Journal of Food Safety and Food Quality 75, Heft 2 (2024), Seiten 35–64

Arch Lebensmittelhyg 75,
44–51 (2024)
DOI 10.53194/0003-925X-75-44

ISSN 0003-925X

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Biofilm formation capacities of *Staphylococcus aureus* strains isolated from döner kebab and effect of acetic acid and citric acid against their biofilms

Biofilmbildungsfähigkeit von Staphylococcus aureus-Stämmen isoliert aus Döner Kebab und Wirkung von Essig- und Zitronensäure gegen diese Biofilme

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Summary

Staphylococcus aureus is a well-known food-borne pathogen causing staphylococcal food poisoning with a capacity to develop biofilms on food contact surfaces. This study aimed at evaluating the capacity of *S. aureus* strains isolated from ready-to-eat döner kebab to produce biofilms on Congo Red Agar (CRA), polystyrene (PST) and stainless steel (SS) surfaces. The effect on *S. aureus* biofilm forming capacity of glucose, Sodium Chloride (NaCl), temperature and acetic and citric acids was also evaluated. At 37°C temperature and 24 h of incubation, only three out of 11 strains tested produced biofilm on CRA, where as all strains tested were considered either strong or moderate biofilm producers on the surface of both the 96-well PST microtiter plates and SS coupons. On the latter surface, the strength of the biofilm was reduced by increasing the incubation time up to 72 h. Biofilms were quantified by crystal violet staining method after exposing the strains to different concentrations of glucose and NaCl. Results showed that the presence of glucose at any concentration tested promoted *S. aureus* biofilm formation at 30°C. Increasing the glucose concentration gave better biofilm growth rate at 37°C but at 40°C no significant influence was recorded. For NaCl addition, concentrations greater than 1% did not affect the biofilm formation at both 30 and 37°C. At 40°C, no effect on the bacterial growth rate by increasing concentration. This study showed that increasing acetic and citric acid concentrations up to 2.5% and 4%, respectively enhanced the elimination of 7-days old *S. aureus* biofilms. Besides, significant reduction was not scored after 5 min of treatment. These results indicate that the environment related conditions could promote *S. aureus* biofilm formation. Also, they showed that citric and acetic acids can be used as an alternative strategy in eliminating food biofilms.

Keywords: döner kebab, *Staphylococcus aureus,* biofilm, glucose, sodium chloride, citric acid and acetic acid

Introduction

The Gram-positive bacterium *Staphylococcus aureus* is not only an environmental microorganism, but also a pathogen causing infections and intoxications. This pathogen is known as the third common cause of foodborne diseases worldwide (Giaouris et al. 2015; Jamali et al., 2015). Generally, *S. aureus* is isolated from various foods, such as meat and meat products, poultry and egg products, milk and dairy products, bakery products, salads, cream cakes, pastries and sandwich fillings (Kowalska et al., 2020; Thiran et al., 2018; Bortolaia et al., 2016; Fetsch et al., 2014; Lee et al., 2014). When growing in foods, *S. aureus* strains can produce staphylococcal enterotoxins causing food poisonings (Kadariya et al., 2014; Hennekinne et al., 2012; Normanno et al. 2007).

Production of biofilms is an universal strategy adopted by bacteria to increase their survival chances against harsh environment including physical and chemical antimicrobial treatments. Biofilms are structured communities of bacterial cells enclosed in a self-produced polymeric matrix and attached to biotic or abiotic surfaces (Da Silva-Meira et al., 2012; Costerton, 1999). Bacterial biofilm formation is a complex dynamic process involving attachment, maturation, and dispersal of cells. Several reports demonstrated that the bacterial adhesion in food environments and consequently biofilm formation may be affected by various factors including, food contact surfaces, nutrient availability, temperature and maturation time (Qiao et al., 2021; Giaouris et al., 2012; Rode et al., 2007). In addition, the presence of biofilms irreversibly attached to surfaces in food environment may affect the quality, functionality and food safety and can cause serious economic problems. Moreover, *S. aureus* is a highly adaptable organism that can live in a self-developed biofilms in a wide variety of environments and on different types of surfaces such as stainless steel, plastic and glass (Avila-Novoa et al., 2018; Unlu et al., 2018; Di Ciccio et al., 2015). In addition, the food area constitutes a suitable environment for the biofilm development and several studies have shown the persistence of *S. aureus* biofilms on food contact surface despite the use of cleaning procedures (Kowalska et al., 2020; Cherif-Antar et al., 2016; Abdallah et al., 2014).

Organic acids, "Generally reconized as Safe" are compounds with antimicrobial activities. Therefore, several studies reported that organic acids including acetic acid and citric acid can be used as an alternative control strategy to eliminate undesirable pathogens especially in their biofilm form (Tsang et al., 2018; Amrutha et al., 2017; Akbas & Cag, 2016; Akbas & Kokumer, 2015).

