Defensive secretions of millipedes *Megaphyllum unilineatum* (C. L. Koch, 1838), *Pachyiulus hungaricus* (Karsch, 1881) and *Cylindroiulus boleti* (C. L. Koch, 1847) (Diplopoda, Julida) as antimicrobial agents in the inhibition of biofilms of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus*

**INTRODUCTION**

**IN RECENT YEARS:** increased resistance of microorganisms to antibiotics

**IMPERATIVE:** discovery of new antimicrobial agents

**OBJECTIVES**

Determine the inhibition and degradation of the formed biofilm of *P. aeruginosa* PAO1 and *S. aureus* by defense secretions of selected millipede species from the family Julidae as well as to determine their antimicrobial activity by MTT assay.

**MILLIPEDES (DIPLOPODA)**
- complex defense against predators
- various chemical compounds
- showed antimicrobial activity
- ORDER JULIDA

**Megaphyllum unilineatum (MUN)**

**Pachyiulus hungaricus (PHU)**

**Cylindroiulus boleti (CBO)**

**METHOD / DESIGN**

**BIOFILM FORMATION** was quantified by the crystal violet staining method

**ANTIMICROBIAL ACTIVITY** was examined using the broth dilution minimum inhibitory concentration (MIC) test.

**ANTIBIOFILM ACTIVITY**

**RESULTS**

**ANTIMICROBIAL ACTIVITY**

- Defensive secretions showed a better antimicrobial activity against *S. aureus* compared to *P. aeruginosa* PAO1, which is most likely due to the high resistance of this bacterial strain.

<table>
<thead>
<tr>
<th>mg/ml</th>
<th>S. aureus</th>
<th>P. aeruginosa (PAO1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>MUN</td>
<td>0.03</td>
<td>&gt;1</td>
</tr>
<tr>
<td>PHU</td>
<td>0.06</td>
<td>1</td>
</tr>
<tr>
<td>CBO</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.006</td>
<td>0.012</td>
</tr>
<tr>
<td>DMSO</td>
<td>&gt;2.5%</td>
<td>&gt;2.5%</td>
</tr>
</tbody>
</table>

**ANTIBIOFILM ACTIVITY**

**A**
- Inhibition of *S. aureus* biofilm formation

**B**
- Degradation of the already formed *S. aureus* biofilm

**C**
- Inhibition of *P. aeruginosa* PAO1 biofilm formation

**D**
- Degradation of the already formed *P. aeruginosa* PAO1 biofilm

Mineral inhibitory concentrations - MIC and Minimum bactericidal concentrations – MBC on *S. aureus* and *P. aeruginosa* PAO1 strains, presented in mg/mL.

**DMSO vs ETHANOL**

- Inhibition of biofilm formation of defensive secretions (A) PHU and (B) MUN and degradation of the already formed *P. aeruginosa* PAO1 biofilm of (C) PHU and (D) MUN; both ethanol (E) and DMSO (D) extracts.

- DMSO extracts showed a stronger antibiofilm effect except in case B, where ethanolic MUN extract showed a stronger effect in biofilm inhibition.

- The antibiofilm effect was observed in all tested defense secretions and was more pronounced against *S. aureus* than against *P. aeruginosa* PAO1.

- The strongest biofilm inhibition of *S. aureus* was at the highest tested concentrations (2 × MIC) while degradation of already formed biofilm was shown at lower tested concentrations (MIC/4).

- The strongest inhibition of *P. aeruginosa* PAO1 biofilm formation was observed at the lowest tested concentrations for PHU (R2 and 54%), and CBO (64.3 and 36.5) while MUN had the strongest activity at the lowest tested concentration of 0.06 mg/mL (34.3%).

- All examined defense secretions had similar degradation activity of *P. aeruginosa* PAO1 biofilm with stronger activity at lower tested concentrations (about 30%).

**CONCLUSIONS**

The defensive secretions of MBO, PHU, and CBO show a good basis for further investigations of their use as antimicrobial agents, especially against *S. aureus*.

Acknowledgements. This work was supported by the Serbian Ministry of Education, Science and Technological Development (Grant No. 451-03-9/2021-14/ 200178).