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Membrane Toxicity of Antimicrobial Compounds from Essential Oils

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Natural antimicrobial compounds perform their action mainly against cell membranes. The aim of this work was to evaluate the interaction, meant as a mechanism of action, of essential oil antimicrobial compounds with the microbial cell envelope. The lipid profiles of *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium, *Pseudomonas fluorescens*, and *Brochothrix thermosphacta* cells treated with thymol, carvacrol, limonene, eugenol, and cinnamaldehyde have been analyzed by gas chromatography. In line with the fatty acids analysis, the treated cells were also observed by scanning electron microscopy (SEM) to evaluate structural alterations. The overall results showed a strong decrease of the unsaturated fatty acids (UFAs) for the treated cells; in particular, the C18:2trans and C18:3cis underwent a notable reduction contributing to the total UFA decreases, while the saturated fatty acid C17:0 raised the highest concentration in cinnamaldehyde-treated cells. SEM images showed that the used antimicrobial compounds quickly exerted their antimicrobial activities, determining structural alterations of the cell envelope.

KEYWORDS: Essential oils; natural antimicrobial compounds; cell envelope alterations; scanning electron microscopy

INTRODUCTION

The antimicrobial activities of some compounds known as constituents of essential oils (EOs) have already been fully discussed by many authors (*1*−*4*). The major active EO components are phenols, terpenes, and aldehydes (*5*), and it is also well-known that the action of these substances is principally performed against the cell cytoplasmic membrane (*5*−*9*). Among the EO constituents, the phenolic compounds were found to possess major antimicrobial activities (*10*). It seems that the presence of the hydroxyl group is related to the inactivation of the microbial enzymes. Most probably, this group interacts with the cell membrane causing leakage of cellular components, a change in fatty acids and phospholipids, and impairment of the energy metabolism and influencing genetic material synthesis (*5*). Similarly to phenolic compounds, the site of action of the terpenes is the cell membrane. They permeate through the membranes causing them to swell, thus inhibiting respiratory enzymes and causing partial dissipation of the pH gradient and electrical potential (*7*). Cinnamaldehyde has the highest antifungal activity among aliphatic aldehydes (*5*), and according to Helander et al. (*2*), it gains access to the periplasm and to the deeper parts of the cells but does not result in the disintegration of the outer membrane as in the case of carvacrol and thymol action. On the other hand, Gill and Holley (*3*) hypothesized a mechanism for cinnamaldehyde activity in which the interaction with the cell membrane causes disruption sufficient to disperse the proton motive force by leakage of small ions without leakage of larger cell components, such as ATP.

Table 1. Bacterial Strains Used in This Study[a]

species	strain
E. coli O157:H7 (nontoxigenic)	ATCC 43888
S. enterica serovar Typhimurium	ATCC 14028
P. fluorescens	NCIMB 10586
B. thermosphacta	NCTC 10822
S. aureus	NCTC 6571

[a] ATCC, American Type Culture Collection; NCIMB, National Collection of Industrial Food and Marine Bacteria; and NCTC, National Collection of Type Cultures.

In this study, we evaluated the membrane damage by analyzing the fatty acid profiles after exposure of *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157: H7, *Pseudomonas fluorescence*, *Brochotrix thermosphacta*, and *Staphylococcus aureus* cells to a high concentration of EO constituents. In addition, scanning electron microscope observations were performed to investigate the occurrence of surface damage on the treated cultures.

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Table 2. Percentage of Total UFAs and Their Changes Occurring in Response to Treatment with EO Compounds[a]

	E. coli O157:H7		*S.* serovar Typhimurium		*P. fluorescens*		*B. thermosphacta*		*S. aureus*	
compounds	UFA[b]	ΔUFA[c]	UFA	ΔUFA	UFA	ΔUFA	UFA	ΔUFA	UFA	ΔUFA
control	55.71 a		59.56 a		67.46 a		58.05 a		70.66 a	
thymol	42.80 b	–12.91	47.50 b	–12.06	30.95 b	–36.52	34.23 b	–23.82	39.73 b	–30.92
limonene	18.38 b	–37.33	25.59 b	–33.98	9.80 b	–57.67	64.70 b	6.66	32.23 b	–38.42
eugenol	43.57 b	–12.14	44.21 b	–15.35	1.66 b	–65.80	48.23 b	–9.82	53.19 b	–17.47
carvacrol	30.99 b	–24.72	49.38 b	–10.19	42.64 b	–24.82	39.66 b	–18.39	19.23 b	–51.43
cinnamaldehyde	14.12 b	–41.58	7.75 b	–51.81	1.13 b	–66.33	2.74 b	–55.31	3.27 b	–67.38

[a] Means in the same column followed by a letter other than the control value differ from this ($P < 0.05$). [b] Values are means of triplicate determinations. [c] UFAs control – UFAs treated cells.

