Journal of Industrial Microbiology (1996) 16, 15–21

Biosurfactants from thermophilic dairy streptococci and their potential role in the fouling control of heat exchanger plates

HJ Busscher, M van der Kuijl-Booij and HC van der Mei

Materia Technica, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands

Recent work on biosurfactant release by thermophilic dairy streptococci is reviewed. There is a suggestion that *Streptococcus thermophilus* isolates may release biosurfactants that stimulate detachment of already-adhering cells and leave an anti-adhesive coating on a substratum. A previously published rapid screening method is described for the identification of biosurfactant-releasing microorganisms, and growth medium supplements to enhance biosurfactant release by thermophilic dairy streptococci are reported. New experimental work described includes the isolation and purification of biosurfactants from dairy isolates by thin layer chromatography. Many compounds isolated were extremely surface-active and reduced the water surface tension to values around 30 mJ m^{-2} at a concentration of 10 mg ml^{-1}. Most importantly, the thin layer chromatograms of various isolates resembled each other, and an adsorbed purified compound from one isolate retarded the deposition to glass of another isolate by a factor of two. Provided our findings implicate that these biosurfactants could also be adsorbed to heat exchanger plates in pasteurizers and thereby retard colonization by thermophilic streptococci, these compounds may have major economic implications. Further work is required, however.

Keywords: biosurfactants; thermophilic streptococci; heat exchanger; *Streptococcus thermophilus*; adhesion; fouling

Introduction

Fouling control in heat exchangers in the dairy industry is essential in order to prolong their uninterrupted operating time (Figure 1) [8,10,11,22]. The composition of the fouling deposits varies in different sections of pasteurizer plants and consists of proteins (15–50%, w/w), minerals (40–75%, w/w) and fat (1–3%, w/w) [10]. Although these fouling deposits are troublesome, reducing the heat transfer efficiency, microbial deposits are even more troublesome. During prolonged operating times, the number of thermoresistant microorganisms in the resulting pasteurized milk gradually increases, which may have adverse effects on dairy products [1,6].

One of the thermoresistant fouling microorganisms of pasteurizers is *Streptococcus thermophilus*. A continuous flow along stainless steel of either raw or pasteurized milk leads to approximately the same initial adhesion of *S. thermophilus* B, but when entering the growth phase of biofilm formation, CFUs of *S. thermophilus* B were found to be about 100 times higher in pasteurized milk than in raw milk [6]. This led to the suggestion that raw milk may contain inhibitory compounds. The propensity of *S. thermophilus* to adhere and grow in pasteurized milk results in its presence in the regenerative section of pasteurizers from which it can contaminate pasteurized milk in even higher concentrations than were originally present in the raw milk [6].

Using a parallel plate flow chamber with *in situ* observation [16, 28], a curious observation was made with regard to the adhesion of *S. thermophilus* B to glass [3]. Initially, *S. thermophilus* adhered in high numbers, and within 80 min 3.4×10^6 cells cm^{-2} were counted. After 80 min, however, virtually all adhering cells gradually detached leaving a 'clean' glass surface. Freshly cultured organisms did not adhere to this glass from which *S. thermophilus* cells had previously detached. The hypothesis was advanced that the adhering *S. thermophilus* cells released a biosurfactant that caused not only the detachment of adhering organisms but that, once adsorbed to glass, also prevented the adhesion of new depositing cells.

Such a role of microbial surfactants has also been described for a biosurfactant released by *Acinetobacter calcoaceticus* RAG-1, an oil-degrading microorganism [26]. *A. calcoaceticus* RAG-1 utilizes relatively long chain *n*-alkanes from oil for growth and accumulates biosurfactants in the form of a minicapsule on its cell surface. After these compounds are utilized, starvation of the organisms causes release of the biosurfactants, providing the hydrophobic oil droplet with a hydrophilic coat and thereby desorbing the starved cell. At the same time, the depleted oil droplet has become hydrophilic [7], which is a marker for other cells not to attach.

