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SUSCEPTIBILITY OF MONOSPECIES AND DUAL-SPECIES BIOFILMS OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* TO ESSENTIAL OILS

F.M. MILLEZI[1,4,5], M.O. PEREIRA[4], N.N. BATISTA[2], N. CAMARGOS[2], I. AUAD[2], M.D.G. CARDOSO[3] and R.H. PICCOLI[2]

[1]Departamentos de Ciências Biológicas – Setor de Microbiologia Agrícola, [2]Ciência dos Alimentos and [3]Química, Universidade Federal de Lavras, Lavras, Brazil
[4]Departamento de Engenharia Biológica, Universidade do Minho, Braga, Portugal

[5]Corresponding author. F.M. Millezi, Avenue Silvio Minecucci, 1695/304, Lavras 37200-000, Minas Gerais, Brazil. TEL: 55-35-91027008; FAX: 55-35-38291401; EMAIL: amillezi@yahoo.com.br

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ABSTRACT

The aim of this study was to investigate the susceptibility of *Staphylococcus aureus* and *Escherichia coli* biofilms formed on the polypropylene surface. The cultures were developed for 240 h, planktonic growth was characterized by colony-forming unit (cfu)/mL and biofilms was characterized by quantifying biomass and cfu/cm^2. Essential oils (EOs) of citronella and lemon were extracted by hydrodistillation and characterized by gas chromatography. Biofilm formation occured after 3 h of contact. In dual-species biofilms, there was competition; *S. aureus* was the number of viable cells damaged by *E. coli* ($P < 0.05$). The EOs disinfectant action was similar in biofilms monospecies, viable cells and biomass decreased significantly. Dual-species biofilms were more resistant to EOs. The action EOs on biofilm suggest promising alternatives to sanitize industrial polypropylene surfaces.

PRACTICAL APPLICATIONS

Historically, the majority of new drugs has been generated from natural products (secondary metabolites) and from compounds derived from natural products. The extracts of higher plants have been and still are widely used to obtain substances with antimicrobial action. However, their low concentration in the extract often makes the purification processes or the synergistic action of the different compounds unfeasible, causing major problems for industries. Seeking to reduce the parameters involved in the isolation and purification of compounds, these essential oils (EOs) have been studied. They present high antimicrobial efficiency, and in appropriate concentrations they are considered safe. The antimicrobial activity of EOs was showed that such substances can be used with sanitizing agents in industrial surfaces against bacterial biofilms. However, this line of research is still very new, so it is important to continue research for the development of industrial sanitizing with EOs.

INTRODUCTION

The term biofilm was created to describe the sessile form of microbial life characterized by adhesion of microorganisms to biotic or abiotic surfaces, with consequent production of extracellular polymeric substances (Nikolaev and Plakunov 2007). In just two decades, we have learned that biofilms comprise highly structured matrix-enclosed communities (Costerton and Stewart 2001) whose cells express genes in a pattern that differs profoundly from that of their planktonic counterparts. Because direct observations show that biofilms constitute the majority of bacteria in most natural (Costerton *et al.* 1978) and pathogenic ecosystems (Costerton *et al.* 1999), it seems unwise to continue to extrapolate from planktonic cultures in studies of these systems.

In the food industry, biofilms cause serious engineering problems such as impeding the flow of heat across a surface, increases in fluid frictional resistance of surfaces, and increases in the corrosion rate of surfaces leading to energy and production losses (Verran and Jones 2000). Pathogenic microorganisms grown on food surfaces and in processing environments can cross-contaminate and cause post-processing contamination (Ganesh and Anand 1998). Several microorganisms are capable of participating in the adhesion processes and biofilm formation. In the food industry, these microorganisms can be classified as spoilage and pathogenic. Among the pathogenic microorganisms, *Staphylococcus aureus* and *Escherichia coli* are able to form biofilms, which are complex structures consisting of surface-attached bacteria surrounded by a self-produced extracellular polymer matrix (Kania *et al*. 2008; Naves *et al*. 2008).

Natural drugs could represent an interesting approach to limit the emergence and the spread of these organisms, which currently are potential sources of contamination that can lead to food deterioration or transmission of foodborne diseases. Recently, there has been considerable interest in the study of plant materials as sources of new compounds for processing into sanitizer agents. One approach may be the use of essential oils (EOs) that have been shown to be potential antibacterial agent (Nostro *et al*. 2007; Oliveira *et al*. 2010b; Millezi *et al*. 2012a,b). The results obtained are promising yet divergent. According to Chorianopoulos *et al*. (2008), the information available on the use of EOs as disinfectants is still limited, pointing to the need of further studies.