In this context, the main objective of this study was to evaluate the biofilm formation ability of *S. aureus* strains isolated from ready-to-eat döner kebab, by phenotypic and biomass quantification on polystyrene and stainless steel surfaces using the crystal violet staining technique. This study aimed also to determine the effect of glucose and sodium chloride under different temperatures on the ability and the process of biofilm formation by *S. aureus,* as well as to assess, the effect of citric and acetic acids at different concentrations in reducing S. aureus biofilms formed on stainless steel surfaces.

Materials and methods

Bacterial strains

A total of 11 *S. aureus* strains was selected and used in this study. The strains from our laboratory collection (LAMAABE), were previously identified at species level by molecular methods through detection the thermostable nuclease *(nuc)* gene, described by Brakstad et al. (1992). All strains were isolated from ready-to-eat döner kebab in Tlemcen city, Algeria. Strains were revived from the glycerol stock cultures at –80°C and cultivated in BHI broth (Liofilchem, Italy) for 24 h at 37°C and then on Baird Parker Agar (PBA) for 24 h at 37°C.

Biofilm formation assays

Phenotype analysis of biofilm formation

The strains were characterized phenotypically for biofilm production by culture on Congo Red Agar (CRA) plates, as described by Freeman et al. (1989). In short, agar plates were prepared by adding 0.8 g of Congo red (Fluka, India) and 50 g saccharose to 1 L of BHI agar, followed by incubation at 37°C for 24 and 48 h. The macroscopic characteristics of the *S. aureus* colonies on the CRA were inspected for slime production and consequently biofilm formation. Following Freeman et al. (1989), crusty black colonies, with dry filamentous appearance, were recorded as biofilm producers; smooth pink colonies as non-producers; and intermediate colony morphology (pink with dark centers resembling bull's eyes) as potential biofilm producers.

Quantification of biofilm formation on microtiter plates

Biofilm production on PTS surfaces was investigated in 96-well microtiter plates, following the quantitative method described by Stepanovic et al. (2000). For each strain, three wells of the microtiter plate were inoculated independently with 10 µL of an overnight-grown culture at 37°C in BHI broth supplemented with glucose at final concentration of 0.25% (w/v) for optimal bacterial growth (cell concentration approximately 10^6 CFU/mL). Then, wells were filled with 200 µL of the same medium and the plates incubated aerobically for 24 h at 37°C. Negative control wells were filled with the broth medium only. Next, to remove the planktonic cells, the content of each well was aspirated and washed two times with phosphate-buffer saline (PBS). Microtiter plates were subsequently dried at room temperature for 15 min prior to staining with a 0.1% crystal violet solution (w/v) for 15 min. The excess stain was rinsed off with sterile distilled water and the microtiter plates were air dried. Adherence of cells was measured as the absorbance released at 596 nm (OD_{596}) using an automatic microtiter plate reader (BioTekELx808, USA) after solubilization of the dye bound to the plates with a 33% acetic acid solution. Based upon the absorbance, biofilm formation capability was interpreted as no biofilm producer (NBP) (OD≤ODc), weak (WBP) (ODc<OD≤2XODc), moderate (MBP) (2XODc<OD≤4XODc) or strong (SBP) (OD>4X ODc) biofilm producer (Stepanovic et al., 2000), where ODc was the optical density measured for the negative control. The experiment was performed in triplicate.

Quantification of biofilm formation on stainless steel surfaces

SS coupons (AISI 304, 2.5 × 1 x 0.1 cm) were used as an experimental surface. The coupons were consecutively cleaned and sterilized according to the method described by Rossoni and Gaylarde (2000). For the biofilm formation, each SS coupon was individually introduced into Petri dishes containing 20 mL of BHI broth supplemented with glucose at a final concentration of 0.25% (w/v). Each dish was inoculated with 5 mL of an overnight-grown culture (cell concentration approximately 10^6 CFU/mL of the

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corresponding strain) and then incubated for 24, 48 and 72 h at 37°C. The S.aureus biofilms were fixed for 30 min at 80°C after removing non-adherent cells by rinsing two times the coupons with PBS. After that, adherence of cells to SS coupons was measured after crystal violet staining as previously described (Stepanovic et al., 2000). Three replicates were performed for each strain. As a negative control, an SS coupon without inoculum was included in all assays.The experiment was performed in triplicate.