Table 3. Changes in the Percentage of Principal Fatty Acids of Cells Treated with the Antimicrobial Compounds

F.A.	control	thymol	limonene	eugenol	carvacrol	cinnamaldehyde
			E. coli			
C6:0	1.32 ± 0.66[a]	ND[b]	54.50 ± 1.99	ND	3.08 ± 2.08	ND
C16:0	18.47 ± 2.67	33.66 ± 1.74	17.53 ± 0.25	37.78 ± 1.22	24.95 ± 0.13	7.37 ± 0.61
C17:0	3.57 ± 1.05	ND	ND	ND	ND	68.79 ± 6.76
C17:1cis	8.25 ± 1.96	15.04 ± 0.61	9.17 ± 0.40	20.80 ± 2.55	11.24 ± 4.24	2.25 ± 0.18
C18:0	4.28 ± 1.32	11.65 ± 0.31	2.71 ± 0.46	7.00 ± 2.49	10.46 ± 1.73	2.97 ± 0.29
C18:1cis	7.07 ± 4.33	15.88 ± 0.68	3.40 ± 0.74	9.79 ± 1.50	13.78 ± 2.17	5.68 ± 0.84
C18:3cis	12.91 ± 4.61	0.90 ± 1.28	0.22 ± 0.31	ND	ND	ND
			S. enterica serovar Typhimurium			
C6:0	0.51 ± 0.72	ND	40.20 ± 4.13	ND	ND	ND
C16:0	25.54 ± 3.92	39.25 ± 0.55	21.95 ± 1.18	43.22 ± 0.61	39.34 ± 1.36	3.17 ± 0.98
C17:0	1.77 ± 2.13	ND	ND	ND	ND	82.51 ± 4.97
C17:1cis	10.47 ± 0.82	20.50 ± 0.39	11.54 ± 0.51	19.08 ± 0.11	23.11 ± 0.76	2.66 ± 0.62
C18:2trans	13.12 ± 4.29	ND	ND	ND	ND	ND
C18:3cis	10.42 ± 3.09	ND	ND	ND	ND	ND
			B. thermosphacta			
C4:0	2.51 ± 0.90	ND	11.8 ± 0.80	ND	ND	ND
C16:0	16.41 ± 2.60	37.91 ± 1.20	11.79 ± 0.71	30.00 ± 5.11	29.38 ± 4.97	1.00 ± 0.07
C17:0	0.16 ± 0.23	ND	0.31 ± 0.44	ND	ND	93.30 ± 1.45
C18:1cis	4.69 ± 0.92	8.19 ± 0.82	19.15 ± 0.92	10.84 ± 1.69	7.69 ± 3.22	0.87 ± 0.59
C18:2trans	21.64 ± 3.54	0.35 ± 0.49	0.08 ± 0.05	1.14 ± 0.21	0.41 ± 0.58	ND
			P. fluorescens			
C14:0	1.59 ± 0.80	8.74 ± 1.01	10.91 ± 0.83	12.75 ± 0.71	7.29 ± 0.52	
C16:0	11.45 ± 2.50	44.86 ± 0.97	10.12 ± 0.13	65.31 ± 0.30	11.98 ± 1.60	0.86 ± 0.08
C17:0	1.18 ± 1.67	ND	ND	ND	ND	85.63 ± 0.62
C17:1cis	0.51 ± 0.72	ND	ND	ND	28.60 ± 10.86	ND
C18:0	3.25 ± 1.37	9.59 ± 0.25	9.91 ± 0.58	14.03 ± 0.46	6.79 ± 0.55	0.23 ± 0.01
C18:1cis	25.19 ± 2.89	18.94 ± 0.73	4.22 ± 0.10	ND	ND	ND
C18:3cis	18.49 ± 1.05	ND	2.67 ± 0.77	1.66 ± 0.02	2.64 ± 1.44	ND
			S. aureus			
C14:1cis	13.19 ± 2.74	12.46 ± 0.24	20.03 ± 0.89	28.23 ± 2.69	16.39 ± 1.05	1.50 ± 0.10
C16:0	3.59 ± 0.94	24.61 ± 0.70	9.35 ± 0.42	10.68 ± 0.68	9.88 ± 0.93	1.72 ± 0.04
C17:0	0.63 ± 0.89	0.72 ± 0.03	ND	ND	ND	87.97 ± 0.16
C18:0	4.04 ± 0.66	16.19 ± 0.72	15.06 ± 0.54	8.82 ± 0.40	14.94 ± 0.91	1.47 ± 0.02
C18:1cis	23.00 ± 0.81	15.67 ± 0.37	2.34 ± 0.48	3.83 ± 0.21	ND	1.06 ± 0.16
C18:3cis	15.42 ± 1.80	ND	ND	4.51 ± 0.49	ND	ND

[a] Means of triplicate determinations ± SD. [b] ND, not detectable.

