Research Article

# Chemical Stimulation of Biopigment Production in Endophytic Fungi Isolated from *Clerodendrum viscosum* L.

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**Abstract**

Biopigments are postulate to render pollution-free environment against chemical colorants. In the rivalry of biopigment production, fungi are the invincible source by having the diligence metabolic profile. Medicinal properties of metabolites from fungi have very preferential to the pharmaceutical and common health care products. Hence the implication of fungal metabolic profiles looking forward to the metabolite such as pigment. In this study, we investigated the biopigment production by implying different chemical constituents in the growth medium for the fungal endophytes, which are isolated from *Clerodendrum viscosum*. Growth of endophytic fungus MECV01 in respective media was examined for its efficacy of pigment production towards the function of different chemical present in media. Morphological observation of the endophytic fungus MECV01was performed using light microscope.

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## Introduction

Endophytic fungi, is the fungi which lives in the tissue of the host plant. Diversity of the endophytic fungi has the major role in plant ecosystem [1]. Endophytic fungi are biologically important microorganisms to produce new pharmaceutical compounds, biological control agents and other useful products [2]. According to their attractive metabolite profile towards bioactivity is improve the field of interest supporting by the evident from the previous research [3]. Exploration of endophytes may help to exploit the new fields of research.

Recently, natural ingredients in consumables have been apparently increased for their marketing benefits. In that crisis, natural products and ingredients made aware about attention to the biopigments [4]. Biopigments are compounds, which has different kind of colors with specialized characteristics. Disconfirming health concerns of synthetic food pigments are reach the user tending on natural colorants [5]. Biopigments from microorganisms induced industrial concern, due to the instability and unavailability of plant and animal sources [6]. Reproducibility of microbial pigments caused special attention to the fungi as potential source [7-10]. Pigment from *Monascus anka* has preferential characteristics for the large scale production [11].Some report proven that species of *Pencillium* and *Monascus* had substantial role in the production of wide range of pigments with high quantities [12-14]. Some of the new fungi shown extraordinary color range of pigment production, while grown in different media and the stability of the pigments may also vary [15].

## Experimental

### *Plant material*

Healthy-looking plant of *Clerodendrum viscosum* was sampled near to Thirparappu falls ($8^0$ 23' 40.35"N, $77^0$ 15' 44.05"E), which is located at Kanyakumari District of TamilNadu in the southern part of India. The plants were collected in the zip-lock bags and stored some of them for further research.

***Isolation of Endophytic fungi***

Leaf and stem parts of sample plant were washed thoroughly in running tap water and allowed to dry. Dried plant samples were transferred to the sterile chamber and surface sterilization was carried out. Leaf and stem twigs were surface sterilized by consecutive immersion of 75% ethanol and 4% Sodium hypochlorite solution in different time interval. Fixed leaf and stem were sliced approximately 5x5 mm respectively. The leaf and stem pieces were then evenly placed in each 90 mm petri dish containing Potato Dextrose Agar supplemented with 1mg/ml Chloramphenicol [15, 16].

***Media for pigment production***

Isolated endophytes are grown in different media, which is used to screen biopigment production [17, 18]. Potato Dextrose (PDA) agar, Czepek-Dox (CDA)Agar, Sabroud Dextrose (SDA) agar, Malt Glucose Yeast Peptone (MGYP) agar, Malt Extract (MEA) agar, Czepek-Dox Yeast autolysate (CYA) agar, Yeast Extract ( YES) agar and Yeast Glucose Trace (YGT) agar were prepared in the 90mm diameter petri dishes. Composition of the media used in this study given in the Table 1.

**Table 1** Different media composition for screening of pigment production in endophytes

| **Media** | **Chemical composition** |
|---|---|
| Potato Dextrose (PDA) | Potato infusion (200gm/L), 20% Glucose |
| Czapek- Dox (CDA) | 3% Sucrose, 0.2% NaNO3, 0.1% $K_2$ $HPO_4$ 0.05% $MgSO_4$, 0.05%KCl, 0.001% $FeSO_4$ |
| Sabroud Dextrose (SDA) | 4% Dextrose and 1% Peptone |
| Malt Glucose Yeast Peptone (MGYP) | 0.3% Malt extract, 0.3% Yeast extract, 0.5% Peptone, 1%Glucose |
| Malt Extract (MEA) | 3%Malt extract and 0.3% Peptone |
| Czapek-Dox Yeast autolysate (CYA) | 0.5% Yeast extract, 3% Sucrose, 0.3% $NaNO_3$, 0.1% $K_2$ $HPO_4$ 0.05% $MgSO_4$, 0.05%KCl, 0.001% $FeSO_4$ |
| Yeast Extract Sucrose (YES) | 2% yeast extract, 15% Sucrose, 0.05% $MgSO_4.7H_2O$ , 0.1% Trace solution (1% $ZnSO_4$.7H2O ,5% CuSO4.5H2O in 10 mL) |
| Yeast Glucose Trace (YGT) | 0.5% Yeast extract, 2% Glucose, 0.1% Trace solution (0.05% $H_3BO_3$, 0.04% $CuSO_4.H_2O$, 0.01% KI, 0.2% $FeCl_3.5H_2O$, 0.4% $MnSO_4.H_2O$, 0.16% $H_2MoO_4$, 0.4% $ZnSO_4.7H_2O$ in 10 mL) |

