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Short communication: Comparison of growth kinetics at different temperatures of *Streptococcus macedonicus* and *Streptococcus thermophilus* strains of dairy origin

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ABSTRACT

Within the genus *Streptococcus*, *S. thermophilus* and *S. macedonicus* are the 2 known species related to foods. Streptococci are widely used as starter cultures to rapidly lower milk pH. As *S. macedonicus* has been introduced quite recently, much less information is available on its technological potential. Because temperature is an important factor in fermented food production, we compared the growth kinetics over 24 h of 8 *S. thermophilus* and 7 *S. macedonicus* strains isolated from various dairy environments in Italy, at 4 temperatures, 30°C, 34°C, 37°C and 42°C. We used the Gompertz model to estimate the 3 main growth parameters; namely, lag phase duration (λ), maximum growth rate (μ_{max}), and maximum cell number at the stationary phase (N_{max}). Our results showed significant differences in average growth kinetics between the 2 species. Among the strains tested, 37°C appeared to be the optimal temperature for the growth of both species, particularly for *S. macedonicus* strains, which showed mean shorter lag phases and higher cell numbers compared with *S. thermophilus*. Overall, the growth curves of *S. macedonicus* strains were more similar to each other whereas *S. thermophilus* strains grew very differently. These results help to better define and compare technological characteristics of the 2 species, in view of the potential use of *S. macedonicus* in place of *S. thermophilus* in selected technological applications.

Key words: starter culture, growth kinetics, Gompertz model

Short Communication

Within the genus *Streptococcus*, *S. thermophilus* and *S. macedonicus* are 2 species related to foods. Although the first is very well known and used in production of a large number of fermented products, *S. macedonicus* is a relatively new species (Tsakalidou et al., 1998) isolated from dairy products. *Streptococcus thermophilus* is widely used in starter cultures with the aim of growing rapidly and decreasing the pH, because changes in pH induce modifications in the bacterial population composition (Bovo et al., 2012; Maragkoudakis et al., 2013). Although much less is known about *S. macedonicus*, several studies have been devoted to its characterization and to the definition of several features relevant for food production; for example, synthesis of exopolysaccharides, bacteriocin production, and response to acid stress (De Vuyst and Tsakalidou, 2008). In addition, some strains have been used for the production of trial cheeses (Settanni et al., 2011, 2013; Guarcello et al., 2016). Because of the presence of such potential technological features, better knowledge of this new species would be useful.

In the present study, we analyzed growth of 8 *S. thermophilus* and 7 *S. macedonicus* strains of dairy origin isolated in northeast Italy (Table 1) at different temperatures to assess and compare their growth kinetics. Strains were stored at −80°C in brain-heart infusion broth (Oxoid, Basingstoke, UK) plus 25% (vol/vol) glycerol and were subcultured twice before use in 10 mL of M17 medium (Oxoid) containing lactose (0.5%) in 15-mL tubes. Cultures were grown at 37°C for 24 h. Growth studies were done in 96-well microtiter plates (SIAL0596, Sigma St. Louis, MO) with a microtiter plate incubator reader (Spark 10M, Tecan GmbH, Grödig, Austria). Overnight cultures (10 mL) were centrifuged at 10,000 × *g* for 10 min. Pellets were then washed twice with 5 mL of PBS and resuspended in 5 mL of sterile PBS.

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Table 1. Bacterial strains of *Streptococcus thermophilus* and *Streptococcus macedonicus* isolated from northeast Italy and used in this study

Strain	Isolation matrix	Geographical origin	Source or reference
S. thermophilus TH1436	Goat raw milk	Friuli Venezia Giulia	Treu et al., 2014a
S. thermophilus TH1435	Goat raw milk	Friuli Venezia Giulia	Treu et al., 2014a
S. thermophilus TH1477	Cow raw milk	Veneto	Treu et al., 2014b
S. thermophilus 1F8CT	Curd from cow raw milk	Veneto	Treu et al., 2014b
S. thermophilus TH982	Buffalo mozzarella curd	Campania	Treu et al., 2014b
S. thermophilus TH985	Buffalo mozzarella whey	Campania	Treu et al., 2014b
S. thermophilus M17PTZA496	Fontina cheese (cow)	Valle d'Aosta	Treu et al., 2014c
S. thermophilus MTH17CL396	Fontina cheese (cow)	Valle d'Aosta	Treu et al., 2014c
S. macedonicus 8SP	Curd of Spressa cheese	Trentino Alto Adige	Lombardi et al., 2004
S. macedonicus 19AS	Natural milk culture for Asiago cheese (cow)	Veneto	Treu et al., 2017
S. macedonicus 62AS	Natural milk culture for Asiago cheese (cow)	Veneto	Lombardi et al., 2004
S. macedonicus 27MV	Monte Veronese cheese (cow)	Veneto	Treu et al., 2017
S. macedonicus 203MA	Malga cow cheese	Veneto	Veneto Agricoltura[1]
S. macedonicus 211MA	Malga cow cheese	Veneto	Treu et al., 2017
S. macedonicus 33MO	Curd of Morlacco cheese (cow)	Veneto	Vendramin et al., 2014