MATERIALS AND METHODS

Bacterial Strains. The strains used in this study (**Table 1**) were routinely grown in tryptone soy broth (Oxoid) supplemented with 0.5% yeast extract (Oxoid) (TSBYE) at an optimal growth temperature for 18−20 h.

Antimicrobial Compounds. All compounds were purchased from Sigma (Milano, Italy); they were 97% (*R*)-(+)-limonene, 99% thymol, 98% carvacrol, 98% cinnamaldehyde, and 98% eugenol, and all of them were EO components. Each component was prepared as a 1 M solution in methanol.

Treatment of Cells. The culture broths of each microorganism were centrifuged for 10 min at 5000*g*, and the cell pellets were harvested and resuspended in 0.02 M phosphate-buffered saline (PBS), pH 7. The compounds were added to each cellular suspension to a final concentration of 0.2 M. The cells were left for 2 h at 30° C and immediately processed for membrane fatty acid extraction. Cells incubated without antimicrobial compounds were used as controls. The experiments were performed in triplicate.

Total Lipid Extraction and Analysis of Fatty Acid Composition. The culture broths of each microorganism were centrifuged for 10 min at 5000*g*, and the cell pellet was harvested and submitted for membrane fatty acid extraction. Extraction of fatty acids from cellular materials was carried out as described by Evans et al. (*11*). Lipid samples were transmethylated for analysis of their acyl groups as fatty acid methyl esters (FAME) by high-resolution gas chromatography (HRGC) analysis (*12*).

Sample Preparation for the Scanning Electron Microscopy (SEM) Analysis. The cells, after being treated with the compounds as above-described, were harvested by centrifugation for 10 min at 5000*g*, washed twice with PBS, and finally resuspended in PBS containing 2.5% glutaraldeyde to fix the cells. This suspension was filtered on a

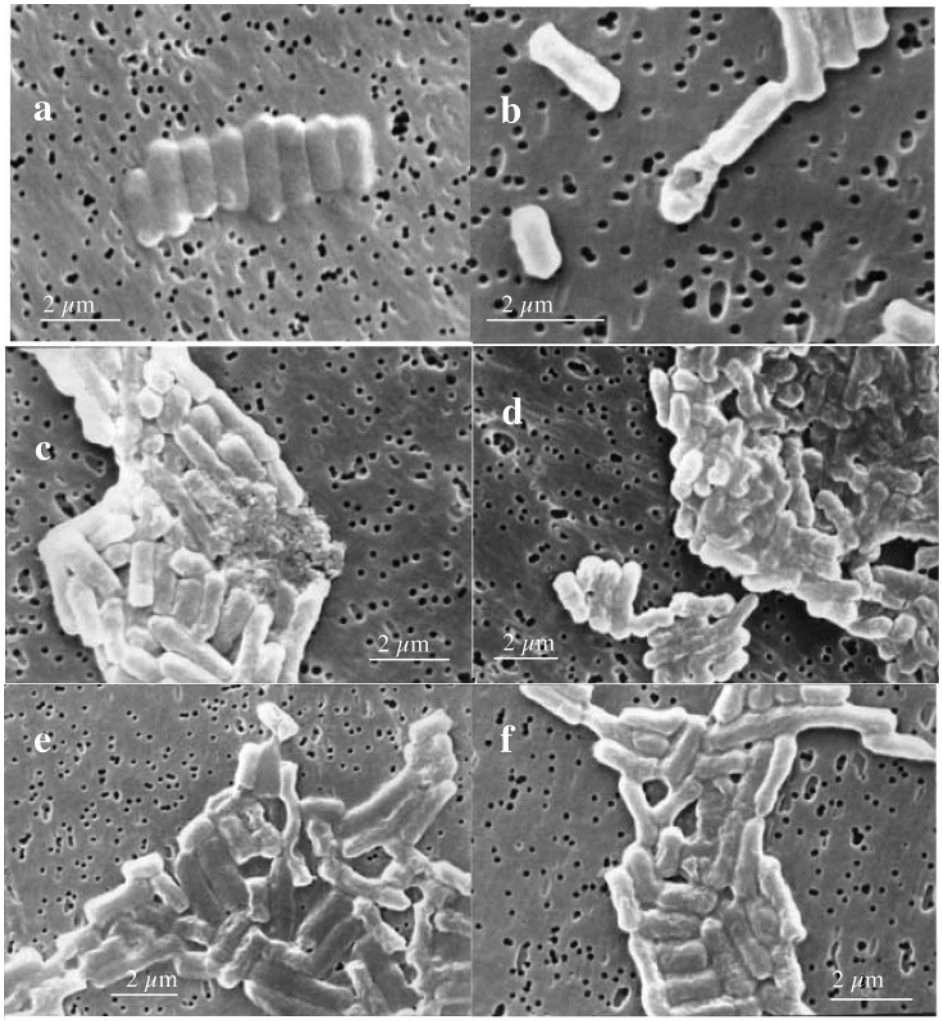

Figure 1. Scanning electron microscope images of *E. coli* O157:H7 cells. (**a**) Untreated cells (10000×) and (**b**) cells after treatment with carvacrol (15000×), (**c**) eugenol (10000×), (**d**) cinnamaldehyde (8000×), (**e**) limonene (8000×), and (**f**) thymol (10000×).

