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Research Article

Biofilm Formation by *Staphylococcus aureus* Isolated from Food Contact Surfaces in the Dairy Industry of Jalisco, Mexico

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Staphylococcus aureus is an important food-borne pathogen able to form biofilms. This pathogen is responsible for outbreaks of food-borne illnesses associated with the consumption of milk and dairy products. The aim of this study was to evaluate the biofilm-forming ability of *S. aureus* isolates, recovered from food contact surfaces in the dairy industry of Jalisco, Mexico. A total of 84 *S. aureus* strains were evaluated. The isolates were characterized phenotypically by culture on Congo red agar plates. The ability of the strains to form biofilms was investigated in 96-well flat-bottomed microtiter polystyrene plates. Stainless-steel coupons were used as an experimental surface. Biofilm formation was observed, using epifluorescence microscopy and scanning electron microscopy. Detection of the *icaADBC* genes in *S. aureus* was performed by the PCR technique. A total of 52.3% (44/84) of the *S. aureus* strains contained the *icaADBC* gene that synthesizes polysaccharide intercellular adhesion (PIA) molecules. On Congo red agar, 75% (63/84) of the *S. aureus* isolates were biofilm producers, 16.6% (14/84) were non-biofilm formers, and 8.3% (7/84) showed a noncharacteristic phenotype. The biofilm production of the *S. aureus* strains SA-4E, SA-9, SA-13, and SA-19 on stainless-steel coupons was investigated at 25°C for 8 days, and the detected cell population density was approximately 7.15–7.82 log CFU cm^{-2}. In addition to the ability of biofilm production, it is important to highlight that these strains are potential enterotoxin producers as *se* genes have been previously detected in their genomes. A part of the ability of biofilm production and the determination of the presence of virulence determinants in the genome of *S. aureus* can contribute to the pathogenicity of strains. Therefore, vigilant food safety practices need to be implemented in the dairy industries regarding FCS to prevent food-borne infections and intoxications due to *S. aureus* contamination.

1. Introduction

In the food industry, biofilms increase bacterial resistance to environmental stresses including cleaning, disinfection, and inhibition, enabling these microorganisms to persist on surfaces and processing equipment, compared to planktonic cells [1–3]. Formation of biofilms can occur on all types of surfaces of technological systems in the dairy industry. The detection of biofilms in the food industry can be related to the presence of pathogenic microorganisms in the industrial settings.

Staphylococcus aureus is a food-borne pathogen that can cause staphylococcal food poisoning. In the USA, staphylococcal food poisoning is estimated to account for 241,188 illnesses, 1,064 hospitalizations, and six deaths, annually [4]. *S. aureus* can adhere to and develop biofilms on food contact surfaces, thereby affecting the quality and safety of food products [5, 6]. The extracellular matrix of *S. aureus* biofilms is usually composed of exopolysaccharide (PIA/PNAG), but the proteinaceous and extracellular DNA matrix can also be present in staphylococcal biofilms [7]. Depending on the environment in which the biofilm was developed, the biofilm matrix can also contain blood components or noncellular materials such as mineral crystals, corrosion particles, and clay or silt particles [8]. PIA

is linked to the irreversible attachment phase [9]. The formation of biofilm of *Staphylococcus aureus* is not only mediated by the PIA-dependent biofilm formation, but it can exist in PIA-independent biofilm. In the PIA-independent biofilm, despite the importance of the *ica* gene locus in biofilm development, biofilms can occur in an *ica*-independent fashion where biofilm-associated protein (Bap) and Bap-related proteins *of S. aureus* can confer biofilm development independently or PIA production through cell-to-cell aggregation and are characterized by their high molecular weight, presence of the bacterial surface, role as a virulence factor, and occasional containment in mobile elements [10, 11].

The main adhesion genes of *S. aureus* that are involved in cellular aggregation and bacterial accumulation within the biofilm are *bap*, *bbp*, *clfA*, *clfB*, *cna*, *ebpS*, *fib*, *fnbA*, *fnbB*, *eno*, *icaAD*, *icaBC*, *sasG*, *sasC*, and *pls* [12].

The aim of this study was to evaluate the biofilm-forming ability of *S. aureus* isolates, recovered from food contact surfaces in the dairy industry of Jalisco, Mexico.

2. Materials and Methods

2.1. S. aureus Isolates. S. aureus (SA1–SA84) strains were isolated from food contact surfaces (FCS) of six dairy industries in the Mexican state of Jalisco [13]. The *S. aureus* strains were identified by the methods described in the Bacteriological Analytical Manual (Gram staining, the coagulase and Voges–Proskauer tests, tests for catalase and thermostable nuclease, and glucose and mannitol utilization test), and finally, PCR was used for confirmation (PCR amplification of genes encoding for 23S ribosomal RNA (rRNA) and thermonuclease (*nuc*)) [13]. The strains were cultivated in tryptic soy broth (TSB; Becton Dickinson Diagnostic Systems) for 24 h at 37°C. All strains were subcultured in TSB with 0.25% glucose (w/v) for 24 h at 37°C for the quantification of biofilm formation and in TSB with 0.5% glucose (w/v) for 8 d at 25°C for biofilm formation on stainless steel. The *S. aureus* strain ATCC 25923, a strong biofilm former, was used as a positive control.

