Article

Quantitative Characterization of *Geotrichum candidum* Growth in Milk

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Abstract: The study of microbial growth in relation to food environments provides essential knowledge for food quality control. With respect to its significance in the dairy industry, the growth of *Geotrichum candidum* isolate J in milk without and with 1% NaCl was investigated under isothermal conditions ranging from 6 to 37 °C. The mechanistic model by Baranyi and Roberts was used to fit the fungal counts over time and to estimate the growth parameters of the isolate. The effect of temperature on the growth of *G. candidum* in milk was modelled with the cardinal models, and the cardinal temperatures were calculated as $T_{\min} = -3.8\text{--}0.0$ °C, $T_{\text{opt}} = 28.0\text{--}34.6$ °C, and $T_{\max} = 35.2\text{--}37.2$ °C. The growth of *G. candidum* J was slightly faster in milk with 1% NaCl and in temperature regions under 21 °C. However, in a temperature range that was close to the optimum, its growth was slightly inhibited by the lowered water activity level. The present study provides useful cultivation data for understanding the behaviour of *G. candidum* in milk and can serve as an effective tool for assessing the risk of fungal spoilage, predicting the shelf life of dairy products, or assessing the optimal conditions for its growth in relation to the operational parameters in dairy practices.

Keywords: *Geotrichum candidum*; predictive microbiology; cardinal models

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1. Introduction

The close connection of *G. candidum* with milk and dairy products was reported as early as 1850, when Fresenius isolated and named this microorganism *Oidium lactis*. *G. candidum* has undergone extensive taxonomic revision since the genus *Geotrichum* was firstly erected by Link in 1809, and it is still evolving [1–5]. Nevertheless, the classification of this species as a filamentous yeast-like fungus is generally accepted [3,4]. *G. candidum* is currently known as a ubiquitous microscopic fungus with a worldwide distribution that is commonly found in soil, water, air, silage, grass, plants, fruits, vegetables, raw milk, and dairy products. It is also a commensal organism of the human and animal digestive tract [2–7].

According to the International Dairy Federation (IDF) and the European Food and Feed Cultures Association (EFFCA), *G. candidum* is included in an authoritative list of microorganisms with a documented history of safe use in fermented foods [8]. The application of this fungus in the manufacturing of fermented dairy products resulted from its common presence in raw milk, regardless of animal origin (cow, goat, sheep, camel, or buffalo) [2,9,10]. However, the concentration of *G. candidum* in raw milk is generally low ($<10^2$ colony-forming units (CFU)·mL^{−1}). In dairy practice, *G. candidum* is used as an adjunct culture in the production of regional fermented milks (e.g., Viili, Kefir) and a wide range of ripened cheeses, including soft mould-ripened cheeses, soft and semi-hard smear-ripened cheeses, and acid-coagulated cheeses [2,5,11–14]. The growth of this milk fungus can be observed on the cheese's surface around the third day of ripening, and it can reach a density of up to $10^5\text{--}10^7$ TFU (thallus-forming units) per gram of cheese [4,15].

G. candidum contributes to the cheese's flavour, aroma, and appearance. Characteristic organoleptic attributes resulting from the presence of *G. candidum* in dairy products include mildly cheesy, mouldy, sweaty, putrid, acidic, yeasty, musty, fermented, cidery, and fruity flavours and a velvety, felted, or lightly fluffy appearance, with an overall good hedonic perception [2,16]. Additionally, this species may contribute to the microbial safety of dairy products. The antimicrobial potential of *G. candidum* against undesirable and pathogenic microorganisms, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Aspergillus ochraceus*, and *Mucor* species has been described [2,4,17,18].

However, another aspect of the relevance of *G. candidum* in the food industry must also be considered. *G. candidum* is known as a spoilage organism in certain dairy products, e.g., butter, cream, and cottage cheese. Uncontrolled growth of this species may lead to the development of off-flavours and cheese defects, such as a slippery rind or toad skin defects [5,7]. *G. candidum* has also been noticed as a machinery mould that can readily grow on all surfaces contacted by food products, and thus, its growth may cause severe economic losses for food producers [5,19].