0.2 μm Nucleopore Track-Etch Membrane (Whatman), air-dried, and then sputter-coated with gold under vacuum; the membrane was ready for the SEM analysis. Samples were examined by using a scanning electron microscope (Leica Cambridge S-360).

Statistical Analysis. *T* tests and standard deviation calculations were performed using Systat software for Macintosh version 5.2.1 to evaluate whether the differences between the means were statistically significant.

RESULTS

Alteration of Membrane Fatty Acids Profiles following Exposure to the Antimicrobial Compounds. Membrane lipid alterations were investigated by GC analysis of FAMEs extracted from the cells after an exposure of 2 h in the presence of antimicrobial compounds.

As shown in **Table 2**, the lipids of the tested strains were composed of more than 50% unsaturated fatty acids (UFAs); in particular, *S. aureus* NCTC 6571 presented the highest concentration of UFAs. It was also noted that all of the strains exhibited a reduction of the UFAs and that in most cases the highest reduction was observed when the cells were treated with cinnamaldehyde.

The major fatty acids and their concentrations before and after the treatments with the antimicrobial substances are reported in the **Table 3**. Preliminary experiments showed no influence of methanol on the microbial growth (*4*) or on cell lipid profiles (data not shown) when tested at the same concentrations used to dissolve the antimicrobial compounds in the growing media.

The palmitic (C16:0), *cis*-10-heptadecenoic (C17:1cis), oleic (C18:1cis), and α-linolenic (C18:3cis) acids comprised the major fatty acids of the *E. coli* lipid profile. When the strain was treated with the phenolic compounds (thymol, eugenol, and carvacrol), these fatty acids underwent an increase, with the exclusion of the α-linolenic (C18:3cis) acid (**Table 3**).

The lipid profiles of *S.* Typhimurium and *E. coli* were very similar (**Table 3**); however, linoleaidic acid (C18:2trans) was only present in the profile of *S.* Typhimurium, while the oleic and stearic acids occurred in *E. coli*. Likewise, for *E. coli*, the phenolic compounds resulted in a complete reduction of the C18 fatty acids, while a notable increase was observed for the palmitic and *cis*-10-heptadecenoic acids.

Remarkable was the change observed when both strains were treated with limonene, a terpenic compound. In this case, the two lipid profiles that resulted were mainly composed of caproic acid (C6:0), which increased to concentrations of 54.50% in *E. coli* and 40.20% in *S.* Typhimurium.

The lipid profile of *B. thermosphacta* was predominantly composed of palmitic acid and linoleaidic (C18:2trans) acid. After the treatment of the strain with the phenolic compounds, the lipid profile that resulted was richest in palmitic acid, and the content of linoleaidic acid was markedly decreased, while an increase of the oleic acid concentration occurred. On the other hand, limonene induced in this strain a significant increase of

