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ORIGINAL ARTICLE

Survival and fatty acid composition of UV-C treated *Staphylococcus aureus*

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Abstract Ultraviolet irradiation (UV-C) is proven to be effective to inactivate microorganisms. The present study investigates the effect of sublethal UV-C rays on the membrane fatty acid profile of four *Staphylococcus aureus* strains isolated from air, patient, food and animal. Our results show that *S. aureus* isolated from patients and air are the most sensitive to UV-C rays and that their inactivation achieves a greater than 2-log reduction after a UV-C exposure of 210 mJ cm^{-2}. However, the strain isolated from food is the most resistant one. The fatty acid analysis indicates that this strain (food) reveals a decrease of branched chain fatty acids (BCFA iso and anteiso) and an increase of straight chain saturated fatty acids (SCFA).

Keywords *Staphylococcus aureus* · Ultraviolet radiation · Fatty acid · Survival

Introduction

Staphylococcus aureus is a Gram-positive and ubiquitous commensal bacterium; the main reservoirs are humans and animals. *S. aureus* colonizes skin, nares, throat and wounds. It can contaminate food but frequently causes severe diseases to human beings (Kluytmans et al. 1997) including meningitis, septicemia, endocarditis, abscesses, toxic shock syndrome and some food intoxications (Lowy 1998; Martineau et al. 1998).

To control the spread of this pathogen, several chemical substances, such as chlorine and chloramines (Andersen et al. 2006), have been used but these antibacterial agents remain very harmful to public health and the environment by the formation of byproducts (Fjellet et al. 2003). Therefore, non-chemical alternatives such as UV-C irradiation are preferred and effectively used . UV-C irradiation is being increasingly applied because of its power to inactivate suspended cells (Hijnen et al. 2006). In addition, UV-C irradiation offers several advantages: the absence of byproducts, low cost and because it is easier and safer to use (Yousef and Marth 1988; Wong et al. 1998).

The UV-C form of electromagnetic radiation is the most effective for germicidal purposes (Gal et al. 1992). It is lethal to microorganisms including bacteria, spores, viruses, yeast and algae. However, the doses which are needed to inactivate them are variable (Nakamura and Ramage 1963; Fino and Kniel 2008).

According to Moss (1981), metabolic products and bacterial cell components offer a good tool for identification and study of the taxonomic classification of bacteria. In fact, fatty acid (FA) profiles are useful for identifying Gram-positive (cell lipids are concentrated in plasma membrane) and Gram-negative bacteria (lipoproteins, polar and non-polar lipids are located in plasma or in outer membrane) (Tranchida et al. 2008). It is well established that bacterial membrane FA composition changes to maintain bacterial viability under varying environmental conditions (Suutari and Laakso 1994). The change of FA profiles requires an efficient system for the regulation of FA metabolism (Choi et al. 2000). Bacteria have evolved responses to survive extreme environmental conditions such as temperature, pH, and gamma rays. These responses include the modification of the membrane fluidity and enhance the activation and/or

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repression of several genes (Kloula et al. 2013). So, bacteria change their lipid profiles (quantitative and qualitative) which influences the ratio of saturated to unsaturated fatty acids, cis to trans unsaturated fatty acids, and branched to unbranched structures.

The aim of this study is to determine the effect of UV-C light on the survival and fatty acid composition of *S. aureus* strains isolated from different sources.

Materials and methods

Staphylococcus aureus isolates

The four strains of *S. aureus* used in this study are isolated from different sources: air (strain 1), patient (strain 2), food (strain 3) and animal (strain 4). After overnight incubation in 5 ml of tryptic soy broth (TSB) (Biolife Italiana, Milan, Italy), then in Chapman agar medium (Biolife Italiana, Milan, Italy) for 24 h at 37 °C, suspected colonies were isolated and inoculated in tryptic soy agar (TSA) (Biolife Italiana, Milan, Italy). The identification of *S. aureus* was already done according to standard protocols: Gram staining, biochemical properties (catalase, tube coagulase and DNase test) and detection of hemolysis (Collee et al. 1996; Mekonnen et al. 2011).

