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Research Article

Shear treatment of starter culture medium improves separation behavior of *Streptococcus thermophilus* cells

A central step in the production of starter cultures is the separation of the cells from the fermentation medium, which is usually achieved by disk centrifuges. In case of microorganisms which produce exopolysaccharides (e.g., various strains of lactic acid bacteria), the properties of the respective exopolysaccharides may interfere with this separation step. By using six strains of *Streptococcus thermophilus* the hypothesis was tested that a shear treatment of the fermented culture medium improves subsequent cell separation markedly. Depending on the type of exopolysaccharides (freely present in the medium, or as capsules around the cells) an energy input of up to 2.5 kJ/mL generated with an Ultra-Turrax affected cell chain length of the strains and viscosity of fermentation medium differently. For bacteria producing capsular exopolysaccharides, space- and time-resolved centrifugation experiments revealed an increase of sedimentation velocity after shear treatment. In general, viability of the microorganisms, detected by flow cytometry measurements and fermentation experiments, was not affected by the shearing procedure. The results therefore indicate that strain-targeted shearing is helpful to improve the separability of cells from the fermented media.

Keywords: Exopolysaccharides / Lactic acid bacteria / Sedimentation

1 Introduction

Starter cultures are a strict pre-requisite for the production of many fermented foods including, for example, beer, bread, or acidified dairy products. For each food type, a broad variety of starter microorganisms is available from the respective manufacturers. Starter cultures for the same target commodity differ in composition and activity of the included strains and hence influence the characteristics of the fermented food. To design the process of starter culture production efficiently, fermentation conditions differ significantly from the conditions during the production of fermented foods. Besides a specific substrate, which is tailored to the demands of the respective organisms, agitation and headspace gas is optimized, and metabolites are either removed (e.g., ethanol) or buffered (e.g., lactic acid) to ensure that high cell densities are obtained. This results in a cell

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Abbreviations: cEPS, capsular EPS; **cFDA**, carboxyfluorescein diacetate; **EPS**, exopolysaccharides; **fEPS**, free EPS; **FSC**, forward scatter; **GE**, glucose equivalent; **PI**, propidium iodide; **SSC**, side scatter; **ST**, Streptococcus thermophilus; **TCA**, trichloroacetic acid

density that is about 10–30 times higher than in classical bulk starters. The cells are usually separated by centrifugation, and arrive at the market as frozen pellets or freeze-dried [1].

The most important requirements for the final starter culture are a high viable count and sufficient cell vitality, and the ability to induce the desired product properties [2]. In case of starters with additional functionality, it is also necessary that this feature remains stable. A functionality that is especially important for the dairy industry is the ability of lactic acid bacteria to produce exopolysaccharides (EPS) [3]. EPS of dairy starters usually comprise more than one sugar monomer (heteropolysaccharides) and act as highly efficient thickeners. When EPS are produced in situ by the starters during, for example, yogurt fermentation, there is no need for declaration in the ingredients list. There are a number of studies which already showed how EPS influence yogurt texture and viscosity (e.g. [4–6]) and also that these effects depend on both EPS type and concentration [7]. A typical product feature associated with EPS formation is ropiness, which means that threads are visible when the product is removed from a beaker by pouring, or by using a spoon.

It has already been demonstrated that EPS production is usually growth associated [8, 9], and there are, until now, no reports available showing how EPS formation can be retarded or switched off. This is, however, a challenge for the producers of

starter cultures as the EPS usually cause an increase of the viscosity of the fermentation substrate, and cell separation is therefore impeded (see Stokes law). For lactic acid bacteria, a shear treatment of the fermentation medium (which might help to reduce viscosity and therefore enhance cell separability) is reported for reducing the length of cell chains prior to the determination of the viable count, which then usually increases [10]. However, turbulent flow and excessive shear energy input have been associated with cell wall disruption, cell lysis and reduced freeze-thaw stability [11, 12].

Observations from the industry point to differences in the cell separation properties depending on whether the bacterial cells liberate the EPS into the medium (further denoted as free or fEPS), or the EPS stick to the cell surface in form of small capsules (cEPS). The aim of the present study was therefore to evaluate to which extent a shear treatment applied prior to centrifugation affects the rheological properties of the fermentation medium and hence cell separability, and whether this procedure impairs cell viability.