This work aimed at testing the susceptibility of biofilms formed by monospecies and dual-species of *S. aureus* and *E. coli* to EOs of lemon and citronella.

MATERIALS AND METHODS

Experiment Execution Sites

The experiment was carried out at the Federal University of Lavras (Lavras – MG, Brazil), in the Food Microbiology, Chromatography and Organic Chemistry, and in the University of Minho (Braga, Portugal), in Applied Microbiology Laboratory.

Extraction, Identification and Quantification of the Compounds of EOs

Fresh leaves of citronella and fresh peels of lemon were collected from Medicinal Plant Nursery of the Federal University of Lavras in Minas Gerais, Brazil. The EOs were extracted by hydrodistillation using a modified Clevenger apparatus (Sovereing, São Paulo, Brazil). Plant materials were chopped and placed with water in a 4-L volumetric flask. The flask was coupled to the modified Clevenger apparatus, and the extraction was performed for 2.5 h with the temperature maintained at approximately 100C. The hydrolate obtained was centrifuged at 321.8 × *g* for 5 min, with the EO being removed with a Pasteur pipette (Labor Import, Osasco, Brazil) and stored at refrigeration temperature in glass flasks wrapped in aluminum foil (Guimarães *et al*. 2008). Qualitative evaluation of EO was performed to gas chromatography coupled with mass spectrometry (GCMS) using the Shimadzu model GCMS – QP2010 Plus apparatus (São Paulo, Brazil). The operational conditions were: fused silica capillary column (30 m × 0.25 mm) with DB5 bonded phase, helium carrier gas, flow rate 1 mL/min, injector temperature 220C, detector temperature 240C, and oven temperature program 40C, increasing 3C/min. The compounds were identified by comparisons with spectra existing in the library Wiley 8 and Flavors and Fragrances of Natural and Synthetic Compounds 1.2, and by the Kovat's index (Adams 2007).

Microorganism Standardization

The microorganism used were *E. coli* American Type Culture Collection (ATCC) 25922 and *S. aureus* ATCC 24922. The standardization of the number of cells was determined by growth curve. Throughout the experiment, the strain was stored under refrigeration in freezing culture medium (15 mL glycerol, 0.5 g bacteriological peptone, 0.3 of yeast extract and 0.5 g NaCl, per 100 mL of distilled water, with the final pH 7.4) and stored at –80C. For strain reactivation and use, an aliquot of the freezing culture medium was transferred to test tubes containing trypticase soy broth (TSB, Merck, Lisbon, Portugal), with two subcultures at 37C for 24 h. The culture was striated in trypticase soy agar (TSA, Merck) added to Petri dishes and incubated at 37C for 24 h. Of the colonies formed on the TSA surface, some were removed and transferred into an Erlenmeyer flask containing 150 mL of TSB, which was incubated at 37C until approximately 10^5 UFC/mL.

Preparation of the Coupons

In order to initiate the bacterial adhesion stage, the polypropylene coupons were previously hygienized and sterilized in autoclave. For cleaning, the coupons were immersed in 0.3% peracetic acid at 50C for 30 min under 50 rpm agitation. After, they were immersed in sterile distilled water at 80C for 15 min. The coupons were autoclaved at 120C for 20 min.

Biofilm Formation on Polypropylene

For biofilm formation, a Petri dish (140 × 20 mm) containing 80 mL of TSB and polypropylene coupons (10 × 20 mm) was used. After the addition of bacterial cultures (concentration 10^5 colony-forming unit [cfu]/mL), the system was incubated at 37C under orbital agitation (50 rpm). Every 48 h, the TSB used as substrate was replaced in the same amount of sterile TSB. At each medium change, the coupons were immersed in

sterile water; nonadherent cells were removed, and the Petri dishes were replaced by sterile dishes. This procedure was carried out to complete 240 h of cultivation. To differentiate the process of adhesion and biofilm, the value of 10^5 cfu/cm^2 as biofilm, which is intermediate to that proposed by Andrade and Macêdo (1998), who set the value of 10^7 cfu/cm^2, and presented by Wirtanen *et al.* (1996) and Ronner and Wong (1993), which they regard as a biofilm adherent cell number of 10^3 and 10^5 cfu/cm^2, was considered.

In monospecies *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were cultured in the previous system, individually, in the approximate amount of 10^5 cfu/mL. In dual-species, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were inoculated together in the approximate concentration of 10^5 cfu/mL of each microorganism.

