

## Co-Cultivation Growth of *Escherichia coli* and *Staphylococcus aureus* as Two Common Dairy Contaminants

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The overgrowth of spoilage and pathogenic bacteria may pose risk to consumers health and cause technological and economic losses. Hence, interactions among *S. aureus* 2064 and *E. coli* BR in dependence of incubation temperature and different initial counts of both microorganisms were quantitatively described based on cultivation experiments and predictive models. Statistically insignificant differences ( $p \geq 0.05$ ) between growth rates of *E. coli* at different initial concentrations suggest that the growth rates of *E. coli* in co-cultivation with *S. aureus* were affected only by incubation temperature. The growth of *E. coli* can be reliably predicted ( $R^2 = 0.968$ ;  $A_r = 1.160$ ) based on the equation  $\sqrt{\mu} = 0.0283 (T - T_{min}) + 0.1038$ . The growth of *S. aureus* during its co-cultivation with *E. coli* was influenced by incubation temperature and the presence of *E. coli* as well. It was documented by relatively high discrepancies indexes ( $D_i$  23.9–43.9%) and also by differences between growth rates at different initial microbial concentrations. These results may help in better understanding of interrelationships during sensitive foods production (e.g. without temperature treatment or those with intensive manual handling).

### INTRODUCTION

The trend for the consumption of raw milk and raw milk products, in the context of “consuming natural” and “purchasing locally” is becoming more and more popular. Taking into account the high nutritious potential of raw milk demonstrated by the presence of various macronutrients (carbohydrates, fats and proteins with easy digestibility), micronutrients (vitamins and minerals), regulators of physiological processes (amino acids), almost neutral pH (6.4–6.7), and high water activity [Claeys *et al.*, 2013; Hahne *et al.*, 2019], it is a valuable food not only for human and animals, but also for microorganisms. The sources of microbial contaminants are versatile, including the animal udder surface, feed, feces, milking equipment [Quigley *et al.*, 2013] or contact with milk handling personnel [Valík, 2013]. Besides technologically important bacteria (e.g. lactic acid bacteria LAB), accompanying species (e.g. *Micrococcus*) also spoilage and pathogenic bacteria may occur. The presence of spoilage bacteria can have considerable negative effects on the quality of milk and dairy products, the presence of pathogens can have more severe repercussions [Quigley *et al.*, 2011; Valík, 2013]. Hence, the risk posed by consuming raw milk, whether due to the rare presence of such pathogenic bacteria that cause severe disease with high mortality rates (e.g. *Listeria monocytogenes*) or due

to frequently occurring bacteria with low severity (e.g. *Campylobacter* spp., *Staphylococcus aureus* or *Escherichia coli*) is still considered high [Valík, 2013].

In our work, we focused on two bacteria: *E. coli* and *S. aureus*, considering their frequent presence in milk and in the environment (raw materials of animal and plant origin, feed, handling personnel, but also wastewater or sludge that can be reused as fertilizer in agricultural soil), their good growth in different nutrient media, genetic properties, and severity of causing diseases. Their growth, survival, and formation of harmful metabolites are dependent not only on the quantity they achieve in the media, but also on conditions of the surrounding environment and also on their mutual interactions. If the environmental conditions create a favorable growth environment, these hygienically and technologically undesirable microorganisms can multiply, produce toxic metabolites, and ultimately endanger the health of consumers [Medvedová & Valík, 2012]. Therefore it is necessary to determine relationships between microorganisms themselves and also between microorganisms and external and internal environmental factors that determine microbial growth, or lead to their inhibition. This is also the main role and contribution of predictive microbiology. Thus, if we obtain a mathematical equation describing the impact of environmental conditions on the behavior of microorganisms in food, it is natural to use such knowledge and quantify (predict) the extent and degree of their impact on microbiological quality and food safety [Ross & McMeekin, 1994]. Additionally, in specific cases,

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mathematical modelling is also required to predict the interaction of antagonist or competitively behaving populations [Vereecken *et al.*, 2000; Brocklehurst *et al.*, 2004; Giménez & Dalgaard, 2004; Valík & Ačai, 2016].

In this context, the aim of our work was to quantitatively describe the mutual relationships between these potentially pathogenic bacteria in dependence on incubation temperature and proportion of their initial counts that can have impact on which population will be dominating in a given environment, even if the initial counts of one of the population will be unusually high or above the legislative limits.