With respect to its relevance in the food industry, the biochemical activity of *G. candidum* has already been deeply investigated [5,7,20–22]. However, there are only a few studies regarding the quantitative approach to the growth dynamics of *G. candidum*. Detailed knowledge of microbial responses to the environmental conditions enables a better control under microbial behaviour, which is an important part of food quality management. Microbial ecology and growth parameters can be quantitatively defined with the use of predictive microbiological tools. The concept of predictive microbiology is based on mathematical models, that enable an objective evaluation of microbial behaviour (growth, survival, inactivation, etc.) in a dependence on environmental factors [23,24], since the responses to a changing environment are reproducible [25]. Therefore, the aim of this study was to characterize the growth of *G. candidum* in milk quantitatively in relation to salt addition and changing temperature, and so the growth of *G. candidum* as a part of adjunct or natural cheese microbiota can be predicted. The data obtained here can be further used for microbial growth control and product quality optimization in dairy practices.

2. Materials and Methods

2.1. Fungal Isolate and Culture Preparation

An isolate J of *G. candidum* was used for all experiments in the present study. The monitored isolate, which was selected from the collection of the Institute of Food Science and Nutrition (Slovak University of Technology in Bratislava, Slovak Republic), originated from the artisanal Slovak “Bryndza” cheese (Turčianske Teplice, Slovak Republic). The identity of the isolate was confirmed based on morphological, biochemical, and molecular characteristics in accordance with Samson et al. [26], De Hoog and Smith [27], and Groenewald et al. [28]. The isolate was stored under refrigeration (5 ± 0.5 °C) on Plate Count Skim Milk Agar (SMA; Merck, Darmstadt, Germany) slant, and it was sub-cultured monthly.

2.2. Inoculum Preparation and Experimental Design

G. candidum was cultivated for 72 h on the upper layer of a horizontal solidified SMA agar in a tube at 25 ± 0.5 °C. Parallel cultivated isolates were then mixed with 10 mL of sterile peptone–saline solution and released through soft rubbing of the agar's surface with a sterile cell scraper. Immediately after the preparation, an appropriate decimal dilution of this culture was used to inoculate 300 mL of pre-tempered ultra-high temperature processed (UHT) milk with an initial level, which ranged approximately from 1.5 to 2.0 CFU·mL^{−1}.

The effect of NaCl addition (0 and 1%, *w/v*) on the kinetic growth behaviour of *G. candidum* isolate J was evaluated in UHT milk (1.5% fat content; Rajo Inc., Bratislava, Slovak Republic) at temperatures of 6, 8, 12, 15, 18, 21, 25, 30, 34, and 37 ± 0.5 °C under static aerobic conditions. The aforementioned temperatures were selected in an attempt to fully cover the growth region of the species to the greatest possible extent. Considering the

salt sensitivity of *G. candidum* contrary to the majority of other dairy yeasts, only addition of 1% NaCl was evaluated. To obtain well-fitting data, three replicates were performed per experiment.

Actual viable counts of the isolate were determined at predefined time intervals based on the incubation temperature by plating on DRBC agar (Biokar Diagnostics, Beauvais, France) according to EN ISO 21527-1 [29] with aerobic incubation at 25 ± 0.5 °C for 5 days. The pH of the milk samples was analysed using a calibrated WTW 720 pH-meter (Inolab, Weilheim, Germany) equipped with a Sen Tix 81 glass electrode (WTW GmbH, Weilheim, Germany) with the same time interval as the microbiological determination.

2.3. Growth Curve Fitting

2.3.1. Primary Modelling

The model proposed by Baranyi and Roberts [30] was fitted to the data of *G. candidum* growth in milk to estimate the kinetic parameters of the growth. The microbial counts obtained from the cultivation experiments were transformed into units of $\log_{10}$ CFU·mL⁻¹ prior to model fitting. The Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA) add-in DMFit (Version 3.5, ComBase, University of Tasmania, Australia, and the USDA Agricultural Research Service, Washington, DC, USA) was employed to fit the log count vs. time data and extract growth parameters, such as the growth rate (*Gr*) or lag phase duration (λ). The maximum specific growth rate ($\mu_{\max}$) was subsequently calculated as:

$$\mu_{\max} = \ln 10 \cdot Gr \quad (1)$$

2.3.2. Secondary Modelling

The cardinal model (CM) by Rosso et al. [31] was used to fit the reciprocal lag time $1/\lambda$ (h⁻¹) as well as the maximum specific growth rate $\mu_{\max}$ (h⁻¹) against the temperature (*T*):

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

$$1/\lambda = 1/\lambda_{\text{opt}} \frac{(T - T_{\max})(T - T_{\min})^2}{(T_{\text{opt}} - T_{\min})[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)]} \quad (3)$$

This secondary predictive model includes only parameters with direct biological meaning, such as μ_{opt} (maximum specific growth rate at the temperature optimum), $T_{\min}$ (theoretical minimum temperature), T_{opt} (optimal temperature for the growth of the studied microorganism), and $T_{\max}$ (maximum temperature above which microbial growth is not likely).