Effect of chemical compounds and temperature on biofilm formation by *S. aureus*

Effect of glucose and incubation temperature

To study the effect of glucose combined with the incubation temperature effect on *S. aureus* biofilm formation, BHI broth was supplemented with glucose to achieve final concentrations of 0.5, 1, 1.5, 2, 2.5 and 3% (w/v). The biofilm was formed by *S. aureus* strains on 96-well PST microtiter plates by filling each well with 200 µL of BHI broth supplemented with different glucose concentration. Afterwards, the wells were inoculated with 10 µL of overnight-grown culture (containing approximately 10^6 CFU/mL of the corresponding strain). Plates were incubated aerobically for 24 h at 25, 37 and 40°C. To quantify the formed biofilm, we applied the crystal violet assay and absorbance was measured as above indicated. Three replicates were performed for each glucose concentration. As a negative control, wells were inoculated with the medium at its initial glucose concentration (0.25%).

Effect of sodium chloride and incubation temperature

To assess the effect of sodium chloride and incubation temperature on *S. aureus* biofilm formation, different concentrations of NaCl were added to BHI broth to achieve final concentrations of 1, 1.5, 2, 2.5, 3 and 3.5% (w/v). The negative control wells were inoculated with the same broth at its initial NaCl concentration (0.5%). The biofilm was quantified as described in the previous sections. Three replicates were performed for each salt concentration

Effect of citric acid and acetic acid on *S. aureus* biofilm

Experimental system for biofilm formation

The experimental system used for biofilm development was inspired from systems used by Cherif-Antar et al. (2016) and Gram et al. (2007). It consists of two SS circles (AISI, 304), in which 12 stainless steel coupons (AISI 304, 2.5 x 1 x 0.1 cm) were held in vertical and radial position. The whole system was consecutively cleaned according to the method described by Rossoni and Gaylarde (2000).

Conditions for biofilm formation

The *S. aureus* strain used for this part of study was selected for its ability to form biofilm from above techniques. A volume of 200 mL sterilized BHI broth was inoculated with 50 mL of an overnight-grown inoculum at a final concentration of approximately 10^6 CFU/mL. The system was incubated for 7 days at 37°C under 100 rpm agitation.

Effect of citric acid and acetic acid on biofilm

To evaluate the effect of citric acid and acetic acid in reducing the *S. aureus* biofilm formed on SS coupons, 10 acid concentrations were tested, 1, 2, 3, 4, 5% (w/v) for citric acid (SIGMA-ALDRICH, USA) and 1, 1.5, 2, 2.5 and 3% (v/v) for acetic acid (Haneywell, Germany). The method applied consists to transfer each SS coupon into glass tube containing 5 mL of acid, in which it was exposed for 5, 10 and 15 min at room temperature. Then, each coupon was rinsed thrice with PBS and transferred into a glass tube containing 10 mL of the saline solution and placed in a 100 MZ Ultrasonic bath (WiseClean) for 2.5 min to recover cells remaining after treatment. Afterwards, cell viability was enumerated by the standard plate count technique on standard agar (PCA, Liofilchem) with incubation for 24 h at 37°C. Three replicates were performed for each acid concentration.

Data analysis

"Student t-test" was used to compare the differences in efficiency between the protocols applied for effect of glucose, NaCl and temperature of *S. aureus* biofilm formation and also the effect of citric acid and acetic acid on elimination of *S. aureus* biofilm.

Results

Biofilm formation assays

All *S. aureus* strains were assessed for biofilm-forming ability by a phenotypic approach. The results are summarized in Table 1. Three out of 11 strains produced slime on CRA as shown in Fig. 1 (A), while the rest did not produce black colonies on the same medium, which qualified them as non-biofilm producers. We did not get intermediate colony morphology in this study. Afterward, biofilms were quantified regarding biomass accumulation on PST and SS surfaces, using a CV staining assay. Strains were classified in four different categories as described in materials and method section. The OD_{596} results showed that all strains (n=11) were MBP ($0.26 \leq OD_{596} < 0.52$) on PST after 24h at 37°Cas showed in Fig. 2. However, 8 out of 11 strains was SBP ($OD_{596} \geq 0.2$) on SS coupons after 24h at 37°C and 3/11 was MBP ($0.1 \leq OD_{596} < 0.2$), as indicated in Fig. 3. After 48 h of exposing the coupons to the inoculum, 9/11

TABLE 1: *Association between biofilm phenotype on CRA, biofilm formation ability on 96-well PST microtiter plates and on SS coupons.*

