Effect of milk temperature and flow on the adherence of Staphylococcus epidermidis to stainless steel in amounts capable of biofilm formation

Jaglic, Cervinkova, Michu, Holasova, Roubal, Vlkova, Babak, Schlegelova

Dairy Sci. & Technol. (2011) 91:361–372
DOI 10.1007/s13594-011-0017-6

ORIGINAL PAPER

Effect of milk temperature and flow on the adherence of *Staphylococcus epidermidis* to stainless steel in amounts capable of biofilm formation

Zoran Jaglic · Dana Cervinkova · Elleni Michu · Martina Holasova · Petr Roubal · Hana Vlkova · Vladimir Babak · Jarmila Schlegelova

Received: 2 August 2010 / Revised: 6 December 2010 / Accepted: 28 December 2010 /
Published online: 18 March 2011

Abstract The adherence of microorganisms to surfaces is a critical precondition for biofilm development. In this study, we evaluated the adherence (in amounts capable of biofilm formation) of *Staphylococcus epidermidis* in milk to stainless steel. During a 6-h time frame, the effect of milk temperature and laminar flow on adherence was analysed. In amounts capable of biofilm formation, the cells adhered within 0.5 h; however, at the milk temperatures promoting cell growth (25 and 28 °C), the cells proceeded to detach from the surface when bacteria started to grow (after 2 h), but only during the milk flow. When the temperatures were below the growth limit (6 and 22 °C) or under static conditions, the cells remained attached during the whole monitoring period. This study showed that temperatures which are suboptimal for growth and static conditions support the adhesion *S. epidermidis* in amounts capable of biofilm formation. On the contrary, at temperatures allowing the growth, adhered cells of *S. epidermidis* can easily be washed away from the stainless steel surface when bacteria begin to grow.

温度和流速对表皮葡萄球菌在不锈钢表面上的粘附以及对生物膜形成的影响

摘要 微生物在表面上粘合是形成生物膜的前提。本文研究了表皮葡萄球菌在不锈钢表面上的粘附作用（也就是形成生物膜的量）。分析了乳在板框中停留 6h 内乳的温度和层流速度对粘附作用的影响。在 0.5h 内就能发生了细胞粘附和形成生物膜，然而随着温度升高到 25°C 和 28°C，微生物的生长速度加快，2h 后由于细菌的大量繁殖使得细胞随着流动的奶开始从表面上脱附下来。但是当乳的温度低于微生物的生长限（6°C 和 22°C）或者是在静态条件下，在整个检

Z. Jaglic (✉) · D. Cervinkova · E. Michu · M. Holasova · H. Vlkova · V. Babak · J. Schlegelova
Veterinary Research Institute, Hudcova 70, Brno, Czech Republic
e-mail: jaglic@vri.cz

P. Roubal
MILCOM a.s., Dairy Research Institute, Ke Dvoru 12a, Prague, Czech Republic

测时间内，细胞粘附于表面。研究结果表明在不利于微生物生长的温度和静态条件下会导致表皮葡萄球菌的粘附和形成生物膜，相反，在有利于微生物生长的温度下，微生物的大量繁殖则使得粘附的细胞很容易从不锈钢表面上脱附下来。

Keywords Food safety · Hygiene · Dairy · Attachment · Detachment

关键词 食品安全 · 卫生学 · 吸附 · 脱附

1 Introduction

Unlike *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS) have not received significant attention. However, their involvement in various types of human infections has been increasingly recognised (Piette and Verschraegen 2009). *Staphylococcus epidermidis*, a member of CoNS and one of the most important opportunistic pathogens of the genus *Staphylococcus*, has already been described as a causative agent of different infections in humans (Piette and Verschraegen 2009; Vuong and Otto 2002). It also has been shown that a novel genomic island encoding for multiple phenol-soluble modulins, a potential virulence factor, may contribute to the evolution of this species from a commensal to a more aggressive pathogen (Gill et al. 2005). In addition to its newly recognised role in pathogenicity, *S. epidermidis* has also been characterised by an increasing antimicrobial resistance rate (Becker et al. 2007). Spread of methicillin-resistant strains represents a serious problem (Jaglic et al. 2010; Piette and Verschraegen 2009).