2.3.3. Statistical Analysis and Validation

The data obtained from the primary modelling were treated with an analysis of variance (ANOVA) to assess if the effects of temperature or salt addition on the kinetic growth of *G. candidum* were significant. A statistical analysis with a least significant difference of 95% was carried out using Microsoft Excel 365.

To evaluate the accuracy of the primary and secondary models' fit, the coefficient of determination (R^2) and root mean square error (RMSE) were calculated:

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i^{\text{exp}} - y_i^{\text{pred}})^2}{\sum_{i=1}^n (y_i^{\text{exp}} - \bar{y}^{\text{exp}})^2} \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i^{\text{exp}} - y_i^{\text{pred}})^2}{n - p}} \quad (5)$$

where y_i^{exp} and y_i^{pred} are the experimental and predicted growth data, respectively; n is the number of experimental observations; and p is the number of model parameters [32–34].

Accuracy (A_f) and bias (B_f) factors, as introduced by Baranyi et al. [35], were used for internal validation of the secondary models according to:

$$A_f = 10 \sqrt{\frac{\sum_{i=1}^n (\log y_i^{pred} - \log y_i^{exp})^2}{n}} \quad (6)$$

$$B_f = 10 \frac{\sum_{i=1}^n (\log y_i^{pred} - \log y_i^{exp})}{n} \quad (7)$$

3. Results

3.1. Primary Modelling of *G. candidum* Growth in Milk

In this study, we used the primary mechanistic model proposed by Baranyi and Roberts [30], which is the most appropriate in comparison with other primary models that are based on statistical indices [34]. Although it was primarily constructed for bacterial growth predictions, it has also been frequently used to describe fungal growth [12,36–40].

The average initial counts ($N_0 \pm$ standard deviation) of *G. candidum* J in milk were $1.55 \pm 0.18 \log \text{CFU} \cdot \text{mL}^{-1}$ (coefficient of variation: $\text{CV} = 11.6\%$) in all experiments without NaCl addition ($n = 30$) and $1.88 \pm 0.19 \log \text{CFU} \cdot \text{mL}^{-1}$ ($\text{CV} = 10.3\%$) in milk with 1% NaCl. This density is in accordance with the inherent presence of *G. candidum* in raw milk [2,13]. The growth curves of the isolate (Figure 1) in milk were characterised by a typical sigmoidal shape, with an exception at 37 °C, when the survival and subsequent decline of *G. candidum* counts were observed. However, during its cultivation in milk with 1% NaCl and in a suboptimal temperature range, we repeatedly observed a slight decline in *G. candidum* counts before the exponential growth phase. Since the lag phase represents the transient period of non-replication during which microorganisms adjust to the new environment, a decrease in the number of viable cells may also occur before the exponential phase [41]. Therefore, for the most appropriate fitting of the growth data by the modelling program (DMFit), we transformed the decreased counts of *G. candidum* into the average of the counts occurring in the lag phase (Figure 2).

Figure 1. Growth curves of *G. candidum* isolate J in ultra-high temperature processed (UHT) milk without NaCl in the temperature ranges of 6–18 °C (a) and 21–37 °C (b). The points represent the observed values of counts of *G. candidum*. The continuous lines represent fitted growth curve according to the DMFit modelling program.

Figure 2. Growth curves of *G. candidum* isolate J in UHT milk with 1% NaCl in the temperature ranges of 6–18 °C (a) and 21–37 °C (b). The points represent the observed values of counts of *G. candidum*. The continuous lines represent fitted growth curve according to DMFit modelling program.