The isolates are checked by polymerase chain reaction PCR for the presence of the *nuc* gene which codes for the thermonuclease using the primers, nuc-F (5′-GCGATTGATG GTGATACGGTT-3′) and nuc-R (5′-AGCCAAGCCTTGAC GAACTAAAGC-3′) as described by Brakstad et al. (1992).

PCR conditions for the *nuc* gene comprise an initial denaturation step at 94 °C for 5 min, followed by 37 cycles at 94 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 for 1.5 min, which is followed by final extension for 3.5 min at 72 °C. The size of PCR product is analyzed by electrophoresis on 1 % (w/v) agarose gel stained with ethiduim bromide (1 mg ml^{-1}) at 90 V cm^{-1} and visualized under ultraviolet transillumination.

Bacterial preparation for UV-C exposure

S. aureus strains are grown in 10 ml of tryptic soy broth (TSB) at 37 °C for 24 h without shaking. After incubation, the optical density (OD) is adjusted to 0.6 at 570 nm (Spectro UV–vis Dual Beam, UVS-2700, Labomed, Inc, Los Angeles, CA, USA). Then, the bacterial cells are harvested by centrifugation at 4,500 rpm for 15 min. The bacterial cells are resuspended in sterile physiological water (0.9 % NaCl) and re-centrifuged.

The pellet is resuspended in sterile saline solution and transferred to a glass Petri dish. The doses of irradiation are given cumulatively using the same bacterial suspension.

Viable cell counts of different samples, before and immediately after UV-C exposition, are estimated by spreading 100 μl on TSA. Plates are then incubated at 37 °C for 24 h. Then, the number of colony forming units (CFU) is determined. Each assay is repeated three times.

UV-C treatment using a photoreactor system

The batch laboratory UV-device was built in cooperation with Guy Daric S.A. (Aubervilliers, France). This prototype contains a sliding rack, with an irradiation board that holds six Petri dishes (90 mm diameter). UV-C exposure is carried out using a germicidal low pressure mercury vapor discharge lamp (length=900 mm, diameter=13 mm, power of UV emission at 253.7 nm=55 W). The incident intensity UV-C ray levels are measured with a selective detector for UV joined to a radiometer (VLX 254, Vilber Lourmat, Norme La Vallée, France). The UV-C doses are calculated as the product of radiation intensity (I = mW cm^{-2}) and the exposure time (t = s) following the formula recognized by Hassen et al. (2000).

$$\text{UV}-\text{C dose}\left(\text{mJ cm}^{-2}\right) = \text{irradiance}\left(\text{mW s cm}^{-2}\right) \times \text{exposure time (s)}.$$

UV-C doses which are used in this study are ranging from 35 to 1,260 mJ cm^{-2}. Light intensity is evaluated several times during the experiments to ensure consistent output of the light and ultraviolet lamps are switched on about 30 min before the measurement to provide complete activation.

Fatty acid extraction

The fatty acids are extracted and derivatized following a standardized procedure called MIDI produced by Microbial ID (MIDI, Newark, DE, USA), and also described by Arcelloni et al. (1989) and Welch (1991) with some modifications. This method uses four reagents and consists of four steps: saponification, methylation, extraction and wash.

Reagent 1 is 45 g of sodium hydroxide, 150 ml of methanol and 150 ml of doubly distilled water. Reagent 2 is 325 ml of 6 M hydrochloric acid and 275 ml of methanol. Reagent 3 is 200 ml of hexane and 200 ml of methyl tertiary butyl ether. Reagent 4 is 10.8 g of sodium hydroxide (NaOH) in 900 ml of doubly distilled water.

Bacterial colonies are harvested and washed (two cycles of centrifugation) at the same conditions using phosphate buffer (75 mM, pH 7.0). Fifty to one hundred milligrams of biomass is transferred into a tube with a Teflon-lined cap using 1 ml of reagent 1, mixed for 5–10 s, heated to 98 °C for 5 min, shaken again and returned to 98 °C for 30 min. The methylation of fatty acids is achieved by adding 4 ml of reagent 2 to the

cooled uncapped tubes, which are then vigorously shaken for 30 s and heated to 80 °C for 10 min. The fatty acid methyl esters (FAMEs) are extracted by adding 1.5 ml of reagent 3 and shaking vigorously for 5 min. Two milliliters of upper phase (organic phase) is transferred to another conical glass tube with Teflon cap and washed with 6 ml of reagent 4 and vigorously shaken for 5 min. Four-hundred ninety microliters of the upper phase are transferred autosampler vials (Teflon caps) and stored at −20 °C for the gas chromatographic (GC) analysis.

