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Isolation and Identification of Fungi from Coal Mines near Hazaribagh and their Diversity Study

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Abstract

The present study was conducted to determine the fungal diversity of soil in coal mine of Charhi (Hazaribagh). Soil samples were collected from five different sites depending upon their richness of coal particles and they were assigned a notion as 1 to 5. Topography, vegetation, mining type, mining area, status of mining operation, biological reclamation activity was considered as the basis of the sample selection. Soil physicochemical characteristics were also analyzed. For microbiological study, soil samples were processed according to standard microbiological techniques. The result showed that the strains found in the soil samples of coal mine were common air borne species, which can grow on these harsh environments and this is done by growing them on agar media supplemented with different concentrations of coal powder and temperature.

Keywords: Thermotolerant; Coal mines; Diversity

Introduction

Coal, is the most abundant fossil fuel resource present in India. Beside crude oil, coal is very important source of basic energy. India is the 3rd largest producer of coal in the world and India has the 4th largest reserves of coal in the world [1]. In India the states are Jharkhand, Odisha, West Bengal, Bihar, Chhattisgarh, Telangana and Madhya Pradesh. [2] Jharkhand is known for its mineral resources and it is the prime center for cooking coal in the country [3].

The province, Chhota Nagpur is a storehouse for the minerals like mica, bauxite, copper, limestone, iron ore and coal. The Damodar valley is rich in coal and it is considered as a good source for cooking coal. This type of land, in which the soil has high concentration of inorganic nitrogen and sulfur, allows microbes to exploit these chemicals, only if they can sustain the high temperature.

The samples were collected from, Tapin North Opencast Project which is a part of CCL, Hazaribagh Area. It is about 7 km east of Charhi village, which lies on NH-33 (Figure 1). The soil of Hazaribagh has sufficient amount of moisture; the moist-heat is very effective in killing microbes as compared to dry heat. So the threshold value for fungi to survive is 60°C. [4], these conditions, plus the presence of organic substances, create a fairly favorable growth environment for microscopic fungi, which can be found around the field [5].

Air is the main source for the colonization of fungi on the coal mine surface, and they adapt themselves to exploit the organic and inorganic substances present in the surrounding [6], some of the airborne fungal species (*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Chaetomium* spp. etc) that occur in the mine. Among them *Chaetomium* spp. are dominantly present. In these habitats, fungi may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions. So the spores can be easily found in the soil samples. The objectives of this study were to determine the diversity of culturable thermophilic and/or thermotolerant fungal species in the soil of coal mines, Charhi, Hazaribagh (Jharkhand), and to determine the optimal *in vitro* growth conditions for these species.

Materials and Methods

Characterization of sites

Jharkhand has been distributed with 29% of the total reserves of Coal in India. We selected Hazaribagh because of its feature being of tropical monsoon type. The coal field is distributed into five zones depending upon their distance from the coal mine. The sites were named as Site 1 (200 m), Site 2 (300 m), Site 3 (400 m), Site 4 (500 m) and Site 5 (600 m), Site 5 was very near to the civilized area and road.

Soil cores

Soil samples were collected in sterile polyethylene bags with the help of a 5 cm diameter stainless steel corer pre-sterilized with 95% ethanol and 15 cm soil cores were obtained. Three soil cores were taken from a single site, mixed thoroughly, stored at 4°C, and processed next day. Soil was prepared by suspending 6 ml (5 g approx) of soil in 100 ml of sterile distilled water, mixing of the suspension for 15 minutes, and allowing the debris to settle. After removing the aliquots for culturing, the pH values of the suspensions were determined with the help of a laboratory digital pH meter. Which were as follows, Site 1 – 6.3, Site 2 – 6.7, Site 3 – 7.1, Site 4 – 6.2, and Site 5 – 5.9.

Isolation, growth and identification of fungi

A pour plate method was employed here for the isolation of the fungi. Soil suspensions were diluted to obtain 40-50 CFUs per plate. The fungi were isolated by plating the soil suspension on the Potato Dextrose Agar (PDA) media. The medium contained 50-mg/L streptomycin, which was added to cooled medium after autoclaving to

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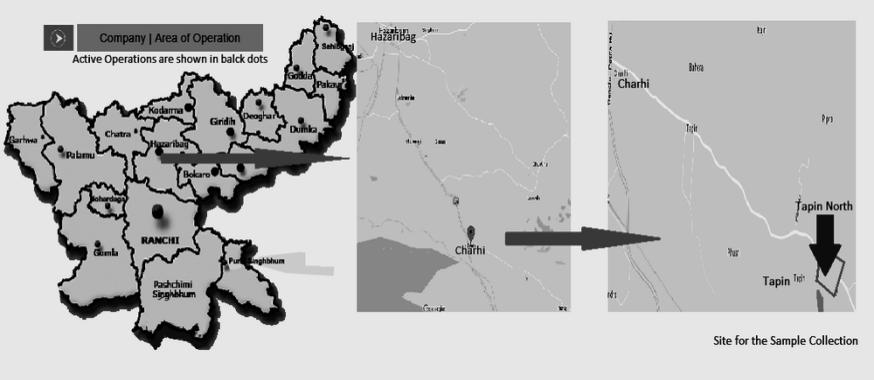

Figure 1: Site for the sample collection, Tapin north of the Tapin Block, Charhi, Hazaribagh, Jharkhand.

inhibit bacterial growth. Three plates from each site were incubated for 24 to 96 hours at 45°C, and each morphologically unique fungal colony was sub-cultured on PDA media. All of the fungal species observed grew on PDA medium, which was used for subsequent experiments and for storage in slants.

