of other metabolites are other important properties of this genus (Vries et al., 2006). Within the genus *Lactobacillus, Lactobacillus plantarum* is a member of facultatively heterofermentative group of lactobacilli. According to Embden-Meyerhof-Parnas pathway it is able to convert hexoses into lactic acid (Arasu et al., 2015). *Lb. plantarum* has also the coding capacity for uptake and utilization of many different sugars, uptake of peptides, and formation of most amino acids. The large number of surface-anchored proteins suggests that *Lb. plantarum* has the potential to associate with many different surfaces and potential substrates for growth. In addition, the relatively high number of genes encoding regulatory functions indicated the ability to adapt to many different conditions. Taken all together, this reflects the potential of *Lb. plantarum* to grow in a large range of environmental niches (Kleerebezem et al., 2003). It is commonly found in the human gastrointestinal tract and can be involved in a variety of dairy, meat, and vegetable fermentations. Furthermore, *Lb. plantarum* can be involved in spoilage of foods, such as meat or wine (Vries et al., 2006). Although the technologically important parameters like optimal pH and temperature for industrially used strains are well known, the alterations in the quantitative growth characteristics like specific growth rate (μ) with changing environmental factors are relatively poorly studied.

As temperature represents an important factor in the microorganism growth, in control of bioprocesses in biotechnology and safe handling of goods, especially in food industry, describing the temperature effect on the microbial growth parameters is required.

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$$\mu_{max} = \frac{\mu_{opt}\left(T - T_{max}\right)\left(T - T_{min}\right)^2}{\left(T_{opt} - T_{min}\right)\left[\left(T_{opt} - T_{min}\right)\left(T - T_{opt}\right) - \left(T_{opt} - T_{max}\right)\left(T_{opt} + T_{min} - 2T\right)\right]} \quad (1)$$

That is why this work deals with the quantification of temperature effect on the growth of *Lb. plantarum* in real growth media.

Material and methodology

Microorganism

Lactobacillus plantarum was isolated from breast milk and identification was provided by the Food Research Institute in Bratislava, Slovakia (Liptáková et al., 2016). The isolate of *Lb. plantarum* was maintained in de Man Rogosa Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France) at 6 ± 0.5 °C.

Substrate inoculation and conditions of cultivation

The standard suspension of the microorganism 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 ultra-pasteurised (UHT) milk with 1.5 % fat content (Rajo, Bratislava, Slovakia) in a concentration of approximately 10^3 CFU · ml^{-1}. Two parallel static cultivations of UHT milk samples were carried out at temperatures ranging from 8 to 40 ± 0.5 °C under aerobic conditions.

Enumeration of bacteria and determination of active acidity

Serial ten-fold dilutions of samples were prepared in a solution of 0.85 % NaCl (w/v) and 0.1 % (w/v) peptone (Biolife, Milan, Italy) at appropriate time intervals. Presumptive numbers of *Lb. plantarum* were estimated using MRS agar (Biokar Diagnostics, Beauvais, France) according to the STN ISO 15214. Inoculated Petri dishes with *Lb. plantarum* were cultivated at anaerobic conditions 37 ± 0.5 °C (5 % CO_2) for 48 hours. The pH values were measured at the same time as the microbiological determination using the pH meter WTW 720 (Inolab, Weilheim, Germany).

Evaluation of growth curves and metabolic parameters

The growth parameters of studied microorganism in inoculated UHT milks were fitted and calculated using the mechanistic model DMFit by Baranyi and Roberts (1994). Growth and metabolic parameters were calculated from each growth curve, from two parallel experiments. Valík et al., (2008) evaluated that the coefficient of variation (CV) of the growth rates for 15 strains of *Staphylococcus aureus* was as low as 7.1 %, despite the errors connected with cultivation methods of determination microbial counts. When 6 growth curves for a single strain were studied in two independent experiments, a CV of 1.2 % for the growth rate was determined.

Specific growth rates μ (h^{-1}) were recalculated from the $\log_{10}$ based growth rates (G_R) according the equation $\mu = \ln 10 \cdot G_R$.