FAME analysis is performed using a GC flame ionization detector (FID) (Varian, CP 3800) for the analysis of the membrane fatty acids, and a capillary column (SPB-5, Supelco; 60 m by 0.25 mm by 0.25 μm) is used to separate the fatty acids.

The injector and the detector are both held at 240 °C. The temperature is programmed from 120 °C (held for 2 min) to 215 °C at a rate of 4.5 °C min^{-1}, then to 225 °C at a rate of 0.5 °C and held at 225 °C for 20 min. The carrier gas is helium, with a rate of 1 ml min^{-1}. Samples (1 μl) are injected directly into the column with a split ratio of 1:10. Component separation is achieved following the method described above. C11 methyl ester is used as internal standard.

Membrane FAs are quantified with internal standards and identified by comparing their retention times of the samples with those of the standard 37-Component FAME Mix and BAME Mix (Supelco, Sigma-Aldrich, Milan, Italy). When necessary, peak identification is also carried out by gas chromatography–mass spectrometry (GC/MS). Concentrations are calculated as percentage relative to the sum of integrals of FAs methyl ester peaks identified in the chromatogram.

Experiments are repeated three times for reproducibility of the fatty acid profiles.

Statistical analysis

Average values of triplicates are given, and the deviation was <5 % of each value (the mean value and standard deviation of replicate value). Significance is assessed using the Student's *t* test.

Results

Identification of *Staphylococcus aureus* isolates

According to cultural and biochemical properties, the four isolates used in the present study could be identified as *S. aureus*. The identification of the isolates is then confirmed by PCR amplification of thermonuclease *nuc* gene. The amplicon of this gene shows a uniform size of approximately 279 base pairs (bp) (Fig. 1).

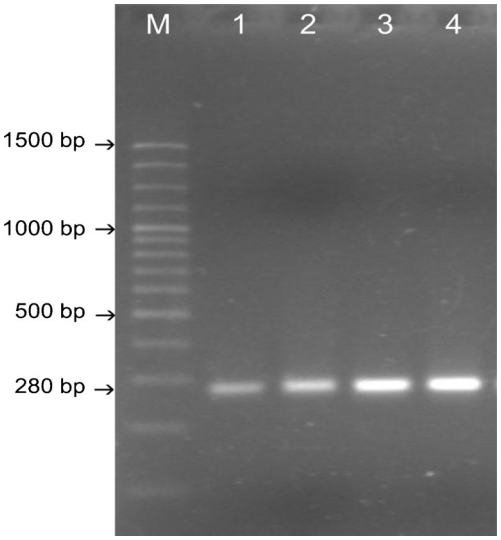

Fig. 1 Agarose gel electrophoresis of specific PCR products obtained from amplification of the nuc gene. M: 100–1,500 base pairs (bp) DNA molecular size marker, *lane 1*: *S. aureus* from air, *lane 2*: *S. aureus* from patient, *lane 3*: *S. aureus* from food and *lane 4*: *S. aureus* from animal

Effect of UV-C on the survival of *Staphylococcus aureus*

The model of Chick–Watson has been proposed to explain the effect of UV on *S. aureus* survival kinetics. The expression of the model is N/N0=A.exp (−kInt). Where N0 and N are the bacterial populations in CFU ml^{-1} prior to and following exposure, respectively, where A is the event corresponding to microorganism retaining viability following UV irradiation, k, the coefficient of lethality, I, the UV-C intensity expressed in mW cm^{-2}, t is the exposure time (s), and n is the model coefficient, which is equal to 1.

The results for the UV-C inactivation of *S. aureus* isolates are shown in Fig. 2. The initial population density for each strain was between 10^6 and 10^7 CFU ml^{-1}.