## MATERIAL AND METHODS

### Bacterial isolates

The isolate of *E. coli* BR was isolated from a Slovakian traditional “Bryndza” cheese. The isolate of *S. aureus* 2064 was isolated from a Slovakian ewes’ lump cheese. Both isolates were maintained in Brain Heart Infusion (BHI) broth (Sigma-Aldrich, St. Louis, Missouri, USA) at  $5 \pm 0.5^\circ\text{C}$  prior to analysis. Their identity was confirmed and described in our previous work [Medvedová *et al.*, 2009, 2018].

### Inoculation, cultivation conditions, and bacterial counts enumeration

A standard suspension of each isolate was prepared from a 24-h old culture grown in BHI broth at  $37^\circ\text{C}$ . These cultures were then diluted in saline-peptone solution and from  $10^{-3}$  dilution 0.3 mL were inoculated into 300 mL of pre-tempered ultra/high temperature treated (UHT) milk (Rajo, Bratislava, Slovakia). The nutritional composition of 100 mL of UHT milk was as follows: 1.5 g of fat, from which 0.9 g were saturated fatty acids, 3.4 g of proteins, and 4.9 g of sugars from which 4.9 g was lactose. The above inoculation procedure was aimed to reach initial concentration of *E. coli* at approximately  $10^3$  CFU/mL and of *S. aureus* at approximately  $10^6$  CFU/mL (3EC+6STA); of *E. coli* at approximately  $10^6$  CFU/mL and of *S. aureus* at approximately  $10^3$  CFU/mL (6EC+3STA); of *E. coli* at approximately  $10^3$  CFU/mL and of *S. aureus* at approximately  $10^3$  CFU/mL (3EC+3STA). The static incubation of three parallel and three replicates experiments was performed at 6, 12, 15, 18, 21, 25, 30, and  $37 \pm 0.5^\circ\text{C}$ .

Sampling was performed at predefined time intervals taking into account the incubation temperature, to reach the best possible fit of model in curvature between lag phase and exponential phase and then between exponential phase and stationary phase. We took into account data obtained during monocultural growth of the isolates. Actual density of the studied isolates was determined by 10-fold dilution in a saline-peptone solution and by using selective nutrient media, Baird-Parker agar (Merck, Darmstadt, Germany) for determination of *S. aureus* 2064 counts according to EN ISO 6888-1 and Chromocult Coliform agar (Merck, Darmstadt, Germany) for determination of *E. coli* BR counts according to National Standard Method F23. At the same time as microbial density was determined, also a pH value of the media was examined by using a WTW Inolab 720 pH meter (Weilheim, Germany).

### Modelling the microbial growth

Growth parameters from each individual growth curves for both microbial isolates were determined by using a primary model of Baranyi & Roberts [1994] using an in-house Excel Add-in package ‘DMFit’ version 3.5 (ComBase managed by United States Department of Agriculture-Agricultural Research Service, Washington, USA; and University of Tasmania Food Safety Centre, Hobart, Australia). Subsequently, the effect of temperature on the growth rates was described by the model of Ratkowsky *et al.* [1982]:

$$\sqrt{\mu_{\max}} = b (T - T_{\min}) \quad (1)$$

where:  $\mu_{\max}$  is specific growth rate (1/h),  $T$  is actual incubation temperature,  $T_{\min}$  is theoretical minimum temperature at which growth was observed ( $^\circ\text{C}$ ), and  $b$  is a Ratkowsky parameter [Ross & McMeekin, 1994; Valík & Ačai, 2016].

### Validation and statistical analysis

To validate mathematical equations describing the temperature effect on the growth rate of the isolates, the accuracy ( $Af$ ), bias ( $Bf$ ), and discrepancy (%  $Df$ ) factors were used as defined by Baranyi *et al.* [1999]. Also the ordinary least-squares criterion and regression coefficient ( $R^2$ ) were used. Finally, the statistical analysis (independent Student  $t$ -test and ANOVA test with statistical significance of  $p < 0.05$ ) was performed using Microsoft Excel tools 2007 (Microsoft, Redmond, USA).