Rapid screening of microbial strains for the release of biosurfactants

A surfactant is a molecule which, by adsorption, is able to alter the surface free energy of an interface, most notably the water–air interface, in which case the term 'surface tension' is used [23]. Here, the term 'biosurfactant' will be used for any microbial product affecting the surface tension of aqueous solutions. The measurement of the surface tension of surfactant solutions by standard methods such as the Wilhelmy plate or du Nouye ring method is difficult because experiments are carried out by expanding the liquid–air interface. Consequently, surfactants have to

Correspondence: HJ Busscher, Materia Technica, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands
Received 20 December 1994; accepted 13 September 1995

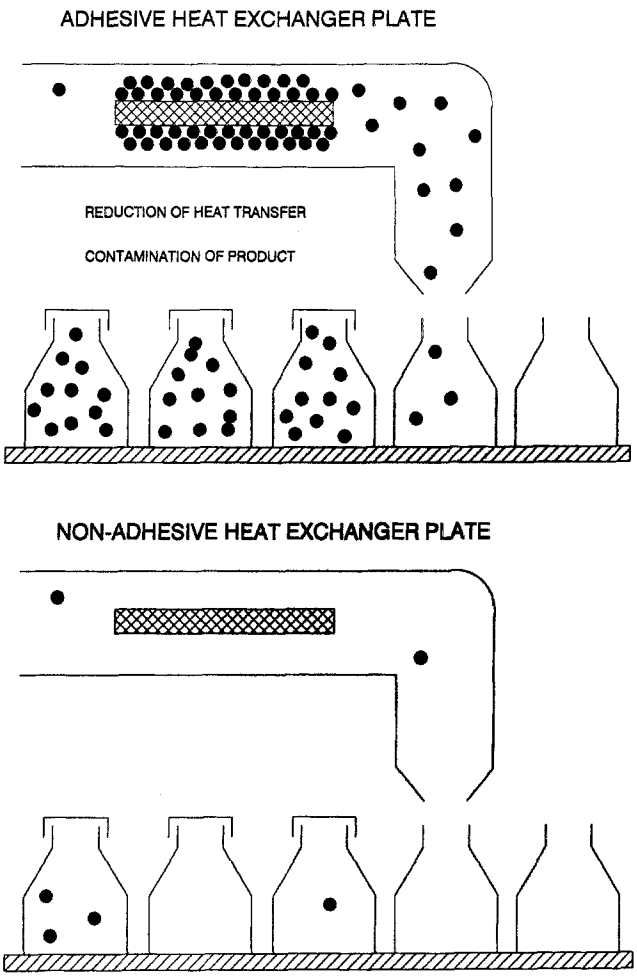

Figure 1 Biofilm formation on heat exchanger plates in pasteurizers may give rise to severe contamination of pasteurized milk (top). This problem would be far less if the heat exchanger plates would be covered with an anti-adhesive, non-fouling coating (bottom)

migrate continuously to the interface in order to exert their surfactive effect.

Axisymmetric Drop Shape Analysis by Profile (ADSA-P) [21,27] is another method to measure liquid surface tensions, circumventing this problem. The method is based on determining the shape of a droplet on a solid surface. High surface tension liquids are more apt to maintain a perfectly spherical shape than low surface tension liquids, an obvious notion mathematically expressed by the Laplace equation:

$$\Delta p = \gamma_{1v}\left(\frac{1}{R_1}+\frac{1}{R_2}\right) \qquad [1]$$

in which Δp is the pressure difference across the interface, γ_{1v} is the liquid surface tension and R_1 and R_2 are the principal radii of curvature.

By fitting the profile of a liquid droplet to the Laplace equation, Eqn 1, one can directly obtain the liquid surface tension. Since in this method the area of the liquid–air interface is kept constant, ADSA-P is suitable for surfactant-containing solutions.

In order to screen microbial strains rapidly for biosurfactant release, we have suggested the employment of ADSA-P [29]. Briefly, 100 μl of a microbial suspension in water or an appropriate buffer is put on a fluoroethylenepropylene surface and the shape of the droplet is monitored as a function of time (Figure 2). Subsequently, the surface tension of such suspension droplets is calculated as a function of time (see Figure 3 for an example). Strains for which the surface tension decrease observed is more than 8 mJ m^{-2} within 2 h are considered as biosurfactant producers.

Using ADSA-P [4] several *S. thermophilus* isolates grown in M17 broth have been identified as biosurfactant producers (Table 1). Generally, larger surface tension decreases were found when sucrose was added to the growth medium as compared to lactose- or glucose-supplemented medium. Of course this rapid screening does not give any information as to whether one or more compounds are involved, the chemical compound(s) making up the biosurfactant, or the surface-active effects of each of the compounds.

Classes of biosurfactants and comparison with other (synthetic) surfactants

There have been a large number of strains and species identified to date as biosurfactant-releasing [5]. These include environmentally important [17] and marine microorganisms [12], medical isolates like *Streptococcus mitis* [25,29] and industrially-used strains for cosmetic [9] and food applications [31]. Chemically-derived synthetic surfactants are generally classified according to the nature of the polar group: cationic, anionic or non-ionic. Biosurfactants are more conveniently classified in terms of the biochemical nature of the surfactant: protein, polysaccharide, lipid or complexes of biomolecules.

