

Purification and characterization of endophytic fungal strains from four different high value medicinal plants of Kashmir valley

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Abstract

This study was designed to isolate, purify and characterize endophytic fungi from four different high value medicinal plants of Kashmir valley as a possible source of bioactive secondary metabolites. A total of 14 morphologically different endophytic strains were isolated from the plants exploited for endophytes. All isolates were identified based on colony morphology, examination of spores and light microscopes. The endophytic fungi were successfully isolated from the plants using the surface disinfection method. The optimized parameters for the optimum growth of the endophytic fungi were obtained as temperature of 28°C, pH5.6, potato dextrose broth medium and rpm at 180-200.

Keywords:Endophyte, secondary metabolite, fungi

1.Introduction

Endophytic fungi are under-explored group of microorganisms that live within a plant's tissue without causing any symptoms or apparent injury to host. Dreyfuss and Chapela[1] predicted that there may be at least one million species of endophytic fungi residing in plants. They are of biotechnological importance as new useful characteristics which may be found by further exploration. A number of endophytic organisms harbor on all plant species[2].The colonization of endophytic fungi in plant tissues occurs in a manner similar to those of plant pathogens and mycorrhiza [3]. Mostly, endophytic fungi had been studied in temperate regions and are found extensively still fungal biodiversity remains ambiguous [4,5,6]. Tropical endophytic fungi are most likely conservative and much less understood.

Endophytes are microorganism that includes bacteria and fungi living within plant tissues without causing and immediate negative effects, and has been found in every plant species examined to date and recognized as the potential source of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products isolated from the microorganism[7]. Endophytes are regarded as an excellent source of bioactive natural products. Some of the endophytes are the chemical synthesizers in inside the plants[8]. Many of them are capable of synthesizing bioactive compounds that can be used for defense against human pathogens and some these compounds have been proven useful for novel drug discovery.

Indeed, endophytic fungi are a very promising source of novel biologically active compounds and have proven to yield a considerable hit-rate of novel compounds when screening larger strain numbers for biological activities[9]. This may be the case because endophytes may have developed close biological associations with and inside their hosts, leading to the production of a high number and diversity of biological derived molecules with a

range of biological activities. In fact, a recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown[10]. In the present study an attempt has been made to isolate the endophytic fungi from these medicinal plants of Kashmir valley as a possible source of bioactive secondary metabolites and to save the natural medicinal plant wealth of Kashmir valley.

2. Materials and methods

2.1. Selection of plant material

Four different plants, *Artimiseaannua*, *Rheum emodi*, *Diocoriadeltodia*, *Hypercumperforatum*, were selected for endophytes isolation from different places of Gulmarg and Sunamarg locations. All the collected plants were transported to the working station in sterile ziplock polythene bags and stored at 4°C for further use.

2.2. Isolation of fungal endophytes

From each plant type, endophytic fungi were isolated as per Arnold *et al.*, [11] with some modifications. Plant materials (leaf, stem and root) were thoroughly washed with running tap water followed by immersion in 70% EtOH for 1 min and in NaOCl (2.5 - 5.25%) for 3 min, drained and immersed in 70% EtOH again for 30 sec. Finally, the samples were rinsed with sterile distilled water and cut under sterile conditions into small pieces (2–3 cm). The plant parts were cut into smaller pieces of 0.5 to 1 cm and plated on different media such as water agar, potato dextrose agar and rose bengal agar (Difco) supplemented with streptomycin sulphate (250 µg/ml, Sigma) at 28°C for 2 -3 weeks.

2.3. Purification of fungal endophytes

The fungi coming out of the plant tissues were taken and grown on PDA plates free of antibiotics as a pure culture. The morphological identification of the endophytic strains were done by preparing microscopic slides stained with lactophenol cotton blue as per the key of Vainio *et al.*, [12] and were examined under light microscope (Olympus, USA).

2.4. Temperature profile of the isolated endophytes

In order to examine the temperature optimization of the isolated fungal endophytic strains, different temperature treatments were given i.e., 20,22,24,26,28,30,32°C. From this temperature profile of all the isolated strains were optimized. All the experiments were performed in triplicates.

2.5. pH profile of the isolated endophytes

In order to check the best pH of the isolated fungal endophytic strains, four different pH treatments were given i.e., 5.2, 5.4, 5.6, 5.8. From this pH profile of all the isolated strains were optimized. All the experiments were performed in triplicates.

2.6. Media optimization of the isolated endophytes

For the optimization of the growth of the isolated endophytic fungal strains three media were used *viz.*, Potato Dextrose agar (PDA), Sabour Dextrose agar (SDA), czapek'sDox+ Yeast extract agar (CYA).

3. Results

3.1. Isolation and purification of the fungal endophytes

A total of 14 morphologically different endophytic fungal strains were obtained from four different types of medicinal plants. The morphologically different isolated are shown in fig. 1. The results of this study showed that endophytes were more prevalent in the roots of the plants as compared to the leaves selected for the endophytic isolation as shown in table 1.

