Growth Characterisation of *Staphylococcus aureus* in Milk: a Quantitative Approach

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Abstract

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Staphylococcus aureus is a pathogenic bacterium that induces several of human illnesses. The staphylococcal enterotoxin (SE) production as the results of previous growth of toxigenic strains is the most crucial problem which may lead to the staphylococcal food poisoning outbreaks in humans. That is why the growth of three strains of *Staphylococcus aureus* was characterised in milk and modelled in dependence of temperature. For the lag phase duration of *S. aureus* 2064, the Davey model was used with the following result: $\ln(1/lag) = 1.973 - 87.92/T + 285.09/T^2$ ($R^2 = 0.962$). The dependence of the growth rate on incubation temperature was modelled by the Ratkowsky square root model and Gibson in sub-optimal and whole temperature range, respectively. The validation of both models showed high significance of the growth rate data fitting. The optimal temperature of $T_{opt} = 38.5$°C was resulted from Gibson model for the *S. aureus* 2064 growth in milk. For practical purpose, the time necessary for the increase of *S. aureus* by 3 log counts was also calculated within the growth temperature range. These data may provide useful information e.g. for the producers using raw milk in their artisanal cheese practice as the specific strains were used in this study.

Keywords: *Staphylococcus aureus*; predictive microbiology, growth parameters

Staphylococcus aureus is a pathogenic bacterium that induces several of human illnesses. The ability to cause a wide range of diseases may be associated with its production of a large spectrum of extracellular toxic compounds and other virulence factors such as toxic shock syndrome 1, exfoliative toxins and enterotoxins (Boynukara *et al*. 2008). From a food safety point of view, the staphylococcal enterotoxin (SE) production is the most crucial problem which leads to the staphylococcal food poisoning outbreaks in humans as the third most common food intoxication in the world (Halpin-Dohnalek & Marth 1989; Baird-parker 2000; Ondrovčík 2003; Boynukara *et al* 2008;). The ability to produce one of the enterotoxins (SEA–SED) was observed in more than 20 % of the *S. aureus* strains examined by Boynukara *et al*. (2008) and Vasiľ *et al*. (2005) even more than 50% of the strains found by Akineden *et al*. (2008) and Normanno *et al*. (2007). Furthermore, 65% up to 84 % of all of these strains were of human origin (Normanno *et al*. 2007). *S. aureus* is mostly found on human skin or mucosa, nostrils, pharynx, in hair, in gastrointestinal and urogenital tracts. For these reasons,

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every hand-operated product is a potential source of staphylococcal contamination. Lack of proper hygienic measures increases the risk of staphylococcal contamination of raw material.

Another natural niche of this organism is the mammalian skin, hull, and mucous membranes. The infected mammary glands of cows and other milk producing animals are the most important reservoirs (Asperger & Zangerl 2003; Charlier *et al*. 2008). In the case of a contaminated udder, *S. aureus* is excreted into the milk during milking. In well drawn milk, its counts are from 100 CFU/ml to 200 CFU/ml (Valík *et al*. 2004), in the case of a contaminated udder, the counts may increase up to 10^4 CFU/ml and even to 10^8 CFU/ml (Lancette & Tatini 1992; Asperger & Zangerl 2003).

During the cheese manufacture, especially in the case of a slower or insufficient acidification of lump cheese, *S. aureus* can also be found in the final products. Poor performance of hygienic precautions can lead to the contamination of thermally treated milk, and this is why it can be found in cheeses made either from raw or pasteurised milk (Lindqvist *et al*. 2002; García *et al*. 2007). According to Asperger and Zangerl (2003), the staphylococci are physically concentrated approximately 10-fold in the curd during the cheese manufacture. Therefore, *S. aureus* content in young cheese is directly dependent on their number in raw milk.

Besides dairy products, *S. aureus* is commonly found in raw, cooked, or roasted meat products, sausages, canned meats, fish and vegetable products, seafood, rice, potatoes and tuna salad, bakery products, pancakes, cream, egg noodles (Halpin-Dohnalek & Marth 1989; Baird-Parker 2000; Normanno *et al*. 2005; Kérouanton *et al*. 2007). It also grew well in pasta during drying until the water activity (a_w) dropped to 0.93 or 0.86, respectively (Valík & Görner 1993).

The European Commission hygiene process criteria for the counts of coagulase-positive staphylococci in dairy products are concerned with cheeses made from raw milk or milk that has undergone milder heat treatment than pasteurisation, unripened soft cheeses, milk and whey powders. Moreover, if numbers above 10^5 CFU/g are detected, the respective food batch has to be tested for staphylococcal enterotoxins (Commission Regulation No. 1441/2007).