2 Materials and methods

2.1 Sample preparation

Starter cultures from industrial production (fermented media with six different *Streptococcus thermophilus* single strains) were provided by Chr. Hansen A/S (Hørsholm, Denmark) in frozen state. These are further denoted as ST-C, ST-D, ST-E, ST-G, ST-H, and ST-I. After defrosting at 4°C for 24 h, cell-free samples were prepared by centrifugation at $19\,000 \times g$ for 15 min at 4°C and subsequent decantation of the supernatant. For defined cell suspensions, cells separated from 30 mL medium by centrifugation were resuspended in 30 mL aqueous sodium chloride (8.9 g/L).

2.2 Characterization of exopolysaccharides

The first step in the EPS isolation and quantification procedure [7] comprised the adjustment of 5.0 g culture medium to pH 7.5 with 1 mol/L NaOH, and incubation with 250 μL of a 4.8 g/L Pronase E solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 37°C for approximately 18 h. A total of 125 μL sodium azide (40 g/L) was added as preservative. After adding 0.7 mL trichloroacetic acid (TCA, 800 g/L) the samples were heated to 90°C for 10 min, cooled in ice water and centrifuged ($19\,000 \times g$, 15 min, 4°C) to remove cells and proteins. The pellets were resuspended in 100 g/L TCA and centrifuged again. The combined supernatants were treated with two volumes of chilled acetone (4°C) overnight. Precipitated EPS were collected through centrifugation ($19\,000 \times g$, 15 min, 4°C), dissolved in demineralized water, and dialyzed (molecular mass cut-off 6–8 kDa; Carl Roth GmbH & Co. KG) for 48 h against demineralized water. The dialysates were then freeze-dried (Alpha 1–2, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). For EPS quantification, the phenol-sulfuric acid method [13]

was used, and EPS content is expressed as milligram per liter glucose equivalent (GE). All assays were performed in duplicate.

Anion-exchange chromatography was applied to determine EPS charge [14]. One milliliter of an EPS solution (approx. 0.5 mg/mL) was eluted through a Fractogel EMD DEAE 650 M column (Merck KgaA, Darmstadt, Germany) with 50 mmol/L sodium phosphate buffer (pH 6.0) of different NaCl content (0, 0.25, and 1.0 mol/L) at a flow rate of 2 mL/min, and GE in the collected fractions was measured.

The average molecular mass of the EPS was determined by size exclusion chromatography (AZURA Assistant ASM 2.1L, Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany) coupled with a Smartline RI detector 2300. For the separation, 3 columns were used: 1 × PSS Suprema 100 Å + 2 × PSS Suprema 3000 Å in combination with a pre-column (PSS-Polymer-Standard-Service GmbH, Mainz, Germany). The eluent was a solution of 50 mmol/L $NaNO_3$ and 7.7 mmol/L NaN_3 in demineralized water. Approx. 1–2 g/L of the samples were dissolved in the eluent and filtered through a 0.2 μm filter before injecting. Calibration was carried out using Pullulan standards with defined molar masses ranging from 342 Da to 2560 kDa.

2.3 Shearing procedure

In a set of test tubes (d = 30 mm, h = 200 mm), an aliquot of 30 mL of each sample was subjected to shearing by using a T25 digital Ultra-Turrax® with the S25N-18G dispersing tool (IKA-Werke GmbH & Co. KG, Staufen, Germany) operating at 11 000, 19 000, or 24 000 rpm for 120 s at room temperature.

2.4 Determination of cell viability with flow cytometry

For live/dead double staining of the cells, propidium iodide (PI) and carboxyfluorescein diacetate (cFDA; both from Sigma-Aldrich Chemie GmbH, Munich, Germany) were used [15, 16]. Before staining, 100 μL cell suspension was diluted in 1 mL phosphate buffered saline (8.9 g/L NaCl, 795 mg/L $Na_2HPO_4*7H_2O$, 144 mg/L KH_2PO_4, pH 7.4), washed twice with this buffer, and adjusted to an optical density of 0.025. For labeling, 1 mL PI solution (80 μmol/L in demineralized water) and 10 μL cFDA (10 mmol/L in dimethylsulfoxide) were added to 1 mL diluted cell suspension and incubated in the dark for 30 min at room temperature. Analysis was carried out with a CyFlow Cube 6 (Sysmex Partec GmbH, Görlitz, Germany) equipped with an argon ion laser emitting 20 mW at 488 nm. Forward scatter (FSC), side scatter (SSC), and two fluorescence signals (FL1 at 530 nm, FL3 at 660 nm) were monitored. Autoclaved water was used as sheath fluid. The analyses were performed at a flow rate of 0.5 μL/s, data collection was for 60 s, and data evaluation was done using the DeNovo software FCS Express 4. Gating on FSC/SSC was used to discriminate bacteria from the background. The numbers of viable and dead cells were estimated from FL1 (cFDA) versus FL3 (PI) dot plots of the gated cells. A 1:1 mixture of stained viable and dead cells (obtained after heating the fermentation medium to 70°C for 10 min) served as control to adjust the detectors.