Strains	Slime production On CRA (24h)	Polystyrene microtiter plates (24h)	Stainless steel coupons		
			24h	48h	72h
Lamaabe4-SA1	–	MBP	MBP	MBP	MBP
Lamaabe4-SA2	–	MBP	MBP	WBP	WBP
Lamaabe4-SA3	–	MBP	MBP	MBP	MBP
Lamaabe4-SA4	+	MBP	SBP	MBP	MBP
Lamaabe4-SA5	+	MBP	SBP	MBP	MBP
Lamaabe4-SA6	–	MBP	SBP	WBP	WBP
Lamaabe4-SA7	–	MBP	SBP	MBP	MBP
Lamaabe4-SA8	+	MBP	SBP	MBP	MBP
Lamaabe4-SA9	–	MBP	SBP	MBP	MBP
Lamaabe4-SA10	–	MBP	SBP	MBP	WBP
Lamaabe4-SA11	–	MBP	SBP	MBP	MBP

SBP: Strong Biofilm Producer; MBP: Moderate Biofilm Producer; WBP: Weak Biofilm Producer

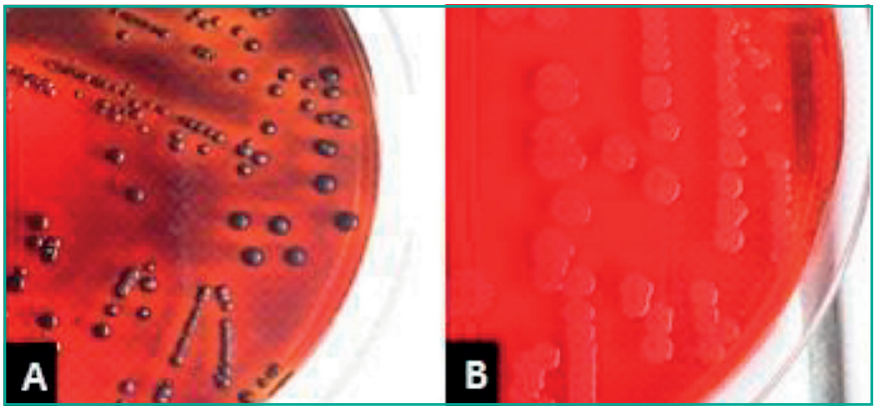

FIGURE 1: *Aspect of S. aureus colonies on Congo Red Agar after 24h of incubation at 37°C; (A): slime producer black colony: biofilm producer (lamaabe4-SA8) and (B): non-slime producer colony: non-biofilm producer (lamaabe4-SA11).*

of strain were MBP ($0.36 \leq OD_{596} < 0.72$), and 2/11 with low biofilm forming capacity ($0.18 \leq OD_{596} < 0.36$). Compared strains capacities after 24 and 48h, 87.5% of SBP after 24h had moderate capacity after 48h and 66.66% of MBP strains after 24h presented the same capacity. When the incubation time was extended to 72h, no SBP neither NBP were detected, instead, 8/11 of strain were MBP ($0.48 \leq OD_{596} < 0.96$) and 3/11 showed low capacity ($0.24 \leq OD_{596} < 0.48$). Compared results obtained after 24h and 72h, 75% of SBP after 24h showed a moderate capacity after 72h and 25% with low capacity, and 66.66% of MBP strains after 24h presented the same capacity.

Effect of glucose, sodium chloride and temperature on *S. aureus* biofilm formation

The bacterial strains were tested for the effect of glucose and sodium chloride on biofilm formation under three different temperatures as described in Materials and methods section and shown in Fig. 4 and 5, respectively. The statistical analysis of the data by fixing significance level $p<0.05$ indicated that glucose concentration enhanced biofilm formation at 30°C ($p<0.05$). All tested concentrations favored *S. aureus* biofilm formation better than the non-supplemented BHI broth. Indeed, we found a significant difference between the biofilms formed at 1% compared with 2.5% and 3%, also at 2% compared with 3% of glucose. The highest value recorded was 0.87 nm at 3% corresponded to lamaabe4-SA9 strain. We observed that increased glucose concentration gave better biofilm growth rate below 37°C with OD_{596}=0.733 nm at 0.5% corresponded to lamaabe4-SA4 strain. The results showed that the glucose concentration had no significant influence on the formation of biofilms at 40°C ($p>0.05$). For some glucose concentrations, the temperature had an impact on biofilm formation. For 0.5% and 1% of glucose concentration, a significant difference was observed by comparing the biofilms growth rate at 37°C and 40°C. For 1.5% the difference was observed at 37°C and 40°C compared to 30°C.

Compared to the medium without added salt, the obtained results indicated that at 30°C, an NaCl concentration greater than 1% did not enhance the biofilm formation. We observed also a significant difference was observed between biofilms formed at 1% and 3% NaCl ($p<0.05$). At 37°C any concentration greater than 1% promoted biofilm formation, and the highest obtained value was 0.84 nm at 1.5% (with lamaabe4-SA4). The increasing concentration of NaCl did not influence biofilm formation. It was found that both NaCl concentrations 2% and 3% presented a difference compared to 1%. At 40°C any concentration greater than 1% promoted biofilm formation. Increasing NaCl concentration did not influence *S. aureus* biofilm biomasses. It was observed that 2% presented a difference compared to 1%. For the effect of incubation temperature on biofilm formation, the difference was observed only in two cases: at 30°C and 37°C for 0.5% of NaCl final medium concentration and at 37°C and 40°C for 1.5%.

