Screening for PKS-I gene cluster from endophytic actinomycetes residing in *Ocimum tenuiflorum* (Tulsi) and *Azadirachta indica* (Neem)

Asma Ilyas¹, Rabia Tanvir¹, Ali Ahmad Sheikh¹ and Wasim Shehzad²

¹University Diagnostic Lab (UDL), Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore 54000, Punjab, Pakistan
²Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences (UVAS), 54000 Lahore, Pakistan

* Corresponding author: rabia.tanvir@uvas.edu.pk; rabiatanvir@outlook.com
Screening for PKS-I gene cluster from endophytic actinomycetes residing in *Ocmium tenuiflorum* (Tulsi) and *Azadirachta indica* (Neem)

Graphical Abstract
Abstract:

Polyketide synthases type I (PKS-I) gene cluster is responsible for the synthesis of highly assorted group of secondary metabolites such as antimicrobial and anticancer agents. In our study, screening was carried out using degenerate primers to determine the presence of PKS-I gene cluster in endophytic actinomycetes isolated from two medicinal plants Ocimum teniflorum (Tulsi) and Azadirachta indica (Neem). A total of 28 endophytes that were isolated and identified from our previous study were further confirmed through 16S rRNA gene sequencing to exhibit a 99% similarity with Streptomyces sp. The molecular screening using PCR revealed the presence of PKS-I gene with a product size of 750bps in the isolates, FHK-1, FHK-2, FHK-3, FHK-4, FHK-5, FHK-6, FHK-7, FHK-8, FHK-9, FHK-11, FHK-13, FHK-16, FHK-18, FHK-20, FHK-21, FHK-23, FHK-25 and FHK-28. These isolates were further checked for their antimicrobial potential using their crude extracts. They displayed prominent bioactivity against ATCC pathogens, Escherichia coli, Proteus vulgaris, Rhodococcus equi, Staphlococcus epidermidis, Enterococcus faecalis and Acinetobacter baumanii. Our study revealed that the endophytes from O. tenuiflorum and A. indica are bioactive and versatile harboring the PKS-I gene cluster.

Keywords: Actinomycetes; Endophytes; Polyketide Synthases type I
Introduction

- Traditional medicine
  - Essential role in the healthcare systems
  - In Asian countries
    - 80% of the population dependent on medicinal practices [1]

- Medicinal plants
  - Attractive targets for discovering novel therapeutic agents [1]

**Ocmium teniflorum**

- Locally known as ‘Tulsi’
- Widespread throughout the Southeast Asian tropics [2]
- Clinical pharmacological properties of oils and extracts from leaves
- Antiemetic, antipyretic, anti-inflammatory, anti-diabetic, antifungal, antibacterial, analgesic and anti stress effects [3]


• **Azadirachta indica** A. Juss

• Locally known as ‘Neem’
• Native to Pakistan, India, Bangladesh, Burma, Malaysia and Sri Lanka [4]
• Clinical pharmacological properties
  • Antifungal, antibacterial, anti-diabetic, antiviral, anthelmintic and sedative properties [5]

---


Introduction (Cont.)

• Actinomycetes
  • Greek ‘aktis’ (a ray) and ‘mykes’ (fungus)
  • Producers of a broad array of secondary metabolites
    • Useful applications in veterinary and human medicine [6]

• Endophytic Actinomycetes
  • Starting platform
  • Antibiotics, enzyme, anticancer agents, immunomodulators, anthelminthic agents,
  • Long-held alliance, plants and endophytic microorganisms develop good information transfer [7]


Introduction (Cont.)

• Polyketides
  • Consequence of the successive condensation of carboxylic acid units [8]

• Polyketide synthases (PKS)
  • Multifunctional enzymes
  • Responsible for the biosynthesis of secondary metabolites
  • Comprising of antibacterial, antifungal, anticancer [8]

Results and discussion

• Total 28 endophytic actinomycetes were isolated [9]

• Frequency of isolation
  • Ocimum tenuiflorum (Tulsi)
    • Roots = 03
    • Roots slurry = 10
    • Shoots = 02
    • Shoot slurry = 06
    • Leaves slurry = 01
  • Total = 22

Results and discussion (Cont.)

- Total 28 endophytic *actinomycetes* were isolated [9]

- Frequency of isolation
  - *Azadirachta indica* (Neem)
    - Roots slurry = 05
    - Shoot slurry = 01
  - Total = 06

Results and discussion (Cont.)

Photo courtesy: Ms. Fatima Nawaz
Results and discussion (Cont.)

- Physiological characterization
  - Esculin hydrolysis [9]
    - Esculin producers = 27
    - Non esculin producers = 01

Results and discussion (Cont.)

- Physiological characterization
  - Hydrolysis of urea [9]
    - Positive = 27
    - Negative = 01

Results and discussion (Cont.)

• Genomic DNA isolation
  • Manual method [10]
  • 28 selected actinomycetes strains

Results and discussion (Cont.)

- 16s Ribosomal RNA gene sequencing
  - FHK-3 and FHK-6
  - 27f and 1522r universal primers [9]
  - 1.5 kb gene sequenced

Results and discussion (Cont.)

- 16s Ribosomal RNA gene sequencing
  - BLAST analysis for FHK-3 and FHK-6
  - 99% homology with *Streptomyces* sp.