Because of the substantial role of *S. aureus* in hygiene and safety of the artisanal cheese production as is the case of the Slovak ewes´ lump and Bryndza cheeses, and along with the lack of the data covering the complete growth temperature range of *S. aureus*, e.g. in Combase database, the quantitative characterisation of the growth of *Staphylococcus aureus* was carried out.

MATERIAL AND METHODS

Microorganisms. Three strains of *Staphylococcus aureus* were used in the study. The strains 2064 and B1 were isolated from the ewes' lump cheese and Slovak Bryndza cheese, respectively, by MVDr. Hanzélyová from the State Veterinary and Food Institution (Prešov, Slovakia), while the strain D1 was isolated from the human milk at the Public Health Institute of the Slovak Republic (Bratislava, Slovakia). The identity of *S. aureus* was confirmed by the API system (BioMérieux, Marcy l'Etoile, France). Additionally, the gram staining and catalase tests were performed.

The detection of staphylococcal enterotoxin production. The detection of staphylococcal enterotoxins (SE) including SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE was performed using enzyme-linked fluorescent assays, the VIDAS Staph Enterotoxin Test (BioMérieux, Marcy l'Etoile, France) with the mini-VIDAS SET automated system. The assays are based on a combination of complementary monoclonal and polyclonal antibodies directed to different antigenic sites of the staphylococcal enterotoxins. The tests were performed on the same samples which had been taken in two parallel growth tests of the D1 strain. The detection limit of enterotoxins in milk growth medium was about 0.1 ng/ml.

Inoculation and cultivation conditions. The strains of *S. aureus* 2064, D1, and B1 were maintained on the slopes of Plate Count Agar (PCA, Imuna, Šarišské Michaľany, Slovak Republic) at 5 ± 1°C. The standard suspension of the strains was prepared from an 18 h culture grown on PCA agar at 37°C. This staphylococcal suspension was inoculated aseptically into 300 ml of pre-tempered ultra high temperature-treated cows' milk (Rajo, Bratislava, Slovak Republic) in order to reach the initial *S. aureus* counts in each sample as constant as possible (approximately 10^3 CFU/ml). We respected the method of inoculation that had been described and validated in our recent work (Valík *et al*. 2008). The static incubation of inoculated milk samples was performed in duplicates at tem-

peratures 7, 8, 12, 15, 18, 21, 25, 30, 35, 39, 43, 46, and 51°C ± 0.5°C.

***Number of** S. aureus **in milk**.* The actual counts of *S. aureus* in parallel ultra-pasteurised milk samples inoculated with a constant concentration of the individual strains, 2064, D1, or B1, were determined at predefined time intervals by ten-fold dilution and cultivation on the Baird-Parker Agar according to the STN ISO 6888-1 standard procedure (1999) in order to obtain the growth curves.

Fitting the growth curves and calculating the growth parameters. The growth data, curves, and parameters of the strains under study were analysed, fitted, and calculated, respectively, using the mechanistic modelling technique of Baranyi *et al.* (1993) which is incorporated in the DMFit tools kindly provided by Dr. J. Baranyi (IFR Norwich, UK). The growth parameters (lag phase duration, growth rate and others) from each individual growth curve were analysed in the secondary phase of modelling by statistic tools of the Microsoft Office 2003 (Microsoft, Redmond, Washington, USA) and the Statistica data analysis software system, Version 7.1 (StatSoft, Inc., Tulsa, USA).

Secondary models. The maximal specific growth rate (μ) and lag phase duration (*lag*) were modelled as a function of the incubation temperature. For that purpose and inspired by the water activity (a_w) transformation by Gibson *et al.* (1994), we applied the following transformation of temperature:

$$T_w = \sqrt{(T_{\max} - T)} \quad (1)$$

where: T_{max} – estimated from the data points in the high-temperature region as recommended by Ratkowsky *et al.* (1983)

Then the natural logarithm of the specific growth rates was modelled by the following quadratic function as introduced by Gibson *et al.* (1994):

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

The coefficients C_0, C_1, and C_2 were estimated by linear regression. From these, the optimum value of T_w for the maximum growth rate was calculated as:

$$T_{w(\text{opt})} = -\frac{C_1}{2C_2} \quad (3)$$

The prediction about the staphylococcal growth rates at a given value of the incubation temperature was obtained in the following way (Gibson *et al.* 1994; Valík & Piecková 2001):

– calculation of T_w from the incubation temperature T using Eq. (1);
– calculation of ln μ using Eq. (2);
– μ = exp(lnμ) was predicted from the specific growth rate.