The attachment of microorganisms to food contact surfaces in dairy plants and subsequent biofilm formation poses a risk of secondary contamination of milk and milk products (Flint et al. 1997; Sharma and Anand 2002). By now, staphylococci (including *S. epidermidis*) have been well recognised as bacteria which may attach, form biofilms and survive on the contact surfaces in both the milk and meat processing plants (Moretro et al. 2003; Sharma and Anand 2002). In general, biofilm formation consists of a few successive steps, beginning with the initial stage of microbial adherence to a contact surface (Gotz 2002). Such adherence is influenced by physicochemical properties of food contact and bacterial cell surfaces including cell-wall-associated adhesins (Lens et al. 2003; Mack et al. 2007). Based on cell-to-cell communication, the adhered cells start to form multilayered cell structures (intercellular adhesion) called biofilms (Vuong et al. 2003). In *S. epidermidis*, the *ica* operon, encoding for the polysaccharide intercellular adhesin, has been recognised as a major factor involved in the intercellular adhesion (Heilmann et al. 1996b). De Araujo et al. (2006) reported the association between biofilm production and multi-resistance in *S. epidermidis* and speculated that increased genetic exchange in the biofilm environment may contribute to the multi-resistance phenotype. Due to the fact that staphylococci are able to form biofilm on inert materials used in the food processing industry, foodstuffs cannot be excluded as one of the possible sources of multi-resistant *S. epidermidis* strains. In our recent work (Schlegelova et al. 2010), we observed that genetically closely related multi-resistant staphylococci may persist in biofilm communities in milk processing plants over time.

In the dairy industry, contact surfaces in transport systems are usually made of stainless steel (Mattila-Sandholm and Wirtanen 1992), which was also used in several studies investigating adherence and biofilm formation of foodborne pathogens such as *Salmonella* Typhimurium, *Listeria monocytogenes* and *S. aureus* (Hilbert et al. 2003; Hood and Zottola 1997; Rieu et al. 2008). In these and some other foodborne pathogens, the adherence and biofilm formation on abiotic surfaces (including stainless steel) were also studied in milk or in the presence of milk proteins as discussed by Kim et al. (2006). Milk or milk proteins have been shown to either enhance or inhibit bacterial attachment to abiotic surfaces depending mainly on tested bacterial species, type of surface, media used and experimental conditions.

To our knowledge, adherence of CoNS to stainless steel in milk has not been studied under experimental conditions. Therefore, *S. epidermidis*, which is one of the most important members of CoNS, was selected for this model study. The effect of milk flow and temperature supporting the bacterial growth on the adherence of *S. epidermidis* to stainless steel in amounts capable of biofilm formation was investigated.

2 Materials and methods

2.1 Bacterial cultures

S. epidermidis field isolate Staph 1597 used in this study originated from a dairy farm equipment surface. The isolate was tested and confirmed as positive for biofilm production in polystyrene microtitration plates according to Heilmann et al. (1996a). The presence of the *ica* operon was confirmed by PCR according to Ziebuhr et al. (1997). The biofilm-positive (*ica*-positive) *S. epidermidis* reference strain CCM 7221 and the biofilm-negative (*ica*-negative) *S. epidermidis* reference strain ATCC 12228 were also included.

2.2 Milk for experimental studies

Cows' milk (fats 3.86%, proteins 3.36%, lactose 4.80%, fat-free dry matter 8.58% and somatic cell count 5 to 6.7×10^4 mL^{-1}) used in all experiments was heat treated (85 °C, 10 min) on the day before inoculation and then cultured onto blood agar (BioRad, Marnes-la-Coquette, France) to check for purity. Before the initiation of each experiment, 1 L of milk was inoculated with 1 mL of an 18-h-old brain–heart infusion (BioRad, Marnes-la-Coquette, France) culture of the tested strains of *S. epidermidis*. The initial concentrations of bacteria in the milk, as quantified by agar plate counting, ranged between 0.4 and 1.3×10^6 colony-forming units (CFU) per millilitre. Sedimentation of the culture in the milk tank was prevented by magnetic stirring and milk temperature was maintained by keeping the milk tank in a water bath with a temperature control unit.

2.3 Experimental set-up

Experimental set-up for studies of *S. epidermidis* adherence during milk flow is shown in Fig. 1. Adherence of *S. epidermidis* was investigated on chips (1×1×

0.06 cm) of stainless steel AISI 304 with a 2B finish (Marcegaglia S.p.A., Gazoldo degli Ippoliti, Italy). A special teflon flow chamber (Masaryk University, Brno, Czech Republic) with a transparent sealing lid and a groove (depth, 1 cm; width, 1.05 cm) with a semi-annular bottom, so that the both lower and upper parts of the horizontally inserted chips may be in contact with milk inoculated as described above, were used for the modelling of adherence under both static and dynamic conditions. To induce flow, a peristaltic pump (Lambda, Brno, Czech Republic) was used. The milk flow in the chamber was laminar and two flow velocities were tested. Velocity v_1 and velocity v_2 corresponded to a mean flow rate of 1.08×10^{-3} $m\cdot s^{-1}$ (340 $mL\cdot h^{-1}$) and 3.12×10^{-3} $m\cdot s^{-1}$ (980 $mL\cdot h^{-1}$), respectively. After flowing through the chambers, the milk was discarded.