[1]Veneto Agricoltura, Istituto per la Qualità e le Tecnologie Agroalimentari, Thiene, Italy.

Cell concentration was standardized to 10^6 cells/mL in M17 medium plus 0.5% lactose at pH 7.0, aliquoted into microtiter wells (200 μL per well), and incubated at 1 of 4 selected temperatures: 30°C, 34°C, 37°C, or 42°C for 24 h inside the plate reader. Growth was measured by automatically reading the absorbance (optical density at 600 nm) every 30 min. All experiments were done using 3 biological and 4 technical replicates. Blank and negative controls were included in all experiments. The Gompertz model (Zwietering et al., 1990) was applied to estimate the main growth parameters; namely, lag phase duration ($\boldsymbol{\lambda}$), maximum growth rate ($\boldsymbol{\mu_{max}}$), and maximum cell number at the stationary phase ($\mathbf{N_{max}}$).

SigmaPlot software version 12.0 (Systat Software, San Jose, CA) was used for statistical analysis. The Shapiro-Wilk test was used to evaluate normal distribution of data. After the normality test, data were analyzed for statistical significance using ANOVA followed using the Tukey test. Data with a non-normal distribution were analyzed by nonparametric test (Dunn method).

Growth curves for *S. thermophilus* and *S. macedonicus* obtained at different temperatures are reported in Figure 1, and growth parameters (λ, μ_{max}, and N_{max}), calculated by applying the Gompertz model, are reported in Table 2. These 3 parameters concisely describe population growth kinetics under specific environmental conditions and are therefore useful for evaluating strain performance in view of their technological use. In general, low λ values indicate that a strain can rapidly begin multiplying, thus hindering the development of the indigenous microbiota. High μ_{max} values indicate that bacteria are colonizing the substrate rapidly and efficiently, and high N_{max} values indicate that a high number of bacteria are present at the end of the growth process, which is a valuable feature of a fermented food.

Although *S. thermophilus* strains can growth at temperatures ranging from about 25°C to 50°C (Vaningelgem et al., 2004), we chose 4 temperatures used for most technological applications, because it is well established that temperature plays a role in the production of substances; for example, benzoic acid (Han et al., 2016) or exopolysaccharide (Li et al., 2016), and affects the technological characteristics of fermented food products (Nor-Khaizura et al., 2014; Westerik et al., 2016). Regarding *S. macedonicus*, only 2 strains have been tested so far for growth at different temperatures. *Streptococcus macedonicus* ACA-198 (Poirazi et al., 2007) was studied for bacteriocin production, which was the highest at 40°C; and strain LC743 (Cho et al., 2010), incubated at 34°C, 37°C and 40°C, showed the highest growth rate at 40°C. Therefore, our results for the strains used in this work add new information concerning this species.

Overall, from the growth curves reported in Figure 1, it can be seen that *S. macedonicus* strains showed less variability, particularly at 30°C, 34°C, and 42°C, with respect to *S. thermophilus*. Clearly, higher temperatures resulted in better growth rates, but no significant ($P < 0.05$) further increase was observed in either species at 42°C (1.55 ± 0.25 h^{-1} at 37°C vs. 1.79 ± 0.62 h^{-1} at 42°C for *S. macedonicus*; 1.46 ± 0.31 h^{-1} at 37°C vs. 1.34 ± 0.35 h^{-1} at 42°C for *S. thermophilus*; Table 2), indicating that this temperature is beyond the optimum. At 30°C, all *S. macedonicus* strains showed a significantly ($P < 0.05$) shorter lag phase and reached a significantly ($P < 0.05$) higher N_{max} compared with *S. thermophilus*. Moreover, the shapes of the *S. thermophilus* growth curves highlighted marked differences among strains, in contrast to strains of *S. macedonicus*. Although 30°C appears nonoptimal for *S. thermophilus* growth, it is the best choice for the development