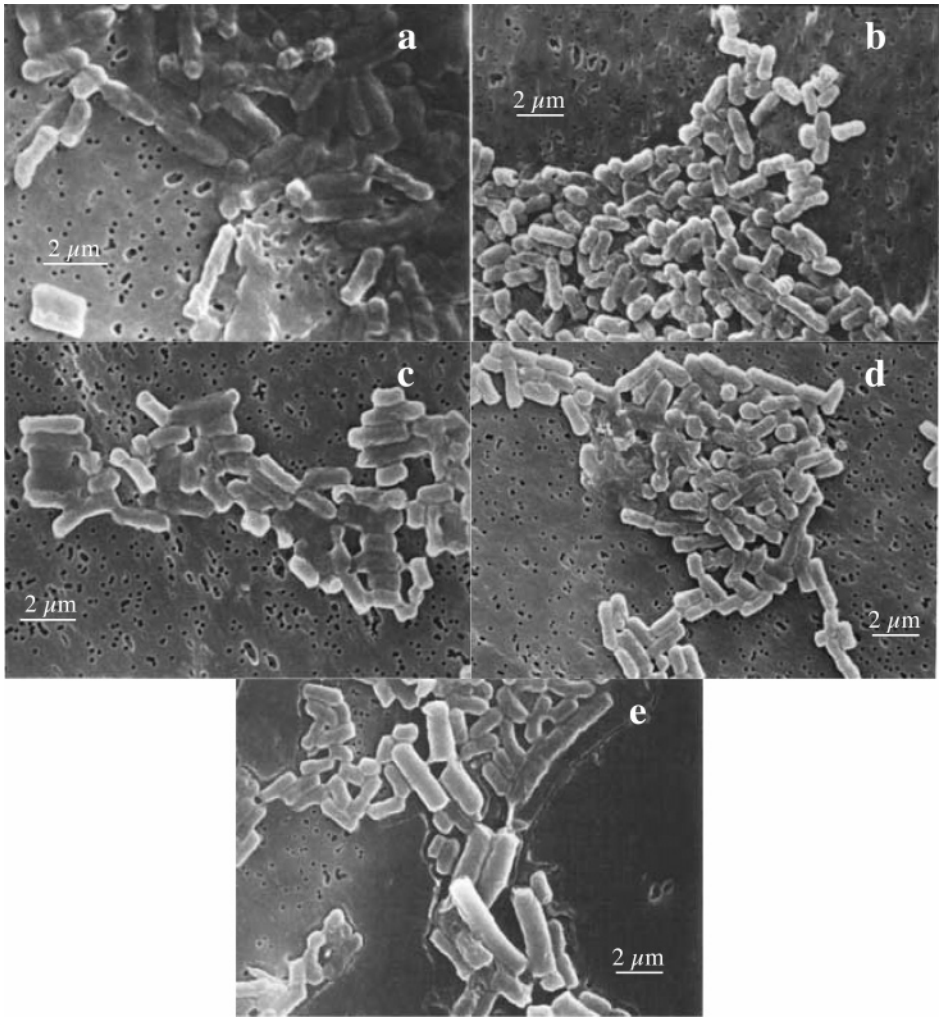

Figure 2. Scanning electron microscope images of *S. enterica* serovar Typhimurium cells. (**a**) Untreated cells (8000×) and (**b**) cells after treatment with cinnamaldehyde (6000×), (**c**) limonene (7000×), (**d**) carvacrol (6000×), and (**e**) thymol (6000×).

the butyric (C4:0) and oleic acids, while the linoleaidic acid underwent a remarkable decrease.

The treatment of the strain of *P. fluorescens* with thymol, eugenol, and carvacrol resulted in a clear increase in concentration of the saturated fatty acids (SFAs), myristic (C14:0), palmitic, and stearic. An increase in myristic and stearic acids also occurred when the strain was treated with limonene as well, while after carvacrol treatment a remarkable increase in *cis*-17-heptadecenoic acid occurred.

The *S. aureus* NCTC 6571 lipid profile was principally composed of myristoleic (C14:1cis), oleic, and α-linolenic acids. After exposure to thymol, the latter two fatty acids underwent a notable decrease; on the other hand, the stearic acid percentage value was detected four times higher than the control. The myristoleic and the palmitic acids were detected at a level concentration higher than the control after the treatment with the phenolic and terpenic compounds. It can be noticed that these compounds determined, in the C18 fatty acids, an increase of the SFA (C18) concentration with a corresponding decrease of the UFAs (C18:1cis and C18:3cis) concentration.

Treatment of cells with thymol, carvacrol, eugenol, and limonene has been shown to change their lipid profiles, but particularly noteworthy was the effect of the cinnamaldehyde. Heptadecanoic acid (C17:0) was found at low concentrations in the control profiles, but when the strains were treated with cinnamaldehyde, its concentration increased to 70% higher than the control value. Overall, a reduction observed in the percentage of long fatty acid (C18) was observed, accompanied by a decrease in the percentage of UFAs.

SEM. Figures 1−5 show the cells before (controls) and after the treatment with the natural antimicrobial compounds. The figures show changes in the appearance of the cells as a result of treatment, most probably exerted via an effect on the outer cell envelope.

Untreated cells of *E. coli* O157:H7 are shown in **Figure 1**. Comparing the control cells with the treated cells (**Figure 1b−f**), differences can be easily noticed in the rod morphology. Particularly interesting is the alteration due to the presence of eugenol when compared with those due to the other compounds. In fact, it seems that this compound is able to disrupt the membrane, allowing the leakage of intracellular constituents, while the other compounds cause just structural alteration of the outer envelope.

S. Typhimurium and *Pseudomonas* spp. strains, after treatment with cinnamaldehyde and limonene, presented external modifications, probably because these compounds penetrate the cell envelope, altering its structure (**Figures 2b**,**c** and **3b**,**c**). In addition, the *S.* Typhimurium membrane was also altered by carvacrol and thymol (**Figure 2d**,**e**). Some of the cells underwent a swelling after treatment with thymol (**Figure 2e**).

Cinnamaldehyde was able to alter the external structure of all of the tested strains. However, cell wall deterioration and a high degree of cell lysis were noticed in *S. aureus* (**Figure 4b**).

