Food Control xxx (2016) 1–6

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

The specific anti-biofilm effect of gallic acid on *Staphylococcus aureus* by regulating the expression of the *ica* operon

Meihui Liu [a], Xiaoxia Wu [a], Jianke Li [a, *], Liu Liu [a, *], Runguang Zhang [a], Dongyan Shao [b], Xiaodan Du [a]

[a] College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an, Shaanxi 710119, China
[b] Key Laboratory for Space Bioscience and Biotechnology, School of Life Sciences, Northwestern Polytechnical University, Xi'an, Shaanxi 710072, China

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form
7 September 2016
Accepted 8 September 2016
Available online xxx

Keywords:
Gallic acid
Staphylococcus aureus
Anti-biofilm
ica operon
Food industry

ABSTRACT

Staphylococcus aureus (*S. aureus*) biofilms are of considerable interest in food safety because biofilms can increase the risk of food contamination and enhance the pathogenicity of bacteria. The *ica*-encoded polysaccharide intercellular adhesin (PIA) plays an important role in biofilm formation. In this study, the MIC of gallic acid against *S. aureus* in suspension and in biofilms was 2 mg/mL and 4 mg/mL, respectively. Quantitative crystal violet staining of biofilms showed that 2 mg/mL gallic acid can effectively inhibit biofilm formation and the ESEM images clearly showed the three-dimensional biofilm morphology of the *S. aureus* and the resulting anti-biofilm effect. The determination of viable bacteria in the biofilm revealed that gallic acid penetrated the biofilm to kill *S. aureus*, the bactericidal effect on the biofilm bacteria was comparable to that of planktonic bacteria. We further explored the influence of gallic acid on *ica* family gene expression and polysaccharide slime formation in *S. aureus* biofilm formation. The results showed that *icaR* was significantly activated that; *icaA* and *icaD* were downregulated in a dose-dependent manner with increasing concentrations of gallic acid; however, the expression of *icaB* and *icaC* was not significantly affected. The polysaccharide slime formation was reduced as well. Based on these results, gallic acid, as a natural substance, may play an important role in the food industry.

1. Introduction

Staphylococcus aureus has frequently been found in food processing plants and has been isolated from dairy products, egg, seafood, and meat (Shi & Zhu, 2009; Rode, Langsrud, Holck, & Moretro, 2007; Melchior, Fink-Gremmels, & Gaastra, 2007). In fact, the nutrients in the residues on the surface of biomaterials that come into contact with foods are important for biofilm formation on food and in food processing plants (Barnes, 1999). Bacteria biofilm possess greater resistances to stress conditions such as sanitizers and can enhance the pathogenicity of pathogenic bacteria, there is no doubt that they can increase the risk for microbial contamination in food safety. (Bridier et al., 2015; Fux, Wilson, & Stoodley, 2004; Singh, Vuddanda, Kumar, Saxena, & Singh, 2014; Souza, 2014). In addition, the occurrence of biofilms can reduce heat transfer and operating efficiency in heat exchange equipment, thereby increasing energy consumption, contributing to, mechanical blockage and accelerating the corrosion of metal surfaces (Kumar, 1998).

Biofilm formation is a very complex process. The properties of the substratum and cell surfaces, surrounding environmental factors, and genetic regulation of bacteria play an important role in the process of biofilm formation (Shi & Zhu, 2009). The extracellular polysaccharide slime is considered an essential component of biofilm formation, because it allows the bacteria to embed into the biofilm to avoid the effect of cleaning agents and antibiotics. The main component of polysaccharide slime is the polysaccharide intercellular adhesin (PIA), a linear glucosaminylglycan that plays a fundamental role in mediating the intercellular adhesion of bacterial cells and biofilm accumulation in *S. aureus* (Mack et al., 1996). PIA synthesis is mediated by the *ica* operon that is present in many species of the *Staphylococcus* genus, including *S. aureus*. Studies found that deletion of the *ica* operon results in a loss of the ability to produce PIA and form biofilms (Cramton, 1999). The *ica* operon is composed of the *icaR* (regulatory) and *icaADBC* (biosynthesis) genes. The first two genes of the *icaA* and *icaD* cluster, *icaA* and *icaD*,