Quantification of Cultivable Planktonic Cells

The planktonic cells number was determined, and aliquots of 1 mL of TSB were removed from the dish after 3, 48, 96, 144, 192 and 240 h. Every 48 h, the plates were replaced by other sterile, and TSB used as the substrate was replaced in the same amount of sterile medium, following the same procedure until the 10th day incubation. Serial dilutions up to 10^{-10} were carried out in test tubes containing 900 µL of peptone solution. Aliquots of 100 µL of each dilution were inoculated in Petri dishes containing TSA using the spread plate technique. The Petri dishes were incubated at 37C for 24 h. The ability to detach and contaminate the sterile substrate was named as biotransfer potential (Oliveira *et al.* 2010a). The values were expressed total number of cfu/mL.

Quantification of Cultivable Cells in Biofilm

Cells adhered on polypropylene coupons were removed using sterile swabs performing standardized smear (100 times) on the coupon on both sides after 240 h of cultivation. The swabs were transferred to tubes containing 0.1% peptone water (v/v) and agitated in a vortex for 2 min. After this procedure was performed, a serial dilution and aliquots of 0.1 mL were removed. The number of viable cells was determined by eosin methylene blue agar to count *E. coli* and Baird Parker agar for *S. aureus* using the technique of surface scattering. The dishes were incubated at 37C/24 h. After, this period took place on plate count, and the values were expressed in total number of cfus per unit area (log cfu/cm^2) (Silva *et al.* 2010). All assays were performed in three separate occasions.

Biomass Quantification by Crystal Violet Staining

Biomass of single and cocultive biofilms were quantified by crystal violet (CV) staining method adapted from Stepanović *et al.* (2000). For fixation of the adhered cells and biofilms, the coupons were added in 12-well microtiter plates (Orange Scientific, Braine-l'Alleud, Belgium), and 2 mL of 99% methanol (Vaz Pereira, Lisbon, Portugal) was added to each well; after 15 min, the methanol was removed, and the coupons were allowed to dry about 25C. Then, 2 mL of CV stain (1% v/v) (Merck) were added to all wells. After 5 min, the excess of CV was removed, and the coupons were gently washed in water. Finally, 1 mL of acetic acid (33% v/v) (Pronalab, Lisbon, Portugal) were added to all wells to dissolve the CV stain, and the absorbance was measured at 570 nm. All assays were performed in triplicate and on three separate occasions.

Polypropylene Coupon Treatment Using Disinfectant Solutions

For the elaboration of the disinfectant solutions based on EOs and control solution (without the EOs), the following proportions and dilutions suggested by Oliveira *et al.* (2010b) were used; with modification, the ethanol was substituted by dimethylsulfoxide (DMSO) 2%. The saline solution was used to provide osmotic concentration adequate to the bacterial cell so that the bactericide effect would be attributed only to the EOs (Oliveira *et al.* 2010b). Tween 80 was used, as well as DMSO, to dilute the EOs. The EOs were initially diluted with DMSO, followed by the addition of the saline solution with 0.5% (v/v) of Tween 80. The amount of EO used in each disinfectant solution was based on previous studies about the bacteriostatic effect (Millezi *et al.* 2012a) on planktonic cell (data not shown) in concentration of 1.0%.

After 240 h, the coupons with biofilms were removed from Petri dishes and immersed in 0.1% peptone water for two consecutive times for the removal of planktonic cells. After, the coupons were dipped in sanitizing solutions for 15 min at 25C. After the treatment, the coupons were removed from solutions and subjected to smear performed with sterile *swabs*. The adhered cells were quantified and expressed in cfu/cm^2.

Statistical Analysis

The data were analyzed using the Prism software package (GraphPad Software, San Diego, CA). *T*-test and one-way analysis of variance test were performed, and $P < 0.05$ was considered significant.

RESULTS

Characterization of EOs and Disinfectant Action on Biofilms Single and Cocultive

The chemical analyses showed that the monoterpenes were major chemical constituents. For EO citronella, the major

TABLE 1. CHEMICAL COMPOSITION OF THE *CYMBOPOGON NARDUS* ESSENTIAL OIL

Constituents	Tr*	%†
Mircene	12.468	0.23
Limonene	13.856	4.19
Eucaliptol	13.962	0.17
Linalool	16.600	0.86
Isopulegol	18.307	2.72
Citronellal	18.671	30.48
Citronellol	21.354	14.32
Neral	21.772	0.67
Geraniol	22.306	17.12
Geranial	22.807	0.87
Citronelil acetate	25.580	2.55
Eugenol	25.756	1.28
Acetato de geranil	26.582	1.91
Elemeno	26.935	1.22
germacrene	29.798	2.08
murolene	30.366	0.46
α – cardinene	30798	0.65
β – cardinene	31.066	2.10
Elemol	31,852	6.11
Naftalemol	32,657	1.64
Others		8.37
Total	–	100.00

* Retention time.

