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Quantification of the Growth Competition of Lactic Acid Bacteria: a Case of Co-culture with *Geotrichum candidum* and *Staphylococcus aureus*

Alžbeta Medveďová, Denisa Liptáková, Anna Hudecová, Ľubomír Valík*

Department of Nutrition and Food Assessment, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovak Republic

* *lubomir.valik@stuba.sk*

Abstract

The work turns the attention to the growth of two microorganisms which are connected with fermentation and contamination of artisanal lump cheeses. The effect of the cultivation temperature and the presence of Fresco culture on the growth of yeast-like microorganism *Geotrichum candidum* and on *Staphylococcus aureus*, the bacterial toxin producing cheese contaminant in ultrapasteurized milk was studied. The culture Fresco with initial concentration from 10^6 to 10^7 CFU.ml^{-1} significantly inhibited growth of both microorganisms. The growth rates of *G. candidum* and *S. aureus* were lower and the lag-phase duration was prolonged in comparison with the data resulted from growth in mono-culture. At all tested temperatures the counts of the Fresco culture observed in stationary phase ranged between 10^8 CFU.ml^{-1} and 10^9 CFU.ml^{-1}, whereas maximal density of *G. candidum* in stationary phase was around 10^5 CFU.ml^{-1} and *S. aureus* grew up only about 0.3 to 0.6 logs with maximal density about 10^4 CFU.ml^{-1}. The growth data found in this work may provide the information basis for food technologists and microbiologists to get the studied organism under the control. This is naturally important, however, each of the organisms play a different role during cheese fermentation.

Key words: *Geotrichum candidum*, lactic acid bacteria, predictive modelling, *Staphylococcus aureus*

Introduction

The interest in biological preservation methods as a significant tool in the ensuring of food safety has been increasing over the past decade. Lactic acid bacteria are most employed for this purpose, mainly because of their preservative effect against wide spectrum of the pathogenic and undesirable microorganisms such as bacteria, yeasts and toxin producing fungi. They take advantage of particular substances production in competition against other microorganism. Besides, these substances are organic acids (lactic, acetic, benzoic, formic, pyroglutamic, phenyllactic), hydroperoxide, diacetyl, acetoin, bacteriocins, proteinases, exopolysaccharides and many other substances that contribute to the flavour, aroma and texture properties of foods. Moreover, these metabolic products act as control tool of the growth and multiplication of spoilage and pathogenic microorganisms synergistically with a lowering of the pH (Schillinger et al. 1996; Caplice and Fitzgerald 1999; Hansen, 2002; Wouters et al., 2002; Valerio et al., 2004; Liptáková et al., 2007). Growth of yeasts can be inhibited by lactic acid bacteria due to their production of cyclic peptides (Nielsen et al., 1998).

Also, the development of cheese fermentation relied on the spontaneous development of lactic acid bacteria, which produce the lactic acid helping the milk to coagulate (Wouters et al., 2002). With concurrent pH value decreasing and development of sensorial substances, the required flavour and shlef life is secured primary and secondary, undesirable microflora is inhibited. Among microorganisms naturally occurring in ewes´ lump cheese, we were focused on *Geotrichum candidum* and *Staphylococcus aureus*, especially.

Geotrichum candidum is yeast-like organism found in soil, grass, fruits, plants, silage and raw milk and belongs to the important part of the microflora of soft cheeses such as Camembert, semihard cheeses such as St. Nectaire and artisanal ewes' and goats' raw milk cheeses (Hayaloglu and Kirbag, 2007; Boutrou and Guégen, 2005; Vasdinyei and Deák, 2003; Cosentino et al., 2001; Fadda et al., 2001). *G. candidum* takes a part in the early stages of ripening of soft cheeses and in typical flavour development due to the proteolytic, peptidolytic and lipolytic activity of some strains. Its aminopeptidases are able to reduce bitterness in Camembert (Wyder and Puhan, 1999; Thammavongs et al., 2000; Marcellino et al., 2001). Yeast-like fungus *G. candidum* is able to assimilate lactate and release ammonia, what contributes to the de-acidification of cheese surface (Fadda et al., 2001; Boutrou et al., 2006).

Commercial strains of *G. candidum* are used as the starter cultures in cheese manufacturing or as the additional culture in fermented milk Viili (Varnan and Sutherland, 1994; Robinson and

Tamine, 1990). In some fresh cheeses like cottage cheese and quark the presence of *G. candidum* may lead to the product spoilage (Fadda et al., 2001; Boutrou and Guégen, 2005).

G. candidum is able to grow in the wide range of temperature and pH, from 5 to 38 °C with optimal temperature around 25 °C and optimal pH between 5.5 and 6.0 or 6.0 and 7.0 (Boutrou and Guégen, 2005). According to van den Tempel and Nielsen (2000) the majority of strains tolerate salt from 1.0 to 2.5 % (w/v) and they are not able to grow in the presence of 4 % (w/v) salt in the environment. Due to the long hyphae formation ability, the maximal counts of *G. candidum* do not exceed 10^6 CFU.g^{-1} in cheese curd (Wyder and Puhan, 1999).

