Prediction of temperature effect on growth of two raw milk cheese isolates of *Escherichia coli* in milk

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Summary
Knowledge on behaviour of pathogenic, opportunistic pathogenic or indicator organisms related with food intrinsic and extrinsic environmental factors is of utmost importance at evaluation of safety and quality of foods. A frequent microbial food contaminant is *Escherichia coli*, which includes common saprophytic as well as a group of pathogenic strains. The aim of this study was to characterize and validate growth of *E. coli* in the entire range of temperatures allowing growth, based on culturing experiments. The growth dynamics was modelled by the Ratkowsky model and cardinal temperature model with inflection (CTMI). The following cardinal values of temperature were determined by CTMI: minimal T_{min} = 3.7 °C, optimal T_{opt} = 40.8 °C, maximal T_{max} = 46.6 °C. Gibson model was used for prediction of the time to increase counts, e.g. by 3 log, for an *E. coli* isolate within the temperature range allowing growth. The prediction data were validated and can be used for growth assessment in artisanal raw milk-based products.

Keywords
Escherichia coli; predictive microbiology; growth; cardinal temperature

Consumption of raw milk cheeses including the fresh (short ripened) ones has a long tradition in the human diet. Although mature raw milk cheeses and those produced from pasteurized milk are considered as safe, some food-borne outbreaks were reported in connection with traditional raw milk cheese consumption [1, 2]. Defenders of pasteurization advocate managing the pathogen risk by applying heat treatment to reduce the microbial load and standardizing the production by inoculation of a few selected strains into milk. On the contrary, defenders of traditional raw milk cheeses recommend maintaining the high microbial diversity in indigenous cheeses arguing that the high diversity of microbial activities is the key for allowing traditional cheeses to develop their particular characteristics, including low pathogen risk [3]. Among the most frequent pathogens causing food-borne outbreaks from cheese consumption are *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus* and also *Escherichia coli*.

The importance of *E. coli* as a cause of diseases both in man and animals increased in the European Union since 2008 and was further strengthened due to the outbreak in Germany in summer 2011. Besides food-borne diseases, enteric diseases or enterohemorrhagic fever, *E. coli* strains are also associated with diarrheal disease in food-producing animals and, moreover, they can represent a threat to humans either by primary or secondary contamination of food or by a direct contact [4]. Pathogenic strains can be present in foods in low prevalence, but saprophytic *E. coli* can occur much more frequently. This is due to its commensally relationship with the intestines of humans and other mammals, and it is commonly present in raw milk, processed milk and dairy products. Raw milk, insufficiently pasteurized or secondary contaminated pasteurized milk are the most frequent sources of *E. coli* [5, 6]. The possibility of *E. coli* transmission through the consumption of raw milk as well as raw milk dairy products was repeatedly documented [1] and that is why the limits for *E. coli* in milk products were set by the EU Regulation.

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The limit for *E. coli* in cheeses manufactured from raw milk or heat-treated whey is 100 CFU·ml^{-1} [7]. Level of *E. coli* contamination in raw milk on the farm is 4.2–10 % [2] and its density in milk samples is sometimes lower than 10^2 CFU·ml^{-1}, but mostly about 10^3–10^4 CFU·ml^{-1} [8]. Chye et al. [9] and Lues et al. [10] detected *E. coli* in 23–65 % of milk samples in the range of 10^4–10^6 CFU·ml^{-1} and, according to Ntuli et al. [11], approximately 10 % of samples were presumptively positive for shigatoxin-producing *E. coli*. If *E. coli* is present in raw milk, it can easily contaminate milking machines and other equipment, and spread contamination to milk and dairy products. Recently, the shigatoxin-producing *E. coli* was isolated also from mozzarella cheese, which is produced with application of hot water at 90 °C [12]. In Slovakian raw milk cheeses, *E. coli* was detected in 20–33 % of samples [13]. The prevalence of *E. coli* in raw milk and raw milk cheeses outside Europe is even higher, ranging from about 32–57 % in India, 26–75 % in Pakistan and Iran, 66–75 % in South Africa and Egypt to almost 96 % in Brazil [14].