† Percentage of the relationship between area and peak.

constituents found were citronellal (30.48%), geraniol (17.12%), citronellol (14.32%) and elemol (6.11%) (Table 1). Limonene (33.67%), ρ-cimene (14.16%), carvone (9.50%) and ciclohexanodiol (7.67%) were the main components for lemon (Table 2).

TABLE 2. CHEMICAL COMPOSITION OF THE *CITRUS LIMONIA* OSBECK ESSENTIAL OIL

Constituints	Tr*	%†
α-pinene	10.257	1.03
β-pinene	11.851	4.30
ρ-cimene	13.702	14.16
limonene	13.866	33.67
menthol	17.365	1.26
pinocarveol	18.035	4.45
pinocarvone	18.926	1.39
mirtenol	20.172	4.20
t – carvoel	20.986	5.65
c-carveol	21.405	2.21
carvone	21.868	9.50
ciclohexanodiol	25.155	7.67
Others		10.51
Total	–	100.00

* Retention time.

† Percentage of the relationship between area and peak.

Growth of Planktonic Cells Associated with the Capacity of Biofilm Formation

The *E. coli* and *S. aureus* planktonic monospecies growth was similar, with no significant difference ($P > 0.05$) and observed similar growth of both bacteria both in co-culture than in monospecies (Fig. 1a). The growth of *E. coli* along the 240 h provided monospecies no significant differences ($P > 0.05$). *S. aureus* after 3 h differed from 48, 96, 144, 192 and 240 h ($P < 0.05$). However, the results shown in Fig. 1b suggest that there was a relationship of competition in which *E. coli* predominated significantly in all times over *S. aureus* ($P < 0.05$). In periods 3, 48 and 96 h, there was a slight increase in the number of planktonic bacteria of *E. coli*, and in 144 and 192 h, there was a decrease in the number of viable cells of *S. aureus* probably prejudiced by *E. coli* (Fig. 1b).

After 48 h of culture was observed the ability of microorganisms adhered in the coupons detached and contaminates the sterile medium, thus leading to potential biotransfer.

The *E. coli* monospecies biofilm at 3 and 48 h was statistically different from 192 and 240 h ($P < 0.05$), and 96, 144 and 192 h were different from 240 h ($P < 0.05$). A monospecies biofilm *S. aureus* formation in 3 h was significantly different from all other periods. For the biomass-accumulated simple biofilm of both bacteria, 3 h was statistically different only at 192 and 240 h, and the times 48, 96, 144 and 192 h were different from 240 h (Fig. 2).

After 3 h of cultivation, both *E. coli* and *S. aureus* biofilm formed on the surface of polypropylene, both in monospecies and in dual-species (Fig. 2). After 240 h of *E. coli* simple biofilm formation, we observed a significant increase only between 3 and 144 h, 3 and 192 h, and 3 and 240 h ($P < 0.05$). In *S. aureus*, simple biofilm after 3 h differed, which was obtained after 48, 96, 144, 192 and 240 h ($P < 0.05$). In the dual-species the cfu/cm^2 number obtained after was different from just 3 and 144 h ($P < 0.05$). Unlike biofilms constituted by bacteria in dual-species biofilm, both *E. coli* and *S. aureus* showed no significant differences in growth along the 240 h ($P > 0.05$).

In monospecies biofilms, the number of *S. aureus* viable cells was higher than *E. coli*; however, the difference was significant only in time 48 h, as well as dual-species culture was also higher than *E. coli* in 48 h ($P < 0.05$) (Fig. 3a). Fig. 3b shows that *E. coli* had a greater number of viable cells, and this difference was significant at 48, 96, 144 and 192 h ($P < 0.05$).

Sanitizing Action of EOs on the Biofilm

The EOs of citronella and lemon had similar disinfectant action on monospecies biofilm *E. coli* and *S. aureus*, and viable cells decreased significantly (Fig. 4a) after treatment. Lemon EO decreased, respectively, 2.99 and 2.49 log cfu of *E. coli* and *S. aureus*, citronella 3.64 and 2.51 log cfu (Fig. 4a).

