Effect of salt and temperature on the growth of *Escherichia coli* PSII

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ABSTRACT

Presence of pathogenic strains of *Escherichia coli* in foodstuffs may pose a health risk for a consumer. Therefore, knowledge on the effect of environmental factors on the growth ability of *E. coli* is of great importance. In this work, the effect of incubation temperature (6–46 °C) and the combined effect of temperature and water activity (0.991–0.930) on the growth dynamic of *E. coli* PSII were analysed. Based on the growth curves obtained, growth parameters were calculated by using the Baranyi D-model. Growth parameters were further analysed in secondary phase of predictive modelling. Using the CM model that describes the effect of combined factors, cardinal values ($T_{\min} = 4.8 \pm 0.4$ °C, $T_{opt} = 41.1 \pm 0.8$ °C, $T_{\max} = 48.3 \pm 0.9$ °C, $a_{\min} = 0.932 \pm 0.001$, and $a_{wopt} = 0.997 \pm 0.003$) for the isolate were calculated. Under optimal conditions, the specific growth rate is $\mu_{opt} = 2.84 \pm 0.08$ h⁻¹. The results obtained may contribute to the assessment of the risk associated with the possible *E. coli* presence in raw materials and to the search for preventive measures with defined degree of accuracy and reliability.

KEYWORDS

E. coli, predictive microbiology, water activity, temperature

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1. INTRODUCTION

Escherichia coli as a pathogen in humans and animals is still of significant importance, since *E. coli* transmission through consumption of raw milk, raw milk dairy products, and minced meat has been repeatedly documented (EU Report, 2013). Though pathogenic strains in foods have low prevalence, saprophytic *E. coli* populations are more common, due to its common occurrence in the intestines of human and other mammals. Lues et al. (2003) and Chye et al. (2004) reported that *E. coli* was detected in 23–65% of raw milk samples ranging from 10⁴ to 10⁶ CFU mL⁻¹. Therefore, the limits for *E. coli* presence in dairy products were set by the EU Regulation No. 1441/2007: e.g., 100 CFU g⁻¹ in cheeses manufactured from raw milk or heat-treated whey; 10 CFU g⁻¹ in butter and cream; 50 CFU g⁻¹ in minced meat and separated meat; and 500 CFU g⁻¹ in meat preparations (EC, 2007).

The fate of heat-labile *E. coli* in raw materials depends on many intrinsic and extrinsic environmental factors (Medvedová et al., 2018), including the interactions with other bacteria as described in our previous works (Ačai et al., 2019; Medvedová et al., 2020). However, some *E. coli* strains exhibit higher tolerance to adverse environmental conditions than other pathogenic and nonpathogenic microorganism. Such a tolerance is given by numerous factors, including production of stress-responding metabolites, harbour of resistance plasmid, and synthesis of protective surface appendages like colonic acid (Chen et al., 2004). In addition, in the case of osmotic stress that limits the availability of water for microbial cells, thus for enzyme functions and cell metabolism, other repair responses, such as production of chaperones and the induction of transport of ions (e.g. potassium glutamate), are immediately induced during lag-phase. Further, osmotic genes such as those corresponding to the osmoprotectant trehalose are expressed. Finally, at the end of lag-phase and at beginning of exponential phase, a change of metabolism, a switch of metabolism from aerobic to anaerobic at a threshold NaCl concentration is observed (Métris et al., 2016).

In this context, our aim was quantify the growth ability of *E. coli* isolate based on cultivation experiments using predictive microbiology principles. Various predictive models were used to compare prediction precisions, and validation with external data was performed to define reliability of models to predict growth responses of food-origin isolate of *E. coli*.

2. MATERIALS AND METHODS

2.1. Microorganism

E. coli PSII was isolated from laboratory-produced pasta-filata cheese from raw cows' milk. Its identity was confirmed by a Gram staining, COLItest and ENTEROtest 24 (Lachema, Brno, Czechia), PCR method, and MALDI-TOF spectroscopy with score 2.397.

2.2. Inoculation and cultivation conditions

The isolate was kept in BHI broth (Sigma-Aldrich, St. Louis, USA) at 5 ± 1 °C prior to analysis. Preparation of a standard suspension and inoculation was performed according to the study by



Medvedová et al. (2018). The effect of temperature was studied in ultra-high temperaturetreated cows' milk (1.5% fat content; Rajo, Bratislava, Slovakia), and the combined effect of temperature and a_w was studied in PCA broth (Sigma-Aldrich, St. Louis, USA). The a_w value was set to final value of 0.99; 0.97; 0.95, and 0.93 by the addition of NaCl, its actual value was measured by a LabMaster-aw (Novasina, Lachen, Switzerland). The static incubation of samples inoculated with isolate PSII was performed at 6, 6.5, 7, 8, 10, 12, 15, 18, 21, 25, 30, 35, 37, 40, 43, and 46 °C \pm 0.5 °C, in three parallels.