From the food technology point of view, the presence of *E. coli* causes early swelling of cheeses with a low-temperature-treated curd (36–40 °C), due to residual lactose fermentation. The fate of heat-labile *E. coli*, but also of other pathogens, in raw milk cheeses depends on many intrinsic and extrinsic environmental factors, from which the temperature conditions during coagulation or fermentation process of cheese curd are crucial. For this reason, knowledge on growth dynamics of *E. coli* isolated from raw milk cheese in milk in relation to temperature is needed. Moreover, most available secondary models describing the effects of main intrinsic and extrinsic food environmental factors on the growth rate of *E. coli* were determined for strains isolated from meat, for pathogenic strains and/or were determined based on turbidity data [15–18]. The aim of this study was to quantitatively characterize the growth of an *E. coli* isolate in milk, based on culturing experiments with subsequent processing of data by predictive microbiology methods. Various predictive models were used to compare the prediction precision. Also, validation with external data was performed to define the reliability of the models to predict the growth of an *E. coli* isolate of food origin.

MATERIALS AND METHODS

Microorganisms

E. coli BR (isolate BR) and *E. coli* LC (isolate LC) were isolated from a Slovakian traditional cheese "Bryndza" and from a ewes' lump cheese (Slovakia), respectively. Their identity was confirmed by Gram staining, the COLItest and the ENTEROtest 24 (Lachema, Brno, Czech Republic), typical growth on the Chromocult agar and on the eosin methylene blue (EMB) agar (both Merck, Darmstadt, Germany). Additionally, the polymerase chain reaction (PCR) identification was performed according to Breum and Boel [19] together with detection of *vtx1*, *vtx2*, *ehx* and *eae* genes. Fermentation tests for utilization of lactose, glucose and saccharose, expressed as production of acids and gas after 24 h at 37 °C, were performed in peptone water with 0.2 g·l^{-1} addition of individual sugars.

Inoculation and culture conditions

The isolates were maintained in brain hearth infusion (BHI) broth (Sigma-Aldrich, St. Louis, Missouri, USA) at 5 °C ± 1 °C prior to analysis. A standard suspension of the isolate was prepared from a 24 h old culture grown in BHI broth at 37 °C. This suspension was inoculated aseptically into 300 ml of pre-tempered ultra high temperature-treated cows' milk (1.5 % fat content, pH 6.7; Rajo, Bratislava, Slovakia) in order to reach as constant initial *E. coli* counts in each sample as possible (approximately 10^3 CFU·ml^{-1}). Sterility of the milk prior to inoculation was confirmed by plating on plate count agar (PCA; Sigma-Aldrich) according to EN ISO 4833-1:2013 [20]. The static incubation of milk samples inoculated with isolate BR isolate was performed at temperatures 8, 10, 12, 15, 18, 21, 25, 30, 35, 37, 40, 43 and 46 °C ± 0.5 °C, in three replicates. The isolate LC was used to validate the growth parameters of *E. coli* BR in milk in a similar way in a temperature range of 10–37 °C.

Counts of *E. coli* in milk

Counts of *E. coli* were determined at time intervals by ten-fold dilution in 0.85 % saline and 0.1 % peptone solution with subsequent cultivation on PCA according to EN ISO 4833-1:2013 [20], using incubation temperature of 37 °C.

Fitting the growth curves and calculating the growth parameters

The growth data, curves and parameters of isolates under study were analysed, fitted and calculated, respectively, using the mechanistic modelling technique of Baranyi and Roberts [21]. The counts were plotted against time and fitted to a model for the estimation of the growth rate (Gr) and the initial (N_0) and maximal (N_{max}) density using an in-house Excel Add-in package 'DMFit'

version 3.5 (ComBase managed by United States Department of Agriculture-Agricultural Research Service, Washington D.C., USA and University of Tasmania Food Safety Centre, Hobart, Australia). The growth parameters from each individual growth curve were analysed in the secondary phase of modelling by statistic tools of the Microsoft Office version 2007 (Microsoft, Redmond, Washington, USA) and the Statistica data analysis software system, version 7.1 (StatSoft, Tulsa, Oklahoma, USA).

Secondary models

The cardinal temperature model with inflection (CTMI) was used to describe the effect of temperature on growth rates of the isolates under study. The effect of temperature on *Gr* is described by the equation:

$$Gr = Gr_{\text{opt}} \frac{a}{b \times c} \quad (1)$$
$$a = (T - T_{\text{max}})(T - T_{\text{min}})^2$$
$$b = (T_{\text{opt}} - T_{\text{min}})$$
$$c = (T_{\text{opt}} - T_{\text{min}})(T - T_{\text{min}}) - -(T_{\text{opt}} - T_{\text{max}})(T_{\text{opt}} + T_{\text{min}} - 2T)$$

where T is actual incubation temperature, T_{min} is the notional temperature below which the growth is not observed, T_{max} is the notional temperature above which the growth is not observed and T_{opt} is the temperature at which the maximal growth (Gr_{opt}) is observed [22]. The advantage of the model is the definition of cardinal values of temperature for the growth of bacteria.