Table 1: Showing isolated fungal endophytes from leaves and roots of the selected medicinal plants

Name of plant	Endophytes from leaves	Endophytes from roots	Total
<i>Artimiseaannua</i>	1	2	3
<i>Rheum emodi</i>	0	4	4
<i>DiocoriaDeltodia</i>	1	3	4
<i>Hypercumperforatum</i>	0	3	3
Total	2	12	14

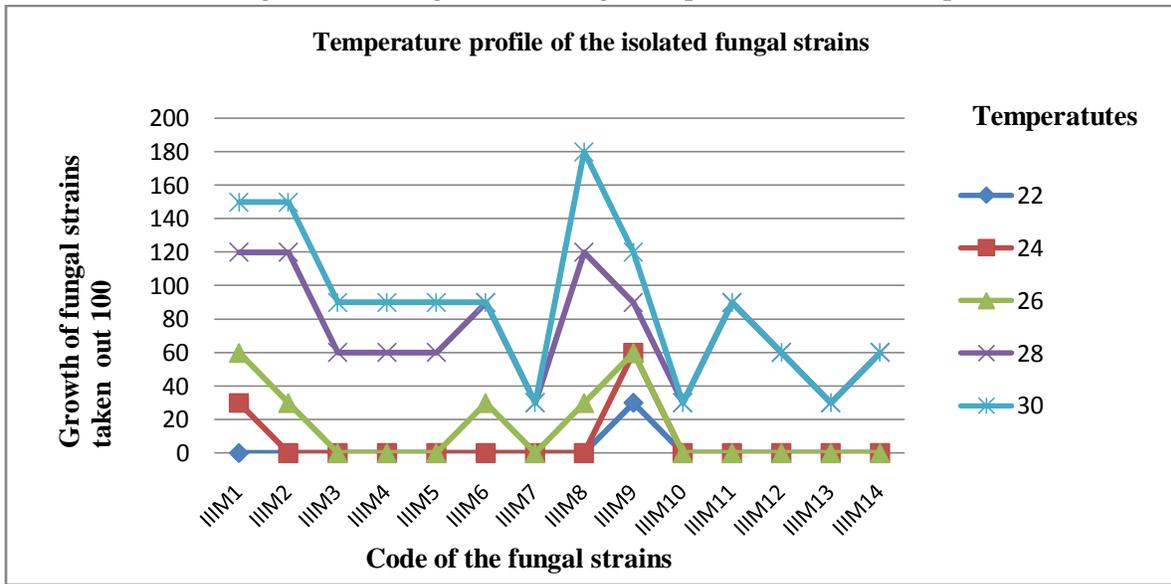
Fig. 1. Isolated fungal endophytes



3.2. Temperature profile of the isolated endophytes

The growths of the isolated endophytes were checked at five different temperatures. The optimum growth was obtained at 28^oc. The temperature profile for the optimum growth is shown in the fig.2.

Figure 2: Showing the different growth profile at different temperature

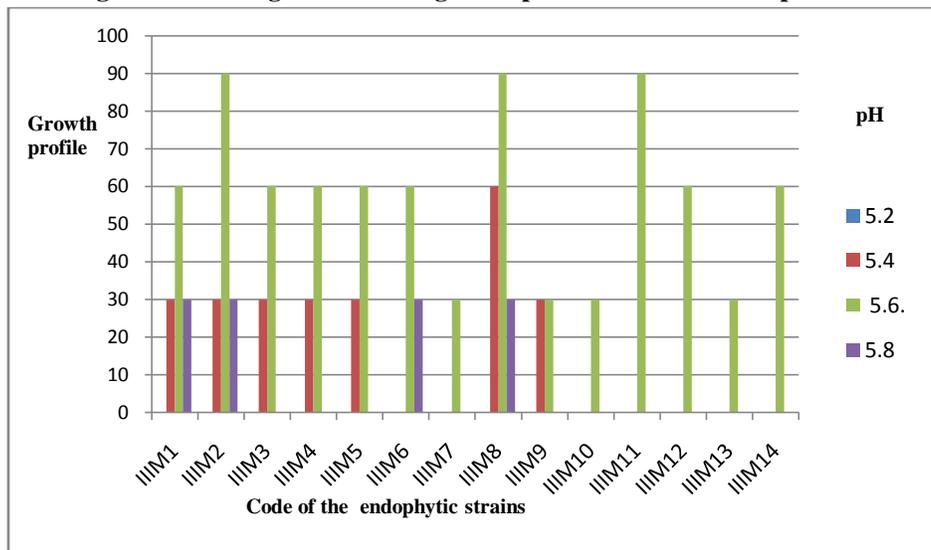


3.3. pH and media optimization of the isolated fungal strains

The isolated endophytes were subjected to different pH and optimum pH for the full growth was found to be 5.6 as shown in fig. 3.

Three media were used for the growth optimization i.e., SDA, PDA, CYA. Thus endophytes showed optimum growth in PDA medium.

Figure 3: Showing the different growth profile at different temperature



4. Discussion

Endophytes have been intensively studied in several unexplored environments around the world. Endophytes were distributed in each and every plant species and were investigated for endophytic microbial components[13]. Endophytes are chemical synthesizers inside plants[14]. A little work in this line has been done from this valley of Jammu & Kashmir state so far in order to harness the potential of hidden treasure of endophytes from indigenous plants. The whole Kashmir valley is naturally endowed with a rich biodiversity of high altitude medicinal aromatic plant species with their long history of traditional use as herbal medicines. In the present study an attempt has been made to isolate the endophytic fungi from the selected high value medicinal plants of Kashmir valley. We identified total fourteen different endophytic fungal species from four different medicinal plants; all these were identified based on the morphology and conidial characteristics, which were supported with other endophytic reports from different hosts[15]. These endophytic fungi may be the source of the potential antimicrobial and anticancer drugs. Single endophytes may be able to produce not one but several bioactive metabolites. Because of these endophytes play an enormous role and hub in the production of novel bioactive compound exploitation in medicine[16]. It has been reported that endophytic organisms that reside in the plant are capable of producing active compounds what plant is producing[9].

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