Before use, the milk tank, flow chambers and chips were thoroughly washed with a cleaning agent (Korsolex Endo-Cleaner; Bode-Chemie, Hamburg, Germany) and rinsed under tap water. The milk tank and flow chamber were then submerged into 75% ethanol for 30 min. In 75% ethanol and during the same time, the chips were sonicated in an ultrasonic bath. After sonication, the chips were washed in acetone followed by 1 $mol\cdot L^{-1}$ NaOH as described previously (Hood and Zottola 1997). To remove potential chemical residuals, all the material was rinsed after cleaning under tap water for 30 min and then ten times in distilled water. In the last distilled water, the material was left overnight and subsequently autoclaved at 121 °C for 20 min. Before use, the silicon tube was autoclaved and after each the experiment discarded.

2.4 Evaluation of adherence

Since biofilms but not primarily adhered cells represent the main problem in cleaning and sanitation of food contact surfaces, we investigated in this study whether the initially adhered cells on the chips were capable of biofilm formation. In

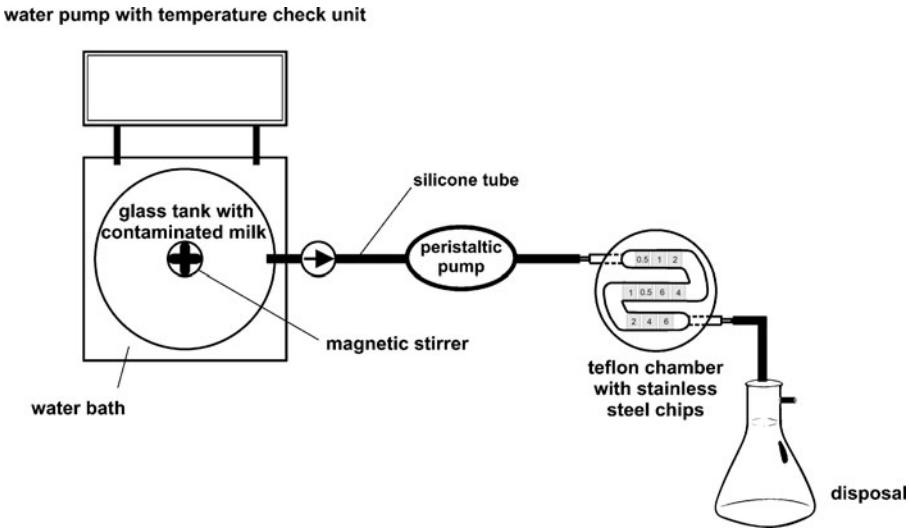

Fig. 1 Experimental set-up for studies of *Staphylococcus epidermidis* adherence during milk flow (chips in the chambers were marked with numbers indicating their exposure time (hours) in milk)

other words, we were interested at which exposure time and under which conditions the cells adhere to the chips in critical amounts, i.e. in the amounts sufficient for biofilm development. To evaluate whether the amounts of adhered cells were sufficient for biofilm development, the chips removed from milk were subsequently incubated in a tryptone soya broth (Oxoid, Basingstoke, Hampshire, England) containing 0.25% glucose (TSBg).

After the exposure time indicated below, the chips were removed from the inoculated milk, rinsed in a moderate stream of 20 mL phosphate-buffered saline (PBS) to remove non-adhered cells, and then separately incubated under aerobic conditions (24 h, 37 °C) in 5 mL of TSBg without agitation. After incubation, the chips were rinsed in 20 mL of PBS and air-dried overnight. The dried chips were stained with 0.1% (*w*/*v*) safranin O (Sigma-Aldrich, St. Louis, MO, USA) for 30 s and sonicated (160 W, 15 min, 30 °C) in 1 mL of distilled water with glass beads in an ultrasonic bath 9LE (Kraintek, Hradec Kralove, Czech Republic). Absorbance of the safranin O eluates was measured at 492 nm by a Helios Gamma spectrophotometer (ThermoSpectronics, Cambridge, UK). The absorbance was compared with a standard curve experimentally prepared in the laboratory which corresponded to the known number of dried and stained cells present on the chips. The standard curve was made for cell numbers ranging between 10^4 and 10^9 CFU on the chips.

