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Influence of temperature and surface kind on biofilm formation by *Staphylococcus aureus* from food-contact surfaces and sensitivity to sanitizers

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ABSTRACT

This study aimed to assess the adhesion, detachment kinetic and biofilm formation of *Staphylococcus aureus* isolates from food services surfaces on stainless steel and polypropylene surfaces when cultivated in a vegetable-based broth at 7 and 28 °C, and the efficacy of peracetic acid (30 mg/L) and sodium hypochlorite (250 mg/L) in removing the bacterial cells from the matrix of the preformed biofilm. The isolates adhered over 4 Log cfu/cm^2 regardless the surface kind and incubation temperature. Cell detachment was around 3 Log cfu/cm^2 over the first six contacts with agar characterizing a high persistence of cells on the tested surfaces. Number of cells (5–7 Log cfu/cm^2) needed for biofilm formation was noted at all experimental systems already after 3 days of incubation. A range of 2.0–3.3 and 1.5 to 2.1 Log cfu/cm^2 was observed in the reduction of cells in biofilm matrix caused by peracetic acid and sodium hypochlorite, respectively. The isolates of *S. aureus* revealed high capability to adhere and form biofilm on the tested surfaces in both assayed incubation temperature.

1. Introduction

Worldwide there is a concern about the impact of microbial foodborne diseases on the human behalf (White, Zhao, Simjee, Wagner, & McDermott, 2002). The importance of contaminated surfaces in spreading pathogenic microorganisms to foods is already well established in food processing, catering and domestic environment (Vasseur, Rigaud, Hébraud, & Labadie, 2001; Vautor, Abadie, Pont, & Thiery, 2008). One of the most common ways for bacteria to live is adhering onto surfaces and forming organized communities named biofilms (Jenkinson & Lappin-Scott, 2001; Malheiros, Passos, Casarin, Serraglio, & Tondo, 2010). Stainless steel, glass, rubber and polypropylene surfaces can be contaminated either by spoilage or pathogenic bacteria, which under certain conditions adhere to these surfaces, initiating the cell growth and leading to the biofilm formation (Murga et al., 2001).

According to Costerton, Steward, and Greenberg (1999) biofilms are cell aggregates embedded in an organic extracellular polymeric matrix that confers resistance to involved microorganisms. Bacteria aggregated to form biofilms have greater resistance to the environmental stress than the planktonic counterparts, including the sensitivity to sanitizers (Fux, Wilson, & Stoodley, 2004; Spoering & Lewis, 2001). Bacterial aggregates detached from biofilms retain the high level of resistance to antimicrobials and may contain enough number of cells to represent a potential infectious dose. The formation of biofilms on food-contact surfaces is known as a potential risk to the consumer's health, particularly, if the cross contamination of food occurs after a bactericidal procedure (Spoering & Lewis, 2001).

Staphylococcus aureus has been frequently found in surfaces of food processing plants being responsible for outbreaks related to the consumption of fresh and processed foods worldwide (Balaban & Rasooly, 2000; Braga et al., 2005; Nostro et al., 2004). The establishment of the food poisoning caused by *S. aureus* depends on the ability of the strain to survive in/on a colonized substrate, multiply under a variety of conditions and produce several extracellular substances (Pastoriza, Cabo, Bernárdez, Sampedro, & Herrera, 2002). Although some researchers have observed the ability to adhere and form biofilm by *Staphylococcus* genera (Hussain, Becker, Von Eiff, Peters, & Hermann, 2001; Kuźman, Różalski, Walenka, Różalska, & Wysokińka, 2007), the most studies have been addressed to clinical aspects related to the

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Table 1
Physico-chemical characteristics of vegetable-based broth.

Physico-chemical parameters	Values
Proteins	0.18%
Fat	0.11%
Moisture	98.32%
Carbohydrates	1.39%
Ashes	–
pH (T: 27.5 °C)	5.69

biofilm formation by *Staphylococcus intermedius* on medical implants and materials (Herrera, Cabo, González, Pazos, & Pastoriza, 2007; Marques et al., 2007).

Currently, there is a lack of information about the capacity of *S. aureus* from food service surfaces of adhering and forming biofilm when exposed to different environmental conditions, and about the efficacy of sanitizers in removing the cells forming the biofilm. Regarding these aspects, this study was carried out with the aim of evaluating the ability of *S. aureus* isolates from food services surfaces to adhere and form biofilms on stainless steel and polypropylene surfaces when cultivated in a vegetable-based broth under different temperatures (7 and 28 °C). Still, it was observed the effect of the sanitizers peracetic acid and sodium hypochlorite in reducing the number of bacterial viable cells on a preformed biofilm.

2. Material and methods

2.1. Test isolates

S. aureus S3, *S. aureus* S28 and *S. aureus* S54 obtained from the Microorganism Collection, Laboratory of Food Microbiology, Health Sciences Center, Federal University of Paraíba (João Pessoa, Brazil) were used as test isolates. The ones were isolated from different surfaces of Food and Nutrition Services by the standard procedures (Downes & Ito, 2001). Stock cultures were kept on Nutrient Agar – NA (Difco, Brazil) slants under refrigeration (7 ± 1 °C).