Staphylococcus aureus is facultatively anaerobic, mesophilic, catalase-positive and oxidase-negative microorganism. It grows within a temperature range from 7 °C to 48 °C and in the pH range from 4.2 to 9.3. This bacterium is halotolerant, the minimal water activity (a_w) for growth ranged from 0.83 to 0.85 (Baird-Parker, 2000; Asperger and Zangerl, 2003; Normano et al., 2007; Bremer et al., 2004).

S. aureus is considered to be one of the ubiquitous contaminants. Typical counts of *S. aureus* in properly drawn ewes' milk are between 100 and 200 CFU.ml^{-1} (Valík et al., 2004). The lack of proper hygienic measures during the preparation of food is a major risk of contamination, and staphylococcal food poisoning is often associated with manually prepared food (Akineden et al., 2008). Strains of *S. aureus* are characterized by production of heat-stable enterotoxins, which may be produced at a_w values as low as 0.86 under aerobic conditions. The critical *S. aureus* cell density higher than 10^6 CFU.ml^{-1} is required to enterotoxin production (Görner and Valík, 2004; Lindqvist et al., 2002; Jay, 2000).

The presence of toxin-producing strains in ewes' lump cheese or Bryndza cheese produced from raw milk is dependent on various factors, including animal health, environmental, hygienic and technological conditions (Valík et al., 2008). The *S. aureus* growth is inhibited by fermentative metabolism of lactic acid bacteria, especially during the fermentation and the ripening of cheeses. However, once formed the thermostable enterotoxins generally retain their biological activity even after killing of cells by pasteurization. Therefore sufficient acidification, especially in the early stages of cheesemaking, must be achieved by the use of an appropriate amount of an active starter culture (Asperger and Zangerl, 2003; Lindqvist et al., 2002; Medveďová et al., 2008).

This work was focused on the characterization of the inhibitory effect of mesophilic Fresco culture on the surviving of *Geotrichum candidum* and *Staphylococcus aureus* in co-culture in the milk substrate at different incubation temperatures.

Materials and Methods

Microorganisms

Geotrichum candidum was isolated from the artisanal ewes' lump cheese and identification was confirmed by Dr. Elena Piecková (Mycological Laboratory, Slovak Medical University). Strain of *G. candidum* was kept on the Plate count skim milk agar (Merck, Darmstadt, Germany) at 5 ± 1 °C.

Staphylococcus aureus strain 2064 originated from ewes' lump cheese was provided by Dr. Hanzélyová (State Veterinary and Food Institution, Prešov). Strain of *S. aureus* was kept on the Plate count agar (Imuna, Šarišské Michaľany, Slovakia) at 5 ± 1 °C.

The mesophilic Fresco culture DVS 1010 is commercial culture of Christian Hansen (Hørsholm, Denmark) and was stored frozen until analysis.

Substrate inoculation

The suspension of *G. candidum* and *S. aureus* used for inoculation were prepared from 24 - 48 h culture grown at defined surface of nutrient agar in tubes by standard rinsing with sterile pepton water/saline water. The ultrapasteurized milk (UHT; pH = 6.7) was used as the substrate for the growth dynamics determination of both tested strains. The inoculum was applied into the ultrapasteurized milk and into the milk co-culture with Fresco so that the initial cell density of *G. candidum* and *S. aureus* reached numbers lower than 10^2 and 10^3 CFU.ml^{-1}, respectively and the initial concentration of the Fresco culture was about 10^6 – 10^7 CFU.ml^{-1}. The trials were repeated in two or three parallel tests at the temperatures range from 12 °C to 30 ± 0.5 °C without shaking at aerobic conditions.

Number of microorganisms in ultrapasteurized milk

Total amounts, expressed as the number of colony-forming units per millilitre of parallel UHT milk samples and reported as CFU.ml^{-1}, of *G. candidum* were calculated according to the Slovak Technical Standard SNT ISO 7954, the STN ISO 6888-1 was used for *S. aureus* numbers expression and the concentration of Fresco culture was determined as number of the mesophilic bacteria grew on the M17 agar according to the STN ISO 4833.

Fitting of growth curves, calculating and validation of growth parameters

The growth parameters were calculated using modelling technique of Baranyi et al. (1993). The dependence of growth parameters on the temperature was modelled by the Ratkowsky model or simple linear mathematical equations.

Results and Disccusion

Growth of G. candidum in milk in co-culture with Fresco at 12 °C, 15 °C, 18 °C and 21 °C.