## RESULTS AND DISCUSSION

Microbial interactions have been described intensively and also several predictive models have been introduced by *e.g.* Giménez & Dalgaard [2004], Vereecken *et al.* [2000] or Brocklehurst *et al.* [2004]. However, all these models focused on the competition or antagonistic behavior of LAB on the growth of spoilage or pathogenic bacteria, as we described also earlier in Valík *et al.* [2018] for *S. aureus* in dependence of LAB and in Ačai *et al.* [2016] for *E. coli* mutual growth with LAB. However, in this paper we would like to draw the attention to the interactions between spoilage/pathogenic bacteria themselves, since *S. aureus* and *E. coli* are frequently present in raw materials [Quigley *et al.*, 2013], in which they can multiply until concentrations resulting in disease outbreaks [Medvedová & Valík, 2012]. Their growth is affected not only by environmental conditions but also by the present microbiota, the pathogenic one included.

The initial concentration of *E. coli* in experiments with 6 EC was  $N_{\text{OEC}} = 6.11 \pm 0.19$  log CFU/mL ( $n=24$ ;  $\text{cv}=3.1\%$ ), and in experiments with 3 EC it was  $N_{\text{OEC}} = 3.27 \pm 0.32$  log CFU/mL ( $n=48$ ;  $\text{cv}=9.9\%$ ). On the other hand, initial counts of *S. aureus* in experiments with 6 STA was  $N_{\text{OSTA}} = 5.98 \pm 0.21$  log CFU/mL ( $n=24$ ;  $\text{cv}=3.5\%$ ) and in experiments with 3 STA it was  $N_{\text{OSTA}} = 3.40 \pm 0.61$  log CFU/mL ( $n=48$ ;  $\text{cv}=17.9\%$ ).

The growth of both microbial partners was positively affected by increased incubation temperature as it is also shown by growth parameters calculated by the model of Baranyi & Roberts [1994] summarized in Table 1. Exemplary

TABLE 1. Growth parameters of *E. coli* (EC) BR and *S. aureus* (STA) 2064 during their co-cultivation in UHT milk in dependence of temperature and parameters of pH value change.