In vitro growth condition and optimal temperature of fungi

The temperature optima of various fungal isolates were determined by measuring the colony diameters after incubation of PDA plates for 24 to 120 hours at 25, 35, 45, 55, and 60°C.

The species that grew at 50°C but not at or below 20°C were considered thermophilic [7].

The coal dust was collected and powdered and mixed with the PDA, and autoclaved. The subsequent sub culturing was done on the medium containing coal powder. The amount of coal powder varies as 5 g, 10 g and 15 g of powder per plate of the PDA.

The isolates were identified by microscopic analysis by using taxonomic guides and standard procedures [8-12].

Growth rates (kd) were determined with the following equation: kd=D/T, where D is the experimentally determined average diameter of the fungal colony in mm exclusively the diameter of the inoculum (8 mm) and T=time period.

Results

From all five sites we cultured 11 different species belonging to different groups on PDA plates at 45°C. All of the species that grew at 45°C were members of the Ascomycetes and Zygomycetes; no members of the Basidiomycetes were found. The optimal growth temperature for most of these fungi was 45°C when the organisms were grown on PDA medium. Four species, *Aspergillus fumigatus*, *Chaetomium senegalense*, *Chrysosporium tropicum* and *Penicillium chrysogenum* had thermotolerant profiles since they did not grow at 55°C but grew when they were incubated at 35°C after exposure to 55°C for 7 days. Seven other species (*Chaetomium thermophile*, *Chaetomium globosum, Chaetomium piluliferum, Curvularia lunata, Melanocarpus albomyces, Scytalidium thermophilum* and *Cladosporium* spp.) grew at 55°C but not below 20°C. These species were classified as thermophiles (7).

Name of the sites are given in the Table 1.

Growth was observed at different temperatures

The fungal discs were cut from the PDA plate and sub-cultured on the PDA plate containing coal powder and incubated at different temperatures. The growth was measured by measuring the diameter of the fungal colonies on the 9th day and from that the optimum temperature and coal concentration is estimated. And the optimum temperature and the concentration of coal are given bellow in Table 2.

S. No.	Name of the Species	Sites
1.	*Chaetomium thermophile*	Site 1
2.	*Chaetomium globosum*	Site 1 and 3
3.	*Chaetomium piluliferum*	Site 1 and 3
4.	*Scytalidium thermophilum*	Site 2
5.	*Curvularia lunata*	Site 2 and 3
6.	*Melanocarpus albomyces*	Site 1 and 2
7.	*Cladosporium spp*	Site 2 and 3
8.	*Aspergillus fumigatus*	Site 3,4 and 5
9.	*Chaetomium senegalense*	Site 3
10.	*Chrysosporium tropicum*	Site 2 and 3
11.	*Penicillium chrysogenum*	Site 3,4 and 5

Table 1: Distance from the site of mining, and the isolates.

S. No.	Name of the Species	Optimum Coal concentration	Optimum temperature
1.	*Chaetomium thermophile*	10 g	50-55 °C
2.	*Chaetomium globosum*	5 g	25-35°C
3.	*Chaetomium piluliferum*	5 g and 10 g	25-30°C
4.	*Scytalidium thermophilum*	5 g and 10 g	55-60°C
5.	*Curvularia lunata*	5 g	25- 35°C
6.	*Melanocarpus albomyces*	5 g and 10 g	>60°C
7.	*Cladosporium spp.*	5 g	25°C
8.	*Aspergillus fumigatus*	5 g and 10 g	35- 45°C
9.	*Chaetomium senegalense*	5 g	30°C
10.	*Chrysosporium tropicum*	5 g	30-35°C
11.	*Penicillium chrysogenum*	5 g	25-30°C

Table 2: Optimum growth condition for the isolates.

Discussion

The coal mines of Charhi seem to provide a little range of habitats for the fungi with thermo-tolerant activity. The evenness of species distribution/genera is significantly low. So, we have found very few species from the different sites around the coal mine. The data supports the smaller diversity and distribution of species along with their capacity to tolerate Coal concentration and temperature. The Maximum numbers of species were found from the *Chaetomium* genus, and they had better tolerance range.

Fungi with higher tolerance range for high temperature and coal concentration were found near the site of mining, which implies that those fungi need inorganic sulfur and nitrogen to grow, and they are able to use them for their benefits. And away from the site of mining, there were fungi which can be considered as air borne and adapted themselves in that environment. The presence of fungal colonies represents the primary succession at the area of mining, and this can be used to treat the soil fertility of such kind of land.

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