* Corresponding authors.
E-mail addresses: jiankel@snnu.edu.cn (J. Li), liuliu@snnu.edu.cn (L. Liu).

http://dx.doi.org/10.1016/j.foodcont.2016.09.015
0956-7135/

respectively, encode for a transmembrane enzyme with *N*-acetylglucosaminyl transferase activity for the synthesis of the poly-*N*-acetylglucosamine polymer, which plays a primary role in exopolysaccharide synthesis (Gerke, 1998). The product of the *icaC* gene appears to translocate the poly-*N*-acetylglucosamine polymer to the bacterial cell surface, and the *icaB* product deacetylaes the molecule (Vuong, 2004). *IcaR*, a regulatory gene, governs the expression of the *ica* locus under the influence of *SarA*, stress sigma σ^B and environmental factors (Cerca, Brooks, & Jefferson, 2008).

To minimize the risk of contamination from *S. aureus*, many synthetic additives are used in industrial food processing. However, consumers are concerned about the potential side effects on health of some synthetic additives, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and phosphoric acid (Dongqin, 2014; Tepe, Daferera, Sokmen, Sokmen, & Polissiou, 2005). From this perspective, it is very important to develop effective and safe methods of controlling food contamination in food processing plants and the environments (Shi & Zhu, 2009). Natural antimicrobials are a potentially interesting solution for the treatment and prevention of biofilm formation on the surface of foodstuffs and processing surfaces, particularly the production of extracts from edible plant tissues because these extracts are thought to be nearly non-toxic. Gallic acid (GA; 3,4,5-triphydroxylbenzoic acid) as a polyhydroxylphenolic compound is widely distributed among various plants, fruits and foods, where it is rarely present in a free form but, more commonly; as a component called gallotannin (Niemetz & Gross, 2005). Particularly, gallnuts, sumac, oak bark, green tea, grapes, strawberries, pineapples, bananas, lemons, red and white wines and apple peels are known to be rich in gallic acid (Chu, 2002; Wolfe, 2003). Various biological activities of gallic acid have been reported, including anti-bacterial (Kang, Oh, Kang, Hong, & Choi, 2008), anti-viral (Jadel Müller et al., 2008) and anti-inflammatory (Kim, 2006).

However, most researchers only focus on gallic acid's inhibitory effect on planktonic bacteria, disregarding its potential impact on biofilm and virulence factors. Therefore, the aim of this work was to characterize the anti-biofilm potential of gallic acid against *S. aureus*.

2. Materials and methods

2.1. Materials and culture

In this study, the standard bacterial strain *S. aureus* (ATCC25923) was stored with 25% glycerol at −20 °C prior to use in biofilm assays. Gallic acid was purchased from the KELON Chemical Reagent Factory (Chengdu, China). *S. aureus* was shaken at 37 °C for 18 h in Luria−Bertani (LB) broth. Gallic acid was dissolved in LB for the assays. To form a biofilm, *S. aureus* was cultured in 1/8 LB with 0.15% glucose to simulate the nutritional conditions that promote biofilm formation.

2.2. Anti-microbial activity of gallic acid on S. aureus

2.2.1. Determination of MIC and MBC in suspension

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined using the 2-fold broth dilution (Costa, Silva, Pina, Tavaria, & Pintado, 2012). *S. aureus* was grown overnight in LB medium and then diluted to an optical density of 0.05 at 600 nm in fresh LB medium, of which 200 μL was added to the wells of a 96-well polystyrene microtiter plate and incubated with various concentrations of gallic acid (0.25, 0.5, 1, 2, 4, and 8 mg/mL) for 24 h at 37 °C. The MIC was defined as the lowest concentration of gallic acid which inhibited visible growth of the bacteria; The sample with the lowest concentration of the gallic acid that showed no growth on LB agar was recorded as the MBC (Jadhav, Shah, Bhave, & Palombo, 2013). Sterile LB was used as a negative control. The samples in each experiment were prepared in six wells (Lin, 2012).