***Growth condition of fungi***

The influence of different media on the diameter growth was examined by growing the endophytes in the positive resulted media during 7days of observation. Discs of cultures (8mm diameter) were cut from active part of PDA plates and evenly place the discs in each medium. Cultures were incubated at $25^0$C and regularly monitor their growth

of each media. For each colony, four sides of growth were measured from the center of colony and plot mean value. In this method, growth of the resulted fungi will monitor in the petri dish for calculating the radial measurements.

***Morphological observation of pigment fungus***

For observing the morphology, the positive endophytes were cultured on PDA plates at $28^0$C for 7 days. The growth rate of the colonies incubated in the same conditions was also recorded. Endophytic fungi were mounted with the Lactophenol cotton blue and lactophenol stains. Labomed LX400 microscope with Sony digital camera DSC-WX7 was employed to capture images.

## Results and Discussions

***Endophytic fungi from Clerodendrum viscosum***

Leaf and stem twigs of *Clerodendrum viscosum* were placed into 6 petri dishes. The plates were monitored regularly and the endophytic fungi initiation was observed after one week. Totally 10 endophytic fungi were isolated from the leaf and stem twigs of *Clerodendrum viscosum.* Total isolates were shown in the Fig 1. Among the 10 endophytic fungi, 6 fungi were from stem and remaining 4 from leaves. This ratio shows that stem portion of the *Clerodendrum viscosum* favorable environment to the fungal survival. Endophytic fungi occurrence in the plant was given in the Table 2.

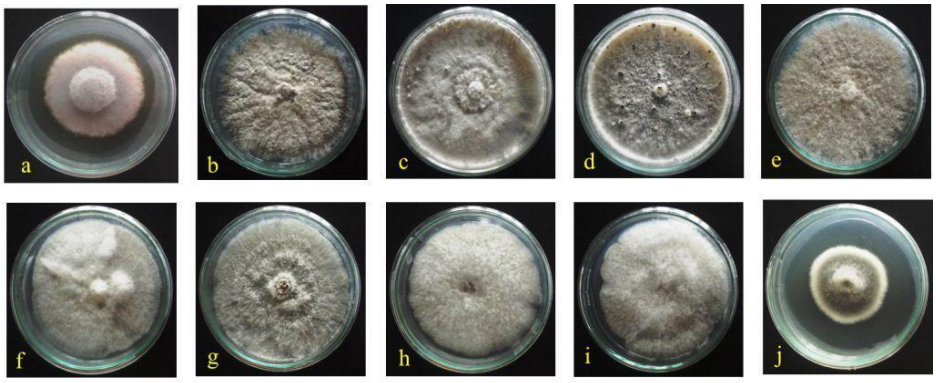


**Figure 1** Endophytic fungi isolated from *Cleordendrum viscosum*. (a)MECV01, (b)MECV02, (c)MECV03, (d)MECV04, (e)MECV05, (f)MECV06, (g)MECV07, (h)MECV08, (i)MECV09, (j)MECV10

**Table 2**. Endophytic fungi isolated from the different parts of *Clerodendrum viscosum*

| Endophytic fungus | MECV 01 | MECV 02 | MECV 03 | MECV 04 | MECV 05 | MECV 06 | MECV 07 | MECV 08 | MECV 09 | MECV 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Clerodendrum viscosum*** | S | L | S | L | S | L | L | S | S | S |

S - Stem; L- Leaf

***Effect of media in the pigment production***

In screening of pigment, 10 endophytes were monitored in 8 different media. Among the 10 endophytes, MECV01 shows the pigment production in four different medium, given in Table 3. Endophytic fungus MECV01 produced red pigment in CDA and CYA medium. Both the medium CDA and CYA have the same chemical composition sucrose, $NaNO_3$, $K_2HPO_4$, $MgSO_4$, KCl and $FeSO_4$. And yellowish red pigment occurrence in MGYP and YGT medium. Yeast extract and glucose were present in MGYP and YGT medium. Compare to the YES medium, YGT medium had