2.5 Viscosity of fermented media

Viscosity of the entire fermented media, the cell-free media and of the cells resuspended in physiological NaCl solution was determined in a double gap geometry (d_o = 44 mm, d_i = 41 mm, $d_{i,stator}$ = 40 mm, h = 59.5 mm) of an AR-G2 rheometer (TA Instruments GmbH, Eschborn, Germany) at 20°C. The shear rate was increased from 0.1/s to 1000/s in a logarithmic ramp with 5 measuring points per decade and a measurement time of 30 s per data point.

2.6 Determination of capsules and bacterial chain length

The presence of capsular EPS was visualized by negative staining [17]. A drop of cell suspension was placed on a glass slide and mixed with one drop of ink. The slide was covered with a cover slip, and inspected in an Axiostar microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) at 400× and 1000× magnification. Capsules appear as a white zone around the cells as ink particles cannot penetrate into the polymeric material [18]. The number of cocci within single cell chains was counted from microscopy images without negative staining using the Fiji package of ImageJ (https://fiji.sc). A minimum of 200 individual cell chains were counted to ensure statistical accuracy. A distribution curve was processed from the histograms, and theoretical x_{90} values calculated.

2.7 Analysis of sedimentation velocity

Sedimentation velocity distribution was measured with a LU-MiSizer (LUM GmbH, Berlin, Germany). This optical analytical centrifuge allows measuring phase separation in liquid samples in rectangular polycarbonate cuvettes (2 × 8 mm² base area). The cuvettes are horizontally rotated while irradiated lengthwise with NIR light, and the transmitted fraction of the light is measured with a CCD-Sensor as a function of time. Consequently, time and place resolved transmission profiles are obtained which provide information on the sedimentation properties of the samples [19, 20]. Fermented medium was diluted with physiological NaCl solution in a ratio of 1:2 and 0.3 mL filled in cuvettes, resulting in a filling height of approximately 20 mm. The measurements were carried out at 3600 rpm (1720 × g) and the sedimentation velocity distribution was calculated with the SEPVIEW software using the constant position method. All experiments were performed in duplicate.

2.8 Acidification activity after shearing

Reconstituted skim milk (120 g/L), prepared by dissolving low heat skim milk powder (Sachsenmilch Leppersdorf, Wachau, Germany) in demineralized water, was heated to 90°C for 10 min, subsequently cooled to 40°C and inoculated with 10 mL/L of the initial fermented medium, either untreated or after the shearing procedure. pH was continuously monitored and time to achieve pH 4.6 was taken as indicator for starter culture activity. Viability of cells in the inoculation medium was analysed by flow cytometry.

2.9 Statistical analysis

When applicable, one-way analysis of variance (factor: *S. thermophilus* strain) was computed using Systat 12 (Systat Software GmbH, Erkrath, Germany).

3 Results and discussion

3.1 Classification of strains

All *S. thermophilus* strains produced exopolysaccharides, and the respective EPS content in the fermentation media ranged from 412 to 1341 mg GE/kg (Table 1). This EPS concentration is significantly higher compared to dairy products (cheese, yoghurt; EPS ~ 50–150 mg/kg (e.g. [5, 21]) due to the specific fermentation conditions (adapted medium composition, fermentation at constant pH). For strains ST-C, ST-G, ST-H and ST-I capsular EPS (cEPS) were visualized by ink staining, and capsules of ST-G and ST-H appeared significantly larger. Anion-exchange chromatography revealed that the EPS of all strains are uncharged.

