Original Article

Comparison of antibacterial effects between antimicrobial peptide and bacteriocins isolated from *Lactobacillus plantarum* on three common pathogenic bacteria

Liu Ming¹, Qian Zhang², Le Yang³, Jian-An Huang⁴

¹Department of Infection, Affiliated Hospital of Nanjing Medical University, Changzhou Second People’s Hospital, Changzhou 213003, China; ²Department of Respiratory, Affiliated Hospital of Nanjing Medical University, Changzhou Second People’s Hospital, Changzhou 213003, China; ³Infection Control Section, Affiliated Hospital of Nanjing Medical University, Changzhou Second People’s Hospital, Changzhou 213003, China; ⁴Department of Respiratory, The First Affiliated Hospital of Suzhou University, Suzhou 215000, China

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Abstract: New strategies for the prevention or treatment of infections are required. The purpose of this study is to evaluate the effects of antimicrobial peptides and bacteriocins isolated from *Lactobacillus plantarum* on growth and biofilm formation of three common pathogenic microbes. The antibacterial properties of the antimicrobial peptide Tet213 and bacteriocins were tested by the disc diffusion method. Tet213 and bacteriocins showed inhibitory effects on biofilm formation for the three organisms, as observed by fluorescence microscopy. Furthermore, Tet213 and the bacteriocins all showed antimicrobial activity against the three bacterial species, with Tet213 having a greater inhibitory effect on *S. aureus* than the bacteriocins (P < 0.05), while the bacteriocins showed stronger antimicrobial activity against *S. sanguis* (P < 0.05).

Keywords: Antimicrobial activity, antimicrobial peptide, bacteriocin, biofilm

Introduction

For many years traditional antibiotics have played an important role in the treatment of infections [1]. However, the extensive use of these “traditional antibiotics” has created significant problems [2]. Many currently used antibiotics are no longer effective at inhibiting or killing certain pathogens. More and more research has focused on the development of new classes of antibiotics, such as antimicrobial peptides (AMPs) or other new compounds with novel mechanisms of action or spectrum of activity [3, 4]. These new antibiotics may hold promise for treating refractory infections and inflammation [5].

Lactobacilli are known for their production of antimicrobial compounds, including bacteriocins and bacteriocin like peptides [6, 7]. Most of the bacteriocins produced by *Lactobacillus* species are small, thermally stable proteins, known as type II bacteriocins [8]. These compounds can induce rupture of the cell membrane, causing leakage of cell contents and playing a role in sterilization [9]. Bacteriocins isolated from *Lactobacillus* species have also been reported to have significant antibacterial effects on common clinical pathogens *in vitro* [10, 11]. However, a comparison of the antibacterial effects of both AMPs and bacteriocins has been rarely reported.

The objective of this study was to compare the antibacterial effect of an antimicrobial peptide (Tet-213) and bacteriocins isolated from *Lactobacillus plantarum* on *Staphylococcus aureus*, *Streptococcus sanguis* and *Pseudomonas aeruginosa*. To our knowledge, this is the first report of the comparison of the antibacterial effect of an antimicrobial peptide and bacteriocins isolated from *Lactobacillus plantarum* on three kinds of pathogenic bacteria.
Comparison between antimicrobial peptide and bacteriocins

Table 1. Tet213 and bacteriocin zone diameters from S. aureus, S. sanguis and P. aeruginosa using a 30 µg disk read at 24 h of incubation (means ± SD)

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>S. sanguis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet213</td>
<td>12.5 ± 3.1a</td>
<td>11.7 ± 1.1a,b</td>
<td>17.5 ± 3.5a,b</td>
</tr>
<tr>
<td>Bacteriocin</td>
<td>17.5 ± 2.9a</td>
<td>18.5 ± 4.4a</td>
<td>10.3 ± 1.7a</td>
</tr>
<tr>
<td>Tinidazole (control)</td>
<td>7.9 ± 1.9</td>
<td>7.6 ± 2.4</td>
<td>8.7 ± 1.6</td>
</tr>
</tbody>
</table>

Note: a means P < 0.05 compared with bacteriocin, b means P < 0.05 compared with control.