Table 2. Growth parameters of strains of *Streptococcus macedonicus* and *Streptococcus thermophilus* estimated by the Gompertz model[1]

Species and strain	30°C			34°C			37°C			42°C		
	λ (h)	μ_{max} (h^{-1})	N_{max} (OD_{600})	λ (h)	μ_{max} (h^{-1})	N_{max} (OD_{600})	λ (h)	μ_{max} (h^{-1})	N_{max} (OD_{600})	λ (h)	μ_{max} (h^{-1})	N_{max} (OD_{600})
S. macedonicus												
8SP	0.03	0.89	1.01	0.44	1.26	1.04	0.20	1.41	1.04	0.59	1.93	1.06
19AS	0.10	0.90	0.80	0.71	1.10	0.76	0.05	1.93	0.95	0.00	0.58	0.60
27MV	0.94	1.05	0.83	1.13	1.24	0.91	0.24	1.73	0.96	0.58	1.73	0.88
203MA	0.72	1.14	1.02	1.13	1.24	1.27	0.67	1.54	1.09	0.60	2.24	1.00
62AS	0.05	0.89	0.79	0.43	1.08	0.75	0.24	1.65	0.97	0.59	1.51	0.96
33MO	0.95	0.86	0.68	0.83	1.31	0.76	2.93	1.17	0.97	1.44	2.10	0.83
211MA	0.74	1.12	1.02	0.62	1.58	1.04	1.09	1.41	1.09	0.66	2.48	0.99
Mean	0.50	0.98	0.88	0.76	1.26	0.93	0.77	1.55	1.01	0.64	1.79	0.90
SD	0.43	0.12	0.14	0.29	0.16	0.20	1.02	0.25	0.06	0.42	0.62	0.15
S. thermophilus												
1F8CT	6.33	0.31	0.07	1.12	0.58	1.07	2.06	1.33	0.84	0.62	0.86	0.51
MTH17CL396	2.89	2.31	0.56	0.74	1.24	0.80	3.48	1.93	0.80	0.63	1.54	0.88
M17PTZA496	2.16	1.00	0.59	0.71	1.02	0.79	2.24	1.87	0.81	0.59	1.61	0.82
TH982	4.02	0.46	0.66	3.10	0.72	1.01	2.94	1.44	0.82	2.06	0.99	0.63
TH98	2.51	0.56	0.46	3.62	0.99	0.54	1.94	1.33	0.80	2.80	1.36	0.57
TH1435	2.40	0.78	0.43	0.85	1.17	0.50	1.98	1.54	0.87	0.13	1.17	0.65
TH1436	2.09	1.03	0.89	0.66	1.33	0.88	2.25	1.17	0.96	0.53	1.93	0.68
TH1477	2.16	0.64	0.70	0.70	1.00	0.82	2.30	1.03	0.90	0.17	1.28	0.89
Mean	3.07*	0.89	0.55*	1.44	1.01	0.80	2.40*	1.46	0.85*	0.94	1.34	0.70*
SD	1.46	0.63	0.24	1.20	0.25	0.20	0.54	0.31	0.06	0.96	0.35	0.14

[1]λ = lag phase duration; μ_{max} = maximum growth rate; N_{max} = maximum cell number at the stationary phase (where OD_{600} = optical density at 600 nm).

*$P < 0.05$: statistically significant differences between species (ANOVA, Tukey's test).