The co-existence of lactic acid bacteria and yeast microorganisms is determined by their adaptation to a substrate and by a number of intrinsic and extrinsic factors including redox potential, water activity, pH and temperature (Holzapfel, 1997). This work was focused on the effect of Fresco culture in initial concentration ranged from 1.6 to 2.2 10^7 CFU.ml^{-1} on the growth of *G. candidum* (N_0 = 7.9 10^2 to 1.3 10^3 CFU.ml^{-1}). The growth kinetics of *G. candidum* in milk inoculated with Fresco culture and cultivated at the temperatures between 12 °C to 21 °C is presented in the Fig. 1 and the growth parameters calculated from D-model are summarized in the Table 1. The incubation temperature and the addition of Fresco into the milk affected the growth of yeast-like microorganism *G. candidum.* For example, the differences between the growth rates of *G. candidum* at the highest (21 °C) and the lowest temperature (12 °C) achieved 63 % for mono- and co-culture. When the starter culture Fresco was added into the milk the growth rates reached about 2 times lower values in the mixed culture than in the pure culture at applied temperatures (Table 1).

Table 1: The growth parameters of *G. candidum* and starter culture Fresco in co-culture and the growth parameters of *G. candidum* in mono-culture in milk

T [°C]	Gc_Fr			Gc			Fr_Gc		
	Gr [logCFU/ml/h]	Lag phase [h]	t_d [h]	Gr [logCFU/ml/h]	Lag phase [h]	t_d [h]	Gr [logCFU/ml/h]	Lag phase [h]	t_d [h]
12	0.034	94.7	9.0	0.056	15.7	5.4	0.039	-	7.7
15	0.051	49.4	5.9	0.077	11.7	3.9	0.064	-	4.7
18	0.066	33.7	4.5	0.138	14.9	2.2	0.123	-	2.4
21	0.093	30.3	3.2	0.154	13.6	2.0	0.179	-	1.7

(Gc_Fr - the growth dynamics of *G. candidum* in co-culture with Fresco, Gc - the growth dynamics of *G. candidum* in mono-culture, Fr_Gc - the growth dynamics of starter culture

Fresco in co-cultivation with *G. candidum*, Gr is the growth rate in log CFU.ml^{-1}.h^{-1}, t_d - double time in h)