The growth parameters from the primary modelling are summarized in Table 1. The longest lag phase durations of *G. candidum* growth in milk without and with 1% NaCl, respectively, were observed at the minimum cultivation temperature used in our study (6 °C) and lasted for almost 3 days. Despite this low incubation temperature, *G. candidum* was able to grow and increased the initial counts by more than three logs within 9 days. Although this cooling temperature is used in practice to minimize microbial growth and to extend the shelf life of non-sterile dairy products [10], it is obvious from our results that the growth of *G. candidum* is enabled under these circumstances, and it may lead to spoilage of dairy products. Organoleptic defects in certain dairy products (e.g., cottage cheese and cream) are obvious when the fungal population is at least 10^4 – 10^5 CFU g⁻¹ [42]. Based on our results, *G. candidum* could reach that density in 7 or 8 days at 6 °C in milk without or with 1% NaCl, respectively. These data should be considered in dairy practices for controlling food quality and effective shelf-life management, including for predictions within consumers' households.

Further increases in the cultivation temperature led to significantly ($p < 0.05$) more intense growth of the isolate in milk. The highest growth rate and the shortest duration of the lag phase were observed at the cultivation temperature of 30 °C. Detailed knowledge of the lag phase duration and the rate of *G. candidum* growth in milk at 30 °C, that is, the temperature of milk coagulation in cheese practices [43], may be helpful in the manufacturing of cheeses with the intentional addition of *G. candidum* culture or in cheese production with raw milk. On the other hand, the effect of temperature on *G. candidum* growth in milk with added salt was less obvious as it was in milk without added salt. There were no significant ($p < 0.05$) differences in growth rate of the isolate at 6 and 8 °C or at 12 and 15 °C. However, the specific growth rate of the isolate significantly ($p < 0.05$) increased with the increase in cultivation temperature from 15 to 25 °C.

Table 1. Growth parameters of *G. candidum* J in UHT milk depending on NaCl addition.

T (°C)	NaCl (%)	$\mu_{\max}$ (h ⁻¹)	λ (h)	$N_{\max}$ (log CFU·mL ⁻¹)
6	0	0.051 ± 0.000 ^{a,x}	70.0 ± 1.46 ^{a,x}	5.01 ± 0.05 ^{a,x}
	1	0.049 ± 0.003 ^{m,x}	66.2 ± 1.06 ^{m,y}	5.32 ± 0.02 ^{a,y}
8	0	0.060 ± 0.000 ^{b,x}	41.5 ± 0.13 ^{b,x}	4.87 ± 0.12 ^{a,x}
	1	0.043 ± 0.004 ^{m,y}	37.2 ± 2.97 ^{n,x}	5.43 ± 0.18 ^{a,y}
12	0	0.106 ± 0.000 ^{c,x}	17.3 ± 1.65 ^{c,x}	5.44 ± 0.08 ^{b,x}
	1	0.166 ± 0.017 ^{n,y}	36.1 ± 3.79 ^{n,y}	5.45 ± 0.01 ^{a,x}
15	0	0.157 ± 0.008 ^{d,x}	30.5 ± 0.59 ^{d,x}	4.61 ± 0.11 ^{c,x}
	1	0.145 ± 0.007 ^{n,x}	21.4 ± 0.51 ^{o,y}	5.59 ± 0.06 ^{b,y}
18	0	0.219 ± 0.002 ^{e,x}	8.8 ± 0.03 ^{e,x}	4.84 ± 0.04 ^{d,x}
	1	0.199 ± 0.021 ^{o,x}	15.1 ± 2.33 ^{p,y}	5.26 ± 0.21 ^{c,y}
21	0	0.266 ± 0.011 ^{f,x}	8.5 ± 0.78 ^{e,x}	4.93 ± 0.07 ^{d,x}
	1	0.267 ± 0.005 ^{p,x}	9.7 ± 0.32 ^{q,x}	5.23 ± 0.09 ^{c,y}
25	0	0.315 ± 0.004 ^{g,x}	5.2 ± 0.38 ^{f,x}	4.40 ± 0.00 ^{e,x}
	1	0.300 ± 0.008 ^{q,y}	7.7 ± 0.20 ^{r,y}	5.28 ± 0.03 ^{c,y}
30	0	0.359 ± 0.005 ^{h,x}	4.6 ± 0.57 ^{f,x}	4.18 ± 0.00 ^{f,x}
	1	0.265 ± 0.008 ^{r,y}	4.3 ± 0.18 ^{s,x}	5.57 ± 0.08 ^{d,y}
34	0	0.293 ± 0.005 ^{i,x}	8.0 ± 1.40 ^{g,x}	3.92 ± 0.02 ^{g,x}
	1	0.254 ± 0.019 ^{r,y}	4.8 ± 0.93 ^{s,y}	4.94 ± 0.12 ^{e,y}
37	0	−0.285 ± 0.017 ^{j,x}	20.1 ± 1.08 ^{h,x}	-
	1	−0.108 ± 0.007 ^{s,y}	13.3 ± 3.89 ^{t,y}	-

