BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2017) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

Biofilm formation by *Staphylococcus aureus* and *Salmonella* spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite

Maricarmen Iñiguez-Moreno, Melesio Gutiérrez-Lomelí, Pedro Javier Guerrero-Medina, María Guadalupe Avila-Novoa *

Universidad de Guadalajara, Centro Universitario de la Ciénega, Departamento de Ciencias Médicas y de la Vida, Ocotlán, Jalisco, Mexico

ARTICLE INFO

Article history:
Received 26 January 2017
Accepted 9 August 2017
Available online xxx
Associate Editor: Solange I. Mussatto

Keywords:
Biofilms
Salmonella spp.
Staphylococcus aureus
Disinfectants
Sensitivity

ABSTRACT

The aim of this study was evaluated the biofilm formation by *Staphylococcus aureus* 4E and *Salmonella* spp. under mono and dual-species biofilms, onto stainless steel 316 (SS) and polypropylene B (PP), and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite. The biofilms were developed by immersion of the surfaces in TSB by 10 d at 37 °C. The results showed that in monospecies biofilms the type of surface not affected the cellular density ($p > 0.05$). However, in dual-species biofilms on PP the adhesion of *Salmonella* spp. was favored, 7.61 ± 0.13 Log_{10} CFU/cm^2, compared with monospecies biofilms onto the same surface, 5.91 ± 0.44 Log_{10} CFU/cm^2 ($p < 0.05$). The mono and dual-species biofilms were subjected to disinfection treatments; and the most effective disinfectant was peracetic acid (3500 ppm), reducing by more than 5 Log_{10} CFU/cm^2, while the least effective was cetrimonium bromide. In addition, *S. aureus* 4E and *Salmonella* spp. were more resistant to the disinfectants in mono than in dual-species biofilms ($p < 0.05$). Therefore, the interspecies interactions between *S. aureus* 4E and *Salmonella* spp. had a negative effect on the antimicrobial resistance of each microorganism, compared with the monospecies biofilms.

Introduction

Salmonella is a zoonotic bacteria, which is one of the most significant enteric foodborne bacterial pathogens[1] that caused losses estimated at 3.3 billion dollars per year in the Unites States.[2] Extra-animal survival is an important parameter for the environmental dissemination of salmonellae, with the ability of these bacteria to survive in the food chain to be largely due to their ability to sense and adapt to a diverse range of adverse environmental conditions.[1] *Salmonella* is able to adhere and form biofilms on a wide range of surfaces, including metal, plastic and rubber, due to their aggregative fimbriae and lipopolysaccharides; it is also able to produce

* *Corresponding author at*: Av. Universidad 1115, Col. Linda Vista, 47820, Ocotlán, Jalisco, Mexico.
E-mail: avila.novoa@cuci.udg.mx (M.G. Avila-Novoa).

http://dx.doi.org/10.1016/j.bjm.2017.08.002

cellulose, leading to bacterial cells being immersed in a hydrophobic network.[3] On the other hand, *Staphylococcus aureus* is a ubiquitous bacterial species commonly found on the skin and hair, as well as in the noses and throats of people and animals.[4,5] *S. aureus* can produce heat-stable enterotoxins, which cause 13–40% of poisonings in countries like the United States, Canada and Japan.[6–8] The ability of *S. aureus* to develop biofilms is strongly linked to the production of polysaccharide intracellular adhesion (PIA), and adhesins called MSCRAMM (microbial surface components recognizing adhesive molecules matrix) on the surface of the microorganism, which have been implicated as major factors in biofilm formation by the *ica*-independent pathway.[9,10]

The biofilms are the dominant lifestyle of bacteria in all environments, either natural or man-made (e.g. food processing).[11] Biofilms can develop on a wide variety of surfaces, including those used within the food industry. The biofilms have been implicated in food spoilage, foodborne diseases and damage to processing equipment in the food industry, including the meat industry, brewing, dairy, fisheries, and other industries.[12–14] In the food industry, the presence of microorganisms, inorganic and organic debris on the surfaces favored biofilm formation.[15] Therefore, without a suitable cleaning treatment followed by the application of disinfectants, the microorganisms can colonize and persist on food contact and non-food contact surfaces. Thus, the food might be contaminated by contact with the contaminated surface.[16–18] Few studies have focused on the evaluation of resistance to disinfectants by multispecies biofilms; most of these studies did not include an assessment of the resistance from each microorganism in the single-species models; therefore, it is impossible to judge whether the interspecies effect affects the individual resistance of each species in the multispecies communities.[14,19–21] Even when these communities in the environment are mainly multi-specie; and the interactions between the microorganisms can affect the biofilm structure and function.[22] In addition, the biofilm formation by bacterial pathogens is important due to its a potential risk, the antimicrobial resistance and bacterial persistence increase.[14,23,24] For this reason, the aim of this study was evaluate the biofilm formation of *S. aureus* 4E and *Salmonella* spp. under mono and dual-species biofilms, onto stainless steel 316 and polypropylene B, and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite.