The viscosity of the medium can be taken as an indicator for the presence of free, viscous EPS (fEPS; [22, 23]). Both fermented medium and cell-free medium of ST-C, ST-D and ST-E showed a slight shear thinning behavior. Media fermented with the other strains were Newtonian, as were all systems with cells resuspended in physiological NaCl solution. It was therefore decided to take apparent viscosity at a shear rate of 10/s ($\eta_{10/s}$) as a representative measure. For all fermented media, $\eta_{10/s}$ was in the range of 1.9–3.3 mPa.s. After removing the cells, the decrease of $\eta_{10/s}$ was approximately 12% in case of ST-E (cEPS negative), and 21–30% for all other strains. For both systems $\eta_{10/s}$ was significantly higher for ST-C, ST-D, and ST-E, indicating that these strains produced highly viscous fEPS. A lower $\eta_{10/s}$ at similar amounts of EPS for ST-G and ST-I points on the additional presence of free but nonviscous EPS besides capsular ones. These large differences disappeared when suspensions of separated cells were measured; the corresponding $\eta_{10/s}$ range was 1.07–1.41 mPa.s.

According to these findings, the strains were classified as (i) producing viscous fEPS (ST-D, ST-E); (ii) producing viscous fEPS and cEPS (ST-C); and (iii) producing nonviscous fEPS and cEPS (ST-G, ST-H, ST-I). The average molecular mass (m_M) of the respective EPS was also strain-dependent. For ST-C, ST-D and ST-E, a larger and a smaller EPS fraction was detected. In general, m_M of the larger EPS fraction varied from 2.0×10^5 to 7.6×10^5 Da, and that of the smaller fraction from 5.2×10^4 to 8.1×10^4 Da (Table 1). The ratio of m_M to the number-average molecular mass m_N [24] indicates that the EPS of ST-H and ST-I occur in a fairly monodisperse distribution, whereas a higher degree of polydispersity or a bimodal distribution is evident for the EPS of ST-C, ST-D, ST-E, and ST-G.
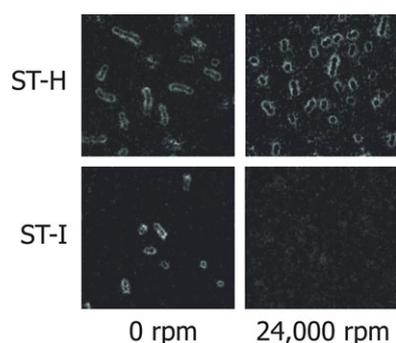
Figure 1. Capsule staining of *Streptococcus thermophilus* ST-H and ST-I in the untreated and the sheared fermented medium (24 000 rpm).

3.2 Influence of the shear treatment on cEPS and cell chain length

Shearing of the fermented media with the T25 Ultra-Turrax at 11 000, 19 000, or 24 000 rpm for 2 min is equivalent to an energy input of approximately 0.26, 1.30, or 2.50 kJ/mL, respectively. Shear forces and energy input are considered important with respect to cell chain length and viability [10–12].

Treatment at 24 000 rpm removed the cEPS from the cell walls of ST-C and ST-I whereas, in case of ST-G and ST-H, capsules were still present. Exemplary, Fig. 1 depicts the microscopy images of ST-H and ST-I before and after shearing.

The x_{90} values of cell chain length decreased for all strains during shearing (Table 1). The distribution of cocci per cell chain before and after shearing at different energy input is depicted in more detail in Fig. 2 for ST-C and ST-D. Before shearing, ST-D showed the longest cell chains with a broad length distribution of
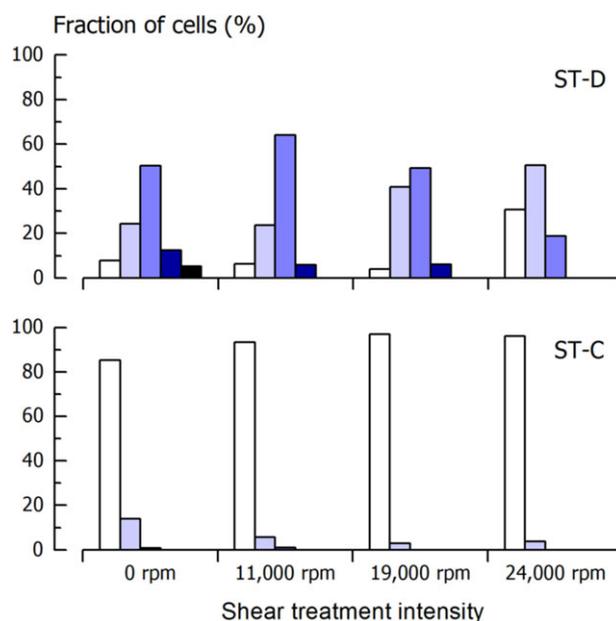

Figure 2. Distribution of cocci per bacterial cell chain for *Streptococcus thermophilus* ST-C and ST-D in the fermented medium as a function of shear treatment intensity (white, 1–2 cells per chain; blue with increasing intensity: 3–4; 5–10; 11–15; 16–20 cells per chain).