2.2. Biofilm Formation Assays

2.2.1. Phenotype Analysis of Biofilm Production. The isolates were characterized phenotypically by culture on Congo red agar (CRA) plates, as described by Arciola et al. [14]. Briefly, agar plates were prepared by adding 0.8 g Congo red (Sigma-Aldrich) and 36 g saccharose (Sigma-Aldrich) to 1 L blood agar (Becton Dickinson Diagnostic Systems), followed by incubation at 37°C for 24 and 48 h. The macroscopic characteristics of the *S. aureus* isolates in the CRA were observed. Crusty black colonies, with a dry filamentous appearance, were recorded as biofilm producers, smooth pink colonies as nonproducers, and intermediate colony morphology (pink with dark centers resembling bull's eyes), as potential biofilm producers [15].

2.2.2. Quantification of Biofilm Formation. The ability of the strains to form biofilms was investigated in 96-well flat-bottomed microtiter polystyrene plates [16]. For each strain, three wells of the microtiter plate were filled with 200 μL bacterial suspension in TSB with 0.25% glucose (w/v) (TSB + 0.25% G). Then, the plates were incubated at 37°C for 24 h. Wells filled with the broth medium (TSB + 0.25% G) were used as negative controls, and *S. aureus* ATCC 25923 was used as the positive control. Next, the content of each well was aspirated and washed three times with phosphate-buffered saline (PBS; 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, and 130 mM NaCl, pH 7.4) to remove the planktonic bacteria. The attached bacteria were fixed with 95% ethanol for 5 min; then, the plates were emptied and left to dry. The plates were stained with 100 μL of 1% (w/v) crystal violet solution per well for 5 min. The excess stain was rinsed off with sterile distilled water, and the microtiter plates were air-dried. The optical density of each well was measured at 570 nm (OD_{570}), using the Multiskan FC (Thermo Fisher Scientific Inc., Madison, WI). Biofilm formation was interpreted as highly positive ($OD_{570} \geq 1$), low-grade positive ($0.1 \leq OD_{570} < 1$), or negative ($OD_{570} < 0.1$).

2.2.3. Detection of icaADBC Genes. Genomic DNA was extracted, using the protocol described by Pu et al. [17]. Detection of the *icaADBC* genes in *S. aureus* was performed, as stated by Diemond-Hernández et al. [18]. The amplifications were performed using the Thermal Cycler (TechNet; Bibby Scientific Ltd., UK). The initial step (94°C for 5 min) was followed by 30 cycles with annealing at 60°C for 1 min (*icaA*), 59°C for 1 min (*icaB*), 42°C for 1 min (*icaC*), or 59°C for 1 min (*icaD*) and a final step at 72°C for 7 min (Table 1). After amplification, the products were electrophoresed on a 2% agarose gel (ultrapure agarose; Invitrogen), containing 0.5 μg/mL ethidium bromide (Sigma-Aldrich), and visualized by transillumination under ultraviolet light. *S. aureus* ATCC 25923 was used as the positive control.

2.3. Conditions for Biofilm Formation. Stainless-steel (SS) coupons (AISI 316, $0.8 \times 2.0 \times 0.1$ cm) were used as an experimental surface. The coupons were consecutively cleaned, according to the method described by Marques et al. [5]. For the biofilm formation, each SS coupon was individually introduced into glass test tubes (20×150 mm) containing 10 mL of TSB with 0.5% glucose (TSB + 0.5% G). The monospecies biofilms were inoculated with 100 μL of cultures incubated at 37°C/24 h (containing approximately 10^8 CFU/mL of the corresponding strains) (Table 2); after that, the biofilms were incubated at 25°C for 8 d. Afterwards, cell viability was determined by the standard plate count technique on standard agar (Becton Dickinson Diagnostic Systems) with incubation at 37°C for 24 h. Biofilm formation was observed, using epifluorescence microscopy and scanning electron microscopy (SEM). Three replicates were performed for each strain. *S. aureus* ATCC 25923 was used as the positive control. As a negative control, an SS coupon without inoculum was included in all assays.

Table 1: Primers used for amplification of the adhesin genes of *Staphylococcus aureus* [18].