Values of minimal and maximal growth temperature were subsequently used in the model of Ratkowsky et al. [23], which enables data in the super-optimal temperature range to be used. The model is described by the equation:

$$\sqrt{\mu} = b(T - T_{\text{min}})\left[1 - exp^{c(T - T_{\text{max}})}\right] \quad (2)$$

where b and c are regression coefficients, T_{min} and T_{max} have the same interpretation as in Eq. 1 and μ is the maximal specific growth rate calculated according to the following equation:

$$\mu = Gr \times \ln 10 \quad (3)$$

With the intention to predict the time needed to increase *E. coli* counts by 1, 2, 3 or 4 log at selected incubation temperatures, a useful application of Gibson model was used. Firstly, the maximal specific growth rate was modelled as a function of the incubation temperature. For that purpose, and inspired by the water activity (a_w) transformation originally introduced by Gibson et al. [24], the following transformation of temperature (T_w) according to Eq. 4 was applied:

$$T_w = \sqrt{(T_{\text{max}} - T)} \quad (4)$$

T_{max} was given by CTMI and was also confirmed by the estimation from data points in the high temperature region as recommended by Ratkowsky et al. [23]. The natural logarithm of the specific growth rates was modelled by the following quadratic function as introduced by Gibson et al. [24]:

$$\ln \mu = C_0 + C_1 T_w + C_2 T_w^2 \quad (5)$$

The coefficients C_0, C_1 and C_2 were estimated by linear regression. Then, the predictions of time ($\ln t_x$) in dependence on incubation temperature T were performed according to the equation:

$$\ln t_x = \frac{x}{\mu} \quad (6)$$

where $x = 1, 2, 3,...$ are counts of *E. coli* expressed as decadic logarithm of colony forming unit per millilitre, and t_x is the time needed to increase counts of *E. coli* by 1, 2, 3 or 4 log.

Validation of the models

To evaluate goodness of fit of the mathematical equations describing *E. coli* BR response to various temperatures, several mathematical and statistical indices were used. The ordinary least-squares criterion and regression coefficient (R^2) were used to fit models to the data. As a measure of the goodness of the fit of the model, percent variance (V) and mean square error were used, as introduced by Daughtry et al. [25], and standard error of prediction as introduced by Zurera-Cosano et al. [26]. Finally, accuracy, bias and discrepancy factors, as introduced by Baranyi et al. [27], were used to validate models with new or predicted data.

RESULTS AND DISCUSSION

Growth of *E. coli* in milk in dependence on incubation temperature

To describe the growth ability of *E. coli* BR, experiments in milk were carried out at temperatures from 8 °C to 46 °C, with the aim to cover the whole temperature range in which the strain was able to grow. The growth curves are shown in Fig. 1. The average initial counts of *E. coli* BR (N_0)

in all experiments were (3.12 ± 0.34) log CFU·ml^{-1} (V = 11.2 %). All the growth curves were characterized by a typical sigmoid shape and were successfully fitted with the model of Baranyi and Roberts [21] at R^2 = 0.983 ± 0.039. At 8 °C, the lowest temperature used, the strain still grew but very slowly. At the rate of 0.005 log CFU·ml^{-1}, they increased by 1 log after 10 days. However, Wang et al. [28] and Van Derlinden and Van Impe [29] estimated minimal growth temperature for their *E. coli* strains in the range of 7.75–8.44 °C. For comparison, Wang et al. [28] also determined that *E. coli* survived at 5 °C for 28 days and grew at 8 °C after 4 days of lag phase in unpasteurized and pasteurized milk, while Sommers et al. [30] defined the temperature of 5.1 °C as the minimal temperature for growth of uropathogenic *E. coli*. On contrary, strain *E. coli* BR in this study was unable to survive at 7 °C and, after 4 days, the cells started to die.