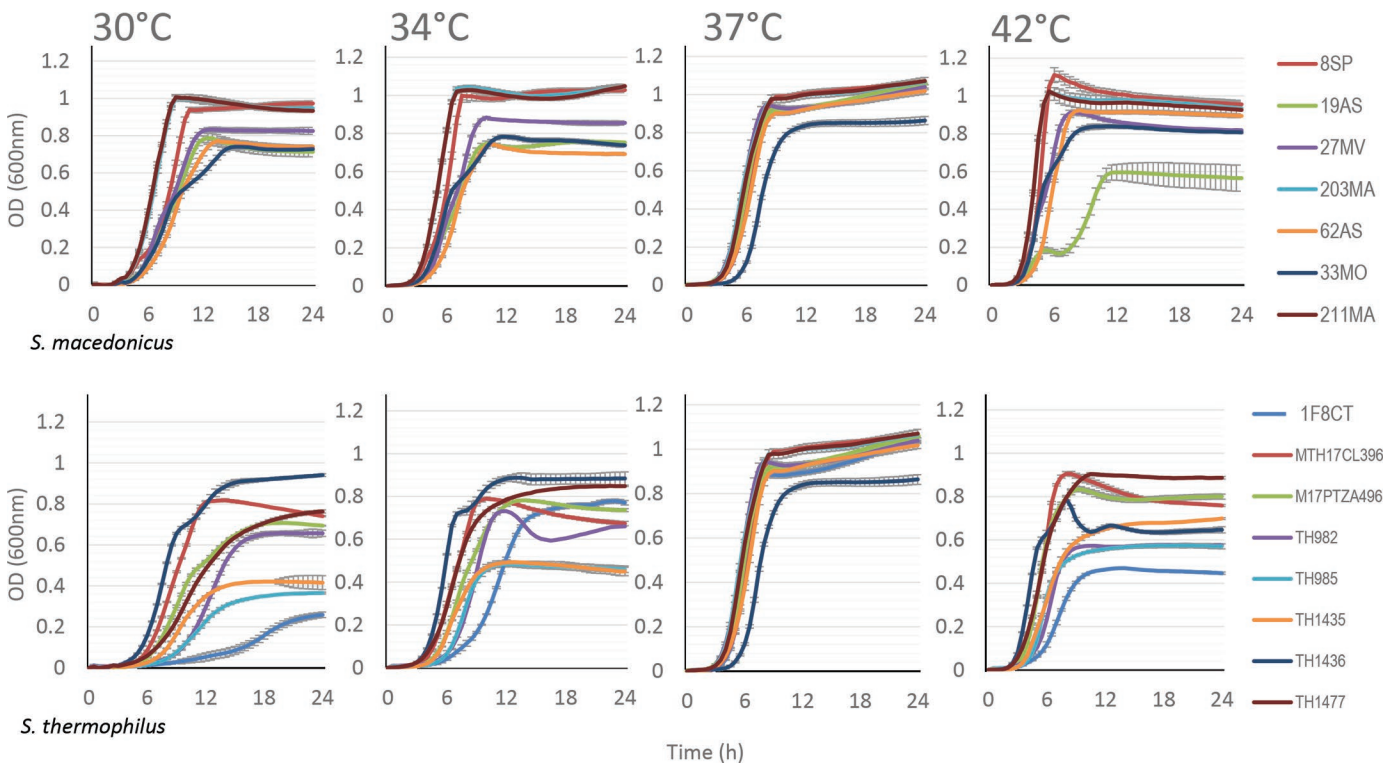

Figure 1. Growth curves (measured as optical density at 600 nm) for strains (colored curves; strain details given in Table 1) of *Streptococcus thermophilus* and *Streptococcus macedonicus* obtained at different temperatures. Vertical bars represent SEM. Color version available online.

of some activities, such as production of bacteriocin (Aktypis et al., 2007) or exopolysaccharides (Purwandari et al., 2007; Kanamarlapudi and Muddada, 2017). At 34°C, mean μ_{max} increased, as expected, in both species, and *S. thermophilus* had a shorter lag phase with respect to that determined at 30°C. From comparison of the growth curves (Figure 1), it is evident that greater diversity existed among *S. thermophilus* strains than among *S. macedonicus*. At 37°C, all strains had similar kinetics and reached their respective optimal growth parameters, but *S. macedonicus* strains showed a significantly ($P < 0.05$) shorter lag phase and higher N_{max}. At 42°C, a generalized decrease in growth performance was observed for both species, indicating suboptimal growth conditions. In particular, the population levels (N_{max}) obtained by *S. thermophilus* strains were significantly lower ($P < 0.05$) than those of *S. macedonicus*. Thus, 37°C could be considered the optimal temperature, among those tested, for all strains, whereas growth becomes strongly strain dependent at temperatures other than 37°C.

All *S. macedonicus* strains tended to increase growth performance with increasing temperature but growth rate did not increase further from 37°C to 42°C; indeed, strain 19AS appeared seriously impaired at 42°C. *Streptococcus thermophilus* strains grew very differently from each other; for instance, TH1436 grew better at lower temperatures, whereas TH1477 and TH1435 grew better at 37°C.

In conclusion, we report for the first time the growth kinetics and optimal temperature for *S. macedonicus* strains and compare kinetics with those of *S. thermophilus* under the same conditions. These data help to better define and compare technological characteristics of the 2 species, in view of the potential use of *S. macedonicus* to replace *S. thermophilus* in selected technological applications. Further studies will be needed on a greater number of *S. macedonicus* isolates to evaluate whether the relatively homogeneous behavior shown by the strains used in this work is due to their relative geographical proximity (all isolated in northeast Italy) or is an intrinsic characteristic of the species.

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