Primers	Sequences (5′–3′)	Product sizes (base pairs)
icaA forward icaA reverse	GAC CTC GAA GTC AAT AGA GGT CCC AGT ATA ACG TTG GAT ACC	814
icaB forward icaB reverse	ATC GCT TAA AGC ACA CGA CGC TAT CGG CAT CTG GTG TGA CAG	526
icaC forward icaC reverse	ATA AAC TTG AAT TAG TGT ATT ATA TAT AAA ACT CTC TTA ACA	989
icaD forward icaD reverse	AGG CAA TAT CCA ACG GTA A GTC ACG ACC TTT CTT ATA TT	371

Table 2: Primers used for the RAPD-PCR method.

Primers	Sequences (5′–3′)
OPL5	ACGCAGGCAC
RAPD5	AACGCGCAAC
P1	CCGCAGCCAA
P2	AACGGGCAGA

2.3.1. Epifluorescence Microscopy. After the incubation at 25°C for 8 d, the SS coupons were removed from the glass test tubes containing 10 mL of TSB with 0.5% glucose using sterile forces. Each coupon was washed with 1 mL PBS for 10 s to eliminate nonadhered cells. The coupons were stained with 5(6)-carboxyfluorescein diacetate (CFDA; 10 μg/mL), rinsed with sterile distilled water, dried in a level II cabinet, and observed under a Nikon Eclipse E400 epifluorescent microscope, using 100× oil immersion lens and the filter BA 515 B2a at 450–900 nm. At least 18 fields were observed. Once inside the cell, the diacetate is hydrolyzed by intracellular nonspecific esterases, producing carboxyfluorescein (CF), which is retained by live cells with an intact plasma membrane [19, 20].

2.3.2. SEM. After the incubation at 25°C for 8 d, the SS coupons were treated as indicated in Section 2.3.1. They were further dried and transferred to 2% glutaraldehyde at 4°C for 2 h to fix the sample [21, 22]. Next, the samples were dehydrated in serial dilutions of ethanol at 30, 50, 60, 70, 90, and 95% at 4°C for 10 min each. Furthermore, three transfers were performed in 100% ethanol for 10 min each. The samples were vacuum-dried and gold-coated for 30 s. Biofilms were observed, using a TESCAN Mira3 LMU scanning electron microscope.

2.4. Genomic Fingerprinting of S. aureus Isolates. The differentiation of the *S. aureus* isolates with genotypic and phenotypic characteristics associated with biofilm formation (presence of *icaADBC* genes, in addition to the presence of virulence determinants in their genome) was performed by the RAPD-PCR method. Primers used for this purpose were OPL5, RAPD5, P1, and P2 (Table 2) according to the method previously described [23, 24]. Strains of *S. aureus* ATCC 25923 and 51811 and *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11778 were included to enable the comparison of genetic variability. RAPD-PCR band patterns from each primer were scanned, and profile grouping (dendrogram) was performed with the PAST (PAleontological Statistics) version 3.20 software (University of Oslo, Noruega), using Jaccard's coefficient and the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis [25].

2.5. Statistical Analysis. Pearson's chi-squared test was employed at the $p < 0.05$ significance level to compare differences between groups. Statistical analysis was performed with SPSS for the Windows software, version 11.0.

3. Results

A total of 84 *S. aureus* strains (SA1–SA84) were studied to estimate their potential to adhere to, and, subsequently, form biofilms on food contact surfaces. Biofilms were quantified regarding biomass accumulation, using the crystal violet staining method. The OD_{570} results showed that 90.4% (76/84) of the strains (SA1 to SA76) isolated from the food contact surfaces were low-grade biofilm formers ($0.1 \leq OD_{570} < 1$), 7.1 % (6/84) were highly positive biofilm formers ($OD_{570} \geq 1$), and 2.3% (2/84) were biofilm negative ($OD_{570} < 0.1$). On CRA, 75% (63/84) of the *S. aureus* isolates were biofilm producers, 16.6% (14/84) were non-biofilm producers, and 8.3% (7/84) exhibit a noncharacteristic phenotype (Figure 1). At least one intercellular adhesion gene was present in 76.1% (64/84) of the *S. aureus* isolates with low-grade biofilm formation (Table 3). Some of the genes of the *icaADBC* locus were detected in most of the strains, with a positive correlation ($r = 0.798$, $p > 0.05$) between the *icaADBC* genes and CRA.

Among 84 *S. aureus* strains, four *S. aureus* (SA-4E, SA-9, SA-13, and SA-19) were examined by epifluorescence and SEM. The four *S. aureus* strains were considered, according to the genotypic and phenotypic characteristics associated with biofilm formation (Table 4). In addition to the ability of biofilm production, it is important to highlight that these strains are potential enterotoxin producers as se genes have been previously detected in their genomes [13]. The genetic variability of these strains of *S. aureus* isolates was determined by RAPD-PCR genotyping using four different primers (Table 2). Strains were grouped into five main clusters (I–V) (Figure 2). Cluster I is composed of strains SA-13 and SA19. Cluster II includes the strain SA-9. Cluster III includes the strain SA-4E. Group IV comprises the strains of *Staphylococcus aureus* ATCC 25923 and 51811. And finally, cluster V comprises the strain of *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11778. The genetic

