Analysis of Antibacterial Compounds Found in *Vitis aestivalis* Fungal Endophytes

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Abstract

With over two and a half million people becoming infected with an antibiotic resistant bacteria each year, antibiotic resistance has become a major world threat and new drugs are needed to combat the growing epidemic. Previous research has shown that fungal endophytes found in Arkansas-grown *Vitis aestivalis* (Norton/Cynthiana) vines – a hardy grape native to the United States – showed antimicrobial activity against two common bacteria, *S. aureus* and *E. coli*. In order to determine which compounds are responsible for the antibacterial activity, the grape endophytic fungi were grown in potato dextrose broth for two weeks in a controlled environment and the compounds were extracted from the broth solution via an ethyl acetate wash. The ethyl acetate solutions were concentrated under reduced pressure and analyzed by GC/MS. Known compounds were identified by comparison of their mass spectra to the mass spectra located in the National Institute of Standards and Technology 2011 database.

Purpose and Hypotheses

- **Purpose:** To determine the structures of the antibacterial compounds present in Arkansas *Vitis aestivalis* grape tissue
- **Hypotheses:** The compounds found in the fungi will be isolated and identified via various chemical methods to determine the types of compounds.

Background Information

- The CDC reports that at least two million people are diagnosed with an antibiotic resistance bacteria each year. (1)
- *V. vinifera* (and other plants) endophytes have been shown to have antibacterial activity. (6)
- In recent years, bacteria have gained an increase in antibiotic resistance culling in the formation of multi-drug resistant bacteria, thus leading to the need of new antibiotics. (7)
- *E. coli* (gram-negative), a common bacteria found in the human intestines, is both a leading cause of food poisoning and can cause Hemolytic Uremic Syndrome which leads to loss of kidney function. (3)
- *S. aureus* (gram-positive) causes food poisoning and health infections. (8,5)
- Species of *Xylaria*, *Coniothyrium*, and *Alternaria* have all been proven to have inhibitory properties against *S. aureus*. (2, 10, 4)
- *E. coli* is currently resistant to tetracycline, ampicillin, and gentamicin. *S. aureus* is resistant to vancomycin, methicillin, and penicillin for. (9,8)
- Previous research indicated that the inhibition by the fungi was significant and that there was at least 3 significant compounds in the ethyl acetate fraction and 5 significant compounds in the aqueous fraction.

Methodology

- Four fungi were chosen. Two showed inhibition against *S. aureus*, one against *E. coli*, and one control. The fungi were grown for one month in potato dextrose broth before being filtered to remove the solids
- The fungal extract aqueous solution was separated via a 1:1 ethyl acetate rinse The compound containing solutions fractions were concentrated to approximately 5-10 mL.
- The concentrated solutions were tested via TLC, HPLC and GC/MS.
- The GC/MS results were then compared to the National Institute of Standards and Technology 2011 Database

Results

<table>
<thead>
<tr>
<th>Plant Tissue</th>
<th>Section</th>
<th>Description</th>
<th>Fungi Name</th>
<th>Soluble in ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf A</td>
<td>8C</td>
<td>Pink/White</td>
<td>Fungal Endophyte <em>Xylaria</em></td>
<td>No</td>
</tr>
</tbody>
</table>

Table 1: Vegetative pieces used, fungal description, likely genus species, and solubility.

Discussion and Conclusions

- From the TLCs performed, it is possible that there are both polar and nonpolar compounds in the solutions.
- All ethyl acetate fractions contained compounds that were similar to known compounds in the Database
- Further research is needed to determine what compounds are found in the aqueous fraction.

References


Acknowledgements

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