Effect of citric acid and acetic acid on *S. aureus* biofilm

According to results, the lamaabe4-SA8 strain was considered maintaining strong and moderate biofilm formation ability on polystyrene and stainless steel surfaces which made it selected for this part. Biofilms formed on SS coupons were exposed to several acetic acid and citric acid to

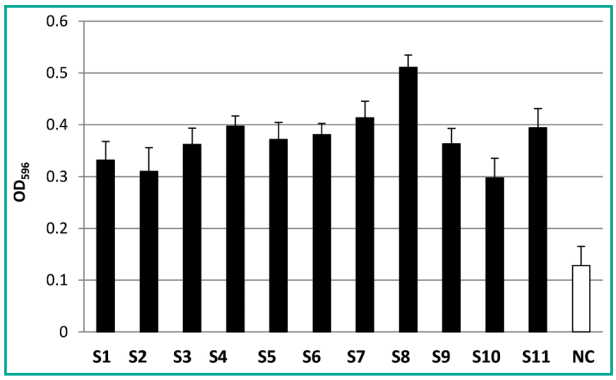

FIGURE 2: *Biofilm formation by S. aureus strains on 96-well PST microtiter plates for 24 h at 37°C with CV assay quantification. S1: lamaabe4-SA1, S2: lamaabe4-SA2, S3: lamaabe4-SA3, S4: lamaabe4-SA4, S5: lamaabe4-SA5, S6: lamaabe4-SA6, S7: lamaabe4-SA7, S8: lamaabe4-SA8, S9: lamaabe4-SA9, S10: lamaabe4-SA10, S11: lamaabe4-SA11, NC: negative control.*

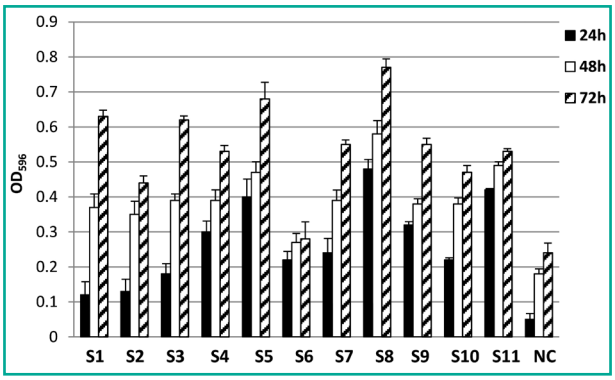

FIGURE 3: *Biofilm formation of S. aureus strains on SS coupons for 24, 48 and 72 h at 37°C with CV assay quantification. S1: lamaabe4-SA1, S2: lamaabe4-SA2, S3: lamaabe4-SA3, S4: lamaabe4-SA4, S5: lamaabe4-SA5, S6: lamaabe4-SA6, S7: lamaabe4-SA7, S8: lamaabe4-SA8, S9: lamaabe4-SA9, S10: lamaabe4-SA10, S11: lamaabe4-SA11, NC: negative control.*

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evaluate their efficiency on biofilm elimination. Treating a 7-days old *S. aureus* biofilm with 1, 1.5, 2, 2.5 and 3% of acetic acid for 5, 10 and 15 min showed that an increasing in acetic acid concentration up to 2.5% had an increase effect on biofilm eradication ($p<0.05$) (Fig. 6). The highest logarithmic reduction was recorded after treatment with 3% acetic acid for 15min and which was 5.80. Moreover, a contact time of *S. aureus* biofilm with acetic acid greater than 5 min did not have a noticeable effect with an exception that we found a difference between 5 and 15 min because after 15 min the value of logarithmic reduction obtained was 5.80 then after 5 min 5.35.

A total of 5 citric acid concentrations were tested on *S. aureus* biofilm 1, 2, 3, 4 and 5% showed in Fig. 7. The obtained results indicated that an increasing in citric acid concentration up to 4% had an increasing effect on biofilm eradication. This means more the concentration increases, the anti-biofilm effect of citric acid is greater. The statistical analysis showed also that a contact time of the biofilm with this acid more than 5 min did not have a significant effect since the highest logarithmic reductions obtained were after exposure biofilm to acid at 4 and 5% for 5 min only (4.53 and 6.14, respectively).