2.5 Experimental studies

2.5.1 Discriminability of adherence

The adherence of field isolate Staph 1597 in milk was tested in two parallel chambers, each containing ten chips, and under the following conditions: 25 °C and flow velocity v_1. Five chips from each chamber were removed after 1 h (first group comprising of ten chips), while the remaining chips were removed after 2.5 h (second group comprising of ten chips). Simultaneously, ten chips placed in the third chamber through which milk without bacteria was flowing served as a negative control group. After the incubation in TSBg, the chips were stained with safranin O and processed (as described above) for the evaluation of potential biofilm development.

2.5.2 Reproducibility of adherence

The adherence of Staph 1597 was tested in three independent experiments performed at 25 °C and flow velocity v_1. In two other experiments, the adherence of reference strains CCM 7221 and ATCC 12228, respectively, was evaluated under the same conditions. During the each experiment, CFU of bacteria in the milk was monitored at 0.5, 1, 2, 4 and 6 h and at the same intervals two chips were removed from the milk and incubated in TSBg. After incubation, one of each of the duplicate chips from one experiment with Staph 1597 and one of each of the duplicate chips from the experiments with the reference strains CCM 7221 and ATCC 12228 were screened by electron microscopy (Tesla B 300 scanning electron microscope, Brno, Czech Republic) using a modified method according to Johnson et al. (1983). Briefly, the chips were fixed in 3% solution of glutaraldehyde in sodium cacodylate buffer (pH 7.3)

and in 1% solution of osmium tetraoxide. The chips were dehydrated in a graded series of acetone concentrations (50%, 70%, 80%, 90% and 100%), dried at the critical point with liquid carbon dioxide in a Polaron E3000 Critical Point Drying Apparatus (Polaron Instruments Inc., Hatfield, PA, USA) and processed by platinum and palladium alloy plating by a Polaron E5100 Sputter Coater (Quorum Technologies, Ringmer, UK). All the remaining chips were stained with safranin O and processed (as described above) for the evaluation of potential biofilm development.

2.5.3 Influence of milk temperature and flow on adherence

In nine independent experiments differing in milk temperature (three experiments at each temperature: 6, 22 and 28 °C), the effect of milk flow (velocity v_2) on adherence of Staph 1597 was investigated in comparison with static conditions. CFU of bacteria in the milk was monitored at 0.5, 1, 2, 4 and 6 h. At the same intervals, two chips were taken out, incubated in TSBg and stained with safranin O and processed (as described above) for the evaluation of potential biofilm development.

2.6 Statistical analysis

After elimination of two outlying values by means of Grubb's test for outliers (1969), the discriminability of adherence (the significance of differences in absorbance between different groups of chips) was assessed using one-way ANOVA and Tukey's multiple comparison post-test (GraphPad Prism 5.00, GraphPad Software, Inc., La Jolla, CA, USA).

3 Results and discussion

3.1 Discriminability of adherence

After their incubation in TSBg, no significant differences ($P>0.05$) in absorbances were found between the chips without adhered cells (negative control group) and the chips which had been removed from the inoculated milk after 2.5 h (Table 1). However, the chips which were removed from the inoculated milk after 1 h had a significantly higher ($P<0.001$) absorbance. Similarly, statistically significant correlation between bacterial adhesion and capability to form biofilm was reported by Sommer et al. (1999). The repeatability of assessment was expressed by the variation coefficient, which ranged between 43.0% and 67.6%. Based on these results, the absorbance of $A_{492nm}=0.170$ was regarded as a threshold discriminating biofilm developed in TSBg from background colour. The presence of 10^7 CFU on the chip corresponded to this value, as determined from the standard curve defined for the known numbers of CFU.

3.2 Reproducibility of adherence

A comparable course of adherence to the chips was observed in three experiments involving field isolate Staph 1597. Adherence, as determined by subsequent biofilm

Table 1 Discriminability of adherence of *S. epidermidis* Staph 1597: descriptive statistics for absorbances ($A_{492\ nm}$) of the safranin O eluates from the chips incubated in TSBg after bacterial adherence in milk

Statistics	Group of chips		
	First (1 h)	Second (2.5 h)	Third (negative control)
n	9[a]	9[a]	10
Range	0.170–0.660	0.022–0.120	0.028–0.168
Mean	0.417	0.068	0.069
SD	0.179	0.033	0.046
CV	43.0	48.5	67.6
CI[b]	0.279–0.555	0.043–0.094	0.035–0.102

SD standard deviation, *CV* coefficient of variation (%)