2–19 cells/chain, and shearing resulted in a significant reduction of chain length: the x_{90} distribution parameter decreased from 12.96 (unsheared) to 5.89 (24 000 rpm) cells/chain ($\sim$ 55%). ST-C, for example, showed relatively short chains (1–5 cocci) in the fermented medium and the distribution in Fig. 2 illustrates that, after shearing at 19 000 and 24 000 rpm, approximately

Table 1. Characterization of the media fermented with different *S. thermophilus* strains

Parameter[1]	Streptococcus thermophilus strain[2]					
	ST-C[3]	ST-D	ST-E	ST-G	ST-H	ST-I
EPS concentration (mg GE/kg)	1062^c ± 117	788^b ± 61	940^c ± 35	733^b ± 109	412^a ± 27	1341^c ± 145
Presence of capsular EPS	+	−	−	+	+	+
Apparent viscosity (mPa.s at 10/s)						
Fermented medium	2.65^b ± 0.15	3.26^c ± 0.06	2.80^b ± 0.15	1.98^a ± 0.04	1.86^a ± 0.05	1.91^a ± 0.02
Cell-free medium	1.88^b ± 0.04	2.61^c ± 0.01	2.50^c ± 0.14	1.38^a ± 0.02	1.45^a ± 0.06	1.45^a ± 0.01
Resuspended cells	1.38^d ± 0.02	1.31^c ± 0.02	1.10^a ± 0.03	1.48^e ± 0.01	1.48^e ± 0.01	1.24^b ± 0.02
EPS molecular mass (m_M, Da)	7.6 × 10^5 [1.43]	2.0 × 10^5 [1.18]	5.6 × 10^5 [1.44]	8.1 × 10^4 [1.36]	5.2 × 10^4 [1.10]	5.2 × 10^4 [1.29]
and ratio [m_M/m_N]	6.7 × 10^4 [1.08]	5.7 × 10^4 [1.43]	5.6 × 10^4 [1.13]			
Cells/chain before/after shearing (x_{90})						
Before shearing	2.99	12.96	3.19	4.51	4.75	2.59
2 min at 11 000 rpm	2.84	9.09	3.10	3.61	3.92	2.63
2 min at 19 000 rpm	2.76	8.81	2.83	3.78	3.80	2.35
2 min at 24 000 rpm	2.77	5.89	2.94	3.43	3.48	2.35

[1] GE, glucose equivalents; m_M/m_N, ratio of weight-average to number-average molecular mass.
[2] Mean values in a row with different superscripts differ significantly at $p < 0.05$.
[3] EPS concentration and apparent viscosity are mean ± half deviation range from duplicate measurements.
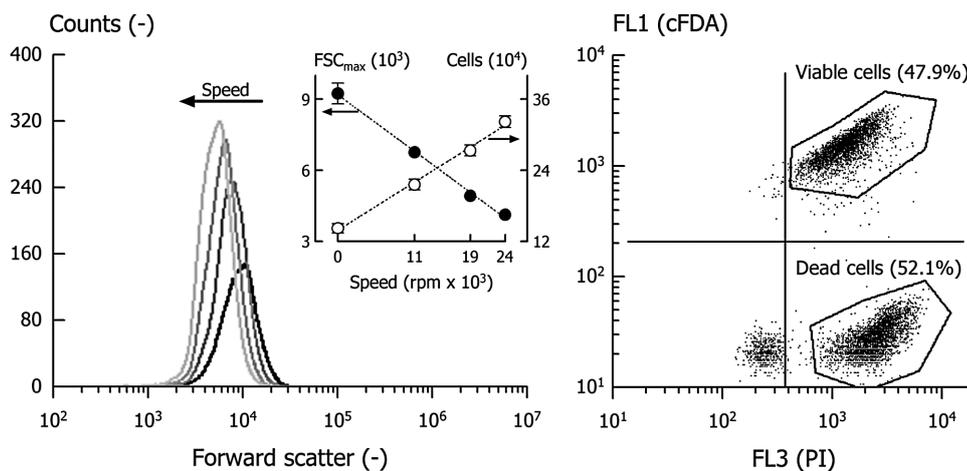a,b,c,d,e statistical difference indicators.