The following types of biosurfactants are commonly distinguished [26]: 1) glycolipids; 2) fatty acids; 3) phospholipids; 4) surface-active antibiotics. Table 2 summarizes the surface-active power of some purified biosurfactants as compared with some surfactive proteins and some synthetic surfactants. Clearly, biosurfactants can be extremely powerful.

Biosurfactants have some special properties as compared to synthetic surfactants, which have provided an incentive for their use in industry, agriculture and medicine [26]: biodegradability and controlled inactivation; selectivity for specific interfaces; potential for surface modification; and diversity. Widespread use of biosurfactants is limited, however, because of difficulties involved in scaling up the production, with the exception of 'emulsan' [18].

At present, our knowledge concerning the biosurfactants released by dairy streptococci is limited, despite the fact that industrial application as an anti-adhesive, non-fouling coating on heat exchangers seems promising. In this paper we describe our first attempts to purify and identify biosurfactants from thermophilic dairy streptococci by thin layer chromatography and to determine the surface-active power of some purified compounds. Also, a preliminary experiment is described, showing the potential of a purified compound from biosurfactants released by one *S. thermophilus* isolate (ATCC 19258) to influence adhesion of another isolate (*S. thermophilus* B).

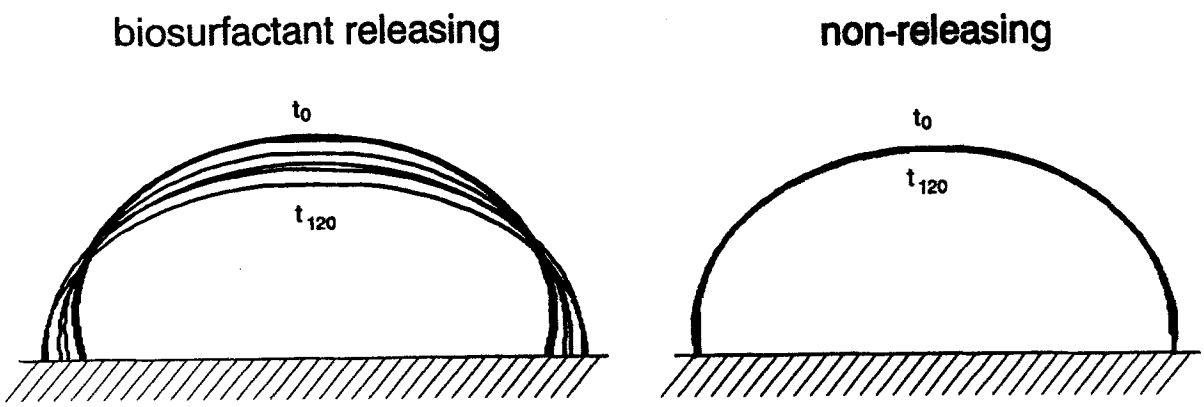

Figure 2 Shape of an axisymmetric drop of an *S. thermophilus* suspension as a function of time for a biosurfactant-releasing strain (left panel) and a non-releasing strain (right panel). t_0 indicates the first profile taken after positioning of the droplet, whereas t_{120} is the profile obtained 120 min after droplet positioning. Using the Laplace equation, the surface tension of these droplets can be calculated

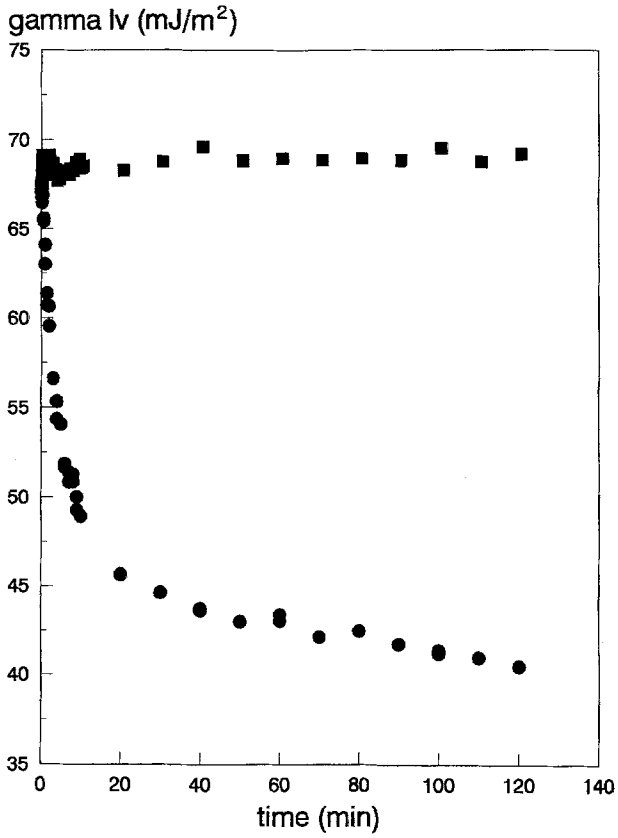

Figure 3 Surface tension of 100 μl *S. thermophilus* suspension droplets (1×10^9 cells ml^{-1}) as a function of time. ●, biosurfactant-releasing strain; ■, non-releasing strain

