Screening of Cinnabar Color Deteriorate Fungi on Shan Style Temple Mural Painting from the 24th Buddhist Era in Chiang Mai

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Shan Style Mural Paintings

Watt Buak Kroo Luang

Watt Tha Karm

Watt Pa Dao

Discussion

A total of 73 isolates of fungi were found on the mural paintings. The most abundant species are Penicillium oxalicum, P. citrinum and Trichoderma caeruleimontis; isolates were identified by molecular biology technique using ITS genes for sequencing, the results showed 6 genera of fungi as follow: Aspergillus sp., Coprinellus sp., Curvularia sp., Fusarium sp., Penicillium sp., and Trichoderma sp. which usually present as normal flora on mural paintings. (Gherna 1999) (Ma et al. 2015) (Duand et al. 2017)

Fungi in the phylum of Ascomycota are responsible for biodeterioration of mural paintings as they can penetrate their hypha deep into mural’s layer and able to utilized substance which makeup the structure of the paintings and some species can produce secondary metabolites as well as colored spores which can alter the aesthetics of the paintings (Senbou 2014) (Gomiou et al. 2015). Four (4) isolates of fungi had shown an ability to deteriorate Cinnabar. When compared to the standard color of cinnabar incorporate PDA; T. caeruleimontis and isolate COBL2 faded the cinnabar away while isolates CBTL3 and CEPL2 produce brown colored-secondary metabolite around their colony, altering the color in that area. However, the Cinnabar were still present on all Cinnabar incorporate PDA. This suggested that the fungi did not utilized the cinnabar directly, but rather produced colored secondary metabolite or penetrated hypha into the media which can also altered the color. As the main composition of Cinnabar is mercuric oxide (HgS), there has been reports on some Aspergillus sp. that could utilized HgS2 but the molecule of the cinnabar might not present in a form that the fungi could easily utilized (Kumarit et al. 2014) (Hindersh 2018).

Microorganisms are the main cause of biodeterioration in mural paintings, among them are fungi which play a huge role in the deterioration of these materials. There are numbers of temples in Chiang Mai which has profoundly intriguing Shan style mural paintings. For instance, Wat Buak Kroo Luang, Wat Tha Karm and Watt Pa Dao. Currently, the deterioration of mural paintings of the mentioned temples are not yet studied. There were total of 73 isolates of fungi on Potato Dextrose Agar (PDA) medium, some of them were identify by morphology and molecular biology technique using ITS genes for sequencing. The results shown Penicillium oxalicum, P. citrinum and Trichoderma caeruleimontis were the most abundant of all the isolates, while only 4 isolates were found to possess an ability to deteriorate color of cinnabar which were observed by change in tone of color form 0.1% cinnabar incorporate PDA media. Isolates of T. caeruleimontis and COBL2 faded the color away while CBTL3 and CEPL2 produced brown color secretion around their colony on the media with average diameters of 3.3 cm and 2.47 cm respectively. When compare to the color of the standard cinnabar incorporate PDA, the change in tone of color may cause by hypha penetration and the secretion of secondary metabolites on the media.

Objectives

To study the diversity of fungi which grow on Chiang Mai’s temple mural paintings.

To study the color deteriorated effect of fungi on Chiang Mai’s temple mural paintings.

Methods

1. Sample Collection: Cotton Swap Technique

2. Isolation: Serial Dilution & Spread Plate Technique

3. Observation and Identification: Slide Culture & Molecular Biology Technique

4. Color Deterioration Test: cinnabar incorporate PDA

Introduction

There were numbers of sites in the province of Chiang Mai which host unique attractions, and some of the places even hold an UNESCO World Heritage site status. One of them are the ancient Mural Painting inside each temples but as ancient as they are, some got deteriorate by microorganism over time. However, no known research has yet established study on this crucial topic on northern part of Thailand.

Results

1. Observation and Identification:

   a. Slide Culture & Molecular Biology Technique
   - Total of 75 Isolates
   - Total of 6 Fungi Genera

Table shows numbers of fungal isolates from each Temple

<table>
<thead>
<tr>
<th>Temples</th>
<th>No. of Isolates</th>
<th>Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wat Buak Kroo Luang</td>
<td>27</td>
<td>Aspergillus sp.</td>
</tr>
<tr>
<td>Muang District</td>
<td>27</td>
<td>Coprinellus sp.</td>
</tr>
<tr>
<td>Wat Ta Kam</td>
<td>25</td>
<td>Penicillium sp.</td>
</tr>
<tr>
<td>Mae Tang District</td>
<td>21</td>
<td>Trichoderma sp.</td>
</tr>
<tr>
<td>Wat Pa Dao</td>
<td>21</td>
<td>Curvularia sp.</td>
</tr>
<tr>
<td>Mae Chaem District</td>
<td>21</td>
<td>Penicillium sp.</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Chart shows Evolutionary Relationship between sample isolates and their closest species

Ascomycota

Basidiomycota

Acknowledgements

This research was performed in 240/200 and 2704 Laboratories, SCB2, Faculty of Science, Chiang Mai University. Thank you Asst. prof. Dr. Terd Disayathanawat, Dr. Nakarin Suwannaram and Mrs. Nattaphun Supaghonal for giving advices and help with the laboratory techniques.

References

Kumarit, S., Bantho, V. 2014. Screening of Fungi from Old Temple Mural Painting in Respect to Ancient Heritage. MSc Thesis. Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.


Table 1: Rates of Fungal Communities on Mural Paintings in Chiang Mai.

<table>
<thead>
<tr>
<th>Fungi Species</th>
<th>Percentage</th>
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<tr>
<td>Aspergillus sp.</td>
<td>47</td>
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<tr>
<td>Coprinellus sp.</td>
<td>38</td>
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<tr>
<td>Penicillium sp.</td>
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</tr>
<tr>
<td>Trichoderma sp.</td>
<td>12</td>
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<tr>
<td>Curvularia sp.</td>
<td>3</td>
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<tr>
<td>Penicillium sp.</td>
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Table 2: Effects of Fungal Isolates on Cinnabar Color Incorporate PDA Control.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>1st Replication</th>
<th>2nd Replication</th>
<th>3rd Replication</th>
<th>Average</th>
</tr>
</thead>
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<tr>
<td>CBTL3</td>
<td>5.6</td>
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<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>CEPL2</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>COBL2</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
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<tr>
<td>Standard Color of Cinnabar</td>
<td></td>
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Table 3: Effect of Fungal Isolates on Cinnabar Color Incorporate PDA.

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