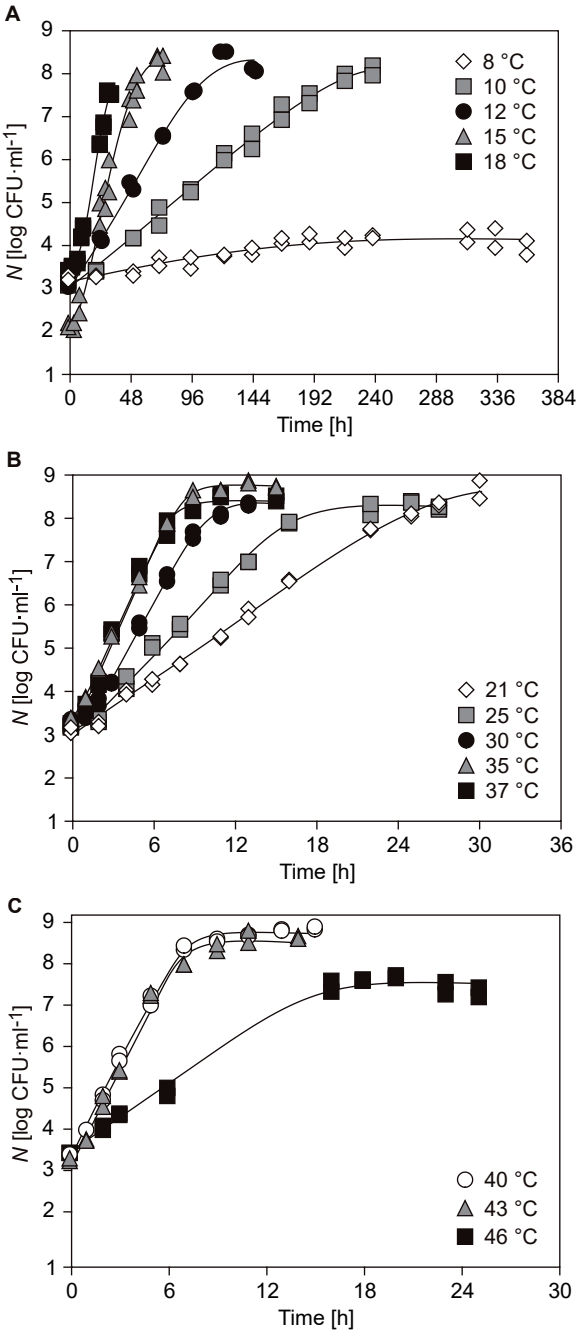

Fig. 1. Growth dynamics of *Escherichia coli* BR in milk in relation to incubation temperature.

A – temperature range 8–18 °C, B – temperature range 21–37 °C, C – temperature range 40–46 °C.
N – counts of *E. coli*.

When the incubation temperature increased to 10 °C, the growth was characterized by a specific growth rate 5-times higher compared to the previous experiment at 8 °C, and maximal culture densities exceeded 8 logs in stationary phase. Naturally, the higher the incubation temperature in the range from 10 °C to 43 °C, the faster growth in exponential phase was noticed (Tab. 1) and the culture densities in stationary phase varied around the average value of (8.46 ± 0.22) log CFU·ml^{-1} (V = 2.6 %). At 46 °C, the growth of isolate decelerated (Fig. 1C), as demonstrated also by the lower N_{max} of (7.51 ± 0.08) log CFU·ml^{-1}.

Secondary modelling

Microbial growth curve that represents the growth of a bacterial culture in time can be simply divided into lag phase, exponential phase and stationary phase. These are characterized by growth parameters such as lag phase duration or growth rate. From the primary growth curves of *E. coli* BR in milk, the growth rate *Gr* (expressed as logarithm of colony forming unit per millilitre and per hour) or the specific growth rate μ (expressed as reciprocal hours) were derived by DMfit tools, their average values calculated from 3 replicate curves at each temperature are summarized in Tab. 1. Individual data were subsequently used in secondary phase of predictive modelling and graphical presentations. Each part of the growth curve is also influenced by environmental factors or by conditions prior to growth analysis, so the secondary models represent an essential approach to describe the influence of the selected factors on microbial growth.

Firstly, the empirical CTMI that is described by Eq. 1 was created. The predicted effect of the incubation temperature on the growth rate of *E. coli* BR in milk is presented in Fig. 2A. It can be seen that the model fitted the experimental data very well. The following cardinal temperatures were estimated by this model: T_{min} = 3.7 °C, T_{max} = 46.6 °C and T_{opt} = 40.8 °C. Moreover,

Tab. 1. Growth parameters of *Escherichia coli* BR in milk in dependence on incubation temperature.