Materials and methods

Microbial strains, growth conditions and biosurfactant production

Eight *S. thermophilus* strains, one ATCC (American Type Culture Collection, Rockville, MD, USA) strain (ATCC 19258) and seven strains isolated from heat-exchanger plates in the downward section of a pasteurizer, were kindly provided by Dr AH Weerkamp (NIZO, Ede, The Netherlands). For each experiment, the bacteria were grown from a frozen stock overnight at 37°C in 10 ml M17 broth (Oxoid, Basingstoke, UK), supplemented with 1% sucrose. From this culture, 2 ml were used to inoculate a second culture (200 ml), which was grown for 20 h. After 20 h, cells were harvested by centrifugation at $6000 \times g$, washed twice in demineralized water and resuspended in demineralized water.

Table 1 Surface tension decreases $\Delta\gamma_{lv}$ of suspension droplets of several *S. thermophilus* isolates (10^9 cells ml^{-1}) observed after 2 h by ADSA-P, and calculated with respect to a water droplet. *S. thermophilus* cells were cultured in M17 broth supplemented with sucrose

S. thermophilus strain	$\Delta\gamma_{lv}$[a] (mJ m^{-2})
A	−13
B	−21
G	−9
G1	−12
IS	−14
K18K	−1
N12	−15
ATCC 19258	−14

[a]Data from Busscher *et al* [4]

Biosurfactants were isolated by stirring a suspension of 5×10^9 cells ml^{-1} in 150 ml demineralized water for 2 h. Subsequently the microorganisms and the biosurfactants released were separated by centrifugation at $15000 \times g$. Biosurfactants were freeze-dried for further experiments.

Thin layer chromatography (TLC)

For TLC, freeze-dried biosurfactants were dissolved in a solvent system consisting of chloroform, methanol and water (1 : 1 : 1, v/v/v). Twenty milligrams of biosurfactant were dissolved in 1 ml of the solvent mixture. TLC was carried out on Silica-gel 60 plates (0.25-mm thick) with concentration zones (Merck, Darmstadt, Germany) using chloroform, methanol and water in a different volume ratio (65 : 25 :4) than that in which the biosurfactants were dissolved.

Staining was done with demineralized water for water-repellent spots, with fluorescein for lipid spots, with ninhydrin for free amino acid-containing spots and with

Table 2 Minimal surface tensions, γ_{1v} and apparent critical micelle concentrations, cmc, of various surface-active molecules in aqueous solution

Compound	γ_{1v} (mJ m^{-2})	cmc (mg L^{-1})	Reference
Synthetic surfactants			
sodium dodecylsulphate	37	2023–2890	[18]
alkylate dodecylbenzene	47	590	[18]
cetyltrimethylammoniumbromide	42		[20]
dihydroamine fluoride	35	475	[2]
oleylamine fluoride	30	270	[2]
Proteins			
bovine serum albumin	49	~5000	[30]
immunoglobuline G	42	~5000	[30]
α-lactalbumine	37	~5000	to be published
Biosurfactants from			
Pseudomonas fluorescens ('viscosin')	27	150	[19]
Pseudomonas fluorescens ('AP-6')	27	<10	[24]
Bacillus subtilis ('surfactin')	27	11	[18]
Pseudomonas aeruginosa ('rhamnolipids')	29	15	[18]
Torulopsis bombicola	37	82	[18]
Rhodococcus erythropolis	37	15	[18]

phenol/sulphuric acid for sugars. All stains were obtained from Merck, Darmstadt, Germany.

Preparative TLC plates (2-mm thick) but without concentration zones were used to obtain purified biosurfactant by applying dissolved biosurfactants across the entire width of the plates. Purified compounds at retention front values (R_f, previously identified on a separate plate by staining) were scraped off the plates, extracted with water and separated from the silica gel by centrifugation. The isolated material was freeze dried for further experiments.