The influence of the incubation temperature, as the sole environmental factor, on the lag phase duration was described by the model developed by Davey (1991) that is based on the Arrhenius model:

$$\ln(1/\text{lag}) = C_0 + C_1/T + C_2/T^2 \quad (4)$$

where:
C_0, C_1, C_2 – coefficients
T – incubation temperature

Assuming that the initial numbers of *Staphylococcus aureus* are 10^3 CFU/ml and that counts higher than 6 log may present a potential risk of staphylococcal enterotoxin production, the time (t_3) necessary for an increase of *S. aureus* counts by about 3 log as compared to its initial numbers was also calculated. For this reason, the linear regression model t_3 was applied according to Gibson *et al.* (1994):

$$\ln t_3 = D_0 + D_1 T_w + D_2 T_w^2 \quad (5)$$

where:
D_0, D_1, D_2 – coefficients calculated by the linear regression method

Validation of the growth parameters. To validate the mathematical equations describing *S. aureus* responses to various temperature conditions, several mathematical and statistical indices were used including the accuracy (A_f), bias (B_f), and discrepancy ($\%D_f$) factors, mean square error (*MSE*), regression coefficient (R^2), per cent variance ($\%V$), and *F*-value. The accuracy (A_f), bias (B_f), and discrepancy ($\%D_f$) factors were calculated as defined by Baranyi *et al.* (1999):

$$A_f = \exp\left(\frac{\sqrt{\sum_{k=1}^{m} [\ln f(\mu^k) - \ln \mu^k]^2}}{m}\right) \quad (6)$$

$$B_f = \exp\left(\frac{\sqrt{\sum_{k=1}^{n} [\ln f(\mu^k) - \ln \mu^k]}}{m}\right) \quad (7)$$

$$\%D_f = (A_f - 1) \times 100 \quad (8)$$

where:
μ – growth rate obtained from the growth curves
$f(\mu^k)$ – growth rate calculated from the equations describing the experimental values
m – number of measurements

The observed and predicted data were compared using the mean square error (*MSE*):

$$\text{MSE} = \frac{\Sigma (\mu_{obs} - \mu_{pred})^2}{n} \quad (9)$$

where:

μ_{obs}, μ_{pred} – observed and predicted values of the growth rates, respectively

n – number of the data points

As a measure of goodness of the fit of the model, the per cent variance accounted for (*%V*) was used as given by Daughtry *et al*. (1997):

$$\%V = \left[1 - \frac{(1 - r^2)(N - 1)}{(N - N_T - 1)}\right] \times 100 \quad (10)$$

where:

N – number of observations

N_T – number of terms

r^2 – multiple regression coefficient

Because the *%V* takes into account the number of terms used in the model, it is considered as more stringent and appropriate test than multiple regression coefficient (R^2)

The comparison between the lack of fit and the measuring error can be quantified statistically by the f testing value as presented by Zwietering *et al*. (1991):

$$f = \frac{[(RSS_{meas} - RSS_{fit})/df_2 - df_1]}{RSS_{meas}/df_1} \quad (11)$$

$$\text{tested against } F_{df_1}^{df_2 - df_1} \quad (12)$$

where:

df_1 – number of degrees of freedom from the general model that equals the total number of data points minus the number of different temperatures measured

df_2 – number of the degrees of freedom from the growth-temperature model that equals the number of data points minus the number of parameters

RSS_{meas} – sum of squares of the deviations between the data and the general model

RSS_{fit} – sum of squares of the deviations between the data and the given growth temperature model

The *F* value is dependent on the numbers of degrees of freedom and numbers of variables of the model. If the model is linear in its parameters, the *f* value is lower than *F*, thus the model is suitable for the microorganism growth prediction (Zwietering *et al*. 1991; McMeekin *et al*. 1993).

RESULTS AND DISCUSSION

Growth of *Staphylococcus aureus* in milk

The growth of three strains of *S. aureus* in milk was studied. All the strains were catalase-positive, gram-positive, and showed reactions in the 'API Staph' kit, with more than 98% probability of *Staphylococcus aureus* identity. Based on the immunofluorescence determination of the enterotoxin production by *S. aureus* strains, only D1 produced enterotoxin SED.