KI, $FeCl_3$, $MnSO_4$, $H_2MoO_4$ and $H_3BO_3$ other than the Yeast extract and Glucose. So, 5 trace elements may influence the pigment production in YGT medium. And endophytic fungus MECV01 showed highly diffusible pigment production in YGT medium. According to these observations of red and yellowish red pigment production, Common chemicals of CDA, CYA, MGYP and YGT caused stimulation in the endophytic fungus MECV01 to produce pigment. Positive resulted pigment plates of endophytic fungus MECV01shown in Fig 2.

**Table 3** Different media for screening the pigment production in endophytic isolates from *Clerodendrum viscosum*

| | PDA | CDA | SDA | MGYP | MEA | CYA | YES | YGT |
|---|---|---|---|---|---|---|---|---|
| **MECV01** | - | **R** | - | **YR** | - | **R** | - | **YR** |
| **MECV02** | - | - | - | - | - | - | - | - |
| **MECV03** | - | - | - | - | - | - | - | - |
| **MECV04** | - | - | - | - | - | - | - | - |
| **MECV05** | - | - | - | - | - | - | - | - |
| **MECV06** | - | - | - | - | - | - | - | - |
| **MECV07** | - | - | - | - | - | - | - | - |
| **MECV08** | - | - | - | - | - | - | - | - |
| **MECV09** | - | - | - | - | - | - | - | - |
| **MECV10** | - | - | - | - | - | - | - | - |

R - red; YR – yellowish red

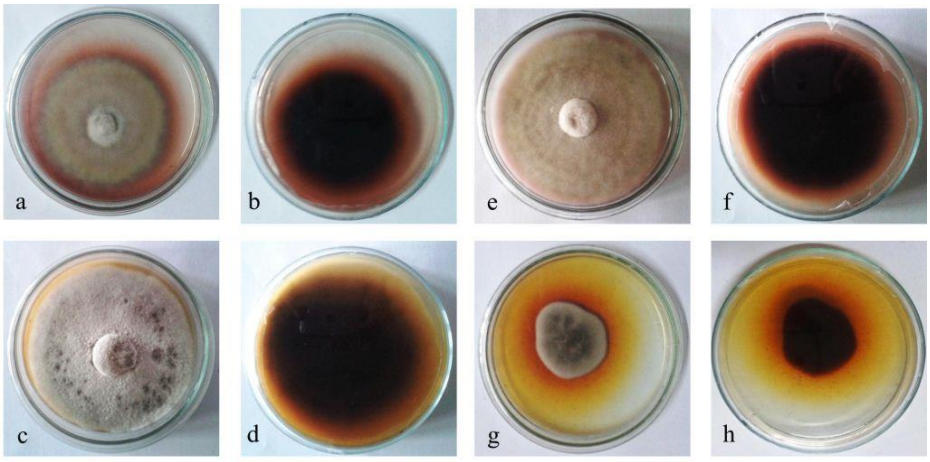


**Figure 2** Positive resulted plates of endophytic fungus MECV01 in screening of pigment. (a, b) CDA medium, (c, d) MGYP medium, (e, f) CYA medium and (g, h) YGT medium.

***Effect of media on the growth models***

Among the 8 different media for endophytic fungus MECV01 four of them showed the pigment production. In this growth model, growth of endophytic fungus MECV01 in PDA medium helps to interpret the results. According to red pigment production in CDA and CYA the chemical which influences are sucrose, $NaNO_3$, $K_2HPO_4$, $MgSO_4$, KCl and $FeSO_4$ and these five molecules with same percent were used in both the medium. But, in CYA medium Yeast extract is additionally over CDA. So, that the growth of MECV01 in CYA medium was higher than the CDA

medium. Yeast extract in CYA medium induce growth of endophytic fungus MECV01. Endophytic fungus MECV01 in MGYP and YGT medium were having very high difference in their growth. In YGT medium, 7 trace elements were influence the growth as well as the pigment production. But, the 7 trace elements are the negative response in the growth. In same case, pigment production was observed in YGT medium over the YES medium in the presence of 4 trace elements (KI, $FeCl_3$, $MnSO_4$, $H_2MoO_4$ and $H_3BO_3$).