Materials and methods

Bacterial strains

The bacterial strains used were *S. aureus* 4E isolated from a stainless steel table from the dairy industry. The strain was confirmed by 23S rDNA according to Straub et al.[25] In addition, the presence of *ica* ABCD operon[26] and gen of bap protein were determined,[27] which are important factors for *ica* dependent and independent biofilm formation, respectively.[8] *Salmonella* spp. were isolated from the meat industry, and was confirmed by PCR using the primer pair ST11 and ST15, specific for *Salmonella* spp.[28] As positive controls for biofilm formation, *S. aureus* ATCC 25923[6,29] and *Salmonella* Enteritidis ATCC 13076[30] were used. Before utilization, the microorganisms were incubated in tryptic soy broth (TSB; Bioxon, Le Pont de Claix, France) for 24 h at 37 °C to give a final concentration of 10^8 CFU/mL.

Biofilm formation of S. aureus and Salmonella spp.

Contact surfaces

Stainless Steel (SS, AISI 316, 0.7 × 0.8 × 0.1 cm) and polypropylene B coupons (PP, 0.8 × 2 × 0.1 cm) were cleaned according to the method described by Rossoni and Gaylarde (2000), modified by Marques et al. (2007). Briefly, the surfaces were immersed in pure acetone (Fermont) for 1 h to remove any debris and grease, immersed in neutral detergent (30 mL/L, provide by CIP & GROUP) for 1 h, rinsed with sterile distilled water, cleaned with ethanol (70%, Hycel), dried for 2 h at 60 °C, and sterilized in autoclave (121 °C for 15 min).

Development of mono and dual-species biofilms and quantification

For the biofilm formation, each coupon was individually introduced into a glass test containing 5 mL of TSB. The monospecies biofilms were inoculated with 50 μL of cultures incubated at 37 °C for 24 h of the corresponding strain and the dual-species biofilms were inoculated with 25 μL of each bacterial suspension, after that, the biofilms were incubated at 37 °C for 10 d.[32,33] Finally, after the incubation period, the coupons were removed from the glass test using sterile forces, and rinsed two times by pipetting 2 mL of Dulbecco's phosphate buffered saline (PBS; Sigma-Aldrich) in order to remove the loosely attached cells.[14] Each coupon was introduced individually into a glass test with 10 mL of casein peptone (BD, Bioxon, Becton Dickinson) (1 g/L), and the biofilms were removed by sonication (1 min, Sonicor Model SC-100th operating 50–60 Hz). Serial dilutions and conventional plating on tryptic soy agar (TSA; Becton Dickinson, Le Pont de Claix, France) for monospecies biofilms and TSA with lactose (10 g/L, Sigma–Aldrich) and phenol red (0.025 g/L, Hycel) for dual-species biofilms were used to estimate the number of microorganisms in the biofilm. The Petri dishes were incubated at 37 °C for 24 h.[34] The colonies of *S. aureus* 4E were yellow due to lactose fermentation and *Salmonella* spp. colonies were colorless.

Biocide resistance assays

The coupons with the biofilms were removed from the culture media described above, and immersed individually in 2 mL aqueous solutions of disinfectants: i) cetrimonium bromide (CB; Sigma–Aldrich) at 100 and 200 ppm, ii) peracetic acid (PAA; Sigma–Aldrich) at 10 and 3500 ppm and iii) sodium hypochlorite (NaClO) at 100 and 200 ppm; the disinfectants were prepared in sterile distilled water. The CB and PAA were applied at 25 °C and 50 °C, while NaClO was assessed at 25 °C and 37 °C; the three disinfectants were evaluated at two times of exposure (10 and 15 min). After the exposure period, each coupon was transferred into 1.5 mL or 3 mL of neutralizer solution (SS and PP, respectively): sodium thiosulfate 1 mol L^{-1} for NaClO, Letheen broth for CB, and Gibson neutralizer (3 g soy lecithin, 30 mL Tween-80, 5 g sodium thiosulfate, 1 g L-

Please cite this article in press as: Iñiguez-Moreno M, et al. Biofilm formation by *Staphylococcus aureus* and *Salmonella* spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite. *Braz J Microbiol.* (2017), http://dx.doi.org/10.1016/j.bjm.2017.08.002