T [°C]	Gr [log CFU·ml^{-1}·h^{-1}]	N_0 [log CFU·ml^{-1}]	N_{max} [log CFU·ml^{-1}]	ΔN [log CFU·ml^{-1}]
8	0.005 ± 0.001	3.17 ± 0.06	4.14 ± 0.13	0.97 ± 0.19
10	0.024 ± 0.001	2.99 ± 0.12	8.23 ± 0.02	5.24 ± 0.11
12	0.049 ± 0.001	3.00 ± 0.08	8.31 ± 0.02	5.31 ± 0.05
15	0.120 ± 0.005	2.12 ± 0.01	8.28 ± 0.07	6.16 ± 0.07
18	0.149 ± 0.001	3.44 ± 0.02	7.71 ± 0.03	4.27 ± 0.01
21	0.218 ± 0.004	2.96 ± 0.02	8.74 ± 0.28	5.78 ± 0.26
25	0.335 ± 0.008	3.16 ± 0.13	8.27 ± 0.04	5.11 ± 0.12
30	0.571 ± 0.009	3.33 ± 0.08	8.36 ± 0.02	5.03 ± 0.10
35	0.684 ± 0.013	3.35 ± 0.07	8.73 ± 0.04	5.38 ± 0.11
37	0.720 ± 0.032	3.04 ± 0.41	8.40 ± 0.03	5.36 ± 0.44
40	0.770 ± 0.012	3.30 ± 0.02	8.74 ± 0.02	5.44 ± 0.04
43	0.779 ± 0.005	3.13 ± 0.05	8.53 ± 0.06	5.40 ± 0.01
46	0.275 ± 0.010	3.43 ± 0.01	7.51 ± 0.08	4.08 ± 0.08

T – incubation temperature, Gr – growth rate, N_0 – initial density of *E. coli* BR, N_{max} – density of *E. coli* BR in the stationary phase, ΔN – growth increment of *E. coli* BR in stationary phase against initial density.

under optimal temperature conditions, the growth rate of 0.804 log CFU ml^{-1}·h^{-1} (or expressed as $\mu = 1.851$ h^{-1} or as doubling time $t_d = 22.5$ min) can be useful for microbiologists and technologists in dairy practice. The advantage of CTMI is that it provides all the cardinal parameters with the simple biological meaning, since the settings of the model parameters are based on their biological interpretation and there is also no structural correlation between them [22]. Similar values of cardinal temperatures were obtained for 9 strains of *E. coli* studied by Salter et al. [18]. The nine strains from their study had T_{min} values of 4.23–6.41 °C, T_{max} values of 47.59–51.26 °C and T_{opt} values of 39.55–42.56 °C. So it can be concluded that the isolate *E. coli* BR is able to grow in a wider temperature range compared to the nine previously studied *E. coli* strains, which were all Shiga toxin-producing *E. coli* (STEC). On the other hand, Van Derlinden and Van Impe [29] calculated almost the same cardinal values, i. e. T_{max} of 46.40–47.40 °C and T_{opt} of 40.70–41.50 °C, but T_{min} of 5.67–8.44 °C was a little bit higher compared to our calculations. Their results were based on different experiments in temperature range from 7 °C to 46 °C, which produced 157 data of dependence of specific growth rate and temperature that were further used for calculation of cardinal temperatures.

For *E. coli* BR, T_{max} of 46.6 °C was also derived from two data points in the high-temperature region as recommended by Ratkowsky et al. [23]. Based on CTMI, T_{min} of 3.7 °C was used for the prediction of the temperature influence on the growth rate in the entire biokinetic range of growth temperatures according to the model of Ratkowsky et al. [23] described by Eq. 7 with R^2 of 0.972.

$$\sqrt{\mu} = 0.0408(T - T_{min})\left[1 - exp^{0.3864(T - T_{max})}\right] \quad (7)$$

Its graphical representation is shown in Fig. 3A. From Fig. 2A and Fig. 3A, it appears that both models fitted the experimental data satisfactorily. A closer look at the minimal temperature range reveals that the experimentally determined data were lower and that both models overestimated growth kinetics at temperatures lower than 15 °C. In spite of this incorrect description in suboptimal temperature range from 10 °C to 30 °C, the fail-dangerous prediction of *E. coli* growth in milk should not be expected contrary to estimations of Van Derlinden and Van Impe [29]. Based on their prediction, a fail-dangerous behaviour of *E. coli* at suboptimal temperatures of approximately 10–30 °C can be expected. On the other hand, they achieved precise prediction in the range beyond T_{opt}, contrary to our results, where both CTMI and Ratkowsky model underestimated growth of *E. coli* BR compared to experimentally obtained data.

