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ARTICLE

Identification of Taxol-producing endophytic fungi isolated from *Salacia oblonga* through genomic mining approach

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Abstract The most promising anti-tumor agent developed in the past three decades is Taxol. It is proven to be effective against many cancers. It is necessary to isolate pharmacologically potent endophytic microbial strains from medicinal plants with special reference to Taxol production. In the current study, endophytic fungi were isolated from the bark of the medicinal plant, *Salacia oblonga*. The isolated endophytes were identified morphologically, and further characterized by ITS-PCR using genomic DNA samples, later the products were sequenced for identification and phylogenetic linkage mapping. The samples were screened for the potential to produce Taxol or taxanes, employing PCR. The resulted data have been sequenced to confirm the presence of the two genes implicated in Taxol biosynthesis, 10-deacetylbaccatin III-10-O-acetyl transferase (DBAT) and C-13 phenylpropanoid side chain-CoA acyltransferase (BAPT). Seven samples showed the amplicons of DBAT gene and one showed the amplicons of BAPT gene. Sequencing of these products was carried out, of which one sample has revealed sequence homology to the original DBAT gene from *Taxus*. The present work confirms and substantiates the potential of genomic mining approach to discover novel Taxol-producing endophytic fungi.

1. Introduction

Salacia oblonga is a tropical woody plant/shrub found in the forests of South Asian countries, including Sri Lanka and India. It is commonly known as 'Saptrangi' or 'Saptachakra'. The roots and stems of *S. oblonga* have been used extensively

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in Ayurveda and traditional Indian medicine for the treatment of Diabetes [1]. *S. oblonga* extracts have long known to have hypoglycemic, hypolipidemic, anti-inflammatory and antioxidant properties [2–4]. It has also been reported to have potential anticancer properties showing antitumor activity against EAT cells [5].

In recent times, molecular techniques are being used extensively in biodiversity studies of endophytes due to their sensitivity and specificity. Further, they are very rapid and economical, these are not affected, or dependent on environmental factors likes culture conditions. Analysis of DNA extracted from fungi has been used widely as a means of identification and screening of fungi for their potential to produce certain desired metabolites [6]. Secondary metabolites are produced from several intermediate products that accumulate, either in the culture media or within the cells (during primary metabolism). Production of secondary metabolites is significantly affected by genetic, developmental and environmental factors [7].

The structural diversity of secondary metabolites is a result of modifications and combinations of reactions from primary metabolic pathways. Secondary metabolites may be found in various species in disparate genera or families, and a variety of metabolites can be expressed from a single species under different environmental conditions [8]. The groups commonly distributed in nature are the polyketides, terpenes, steroids, shikimic acid and alkaloids. Most secondary metabolites are low molecular weight compounds having molecular masses less than 1500 Da [9]. Conventional morphological characterization of fungal endophytes has the drawback of difficulty in identifying the species which have structural similarity. Furthermore, these are very difficult in fungal isolates that fail to sporulate in culture [10]. Molecular methods were successfully employed in identifying microorganisms at diverse hierarchical taxonomic levels due to their high sensitivity, specificity and quicker procedures. Most of the endophytic fungi can be detected and identified based on comparative analyses of the ribosomal DNA sequences, especially the ITS region [11–13].

The diterpenoid "Taxol" (Paclitaxel) have gained more attention and interest than any other drug since its discovery mainly due to its unique mode of action compared to any other anticancer agents. This compound affects the multiplication of cancer cells by interfering with the cell cycle, hence, reducing their growth and spread. Food and drug administration (FDA) has approved Taxol for the advanced treatment of various cancers [14].

Taxol is a diterpenoid originally isolated from the stem/bark of the Pacific Yew tree (*Taxus brevifolia*, Nutt.; Taxaceae family). Several other species of *Taxus* have also been reported to produce Taxol [15]. Taxol has become a widely used anticancer drug for the treatment of various cancers. Besides, it is also effective against non-cancerous conditions like polycystic kidney diseases [16]. The production of Taxol from the *T. brevifolia* bark is limited (0.01–0.05%) because, the plant is not abundantly found in nature and grows slowly and also it results in low yield of Taxol per gram. Further, it results in the death of the tree due to the removal of the bark. Hence, alternate methods for Taxol production have been explored, such as production by chemical synthesis, semi-synthesis (chemical modification of Taxol precursors), plant cell and tissue culture and also by fermentation using endophytic fungi [17]. Extraction of Taxol from endophytic fungus has established high potential in increasing the efficiency of Taxol production and other cancer treatment products [18–19].

Taxol is a polyoxygenated cyclic diterpenoid characterized by the taxane ring system and it differs from other known taxanes either in the substitution pattern, the nature of the ester side chains, or in the presence of the oxetane ring (D-ring) system. Potent antimitotic activity seems to be restricted to taxanes, like Taxol, which possess an N-benzoyl-3-phenyliso serineside-chain at C-13, the oxetane ring function, and a benzoyl group at C-2. Similar to other terpenoids of plastid origin, Taxol biosynthesis follows the mevalonate-independent (1-deoxy-D-xylulose-5-phosphate) pathway, which operates in parallel with cytosolic acetate/mevalonate pathway for the biosynthesis of isopentenyl diphosphate (IPP). On the other hand, the IPP derived from the plastid 1-deoxy-D-xylulose-5-phosphate pathway is used in the biosynthesis of carotenoids, phytol, plastoquinone, isoprene, monoterpenes, and diterpenes [20].

Due to the increased demand of the Taxol production the current study has been undertaken to identify the Taxol producing gene from the fungal endophytes isolated from *S. oblonga*.

2. Materials and methods

2.1. Sampling

Plant materials *S. oblonga* was collected in the early monsoon season and brought to the laboratory in polythene bags from the village Kigga, Sringeri Taluk, Chikmagalore district, Karnataka, India (Western Ghats).

2.2. Isolation of endophytes

Samples of *S. oblonga* were washed in running tap water to remove adhered soil particles to it. Then, the plants samples were processed under laminar chamber using 70% (v/v) ethanol for 30 s and 3.5% (v/v) sodium hypochlorite for 3–5 min for the surface sterilization. Later, they were washed thoroughly using sterile distilled water. The plant materials were aseptically cut into small pieces and were plated on water agar medium (0.15%, w/v) containing 1% (w/v) Chloramphenicol. The plates were incubated at 25 ± 3 °C for 21 days.

Pure cultures of 34 endophytic fungal samples were isolated from the bark of *S. oblonga*. Pure cultures of samples were grown on Potato Dextrose Agar (PDA), the culture was used for identification, characterization and further genetic analysis.

2.3. Endophyte characterization and identification

All 34 endophyte cultures were used for identification. Morphological characteristics of the various endophytic colonies were noted. The colony characteristics and morphology were examined by microscopy (Zeiss AX10 Imager A2, Zeiss, Germany) using lactophenol cotton blue staining. Some of their characteristics were identified with the help of the standard manual for identification of endophytes [21].

2.4. Subculturing of endophytes for DNA extraction

The 34 isolates were subcultured into the flasks containing 40 ml of potato dextrose broth (PDB) containing Ampicillin (0.5 mg/L). The bottles were left standing undisturbed for