Axisymmetric Drop Shape Analysis by Profile (ADSA-P)

In this study ADSA-P was performed as described by Noordmans and Busscher [21]. Briefly, a liquid droplet on a solid surface is digitized with a contour monitor, especially designed for contact angle measurements. Exact x and y magnification factors were calculated from profiles of spherical stainless steel balls. Droplets with 10 mg ml^{-1} biosurfactant dissolved in demineralized water with a volume of approximately 100 μl were placed on the FEP-Teflon substrates. Measurements on one solution droplet were done as a function of time up to 2 h in an enclosed chamber at room temperature. In order to prevent evaporation, a small water reservoir was placed in the chamber to create conditions of saturated water vapor. Each liquid profile was recorded twice with a minimal (<0.5-s) time interval in between, and the ADSA-P surface tensions were averaged. This procedure was carried out three times with separate liquid droplets.

Several fractions of biosurfactants obtained after purification by TLC from various *S. thermophilus* strains were used. Due to the limited availability of purified compounds, only one fraction of biosurfactant from *S. thermophilus* B was measured as a function of biosurfactant concentration up to 10 mg ml^{-1}.

Flow chamber deposition

Deposition of one *S. thermophilus* strain (strain B) was studied in a flow chamber system described in detail previously [16,28]. Briefly, a pulse-free flow (0.08 ml s^{-1}) of bacterial suspension (3×10^8 cells ml^{-1}) was established in a parallel plate flow chamber ($5.5 \times 3.8 \times 0.06$ cm) with the bottom plate made out of glass, yielding a shear rate of 35 s^{-1}.

With the aid of a phase contrast microscope, equipped with an ultra-long working distance objective, real-time image analysis (PC-Vision, Imaging Technology, Woburn, USA) was carried out on one field of 16×10^{-5} cm^2 per sample to obtain the number of adhering cells per cm^2 as a function of time, t (Figure 4). The initial deposition rate, j_0 (the number of microorganisms adhering per cm^2 per second) was determined from the data obtained during the first 15 min of the experiment. Experiments were done on bare glass and on glass pre-coated with an adsorbed layer of a biosurfactant fraction of *S. thermophilus* ATCC 19258.

Results

Thin layer chromatography

Figure 5 shows schematically the thin layer chromatograms of the biosurfactants released by the various *S. thermophilus* strains employed in this study. As can be seen, the products released are mixtures of several compounds. Staining so far has not given an adequate identification of the compounds released, although a lipoproteinaceous character of most compounds appears likely. With respect to the surface-active properties, the water-repellent spots might be of special interest.

Thin layer chromatography was done 3–5 times on biosurfactants released from different cultures. The chromatograms as presented are considered to be representative for a given *S. thermophilus* isolate, but it must be noted that

