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NO INDUCTION OF ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES DURING CONTINUOUS EXPOSURE TO EUGENOL AND CITRAL

Annotation: *Adaptation to eugenol and citral was done by sequential exposure of the pathogens to increasing concentrations of the essential oils. The M2-A9 standard was used to determine the antibiotic susceptibility. The effect of eugenol and citral on the adherence ability was evaluated by the crystal violet assay. The impact of adaptation to eugenol on virulence was estimated using the Galleria mellonella model. No development of resistance to the components and antibiotics was observed in the adapted cells of S. aureus, MRSA, and L. monocytogenes.*

Keywords: *essential oil, adaptation, resistance, virulence.*

ОТСУТСТВИЕ ПРОТИВОДЕЙСТВИЯ АНТИМИКРОБНОЙ УСТОЙЧИВОСТИ У МОНОЦИТОГЕНОВ STAPHYLOCOCCUS AUREUS И LISTERIA ПРИ НЕПРЕРЫВНОМ ВОЗДЕЙСТВИИ ЕВГЕНОЛА И ЦИТРАЛА

Аннотация: *Адаптация к эвгенолу и цитралю осуществлялась путем последовательного воздействия на патогены увеличивающихся концентраций эфирных масел. Стандарт M2-A9 использовали для определения восприимчивости к антибиотикам. Влияние эвгенола и цитраля на адгезионную способность*

оценивали методом кристаллического фиолетового. Влияние адаптации к эвгенолу на вирулентность оценивали с использованием модели Galleria mellonella. В адаптированных клетках S. aureus, MRSA и L. monocytogenes развития устойчивости к компонентам и антибиотикам не наблюдалось.

Ключевые слова: *эфирное масло, адаптация, устойчивость, вирулентность.*

1- Introduction:

Either in clinical or in food-processing environment, the bacterial pathogens encounter a myriad of stresses (to name a few; exposure to antimicrobial agents, acids, bases, different osmotic pressures, different pH and water activity values, heat, freezing and thawing). The action of these stresses compromises the cell viability but at the same time, the cells experience a gene expression alteration directed to protect them against each individual stress or even mount a cross-protection response that not only provide the cell with the means to defeat the individual stress but also a complex of stresses injuries(1,C142–150).The threatening emergence of antibiotic-resistant bacteria is of great concern worldwide, and the possibility of food microbiota being reached by antibiotic resistance is greatly disturbing (2, 1646–1653).

1-1 Adaptation to eugenol and citral

The bacterial strains were grown in BHI agar plates during 24 h at 37 or 30 °C. From each plate, a loop was used to inoculate 10 mL of BHI broth, and the culture was incubated overnight. From the overnight, 300 μL culture was centrifuged (2790 × g, 5 min at 4 °C). The pellet was resuspended in 300 μL BHI supplemented with 0.05 mg mL−1 eugenol or citral and used to inoculate 10 mL of BHI (supplemented with the same concentration). Cultures were incubated at 37 °C during 18–24 h. The culture was used to inoculate fresh medium supplemented with eugenol or citral (0.05 mg mL−1). During 4 days, bacteria were transferred sequentially at the same concentration. At each serial passage, the bacterial growth was followed by optical density (OD600 nm). Following the four sequential passages, bacteria were transferred to BHI at higher concentration (increment

of 0.02 mg mL−1). The process was repeated until bacteria stop growing. Control cultures (bacteria were grown in medium with no EO component) were maintained in parallel. Three independent replicates were carried out (1,C142–150).

1-2 Influence of eugenol and citral on adherence

The impact of eugenol and citral on adherence of S. aureus and L. monocytogenes was carried out according to Adrião et al. (2008). Bacterial suspensions (OD600 nm = 0.4–0.5) in BHI supplemented with 0.05 and 0.1 μg mL−1 of eugenol or citral were allowed to adhere for 30 min. Nonadherent cells were removed by washing the wells twice with sterile phosphate-buffered saline (PBS). Wells were air-dried and adherent cells heat-fixed at 80 °C for 30 min and stained with 0.1% crystal violet (3,C1928–1939).

2- Results:

Susceptibility to eugenol and citral

The observed MIC values for eugenol and citral determined using the agar dilution and the microdilution methods are indicated in Table1. Differences were observed between the MIC values either of eugenol or of citral, in particular for S. aureus strains with trend to a lower MIC values obtained by the microdilution method; the range of the MIC value for eugenol was 0.10–0.15 mg mL−1 for S. aureus strains determined by the agar dilution method, whereas the MIC value determined by the microdilution method ranged from 0.06 to 0.08 mg mL−1. The susceptibility of S. aureus strains to citral was similar to eugenol (Table 1). In contrast to S. aureus, no differences were observed in the MIC values of eugenol for L. monocytogenes determined by the two methods (Table 1).

Table 1

Minimum inhibitory and minimum bactericidal concentrations of eugenol and citral

Bacteria	**Eugenol**		**Citral**			
MIC (mg mL^{-1})	**MBC (mg mL^{-1})**		**MIC (mg mL^{-1})**	**MBC (mg mL^{-1})**		
Agar dilution	**Microdilution**		**Agar dilution**	**Microdilution**		
Staphylococcus aureus ATCC 6538	0.1	0.06***	0.1	0.1	0.08***	0.1
Staphylococcus aureus methicillin-resistant 4 (MRSA 4)	0.1	0.08***	0.1	0.1	0.06***	0.1
Staphylococcus aureus methicillin-resistant 12 (MRSA 12)	0.15	0.08***	0.1	0.15	0.08***	0.1
Listeria monocytogenes EGD	0.08	0.08	0.1	0.1	0.08***	0.1
Listeria monocytogenes ScottA	0.08	0.08	0.1	0.08	0.06***	0.08
Listeria monocytogenes C882	0.08	0.08	0.1	0.1	0.08***	0.1

References:

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