[a] one outlying value removed by means of Grubb's test for outliers (1969)

[b] CI 95%; confidence interval for the mean

formation, occurred within 0.5 h and peaked between 1 and 2 h after initiation of the experiments. Amounts of adhered cells decreased after 2 h and no visible biofilm formation was observed on the chips removed at 6 h. Concurrently with decreased amounts of adhered cells, increased counts of planktonic cells were observed in the milk (Fig. 2a). The course of the adherence of the biofilm-positive reference strain CCM 7221 was similar to those observed with Staph 1597 (data not shown). No biofilm development was observed with the biofilm-negative reference strain ATCC 12228 after the incubation of the chips in TSBg. The electron microscopy findings correlated with the absorbance of the biofilms that were stained and sonicated at the same time. Typical three-dimensional biofilm structures as well as extracellular polymers were only observed on the chips which were removed from the inoculated milk within the first 2 h of the experiments (Fig. 3).

3.3 Influence of milk temperature and flow on adherence

Although milk temperatures in dairy plants are generally low (3–6 °C), higher temperatures (more than 30 °C) may occur in some specific production lines such as those producing cheese and yogurts. Especially risky may be surfaces of plate heat exchange pasteurizers (cooling sections) where bacterial biofilms may grow and thus contaminate the product stream with more than 10^6 CFU of bacteria per millilitre (Flint et al. 1997; Knight et al. 2004). In our preceding work (Michu et al. 2010), we experimentally showed that *S. epidermidis* is able to grow in a biofilm form on stainless steel in milk under static conditions, especially in the presence of glucose and NaCl. In the current study, however, we found that detachment of *S. epidermidis* cells from the chips' surfaces at a higher temperature (25 °C) and during milk flow correlated with the initiation of planktonic cell growth in the milk. Therefore, to determine whether such detachment is under the influence of bacterial growth, we performed additional experiments involving three different temperatures: 6 °C, which is a milk-handling temperature; 22 °C, which was closely below the growth limit under the conditions used in this study and 28 °C, which supports the growth.