Materials and methods

Strains and culture conditions

Lactobacillus plantarum ATCC 8014 was purchased from ATCC. The three strains used for the experiments were P. aeruginosa ATCC 90271, S. sanguis ATCC10556 and S. aureus ATCC 25923. The three strains were incubated on Columbia sheep blood agar (BioMérieux, France) at 37°C under microaerophilic conditions (6% CO₂) for 24 h. The in vitro experiments were performed in Brian Heart Infusion (BHI) broth and Brian Heart Infusion agar (Oxoid, UK).

Peptides

Tet-213 (amino acid sequence: KRWW KWW-RRC) was synthesized by Shanghai Apeptide Co. Ltd (Shanghai, China) (~94% purity by HPLC).

Production of culture supernatants

Lactobacillus plantarum ATCC 8014 was grown in MRS broth at 37°C for 24 h. Supernatants were harvested by centrifugation (7000 g for 10 min), adjusted to pH 6.5, treated with catalase (5 mg/ml), to eliminate the inhibitory activity due to hydrogen peroxide, and filtered through a 0.22 µm pore size filter (Millipore, USA).

Isolation of bacteriocins

The bacteriocins from Lactobacillus plantarum ATCC 8014 was isolated according to the methods by Lash et al [12]. In brief, 100 ml culture supernatants of L. plantarum were precipitated using 60 g ammonium sulfate. The crude precipitate was centrifuged for 20 min at 10,000 × g at 4°C. The resulting pellet was resuspended in 2 ml of 10 mM Tris-HCl pH 7.4. The resuspended pellet was concentrated by using an Amicon Ultra-4 Centrifugal Filter device (Millipore, USA) with a molecular weight (MW) cut-off of 10 kDa [13] to a final volume of 0.5 ml at 4°C and then the final suspension was concentrated by freeze-drying [14] and stored at 4°C.

Antimicrobial activity

The antimicrobial activity of Tet-213 and bacteriocins were tested by the agar disc diffusion method on BHI agar according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [15]. The test strains P. aeruginosa ATCC 90271, S. sanguis ATCC10556 and S. aureus ATCC 25923 used in this study were the fully sequenced strains. The following antibiotics were tested: Tet213 30 µg, bacteriocin 30 µg, ceftazidime 30 µg. Disks were incubated for 24 h at 35°C. Inhibition zone diameters for antimicrobial peptide and bacteriocin were noted and compared.

Inhibitory effects of the test compounds on biofilm formation in the three test strains

Biofilm formation was conducted according to previously published methods [16]. In detail, the S. aureus, S. sanguis and P. aeruginosa cultures were incubated in BHI broth and grown under microaerophilic conditions for 24 h at 37°C. The cells were washed three times with PBS and then adjusted with PBS to 0.5 McFarland standard (1.5 × 10⁶ CFU/ml) by using a Densicheck (BioMérieux, France) [17]. Individual Petri dishes were filled with 10 ml of BHI broth, and a sterile coverslip (18 mm diameter) was added to each dish. 100 µl of individual bacterial suspensions were mixed with 500 µl of antimicrobial peptide or bacteriocin suspensions (100 µg/ml) in the dishes. PBS was used as a control. All Petri dishes were incubated under microaerophilic conditions at 37°C for 24 h.

After 24 h of biofilm formation, each coverslip was washed with 10 ml of PBS to remove unattached cells. After fixation with 1% formaldehyde, each coverslip was stained with a 0.01% acridine orange solution [18] (Sigma, USA) and then observed with a Nikon 80i microscope (using the green fluorescence channel). Image analysis was conducted by Image-Pro Plus 4.5 software (Media Cybernetics, USA) [19].
Comparison between antimicrobial peptide and bacteriocins

**Statistics**

All tests were performed in triplicate. SPSS 14.0 software for Windows was used for data analysis. A one-way analysis of variance (ANOVA) was performed first, and then data comparisons were performed with paired t-test.