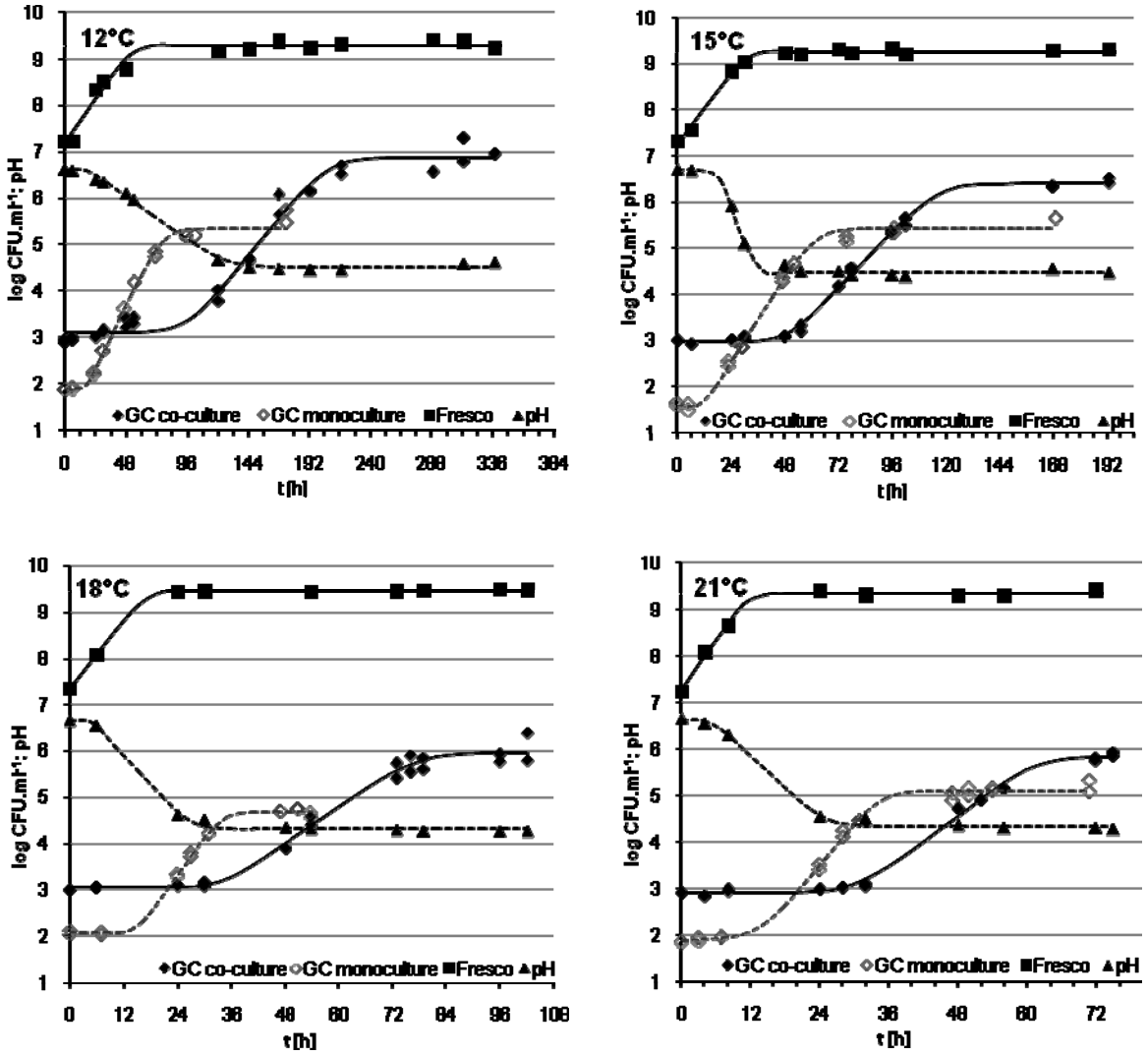

Fig. 1: Growth of *Geotrichum candidum* in milk in co-culture with Fresco culture at temperatures 12 °C, 15 °C, 18 °C and 21 °C

The growth dynamics of Fresco culture in co-culture in milk at 12 °C was faster in comparison with *G. candidum* growth in milk co-culture (Fig. 1). The growth rate of *G. candidum* in mono-culture at pH 6.7 was Gr_{Gc} = 0.056 log CFU.ml^{-1}.h^{-1} and in co-culture Gr_{Gc_Fr} = 0.034 log CFU.ml^{-1}.h^{-1}, that represented the decrease about 39 %, and the lag-phase was prolonged 6 times in comparison with the pure cultivation. The final counts of Fresco in co-culture with *G. candidum* were ranged between 10^8 till 10^9 CFU.ml^{-1} and the maximal counts of *G. candidum* were closed to 10^5 to 10^6 CFU.ml^{-1} what was in compliance with the study of Álvarez-Martín et al. (2008). With increase of temperature to 15 °C the lag-phase of mould decreased to 2 d and thereafter *G. candidum* started to proliferate with growth rate of 0.051

log CFU.ml^{-1}.h^{-1}. The decrease of the growth dynamics of *G. candidum* in co-cultivation with Fresco was about 34 % in comparison with the growth parameters determined in the mono-culture. The acidity of milk, the pH value, observed during the co-cultivation decreased from 6.7 to 4.5 during 2 days, approximately. At 18 °C the duration of the *G. candidum* lag-phase in co-culture with Fresco was 2.4 times longer (36 h) in comparison with the lag-phase observed in mono-culture (15 h) and the growth rate of *G. candidum* in co-culture decreased about 52 % compared with mono-culture (Gr_{Gc_Fr} = 0.066 log CFU.ml^{-1}.h^{-1}, Gr_{Gc} = 0.138 log CFU.ml^{-1}.h^{-1}). Express multiplication of Fresco culture, which achieved stationary phase after 24 h approximately, led to the drop of the pH from 6.7 to 4.5.

The last selected temperature for the growth dynamics study of *G. candidum* was 21°C. At this temperature Fresco decreased the growth kinetics of *G. candidum* alike as in the past cases, during which reached the stationary phase after 14 h (N = 2.1 10^9 CFU.ml^{-1}) and caused the pH value drop from 6.6 to 4.5. Yeast-like strain *G. candidum* grew with the growth rate of 0.093 log CFU.ml^{-1}.h^{-1}, which represented the decrease about 40 % in comparison with mono-culture and the previous lag-phase was 30 h long.

Further, the growth rates were analysed in relation to the temperature in mono- and co-culture of *G. candidum* and Fresco within second part of mathematical modelling. The Ratkowsky square root model linearizes the dependence of the microbial growth rate on the incubation temperature according to the Eq. 1 for pure culture and the Eq. 2 for mixed culture (Fig. 2, 3). This linear model enabled us to calculate the growth rate at different temperatures and predict the growth potential of the mould in milk also under conditions which were not included in the experiments.

$$\sqrt{Gr_{Gc}} = 0.0187T + 0.0106 \qquad R^2_{Gr} = 0.9482 \qquad \text{(Eq. 1)}$$

$$\sqrt{Gr_{Gc\_Fr}} = 0.0133T + 0.0235 \qquad R^2_{Gr} = 0.9945 \qquad \text{(Eq. 2)}$$