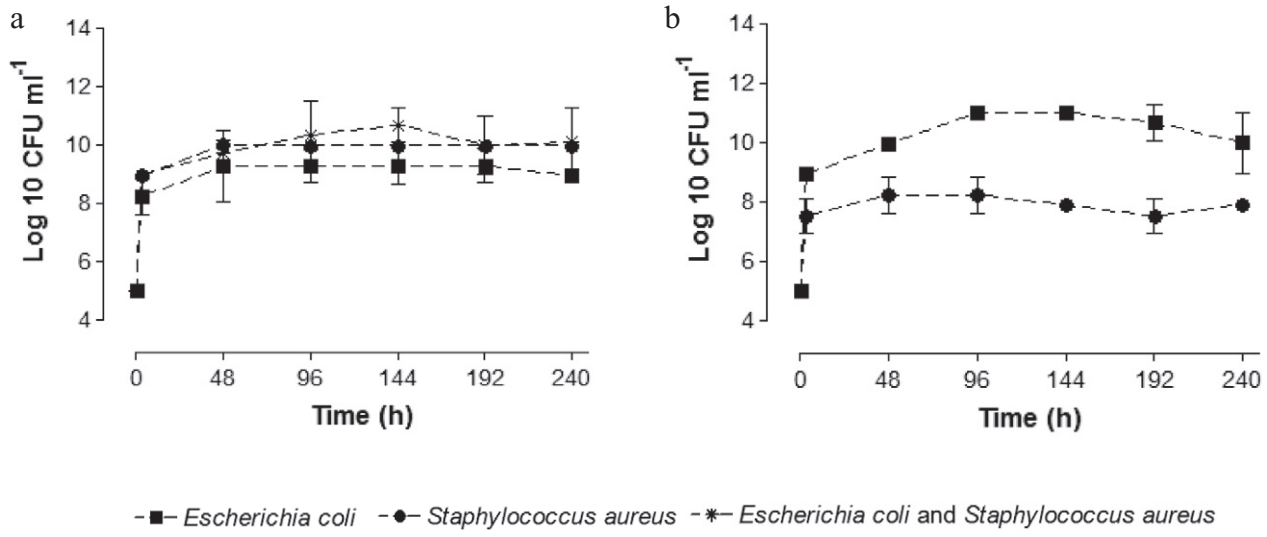

FIG. 1. CULTIVABLE PLANKTONIC CELLS OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* IN TRYPTICASE SOY BROTH UNDER CULTIVATION SIMPLE AND MIXED (A) AND ONLY MIXED (B) AT 37C OVER 240 H

The accumulated biomass was also reduced significantly by comparing the results of the control (Fig. 4b).

Despite the interesting results, dual-species biofilm showed that there was greater resistance to EOs (Fig. 5). The lemon EO was more effective in reducing cultivable cells ($P < 0.05$) with a reduction of 4.63 log cfu, and the treatment with citronella did not differ from control ($P > 0.05$), which reduced 2.72 log cfu (Fig. 5a). Biomass again, lemon oil was more efficient; however, citronella biomass also decreased significantly ($P < 0.05$) (Fig. 5b).

In dual-species biofilm, *E. coli* and *S. aureus* were sensitive to EOs, but the action was better for *E. coli*, reduced lemon 4.65 log cfu and citronella 4.86 log cfu/cm^2. For *S. aureus*, there was reduction of only 1.52 and 1.75 log cfu/cm^2 through the action of EOs lemon and citronella, respectively (Fig. 6).

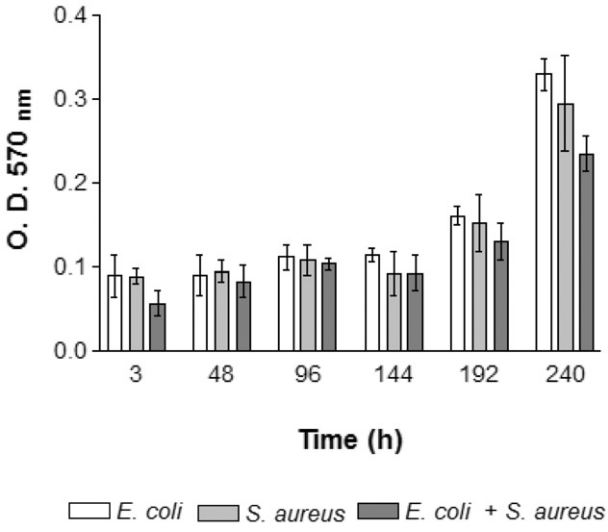

FIG. 2. OD_{570} VALUES AS A MEASURE OF SIMPLE AND DUALSPECIES BIOFILM MASS OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* The means and standard deviations for at least three replicates are illustrated.

DISCUSSION

The biofilm formation is serious risk to the food industry because the removal of irreversibly adhered cells is difficult and requires the application of strong mechanical force or chemical interruption of the microbial adhesion using surfactants, sanitizers or heat. Thus, there is a high probability that the irreversibly adhered cells will remain even in the surfaces after sanitation. This is one of the main reasons for biofilm formation on surfaces in contact with food. This risk is aggravated by *E. coli* and *S. aureus* because this study observed that these bacteria have the capacity of rapidly adhering to polypropylene, being able to reach an irreversible stage in a few hours.