Figure 3. Left: Histogram of particle counts versus the forward scatter (FSC) of untreated and sheared fermented medium of ST-D in flow cytometry measurements (arrow indicates increase of shear intensity from 0; 11 000; 19 000 to 24 000 rpm). Insert: FSC_{max} and amount of cells as a function of shear speed. Right: Validation dot plot of FL1 (cFDA) and FL3 (PI) after double staining of a 1:1 mixture of viable and dead cells (obtained by heating at 70°C, 10 min).

97% of the cells are present in small units (1–2 cells/chain). For ST-C, ST-E and ST-I the decrease of x_{90} was approximately 10%, for ST-G and ST-H 30%.

These results can be linked to apparent viscosity. The viscosity change in the fermented medium induced by cell removal was relatively low in case of ST-E (cEPS negative, $x_{90} = 3.19$ cells/chain) compared to ST-D (also cEPS negative, $x_{90} = 12.96$ cells/chain). In case of strains with capsular EPS and short chains ($x_{90} = 2.59$–4.75 cells/chain), the reduction of viscosity after cell removal has consequently to be attributed to the cEPS which are removed with the cells.

Evaluation of the forward scatter signal (FSC) from the flow cytometry measurements also gives evidence on differences in the size of the detected particles [25–27]. Figure 3 (left) exemplary shows the distribution of the particle count versus FSC for untreated and sheared samples of ST-D. In case of the untreated sample, forward scatter at the particle count peak (FSC_{max}) was highest (9.2×10^3). FSC_{max} decreased continuously with increasing shear intensity to approximately 4.2×10^3 after shearing at 24 000 rpm, indicating a reduction of the average particle size. In line with this decrease, the number of particles (either single cocci or cell chains; estimated from the area under the FSC peak) increased ($R^2 = 0.98$). FSC_{max} for the other ST strains showed a lower size reduction (~10–20%), and the number of particles increased by a similar amount (data not shown). Although a precise determination of cell chain length was not possible, flow cytometry confirmed the trends that were evident from microscopic analysis.

3.3 Influence of the shear treatment on the viability of the strains

Live/dead assays with cFDA/PI double staining were already successfully used to distinguish between viable and dead cells of various bacteria strains [28,29]. PI is a hydrophilic cationic molecule that can only pass membranes of damaged cells where it stains nucleic acids, whereas cFDA passes the cell membrane of most viable cells and is enzymatically hydrolyzed to a polar, fluorescent carboxyfluorescein which accumulates in the cytoplasm [30]. To check the reliability of the staining procedure we divided a sample into two parts and heated one part to 70°C for 10 min to obtain dead cells; subsequently this sample was mixed with an unheated fermented medium in a ratio of 1:1. As illustrated in Fig. 3 (right) double stained populations labeled with cFDA and PI could be successfully resolved in dot plots of FL1 versus FL3. The events can be grouped into living cells in the first quadrant, dead cells in the fourth quadrant, and unstained debris in the third quadrant. The percentage of viable and dead cells was calculated after subtraction of the debris subpopulation.

The fraction of viable cells in the untreated starter cultures was at least approximately 90% (Fig. 4). After shearing, this fraction remained almost unchanged, and the lowest observed value was 85% for ST-E, sheared at 24 000 rpm. For most of the strains, shearing resulted in an increase of the total number of counted particles, which is attributable to the reduction of cell chain length. This increase was most pronounced for ST-D–the number of living cells after shearing was twice as high as in the untreated sample–and moderate but still significant for ST-C (20%), ST-G (45%), and ST-H (25%).