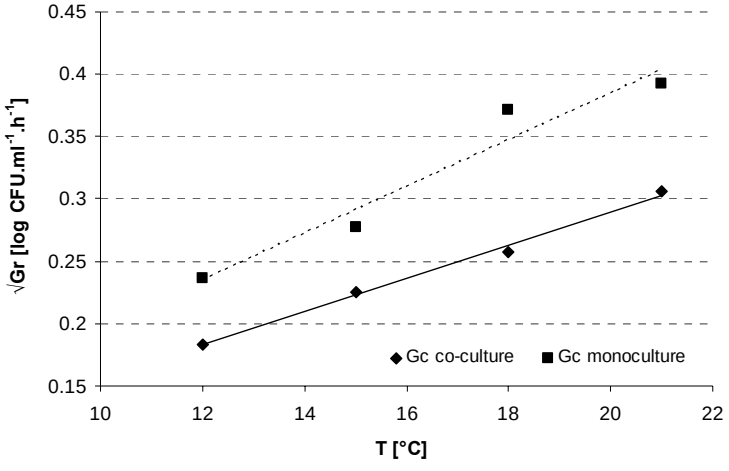

Fig. 2: The effect of the temperature on the growth rate of *Geotrichum candidum* ($\sqrt{Gr_{Gc}}$) in milk mono-culture (■) and in co-culture with Fresco (♦)

The Ratkowsky square root model (Eq. 1 and 2) was consequently used for time prediction when *G. candidum* would be able to increase its number by 3 log in milk. At this concentration yeast-like strain of *G. candidum* created mouldy layer on the surface of the milk, which resulted to the spoilage. We calculated these times for the pure culture and also for the mixed culture with Fresco at each temperature, respectively (Tab. 2). The mould needed approximately 1.7 times longer time to increase by 3 log in the co-culture than in the pure culture in accordance with the previous results (Fig. 3). Even if the time for multiplication was longer during mixed culture the mould finally reached higher counts than in the pure culture, at each temperature. This could be a result of the milk coagulation by Fresco activity. The mould is better adapted to grow on the solid substrates thanks to the higher presence of oxygen than in liquid media and created the mycelium on the surface of the coagulum.

Tab. 2: The time needed for *G. candidum* to increase its number by 3 log in milk

T [°C]	t_{3RTK} Gc [d]	t_{3RTK} Gc_Fr [d]
12	2.3	3.7
15	1.5	2.5
18	1.0	1.8
21	0.8	1.4

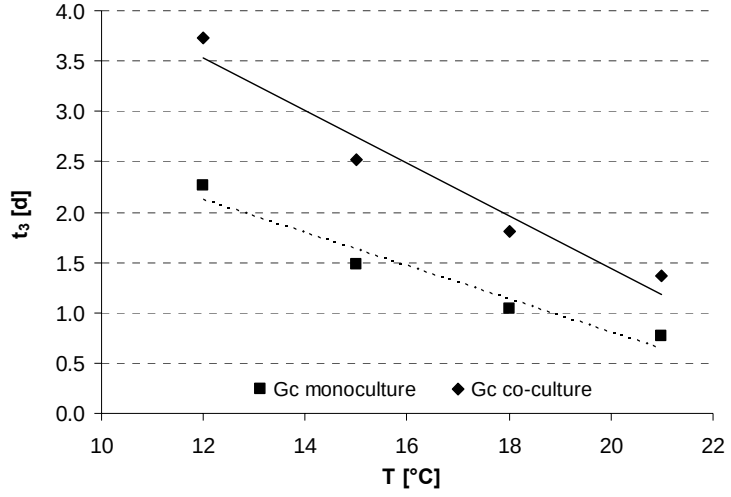

Fig. 3: The effect of temperature on the time needed to increase of *Geotrichum candidum* by 3 log in milk mono-culture (■) and in co-culture with Fresco (♦)

Our experimental results refer to the fact that the antagonistic relationship between Fresco culture and *G. candidum* was the consequence of the primary product of metabolism, lactic acid, the decreasing of pH-value and it was influenced as well by other factors, such a competition for nutrients and oxygen and by production of secondary antimicrobial substances. *G. candidum* was able to adjust to the new environmental conditions after prolonged lag-phase and started to grow and multiply with lower growth rate compared with its mono-culture growth rates. These findings are in accord with well-developed systems for maintaining intracellular pH known in yeast cell physiology. Intracellular pH and the H^+ pump are thought to play an important role in yeast growth. The plasma membrane ATPase that forms the transmembrane H^+ gradient driving force in nutrient transportation plays an essential role in the yeast physiology, regulation of the cell metabolic systems, lower permeability of the plasma membrane to the acid, active extrusion of the preservative, mediated transport and conversion of the preservatives (Imai and Ohmo, 1995; Henriques et al., 1997). H^+-ATPase enzyme consumes 40 to 60 % of total ATP pool, what can lead to the depletion of cell energy needed for growth and multiplication of yeasts (Imai and Ohmo, 1995, Brul and Coote, 1999).

Growth of S. aureus in milk co-culture with Fresco at temperatures between 12 °C and 30 °C.

The influence of lactic acid bacteria on the *Staphylococcus aureus* 2064 growth was studied at temperatures 12, 15, 18, 21, 25 and 30 °C in the ultrapasteurized milk. The average initial concentration of *S. aureus* in co-cultivation was 3.18 log $CFU.ml^{-1}$.

The growth rate of *S. aureus* in milk mono-culture had been increasing with successively escalating of incubation temperature. This interdependency is shown on the Figure 4 (dashed line) and described also by the equation 3.