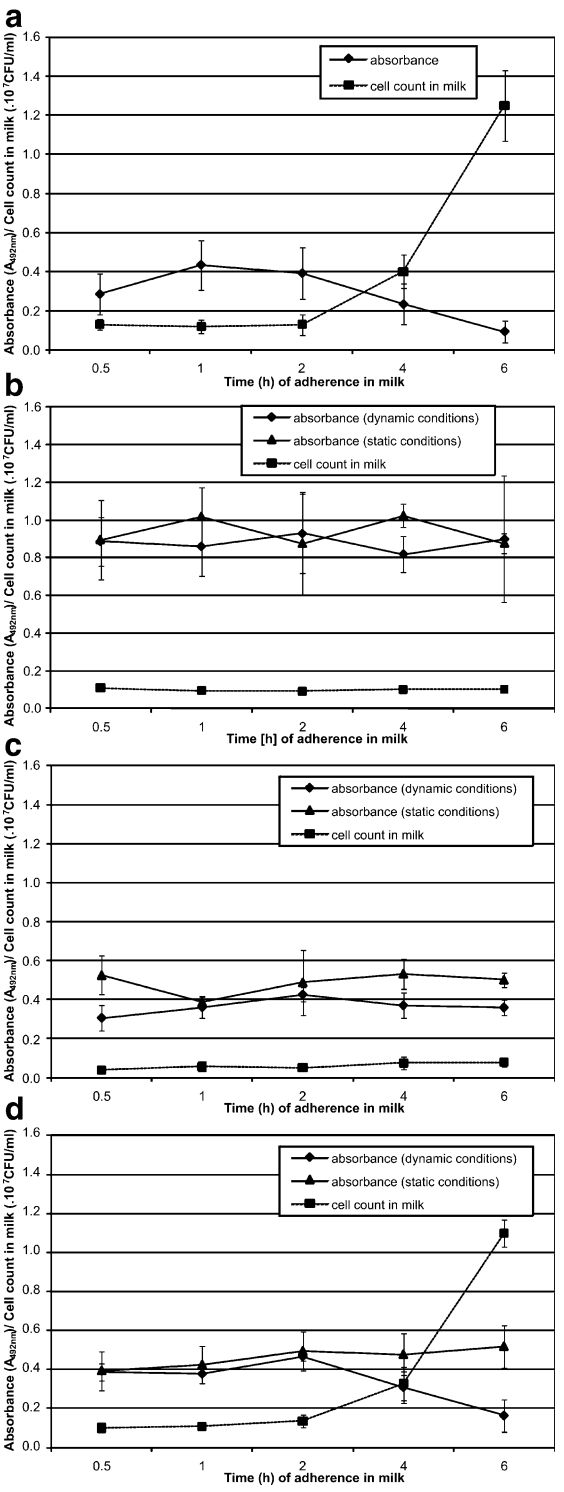

Fig. 2 Adherence of *Staphylococcus epidermidis* Staph 1597 in milk at 25 °C and flow rate v_1 (**a**); at 6 °C and flow rate v_2 compared with static conditions (**b**); at 22 °C and flow rate v_2 compared with static conditions (**c**); and at a temperature of 28 °C and flow rate v_2 compared with static conditions (**d**). Absorbances ($A_{492\ nm}$) of the safranin O eluates from the chips incubated in TSBg are shown in relation to their exposure time (time of adherence) in milk. Standard deviations of the mean of three independent experiments are indicated by *error bars*

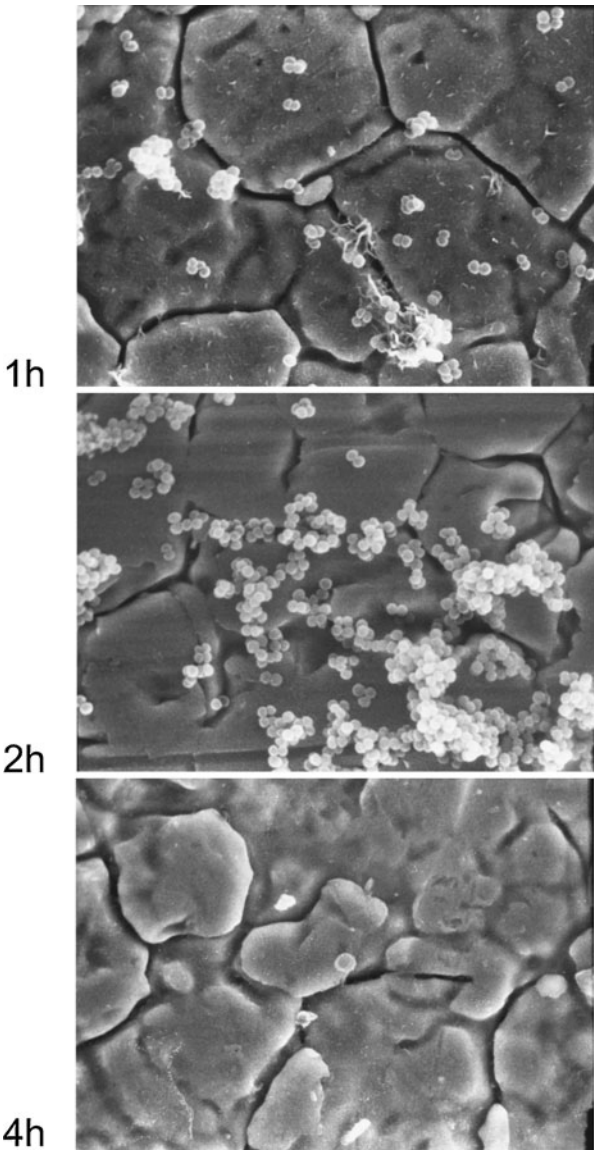

Fig. 3 Electron microscopy of *Staphylococcus epidermidis* CCM 7221 biofilms after incubation of the chips in TSBg. Exposure time (hours) of chips in milk is indicated on the left side (2250× magnification)

Furthermore, to assess the influence of flow, a flow rate of velocity v_2 was compared with static conditions. At 6 and 22 °C, the bacterial cells remained attached to the chips in amounts capable of biofilm formation during the whole period (6 h), under both static and dynamic conditions. At these temperatures, the number of planktonic cells (CFU) in the milk did not substantially increase (Fig. 2b, c). On the contrary, at 28 °C, a decrease in the adherence occurred after 2 h, which was similar to the findings observed at 25 °C, but only under dynamic conditions. This was also accompanied with an increased number of planktonic cells in the milk (Fig. 2d).