The growth and SED detection assays of D1 strain in milk were performed at temperatures from 12°C to 21°C selected in connection with the typical temperatures of milk and artisanal cheese fermentation. The growth curves showed the typical sigmoid shape and were successfully fitted with the Baranyi model at the average of R^2 = 0.994 (Figure 1). The range of the toxin production is also demonstrated in this figure and the average growth parameters are summarised in Table 1. According to Balaban and Rasooly (2000), Delbes *et al*. (2006) and Normanno *et al*. (2007), SED was the second most common serotype of enterotoxins among staphylococcal strains isolated from dairy products associated with food poisoning. Figure 1

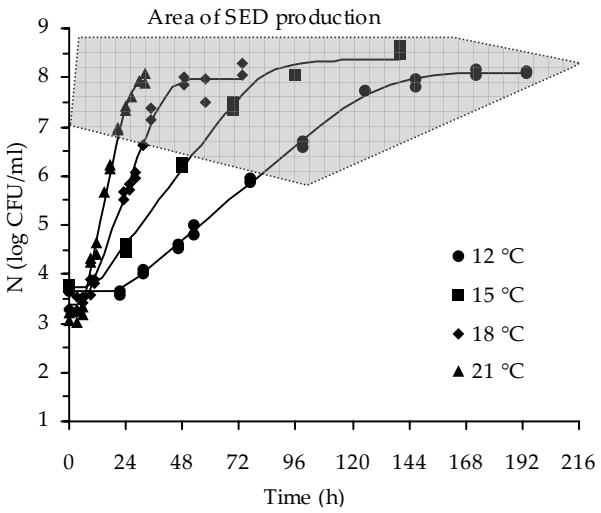

Figure 1. Growth of *S. aureus* D1 in milk at incubation temperatures 12, 15, 18, and 21°C, and the area of the SED production

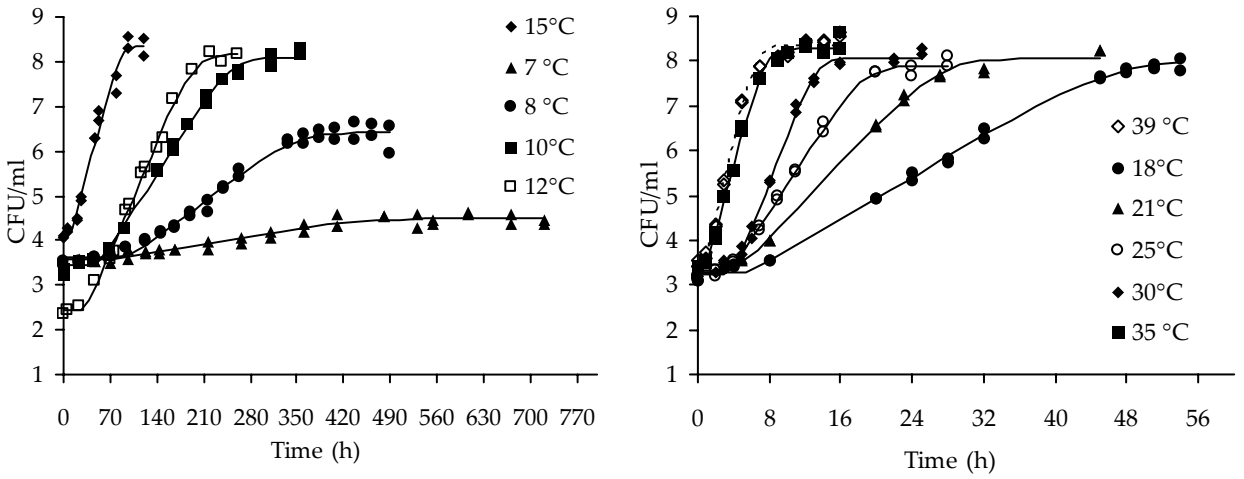

Figure 2. Growth of *S. aureus* 2064 in milk at incubation temperatures from 7°C to 39°C

indicates the fact that SED was already detected at the level of *S. aureus* 10^6 CFU/ml at the lower temperature of 12°C. At the higher temperatures of 18 and 21°C, SED toxin was detectable when *S. aureus* reached the density of 10^7 CFU/ml. Based on the previous literature data of Lindqvist *et al.* (2002), Asperger & Zangerl (2003), Delbes *et al.* (2006), Fujikawa and Morozumi (2006), Charlier *et al.* (2008) the generally accepted minimal concentration of *S. aureus* 10^6 CFU/ml was confirmed as anticipating the presence of staphylococcal enterotoxin by food practice.