| Experimental variant | $\mu$                     |                           | lag                      |                           | $N_{max}$                 |                           | pH         |                   |
|----------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|------------|-------------------|
|                      | <i>S. aureus</i>          | <i>E. coli</i>            | <i>S. aureus</i>         | <i>E. coli</i>            | <i>S. aureus</i>          | <i>E. coli</i>            | <i>k</i>   | lag <sub>pH</sub> |
| Temperature 6°C      |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.02±0.00                 | -0.01±0.00                | 138.3±3.9                | 0±0.0                     | 5.11±0.06                 | 1.25±0.07                 | -0.01±0.00 | 0±0.0             |
| 3STA+6EC             | 0.12 <sup>a</sup> ±0.02   | -0.01±0.00                | 226.6 <sup>a</sup> ±27.3 | 0±0.0                     | 3.50 <sup>a</sup> ±0.14   | 3.91 <sup>a</sup> ±0.09   | -0.01±0.00 | 0±0.0             |
| 6STA+3EC             | 0.01 <sup>c</sup> ±0.00   | -0.01±0.00                | 0 <sup>b,c</sup> ±0.0    | 35.9 <sup>b,c</sup> ±14.6 | 6.03 <sup>b,c</sup> ±0.17 | 0.97 <sup>b,c</sup> ±0.05 | -0.01±0.00 | 300.4±5.4         |
| Temperature 12°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.10±0.01                 | 0.14±0.00                 | 23.9±0.7                 | 21.0±0.6                  | 8.08±0.06                 | 8.65±0.04                 | -0.22±0.02 | 128.8±2.4         |
| 3STA+6EC             | 0.51 <sup>a</sup> ±0.02   | 0.13±0.00                 | 121.7 <sup>a</sup> ±0.3  | 18.7 <sup>a</sup> ±0.2    | 8.07±0.31                 | 8.72±0.02                 | -0.03±0.01 | 130.4±3.5         |
| 6STA+3EC             | 0.12 <sup>c</sup> ±0.02   | 0.14±0.00                 | 75.2 <sup>b,c</sup> ±7.7 | 19.9±1.9                  | 7.99 <sup>b,c</sup> ±0.02 | 8.70±0.06                 | -0.01±0.00 | 0±0.0             |
| Temperature 15°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.30±0.00                 | 0.31±0.01                 | 15.0±0.2                 | 5.5±1.6                   | 8.54±0.01                 | 8.85±0.04                 | -0.26±0.02 | 44.9±2.4          |
| 3STA+6EC             | 0.01 <sup>a</sup> ±0.00   | 0.21 <sup>a</sup> ±0.00   | 64.1 <sup>a</sup> ±27.9  | 6.6±1.2                   | 3.67 <sup>a</sup> ±0.07   | 8.62 <sup>a</sup> ±0.03   | -0.07±0.01 | 24.2±1.6          |
| 6STA+3EC             | 0.30 <sup>c</sup> ±0.01   | 0.21 <sup>b</sup> ±0.00   | 16.5 <sup>b,c</sup> ±1.5 | 3.8 <sup>b,c</sup> ±1.1   | 8.69 <sup>b,c</sup> ±0.06 | 8.55 <sup>b</sup> ±0.00   | -0.06±0.01 | 21.9±2.1          |
| Temperature 18°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.31±0.00                 | 0.35±0.02                 | 2.5±0.8                  | 5.2±1.1                   | 7.88±0.03                 | 8.26±0.14                 | -0.05±0.01 | 25.1±2.5          |
| 3STA+6EC             | 0.10 <sup>a</sup> ±0.00   | 0.29 <sup>a</sup> ±0.01   | 17.9 <sup>a</sup> ±0.2   | 4.6±0.6                   | 4.85 <sup>a</sup> ±0.00   | 8.62 <sup>a</sup> ±0.06   | -0.44±0.08 | 19.3±1.8          |