A reversible non-specific adsorption of microorganisms to a solid surface is followed by attaching in an irreversible specific way, which leads to the formation of

complex colonies known as biofilms (Genigeorgis 2004). Different bacterial exopolymers have been shown to be involved in a time-dependent irreversible phase of adherence (Lens et al. 2003). It has been reported that the irreversible phase of adherence to a stainless steel surface can appear very soon, in some cases within 1 min (Frank 2001). Under the conditions tested in this study, the cells of *S. epidermidis* adhered to the stainless steel (in a count capable of biofilm formation) within 0.5 h, however, the persistence of such adherence depended on temperature and flow. Pompermayer and Gaylarde (2000) reported that low temperatures (those below 12 °C) positively affected the adherence of *S. aureus* to inert surfaces. This is in accordance with the findings of the current study where biofilm development was most intensive after the adherence performed at 6 °C (Fig. 2b). Conversely, a reduction in the number of adhered cells at temperatures supporting the growth has been reported for the foodborne *Salmonella* Typhimurium, *Escherichia coli* O157: H7 and *Pseudomonas fragi* (Hood and Zottola 1997). In accordance with these findings, we detected a persisting adherence of *S. epidermidis* at the temperature slightly below the growth limit (22 °C), while higher temperatures (25 and 28 °C) allowing bacterial growth led to the detachment of the adhered cells but only during the milk flow (Fig. 2a, c, d).

Detachment of *S. epidermidis* over time under dynamic conditions correlated with the initiation of planktonic cell growth in the milk. Although this phenomenon has not yet been fully explained, Hood and Zottola (1997) speculated that the ability to adhere may differ depending on the growth phase of microorganisms. While entering the early exponential phase of growth, adhered cells could change the cell surface qualities as a consequence of the expression of other genes, than those which are responsible for biofilm formation. It has been shown that expression of the *agr* quorum-sensing system results in rapid detachment of *S. aureus* cells. *S. aureus* cell wall-attached proteins and some surface adhesins that mediate adherence to a variety of substrates are cleaved by the native secreted proteases regulated by the *agr* system (Boles and Horswill 2008). In *S. epidermidis*, *agr* negatively regulates the attachment ability to polystyrene through repressing the AtlE autolysin and ClpP protease, the factors specifically involved in adhesion (Vuong and Otto 2002; Wang et al. 2007). The results of the current work indicate that under the temperatures supporting the growth of *S. epidermidis* cells, the bacteria adhered to the stainless steel in milk in a reversible way and could easily be washed away during the milk flow. Even in the case of such low laminar flows tested in this study (in milk processing plants, the milk flow is generally turbulent), these flows were able to markedly reduce the amounts of adhered cells. Consequently, the ability of the remaining cells to form a biofilm on the chips was negatively affected since it is dependent on the intercellular signalling through the quorum-sensing system which requires a certain degree of bacterial density (Vuong et al. 2003).

4 Conclusion

Temperatures suboptimal for growth and stationary conditions represent the risk factors for adhesion of *S. epidermidis* to stainless steel in milk in amounts sufficient for biofilm formation. Conversely, temperatures allowing growth led to the cell

detachment during milk flow when bacteria started to grow. This means that *S. epidermidis* can easily be washed away from the stainless steel surface when it starts to grow.

Acknowledge The authors thank Mr. Jiri Kudrna (Veterinary Research Institute, Brno, Czech Republic) for preparation of specimens and images from scanning electron microscope, Mgr. Maria Vass, PhD. for English proofreading and Prof. Anping Deng, Ph.D. for Chinese translation of the abstract. Supported by the Ministry of Agriculture of the Czech Republic (project MZe0002716202) and Ministry of Education, Youth and Sports of the Czech Republic (projects 2B08074 and CZ.1.05/2.1.00/01.0006; ED0006/01/01).

References

Becker K, Bierbaum G, von Eiff C, Engelmann S, Gotz F, Hacker J, Hecker M, Peters G, Rosenstein R, Ziebuhr W (2007) Understanding the physiology and adaptation of staphylococci: a post-genomic approach. Int J Med Microbiol 297:483–501

Boles BR, Horswill AR (2008) *agr* – mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathog 4:1–13

de Araujo GL, Coelho LR, de Carvalho CB, Maciel RM, Coronado AZ, Rozenbaum R, Ferreira-Carvalho BT, Sa Figueiredo AM, Teixeira LA (2006) Commensal isolates of methicillin-resistant *Staphylococcus epidermidis* are also well equipped to produce biofilm on polystyrene surfaces. J Antimicrob Chemother 57:855–864

Flint SH, Bremer PJ, Brooks JD (1997) Biofilms in dairy manufacturing plant—description, current concerns and methods of control. Biofouling 11:81–97

Frank JF (2001) Microbial attachment to food and food contact surfaces. Adv Food Nutr Res 43:319–370

Genigeorgis CA (2004) Reducing the role of contact materials in the contamination of foods of animal origin. In: Smulders FJM, Collins JD (eds) Safety assurance during food processing: food safety assurance and veterinary public health, vol 2. Wageningen Academic Publishers, Wageningen, pp 279–315

Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM (2005) Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J Bacteriol 187:2426–2438

Gotz F (2002) Staphylococcus and biofilms. Mol Microbiol 43:1367–1378

Grubbs F (1969) Procedures for detecting outlying observations in samples. Technometrics 11:1–21

Heilmann C, Gerke C, Perdreau-Remington F, Gotz F (1996a) Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect Immun 64:277–282

Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F (1996b) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol Microbiol 20:1083–1091

Hilbert LR, Bagge-Ravn D, Kold J, Gram L (2003) Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance. Int Biodeterior Biodegrad 52:175–185

Hood SK, Zottola EA (1997) Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Food Microbiol 37:145–153

Jaglic Z, Michu M, Holasova M, Vlkova H, Babak V, Kolar M, Bardon J, Schlegelova J (2010) Epidemiology and characterization of *Staphylococcus epidermidis* isolates from humans, raw bovine milk and a dairy plant. Epidemiol Infect 138:772–782

Johnson AP, Clark JB, Osborn MF (1983) Scanning electron microscopy of the interaction between *Haemophilus influenza* and organ cultures of rat trachea. J Med Microbiol 16:477–482

Kim H, Ryu JH, Beuchat LR (2006) Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. Appl Environ Microbiol 72:5846–5856

Knight GC, Nicol RS, McMeekin TA (2004) Temperature step changes: a novel approach to control biofilms of *Streptococcus thermophilus* in a pilot plant-scale cheese-milk pasteurisation plant. Int J Food Microbiol 93:305–318

Lens P, Moran AP, Mahony T, Stoodley P, O'Flaherty V (2003) Biofilms in medicine, industry and environmental biotechnology. Characteristics, analysis and control, 1st edn. IWA Publishing, London

Mack D, Davies AP, Harris LG, Rohde H, Horstkotte MA, Knobloch JKM (2007) Microbial interactions in *Staphylococcus epidermidis* biofilms. Anal Bioanal Chem 387:399–408

Mattila-Sandholm T, Wirtanen G (1992) Biofilm formation in the food industry: a review. Food Rev Int 8:573–603

Michu E, Cervinkova D, Babak V, Kyrova K, Jaglic Z (2011) Biofilm formation on stainless steel by *Staphylococcus epidermidis* in milk and influence of glucose and sodium chloride on the development of ica-mediated biofilms. Int Dairy J 21:179-184

Moretro T, Hermansen L, Holck AL, Sidhu MS, Rudi K, Langsrud S (2003) Biofilm formation and the presence of the intercellular adhesion locus *ica*, among staphylococci from food and food processing environments. Appl Environ Microbiol 69:5648–5655

Piette A, Verschraegen G (2009) Role of coagulase-negative staphylococci in human disease. Vet Microbiol 134:45–54

Pompermayer DMC, Gaylarde CC (2000) The influence of temperature on the adhesion of mixed cultures of *Staphylococcus aureus* and *Escherichia coli* to polypropylene. Food Microbiol 17:361–365

Rieu A, Lemaitre JP, Guzzo J, Piveteau P (2008) Interactions in dual species biofilms between *Listeria monocytogenes* EGD-e and several strains of *Staphylococcus aureus*. Int J Food Microbiol 126:76–82

Schlegelova J, Babak V, Holasova M, Konstantinova L, Necidova L, Sisak F, Vlkova H, Roubal P, Jaglic Z (2010) Microbial contamination after sanitation of food contact surfaces in dairy and meat processing plants. Czech J Food Sci 28:450–461

Sharma M, Anand SK (2002) Characterization of constitutive microflora of biofilms in dairy processing lines. Food Microbiol 19:627–636

Sommer P, Martin-Rouas C, Mettler E (1999) Influence of the adherent population level on biofilm population, structure and resistance to chlorination. Food Microbiol 16:503–515

Vuong C, Otto M (2002) *Staphylococcus epidermidis* infections. Microbes Infect 4:481–489

Vuong C, Gerke C, Somerville GA, Fischer ER, Otto M (2003) Quorum-sensing control of biofilm factors in *Staphylococcus epidermidis*. J Infect Dis 188:706–718

Wang C, Li M, Dong D, Wang J, Ren J, Otto M, Gao Q (2007) Role of ClpP in biofilm formation and virulence of *Staphylococcus epidermidis*. Microbes Infect 9:1376–1383

Ziebuhr W, Heilmann C, Gotz F, Meyer P, Wilms K, Straube E, Hacker J (1997) Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect Immun 65:890–896