Because we were not satisfied with the results of the first approach, we used further the model introduced by Gibson et al. [24] within an empirical approach to model the effect of the incubation temperature on the growth rate of *E. coli* BR. This

model provides also some practical applications. In the original equation, a useful water activity transformation of (Eq. 8) was used, in which the value of 1 represents maximal water activity.

$$b_w = \sqrt{(1 - a_w)} \quad (8)$$

Taking into account the effect of incubation temperature on the growth rate, analogical transformation (Eq. 4) was used. Such transformation was already used in our previous study [31]. The temperature transformation was applied with T_{max} of 46.6 °C, which had been previously derived and verified by CTMI and Ratkowsky model. In the next step, values of the specific growth rate of *E. coli* BR were plotted against the calculated T_w values and fitted with a regression model (Eq. 3) represented by Eq. 9 with good fitting in the range beyond T_{opt} (R^2 = 0.967).

$$\ln \mu = -0.296T_w^2 + 1.655T_w - 1.606 \quad (9)$$

From the food practice point of view, predictive microbiology is able to provide some easily interpretable data, such as how fast can contaminants grow in a product or when a microorganism can reach the stationary phase. As the initial counts of *E. coli* in milk can vary, and also the ability of *E. coli* to multiple is dependent on the temperature or accompanying microbiota, the maximal

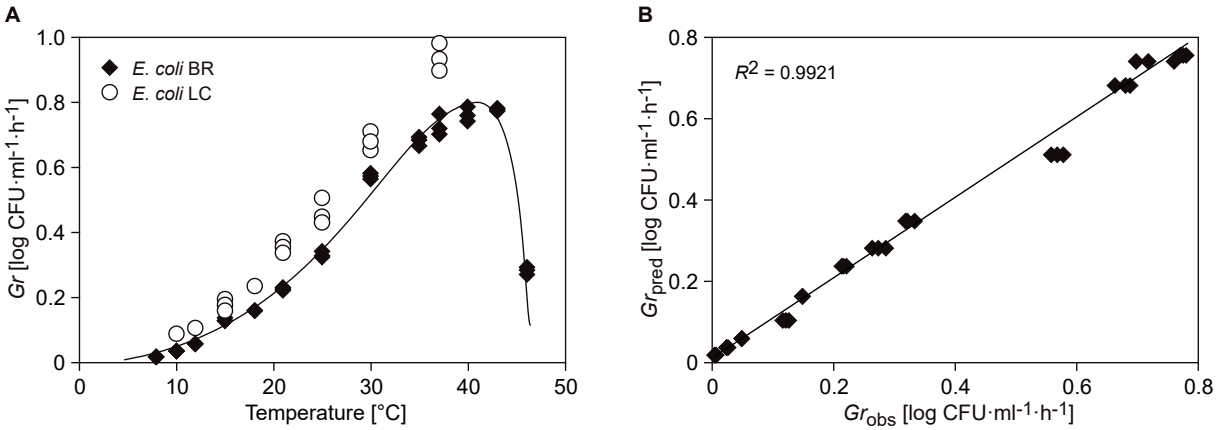

Fig. 2. Prediction of growth rate of *Escherichia coli* BR as a function of incubation temperature according to cardinal temperature model with inflection.

A – growth rate values fitted with cardinal temperature model with inflection, B – comparison of predicted and observed growth rates.
Gr – growth rate, Gr_{pred} – predicted growth rate, Gr_{obs} – observed growth rate.