Inocula used in antimicrobial assays were obtained from overnight cultures grown on NA slants at 37 °C. A loopfull of the culture was diluted in sterile saline solution (0.85 g/100 mL) to have a final concentration of approximately 8 Log of colony forming unity per mL (cfu/mL) adjusted according to the turbidity of 0.5 McFarland standard tube (Oliveira, Stamford, Gomes Neto, & Souza, 2010).

2.2. Test surfaces and experimental conditions

AISI 304 stainless steel (2 × 2 × 0.2 cm) and polypropylene coupons (2 × 2 × 0.4 cm) were used as test surfaces. The coupons were individually cleaned, sanitized and sterilized according to procedure described by Marques et al. (2007).

The adherence, detachment and biofilm formation of the test isolates on polypropylene and stainless steel surfaces and inoculated in a vegetable-based broth was assessed in two different incubation temperatures, 7 and 28 °C.

2.3. Preparation of vegetable-based broth

A mixture (1:1:1) of vegetables (carrot, lettuce and tomato) containing 300 g was mashed with 600 mL of distilled water using a domestic blender and vacuum filtered using Whatman no.1 filter paper. The material was sterilized by filtration using a Millipore 0.22 μm. The obtained broth was stored at −20 °C in aliquots of 50 mL and when required one aliquot was thawed under refrigeration (7 ± 1 °C) and used for the experimental assays.

The vegetable broth was characterized regarding its physico-chemical characteristics (moisture, protein, fat, carbohydrate, ashes and pH value) according to procedures described by IAL (2005). Physico-chemical characteristics of the vegetable-based broth used in the assays are shown in Table 1.

2.4. Adhesion to surfaces and quantification of adhered cells

An aliquot of 100 μL of the growth media was mixed to 50 μL of the bacterial inoculum, plated onto the center of each coupon and incubated under the pre-established temperatures. After 24, 48 and 72 h of incubation, coupons (two for each treatment) were withdrawn and immersed in sterile peptone water – SPW (0.1 g/100 mL) during 15 s for releasing non-adhered cells. The cells adhered to the coupons were collected by thoroughly rubbing their surfaces with two moistened swabs, which were resuspended in SPW by vigorously vortexing for 30 s. The mixture was serially diluted (10^{-1}–10^{-5}) in SPW and aliquots of 100 μL were spread plated onto sterile NA plates. The plates were incubated for 24 h at 37 °C (Herrera et al., 2007; Rode, Langsrud, Holck, & Moretto, 2007). After the incubation period, the number of viable cells was counted and the results were expressed in Log cfu/cm^2.

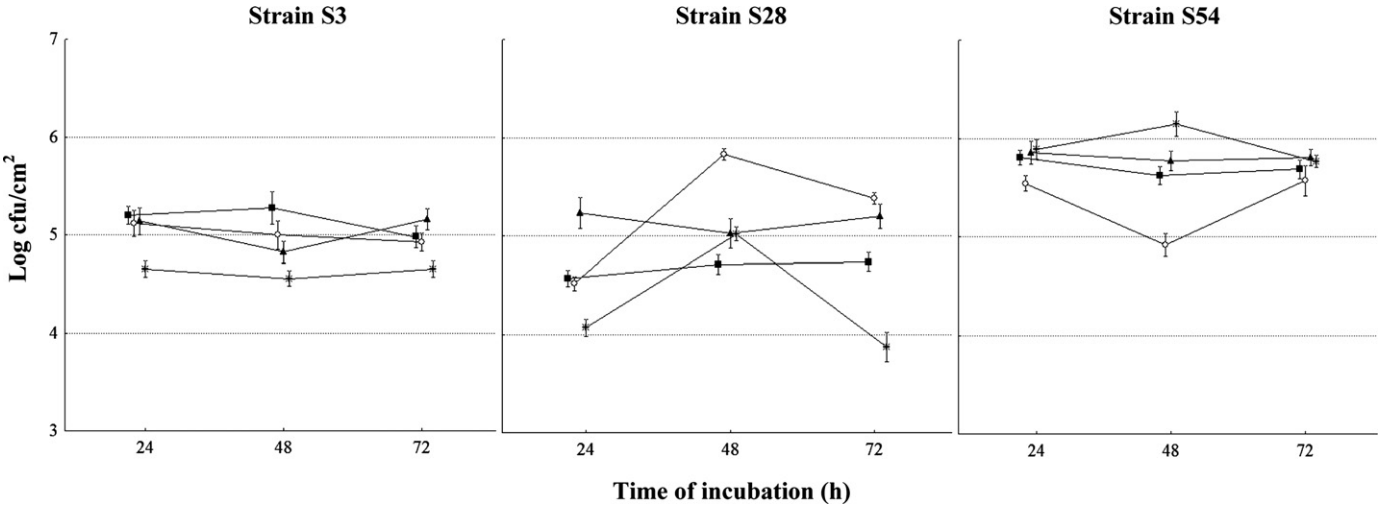

Fig. 1. Kinetics of adhesion of *S. aureus* S3, S28 and S54 to polypropylene and stainless steel surfaces in vegetable-based broth at 7 °C and 28 C over 72 h of incubation (■: polypropylene 7 °C, ○: polypropylene 28 °C, ▲: stainless steel 7 °C, ✳: stainless steel 28 °C).