T—incubation temperature; $\mu_{\max}$ —maximum specific growth rate; λ —lag phase duration; N_0 —initial counts; $N_{\max}$ —maximum counts in the stationary phase. ^{a–j}—different superscript letters among data observed in milk with 0% NaCl indicate statistical significance with increasing temperature ($p < 0.05$); ^{m–t}—different superscript letters between data observed in milk with 1% NaCl indicate statistical significance with increasing temperature ($p < 0.05$); ^{x,y}—different superscript letters indicate statistical significance between data in milk with 0% NaCl and 1% NaCl ($p < 0.05$).

Higher temperatures than 30 °C caused a decline in the growth rate in both cases—*G. candidum* growth in milk without and with 1% NaCl. As already mentioned, at the cultivation temperature of 37 °C, the *G. candidum* log counts continuously decreased after lag phase durations that were shorter than 24 h, regardless of the salt content in the milk. This result is in accordance with that of Koňuchová and Valík [44], who did not observe any growth of *G. candidum* isolate I on the surface of skim milk agar at 37 °C. The studies of other cheese-related yeasts demonstrated similar patterns of response to temperature [16,45,46]. In addition, Agarbati et al. [47] reported *Debaryomyces hansenii* MM6 1194 and PCF1 1148 or *Candida zeylanoides* 7 as unable to grow at 37 °C. Although we observed significantly ($p < 0.05$) slower inactivation of *G. candidum* cells in milk with 1% NaCl in contrast to the rate of devitalisation of the isolate in milk without salt, more experiments would be needed to understand the protective effect of salt against the higher temperature in this specific case. However, the effect of decreasing water activity, which increases the microbial tolerance and even the resistance to higher temperatures, is generally known [48].

In contrast to the lag phase duration and growth rate of *G. candidum* J, counts of the isolate in the stationary phase were almost equal in all experiments (excluding the growth curves at 37 °C) at the level of 10^4 – 10^5 log CFU·mL⁻¹. In addition, the pH of the milk growth medium was stable during all of the experiments performed, with an average initial value of 6.54 ± 0.05 (CV = 0.8%, $n = 10$) and average final value of 6.53 ± 0.08 (CV = 1.2%). Similarly, the pH of milk with 1% NaCl had the average initial value of 6.50 ± 0.02 (CV = 0.3%) and final value of 6.52 ± 0.04 (CV = 0.6%). This may result from the fact that *G. candidum* is unable to ferment lactose [2,7]. Although the optimal pH for *G. candidum* growth was reported to be in the range of 5.5–6.0 [4,5,49], according to Šípková

et al. [50], the pH range of 5.5–6.6 has no significant impact on the growth of *G. candidum* in milk.

3.2. Secondary Modelling of *G. candidum* Growth in Milk

In general, temperature is the most significant external environmental factor that affects microbial growth. That is why the secondary modelling in terms of the quantitative evaluation of temperature's effect on *G. candidum* growth was performed.

As shown in Figure 3, the duration of lag phase decreased with increasing temperature and slightly increased in the area beyond the optimum towards the maximum values of the temperature. The qualitative growth study of Koňuchová and Valík [44] demonstrated a similar pattern of surface growth responses to temperature in the *G. candidum* lag phase duration.

Figure 3. Plots of the reciprocal lag phase duration of the *G. candidum* isolate in relation to the temperature (6–37 °C) in milk (blue line) and milk with 1% NaCl (yellow line) according to the cardinal model (CM, solid line). The symbols indicate the experimental values of $1/\lambda$ estimated from the primary model at each incubation temperature. Solid lines represent the fitted estimates according to the model. Dashed lines represent the 95% confidence intervals.