In comparison to the PDA medium growth, MGYP and CYA are the significant growth medium for endophytic fungus MECV01. Growth of endophytic fungus MECV01in CDA medium depicted as next significant growth medium. Although pigment production in the YGT medium, growth of the endophytic fungus was much more less while compare the growth in PDA medium. Growth model of endophytic fungus MECV01 in different media responsible for pigment production was shown in Fig 3.

***Morphological observation of endophytic fungus MECV01***

Endophytic fungus MECV01 has observed under the microscope mounted with lactophenol cotton blue and the images are shown in Fig 4. The observed morphological data similar to the *Phoma* sp. by having ellipsoid, slightly curved and chlamydospore chain formation [19].

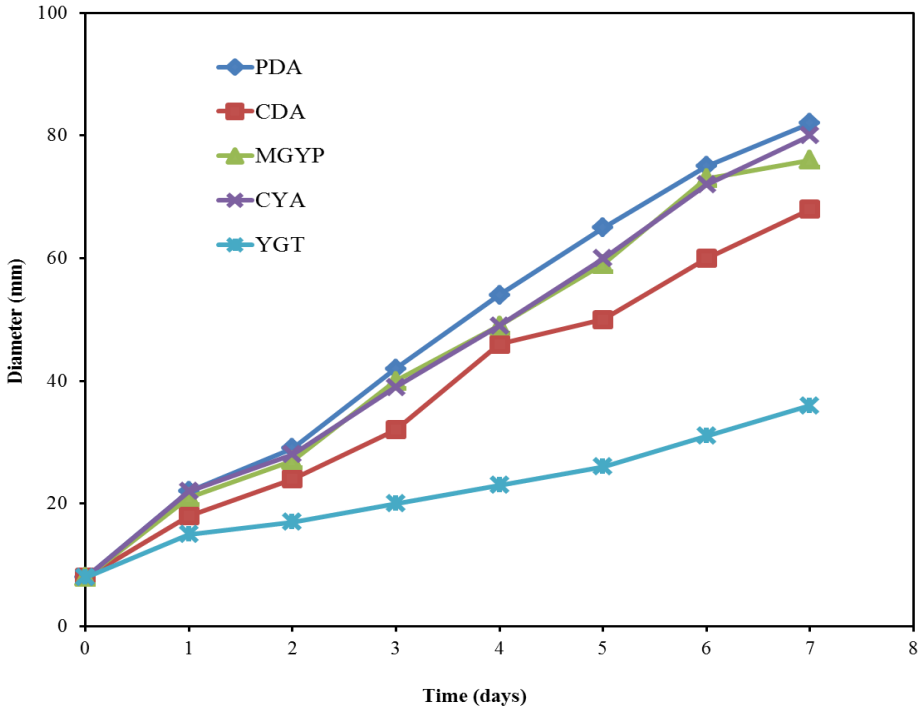


**Figure 3** Growth models of MECV01 isolate in CDA medium, MGYP medium, CYA medium, YGT medium and PDA medium as control medium during 7 days.

## Conclusion

In conclusion, this study shows that chemical influence may produce new compounds from unexplored fungi related to pigments. Different media usage was the major core in screening the pigment production and bioactive compounds. In this study, MGYP and CYA medium showed yellowish red and red pigment production at significant growth. And the endophytic fungus MECV01 was morphologically observed and state that as *Phoma* sp. Further work design to

quantify the pigment production in each media and characterize the pigment. And also morphological confirmation by cross-sectioning observation of conidia and molecular characterization of endophytic fungus MECV01 will perform for the species confirmation.

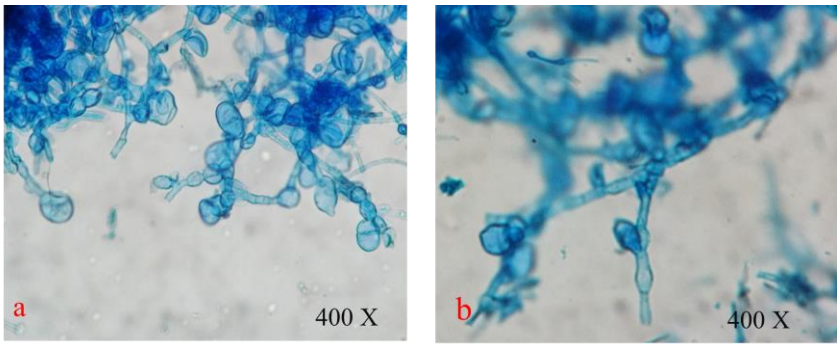


**Figure 4** Microscopical observation of endophytic fungus MECV01 mounted with Lactophenol cotton blue (a, b).

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