| 6STA+3EC             | 0.26 <sup>b,c</sup> ±0.00 | 0.41 <sup>c</sup> ±0.01   | 2.8 <sup>c</sup> ±0.3    | 7.5 <sup>c</sup> ±0.0     | 8.38 <sup>b,c</sup> ±0.08 | 6.71 <sup>b,c</sup> ±0.00 | -0.14±0.04 | 15.9±1.9          |
| Temperature 21°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.47±0.01                 | 0.64±0.01                 | 1.8±0.0                  | 3.7±2.3                   | 8.47±0.01                 | 8.81±0.03                 | -0.49±0.12 | 27.2±5.4          |
| 3STA+6EC             | 1.40 <sup>a</sup> ±0.39   | 0.65±0.00                 | 7.7±4.4                  | 3.9±0.1                   | 4.76 <sup>a</sup> ±0.05   | 8.99 <sup>a</sup> ±0.00   | -0.10±0.01 | 23.7±4.8          |
| 6STA+3EC             | 0.69 <sup>b,c</sup> ±0.01 | 0.55 <sup>b,c</sup> ±0.02 | 2.9±0.1                  | 2.2±1.7                   | 8.82 <sup>b,c</sup> ±0.01 | 8.58 <sup>b,c</sup> ±0.03 | -0.22±0.04 | 49.2±6.9          |
| Temperature 25°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 0.78±0.00                 | 1.09±0.00                 | 1.3±0.2                  | 2.0±0.1                   | 8.73±0.02                 | 9.03±0.04                 | -0.61±0.15 | 5.1±0.4           |
| 3STA+6EC             | 0.05 <sup>a</sup> ±0.00   | 0.41 <sup>a</sup> ±0.00   | 0 <sup>a</sup> ±0.0      | 0 <sup>a</sup> ±0.0       | 4.89 <sup>a</sup> ±0.00   | 8.81 <sup>a</sup> ±0.03   | -0.24±0.08 | 9.8±1.1           |
| 6STA+3EC             | 1.55 <sup>b,c</sup> ±1.26 | 0.89 <sup>b,c</sup> ±0.01 | 2.7 <sup>b,c</sup> ±0.0  | 1.2 <sup>b,c</sup> ±0.3   | 8.95 <sup>b,c</sup> ±0.04 | 8.69 <sup>b,c</sup> ±0.02 | -0.11±0.01 | 4.2±0.7           |
| Temperature 30°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 1.03±0.01                 | 1.09±0.01                 | 1.4±0.3                  | 0.4±0.2                   | 7.47±0.06                 | 8.32±0.01                 | -0.12±0.01 | 8.3±1.2           |
| 3STA+6EC             | 0.28 <sup>a</sup> ±0.00   | 1.08±0.07                 | 0 <sup>a</sup> ±0.0      | 1.2±0.8                   | 4.94 <sup>a</sup> ±0.00   | 8.91 <sup>a</sup> ±0.06   | -0.10±0.01 | 3.6±0.5           |
| 6STA+3EC             | 0.86 <sup>b,c</sup> ±0.04 | 1.21 <sup>b</sup> ±0.01   | 0 <sup>b</sup> ±0.0      | 0±0.0                     | 8.95 <sup>b,c</sup> ±0.02 | 8.20 <sup>b,c</sup> ±0.01 | -0.11±0.01 | 4.3±0.2           |
| Temperature 37°C     |                           |                           |                          |                           |                           |                           |            |                   |
| 3STA+3EC             | 1.43±0.01                 | 1.82±0.01                 | 0±0.0                    | 0.7±0.0                   | 8.28±0.03                 | 8.89±0.08                 | -0.26±0.17 | 6.9±1.5           |
| 3STA+6EC             | 0.34 <sup>a</sup> ±0.09   | 2.06±0.12                 | 0±0.0                    | 1.1±0.0                   | 4.78 <sup>a</sup> ±0.12   | 8.78±0.01                 | -0.42±0.21 | 4.7±1.1           |
| 6STA+3EC             | 0.88 <sup>b,c</sup> ±0.01 | 2.19±0.11                 | 1.1 <sup>b,c</sup> ±0.3  | 1.5±0.3                   | 8.95 <sup>b,c</sup> ±0.15 | 8.79±0.03                 | -0.34±0.12 | 9.1±2.4           |