Based on this model, our results showed that UV-C irradiation caused a bacterial reduction. S. *aureus* strains isolated from patient and air present the highest level of inactivation with more than 2-log reduction following UV-C exposure for 30 s (Fig. 2). However, *S. aureus* isolated from animal exhibit a moderate resistance to UV-C and required a dose of 360 mJ cm^{-2} for the eradication of 99.9 % of the initial inoculum. A 1.3-log reduction is recorded following 180 s exposure time for *S. aureus* isolated from food.

Fatty acids profile analysis

FAMEs from untreated and UV-C treated *S. aureus* are separated by gas chromatograph. Our data prove that *S. aureus* is characterized by a complex fatty acid composition constituting straight chain saturated fatty acids (SCFAs) and branched chain fatty acids (BCFAs). In addition, a total of 15 recognizable components are detected. The major components of this

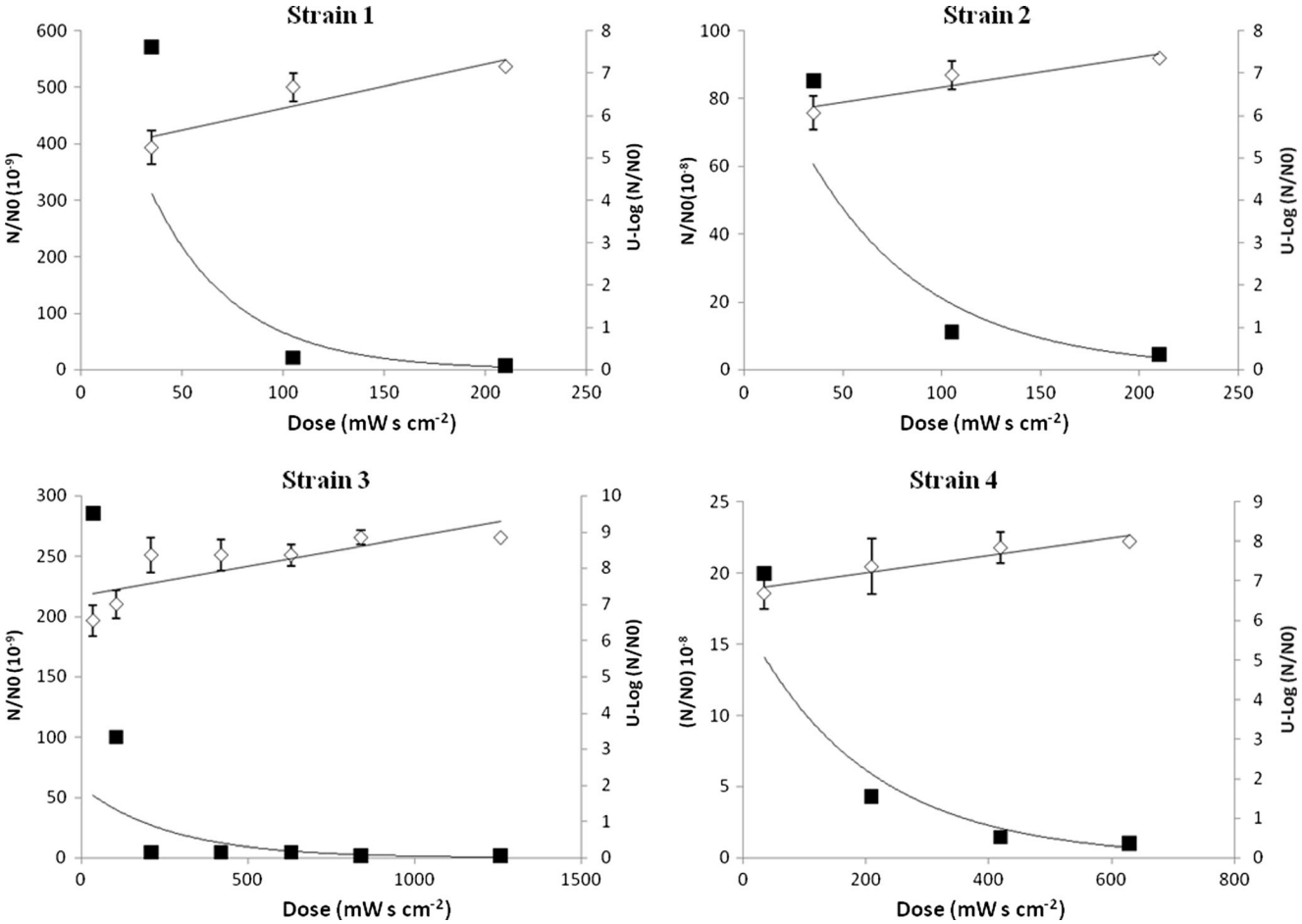

Fig. 2 Kinetic inactivation of *S. aureus* by UV-C radiation. (*Strain 1*: from air, *Strain 2*: from patient, *Strain 3*: from food and *Strain 4*: from animal), *N* number of bacteria after UV-C exposure, *N0* number of bacteria before UV-C exposure

mixture have retention times corresponding to authentic iso C14:0, C15:0, and anteiso C15:0, C18:0, and C20:0.

As expected, the fatty acid profiles demonstrate the presence of branched iso fatty acids (iso C14:0, iso C15:0, iso C16:0, iso C17:0 and iso C19:0), where the methyl group is on the second to last carbon in the chain, and branched anteiso fatty acids (anteiso C15:0, anteiso C17:0 and anteiso C19:0), where the methyl group is on the third to last carbon in the chain.