One of the great biofilm formation issues in the food industry or other areas is cell detachment, which makes it a constant source of microorganism contamination in food, water or new infection processes. Thus, the evaluation of the biotransfer potential of microorganisms is interesting. In present study, this can be observed from the values found after 48 h of biofilm formation.

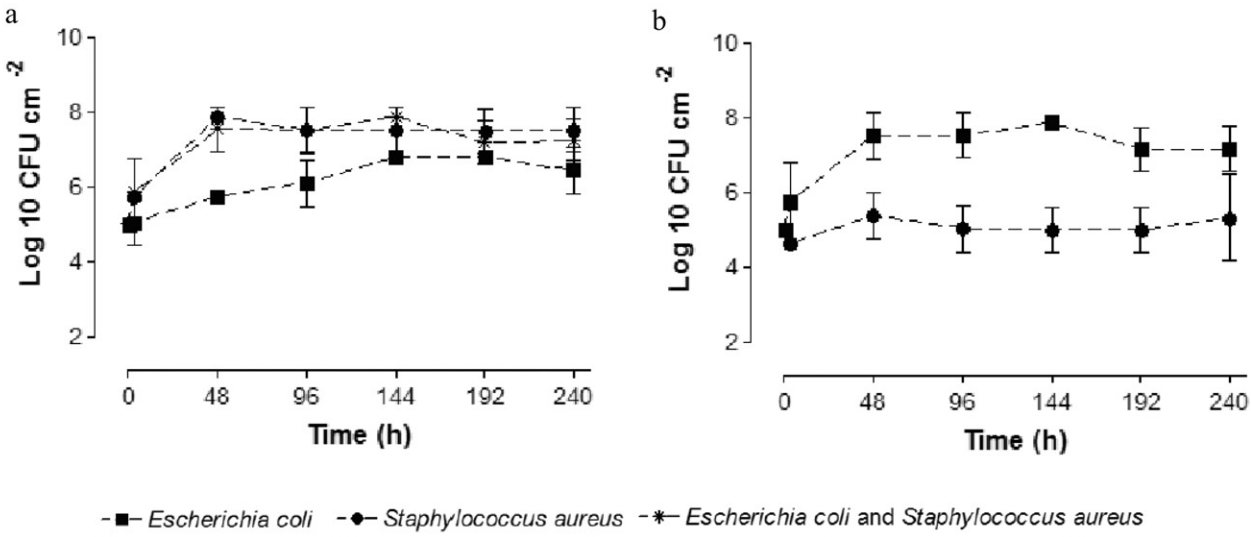

FIG. 3. BIOFILM CULTIVABLE CELLS OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* IN TRYPTICASE SOY BROTH UNDER MONOSPECIES AND DUAL-SPECIES CULTIVATION (A) AND ONLY DUAL-SPECIES (B) AT 37C OVER 240 H

Significant differences are reported between different characteristics between planktonic bacteria and sessile. Ronner and Wong (1993) report up to 5 log cfu/cm^2 occurring genotypic and phenotypic processes that differentiate sessile from planktonic cells, indicating the formation of biofilms. According to Shank and Kolter (2009), many microorganisms can grow better in combination with other microorganisms. The present results show that the dual-species association was a different situation compared with simple-species. In the present study, there was a competitive relationship in which *S. aureus* had the number of viable cells in biofilm hampered by the presence of *E. coli* ($P < 0.05$).

Results similar to those found in this study were reported by Pompermayer and Gaylarde (2000) who investigated the adherence of *S. aureus* and *E. coli*, a condition that simple-species and dual-species; they concluded that there is competition between bacteria, and the growth of *E. coli* is favored in dual species cultures.

Most research into interspecies interactions within biofilms have focused on the beneficial aspects of these relationships. However, not all interactions will be advantageous for the several interacting microorganisms. Antagonistic interactions may play an important role in the development and structure of microbial communities. Competition for substrates is considered to be one of the major evolutionary