The cardinal temperature model with inflection (CTMI) was used to describe the influence of selected environmental factor on the data. The effect of temperature on the specific growth rate is described by the equation (1), where T_{min} (°C) is the temperature below which no growth is observed, T_{max} (°C) is the temperature above which no growth occurs and T_{opt} (°C) is the temperature at which the maximum specific growth rate equals its optimal value (μ_{opt}) (Rosso et al., 1993).

Results and discussion

Growth trials with *Lb. plantarum* in UHT milk were performed at 8, 12, 15, 18, 21, 25, 30, 34, 35, 37, 38 and 40 ± 0.5 °C. A temperature range was selected in order to detect the entire growth ability of the microorganism. Growth curves at all temperatures are shown in Fig. 1; at 8, 12, 15, 18, 21 and 25 at Fig. 1a) and 30, 34, 35, 37, 38 and 40 at Fig. 1b). Calculated growth parameters at 8, 12, 15, 18, 21, 25 ± 0.5 °C are summarized in Table 2 and 30, 34, 35, 37, 38 and 40 ± 0.5 °C in Table 3.

During the growth and multiplication of *Lb. plantarum* no significant changes of pH values (Tab. 1) were recorded in comparison to initial state in dependence on temperature (0.00—0.24 units). This can be explained by the low ability of *Lb. plantarum* to utilize lactose and convert pyruvate to lactate in a rate to match the glycolysis (Jyoti et al., 2004). Smetanková et al., (2012) reported faster decrease of pH values in MRS broth under anaerobic than under aerobic conditions in case of *Lb. plantarum* 115. In comparison, Salmerón et al. (2014) evaluated the growth and metabolism of *Lb. plantarum* in cereal beverages (oat, barley and malt substrates), where pH values decreased below 3.7 after 10 h of fermentation.

Despite the slight acid production, *Lactobacillus plantarum* showed good growth in UHT milk with specific growth rates ranging between 0.0049 to 0.7683 h^{-1}. In the stationary phase, *Lb. plantarum* reached counts in average 10^7 CFU · ml^{-1} at studied temperatures, except for marginal ones (8 and 40 °C) from initial 10^2—10^3 CFU · ml^{-1}.

Tab. 1. The effect of temperature on pH values in UHT milk.

Temperature [°C]	pH_0	pH_{end}	k_{pH} [h^{-1}]
8	6.60	6.67	0.0001
12	6.72	6.51	–0.0036
15	6.70	6.54	–0.0028
18	6.58	6.48	–0.0022
21	6.64	6.40	–0.0019
25	6.59	6.52	–0.0016
30	6.48	6.36	–0.0068
34	6.45	6.45	–0.0003
35	6.50	6.33	–0.0038
37	6.53	6.40	–0.0161
38	6.48	6.37	–0.0034
40	6.58	6.35	–0.0022