To describe the growth of *S. aureus* and to cover the whole temperature range, similar experiments with the strain 2064 were carried out. The growth curves of *S. aureus* 2064 at all studied temperatures from 7°C to 51°C are shown in Figures 2 and 3. Except for the case of 51°C, all the growth curves were characterised by the typical sigmoid shape. The average initial counts of *S. aureus* 2064 ($N_{0\ STA\ 2064}$)

Table 1. Specific growth rates and t_d of *S. aureus* strain D1, B1 and 2064 in milk

T (°C)	Stran D1		Strain B1		Strain 2064	
	μ (h^{-1})	t_d (h)	μ (h^{-1})	t_d (h)	μ (h^{-1})	t_d (h)
7					0.006	120.3
8					0.026	27.0
10					0.055	12.7
12	0.103	6.8			0.082	8.5
15	0.148	4.7	0.051	13.5	0.145	4.8
17			0.139	5.0		
18	0.313	2.2			0.264	2.6
20			0.282	2.5		
21	0.545	1.3			0.484	1.4
25			0.399	1.7	0.711	1.0
30					1.215	0.6
35			1.311	0.5	1.664	0.4
39					1.931	0.4
43					1.903	0.4
46					0.562	1.2

μ – specific growth rate, t_d – time to double

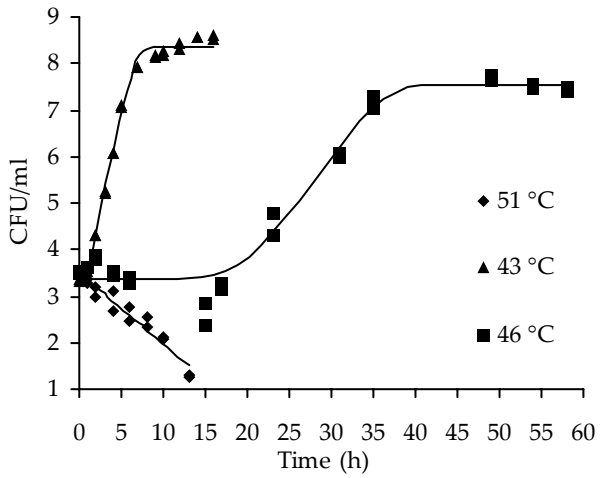

Figure 3. Growth of *S. aureus* 2064 in milk at incubation temperatures 43°C, 46°C, and 51°C

varied in the range of 3.39 ± 0.35 log CFU/ml. At 7°C, the lowest temperature used, the strain 2064 still grew but very slowly, with the specific growth rate only μ = 0.006 h^{-1} reaching maximal density in the stationary phase of 4.5 log after 30 days of incubation which was just 1 log higher than the initial number of *S. aureus* 2064. When the temperature increased by as little as 1°C, the growth curve of this strain was characterised with specific growth rate twice higher and maximal densities exceeding 6 logs in the stationary phase.

Despite the slow growth of the strain 2064, the temperature of 7°C can be considered as the minimal temperature for the growth of *Staphylococcus aureus* 2064 as proposed by Tatini (1973). However, Dengremont and Membré (1995) and Notermans and Heuvelman (1983) did not observe *S. aureus* growth at 8°C even after 1 week of incubation. On the other hand, other literature sources mentioned the lowest *S. aureus* growth temperature as T_{min} = 6.5–7.0°C (Halpin-Dohnalek & Marth 1989; Baird-Parker 1990; Jay 2000; Asperger & Zangerl 2003; Bremer 2004).

At 46°C (Figure 3), the viable *S. aureus* population propped from its initial counts until reaching the counts of 2.61 log CFU/ml during the first 15 hours. After that, *S. aureus* began to grow with the specific growth rate of μ = 0.56 h^{-1}. The survival line, with the rate of –0.15 log CFU/ml/h (–0.35 h^{-1}) which meant the D-value of 6.7 h, was observed at 51°C.

Secondary modelling

The microbial growth curve that represents the growth of a bacterial culture in time can be simply divided into the lag, exponential, and stationary phases. Each of these parts is also influenced by environmental food factors or the conditions prior to the growth analysis. Baranyi and Roberts (1995) emphasised that the lag phase is a period of adjustment to the environment during which only the intracellular conditions change. There are many different factors influencing the lag phase duration (Dens *et al.* 2005) e.g. the medium, temperature, physiological state of the cells, etc. When the temperature is the only modifying environmental factor, lag phase can be modelled by means of the model developed by Davey (1991). In this study, the so called "Davey" model was applied to 35 lag data set of *S. aureus* 2064 in the range from 8 to 43°C with the following three-parameter Eq. 13, where *lag* was in hours and temperature (*T*) in °C.

$$\ln (1/lag) = 1.973 - 87.92/T + 285.09/T^2 \quad R^2 = 0.962 \qquad (13)$$

Per cent variance (*%V*) of 95.8 indicated a high degree of fit that was comparable to the data of Daughtry *et al.* (1997). If a sufficient number of observations was taken into account, that means much more than the number of terms in Eq. 13, than $\%V \sim R^2$.