Discussion

The present study evaluated the biofilm-forming ability of 11 *S. aureus* strains isolated from döner kebab by phenotypic revelation on CRA and by measuring biomass formed

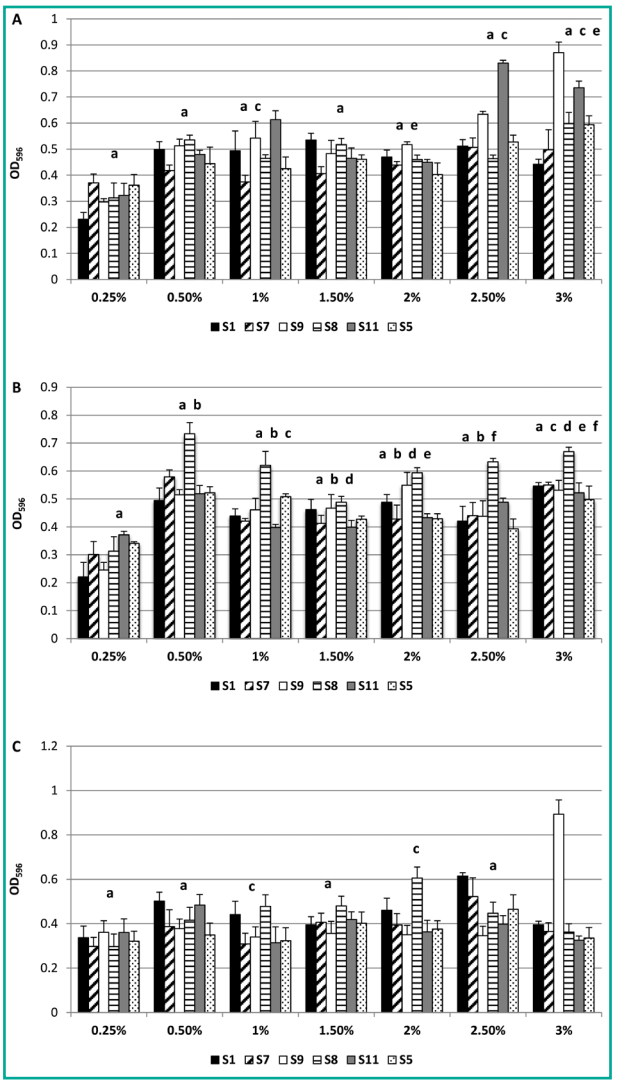

FIGURE 4: *The effect of different glucose concentrations on S.aureus biofilm formation. Biofilm were formed on 96-well PST microtiter plates for 24 h at A): 30°C, B): 37°C, C): 40°C and quantified by CV assay. The data represents the average of experiments performed in triplicate and standard deviations. Letters indicate significant difference (P<0.05): a): compared with 0.25%, b): compared with 0.5%, c): compared with 1%, d): compared with 1.5%, e): compared with 2%, f): comparied with 2.5%. S1: lamaabe4-SA1, S7: lamaabe4-SA7, S9: lamaabe4-SA9, S8: lamaabe4-SA8, S11: lamaabe4-SA11, S5: lamaabe4-SA5.*