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces</em> sp. strain RB110 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY58688.2</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain D7 16S ribosomal RNA gene, partial sequence</td>
<td>2361</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MG134505.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> purpureus strain NRIC 12311 (T) 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MG134505.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> mediocris strain RFB25 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MH209233.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain M6 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MH141052.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain MS16129 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MF448017.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain W775-2 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY206612.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> californicus strain T1504 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>MF82217.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain W7169 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY008561.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> victorii strain S21, complete genome</td>
<td>14168</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.24%</td>
<td>CP026720.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain RB99 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY58687.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain 3C-H12 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY777595.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> microflavus strain 36_3 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY007180.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> cayennensis strain 19_3 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KY007172.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. NEAU-107 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KP267985.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. NEAU-JF11 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KP267986.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. strain ED129 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KP209458.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. JCG212 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KP097429.1</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. KC214 16S ribosomal RNA gene, partial sequence</td>
<td>2351</td>
<td>2561</td>
<td>89%</td>
<td>0.0</td>
<td>99.09%</td>
<td>KP831582.1</td>
</tr>
</tbody>
</table>
Results and discussion (Cont.)

• Screening for PKS-I gene cluster
  • Degenerate primers MDPQQRF and HGTGTr [11]
  • 750 bp product size
  • 18 strains positive (64.2%)

Results and discussion (Cont.)

- Antimicrobial activity of strains containing the PKS-1 gene against ATCC pathogens using agar well method [12]
  - Crude extraction
    - 1:1 ethyl acetate [13]
    - Extracts stored in absolute methanol


Results and discussion (Cont.)

- Preliminary antimicrobial activity against Gram positive and Gram negative ATCC pathogens
  - Broad spectrum activity
  - Maximum zones of inhibition of 18mm

Photo courtesy: Ms. Fatima Nawaz
Results and discussion (Cont.)

- *In vitro* antimicrobial testing for the strains containing PKS-1 gene

<table>
<thead>
<tr>
<th>Strain code</th>
<th>E. coli</th>
<th>P. vulgaris</th>
<th>R. equi</th>
<th>S. epidermidis</th>
<th>E. faecalis</th>
<th>A. baumanii</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHK-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.6</td>
<td>10.6</td>
<td>-</td>
</tr>
<tr>
<td>FHK-2</td>
<td>6.3</td>
<td>-</td>
<td>6.3</td>
<td>10</td>
<td>17.6</td>
<td>-</td>
</tr>
<tr>
<td>FHK-3</td>
<td>6.3</td>
<td>11</td>
<td>8.6</td>
<td>11</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>FHK-4</td>
<td>5.3</td>
<td>9.6</td>
<td>8.3</td>
<td>9</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>FHK-5</td>
<td>6.6</td>
<td>10.6</td>
<td>6.6</td>
<td>6.6</td>
<td>10.3</td>
<td>-</td>
</tr>
<tr>
<td>FHK-6</td>
<td>10.6</td>
<td>13.6</td>
<td>8.3</td>
<td>8.3</td>
<td>12.3</td>
<td>-</td>
</tr>
<tr>
<td>FHK-7</td>
<td>4</td>
<td>6</td>
<td>6.6</td>
<td>6.6</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>FHK-8</td>
<td>6</td>
<td>10</td>
<td>11.3</td>
<td>11.3</td>
<td>7.6</td>
<td>4.3</td>
</tr>
<tr>
<td>FHK-9</td>
<td>11.3</td>
<td>9.6</td>
<td>8.6</td>
<td>8.6</td>
<td>9.6</td>
<td>11.3</td>
</tr>
<tr>
<td>FHK-11</td>
<td>6.6</td>
<td>9.6</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>6.6</td>
</tr>
<tr>
<td>FHK-13</td>
<td>3.6</td>
<td>12.3</td>
<td>12.6</td>
<td>12.6</td>
<td>10.6</td>
<td>5.6</td>
</tr>
<tr>
<td>FHK-16</td>
<td>7.8</td>
<td>-</td>
<td>8.7</td>
<td>13</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>

*Key:* E. coli = *Escherichia coli*; P. vulgaris = *Proteus vulgaris*; R. equi = *Rhodococcus equi*; S. epidermidis = *Staphlococcus epidermidis*; E. faecalis = *Enterococcus faecalis*; A. baumanii = *Acinetobacter baumanii*
Results and discussion (Cont.)

- *In vitro* antimicrobial testing for the strains containing PKS-1 gene

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Zone of inhibition (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>P. vulgaris</em></td>
</tr>
<tr>
<td>FHK-18</td>
<td>7.8</td>
<td>-</td>
</tr>
<tr>
<td>FHK-20</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>FHK-21</td>
<td>9.3</td>
<td>8</td>
</tr>
<tr>
<td>FHK-23</td>
<td>7.9</td>
<td>7.3</td>
</tr>
<tr>
<td>FHK-25</td>
<td>6.7</td>
<td>12</td>
</tr>
<tr>
<td>FHK-28</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

Key: *E. coli* = *Escherichia coli*; *P. vulgaris* = *Proteus vulgaris*; *R. equi* = *Rhodococcus equi*; *S. epidermidis* = *Staphylococcus epidermidis*; *E. faecalis* = *Enterococcus faecalis*; *A. baumanii* = *Acinetobacter baumanii*
Conclusions

• Endophytic actinomycetes from *Ocimum teniflorum* (Tulsi) and *Azadirachta indica* (Neem)
  • Rarely studied before

• Our study
  • Diversity of actinomycetes reside in different plant parts
  • Biological screening revealed
    – Broad spectrum activity
    – Bioactive against gram positive and gram negative pathogens
      » *Escherichia coli*, *Proteus vulgaris*, *Rhodococcus equi*, *Staphlococcus epidermidis*, *Enterococcus faecalis*, *Acinetobacter baumanii*
Conclusions (Cont.)

• Contain PKS-1 gene cluster
  • 18 strains
  • Potential for novel antibiotics

• Further exploration of these strains
  • Large scale study
  • Further bioactivity screening
  • HPLC-MS and NMR