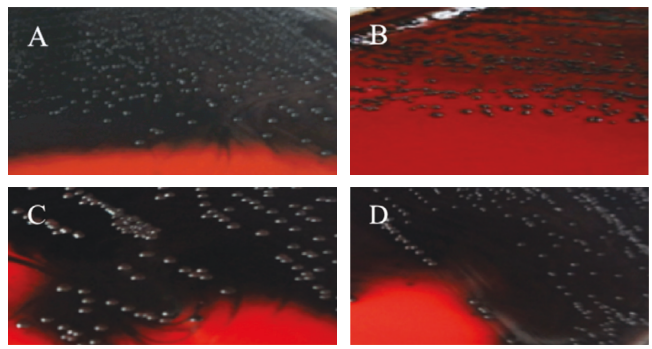

Figure 1: CRA plate test. Black colonies of the slime-producing *S. aureus*: (A) *S. aureus* 4E; (B) *S. aureus* 9; (C) *S. aureus* 13; (D) *S. aureus* 19.

Table 3: *icaADBC* genes in *Staphylococcus aureus* isolates from food contact surfaces.

Strain	Number of isolates	*icaB*	*icaD*	*icaA* + *icaD*	*icaB* + *icaC*	*icaADBC*
S. aureus (SA1–SA84)	64	1	2	16	1	44

SA = *S. aureus*.

Table 4: Association between the biofilm phenotype on Congo red agar, slime production, adherence assay, and the presence of *icaADBC* genes in *Staphylococcus aureus*.

Bacterial strain	Source	Presence of *icaADBC*				Adherence state	Biofilm phenotype on CRA	Slime production
		icaA	*icaB*	*icaC*	*icaD*			
SA-4E *sec*, *sed*, *seg*, *sej*, *nuc**	FSC-stainless steel	+	+	+	+	Low-grade positive	Black	Figure 1(A)
SA-9 *sec*, *sed*, *seh*, *sej*, *nuc**	FSC-stainless steel	+	+	+	+	Low-grade positive	Black	Figure 1(B)
SA-13 *seb*, *sed*, *sei*, *nuc**	FSC-stainless steel	+	+	+	+	Highly positive	Black	Figure 1(C)
SA-19 *sea*, *sej*, *nuc**	FSC-stainless steel	+	+	+	+	Low-grade positive	Black	Figure 1(D)

*Virulence determinants in the genome of the strains of *S. aureus* [13].

variability of the strains of *S. aureus* was demonstrated by RAPD-PCR analysis.

The four *S. aureus* strains showed the ability to form single-species biofilms on SS coupons at 25°C; cell adhesion was visualized during biofilm maturation by epifluorescence microscopy (Figure 3). With this technique, it is possible to observe the presence of metabolically active living cells, and the diacetate is hydrolyzed by intracellular nonspecific esterases, producing carboxyfluorescein (CF) that indicates the integrity of the plasma membrane and esterase activity. Moreover, in the SEM microphotographs, the surface of microcolonies of the biofilm of the four *S. aureus* strains was visualized as well as probably the presence of the EPS (Figure 4). All isolates evaluated in this study had a concentration ranging from 7.15 ± 0.15 to 7.82 ± 0.25 log CFU cm^{-2} on the SS coupons, and no significant differences ($p > 0.05$) were observed among them.

4. Discussion

Biofilms formed on food contact surfaces can lead to significant health problems. Biofilms reduce the effectiveness of sanitizers, cause economic losses to industries, and contaminate food and can increase the level of antimicrobial resistance [26]. Our results indicated that most of the examined *S. aureus* strains had at least one intercellular adhesion gene involved in the formation of PIA. Of note, 44 strains harbor the 4 genes of the *icaADBC* locus, which support their ability to produce biofilms. Most of the *S. aureus* strains formed the biofilm in an *ica*-dependent mechanism. This finding is consistent with results reported by Tang et al. [12], who detected *icaAD* and *icaBC* in 87.5% ($n = 57$) of *S. aureus* strains isolated from several sources (chicken, food samples, and goats). Gutiérrez et al. [27] also showed that 100% of *S. aureus* ($n = 63$) strains collected from various food contact surfaces in the dairy, meat, and seafood industries were positive for the *icaA* and *icaD* genes.