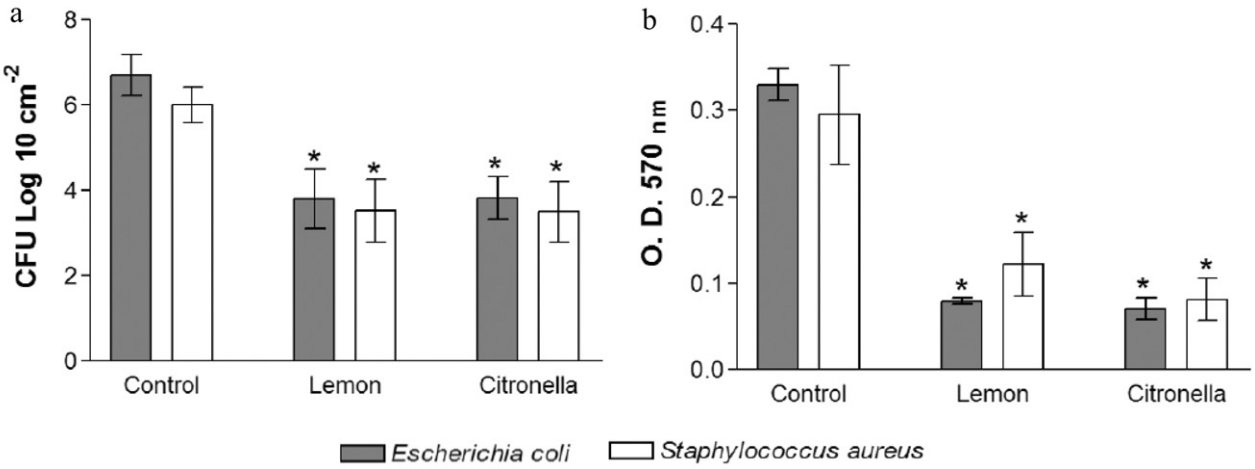

FIG. 4. EFFECT OF ESSENTIAL OILS ON CULTIVABLE CELLS (A) AND BIOMASS (B) OF SIMPLE-SPECIES BIOFILMS
The values are means of three separate assays, and the bars indicate standard deviation. *$P < 0.05$ in one-way analysis of variance test.

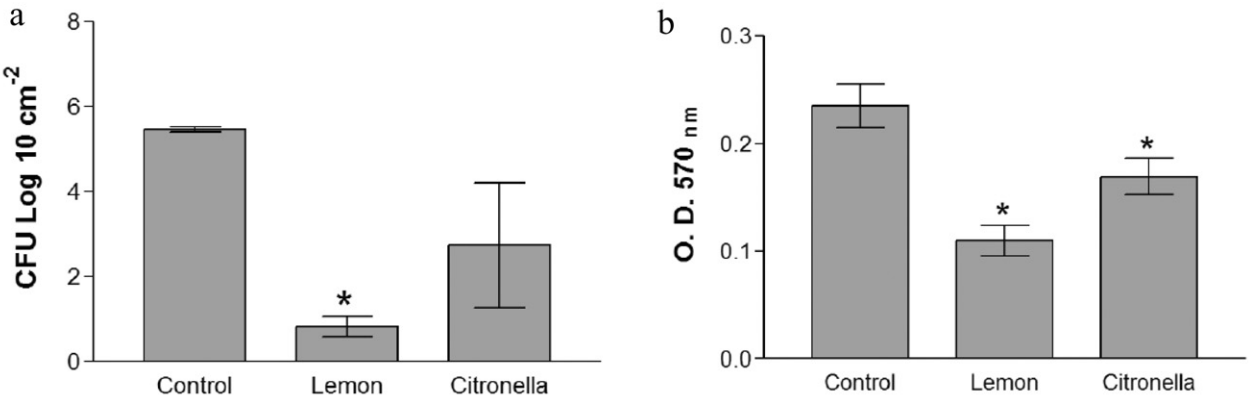

FIG. 5. EFFECT OF ESSENTIAL OILS ON CULTIVABLE CELLS (A) AND BIOMASS (B) OF DUAL-SPECIES BIOFILM
The values are means of three separate assays, and the bars indicate standard deviation. **P* < 0.05 in one-way analysis of variance test.

driving forces in the bacterial world, and numerous experimental data obtained in the laboratory under well-controlled conditions show how different microorganisms may effectively outcompete others as a result of a better utilization of a given energy source (Christensen *et al.* 2002; Komlos *et al.* 2005; Rao *et al.* 2005). The production of antagonistic compounds also seems to be a common phenomenon for some bacteria (Tait and Sutherland 2002; Rao *et al.* 2005; Bhattarai *et al.* 2006). Boari *et al.* (2009) investigated dual-species biofilms of *S. aureus* and *Aeromonas hydrophila*, and similar behavior occurred in the present research; *S. aureus* was approximately two log cycles lower than the simple biofilm.