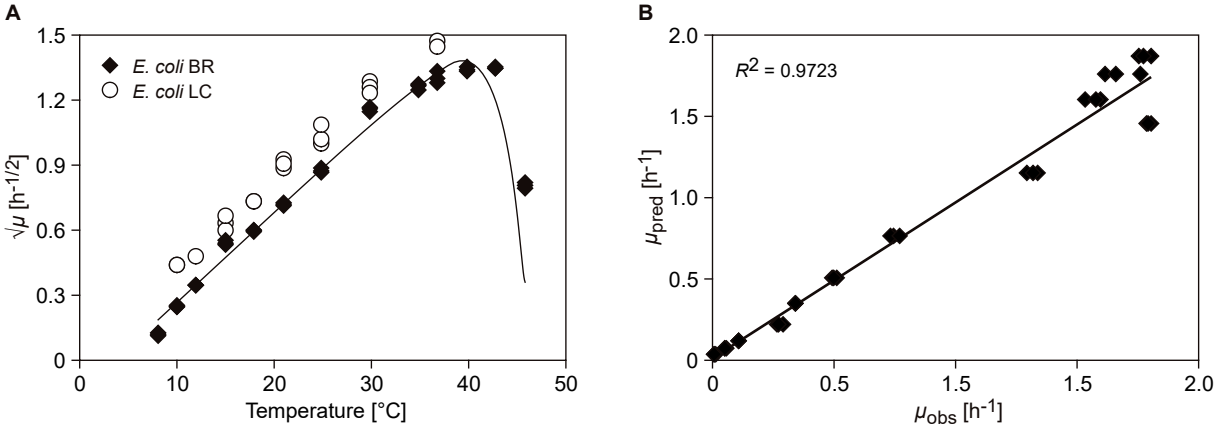

Fig. 3 Prediction of specific growth rate of *Escherichia coli* BR as a function of incubation temperature according to Ratkowsky model.

A – Specific growth rate values fitted with the Ratkowsky model, B – comparison of predicted and observed specific growth rates.
μ – specific growth rate, μ_{pred} – predicted specific growth rate, μ_{obs} – observed specific growth rate.

density of *E. coli* in stationary phase is strongly influenced by these variables. That is why the predictions of the time (t_x) needed for increase of *E. coli* in milk by *x* logarithmic counts at a selected temperature can be a useful output of the Gibson model. The graphical representation of the equation allowing the prediction of the time needed to increase *E. coli* counts in milk from its selected hypothetical initial counts by 1, 2, 3 or 4 log is shown in Fig. 4. In this approach it was assumed that time t_x is inversely related to ln μ of *E. coli* described by the Gibson model with the equation mentioned earlier. For example, the increase of *E. coli* counts by 3.42 log CFU·ml^{-1} at 25 °C during 104 h and at 30 °C during 6.7 h is expected. These expectations are supported by the results of Ioanna et al. [32] who observed an increase in *E. coli* O157:H7 counts by 3.42 log CFU·ml^{-1} during first 24 h of ripening of Carioricotta raw goats' milk cheese at laboratory temperature. The temperature range of 25–30 °C for calculation of the time necessary to increase counts of *E. coli* by 3.42 log CFU·ml^{-1} was considered due to its connection with the temperature profile of draining of whey from the cheese curd.

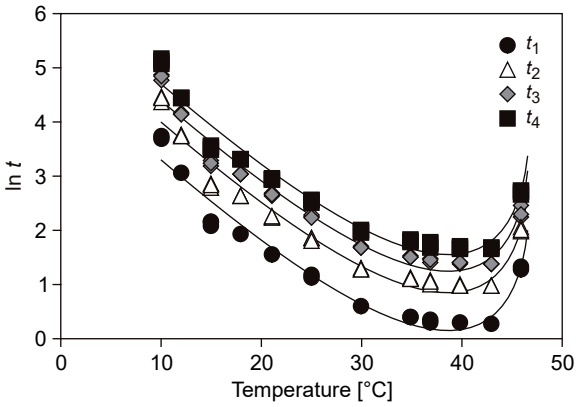

Fig. 4. Prediction of time to increase of *Escherichia coli* BR counts in increments according to Gibson model.
t_1, t_2, t_3, t_4 – time in hours to increase counts by 1, 2, 3 and 4 log, respecively.

Validation of the models

The values of growth rates calculated according to each model were first graphically compared to the experimentally observed growth rates of *E. coli* BR. As can be seen in Fig. 2B and Fig. 3B, the regression coefficients were 0.992 for CTMI and 0.972 for the Ratkowsky extended model. Subsequently, an internal (precision of the model to fit the experimental data) and external (suitability, accuracy and correctness of the model to predict the growth of *E. coli* in milk) validation was performed. For the external validation of the model, the isolate *E. coli* LC was used and its growth parameters were obtained in a similar way as for the BR isolate, but in the temperature range of 10–37 °C. The indices of internal validation are summarized in Tab. 2. Tab. 3 summarizes the indices for external validation performed with the LC isolate. The graphical comparison of the growth dynamics of BR isolate predicted by each model with external data for LC isolate is also depicted in Fig. 2A and Fig. 3A.