It was also obvious that the CM line representing the dependence of $1/\lambda$ on temperature with 1% NaCl moved down to lower values of $1/\lambda$ and toward a higher temperature range. Specifically, this means that the lag phase durations were prolonged, the differences between $1/\lambda$ values increased gradually with the temperature, and the predicted T_{opt} and T_{max} were higher by 4.3 and 2.0 °C (Table 2), respectively, as a result of the addition of salt in the milk. On the other hand, the cardinal temperatures that resulted from CM when applied to the specific growth rate of the isolate in milk with and without added salt were very close to each other. Similar cardinal temperatures ($T_{min} = 1.18$ °C, $T_{opt} = 28.9$ °C, $T_{max} = 37.3$ °C) for *G. candidum* growth in milk were also reported by Šípková et al. [50]. The optimal specific growth rate of the isolate slightly decreased in the milk medium with 1% NaCl addition in contrast to its optimal growth rate in milk. These results indicate the slower growth of *G. candidum* at 1% NaCl, which is in accordance with the findings of Eliskases-Lechner et al. [7] and Uraz and Özer [51]. However, strain-dependent sensitivity of *G. candidum* on salt concentration should also generally be considered [2,4,44].

Table 2. Cardinal parameters of the growth of *G. candidum* J in milk depending on NaCl presence as a result of CM applied to the specific growth rate (CM_μ) and lag phase ($CM_{1/\lambda}$).

Model	0% NaCl		1% NaCl	
	$CM_{1/\lambda}$	CM_μ	$CM_{1/\lambda}$	CM_μ
$T_{\min}$ (°C)	-0.81 ± 0.01	0 (fixed)	-3.75 ± 0.05	0 (fixed)
T_{opt} (°C)	30.32 ± 0.27	28.00 ± 0.06	34.63 ± 0.44	28.27 ± 0.14
$T_{\max}$ (°C)	35.21 ± 0.03	35.20 ± 0.05	37.17 ± 0.12	36.62 ± 0.12
$1/\lambda_{\text{opt}}$ (h ⁻¹)	0.229 ± 0.022	-	0.202 ± 0.029	-
μ_{opt} (h ⁻¹)	-	0.358 ± 0.008	-	0.325 ± 0.016

$T_{\min}$ —minimum temperature (theoretical value); T_{opt} —optimal temperature; $T_{\max}$ —maximum temperature; $1/\lambda_{\text{opt}}$ —reciprocal lag phase duration at the optimal temperature; μ_{opt} —maximum specific growth rate at the optimal temperature; $CM_{1/\lambda}$ —cardinal model applied to its lag phase data; CM_μ —cardinal model applied to its specific growth rate.

According to Eliskases-Lechner et al. [7], *G. candidum* species grow at temperatures ranging from 5 to 35 °C, which is in accordance with our observations, although we predicted a slightly higher maximum temperature for *G. candidum* growth in milk at the level of 36 °C. The optimal temperature for *G. candidum* growth is generally considered to be around 25 °C [2,7]. However, based on our results, the optimal temperature was predicted to be close to 30 °C. These data illustrate the variability in the optimal temperature for growth, which can at least be partially explained through the variability of strains and culture conditions, as also reported by Koňuchová and Valík [44].

Generally, within the experimental limits, the maximum specific growth rates of the isolate in milk with higher a_w level (0% NaCl) increased significantly ($p < 0.05$) with the increase in temperature up to an optimum, and then decreased beyond the physiological limits for the isolate. The graphical evaluation of the fitted curves in Figure 4 at temperatures below 21 °C indicates that when a_w becomes more stressful (as a result of 1% NaCl addition), *G. candidum* exhibits a slightly faster growth rate compared to the growth predictions in milk without salt. A previous qualitative study by Hudecová et al. [52] showed a similar phenomenon. They investigated the effect of NaCl content on the radial growth of *G. candidum* and reported a similar trend, where kinetic growth over the entire temperature range (from 8 to 37 °C) was positively influenced by 1% NaCl content in the growth medium. However, in Figure 4, it can also be noticed that, in the temperature interval from 25 to 34 °C, increased osmotic stress resulted in decreased growth potential, which was already indicated by the results of the primary modelling. In addition, both $T_{\max}$ and μ_{opt} were lower for lower a_w values.