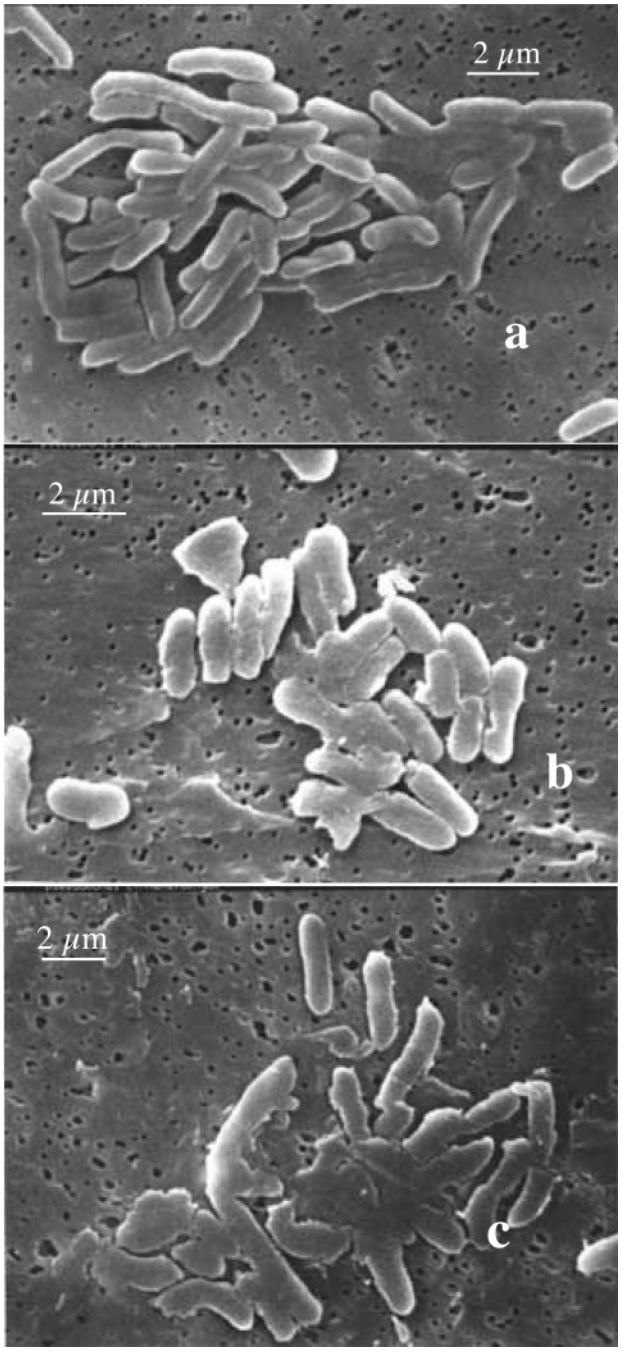

Figure 3. Scanning electron microscope images of *P. fluorescens* cells. (**a**) Untreated cells (7000×) and (**b**) cells after treatment with limonene (8000×) and (**c**) cinnamaldehyde (6000×).

B. thermosphacta showed visible alterations when treated with eugenol, cinnamaldehyde, and limonene (**Figure 5b−d**). The eugenol often resulted in a swelling of the cells and sometimes disruption of the external envelope.

DISCUSSION

The lipids biosynthesis pathway appears to be an important target for the development of novel antimicrobials (*13*−*15*). Active compounds from EOs are well-known to possess antibacterial activity (*10*, *16*, *17*).

Because of the hydrophobic character of the EO constituents, the cytoplasmic membrane appears to be a suitable site of their action, influencing the percentage of UFAs and altering its structure (*10*, *18*). In this research, the cells were treated with

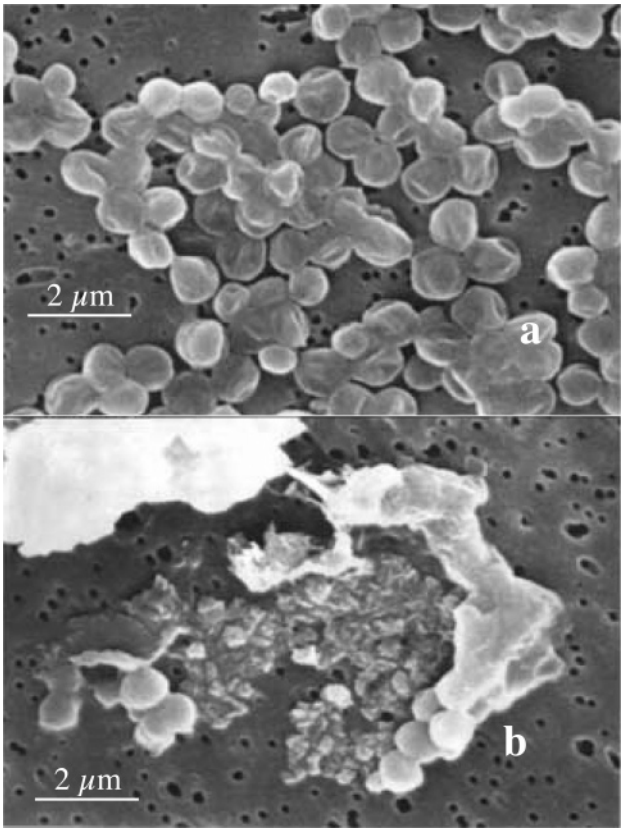

Figure 4. Scanning electron microscope images of *S. aureus* cells. (**a**) Untreated cells (10000×) and (**b**) cells after treatment with cinnamaldehyde (10000×).

a concentration of antimicrobials higher than minimum inhibitory concentrations (MICs) (*4*), to verify whether the action of the compounds was exerted on the fatty acid profiles. Among the substances used, thymol, eugenol, and carvacrol are phenolic compounds, limonene is a terpene, and cinnamaldehyde is an aliphatic aldehyde.