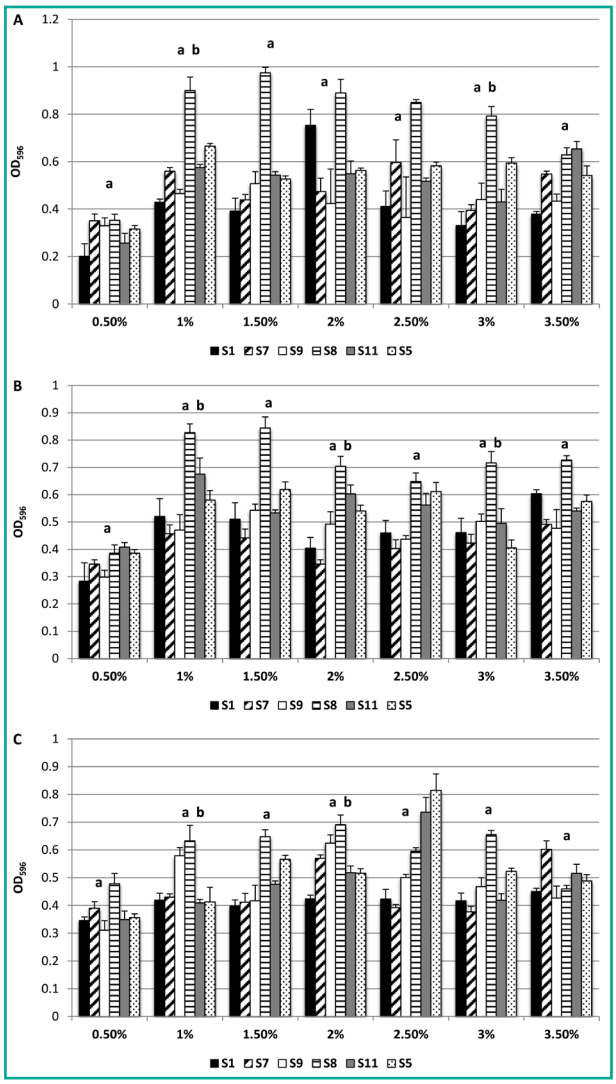

FIGURE 5: *The effect of different chloride sodium concentrations on S.aureus biofilm formation. Biofilm were formed on 96-well PST microtiter plates for 24 h at A): 30°C, B): 37°C, C): 40°C and quantified by CV assay. The data represents the average of experiments performed in triplicate and standard deviations. Letters indicate significant difference (P<0.05): a): compared with 0.5%, b): compared with 1%. S1: lamaabe4-SA1, S7: lamaabe4-SA7, S9: lamaabe4-SA9, S8: lamaabe4-SA8, S11: lamaabe4-SA11 and S5: lamaabe4-SA5.*

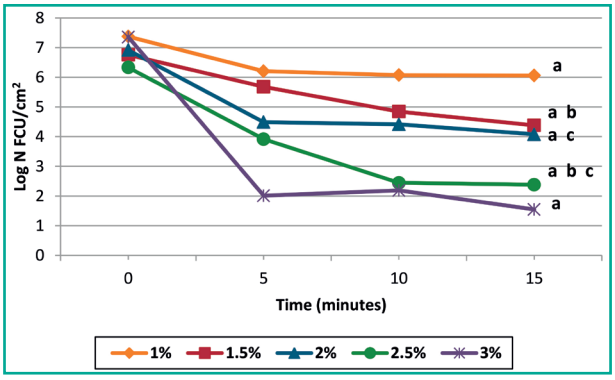

FIGURE 6: *Effect of acetic acid on seven-days S. aureus biofilm formed on SS coupons. The data represents the average of experiments performed in triplicate and standard deviations. Letters indicate significant difference (P<0.05): a): compared with 1%, b): compared with 1.5%, c): compared with 2%.*

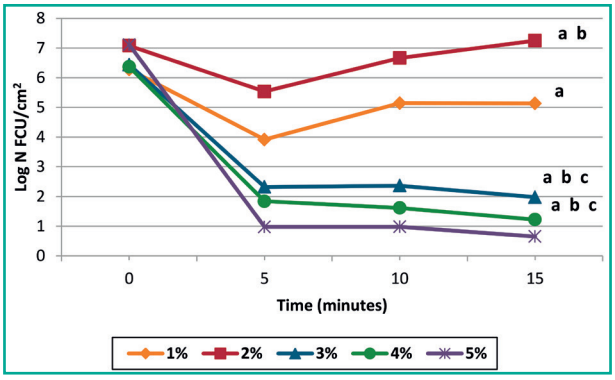

FIGURE 7: *Effect of citric acid on seven-days S. aureus biofilm formed on SS coupons.The data represents the average of experiments performed in triplicate and standard deviations. Letters indicate significant difference (P<0.05): a): compared with 1%, b): compared with 2%, c): compared with 3%.*

on polystyrene microtiter and on stainless steel coupons using CV staining assay. *S. aureus,* usually known for their slime production, did not produce black colonies on CRA except three strains (27.27%). These observations are entirely in disagreement with those reported by previous studies (Melo et al., 2013; Freeman et al., 1989).In addition, this method has been previously compared to the microtitre plate test, which was considered to be the gold standard which is not the case in our study (Jain & Agarwal, 2009; Stepanovic et al., 2000). Our results agree with those obtained by Mathur et al. (2006) who were unable to recommend this method for detection of *S. aureus* biofilm formation. It is well-known that the CRA plate test is not a quantitative assay because it can directly interact with certain polysaccharides, forming colored pigments which remain unchanged in color, so it is based on a subjective chromatic evaluation and some metabolic reactions that form secondary products with the dye can influence the formation of dark colonies (Jain & Agarwal 2009; Arciola et al., 2001; Freeman et al., 1989).