2.3. Enumeration of E. coli

The actual counts of *E. coli* were determined at predefined time intervals with respect to the incubation temperature according to ISO 4833-1:2013 standard procedure with incubation at 37 $^{\circ}$ C to gain the growth curves.

2.4. Fitting the growth curves and calculating the growth parameters

The growth data, curves and parameters of the isolate were analysed, fitted, and calculated, respectively, using the mechanistic modelling technique of Baranyi and Roberts (1994). The growth response of *E. coli* PSII was plotted against time and fitted to a model for the estimation of the specific growth rate (μ) and maximal (N_{max}) density using an in-house Excel Add-in package 'DMFit' version 3.5 (ComBase managed by USDA ARS, Washington D.C., USA and University of Tasmania, Hobart, Australia).

2.5. Secondary models

The growth parameters from each individual growth curve were analysed in the secondary phase of modelling by statistic tools of Microsoft Office v. 2010 (Microsoft, Redmond, Washington, USA) and Statistica v. 10.0 data analysis software system (StatSoft, Tulsa, Oklahoma, USA). The specific growth rate (μ) as a function of temperature (T) was modelled according to the Ratkowsky extended model (RTKext; Ratkowsky et al., 1983). Cardinal model (CM; Rosso et al., 1993) was used to describe the influence of T or a_w on specific growth rate. Finally, the combined effect of T and a_w based on individual cardinal models was determined according to the gamma concept (Zwietering et al., 1991).

2.6. Model validation

To validate the mathematical equations describing *E. coli* PSII responses to various *T* and a_w conditions, some mathematical and statistical indices were used. For the internal validation (model's precision to fit the experimental dataset of PSII isolate) standard error of prediction (*SEP*), root mean square error (*RMSE*; Zurera-Cosano et al., 2006), and regression coefficient (R^2) were calculated. For external validation (model's suitability, accuracy, and correctness to predict the *E. coli* growth) accuracy (A_f) and bias (B_f) factors (Baranyi et al., 1999) were calculated based on growth parameters dataset of *E. coli* BR isolate (Medvedová et al., 2018). In addition, the comparison (Fig. 2) was performed with ComBase (*E. coli* growth in broth), PMP (*E. coli* O157:H7 aerobic growth in broth), and MPV (non-pathogenic *E. coli* growth in milk) databases.



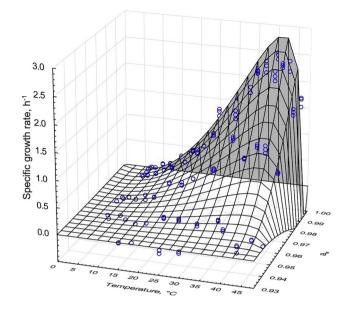


Fig. 1. Plots of the specific growth rates (μ) versus temperature (*T*) and water activity (a_w) for *E. coli* PSII. Symbols indicate calculated μ from growth curves at each *T* and a_w value. The network indicates fitted μ versus (*T*, a_w) function according to gamma concept

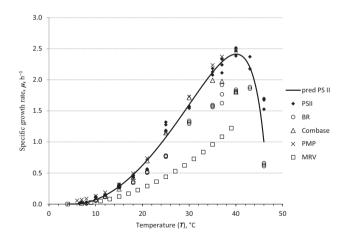


Fig. 2. The comparison of E. coli PSII (◆ growth in milk) modelled with CM model (continuous line) with E. coli BR (○ growth in milk), ComBase database (∆ E. coli growth in broth), PMP database (x E. coli O157:H7 aerobic growth in broth), and MRV (□ non-pathogenic E. coli growth in milk)



3. RESULTS AND DISCUSSION

In our previous work (Medvedová et al., 2018), the growth of two *E. coli* isolates (BR and LC) in milk as a function of incubation temperature by the use of predictive models was described. The Ratkowsky and CM models were suitable for estimation of *E. coli* growth dynamics. Consistent to study by Garre et al. (2020), where the strain variability in microbial responses to environmental factors was highlighted, we focused on describing the effect of temperature on the growth of *E. coli* PSII from pasta-filata cheese from raw cows' milk with the use of the above mentioned predictive models. Further, we described the effect of a_w on *E. coli* PSII growth at the same temperatures.