The specific growth rates μ in h^{-1} were derived from the growth curves of *S. aureus* by DMfit. Their average values calculated from 3 to 5 curves of the strains at each temperature are summarised in Table 1. The individual data were used in model-

Table 2. The indices of the internal performance of the G-model for the *S. aureus* 2064 in milk

Model	A_f	B_f	$\%D_f$	*MSE*	*F*-value	*f* value
$\ln\mu = \exp^{(-0.378T_w^2 + 2.202T_w - 2.371)}$	1.214	1.023	21.4	0.084	4.11	0.337

μ – the specific growth rate of *S. aureus* 2064; $T_w = \sqrt{(T_{max} - T)}$, $T_w = \sqrt{(T_{max} - T)}$; T_{max} – maximal growth temperature for *S. aureus* 2064; *T* – incubation temperature; A_f – the accuracy factor; B_f – the bias factor; D_f – discrepancy factor; *MSE* – mean square of error; *F*- and *f*-value – values for the goodness-of-fit test by using *F* ratio test

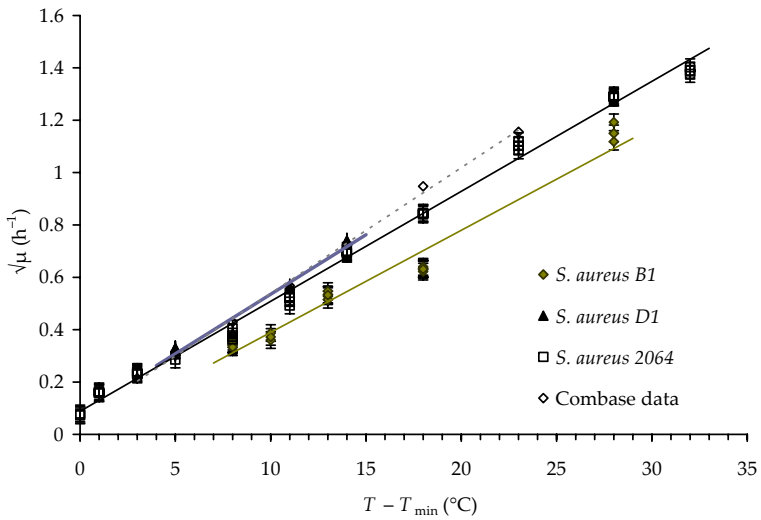

Figure 4. Comparison between the Ratkowsky model applied to the strains of *S. aureus* and the selected data from the Combase Predictor at the sub-optimal growth temperatures

ling and graphical presentations. All the specific growth rates at the temperatures from 7°C to 39°C showed high linearity with the correlation coefficient R^2 from 0.962 to 0.995 when modelled with square root model (Ratkowsky 1982).

The following equations resulted from fitting the growth rates with square root model in the temperature range from 7°C to 39°C for the strains 2064, D1, and B1, respectively:

$\sqrt{\mu}_{206} = -0.2057 + 0.042T \quad R^2 = 0.9948;\ \%V = 99.46$

$\sqrt{\mu}_{D_1} = -0.2381 + 0.0455T \quad R^2 = 0.9784;\ \%V = 97.73$

$\sqrt{\mu}_{B_1} = -0.2739 + 0.039T \quad R^2 = 0.9623;\ \%V = 95.85$

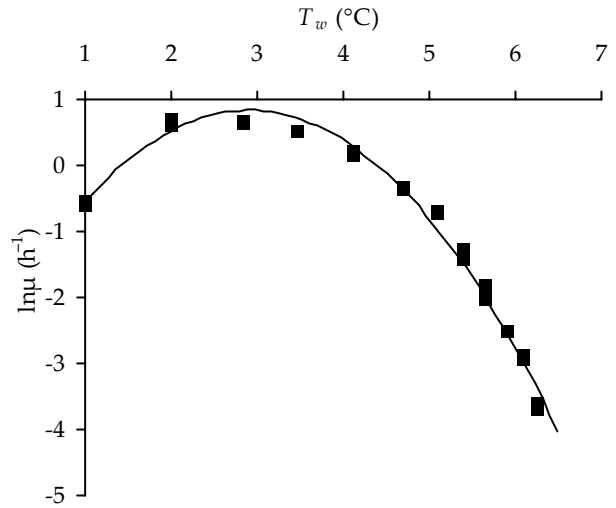

Figure 5. Plots of the natural logarithm of specific growth rates (ln μ) versus $T_w = \sqrt{(T_{max} - T)}$ for *S. aureus* 2064. The symbols indicate the natural logarithm of the specific growth rate calculated from the growth curves at each incubation temperature. The continuous line indicates the fitted ln μ vs. T_w function, where $\ln\mu = -0.378T_w^2 + 2.202T_w - 2.372$ ($R^2 = 0.987$)

From the testing of the goodness of fit, the per cent variance (%V) confirmed high correlation coefficients R^2 (above) for the strains of 2064, D1, and B1, respectively. The graphical representations of these regression lines are shown in Figure 4 including the comparison with Combase data for *S. aureus* in the range from 10°C to 30°C. Their model coefficients b ($\sqrt{\mu} = a + bT$), except for the B1 strain, were very close not only to each other but also to the coefficient of Combase line b_{Comb} = 0.048 or b = 0.0442 found by Fujikawa and Morozumi (2006).

In an empirical approach to modelling the effect of the incubation temperature on the growth rate of *S. aureus* 2064, the model by Gibson *et al.* (1994) was used to include the data beyond the growth optimum. In the original equation, Gibson *et al.* (1994) introduced a useful transformation of $b_w = \sqrt{(1 - a_w)}$, in which the value of 1 represents maximal water activity. In our case, the following T_w transformation ($T_w = \sqrt{(T_w - T)}$) was analogically used and T_{max} = 47°C for *S. aureus* 2064 was derived from two data points in the high-temperature region as recommended by Ratkowsky *et al.* (1983).

In the next step, the values of the specific growth rate of *S. aureus* 2064 were plotted against the calculated T_w-values and fitted with the regression model (Eq. 2) that was represented by the following C_i coefficients of the equation $\ln\mu = -0.37\ T_w^2 + 2.202\ T_w - 2.372$ ($R^2 = 0.987$). By using Eq. 3, $T_{w(opt)}$ and, subsequently, T_{opt} = 38.5 °C for *S. aureus* 2064 were calculated. According to the literature sources, the optimal temperature for the growth of *S. aureus* was similarly found or mentioned in the range from 37°C to 40°C by Halpin-Dohnalek &

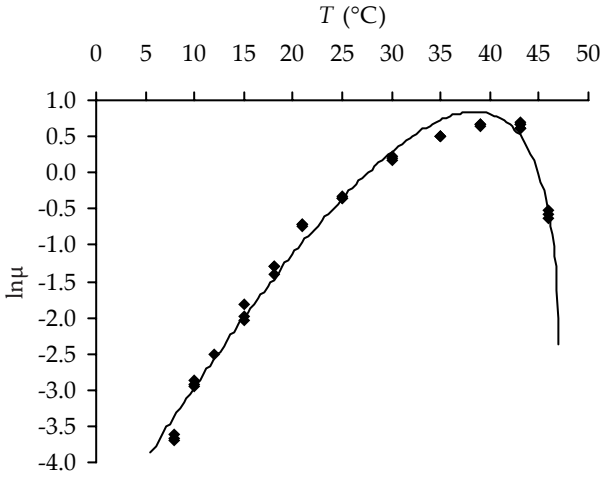

Figure 6. Plots of the natural logarithm of the specific growth rates (ln μ) versus T for *S. aureus* 2064. The symbols indicate the natural logarithm of the specific growth rate calculated from the growth curves at each incubation temperature. The continuous line indicates the fitted ln μ vs. T function, where $\mu = \exp^{(-0.378\ T_w^2 + 2.202 T_w - 2.372)}$ and $T_w = \sqrt{(T_{max} - T)}$