Upon comparison of the whole cell, fatty acid profiles, before and after UV-C treatment (summarized in Table 1), *S. aureus* shows moderate changes in the fatty acid profiles following UV-C treatment.

Discussion

The aims of our study are to determine the effects of UV-C radiations on the survival and the fatty acid composition of *S. aureus* strains isolated from diverse Tunisian sources. The characterization and identification of isolates are based on routine methods (cultural and biochemical properties) as well as PCR technology (by amplification of the *nuc* gene, which is specific to *S. aureus*). A single and specific 279 bp amplicon corresponding to the *nuc* gene is obtained for all *S. aureus* isolates. These results are in agreement with several previous studies (Brakstad et al. 1992; Pinto et al. 2005) for the staphylococcal thermonuclease. Furthermore, the *nuc* PCR identification approach, used as a complementary molecular method to check the accuracy of the phenotypic tests, is shown to be simple, rapid and reproducible on testing *S. aureus* isolated from various sources and gives promising results for the usefulness of this method.

The survival after UV-C exposure of the four strains was then studied. The UV-C inactivation curves show that *S. aureus* isolated from patient and air present the highest level of inactivation with more than a 2-log reduction. However, *S. aureus* isolated from animal exhibited an average resistance to UV-C and required 90 s for a destruction of 99.9 % of the initial inoculums. However, the strain isolated from food is more resistant to UV-C exposure having a lower inactivation rate constant and needs a higher UV-C dose. These findings suggest a relationship between the origin of *S. aureus* and UV-C resistance.

Table 1 Fatty acid profiles of S. aureus isolated from different origins with and without UV-C treatment