$\mu$  – specific growth rate (1/h), lag – duration of lag phase (h),  $N_{max}$  – counts of isolates in the stationary phase (log CFU/mL), *k* – rate of pH value decrease (1/h), lag<sub>pH</sub> – duration of pH value lag phase (h); \* – results only for the first growth phase; statistical significant differences ( $p < 0.05$ ) within parameters <sup>a</sup> – in experiments 3STA+3EC and 3STA+6EC, <sup>b</sup> – in experiments 3STA+3EC and 6STA+3EC, <sup>c</sup> – in experiments 3EC+6STA and 6STA+3EC.

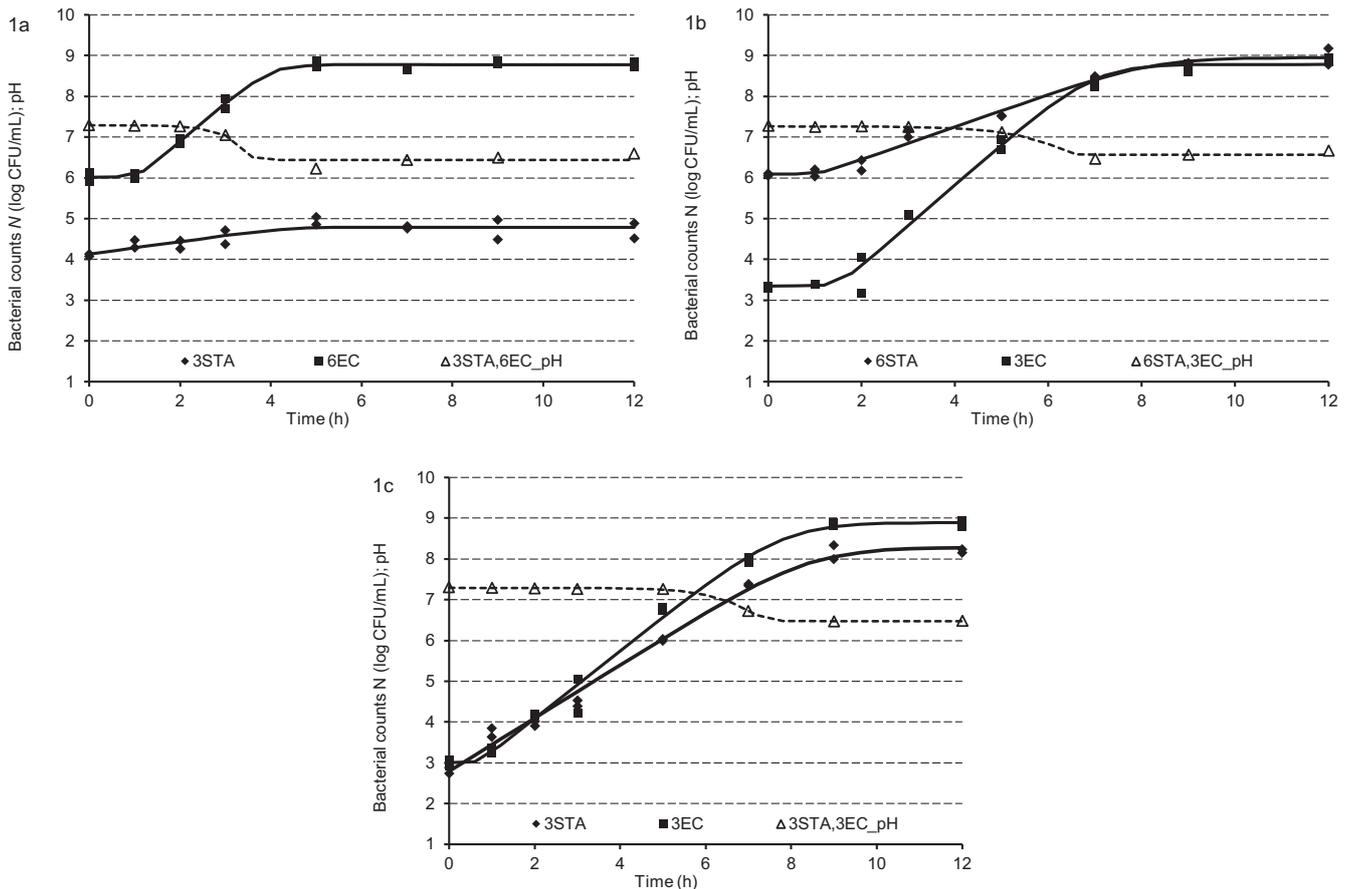


FIGURE 1. Growth of *S. aureus* 2064 and *E. coli* BR during co-cultivation in UHT milk in dependence on their initial counts (1a – 3STA+6EC; 1b – 6STA+3EC, 1c – 3STA+3EC) and subsistent change of pH value at 37°C.

growth curves at 37°C are depicted in Figure 1a-c, where one can see that if the initial concentration of *E. coli* and *S. aureus* was almost equal (3STA+3EC), both microorganisms grew very similarly to their typical final densities of about 8–9 log CFU/mL. Also in the case of dominating *S. aureus* counts (6STA+3EC), the growth of *E. coli* was affected only by the incubation temperature and not by the presence of *S. aureus*, as it is expressed by statistically insignificant differences ( $p \geq 0.05$ ) at 6, 12, 18, and 37°C between growth rates of *E. coli* during co-culture with *S. aureus* in experiments 3EC+3STA and 3EC+6STA (missing superscript b in Table 1). Also in these experiments, *E. coli* was able to reach final densities in the stationary phase of at least 7 log CFU/mL. However, when there was an excess of *E. coli* in the media (3STA+6EC), the growth of *S. aureus* was inhibited and resulted in significantly lower final counts of *S. aureus*. The maximal *S. aureus* counts in the stationary phase in the case of *E. coli* prevalence in the media were at densities lower than 5 log CFU/mL.

Based on co-cultivation growth of *S. aureus* and *E. coli* and based on comparing their mono-cultural growth [Medvedová & Valík, 2012; Medvedová *et al.*, 2018], it can be concluded that the *S. aureus* 2064 growth is inhibited in the presence of *E. coli* BR abundance. It can probably be due to faster nutrient consumption by *E. coli* and also to the production of metabolites with antistaphylococcal properties, e.g. lactic acid, citric acid, and other organic acids, lower fatty ac-

ids. All these substances were identified in *E. coli* by Kim & Kim [2017] who reported about 1000 intracellular metabolites in *E. coli*. Even so, the actual produced substances are dependent on completeness of the media and other factors affecting *E. coli* biosynthesis processes [Tokuyama *et al.*, 2019], in the case of *E. coli* cultivation in milk, the completeness of media is unchallenged [Claeys *et al.*, 2013; Hahne *et al.*, 2019].

The intensiveness and dominance of *E. coli* growth is obvious also in Figure 2. Values of its specific growth rates were in all experiments and at all temperatures higher than the specific growth rates of *S. aureus*, as the linear curve of *E. coli* specific growth rates affected by temperature was above linear curve of *S. aureus* in experiments 3STA+3EC. We may suppose that *E. coli* started to grow earlier than *S. aureus* (majority of *E. coli* lag phases are shorter than those of *S. aureus*), subsequently it started to consume nutrients and produced metabolites. Taking together, we may assume that *E. coli* was more effective in nutrient competition and together with probable antistaphylococcal metabolites it led to *S. aureus* 2064 growth inhibition during their co-cultural growth.