The concentration of *S. aureus* at least 10^6 $CFU.ml^{-1}$, which is necessary to presumably enterotoxins production, was reached after 30 h at 18 °C and even after 12 h at 25 °C of incubation in milk mono-culture.

The minimal pH value for *S. aureus* growth is between 4.0 and 4.8, in dependence on the type and form of present acid (Normano et al., 2005; Asperger and Zangerl, 2003; Baird-Parker, 2000). According to Charlier et al. (2008), for pH lower than 5.0, the growth rate of *S. aureus* strongly decreased and no growth occurred at pH 4.5. With the respect to the *S. aureus* inability to growth under the acid conditions and to the necessity of fast pH dropping on values lower than 5.0 in the early hours of fermentation, the inhibitory potential of Fresco culture addition against *S. aureus* was studied in milk co-culture.

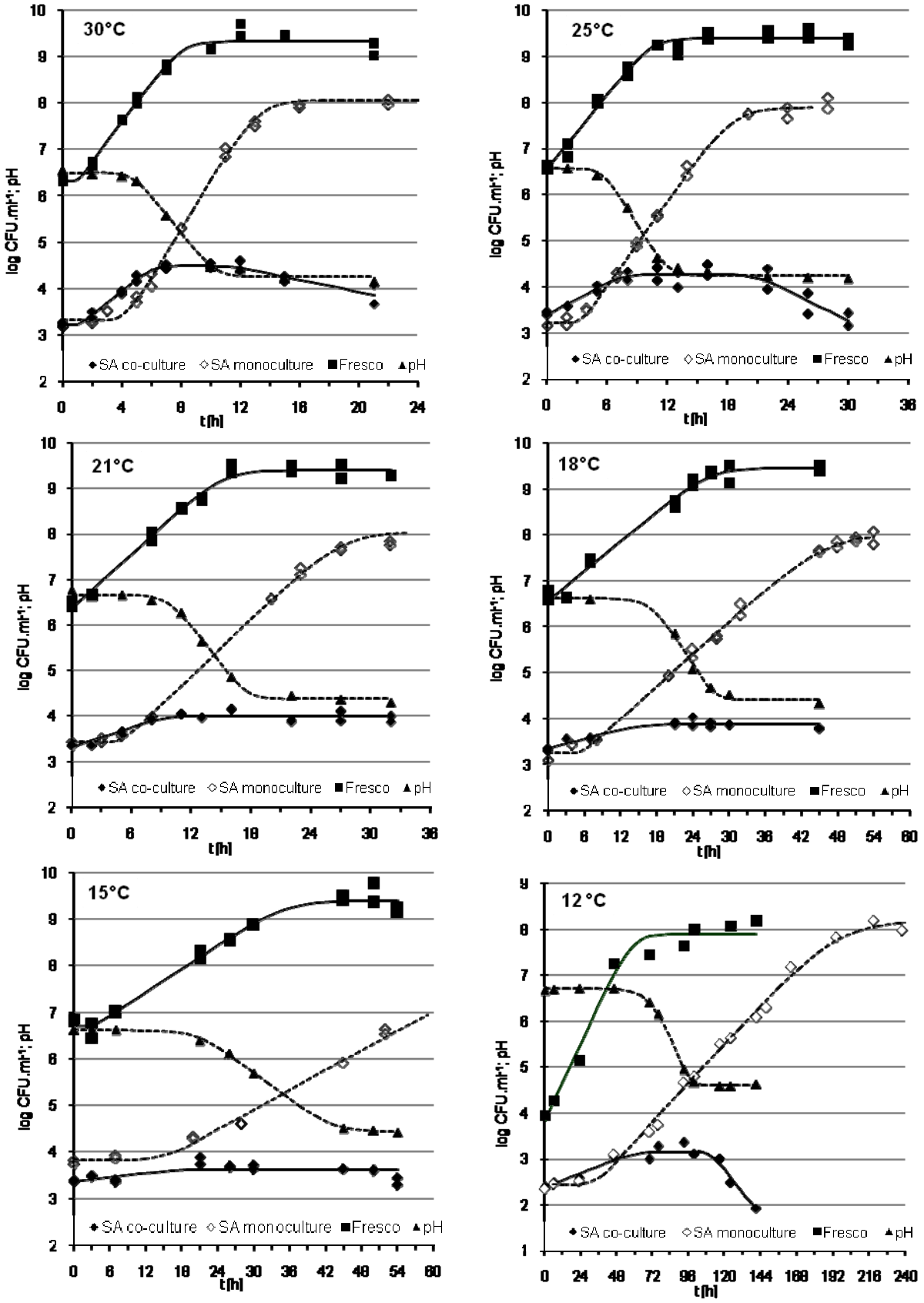

Fig. 4: Growth of *Staphylococcus aureus* 2064 in milk in co-culture with Fresco culture at the temperatures from 12 °C to 30 °C