	Strain 1 (air)		Strain 2 (patient)		Strain 3 (food)		Strain 4 (animal)	
	NT	T (30 s)	NT	T (30 s)	NT	T (30 s)	NT	T (30 s)
C12:0	1.93±0.13^{a}	3.31±0.32^{b}	1.6±0.45^{c}	4.28±0.12^{d}	3.6±0.23^{e}	9.59±0.71^{f}	0.68±0.11^{g}	1.38±0.55^{h}
isoC14:0	11.09±0.10^{a}	5.97±0.21^{b}	6.27±0.49^{c}	6.76±0.19c	8.38±0.47^{e}	10.67±0.38^{f}	6.89±0.39^{g}	7.6±0.24^{h}
C14:0	1.99±0.20^{a}	0.94±0.07^{b}	0.97±0.04^{c}	1.77±0.17^{d}	2.22±0.09^{e}	2.96±0.37^{f}	2.4±0.28^{g}	2.23±0.19^{g}
isoC15:0	12.06±0.07^{a}	14.01±0.5^{b}	14.62±0.12^{c}	15.14±0.09^{d}	9.82±0.23^{e}	7.84±0.26^{f}	14.06±0.17^{g}	16.51±0.07^{h}
anteiC15:0	31.46±0.16^{a}	29.1±1.31^{b}	31.39±0.31^{c}	30.96±0.84^{c}	30.92±0.45^{e}	18.58±0.53^{f}	32.79±0.21^{g}	38.98±0.08^{h}
isoC16:0	2.85±0.19^{a}	2.96±0.12^{a}	3.17±0.10^{c}	2.92±0.15^{c}	1.97±0.12^{e}	1.69±0.45^{e}	1.94±0.10^{g}	2.43±0.40^{g}
C16:0	2.72±0.18^{a}	2.63±0.13^{a}	2.7±0.35^{c}	3.14±0.15^{c}	4.17±0.33^{e}	5.92±0.16^{f}	4.44±0.30^{g}	4.87±0.83^{g}
isoC17:0	3.43±0.78^{a}	3.53±0.49^{a}	2.67±0.22^{c}	2.79±0.05^{c}	1.9±0.01^{e}	1.4±0.43^{f}	2.81±0.29^{g}	3.28±0.34^{g}
anteiC17:0	3.15±0.17^{a}	3.96±0.58^{b}	3.21±0.36^{c}	3.43±0.16^{c}	2.05±0.02^{e}	1.86±0.36^{e}	3.58±0.26^{g}	4.39±0.17^{h}
C17:0	0.61±0.05^{a}	0.63±0.01^{a}	0.74±0.05^{c}	0.83±0.08^{c}	0.31±0.08^{e}	0.67±0.03^{f}	1.04±0.08^{g}	0.86±0.06^{h}
C18:0	11.12±0.22^{a}	11.66±0.43^{a}	11.58±0.12^{c}	11.25±0.23^{c}	11.36±0.13^{e}	11.31±0.22^{e}	12.37±1.21^{g}	7.76±0.52^{h}
isoC19:0	0.91±0.17^{a}	2.67±0.36^{b}	2.05±0.04^{c}	1.13±0.15^{d}	0.79±0.28^{e}	0.63±0.10^{e}	1.14±0.45^{g}	1.36±0.38^{g}
anteiC19:0	0.65±0.14^{a}	1.55±0.24^{b}	1.24±0.01^{c}	0.66±0.14^{d}	0.65±0.09^{e}	0.58±0.13^{e}	0.83±0.24^{g}	0.84±0.03^{g}
C19:0	2.59±0.16^{a}	3.27±0.28^{b}	3.39±0.26^{c}	2.34±0.16^{d}	1.82±0.17^{e}	2.46±0.7^{e}	2.83±0.59^{g}	1.77±0.08^{h}
C20:0	13.44±0.26^{a}	13.8±1.29^{a}	14.39±0.96^{c}	12.6±0.09^{d}	20.04±0.17^{e}	23.85±0.62^{f}	12.19±0.19^{g}	5.73±0.41^{h}
$\sum BCFA_{anteiso}$	35.26	34.61	35.84	35.05	33.62	21.02	37.20	44.21
$\sum BCFA_{iso}$	30.34	29.14	28.78	28.74	22.86	22.23	26.84	31.18
∑SCFA	34.40	36.24	35.37	36.21	43.52	56.76	35.95	24.60

Only fatty acids with a concentration greater than 0.3 % are tabulated. Values are mean of percentage of total fatty acids identified. *FAs* fatty acids, *BCFA* branched chain fatty acid, *SCFA* straight chain saturated fatty acid, *NT* untreated, T (30 s) UV-C treated for 30 s

Each value is the mean obtained from three separate analyses. The data presented are shown as the mean value and standard deviation of three replicates. Statistical analysis was done using the Student's t test ($P<0.05$). Comparison was done for each fatty acid of untreated and treated strains. Different letters mean significant difference ($P<0.05$) for each fatty acid before and after UV-C treatment in each strain. Same letters means no significant difference