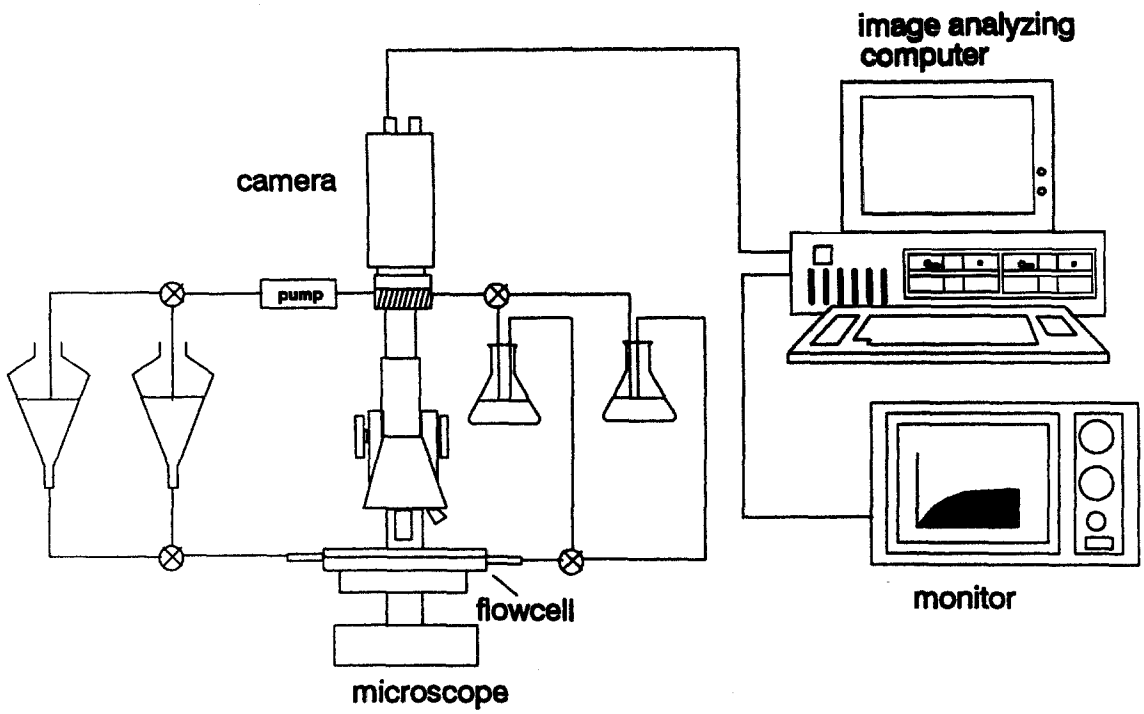

Figure 4 Schematic overview of the parallel plate flow chamber and additional equipment used to study the deposition of *S. thermophilus* cells to solid substrata. (For detailed description, see [16,28])

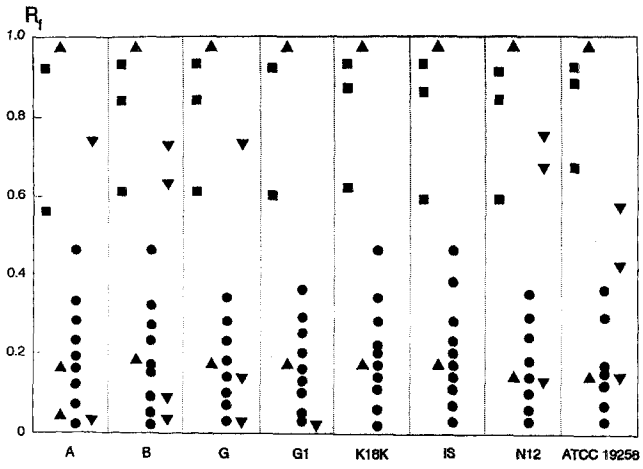

Figure 5 Schematic presentation of water-repellent spots (■), fluorescein-stainable spots (▲), ninhydrine-stainable spots (●) and phenol/sulphuric acid-stainable spots (▼) on TLC plates with biosurfactants from various *S. thermophilus* isolates

minor variations in the positions of the major spots sometimes occur. Also, some of the minor spots have been found to be absent in biosurfactants prepared from 1 or 2 of the cultures.

Axisymmetric Drop Shape Analysis by Profile

Using preparative TLC plates, the biosurfactants released (Figure 5) were purified. For the purified biosurfactant compound from *S. thermophilus* B, detected by its water repellency at $R_f = 0.85$, the surface tension was measured as a function of concentration. Figure 6 shows that this compound is able to decrease the water surface tension from 72 mJ m^{-2} to approximately 42 mJ m^{-2} with a critical micelle concentration around 0.1 mg ml^{-1}.

Table 3 summarizes surface tensions of purified compounds in 10 mM potassium phosphate (pH 7.0), presumably above the cmc. Note that the compounds found at the highest R_f values are not necessarily the most surface-active. Some compounds, especially $B_{0.95}$ and $Gl_{0.84}$, are extremely surface-active.

Flow chamber deposition

A single experiment has been carried out in which the initial deposition rates of *S. thermophilus* B from a 40-mM potassium phosphate buffer (pH 7.0) to glass with and without a coating of ATCC $19258_{0.93}$ were compared. *S. thermophilus* B adhered extremely rapidly to bare glass (j_0 = 3222 cm^{-2} s^{-1}). Deposition was, however, greatly reduced in the presence of an ATCC $19258_{0.93}$ coating on the glass to an initial deposition rate of 1867 cm^{-2} s^{-1}.

Discussion

Biosurfactants were isolated from thermophilic dairy streptococci with the aim of establishing an anti-adhesive, non-fouling coating for heat exchanger plates in pasteurizers. Several purified compounds from biosurfactants released by