At the studied temperatures, apart from 12 °C, the initial concentrations of Fresco culture were 6.51 log counts. At 12 °C the starter culture addition was only about 3.83 log counts. The lactic acid bacteria grew without the lag-phase, only at 30 °C the lag-phase of 1 hour was recorded. The growth rate of Fresco culture accelerated with increasing of incubation temperature according to the equation 4.

$$\sqrt{Gr}_{\mathrm{SA\_mono}} = 0.030T - 0.199 \qquad R^2_{Gr} = 0.994 \qquad \text{(Eq. 3)}$$

$$ln\ Gr_{\mathrm{FR}} = 2.530 - 126.2/T + 766.8/T^2 \qquad R^2_{lnGr\_\mathrm{Fr}} = 0.988 \qquad \text{(Eq. 4)}$$

The highest growth rate of Fresco culture of 0.427 log $CFU.ml^{-1}.h^{-1}$ was at 30 °C, obviously. On the other hand, the growth rate of Fresco at 12 °C was 6-times slower. Except the experiment at 12 °C, the maximal Fresco concentrations exceed 10^9 $CFU.ml^{-1}$. Its growth was attended by lactic acid production. As soon as the Fresco culture had reached the stationary phase, the pH lag-phase had stopped and pH values followed to decline.

In general, duration of the pH lag-phase is influenced by the lactic acid bacteria metabolism rate. The adaptation of microorganisms to the new environmental conditions (the lag-phase) is as quicker as higher the incubation temperature is. Because of fermentative metabolism of lactic acid bacteria, lactose is largely depleted in the first hours of incubation and lactic acid production with decreasing of pH values occurs. In temperature range from 12 °C to 30 °C, time necessary to achieve the minimal pH value had been compressing with the temperature increasing. At 12 °C, after 75 hours lasted pH lag-phase, the minimal pH value of 4.62 was attained. On the other hand, at 30 °C minimal pH value of 4.26 was achieved after the pH lag-phase of 5 h. The lactic acid production had a negatively effect on the pathogenic and undesirable microorganisms, including *Staphylococcus aureus*.

Tab. 3: Growth parameters of *S. aureus* 2064 and Fresco culture in milk co-cultivation in dependence on the incubation temperature and the Fresco culture addition

T [°C]	N_{o_Fr} [log CFU.ml^{-1}]	Gr_{Fr} [log CFU.ml^{-1}.h^{-1}]	$N_{max\_SA} - N_{o\_SA}$ [log CFU.ml^{-1}]	Gr_{SA} [log CFU.ml^{-1}.h^{-1}]
30	6.34	0.427	1.27	0.257
25	6.56	0.271	0.88	0.114
21	6.38	0.199	0.69	0.073
18	6.56	0.108	0.53	0.035
15	6.72	0.084	0.25	0.014
12	3.83	0.070	0.78	0.013

T – the incubation temperature, N_{o_Fr} – initial number of Fresco culture, Gr_{Fr} – growth rate of Fresco, Gr_{SA} – growth rate of *S. aureus*, $N_{max\_SA} - N_{o\_SA}$ – increase of *S. aureus* counts in stationary phase

From Fig. 4 is noticeable that growth of *Staphylococcus aureus* was possible only during the pH lag-phase. The addition of lactic acid bacteria was the lowest at 12 °C, only about 2.5 log counts higher than the concentrations of *S. aureus*. Although the *S. aureus* lag-phase was not detected at this Fresco concentration, the growth of *S. aureus* was inhibited effectively. Growth rate in the exponential phase was 0.013 log CFU.ml^{-1}.h^{-1}. In co-culture, growing up of *S. aureus* had lasted only 2 d. After next 3 d of stationary phase *S. aureus* started to pass away with the inhibition rate of 0.042 log CFU.ml^{-1}.h^{-1}. If *S. aureus* was kept in milk mono-culture, the growth rate was thrice higher than in co-culture with lactic acid bacteria, at temperature 12 °C.

Increase of incubation temperature about 3 °C caused rising up of *S. aureus* growth rate in mono-culture about 60 %, but the growth rate in co-culture was similar as at 12 °C (0.014 log CFU.ml^{-1}.h^{-1}). Although in this case, the addition of Fresco culture was higher about 2.5 log counts than at 12 °C, the net density of *S. aureus* in stationary phase ($N_{max\_SA} - N_{0\_SA}$) was about 0.5 log counts lower.