In a previous study (*12*), the adaptation of the cells to the presence of these compounds, at a concentration lower than the MIC, resulted in a sensitive increase of the percentage of the UFAs, which are well-known to give a high degree of fluidity to the membrane (*19*). In this work, after just 2 h of exposure of the cells to the compounds, the percentage of UFAs was found to be lower than SFAs. This result would support a mechanism of action of these compounds against the outer cell envelope, most probably interacting with the membrane lipid profile and causing membrane structural alterations appreciable by SEM examination.

The reorganization of the lipid profile shown after the treatments of the resting cells is strictly related to the presence of the EO compounds. Not much has been found in the literature to explain the increase of SFAs in cells treated with natural antimicrobials, even though many authors describe the bacterial fatty acid synthesis as an optimal target of many antibacterial agents (*13*−*15*, *20*).

The cells, after they were treated with thymol, eugenol, and carvacrol compounds belonging to the class of phenols, showed similar modifications of the lipid profile: an increase of saturated C16 (and shorter length) fatty acids, an increase of the saturated C18, and a corresponding decrease of the unsaturated C18 fatty acids. When the saturated C18 was not present in the control samples, we noticed that a decrease in C18:2trans and C18:3cis occurred in treated cells of *S.* Typhimurium while

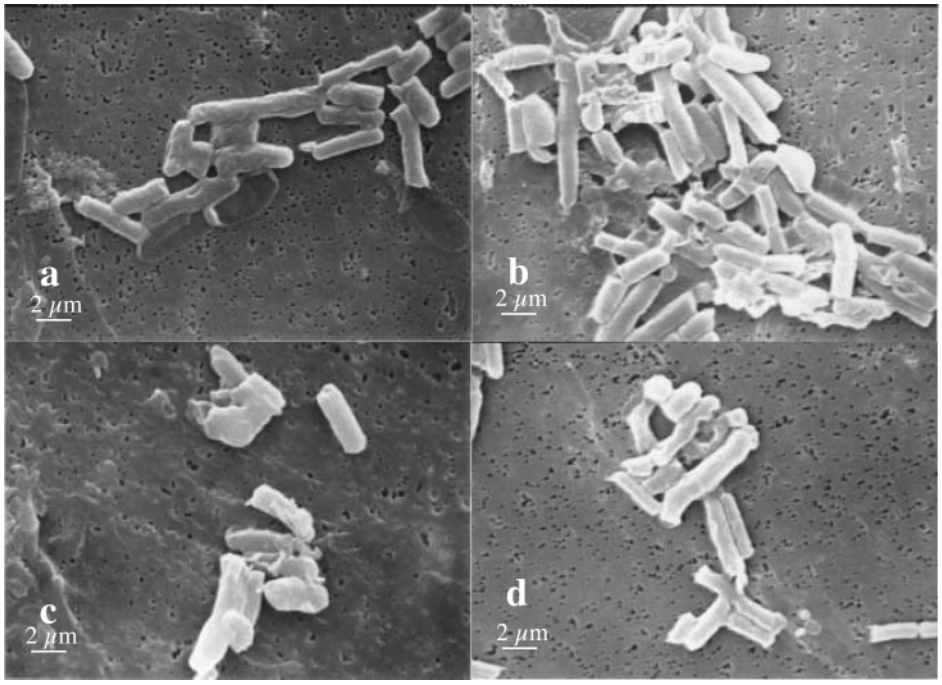

Figure 5. Scanning electron microscope images of *B. thermosphacta* cells. (**a**) Untreated cells (5000×) and (**b**) cells after treatment with eugenol (5000×), (**c**) cinnamaldehyde (5000×), and (**d**) limonene (5000×).

an increase of C18:1cis and a corresponding decrease of C18:2trans were observed in treated cells of *B. thermosphacta*.

A multicomponent membrane desaturase enzyme is employed by cells to produce the SFAs (*21*, *22*). Many authors (*2*, *9*, *23*) have demonstrated that phenolic and terpenic compounds act on the outer membrane, increasing its permeability. As a result of cell leakage, a dispersion of the desaturase enzymes in the suspension may be hypothesized, with a consequent action of these enzymes on the membrane fatty acids.