In the current study, most of the evaluated strains were *S. aureus* biofilm producers. Similar results were obtained by Szczuka et al. [9], who reported that, of 74 biofilm-positive strains, 56 carried the *icaA* (76%) gene and produced slime on CRA. However, the variation between phenotypic and genotypic methods for detection of the biofilm produced by *S. aureus* has been reported, regarding CRA [28]. Congo red can directly interact with certain polysaccharides, forming

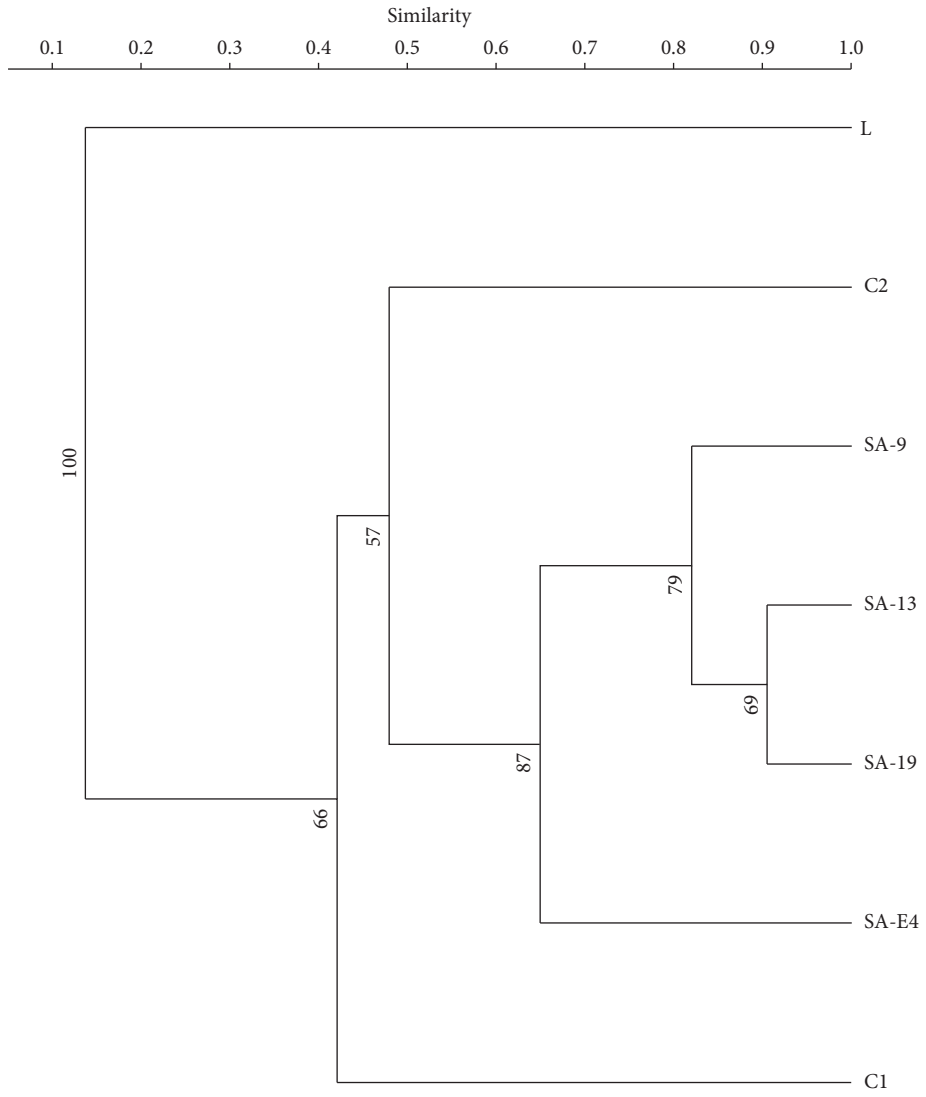

Figure 2: Dendrogram of isolated strains based on Jaccard's similarity coefficient. Strains: SA-4E, SA-9, SA-13, and SA-14 (*Staphylococcus aureus* isolated from food contact surfaces); C1 (*Staphylococcus aureus* ATCC 25923); C2 (*Staphylococcus aureus* ATCC 51811); L (*Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11778).

colored pigments [29], and some metabolic reactions that form secondary products with the dye can influence the formation of dark colonies [14]. Nevertheless, Kim et al. [30] determined slime production under various environmental conditions (1% dextrose, 5% NaCl, and their combination) in four *S. aureus* strains (ATCC 12600, D8, D29, and C52), but the results did not indicate any influence of the tested conditions on slime production.

Consequently, the CRA technique could be used as the presumptive test for the formation of a biofilm. However, Arciola et al. [14] suggest that the phenotypic change may be caused by a deletion of the *ica* operon rather than an insertion event which inactivates the *ica* genes. The type of the food contact surface and diverse environmental factors, such as osmolarity, nutrient content, and temperature, and genetics, such as the presence of *sarA*, *ica*, and *agr* genes [31], may influence the development of a biofilm by *S. aureus* and, consequently, its persistence on contact surfaces within the food industry [32]. Moreover, the *ica* operon expression is strongly influenced by environmental factors, such as glucose, temperature, osmolarity, and growth under anaerobic conditions [33]. Li et al. [34] reported that, besides *icaAD* and *icaBC*, other virulence regulators including *bap*, *sigB*, and *sar* might be crucial biofilm-associated genes because these genes are expressed more often in biofilm-positive strains than in biofilm-negative strains.