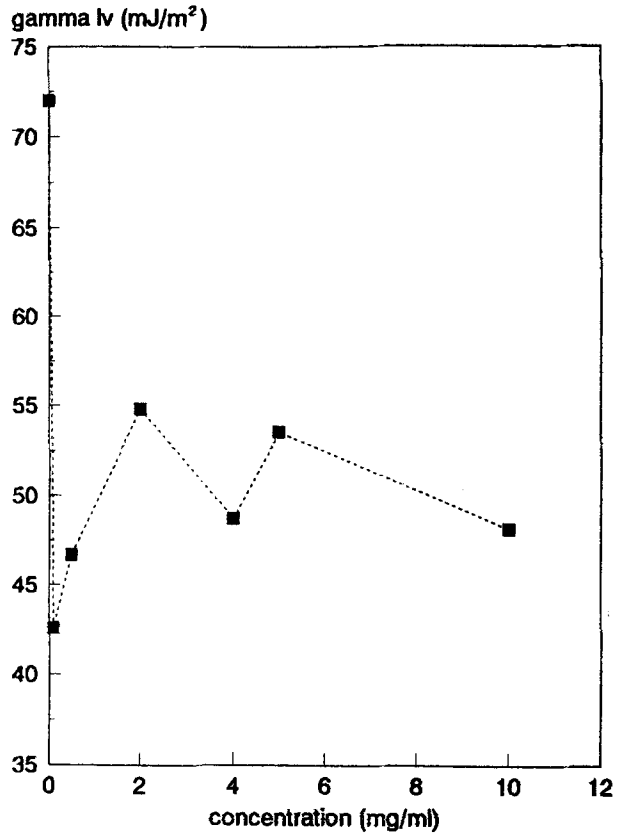

Figure 6 Surface tension of $B_{0.85}$ in water as a function of concentration. $B_{0.85}$ denotes a purified biosurfactant compound, detected on a TLC plate at $R_f = 0.85$ of biosurfactants released by *S. thermophilus* B

Table 3 Surface tensions γ_{lv} of various purified biosurfactant compounds dissolved in water. The concentration used was 10 mg ml^{-1}

Biosurfactant	γ_{lv}[b] (mJ m^{-2})
$B_{0.95}$[a]	30.2
$B_{0.87}$	64.1
$B_{0.80}$	48.7
$B_{0.51}$	44.8
$G1_{0.96}$	70.5
$G1_{0.84}$	34.3
$G1_{0.73}$	58.3
$N12_{0.95}$	50.5
$N12_{0.87}$	46.9
$N12_{0.52}$	37.5

[a]The code refers to the strain code of the *S. thermophilus* isolate and the R_f value on the TLC plate from where the biosurfactant compound was isolated
[b]All data are from single experiments

various isolates were extremely surface-active (Table 3). It is questionable, however, to what extent the biosurfactants have really been purified to a single compound, because the $\gamma_{lv}(c)$ curve for $B_{0.85}$ shows a distinct maximum at lower concentrations, indicative of the presence of impurities [13–15]. Further work is therefore required to purify the biosurfactants and also to identify them. It is interesting to note from the thin layer chromatograms that there is a broad similarity between the retention front values R_f for spots from different isolates, suggesting that all isolates release more or less similar products.

Previously it was shown that *S. thermophilus* B cells could cause their own detachment, presumably by the release and adsorption of biosurfactants [3]. Here it is demonstrated that the initial deposition rate of adhering *S. thermophilus* B cells can be reduced by coating the substratum with a purified biosurfactant compound of another producer strain. This indicates that, whereas it may not be possible to inhibit adhesion of thermophilic dairy streptococci by a biosurfactant coating, it appears possible to retard the adhesion by a factor of two. Clearly, since this would indicate that the uninterrupted operating time of pasteurizers would also be doubled, these biosurfactants may have major economic implications. Further work is required to pursue this approach.

Acknowledgements

The authors greatly acknowledge the contributions of Daan Aubrey, Saskia Tempelman and Jitske Groenenstein, students from the Groenhorst College, Ede, The Netherlands for skilfully carrying out the experiments and of Mrs Marjon Schakenraad-Dolfing for manuscript preparation.

References

1 Bouman S, DB Lund, FM Driessen and DG Schmidt. 1982. Growth of thermoresistant streptococci and deposition of milk constituents on plates of heat exchangers during long operating times. J Food Prot 45: 806–812.
2 Busscher HJ, HM Uyen, GAM Kip and J Arends. 1987. Adsorption of aminefluorides onto glass and the determination of surface free energy, zeta potential and adsorbed layer thickness. Coll Surf 22: 161–169.
3 Busscher HJ, MN Bellon-Fontaine, N Mozes, HC Van der Mei, J Sjollema, O Cerf and PG Rouxhet. 1990. Deposition of *Leuconostoc mesenteroides* and *Streptococcus thermophilus* to solid substrata in a parallel plate flow cell. Biofouling 2: 55–63.
4 Busscher HJ, TR Neu and HC Van der Mei. 1994. Biosurfactant production by thermophilic dairy streptococci. Appl Microbiol Biotechnol 41: 4–7.
5 Desai JD and AJ Desai. 1993. Production of biosurfactants. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 65–97, Marcel Dekker, New York.
6 Driessen FM, J de Vries and F Kingma. 1984. Adhesion and growth of thermoresistant streptococci on stainless steel during heat treatment of milk. J Food Prot 47: 848–852.
7 Gerson DF. 1993. The biophysics of microbial surfactants: growth on insoluble substrates. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 269–286, Marcel Dekker, New York.
8 Gilmour A and TW Fraser. 1991. The study of the interaction of microorganisms with dairy equipment surfaces using scanning electron microscopy. Biofouling 5: 133–141.
9 Klekner V and N Kosaric. 1993. Biosurfactants for cosmetics. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 373–390, Marcel Dekker, New York.
10 Lalande M, JP Tissier and G Corrieu. 1984. Fouling of a plate heat exchanger used in ultra high temperature sterilization of milk. J Dairy Res 51: 557–568.
11 Lalande M, F Rene and JP Tissier. 1989. Fouling and its control in heat exchangers in dairy industry. Biofouling 1: 233–250.
12 Lang S and F Wagner. 1993. Biosurfactants from marine microorganisms. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 391–418, Marcel Dekker, New York.