After inoculation of skim milk with the untreated and sheared samples, acidification activity at 40°C was on a comparable level within each strain, indicating that the shearing procedure did not have a significant effect on their acidification activity. Independent of the pretreatment of the samples, time to reach pH 4.6 was 9.04 ± 0.38 h for ST-C, 12.37 ± 0.32 h for ST-E, 4.15 ± 0.02 h for ST-G, $4.55 \pm$
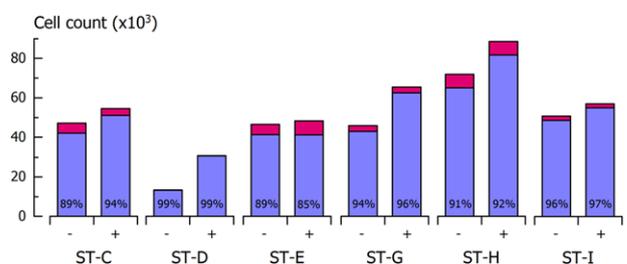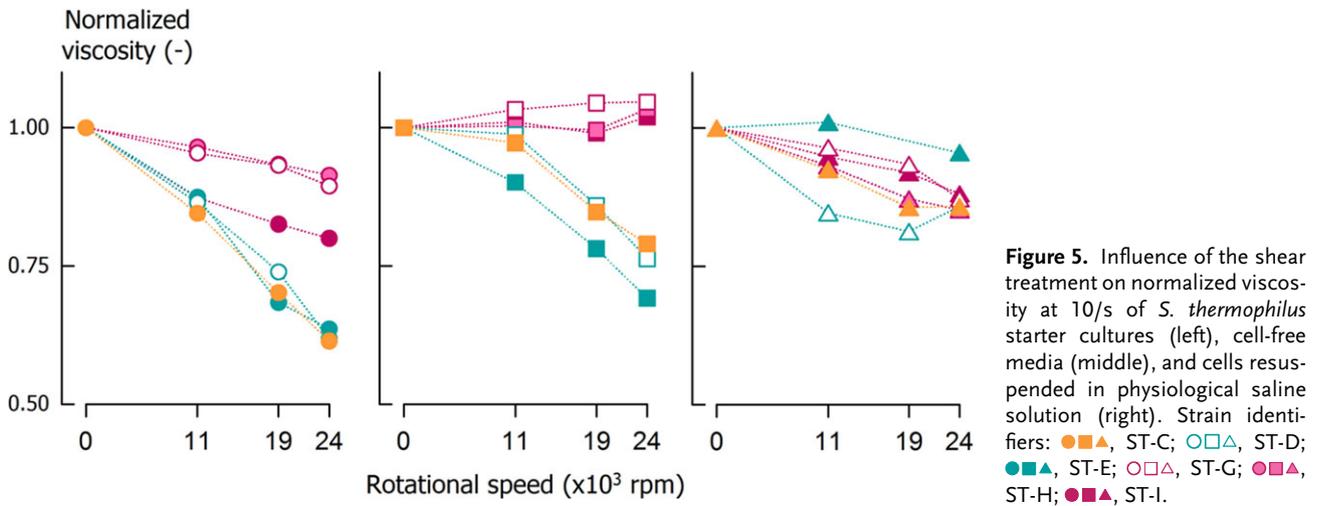

Figure 4. Influence of the shear treatment (–, unsheared; +, sheared at 24 000 rpm for 2 min) on the amount of living (■) and dead (■) *Streptococcus thermophilus* cells. Numbers refer to the percentage of living cells.

Figure 5. Influence of the shear treatment on normalized viscosity at 10/s of *S. thermophilus* starter cultures (left), cell-free media (middle), and cells resuspended in physiological saline solution (right). Strain identifiers: ●■▲, ST-C; ○□△, ST-D; ●■▲, ST-E; ○□△, ST-G; ●■▲, ST-H; ●■▲, ST-I.

0.09 h for ST-H and 4.86 ± 0.08 h for ST-I; for ST-D pH after 18-h fermentation was approximately 4.80.