2.2.2. Determination of the MIC and MBC in biofilm

The biofilm model was established using the micro plate method (Dosler & Karaaslan, 2014; Lee et al., 2014). *S. aureus* was grown overnight in LB medium and then diluted to an optical density of 0.05 at 600 nm, of which 200 μL was added to the wells of a 96-well polystyrene microtiter plate and incubated for 24 h at 37 °C. After biofilm formation, each well was gently washed with PBS to remove planktonic bacteria, gallic acid (0.25−32 mg/mL) was added and plate was incubated for additional 24 h at 37 °C. The antimicrobial agents were then removed and washed with PBS. Biofilms were recovered from the wells by scraping and washing with 250 μL of sterile saline for plate counting. The MIC was defined as the lowest concentration of gallic acid which inhibited visible growth of the bacteria; The MBC was determined to be the lowest concentration demonstrating no bacterial growth on LB agar. Sterile saline was used as a positive control.

2.3. The anti-biofilm activity of gallic acid on S. aureus biofilm formation

The biofilm formation in each well was determined using the crystal violet staining method (Duan, 2008). After biofilm formation, the culture medium was discarded, and the wells were gently washed three times with PBS to remove planktonic bacteria. Next, 200 μL of methanol was added to fix the biofilm and then discarded 15 min later. After air-drying, biofilms were stained with 2% crystal violet for 5 min, rinsed under running water to remove excess stain, and then air-dried. The stained biofilm were dissolved in 33% glacial acetic acid and analyzed by reading the optical density at 570 nm with a Multiskan Spectrum (Thermo, Multiskan Go, USA). Wells with no gallic acid added were used as positive controls (PC).

2.4. Inverted fluorescence microscope studies

Biofilm cells were stained with a BacLight LIVE/DEAD bacterial viability staining kit according to the manufacturer's instructions (Molecular Probes, Invitrogen, France). Gallic acid (0.25, 0.5, 1, 2 and 4 mg/mL) was added after biofilm formation in a 6-well polystyrene microtiter plate at 37 °C. The samples without gallic acid were used as a control. After 24 h, the polystyrene plates were washed three times with sterile PBS and stained for 15 min in the dark at room temperature with diluted PI (1:8000 diluted with PBS) and SYTO 9(1:8000). Stained biofilms were examined using a fluorescence microscope (Leica, DMI3000B; Leica Microsystems CMS GmbH, Mannheim, Germany). For each sample, at least 10 random positions in two independent cultures were chosen for analysis with an inverted microscope (Kim, Lee, Kim, Baek, & Lee, 2015; Vassena et al., 2014).

2.5. Environment scanning electron microscopy (ESEM) studies

To visualize of the *S. aureus* biofilm architecture, ESEM images were taken. First, sterile cover glass (4 mm × 4 mm) was added to LB containing *S. aureus* (OD_{600} = 0.05) to form biofilms in 6-well polystyrene microtiter plates. Various concentrations of gallic acid (0.25, 0.5, 1, 2 and 4 mg/mL) were added to incubate at 37 °C for 24 h. The samples without gallic acid were used as a control. After incubation, the glass covers were washed three times with sterile PBS, and the cells were fixed in 2.5% (v/v) glutaraldehyde for 2 h at 4 °C. Following fixation, the samples were washed three times with

Please cite this article in press as: Liu, M., et al., The specific anti-biofilm effect of gallic acid on *Staphylococcus aureus* by regulating the expression of the *ica* operon, *Food Control* (2016), http://dx.doi.org/10.1016/j.foodcont.2016.09.015