13 Lunkenheimer K, R Miller, G Kretschmar, KH Lerche and J Becht. 1984. Investigations on the possibility of purifying surfactants/solutions by adsorption on solids. Coll Polym Sci 262: 662–666.
14 Lunkenheimer K and R Miller. 1987. A criterion for judging the purity of adsorbed surfactant layers. J Coll Interf Sci 120: 176–183.
15 Lunkenheimer K, HJ Pergande and H Krüger. 1987. Apparatus for programmed high-performance purification of surfactant solutions. Rev Sci Instrum 58: 2313–2316.
16 Meinders JM, HC Van der Mei and HJ Busscher. 1992. In situ enumeration of bacterial adhesion in a parallel plate flow chamber—elimination of in focus flowing bacteria from the analysis. J Microbiol Meth 16: 119–124.
17 Müller-Hurtig R, R Blaszczyk, F Wagner and N Korasic. 1993. Biosurfactants for environmental control. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 447–470, Marcel Dekker, New York.
18 Mulligan CN and BF Gibbs. 1993. Factors influencing the economics of biosurfactants. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 329–372, Marcel Dekker, New York.
19 Neu TR, T Härtner and K Poralla. 1990. Surface active properties of viscosin: a peptidolipid antibiotic. Appl Microbiol Biotechnol 32: 518–520.
20 Neu TR and K Poralla. 1990. Emulsifying agents from bacteria isolated during screening for cells with hydrophobic surfaces. Appl Microbiol Biotechnol 32: 521–525.
21 Noordmans J and HJ Busscher. 1991. The influence of droplet volume and contact angle on liquid surface tension measurements by axisymmetric drop shape analysis-profile (ADSA-P). Coll Surf 58: 239–249.
22 Nordman-Montelius M and I von Bockelmann. 1985. Analyses of raw milk deposits on non-treated polymer surfaces. In: Fouling and Cleaning in Food Processing (Lund D, E Plett and C Sandu, eds), pp 276–285, Madison, Wisconsin.
23 Ottewil RH. 1984. Introduction to surfactants. In: Surfactants (Tadros ThF, ed), pp 1–18, Academic Press, London.
24 Persson A, E Österberg and M Dostalek. 1988. Biosurfactant production by *Pseudomonas fluorescens* 378: growth and product characteristics. Appl Microbiol Biotechnol 29: 1–4.
25 Pratt-Terpstra IH, AH Weerkamp and HJ Busscher. 1989. Microbial factors in a thermodynamic approach of oral streptococcal adhesion to solid substrata. J Coll Interface Sci 129: 568–574.
26 Rosenberg E. 1986. Microbial surfactants. CRC Crit Rev Biotechnol 3: 109–131.
27 Rotenberg Y, L Boruvka and AW Neumann. 1983. Determination of surface tension and contact angle from the shape of axisymmetric fluid interfaces. J Coll Interf Sci 93: 169–183.
28 Sjollema J, HJ Busscher and AH Weerkamp. 1989. Real-time image analysis of adhering microorganisms in a parallel plate flow cell using automated image analysis. J Microbiol Meth 9: 73–78.
29 Van der Vegt W, HC Van der Mei, J Noordmans and HJ Busscher. 1991. Assessment of bacterial biosurfactant production through axisymmetric drop shape analysis by profile. Appl Microbiol Biotechnol 35: 766–770.
30 Van der Vegt W, HC Van der Mei and HJ Busscher. 1993. Interfacial free energies in protein solution droplets on FEP-Teflon by axisymmetric drop shape analysis by profile—IgG versus BSA. J Coll Interface Sci 156: 129–136.
31 Velikonja J and N Kosaric. 1993. Biosurfactants in food applications. In: Biosurfactants—Production, Properties, Application (Kosaric N, ed), pp 419–446, Marcel Dekker, New York.