Rode et al. [32] demonstrated that temperatures suboptimal for growth increased the biofilm formation in eleven *S. aureus* strains and the highest biofilm production occurred at 25, 30, and 46°C, whereas, in general, biofilm formation was low at 42°C. Da Silva Meira et al. [22] evaluated the biofilm formation of three food industry-associated *S. aureus* isolates on SS and polypropylene surfaces, incubated in a vegetable-based medium at two temperatures (7 and 28°C/15 d), deducing that the biofilm development was favored at 28°C, without significant differences between the type of surface.

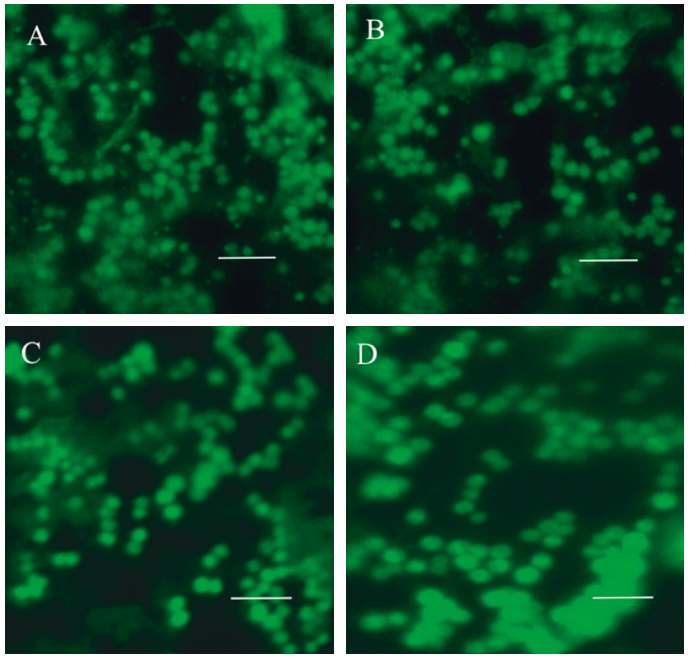

Figure 3: Epifluorescence photomicrograph of biofilms of *Staphylococcus aureus* isolates from food contact surfaces. Biofilms were developed on SS by 8 days of incubation in TSB with 0.5% glucose at 25°C: (A) *S. aureus* 4E; (B) *S. aureus* 9; (C) *S. aureus* 13; (D) *S. aureus* 19. The white bar scale indicates 10 μm.

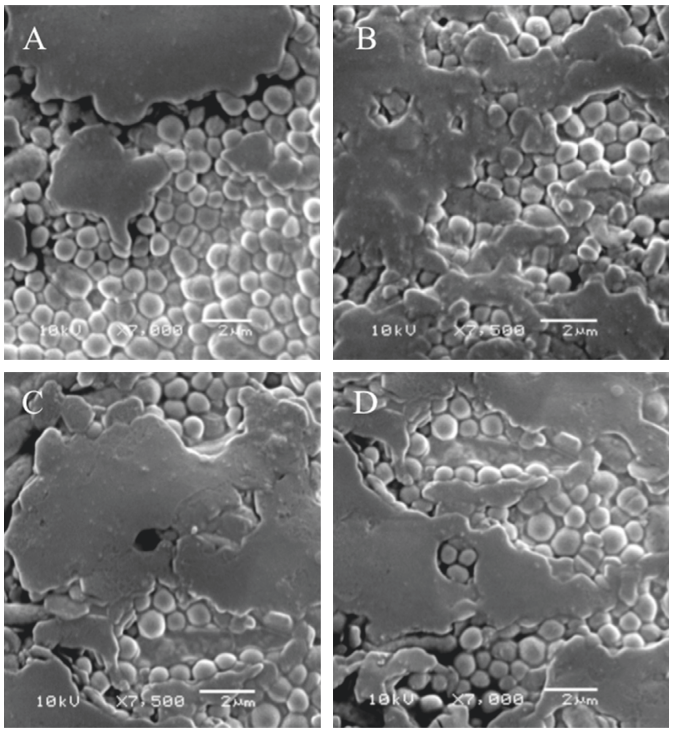

Figure 4: Scanning electron photomicrograph of biofilms of *Staphylococcus aureus* isolates from food contact surfaces. Biofilms were developed on SS by 8 days of incubation in TSB with 0.5% glucose at 25°C: (A) *S. aureus* 4E; (B) *S. aureus* 9; (C) *S. aureus* 13; (D) *S. aureus* 19. Bar = 2 μm.