3.4 Influence of the shear treatment on the rheological behavior

The applied shearing procedures resulted in a significant decrease of the apparent viscosity of all fermented media. Figure 5 illustrates that the relative decrease of $\eta_{10/s}$ was most pronounced for strains which produced viscous fEPS (ST-C, ST-D, and ST-E). This decrease appears as being independent from cell chain length and the presence of capsular EPS, which suggests that the shearing procedure induced changes in molecule size or conformation of fEPS. This was for example found for konjac glucomannans after treatment with power ultrasound [34]. A less pronounced viscosity decrease was observed for the strains that produced both non-viscous fEPS and cEPS (i.e., ST-G, ST-H, and ST-I). For these samples, the normalized viscosity of the respective cell-free medium was completely unaffected by the energy input through shearing. Consequently it can be stated that for these strains their capsular EPS exhibited the highest impact on the behavior of the fermentation media. It is known that cEPS bind a high amount of water [31, 32] so that a relatively thick layer is formed around the cells. The shear-induced removal of the capsules from the cells reduced apparent viscosity of the fermented medium, but also that of NaCl solution with resuspended cells. In case of the non cEPS producing ST-D, the shear-induced reduction of $\eta_{10/s}$ in the cell-containing medium is mainly attributable to the reduction of cell chain length (Table 1). Resuspended cells of ST-E (cEPS negative, short cell chains) showed the lowest absolute $\eta_{10/s}$, and were therefore hardly affected by shearing at all.

3.5 Changes of sedimentation behavior after the shear treatment

According to Stokes law [33], sedimentation velocity (v_{sed}) in dispersions is affected by size, density and shape of the particles (or
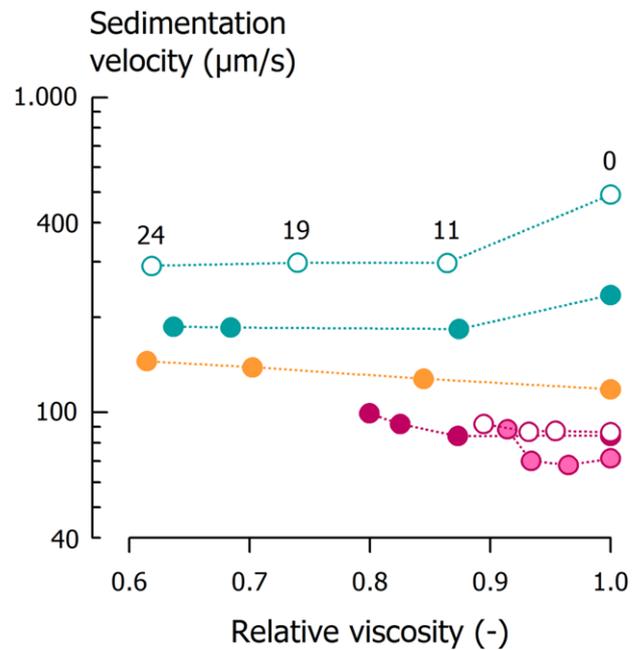

Figure 6. Relationship between sedimentation velocity, shear intensity and relative viscosity. Strain identifiers: ●, ST-C; ○, ST-D; ●, ST-E; ○, ST-G; ●, ST-H; ●, ST-I. Shear intensity (in 10^3 rpm) is indicated for sample ST-D.

cells), and by density and viscosity of the continuous phase. For the present samples it seems that viscous fEPS, which increase medium viscosity, have only a minor effect on the sedimentation behavior of the respective strains (ST-D and ST-E; Fig. 6). Especially for ST-D, the decrease of v_{sed} after shearing can be mainly attributed to the pronounced decrease of cell chain length (Table 1). For the capsule producers ST-C, ST-G, ST-H and ST-I, an increase of v_{sed} was observed after shearing, and this increase was lower for the strains with the thickest cEPS layer around the cells (ST-H, ST-G). Cell chain destruction through shearing was relatively low for these strains, which leads to the following

hypothesis: we assume that the cEPS around the bacteria cells act as a kind of 'friction pad' during sedimentation, so that shearing off this layer reduces the retarding effect and hence causes an increase in sedimentation velocity.

4   Concluding remarks

By using fermentation media with six strains of *S. thermophilus* we demonstrate for the first time that applying a shearing procedure improves the subsequent cell separation without having an impact on cell viability. Especially for strains that produce capsular EPS the sedimentation velocity increased after shearing off the EPS from the bacteria cells. First results on the scaling-up with an industrial homogenizer confirm the high application potential of this method for the starter culture industry.

Practical application

The separability of starter culture cells depends, among others, on the viscosity of the fermented medium. In case the bacteria produce exopolysaccharides, the separation might be negatively affected. To overcome this drawback, a targeted shearing procedure might be applied. Using six strains of *S. thermophilus* that produce different types of exopolysaccharides and shearing regimes with different energy input, the results of the study demonstrate that such a shearing procedure is able to improve cell separation without having an impact on cell viability.

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