We next examined the fatty acid composition of each strain before and after UV-C treatment. Cellular fatty acid composition data of the four strains is typical for *S. aureus*, showing a complex mixture of BCFAs and SCFAs (Schleifer and Kroppenstedt 1990) with BCFAs accounting for about 55 to 65 % of the total FAs, where anteiso C15:0 is the major BCFA (about 30 %). BCFAs in general, and anteiso C15:0 in particular, are major determinants of the membrane fluidity in *S. aureus*. Quantitative fatty acid profile analysis is performed to find out if any changes have occurred in the lipid content of these strains after UV-C treatment. Data obtained and reported in Table 1, show that the exposition to sublethal UV-C dose irradiation led to considerable changes on FA composition of the unbound lipids. UV-C treatment disturbs

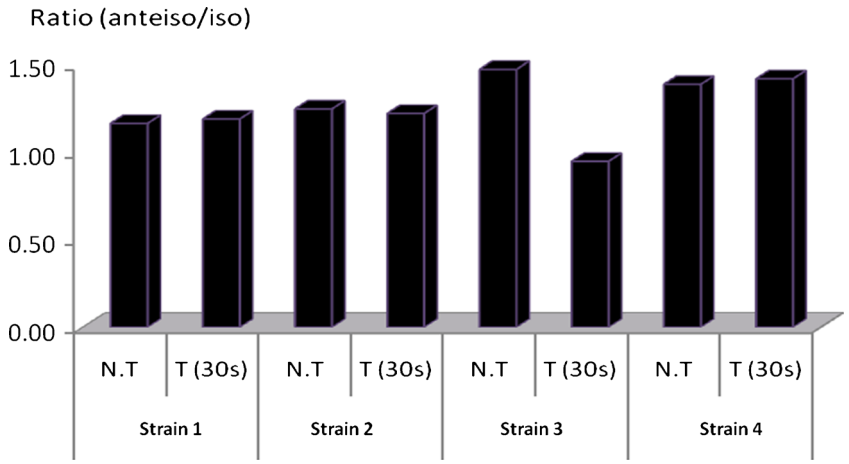

Fig. 3 The anteiso/iso ratios of S. *aureus* isolates before and after UV-C treatment. *N.T* untreated, *T* treated

some fatty acid concentrations (C12:0, iso C14:0, iso C15:0, anteiso C15:0, C19:0 and C20:0) by increasing or decreasing them. Significant decrease of C20:0 and C18:0 for the strain isolated from animal was obtained. On the other hand, the other fatty acids amounts are slightly affected such as iso C16:0, iso C17:0, anteiso C17:0, C17:0 and C18:0. However, a trend toward a decrease in the amount of the BCFAs and the anteiso C15:0, except the strain isolated from animal, is established. The anteiso C15:0 decreased significantly in the strain isolated from food and increased significantly in the strain isolated from animal. Figure 3 shows that strain isolated from food presents the greatest changes on ratio (anteiso/iso) after 30 s UV-C exposition compared to the three other strains. An interesting issue of the present study is that strain 3 (isolated from food) and 4 (isolated from animal) adapt more to UV-C stress than strain 1 (isolated from air) and 2 (isolated from patient) by changing the fatty acid concentration profiles because they exhibit the most remarkable variations. Consequently, *S. aureus* strains have rendered necessary fatty acid adaptation and, therefore, acquire an ability to develop resistance responses to survive and grow under UV-C sublethal conditions. The analysis of these findings suggests that the high resistance of *S. aureus* to UV-C may explain distinguished changes on fatty acid profiles. The findings which are discussed in this paper on *S. aureus* are in agreement with those found by Korachi et al. (2010) who reported a high resistance of this microorganism to plasma discharge. Our results suggest that *S. aureus* strains regulate their fluidity by changing the proportion of iso- and anteiso-branched fatty acids to reduce the effects of environmental changes on the membrane.

In conclusion, the results of this study credibly show not only the reduction rate of *S. aureus* strains in logarithmic scale but also changes of fatty acid amounts caused by ultraviolet C light. Consequently, this phenomenon (fatty acid amount shifts) is important because it offers a selective benefit for bacterial survival as a response to adverse environmental conditions.

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