Bae et al. [35] found that populations of five food-borne pathogens including *S. aureus* formed biofilms with 8.8–9.3 and 9.4–10.3 log CFU/coupon on SS and polypropylene surfaces, respectively. Consequently, these isolates of *S. aureus* (SA-4E, SA-9, SA-13, and SA-19) have the ability to form biofilms on food contact surfaces.

5. Conclusion

In conclusion, this study showed the biofilm-forming ability of *S. aureus*, isolated from food contact surfaces in the dairy industry. Biofilm formation can cause public health problems and economic losses, associated with food contamination by the pathogen and equipment damage, by favoring equipment corrosion or resistance to hygiene treatments of food contact surfaces.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] M. Kostaki, N. Chorianopoulos, E. Braxou, G. J. Nychas, and E. Giaouris, "Differential biofilm formation and chemical disinfection resistance of sessile cells of *Listeria monocytogenes* strains under monospecies and dual-species (with *Salmonella enterica*) conditions," *Applied and Environmental Microbiology*, vol. 78, no. 8, pp. 2586–2595, 2012.

[2] K. Laird, D. Armitage, and C. Phillips, "Reduction of surface contamination and biofilms of *Enterococcus* spp. and *Staphylococcus aureus* using a citrus-based vapour," *Journal of Hospital Infection*, vol. 80, no. 1, pp. 61–66, 2012.

[3] A. Bridier, P. S. Vizuete, M. Guilbau, J. C. Piard, M. Naitali, and R. Briandet, "Biofilm-associated persistence of food-borne pathogens," *Food Microbiology*, vol. 45, pp. 167–178, 2015.

[4] E. Scallan, R. M. Hoekstra, F. J. Angulo et al., "Foodborne illness acquired in the United States-major pathogens," *Emerging Infectious Diseases*, vol. 17, no. 1, pp. 7–15, 2011.

[5] S. C. Marques, J. G. Silva-Rezende, L. P. de Freitas-Alves et al., "Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitiziers," *Brazilian Journal of Microbiology*, vol. 38, no. 3, pp. 538–543, 2007.

[6] S. Srey, I. J. Jahid, and S. D. Ha, "Biofilm formation in food industries: a food safety concern," *Food Control,* vol. 31, no. 2, pp. 572–585, 2013.

[7] B. R. Boles, M. Thoendel, A. J. Roth, and A. R. Horswill, "Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation," *PLoS One*, vol. 5, no. 4, Article ID e10146, 2010.

[8] R. M. Donlan, "Biofilms: microbial life on surfaces," *Emerging Infectious Diseases*, vol. 8, no. 9, pp. 881–890, 2002.

[9] E. Szczuka, K. Urbanska, M. Pietryka, and A. Kaznowski, "Biofilm density and detection of biofilm-producing genes in methicillin-resistant *Staphylococcus aureus* strains," *Folia Microbiology*, vol. 58, no. 1, pp. 47–52, 2013.

[10] I. Lasa and J. R. Penades, "Bap: a family of surface proteins involved in biofilm formation," *Research inMicrobiology*, vol. 157, no. 2, pp. 99–107, 2006.

[11] N. K. Archer, M. J. Mazaitis, J. W. Costerton, J. G. Leid, M. E. Powers, and M. E. Shirtliff, "*Staphylococcus aureus* biofilm properties, regulation and roles in human disease," *Virulence*, vol. 2, no. 5, pp. 445–459, 2011.

[12] J. Tang, J. Chen, H. Li, P. Zeng, and J. Li, "Characterization of adhesin genes, staphylococcal nuclease, hemolysis, and biofilm formation among *Staphylococcus aureus* strains isolated from different source," *Foodborne Pathogens and Diseases*, vol. 10, no. 9, pp. 757–763, 2013.

[13] M. G. Avila-Novoa, M. Iñiguez-Moreno, J. P. González-Gómez et al., "Detection of enterotoxin genes of *Staphylococcus aureus* isolates from food contact surfaces in the dairy industry of Jalisco, Mexico," *Biotecnia*, vol. 20, no. 8, pp. 72–78, 2018.

[14] C. R. Arciola, L. Baldassarri, and L. Montanaro, "Presence of icaA and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections," *Journal of Clinical Microbiology*, vol. 39, no. 6, pp. 2151–2156, 2001.

[15] C. R. Arciola, D. Campoccia, S. Gamberini, L. Baldassarri, and L. Montanaro, "Prevalence of *cna*, *fnbA* and *fnbB* adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant," *FEMS Microbiology Letters*, vol. 246, no. 1, pp. 81–86, 2005.

[16] B. Kouidhi, T. Zmantar, H. Hentati, and A. Bakhrouf, "Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in *Staphylococcus aureus*," *Microbial Pathology*, vol. 49, no. 1-2, pp. 14–22, 2010.

[17] S. Pu, F. Wang, and B. Ge, "Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats," *Foodborne Pathogens and Diseases*, vol. 8, no. 2, pp. 299–306, 2011.