3.1. Effect of temperature on E. coli PSII growth

To compare the growth ability of *E. coli* PSII in the temperature range 6–46 °C, it was necessary to inoculate with as constant initial counts as possible. The average initial *E. coli* PSII counts in all experiments were $3.1 \pm 0.4 \log \text{ CFU mL}^{-1}$ (%V = 12; n = 48). All growth curves were characterised by typical sigmoid shape and were successfully fitted with the model of Baranyi and Roberts (1994) with the average of $R^2 = 0.978 \pm 0.058$. Obtained growth parameters are summarised in Table 1. At 6 °C, no growth of the isolate could be detected in 13 days; however, at 7 °C, the increase of approximately 4 log CFU mL⁻¹ was observed. Therefore, the growth of the isolate at 6.5 °C was also studied, with final counts being 0.9 log CFU mL⁻¹ lower compared to counts at 7 °C. Further increase in incubation temperature led naturally to more intensive growth until 40 °C was reached, at which the maximal growth rate was obtained. Temperature

	μ specific growth rate (h ⁻¹) or k inhibition rate									
	(h^{-1})				N_{max} (log CFU mL ⁻¹)					
<i>T</i> (°C)	0%	1.5%	5%	8%	10%	0%	1.5%	5%	8%	10%
6	-0.02	-0.01	-0.01	/	/	1.4	-3.0	-1.4	/	/
6.5	0.01	-0.01	-0.01	/	/	4.1	-2.9	-1.7	/	/
7	0.03	0.03	-0.01	-0.01	/	7.2	4.3	-2.2	-2.1	/
8	0.03	0.04	-0.01	-0.01	/	7.6	4.5	-2.2	-1.3	/
10	0.11	0.08	-0.01	-0.01	/	8.6	5.3	-2.6	-2.2	/
12	0.15	0.14	-0.04	-0.01	/	8.5	5.0	-1.3	-2.2	/
			0.05					5.3		
15	0.29	0.29	0.10	-0.02	-0.03	8.5	5.3	4.1	-2.6	-1.5
18	0.44	0.39	0.32	0.04	-0.02	8.7	5.3	5.2	4.0	-1.2
21	0.56	0.59	0.29	0.05	-0.03	8.9	5.2	5.2	4.0	-1.6
25	1.28	0.94	0.54	0.08	-0.12	8.9	5.6	5.2	4.5	-1.2
30	1.57	1.32	0.74	0.04	-0.01	8.7	5.4	5.0	4.7	-1.4
35	2.13	1.98	1.38	0.17	-0.07	8.8	5.6	4.1	4.4	-1.4
37	2.33	2.28	0.95	0.08	-0.62	8.7	5.2	5.0	3.6	-1.7
40	2.52	2.13	1.19	-0.22	-0.20	8.8	5.3	5.0	-2.6	-2.5
43	2.37	2.45	1.25	-0.16	-0.46	8.5	5.3	5.5	-2.0	-2.4
46	1.53	1.62	0.83	*	-0.22	8.3	4.7	2.9	*	-3.1

Table 1. Growth parameters of E. coli PSII in dependence on incubation temperature and NaCl addition



above 40 °C resulted in a slowdown in growth dynamics. At 43 and 46 °C, the decrease in growth rate by 6 and 30%, respectively, was noticed. The growth rate at 46 °C was comparable to growth rate at 30 °C. In contrast, *E. coli* BR grew fastest at 43 °C, and the growth rate at 46 °C was comparable to rate at 21 °C (Medvedová et al., 2018).

3.2. Effect of water activity and temperature on *E. coli* PSII growth

To study the effect of a_w adjusted by NaCl addition to 1.5% ($a_w = 0.991 \pm 0.002$; $c_v = 41\%$; n = 48); 5% ($a_w = 0.970 \pm 0.002$; $c_v = 53\%$; n = 48); 8% ($a_w = 0.950 \pm 0.002$; $c_v = 46\%$; n = 40), and 10% ($a_w = 0.930 \pm 0.002$; $c_v = 43\%$; n = 24), the initial *E. coli* PSII counts were 3.07 ± 0.48 log CFU mL⁻¹ (%V = 16).