The conversion of cis fatty acids into their trans isomers is known to be an adaptive mechanism of the cells to the environmental stresses affecting the membrane function, and it is regulated by the activation of the enzymatic system cis trans isomerase (*Cti*) during stress exposition (*24*). The compounds used in this study did not cause the activation of the *Cti* system. This is in agreement with the results obtained by Neumann et al. (*25*), who found that stress conditions, such as osmotic stress caused by glycerol, cold shock, and high pH, did not activate the cellular K^+ uptake, which is the first cellular reaction to membrane damage leading to increased permeability. According to their study, Neumann et al. (*25*) concluded that in spite of the stress imposed, the cells did not activate the *Cti* system; the same appears to have happened in our case. Observing the results, it is clear that the major enzymatic system activated by the cells is the desaturase system. Moreover, its action is probably contemporaneous to a pool of enzymes acting on the fatty acids, leading to the increase of cis isomers, reduction of the chain length, and a general decrease of UFAs.

A high degree of SFAs in the membrane lipid bilayer is related to a decrease of membrane fluidity and to a consequent increase of its rigidity (*19*). Cronan (*26*) described that *E. coli* cells defective in UFAs continued the growth and phospholipids synthesis in the absence of a supply of UFAs but the cells soon began to leak metabolites and then lysed.

More lipophilic solvents that are soluble in water at low concentrations (benzene, aniline, amyl acetate, etc.) can induce an enrichment of SFAs and an increase in the total phospholipid concentration. An explanation for this reaction might be that the SFAs show a higher degree of membrane ordering, which also allows a higher surface density. These effects are known to oppose the partitioning of lipophilic solutes to a lipid bilayer, especially for compounds that are rather polar but not water miscible; this adaptation may be advantageous (*1*). For more apolar compounds, the membrane partition coefficients are such that mechanical exclusion does not suffice to withstand these compounds, and cells require additional adaptation mechanisms (*7*).

The strong reduction of the trans C18 fatty acid detected for *B. thermosphacta* and *S.* Typhimurium was not accompained by an increase of its cis isomer, but the increase of the C18:1cis and C17:1cis fatty acids, respectively, may be related to such a decrease.

The substantial fatty acid changes detected by GC analysis are probably related to the membrane alteration observed by SEM. Studies with liposome model systems confirmed that cyclic terpene hydrocarbons accumulate in the membrane, causing a loss of membrane integrity and dissipation of the proton motive force (*7*). Burt and Reinders (*18*) found, by electron microscope observation, that treating *E. coli* O157:H7 with oregano EO, which is rich in thymol and carvacrol, resulted in the collapse of cells after loss of their contents. Oussalah et al. (*27*) found important morphological damage and a disrupted membrane when *E. coli* O157:H7 and *Listeria monocytogenes* cells were treated with Spanish oregano, Chinese cinnamom, and savory oils. In particular, the authors found that the *E. coli* cells after treatment with the oils showed holes or white spots on the cellular wall. We found important surface alteration of *E. coli* O157:H7 and *S.* Typhimurium strains after they were treated with thymol and carvacrol.

In our experiments, eugenol caused a noteworthy change only in the fatty acid profile of *P. fluorescens*, it caused cell wall deterioration and a high degree of cell lysis in *E. coli*, and it caused deformity in the cellular shape of many *B. thermosphacta* cells. The hydroxyl group of eugenol is thought to bind to proteins, so most probably the slight effect of eugenol on fatty

acids and on the membrane is due to a different mechanism of action, which probably involves membrane proteins not visible by SEM.

In a study on *Saccharomyces cerevisiae*, it was demonstrated that *trans*-cinnamaldehyde caused a partial collapse of the integrity of the cytoplasmic membrane, leading to excessive leakage of metabolites and enzymes from the cell and finally loss of viability (*28*). Helander et al. (*2*) suggested that a possibility for the *trans*-cinnamaldehyde to penetrate into the cells was through OM-traversing porin proteins.

The high variation of UFAs in all of the strains tested in the presence of cinnamaldehyde suggests that this compound acts on the membrane, altering its lipid profile, increasing the surface areas of the membrane, and altering its structure. However, it is also able to penetrate the deeper part of the cell, leading them to death. The action of cinnamaldehyde does not result in the disintegration of the membrane; in fact, the images show that just the *S. aureus* (**Figure 4**) cells result in collapse, while the other cells show swelling on the surface, perfectly visible on *B. thermosphacta* (**Figure 5**).

According to Rhayour et al. (*29*), the different types of damage observed by SEM could be different from one bacterium to another when treated with the same EO. In light of our findings, the antimicrobial action of the tested compounds takes place in different ways, and each one is involved in increasing the membrane disorder. The mechanism of action implicates the alteration of fatty acid profiles along with damage, alteration, or disintegration of the cell envelope structure.

A better understanding of the interaction between EO compounds and cell target molecules is going to be fundamental to work out the best environmental conditions to be used to ensure an effective antimicrobial activity.

ACKNOWLEDGMENT

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