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Modulation of drug resistance and biofilm formation of *Staphylococcus aureus* isolated from the oral cavity of Tunisian children

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ABSTRACT

Objectives: This study aims to investigate the antimicrobial and the anti-biofilm activities of *Lactobacillus plantarum* extract (LPE) against a panel of oral *Staphylococcus aureus* ($n = 9$) and *S. aureus* ATCC 25923. The *in vitro* ability of LPE to modulate bacterial resistance to tetracycline, benzalchonium chloride, and chlorhexidine were tested also.

Methods: The minimum inhibitory concentrations (MICs) and the minimal bactericidal concentrations of *Lactobacillus plantarum* extract, tetracycline, benzalchonium chloride and clohrhexidine were determined in absence and in presence of a sub-MIC doses of LPE (1/2 MIC). In addition, the LPE potential to inhibit biofilm formation was assessed by microtiter plate and atomic force microscopy assays. Statistical analysis was performed on SPSS v. 17.0 software using Friedman test and Wilcoxon signed ranks test. These tests were used to assess inter-group difference ($p < 0.05$).

Results: Our results revealed that LPE exhibited a significant antimicrobial and anti-biofilm activities against the tested strains. A synergistic effect of LPEs and drug susceptibility was observed with a 2–8-fold reduction.

Conclusion: LPE may be considered to have resistance-modifying activity. A more detailed investigation is necessary to determine the active compound responsible for therapeutic and disinfectant modulation.

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Introduction

Staphylococci are important causes of infections associated with various devices. *Staphylococcus aureus* is one of the most frequent human pathogen associated to medical implant.[1] It has been isolated from parotitis,[2] gingival pockets,[3] periodontitis,[4] carious lesions,[5] and gingivitis.[6] Orthodontic and other oral appliances may act as reservoir of resistant opportunistic *S. aureus*.[7]

S. aureus has the capacity to adhere to various medical devices and form biofilm.[8] Biofilm is associated to the *ica* gene encoding for polysaccharide intercellular adhesion (PIA)[9] which leads to increased bacterial drug resistance compared to planktonic cells.[10,11]

S. aureus is able to acquire resistance to antibiotics and to antiseptic agents *via* efflux pumps (TetK, msrA transporters) systems, which export certain tetracyclines and macrolides molecules outside the cells. On the other hand, some multidrug resistance (MDR) proteins like NorA and QacA confer resistance to a wide range of structurally unrelated antiseptics.[12] In clinical practice, chlorhexidine (CHX) and quaternary ammonium compounds (QAC) are the most frequently used disinfectants to reduce and prevent the spread of pathogens. Multidrug resistance pumps have been recognized as mediators of a number of commonly used ammonium compounds and detergents.[13] However, the use of these disinfectants in hospitals may contribute to the rise of disinfectant-resistant bacteria[14,15] due to QAC-resistant genes (*qac*A, *qac*B, and *qac*C), which have been identified in several staphylococcal species.[16–18]

In this study, the antibacterial activity of *Lactobacillus plantarum* extract (LPE) was investigated. In addition, the ability of LPE to modulate the susceptibility of *S. aureus*, isolated from the oral cavity of Tunisian children, to tetracycline (TET), benzalchonium chloride (BC) and clohrhexidine (CHX) was tested. Secondly, the effects of disinfectant and antibiotic associated with LPE were tested for biofilm inhibition of *S. aureus* to polystyrene and glass using microtiter plate and atomic force microscopy assays, respectively.

Material and methods

Antibacterial activity of L. plantarum

L. plantarum strain was isolated from Tunisian traditional fermented milk (ricotta cheese), identified with a conventional method using Api-50 CHL system (BioMerieux, Marcy-l'Étoile, France) and by polymerase chain reaction (PCR) technique using *L. plantarum*-specific primers: forward IDL04F: 5′-AGGGTGAAGTCGTAACAAGTAGCC-3′ and reverse IDL62R 5′-CTAGTGGTAACAGTTGATTAAAACTGC-3′, giving a product size of about 428 bp as described previously.[19]

Cell-free supernatant was obtained by centrifuging (10,000 × *g* for 10 min at 4 °C) *L. plantarum* culture, grown in MRS broth for 16 h at 30° C. The supernatant was adjusted at neutral pH and filter-sterilized (0.22 μm, Millipore). The obtained *L. plantarum* extract (LPE) was conserved at 4 °C until use.

Antibacterial activity of LPE was tested against nine strains (Table 1) isolated from the oral cavity of Tunisian children and *S. aureus* ATCC 25923 using the broth microdilution method.[20,21] The LPE was tested alone or in combination with antibiotics.

Microorganisms

The *S. aureus* strains ($n = 9$) used in this study were isolated from the oral cavity of Tunisian children from the dental clinic of dentistry (Monastir, Center of Tunisia).

The criteria for inclusion were: no antibiotic treatment four weeks prior to sampling, no use of mouth rinses or any other preventive measure that might involve exposure to antimicrobial agents, and no systemic disease. A sterile swab was used for sample collection from the oral cavity of each patient. After incubation (24 h at 37 °C), swabs were plated on blood agar plates supplemented with 5% sheep blood (24 h at 37 °C) and bacterial identification was achieved using conventional methods.

Minimal inhibitory concentration determination

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of LPE (5–90%, v/v), BC (Acros organics, USA) (0–1024 μg/mL), CHX (Sigma–Aldrich, USA) (0–1024 μg/mL) and tetracycline (TET) (0–1024 μg/mL) according to the Clinical and Laboratory Standards Institute (2006) guidelines.[22]

After 24 h of incubation, bacterial growth was evaluated by the presence of turbidity and a pellet on the well bottom. MIC was defined as the lowest concentration of the compound that had no macroscopically visible growth. All experiments were carried out three times.

Minimal bactericidal concentration determination (MBC)

To determine the MBC values, 10 μL of each well medium, with no visible growth was removed and inoculated in Muller Hinton agar plates. MBC was defined as the lowest concentration at which 99% of the bacteria were killed. Each experiment was repeated at least twice.[23]

Modulation of S. aureus susceptibility to BC, CHX and TET by LPE

To determine the potential effect of LPE to modulate drug resistance of *S. aureus*, MICs of BC, CHX and TET (ranging from 0.5 to 2048 μg/mL) were determined alone and combined with a sub-MIC of LPE (1/2 MIC, v/v) using the microtiter plates assay.[24]

Determination of anti-biofilms activity by microtiter plates assay

The anti-adhesion properties of LPE (ranging from 10% to 90%, v/v) to *S. aureus* strains were tested as previously described by Merritt et al.[25] Briefly, the bacterial culture was grown in Tryptone soy broth (TSB) at 37 °C for 24 h. Two microliters were disposed into each well of 96-well plates in the presence of