The accuracy indices for the model predictions compared to the original data of *E. coli* BR were from 1.219 to 1.373 for the Gibson model and CTMI model, respectively. Ross et al. [33] proposed that, as a "rule of thumb", the relative error in growth rate estimates under controlled laboratory conditions is around 10 % per independent variable. So, if the temperature was the only affecting factor, the best accuracy factor expected was approximately 1.1. This value may represent e.g. the overestimations of models at temperatures lower than 15 °C and underestimation in the range beyond T_{opt}. However, all models applied in this work can be acceptable since, as proposed by Zurera-Cosano et al. [26], the model will be considered as good if the bias factor is in the range of 0.95–1.01. The bias factor higher than 1.0 also reveals overestimation of the growth rate that would lead to the slower real growth of *E. coli* in milk than it is predicted by the models [21].

According to indices of external validation based on data for LC isolate (Tab. 2), this isolate grew slower than predicted by Ratkowsky model and, on the other hand, CTMI and Gibson model underestimated growth dynamics of this strain. Also, when the growth predictions based on *E. coli* BR models are compared to previously published data for other *E. coli* strains are either underestimated, e.g. data from Microbial Response Viewer (MRV) database (Free Software Foundation, Boston, Massachusetts, USA), data from Van Derlinden and Van Impe [29] and some strains from the study of Salter et al. [18], or overestimated, e.g. data from Combase database, Pathogen Modellling Program (Wyndmoor, Pennsylvania, USA) and some strains from the study of Salter et al. [18]. This might be caused by the different origin of strains, their different biochemical properties, different growth media or due to the use of a different model. Ross et al. [33] mentioned that systematic differences between growth rate estimates can be recognized from turbidimetric data and when the growth data are fitted to the Gom-

Tab. 2. Indices of internal validation for the growth data of *Escherichia coli* BR fitted by various models.

	BR_{CTMI}	BR_{RTK}	BR_{Gibson}
Number of observations	39	39	36
Mean square error	0.0258	0.1141	0.1481
Standard error of prediction [%]	7.1	13.5	16.4
Accuracy factor	1.373	1.353	1.219
Bias factor	1.113	1.051	1.004
Discrepancy factor [%]	37.3	35.3	21.9

BR_{CTMI} – *E. coli* BR data calculated by cardinal temperature model with inflection, BR_{RTK} – *E. coli* BR data calculated by the Ratkowsky model, BR_{Gibson} – *E. coli* BR data calculated by the Gibson model.

Tab. 3. Indices of external validation for the growth data of *Escherichia coli* LC fitted by various models.

	LC_{CTMI}	LC_{RTK}	LC_{Gibson}
Number of observations	24	24	21
Mean square error	0.1178	0.3594	0.3309
Standard error of prediction [%]	43.3	41.6	34.0
Accuracy factor	1.637	1.368	1.543
Bias factor	0.642	1.019	0.697
Discrepancy factor [%]	63.7	36.8	54.3

LC_{CTMI} – *E. coli* LC data calculated by cardinal temperature model with inflection, LC_{RTK} – *E. coli* LC data calculated by the Ratkowsky model, LC_{Gibson} – *E. coli* BR data calculated by the Gibson model.

pertz model instead of Baranyi model. This also supports previously published information [34] that evaluation of predictive models by comparison to published microbial growth data may be inappropriate because of limitations in those data. Therefore, the indices of internal validation provide an objective and readily interpretable summary of the model performance and may serve as the first step towards an objective growth analysis.

CONCLUSIONS

The results demonstrated that *E. coli* BR is able to grow well in milk in the temperature range from 8 °C to 46 °C with the optimal temperature of 39–41 °C. The other cardinal temperatures for growth of this strain in milk were determined according to CTMI, such as T_{min} of 3.7 °C and T_{max} of 46.6 °C. The growth rate of *E. coli* BR in milk at optimal temperature was 0.804 log CFU $ml^{-1} \cdot h^{-1}$ (corresponding to a doubling time of 51.7 min). The results of predictive analysis provide useful information for producers of artisanal cheeses. The used models were found suitable for estimation of growth dynamics of *E. coli* in dairy products and we suggest that they can be applied to shelf-life estimations for these products.

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