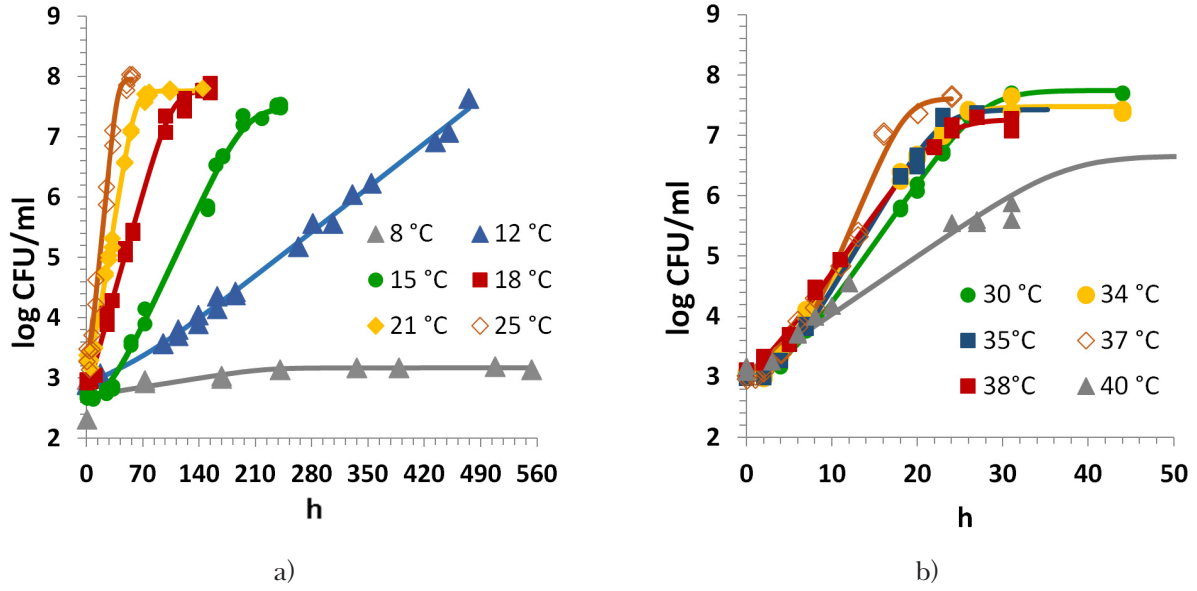
pH_0 – initial pH value, pH_{end} – final pH value, k_{pH} – rate constant for the decrease of pH.

Similar results reported Pelikánová et al., (2011) in *Lactobacillus paracasei* subsp. *paracasei* 1753 in UHT milk. At the lowest temperature (8 °C), *Lb. plantarum* grew, although slowly (μ = 0.0049 h^{-1}; t_d = 141.5 h) without previous lag-phase. In this case, the experiment lasted for 23 days. Valík et al., (2008) in their study reported about 93 % higher specific growth rate in *Lb. rhamnosus* GG in UHT milk (0.069 h^{-1}) that reached stationary phase after 9 days of incubation. Increase of incubation temperature (12 °C) resulted into increase of specific growth rate (0.0242 h^{-1}) and the stationary phase was reached on the 20th day of aerobic cultivation. At the temperature of 15 °C, the specific growth rate increased about 63 % in comparison to 12 °C and about 93 % than at 8 °C. At 15 °C, specific growth rate of *Lb. plantarum* was characterized as the same as the specific growth rate of *Lactobacillus rhamnosus* VT1 evaluated in study Liptáková et al., (2007).

At 18 °C, the stationary phase was reached after 6 days of incubation (μ = 0.1082 h^{-1}), and the maximal density of *Lb. plantarum* was 7.8 log counts. Aerobic cultivation of *Lb. plantarum* at 21 °C decreased lag phase duration almost 10 times in comparison with that at 12 °C. Pelikánová et al., (2011) observed specific growth rate of *Lactobacillus paracasei* subsp. *paracasei* in UHT milk 0.216 h^{-1} at 21 °C that represents 14 % difference in comparison with our results.

Similar trends were observed at temperatures ranging from 30 °C to 37 °C with an acceleration of the specific growth rate upon increasing the incubation temperature and the lag phase duration was shortened (Table 3). The time at which *Lb. plantarum* reached stationary phase also decreased. At 37 °C, the shortest time necessary to reach the stationary phase (24 h) was achieved and the maximal specific growth rate was observed (0.7683 h^{-1}). With further increasing the incubation temperature, this time was prolonged. The given experimental temperature was expected to be optimal for the growth and multiplication of *Lb. plantarum*.

Within the last selected temperature (40 °C) the specific growth rate decreased by about 62 % in comparison to 37 °C, during which *Lb. plantarum* reached the stationary phase after 4 days of incubation (N_{max} = 6.78 log CFU · ml^{-1}). Valík et al., (2008) reported about 88 % higher specific growth rate in case of *Lb. rhamnosus* GG at 41 °C (1.978 h^{-1}).



a) b)

Fig. 1. Growth dynamics of *Lb. plantarum* with respect to incubation temperature.

Tab. 2. Growth characteristics of *Lb. plantarum* at 8, 12, 15, 18, 21, 25 ± 0.5 °C.