The only exceptions of this trend were experiments at 6 and 12°C. At 12°C, and in the case of 3STA+6EC experiment, *S. aureus* reached in the stationary phase counts of 8.07 log CFU/mL. It may be a result of slower pH value decrease ( $k = -0.03$  1/h) compared to its decrease at other temperatures, where the slowest decrease ( $k = -0.07$  1/h) was observed at 15°C; even it was 2.3-times faster.

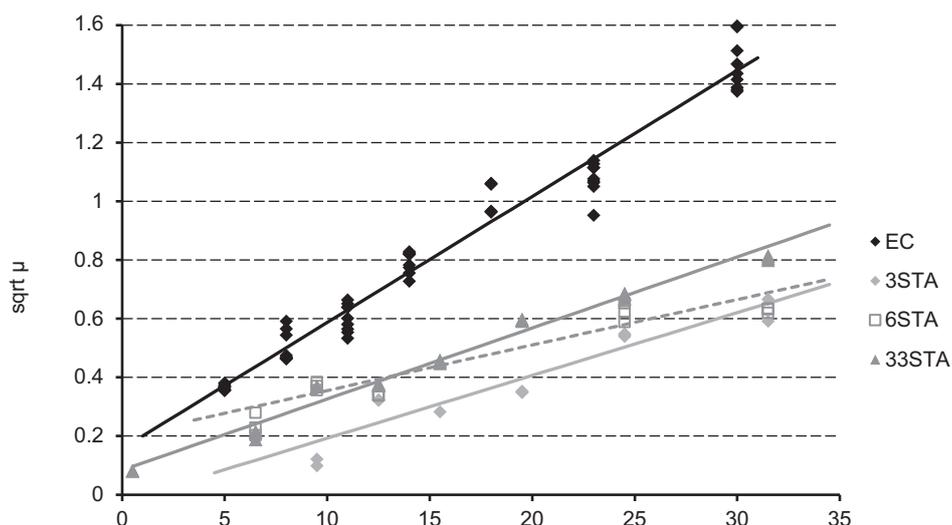


FIGURE 2. Effect of incubation temperature (expressed as  $T - T_{min}$ ) on the specific growth rate (expressed as square root of specific growth rate  $\mu$ ) of *E. coli* (EC) BR in mono-culture (◆) and during its co-cultivation with *S. aureus* (STA) 2064 in 3STA+3EC (●), in 6STA+3EC (□) and 3STA+6EC (▲) in UHT milk according to Ratkowsky model. 3STA or 3EC stands for initial bacterial density of  $10^3$  CFU/mL, 6STA or 6EC stands for initial bacterial density of  $10^6$  CFU/mL.

A specific trend of bacterial behavior was observed also at  $6^\circ\text{C}$ , since only rotting of *E. coli* was observed at all mutual initial counts combinations. Contrary, *S. aureus* was able either to maintain its counts (6STA+3EC) or even increase its counts by about 2.59 log CFU/mL (3STA+3EC) or increase them by about 0.9 log CFU/mL in the first phase (marked with \* in Table 1) and after the second lag phase lasting for 242 h, the decrease of its counts was noticed with the rate of  $-0.01$  1/h (growth parameters from the second phase are not shown in Table 1). The ability of *S. aureus* to grow at  $6^\circ\text{C}$  in experiment 3STA+3EC with the specific growth rate of  $\mu=0.02$  1/h is remarkable, since in ultra-high temperature treated milk the same isolate was unable to grow at  $6^\circ\text{C}$  and it started to grow only at  $7^\circ\text{C}$  (with  $\mu=0.006$  1/h) [Medvedová & Valík, 2012]. It is also worthy of mention that at  $6^\circ\text{C}$  the *S. aureus* isolate was able to maintain its counts in experiment 6STA+3EC at density of 6 log CFU/mL for 36 days. It is undesirable in the case of enterotoxigenic strains as 5 log CFU/mL are the minimal counts needed for staphylococcal enterotoxins production [Delbes et al., 2006; Charlier et al., 2008]. Therefore, it seems that the presence of *E. coli* helped *S. aureus* to withstand such a low temperature better than during its mono-cultural growth.