With next increasing of incubation temperature, the growth rate of *S. aureus* was significantly positively related to increase of incubation temperature as follows the equation 5. This equation is applicable at initial Fresco culture concentration of 6 log counts. On the other hand, increase about 1 log counts of *S. aureus* was secured by the addition of Fresco culture higher than 10^6 CFU.ml^{-1}at all studied temperatures apart from 30 °C (Fig. 5).

$$\sqrt{Gr}_{SA\_co\text{-}culture} = 0.022T - 0.194 \qquad R^2_{Gr} = 0.962 \qquad \text{(Eq. 5)}$$

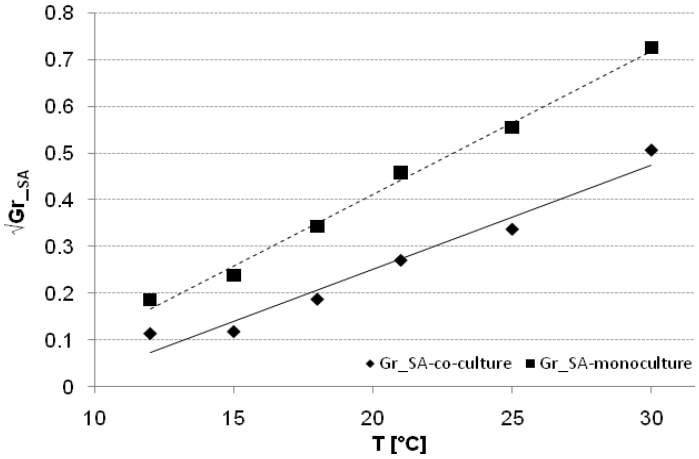

Fig. 5: The effect of the incubation temperature on the growth rate of *S. aureus* ($\sqrt{Gr}_{SA}$) in milk mono-culture (▲) and in co-culture with 6 log counts of Fresco (■)

The inhibition of *S. aureus* growth with Fresco culture was the more effective the higher addition of competitive culture and the lower the incubation temperature was. The pH lag-phase duration influenced the time necessary to reach stationary phase by *S. aureus*. The unchanged pH conditions in milk were determined with growth dynamics of lactic acid bacteria. The higher the incubation temperature was the shorter time was needed to sufficient lactic acid production and pH values depletion.

According to the European regulation No. 1441/2007, the total amounts of *S. aureus* in raw milk cheeses should not exceed 10^4 CFU.ml^{-1}. To keep counts of *S. aureus* under this limit the addition of Fresco culture in concentration higher than 10^6 CFU.ml^{-1} is recommended. Our results confirmed that for control of *S. aureus* growth is necessary to reduce the lactic acid bacteria lag-phase and the time of pH lag-phase what was crucial together with temperature of fermentation. Only at 25 °C and 30 °C the advisable concentration of *S. aureus* was exceed. However, the increases in *S. aureus* counts were lower than 2 log and therefore the critical concentration for enterotoxin production should not be implemented on the assumption of well drawn milk.

The effect of increasing addition of Fresco culture on *S. aureus* (N_{max_SA} - N_{0_SA}) up-growing is shown on the Fig. 6 at temperatures from 15 °C to 30 °C. With respect to the conditions at ewes´ lump cheese artisanal production, to keep the net growth of *S. aureus* ($N_{max\_SA} - N_{0\_SA}$) at the level lower than 2.0 log at 21 °C or 18 °C, the initial number of Fresco should be higher than 4.0 or 2.5 log CFU.ml^{-1}, respectively.

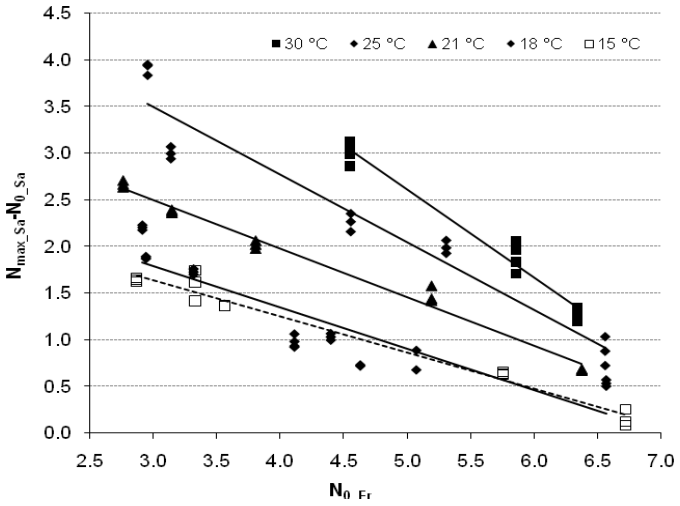

Fig. 6: The dependence of various initial concentration of Fresco on the up-growth of *S. aureus* at incubation temperature from 15 °C to 30 °C

Conclusion

Main goal of our work was to characterize the growth and multiplication of *Geotrichum candidum* and *Staphylococcus aureus* in model UHT milk substrate in relation to the addition of Fresco culture and temperature. The Fresco culture showed the inhibitory effect on both tested microorganisms at the higher initial concentration. Behaviour of the studied microorganisms, the decrease of the pH value below 5.0 and temperatures within 18 to 21 °C could be taken into account as the conditions during lactose fermentation in ewes' cheeses. The found growth data can provide the sound predictions for dairy practice. In the future, the co-existence of another lactic acid bacteria native present in sheep cheeses and *S. aureus* and *G. candidum* could be determined to ensure hygienic safe food products.

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