Temperature [°C]	μ [h^{-1}]	N_0 [log CFU·ml^{-1}]	N_{max} [log CFU·ml^{-1}]	λ [h]	t_d [h]
8	0.0010*	2.97	3.14	–	693.1
8	0.0212*	2.32	3.14	–	32.7
8	0.0049°	2.65	3.14	–	141.5
12	0.0247	2.90	7.64	51.7	28.1
12	0.0237	2.88	7.64	32.2	29.2
12	0.0242	2.89	7.64	42.3	28.6
15	0.0583	2.73	7.54	22.3	11.9
15	0.0679	2.67	7.48	29.6	10.2
15	0.0661	2.70	7.41	25.7	10.5
18	0.1114	2.92	7.88	–	6.22
18	0.1053	2.97	7.78	–	6.58
18	0.1082	2.95	7.81	–	6.41
21	0.1739	3.30	7.69	–	3.98
21	0.1869	3.38	7.74	4.9	3.70
21	0.1860	3.34	7.72	4.3	3.73
25	0.2879	3.28	8.04	–	2.41
25	0.2841	3.49	8.00	–	2.44
25	0.3039	3.39	8.02	2.3	2.28

μ – specific growth rate, N_0 – initial numbers of *Lb. plantarum*, N_{max} – numbers in stationary phase, λ – lag phase duration, t_d – time to double, *data from 2 parallel experiments, °data from results of 2 parallel experiments.

Tab. 3. Growth characteristics of *Lb. plantarum* at 30, 34, 35, 37, 38, 40 ± 0.5 °C.

Temperature [°C]	μ [h^{-1}]	N_0 [log CFU·ml^{-1}]	N_{max} [log CFU·ml^{-1}]	λ [h]	t_d [h]
30	0.4653*	3.00	7.71	3.7	1.49
30	0.4669*	2.99	7.70	4.0	1.48
30	0.4658°	3.00	7.70	3.8	1.49
34	0.4602	3.07	7.38	–	1.51
34	0.4922	3.04	7.43	3.3	1.41
34	0.4899	3.06	7.41	3.7	1.41
35	0.5485	2.98	7.34	3.5	1.26
35	0.5434	3.04	7.46	3.9	1.28
35	0.5461	3.01	7.36	3.7	1.27
37	0.7441	3.02	7.65	2.1	0.93
37	0.7443	2.96	7.62	2.0	0.90
37	0.7683	2.99	7.64	2.1	0.90
38	0.4249	3.11	7.08	–	1.63
38	0.4282	3.08	7.28	–	1.62
38	0.4568	3.09	7.18	1.50	1.52
40	0.2133	3.18	6.83	–	3.24
40	0.2316	3.08	6.72	–	2.99
40	0.2224	3.13	6.78	8.0	3.11

μ – specific growth rate, N_0 – initial numbers of *Lb. plantarum*, N_{max} – numbers in stationary phase, λ – lag phase duration, t_d – time to double, *data from 2 parallel experiments, °data from results of 2 parallel experiments.

In a study of Medveďová et al., (2016) *Lactobacillus acidophilus* NCFM in UHT milk reached about 73 % higher specific growth rate (0.827 h^{-1}) in comparison with our results at 40 °C.

Conclusion

Since the growth of microorganisms is strain dependent, it is necessary to know the growth dynamics of specific strains in dependence on environmental factors. Thus, the growth responses of the potentially probiotic *Lactobacillus plantarum* in UHT milk as affected by the incubation temperature were studied. *Lb. plantarum* showed good growth properties in the temperature range from 8 to 40 °C. Cardinal temperatures using CTMI model (T_{opt} = 34.7 °C, T_{min} = 7.8 °C, T_{max} = 41.1 °C) were evaluated with μ_{opt} = 0.522 h^{-1}. Due to the pH stability during its growth at evaluated incubation temperatures, could be added into pasteurized milk. In this case, pasteurized milk could be a carrier of potentially probiotic microorganism, because any significant pH changes were not recorded at all temperatures. For such a purpose it is important, that growth dynamics of used isolate in the applications is known.

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