[18] B. Diemond-Hernández, F. Sólorzano-Santos, B. Leaños-Miranda, L. Peregrino-Bejarano, and G. Miranda-Novales, "Production of i*caADBC*-encoded polysaccharide intercellular adhesin and therapeutic failure in pediatric patients with staphylococcal device-related infections," *BMC Infectious Diseases*, vol. 10, no. 68, pp. 1–6, 2010.

[19] E. Gorokhova, L. Mattsson, and A. M. Sundstrom, "A comparison of TO-PRO-1 iodide and 5-CFDA-AM staining methods for assessing viability of planktonic algae with epifluorescence microscopy," *Journal of Microbioligical Methods*, vol. 89, no. 3, pp. 216–221, 2012.

[20] Y. Pan and L. Kaatz, "Use of image-based flow cytometry I bacterial viability analysis using fluorescent probes," *Current Protocols in Microbiology*, vol. 27, no. 1, pp. 1–11, 2012.

[21] M. Alhede, K. Qvortrup, R. Liebrechts, N. Hoiby, M. Givskov, and T. Bjarnsholt, "Combination of microscopic techniques reveals a comprehensive visual impression of biofilm structure and composition," *FEMS Inmmunology and Medical Microbiology*, vol. 65, no. 2, pp. 335–342, 2012.

[22] Q. G. Da Silva Meira, I. D. M. Barbosa, A. A. Athayde, J. P. Siqueira-Júnior, and E. L. Souza, "Influence of temperature and surface kind on biofilm formation by *Staphylococcus aureus* from food-contact surfaces and sensitivity to sanitizers," *Food Control*, vol. 25, no. 2, pp. 469–475, 2012.

[23] D. Gutiérrez, A. M. Martín-Platero, A. Rodríguez, M. Martínez-Bueno, P. García, and B. Martínez, "Typing of bacteriophages by randomly amplified polymorphic DNA (RAPD-PCR) to assess genetic variability," *FEMS Microbiology Letters*, vol. 322, no. 1, pp. 90–97, 2011.

[24] T. B. Salgado-Ruiz, A. Rodríguez, D. Gutiérrez et al., "Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* from small-scale dairy systems in the highlands of Central México," *Dairy Science and Technology*, vol. 95, no. 2, pp. 181–196, 2015.

[25] M. J. Struelens, "Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems," *Clinical Microbiology and Infection*, vol. 2, no. 1, pp. 2–11, 1996.

[26] T. F. Mah, "Biofilm-specific antibiotic resistance," *Future Microbiology*, vol. 7, no. 9, pp. 1061–1072, 2012.

[27] D. Gutiérrez, S. Delgado, D. Vázquez-Sánchez et al., "Incidence of *Staphylococcus aureus* and analysis of associated bacterial communities on food industry surfaces," *Applied and Environmental Microbiology*, vol. 78, no. 24, pp. 8547–8554, 2012.

[28] R. Yazdani, M. Oshaghi, A. Havayi et al., "Detection of icaAD gene and biofilm formation in *Staphylococcus aureus* isolates from wound infection," *Iranian Journal of Public Health*, vol. 35, no. 2, pp. 25–28, 2006.

[29] A. Jain and A. Agarwal, "Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci," *Journal of Microbiological Methods*, vol. 76, no. 1, pp. 88–92, 2009.

[30] B. R. Kim, Y. M. Bae, and S. Y. Lee, "Effect of environmental conditions on biofilm formation and related characteristic of *S. aureus*," *Journal of Food Safety*, vol. 36, no. 3, pp. 412–22, 2016.

[31] J. P. O'Gara, "ica and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*," *FEMS Microbiological Letters*, vol. 270, no. 2, pp. 179–188, 2007.

[32] M. T. Rode, S. Langsrud, A. Holck, and T. Moretro, "Different patterns of biofilm formation in *Staphylococcus aureus* under food-related stress conditions," *International Journal of Food Microbiology*, vol. 116, no. 3, pp. 372–383, 2007.

[33] J. Kim, C. Kim, J. Hacker, W. Ziebuhr, B. K. Lee, and S. Cho, "Molecular characterization of regulatory genes associated with biofilm variation in a *Staphylococcus aureus* strain," *Journal of Microbiology and Biotechnology*, vol. 18, no. 1, pp. 28–34, 2008.

[34] L. Li, H. J. Yang, D. C. Liu et al., "Analysis of biofilm formation and associated gene detection in *Staphylococcus* isolates from bovine mastitis," *African Journal of Biotechnology*, vol. 11, no. 8, pp. 2113–2118, 2012.

[35] Y. M. Bae, S. G. Heu, and S. Y. Lee, "Inhibitory effect of dry-heat treatment and chemical sanitizers against foodborne pathogens contaminated on the surfaces of materials," *Journal of Korean Society of Food Science and Nutrition*, vol. 38, no. 9, pp. 1265–1270, 2009.