The predicted growth kinetics of *G. candidum* J, which were influenced by temperature in Figure 4, demonstrated a similar growth response framework to that reported by Šípková et al. [50]. However, they observed slightly higher μ_{opt} values associated with the temperature of the *G. candidum* isolate A (0.535 ± 0.011 h⁻¹). Since they used the same experimental research design, the differences between the estimated μ_{opt} of *G. candidum* may be related to the use of different isolates. This is in accordance with the polymorphism, as well as morphological and phenotypic variability, of *G. candidum* observed by some authors [4,39].

Figure 4. Plots of the maximum specific growth rate of *G. candidum* J versus temperature (6–37 °C) in milk (blue line) and milk with 1% NaCl (yellow line) according to the CM (solid line). The symbols indicate the experimental $\mu_{\max}$ estimated from the primary growth curves at each incubation temperature. Solid lines represent the fitted model estimates. Dashed lines represent the 95% confidence intervals.

3.3. Statistical Evaluation and Validation of Models

The reliability of the predictive models used was assessed with the mathematical indices that are summarised in Table 3. Although the most common statistical index, R^2 , was higher than 0.8 in all cases of the predictions in our study, this index is primarily adequate for linear models [53]. Therefore, the suitability of the primary predictive model used was also assessed with the RMSE index, which was higher in the case of *G. candidum* growth in milk with 1% NaCl in comparison with the RMSE index of the model used in the predictions of the growth of the isolate in milk without salt. However, the values were still close to zero. The model is considered to be more suitable, as the RMSE value is closer to zero [54].

Table 3. Mathematical indices and validation factors of the models used, which describe the effects of temperature and NaCl presence on *G. candidum* J growth in milk.

	Model	% NaCl	R^2	RMSE	Af	Bf
Primary models	BR	0	0.985–0.997	0.092–0.027	-	-
		1	0.966–0.999	0.166–0.062	-	-
Secondary models	$CM_{1/\lambda}$	0	0.983	0.127	1.116	1.007
		1	0.926	0.238	1.237	0.998
	CM_{μ}	0	0.981	0.015	1.259	0.902
		1	0.888	0.035	1.296	0.909

RMSE—root mean square error; R^2 —coefficient of determination; Af—accuracy factor; Bf—bias factor; BR—Baranyi and Roberts model; $CM_{1/\lambda}$ —cardinal model applied to its lag phase data set; CM_{μ} —cardinal model applied to its growth rate.

A similar pattern of higher RMSE index values in the case of *G. candidum* growth in milk with 1% NaCl was also demonstrated in the secondary modelling. The CM showed better performance for modelling of the lag phase than for the growth rate based on the RMSE indices, and the values were in the range of or lower than the values of the RMSE reported by Garcia et al. [55], who applied extended Davey and general polynomial models to study fungal radial growth. Satisfactory predictions of fungal growth in relation to temperature with the use of the CM were also reported by other authors [56,57].

Ideally, predictive models would have $A_f = B_f = 1$. However, A_f can increase by 10–15% for every variable in the model [58]. Therefore, if the model predicts the effect of the temperature (as the only factor) on the microbial growth, the best expected A_f would be 1.1–1.2 [58,59]. In our study, the A_f factors were slightly higher than 1.2. The satisfactory B_f factor limits were related to the specific application of the model. If $B_f < 1$, then the model overpredicts the observed growth rate and, thus, the model is considered to be “fail-safe” in terms of the growth rate predictions for spoilage or pathogenic microorganisms [58]. However, an acceptable interval of the B_f factor for predictive models is 0.75–1.25 [34]. All B_f factors calculated in our study were in the acceptable interval.

4. Conclusions

The present study provides quantitative growth data on the behaviour of a dairy isolate in milk under a wide range of temperature conditions. The effect of 1% NaCl on the growth kinetics of the studied microscopic fungus was also expressed quantitatively. The outputs of the *G. candidum* growth modelling can be used for several types of prediction in dairy practice. Firstly, it may assist in predicting conditions under which the growth of *G. candidum* is unfavourable from the point of view of food spoilage. Based on the presented data, the food technologists may also estimate the time that is required for *G. candidum* growth to reach the hygienic relevant limit. It may also be considered in the effort to extend the shelf life of raw milk dairy products or to adequately define their “use by” date. On the other hand, the results can be applied as a tool to evaluate the temperature conditions for achieving a specific growth rate, e.g., in an artisanal cheese making process, according to the operational parameters.

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