Therefore, a quantitative assay was conducted on 96-well PST microtiter and on SS coupons. The microtitre plate test is a convenient and economical quantitative technique for the identification of critical factors and optimal culture conditions for biofilm formation. This technique is used for direct detection of polysaccharide production because spectrophotometric measurements provide quantitative information on the ability of bacterial strains to rapidly grow while adhering to the substratum (Stepanovic et al., 2000). Biofilm formation was also investigated on stainless steel coupons, which is the most commonly used contact material in food processing environment (Olszewska, 2013). The present study showed that *S. aureus* strains tested were capable to form biofilm with a capacity varying between strong, moderate and weak showing that the incubation time influenced the biofilm formation because the results obtained indicated that after increasing incubation time, biofilm formation decreased and bacteria took their maximum rate of biofilm production just after 24 hours of incubation. In addition, several studies reported that incubation conditions including the composition and the pH of the growth medium, the incubation temperature and time can influenced *S. aureus* biofilm formation (Kowalska et al., 2020; Miao et al., 2019; Avila-Novoa et al., 2018; Fernandes et al., 2018; Kim et al., 2016).

In this study, *S. aureus* strains isolated from döner Kebab produced biofilm in BHI broth supplemented with different concentrations of glucose and NaCl at 30 and 37°C but there was no significant effect at 40°C. This means that *S. aureus* biofilm temperature growth optimum ranged from 30 to 37°C but was sensitive to 40°C. The obtained results were consistent with several previous studies showing that the enriched growth medium and the optimum growth temperature promote the growth of bacterial biofilms (Roy et al., 2021; Miao et al., 2019; Sar & Akbas, 2019; Manandhar et al., 2018; Rode et al., 2007; Stepanovic et al., 2000). Generally, the environmental factors including the presence of glucose, salt, temperature and pH could be effective facilitators of biofilm formations. The above conditions lead to changes in the composition of bacterial cell wall and physicochemical properties of surface such as hydrophobicity and electron donor/acceptor properties (Zou &Liu, 2020; Miao et al. 2019; Khangholi & Jamalli, 2016; Kyoui et al., 2016; Michu et al., 2011; Giovannacci et al., 2000). Glucose serves as an important carbohydrate for the growth of *S. aureus.* This bacterium grown in medium usually forms weak biofilm and adding the glucose to growth medium is a common practice to simulate biofilm formation *in vitro* (Sar & Akbas, 2019; You et al., 2014). Moreover, Changes in the nature of bacterial surrounding environments cause changes in bacterial cell surfaces and consequently biofilm formation (Khangholi & Jamalli, 2016; Jana et al., 2000; Costerton et al., 1995).

In this research, the antibiofilm activity of citric acid and acetic acid treatment on *S. aureus* strain isolated from döner kebab was performed against 7-days age biofilms formed on SS coupons. The obtained results illustrated that both tested organic acids were effective to achieve maximum removal *S. aureus* biofilm at low concentrations and in a shorter contact time with varied effect from acid to acid (Akbas & Cag, 2016; Akbas & Kokumer, 2015). Our findings getting closer to those elaborated by Akbas & Kokumar (2015) who demonstrated that the prevention and removal of *S. aureus* biofilm formation and the number of strains prevented or removed were increased by increasing the concentration of citric acid treatment from 2% to 10% (w/v) for 20 min. Even though the assay results indicated that 10% (w/v) citric acid treatment was generally more effective than 2% (w/v) and 2% (w/v) citric acid treatment can be effectively used in practice to control biofilm for-

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mation. Other results were in total agreement with ours, based on treatment of *S. aureus* biofilm with 0.5% acetic acid reduced the number of viable cells, whereas complete eradication was obtained using 1.0% acetic acid (Jarnsholt et al., 2015). Similar results have also been obtained by a recent study conducted by Kundukad et al. (2020) demonstared that Weak acids such as acetic acid and citric acid can effectively eradicate *S. aureus, Klebsiella pneumoniae* and *Pseudomonas putida* biofilms on hard surfaces due to their ability to penetrate the biofilm matrix and the cell membrane without being toxic to human cells. The use of natural antimicrobial agents is an effective alternative for controlling microorganisms. Among them, the organic acids naturally present in various fruits and vegetables are considered safe for human and animal health without toxic residues. Theses organic acids are commonly used in food preparations that are generally recognized as safe and approved for use in the manufacture of fresh and processed meats and poultry at specific concentrations (USDA-FSIS, 2010). It was also shown that 3% (w/v) citric acid did not change organoleptic properties of poultry skin for longer periods of time (González-Fandos et al., 2009).

Conclusion

Under the conditions tested in our research, it can be concluded that *S. aureus* strains isolated from döner kebab can form biofilms on SS and PST surfaces. The use of growth medium supplemented with glucose or NaCl and optimal growth temperature are important factors promoting biofilm formation. Understanding the effect of environmental conditions on biofilm formation is essential to develop effective control strategies. The findings of this study highlight also the importance of using natural antimicrobial agents including organic acids like citric and acetic acids as an effective alternative for food biofilms control. Further studies are needed to determine the effects of other organic acids on mixed bacterial biofilms. Future research should be extended to control biofilm formation on other surfaces commonly used in the food environment.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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