Effect of lupinifolin on the proteome of multidrug-resistant *Enterococcus faecium*

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Introduction

Vancomycin-resistant enterococci (VRE) is a major concern of a global public health because it has been a leading cause of healthcare-associated infections (Belkuz et al., 2019). Currently, bioactive compound have gained popularity and played a key role as an alternative treatment against infectious diseases, including antibiotic drugs (Atamasov et al., 2021).

- Lupinifolin is a purified flavonoid in Thai traditional herbs.
- The bioactive compound used in this study was isolated from *Albizia myriophylla* Benth.
- Lupinifolin showed the potential antibacterial activity against Gram-positive bacteria including *Enterococcus* (Joycharat et al., 2013; Joycharat et al., 2016; Sianglum et al., 2019).
- Lupinifolin is a promising new antibiotic.
- However, the mechanism of action underlying antibacterial effects of this compound is not yet understood.

Material and methodology

**Antibacterial activity**

- Clinical isolate, *E. faecium* HTY0236 was collected from urine sample.
- *E. faecalis ATCC29212* was used as a control quality.
- The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin, vancomycin and penicillin G were determined by broth microdilution method (CLSI, 2019).

**Proteomic analysis**

- The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin, vancomycin and penicillin G were determined by broth microdilution method (CLSI, 2019)
- Amino acid sequences were compared and generated using mass spectra by LC-MS/MS.
- Protein quantitation was performed using a proteomics analysis software (Progenesis, LCC, 2017).
- The resulting data were analyzed using the Genomic Interpretation Database (GND).
- The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin, vancomycin and penicillin G were determined by broth microdilution method (CLSI, 2019)

**Bioinformatic data analysis**

- Protein sequences were analyzed using the Genomic Interpretation Database (GND).
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- The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin, vancomycin and penicillin G were determined by broth microdilution method (CLSI, 2019)

Results

**Table 1** MIC and MBC of *E. faecium* HTY0236 and *E. faecalis* ATCC29212

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Lupinifolin (μg/ml)</th>
<th>Vancomycin (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td><em>E. faecium</em> HTY0236</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC29212</td>
<td>0.5</td>
<td>2</td>
</tr>
</tbody>
</table>

**Figure 1.** Proteomic profiling of *E. faecium* HTY0236 after lupinifolin exposure. RIEG pathway enrichment analysis of differentially expressed proteins (DEPs). DEPs were functionally sorted into 17 functional categories according to the gene ontology annotation.

- Proteins from same pathway probably carried out their biological function together.
- RIEG database was applied to analyze the biological pathways of the lipopolysaccharide treatment, DEPs were mapped to RIEG pathway database in control vs. treatment group.
- Among these pathways, the majority of pathways were related to transporter membrane, carbohydrate metabolism, cell wall organization and replication, and DNA repair.
- The results suggested that lupinifolin mainly affected the transporter membrane, carbohydrate metabolism, cell wall organization and replication, and DNA repair.

Conclusions

- These findings mainly suggest the comprehensive proteomic profiling related to action of lupinifolin against vancomycin-resistant *Enterococcus*.
- Our works provide further evidence to support therapeutic efficiency of lupinifolin which could lead to the development of a new effective drug for treatment of multidrug resistant infections.

References


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