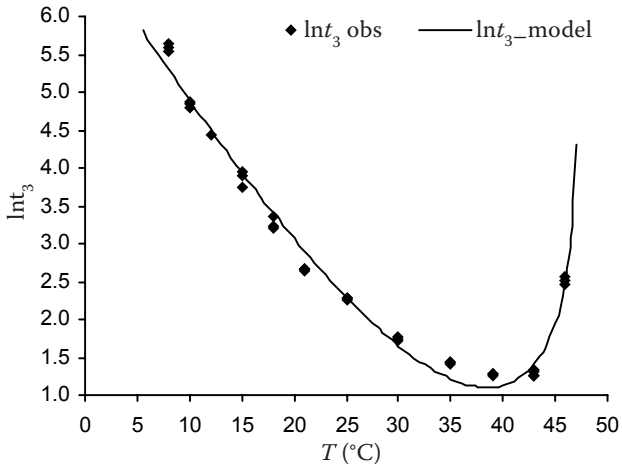

Figure 7. Plots of natural logarithm of time (t_3) necessary for an increase of *S. aureus* 2064 counts by about 3 log as compared to its initial numbers versus incubation temperature T. The symbols indicate the natural logarithm of time (t_3) calculated from the growth curves at each incubation temperature. The continuous line indicates the fitted ln t_3 vs. T function, where ln $t_3 = 3/\mu$ and $\mu = \exp^{(-0.378\ T_w^2 + 2.202 T_w - 2.372)}$

Marth (1989), Baird-Parker (1990), Asperger and Zangerl (2003), Bremer *et al.* (2004). The graphical representation of this modelling is shown in Figure 5 and, subsequently, the previous data were transformed into the more useful plot of lnμ against temperature (Figure 6).

Food practice is interested in easily interpretable data. As the level 10^6 CFU/g or ml is generally accepted for the anticipation of the staphylococcal enterotoxins, the time (t_3) needed for the increase of *S. aureus* counts by 3 log can be useful. Moreover, in coincidence with the *S. aureus* numbers in foods, Zárate *et al.* (1997), Kérouanton *et al.* (2007) and Akineden *et al.* (2008) considered the numbers about 10^3 CFU/g or ml as prevalent. That is why this prediction against temperature is shown in Figure 7.

Validation

The internal validation of the model used in this work was performed in accordance with the equations described in the Material and Methods part (Eqs 6–12). The results of the internal validation are summarised in Table 2. The indices bias and ac-

Table 3. Growth parameters of different strains of *Staphylococcus aureus* in various media

Observed values of *S. aureus* 2064				Dengremont and Membré (1995)		Charlier *et al.* (2008)			Sutherland *et al.* (1994)	
T (°C)	lag (h)	μ (h^{-1})	t_d (h)	T (°C)	μ (h^{-1})	T (°C)	lag (h)	M (h^{-1})	T (°C)	t_d (h)
15	11.6	0.131	5.25	14	0.053	15.5	11	0,4	–	–
25	3.4	0.715	0.97	25	1.05	27	3	1.6	25	0.63
30	4.3	1.215	0.57	–	–	–	–	–	30	0.51
35	0.9	1.663	0.42	–	–	–	–	–	33	0.44
39	0.8	1.930	0.36	37	0.44	37	1	3.1	37	0.39

T – incubation temperature; lag – lag phase duration; μ – specific growth rate; t_d – time to double

curacy indicate a good fit between the predictions with 21% of discrepancy. The accuracy factor based on the comparison of the model with the Combase or D1 strain growth data indicates that our predictions may differ from these values by 30–34%.

There is some evidence in the literature concerning the growth of *S. aureus* in the dependence on the incubation temperature. In Table 3, the values of the growth parameters of *S. aureus* are summarised as observed by various authors. Dengremont & Membré (1995) studied the growth of *S. aureus* in a synthetic culture medium at pH = 7.4, and the different medium can be the reason for the growth rates being half in comparison to our values. Charlier *et al.* (2008) performed an experiment using Tryptic Soy Broth with *S. aureus* MF31. The values of the lag phase duration are close to our data, but the values of the specific growth rates are twice as high than the values in milk observed by us. On the other hand, Sutherland *et al*. (1994) studied the growth of *S. aureus* in sterilised milk at pH = 6.5 and observed the values of time to double that are comparable with our values.

CONCLUSION

Focusing on the growth data obtained with two strains of *S. aureus* isolated from artisanal ewes' cheese, we would like to provide reliable data that could be taken into account when considering the safety of these fresh or short ripened soft cheeses.

The results demonstrated that the strains of *Staphylococcus aureus*, including the strain of human origin, were able to grow well in milk in the temperature range from 8°C to 46°C, the optimal temperature being about 38.5°C. As specific strains were used in the study, the growth data may provide useful information for the artisanal cheese practice. Taking into account that the initial *S. aureus* numbers in raw milk varied between 2–3 log CFU/ml, artisanal ewes´ lump cheese producers may apply our prediction of the time (t_3) directly as the t_3 time values are in coincidence with the time to reach the critical density of 10^6 CFU/ml for the possible enterotoxin presence.

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