In case of 1.5% NaCl addition, *E. coli* PSII at 7 °C only started to grow after 21 days, but its final counts and the growth rate were higher about 14% than in the medium without added NaCl. Similarly, at 8, 43, and 46 °C, the addition of NaCl led to higher growth rates by 30, 4, and 6%, respectively. It may be a result of *E. coli* response to osmotic stress on the membrane that induced production of chaperones and the induction of transport ions (Métris et al., 2016). Moreover, accumulation of compatible solutes (betain, prolin, etc.) at lower a_w values can support more intensive growth of *E. coli* (O'Byrne and Booth, 2002) compared to its growth in the medium without added NaCl.

With 5% NaCl addition, *E. coli* PSII growth could be observed at temperatures above 12 °C. However, at 12 °C, first an inhibition after 80 h was observed, and only after further 11 days it started to grow to a final density of 7 log CFU mL⁻¹. In temperature range from 15 to 46 °C the growth was slower by 30–66% compared to growth at 1.5% NaCl addition.

At 8% NaCl addition, the growth of *E. coli* PSII could only be detected from 18 to 40 °C. The growth was slower by 83–94% compared to its growth at 5% NaCl addition. Interestingly, at 46 °C, the viable cell concentration decreased with k = -0.20 h⁻¹ immediately after inoculation to a level of 0.4 log CFU mL⁻¹. However, after 20 h, bacteria started to grow with $\mu = 0.88$ h⁻¹ to 1.3 log CFU mL⁻¹ concentration, which was maintained for 80 h, then started to decrease again with k = -0.01 h⁻¹. As such a complicated process was observed in this case, a symbol * is used in Table 1.

Finally, at 10% NaCl addition, growth inhibition of the isolate was observed during the whole experiment, thus those values were excluded from secondary modelling.

3.3. Secondary modelling and validation

From 3 parallel primary growth curves, the specific growth rate (μ) and maximum counts in stationary phase (N_{max}) were derived by DMfit tools, and their average values at each temperature are summarised in Table 1. Individual data were subsequently used in secondary phase of predictive modelling to describe the influence of selected factors on microbial growth.

To predict the effect of incubation temperature on the specific growth rate of *E. coli* PSII at selected NaCl addition or without NaCl, the RTK_{ext} model and the CM model were used. The advantage of the CM model is that it provides not only T_{\min} and T_{\max} calculations (as in case of RTK_{ext} model) but also calculation of T_{opt} . Moreover, all cardinal parameters are defined with the simple biological meaning, since the settings of the model parameters are based on their biological interpretation with lack of structural correlation between them (Rosso et al., 1993). Based on these models, cardinal temperatures, presented in Table 2, were calculated. At the



NaCl addition; equation						
Model;	R^2	RMSE	%SEP	T_{\min}	$T_{\rm opt}$	$T_{\rm max}$
RTK _{ext} ; 0% NaCl; µ _{max}	c = 0.052(T -	$(T_{min})^2 \{1 - exp\}$	$[0.137(T - T_{max})]$	x)]}		
	0.992	0.08	5.2	4.7	-	49.2
RTK _{ext} ; 1.5% NaCl; μ_m	$_{nax} = 0.050(T)$	$-T_{min})^2 \{1 - ex$	$p[0.157(T - T_n)]$	nax)]}		
	1.000	0.002	10.4	4.8	-	49.2
RTKext; 5% NaCl; µmax	c = 0.030(T -	T_{min}) ² {1 - exp	$[0.302(T - T_{max})]$	x)]}		
	0.971	0.087	10.4	2.2	-	48.7
RTK _{ext} ; 8% NaCl; µ _{max}	c = 0.015(T -	$(T_{min})^2 \{1 - exp\}$	$[4.844(T - T_{max})]$	x)]}		
	0.891	0.018	23.5	7.8	-	37.1
CM; 0% NaCl						
	0.959	0.184	12.1	5.1	40.1	47.0
CM; 1.5% NaCl						
	0.984	0.111	10.9	5.0	41.7	47.7
CM; 5% NaCl						
	0.920	0.126	17.7	7.3	40.3	48.9
CM; 8% NaCl						
	0.943	0.037	43.1	10.7	31.6	40.8

 Table 2. RTK_{ext} model equations, validation indices for RTK_{ext} and CM model, and cardinal values for E.