**Results**

**Antimicrobial activity**

Results for the antimicrobial activity of Tet213 and bacteriocins in the disc diffusion assay are shown in **Table 1**. Tet213 and the bacteriocins all showed greater antimicrobial activity against *P. aeruginosa*, *S. aureus* and *S. sanguis* compared with the control (*P* < 0.05). For *S. aureus*, Tet213 showed a greater inhibitory effect compared with the bacteriocins (*P* < 0.05) while the bacteriocins showed stronger antimicrobial activity against *S. sanguis* (*P* < 0.05). Both Tet213 and the bacteriocins showed no significant inhibitory effect on the growth of *P. aeruginosa* (*P* > 0.05). The inhibition zone tests are shown in **Figure 1**.

**Inhibitory effects on biofilm formation**

Individually, Tet213 and the bacteriocins showed strong inhibitory effects on biofilm formation in *P. aeruginosa*, *S. aureus* and *S. sanguis* (*P* < 0.05, **Figure 2**), but there was no significant difference in biofilm formation of these strains, when the effects of Tet213 and the bacteriocins were compared (*P* > 0.05) (**Figure 1**). Fluorescent images of these biofilms are shown in **Figure 3**.

**Discussion**

AMPs have a wide antimicrobial spectrum, including activity against many multi-drug resistant bacteria [20]. Furthermore, resistance does not develop during bacterial killing, making these compounds attractive candidates for drug development [21, 22]. Our results showed that Tet213 had strong antibacterial activity against *P. aeruginosa*, *S. aureus* and *S. sanguis*, which agree closely with previous experimental results [23-25].

AMPs and bacteriocins from lactobacilli are known to be more active against Gram-positive than Gram-negative bacteria [26]. Our results, however, showed that there was no obvious difference in antibacterial activity against the three strains. Bacteriocins isolated from...
Lactobacillus species usually have a broad antimicrobial spectrum [27] and can inhibit common pathogenic bacteria that are responsible for food spoilage or human diseases. S. aureus can cause suppurative infections, and also can produce enterotoxins, which result in food poisoning [28]. P. aeruginosa can cause wound infections after surgery [29] and is especially likely to develop resistance [30]. S. sanguis causes endocarditis [31], an infection where bacterial biofilms play a prominent role and are often responsible for treatment failure [32]. Our research shows that Tet213 and bacteriocins all have good inhibitory effects on biofilm formation, which indicates that Tet213 and bacteriocins may be effective in treating the above mentioned diseases. In this study, there was little difference in the antimicrobial activity between Tet213 and bacteriocins, while differences in inhibitory effects of these compounds on biofilm formation were not obvious. However, our studies were based on a small number of test strains and in future experiments, we will utilise a larger panel of experimental bacteria.

In conclusion, an antimicrobial peptide and bacteriocins isolated from Lactobacillus plantarum were able to inhibit the growth and biofilm formation of S. aureus, S. sanguis and P. aeruginosa. Their antibacterial activity against S. aureus, S. sanguis and P. aeruginosa is slightly different, suggesting that these compounds may be one promising way to control infectious diseases.

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Disclosure of conflict of interest

None.
Comparison between antimicrobial peptide and bacteriocins

Address correspondence to: Dr. Jian-An Huang, Department of Respiratory, The First Affiliated Hospital of Suzhou University, Suzhou 215000, China. Tel: 0086-512-67780050; Fax: 0086-512-67780050; E-mail: huangjianan1977@126.com

References

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determinants,’ total hydrophobicity, hydrophobe type and location as design parameters to improve the therapeutic ratio. Chem Biol Drug Des 2011; 77: 225-240.