In the food industry, a considerable number of surfaces such as stainless steel, glass, low density polyethylene, cast iron, rubber, polycarbonate and polypropylene are susceptible to microbial adhesion. However, the surface characteristics such as electric charge, water retention capacity, free energy and topography have an important role in the accession process (Ploux *et al.* 2007). Shi and Zhu (2009) mention that the cells adhere better on hydrophilic surfaces (stainless steel, glass) than on hydrophobic surfaces (rubber and plastics). Currently, the use of polypropylene in the industry to build tanks, fittings, pipes and surfaces of food processing has grown rapidly (Lugão *et al.* 2007). According to Pompermayer and Gaylarde (2000), *E. coli* and *S. aureus* adhere to the polypropylene surface in 8 h at 12C and 30C, but the adherence of *E. coli* was greater than *S. aureus* at both temperatures; in the present study, in 3 h, there have been similar adherence in both microorganisms, demonstrating that adherence can be very fast on inadequately sanitized surfaces of polypropylene, with conditions favorable for the formation of biofilms.

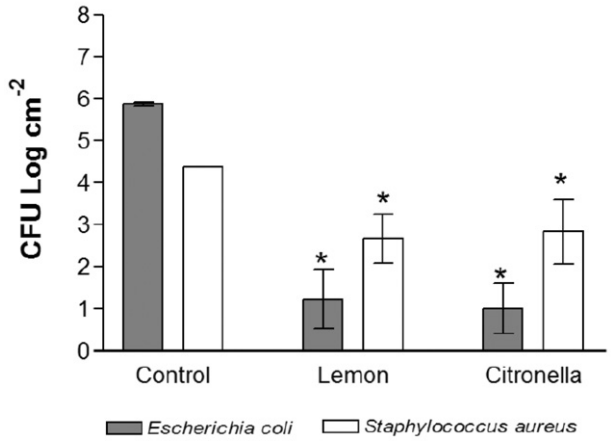

FIG. 6. EFFECT OF ESSENTIAL OILS ON CULTIVABLE CELLS OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN DUAL-SPECIES BIOFILM
**P* < 0.05 in one-way analysis of variance test 240 h.

Given the rapid acceptance and training biofilms *E. coli* and *S. aureus* are necessary new strategies in sanitizing surfaces used in the food industries; in this view are the natural antimicrobial agents derived from plant secondary metabolism: EOs.

The effectiveness of disinfectants is frequently determined by the number of surface-adhered cells they are capable to reduce, obtained by standard plate count. This work showed good results using the EOs of citronella leaves and lemon peels; we demonstrated that the EOs of lemon and citronella reduce *E. coli* 4 log cfu and *S. aureus* 2.5 log cfu. The effectiveness of EOs was similar to reduction in cfu of biofilms simple-species, although dual-species biofilms were more resistant to citronella oil. In dual-species, *E. coli* was more sensitive to the action of the oils, and there was less cfu reduction of *S. aureus*, a little more than 1 log cfu. Wide-spectrum antibacterial activities of EOs against gram-positive and gram-negative bacteria are well documented (Chorianopoulos *et al.* 2008; Sandasi *et al.* 2008; Oliveira *et al.* 2010b; Millezi *et al.* 2012a).

Research on biofilm formed by other bacteria have also shown effective results; Oliveira *et al*. (2010b) achieved a reduction of 3.28 log cfu of *Listeria monocytogenes* biofilm in the stainless steel surface disinfecting action of the EO of citronella.

The biologic activity of EOs on biofilms can be attributed to the compounds, majority of EOs. In this research, the chemical characterization of EOs is in accordance with the records of literature for the lemon oil; the compound majority limonene (Simões *et al*. 2004; Fisher and Phillips 2006) and citronella oil are the majority citronellal, citronellol and geraniol (Oliveira *et al*. 2010b), all belonging to the group of monoterpenes.

The mechanism of action of the monoterpenes (limonene, citronellal, citronellol, geraniol) involves mainly toxic effects on the structure and function of the cell membrane. As a result of their lipophilic character, the monoterpenes will preferably dislocate from the aqueous phase toward the membrane structures (Sikkema *et al*. 1995). Accumulation of the EO constituents in the lipid double layer of the cytoplasm membrane will confer a characteristic of permeability. In bacteria, cytoplasmic membrane permeabilization is associated to dissipation of the proton motive force regarding reduction of the adenosine triphosphate pool, internal pH and electric potential, and loss of ions such as potassium and phosphate ions (Bakkali *et al*. 2008).

Another fact observed in this study was the significant reduction of biomass accumulated, suggesting that the EOs interact with the matrix of exopolysaccharide (EPS) that is disrupted (Nostro *et al*. 2007).

Thus, it was concluded from the conditions studied that EOs lemon and citronella are new alternatives to sanitize industrial polypropylene surfaces contaminated by *E. coli* and *S. aureus*. We suggest further research on search strategies using natural antimicrobials against bacterial biofilms; there are few studies on this perspective.

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