 coli PSII growth in dependence on NaCl addition

optimal temperature, the specific growth rate of $\mu = 2.51 \text{ h}^{-1}$, $\mu = 2.41 \text{ h}^{-1}$, $\mu = 1.24 \text{ h}^{-1}$, and $\mu = 0.12 \text{ h}^{-1}$ was calculated by CM model at 0, 1.5, 5, and 8% NaCl addition, respectively. Those values can be useful for microbiologists and technologists in practices after recalculating to time to double ($t_d = ln2 / \mu$). Finally, the gamma concept (Fig. 1) combining the mutual effect of temperature and a_w on *E. coli* PSII specific growth was used, and cardinal values of temperature ($T_{\min} = 4.1 \text{ °C}$, $T_{\text{opt}} = 41.8 \text{ °C}$, $T_{\max} = 47.7 \text{ °C}$) and $a_w (a_{w\min} = 0.932 \pm 0.001$, $a_{wopt} = 0.997 \pm 0.001$, $a_{w\max} = 1.000 \pm 0.000$) were defined. At optimal conditions, the isolate will grow with $\mu = 2.84 \pm 0.08 \text{ h}^{-1}$.

In contrast, lower cardinal values for temperature were published for *E. coli* BR ($T_{min} = 3.7 \,^{\circ}$ C, $T_{opt} = 40.8 \,^{\circ}$ C, $T_{max} = 46.6 \,^{\circ}$ C; Medvedová et al., 2018) and for another 157 experimental *E. coli* strains ($T_{min} = 5.7-8.4 \,^{\circ}$ C, $T_{opt} = 40.7-41.5 \,^{\circ}$ C, $T_{max} = 46.4-47.4 \,^{\circ}$ C; Van Derlinden and Van Impe, 2012). Consistent to our findings, cardinal values ($T_{min} = 4.2-6.4 \,^{\circ}$ C, $T_{opt} = 39.6-42.3 \,^{\circ}$ C, $T_{max} = 47.6-51.3 \,^{\circ}$ C) were reported for 9 strains of Shiga toxin-producing *E. coli* (Salter et al., 1998). In addition, Sommers et al. (2018) defined $T_{min} = 5.1 \,^{\circ}$ C and Wang et al. (1997) reported $T_{min} = 7.8-8.4 \,^{\circ}$ C. Such differences in T_{min} for *E. coli* growth highlight the need to study the variability in growth dynamics of several *E. coli* representatives.

To determine the accuracy and suitability of models used, internal and external validation were performed. The validation factors and mathematical indices are summarised in Table 2. Taking into account %SEP (5.2–23.5 in case of RTK_{ext} model and 12.1–43.1 in case of CM model) and also low *RMSE* values (0.002–0.184), the predictions of *E. coli* PSII growth rate can be considered as acceptable. The external validation was performed with the dataset for *E. coli* BR (Medvedová et al., 2018) with the same experimental design. Based on obtained validation factors it can be concluded that both models overestimate the growth of *E. coli* BR, in case of the

RTK_{ext} model by 44.8% ($A_f = 1.448$, $B_f = 1.350$) and in case of CM model by 37.6% ($A_f = 1.376$, $B_f = 1.323$); however, it provides options for preventive measures to be taken during production of risky foods. Comparing the growth data obtained from ComBase database (growth of non-pathogenic *E. coli* in broth) and MRV database (growth of non-pathogenic *E. coli* in milk), the CM model overestimates the parameters obtained. In the case of the growth of pathogenic *E. coli* 0157:H7 in broth, comparable specific growth rate values were found in PMP database. Taking this into account, the predictions based on PSII isolate will be reliable and will reliably provide *E. coli* responses to changing environmental factors, especially temperature, water activity, and their mutual combinations.

4. CONCLUSIONS

The growth of *E. coli* PSII isolated from pasta-filata cheese from raw cows' milk was described in dependence on temperature (6–46 °C) and NaCl addition (1.5, 5, 8, 10%) by the use of predictive models. Based on RTK_{ext}, CM model, and gamma concept, the cardinal values for the *E. coli* PSII growth were defined as $T_{\text{min}} = 4.8 \pm 0.4$ °C, $T_{\text{opt}} = 41.1 \pm 0.8$ °C, $T_{\text{max}} = 48.3 \pm 0.9$ °C, $a_{\text{wmin}} = 0.932 \pm 0.001$, and $a_{\text{wopt}} = 0.997 \pm 0.001$. At optimal conditions, the isolate grows with $\mu = 2.84 \text{ h}^{-1}$ ($t_d = 14.6 \text{ min}$). Based on validation factors and mathematical indices, all models used are suitable for the estimation of growth dynamic of *E. coli* and can be applied to shelf-life estimations of selected foods.

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