The effect of temperature on the microbial growth in sub-optimal temperature range can be described by Ratkowsky

model. As it is shown in Figure 2 and by equation 1 (Table 2), there was a linear trend in *E. coli* growth rate increase in dependence on temperature and it was not influenced by the presence of *S. aureus*. On the other hand, the growth rate of *S. aureus* was influenced not only by the incubation temperature but also by the presence of *E. coli* and mutual ratio between them (Figure 2). It is also obvious in Table 2, where 3 different equations describing the growth rate of *S. aureus* in dependence of *E. coli* initial counts are presented. An inevitable part of predictive modelling is also validation. Subsistent validation indices are also mentioned in Table 2. Since the discrepancy coefficient for the Ratkowsky model describing the growth of *E. coli* is only 16%, it is also clear that its growth was influenced only by the incubation temperature. It is also emphasized by statistically insignificant discrepancies ( $p \geq 0.05$ ) in almost all experiments between *E. coli* growth rates at different *S. aureus* initial counts and their combinations. Moreover, also in the case of *E. coli* lag phase there were only few cases when its values were significantly ( $p \geq 0.05$ ) influenced by *S. aureus* initial density. It was also the case of *E. coli* final densities in the stationary phase. On the other hand, discrepancy indices of 23.9–43.9% are referring to a significant effect of *E. coli* presence in the cultivation media, mutual ratio between *E. coli* and *S. aureus*, and also to the effect

TABLE 2. Validation of Ratkowsky model describing the dependence of temperature on specific growth rate of *E. coli* BR and *S. aureus* 2064 during their mutual co-cultivation.

| Experimental variant | Equation describing dependence             | $A_f$ | $B_f$ | $\%D_f$ | $R^2$ |
|----------------------|--|-------|-------|---------|-------|
| 1: EC in all         | $\sqrt{\mu} = 0.028 (T - T_{min}) + 0.104$ | 1.160 | 0.994 | 16.0    | 0.968 |
| 2: STA in 3STA+3EC   | $\sqrt{\mu} = 0.036 (T - T_{min}) + 0.133$ | 1.239 | 0.953 | 23.9    | 0.980 |
| 3: STA in 3STA+6EC   | $\sqrt{\mu} = 0.021 (T - T_{min}) - 0.021$ | 1.439 | 1.015 | 43.9    | 0.883 |
| 4: STA in 6STA+3EC   | $\sqrt{\mu} = 0.024 (T - T_{min}) + 0.259$ | 1.411 | 0.960 | 41.1    | 0.904 |

of incubation temperature. Besides *S. aureus* specific growth rate, also its lag phase duration and also counts in the stationary phase were significantly influenced by the presence of the second bacterial population.

Regarding the pH value of the growth media, its value in all performed experiments started to decline from an average value of  $7.40 \pm 0.11$  ( $n=96$ ;  $cv=1.5\%$ ) after a given time (pH lag phase) that was as shorter as the incubation temperature was higher. The pH value decrease was observed during the growth phase of *E. coli* to an average value of  $pH\ 6.31 \pm 0.31$  ( $n=96$ ;  $cv=5.0\%$ ). The lowest pH value observed in time of reaching the stationary phase by *E. coli* indicates that its growth was accompanied by the production of acid with subsequent pH value decrease. Contrary, in the case of slower *E. coli* growth (experiments with lower values of *E. coli* specific growth rates) also its metabolic activity was slower that indicates lower pH value decrease rates  $k$  (Table 1). Finally, during the stationary phase of *E. coli*, the pH value started to increase again to a final value of about  $6.62 \pm 0.33$  ( $n=96$ ;  $cv=5.0\%$ ). As the pH value was determined only in one of parallels, the statistical analysis of pH value parameters was not performed.

## CONCLUSION

To conclude, the interactions between two frequent contaminants of raw materials, *E. coli* and *S. aureus*, as affected by temperature and their mutual ratios were described. The growth of *E. coli* isolate was affected and positively stimulated by the incubation temperature. During its growth, pH value decrease was observed as a result of *E. coli* metabolic activity. pH decrease together with competition for nutrient led to *S. aureus* growth inhibition, even its growth was positively stimulated by increasing incubation temperature. Presented results may help during production of mainly foods with minimal heat treatment with the aim to increase their safety and harmlessness. However, as many lactic acid bacteria cultures are used in the food industry, their role in spoilage / pathogenic bacteria growth is indisputable; the problem of microbial behavior and interactions is more complex and need to be described deeply. Furthermore, mathematical modelling cannot be a compromise or alternative to the established hygiene norms. Using high quality raw material is crucial in dairy technology especially in “traditional ways of production using raw milk” to increase safety of those products.

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## CONFLICT OF INTEREST

None of the authors of this manuscript has a financial or personal relationship with other people or organizations that could inappropriately influence the content of this work.

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