Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides

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Abstract

Twenty three soil samples were characterized for the incidence of fungal strains from pesticides contaminated agricultural soils. A total of 59 fungal strains were isolated and 33 fungi were characterized using various isolation and identification methods. Soil samples were also characterized for physiochemical properties. The isolated fungal strains were successfully identified belonging to the phylum ascomycota (7 genera), deuteromycota (2) and zygomycota (1). Alternaria, Aspergillus, Drechslera and Fusarium were predominant genera. Curvularia, Exserohilum, Humicola, Rhizopus and Torula were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used incubation conditions. Such strains were designated as Mycelia sterilia. Further, these species will be used in biodegradation of commonly used pesticides.

Key words: Ascomycota, deuteromycota, zygomycota, mycelia sterilia, physiochemical, pesticides.

Introduction

Increasing environmental awareness has resulted in regulatory measures that aim to remedy past mistakes and protect the environment from future contamination and exploitation. These measures intend to preserve the environment and protect human health. Some of the pollutants of concern are chemical from pesticide, were banned when it was discovered that they were hazardous to human health. India is the largest consumer of pesticides in South Asian countries where maximum (44.5%) consumption of the total pesticides is by cotton crop.

Pesticides get sorbed to soil and sediments owing to its hydrophobic nature. This makes it persistent in soil and sediments owing to its hydrophobic nature. This makes it persistent in soil and sediments and water. This Results in rapid increase in aquatic and soil environments lead to accumulation in crop wastes, macrophytes, phyto-plankton, fishes, vegetables, milk, and milk products. Pesticides and their degradation products, generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation.

In our country, about 99 per cent of the pesticides are imported in bulk and in concentrated form (based on 1996 statistic). They are diluted and/or mixed with other chemicals by local manufacturers to obtain the formulation desired for local conditions. Unfortunately, in many cases, these compounds are also persistent in nature. Long after their use has been discontinued, these chemicals remain in soils and sediments where they can enter the food chain directly or percolate down to the water table. Once in the groundwater, these pollutants can enter drinking water wells and cause health problems.

Indirect accumulation in higher trophic level organisms, such as mammals, may cause health problems over time because of the increasing levels of toxic compounds within the body. There are two main reasons that these compounds persist in nature. First, the conditions necessary for their biodegradation are not present. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site (Frazar, 2000). If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavorable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation.

However, there were some microorganisms that can survive in pesticide contaminated site. Pesticide may affect soil microbial populations, stimulating growth of certain microorganisms and exerting toxic effects and inhibiting growth of others. So, identification and characterization of these microbial species is important to study about its potential candidate used in bioremediation. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. Therefore, in present investigation an attempt has made to
Material and Methods

Collection of soil samples: Twenty three soil samples collected from different sites having history of repeated pesticides application were used for the isolation of fungal organisms. Samples were collected in sterile zipper polyethylene bags and stored at 4 °C before processing.

Reagent and chemicals: All media components and chemicals used in the studies were of analytical grade and purchased from Hi-media laboratory Pvt. Ltd, Mumbai and Sigma–Aldrich, USA.

Physicochemical characterization of soil samples: Physicochemical parameters include organic carbon/nitrogen, pH, water content and temperature etc., microbial population density generally decreases with depth as a function of the availability of organic carbon and molecular oxygen, parameters which typically decrease with depth. Temperature and color of the soil samples was recorded on the spot. Moisture content, pH and % organic carbon and % organic nitrogen was measured according to standard procedure as given below:

Moisture content: 10 g of soil samples collected from pesticides contaminated fields were dried at 60°C for 72 h in oven and then the moisture content was calculated. Dry weight of the sample was taken till it showed its constant weight. The percent moisture was expressed as follows:

\[
\text{Moisture} \% = \frac{W_1 - W_2}{100} \times 100
\]

Where W1 = Weight of soil before oven drying
W2 = Weight of soil after oven drying

pH of soil sample: Soil sample were dried at 60 °C for 72 h, powdered in pestle and mortar and filtered through 2 mm sieve and the sieved soil were dissolved in distilled water (2.5w/v) and vortexing for 5 minutes at 120 rpm then pH was measured by digital pH meter. The results revealed that the soil samples from various sites were shown heterogeneity in physicochemical parameters presenting in table 1.

Percent organic Carbon / Nitrogen: One gram soil sample was mixed with 10 ml potassium dichromate (1 N) and 20 ml concentrated H2SO4. Then 150 ml distilled water and 25 ml FeSO4 (0.5 M) were added and the excess was titrated against potassium permanganate (0.1 N) solution to pink end point. The organic carbon content of soil was determined using following equation:

\[
\% \text{ Organic carbon} = \frac{A - B}{C} \times 0.3 \times 1.33
\]

Where
i. Volume of K2Cr2O7 X Normality of K2Cr2O7, ii. Volume of KMnO4 X Normality of KMnO4, iii. Weight of sample

Soil organic nitrogen was calculated using following equation: Organic nitrogen (%) = 0.862 × % organic carbon

Isolation and characterization of fungi: Serial dilution agar plating (Apinis, 1963), Warcup’s soil plate and Waksman Direct inoculation methods were employed for the isolation of soil microbe; suspension was diluted up to 10−5. The aliquots were cultured for fungus on Czapek Dox Agar (NaN03 2.0 g, KCl 0.5 g, K2HPO4 1.0 g, MgSO4 7H2O 0.5 g, FeSO4 7H2O 0.01 g); and Potato Dextrose Agar (Peeled potato200.0 g, Dextrose 20.0g) media. For primary isolation Rose Bengal (30mg/L) was also added to the medium. Three plates from each soil samples were incubated for 24-96 h at 25±2 °C, and each morphologically unique fungal colony was sub-cultured and purified using standard techniques. The fungal species were identified and characterize based on their morphological characters and microscopic analysis by using taxonomic guides and standard procedures. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc. The procedure of isolation and characterization of fungal species is shown in flow chart:

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

Results and Discussion

Characterization of physiochemical parameters of soil samples: The physicochemical properties of soil used for isolation of microbial species were analyzed in the present study. The color of soil samples was brown to black, with variation in pH (7.82 - 8.65). The temperature of the soil was high (30.2 – 33.2 °C) with great variation in percent moisture content (0.35 – 0.95), organic carbon (0.2568 – 0.4125) and percent organic nitrogen (0.2213 – 0.3555). The results revealed that the soil samples from various sites were shown heterogeneity in physicochemical parameters presenting in table 1.

Soil properties like organic matter, pH and moisture content etc., affects the density and diversity of microbes in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. The moisture content in soil acts as solvent and is essential for microbial functioning. A certain minimum level of organic matter and moisture content is essential to ensure the presence of an active microbial population in the soil. In the present study, the important
Physicochemical properties of the soils, used for the evaluation of natural fungal density, were determined.

Isolation and characterization of fungal strains: A total of 59 fungal isolates were obtained from the analyses of 23 soil samples taken from pesticide contaminated soils through soil dilution agar plating. Warcups soil plate and Waksman Direct inoculation method. All fungal isolates were obtained in pure cultures by using standard techniques. The photomicrographs of all the fungal isolates were taken helps in identification of the fungal isolates. The cultural characteristics and the sporulating structures of these isolates are presented in figure 1. Thirty three fungal isolates were identified as, Aspergillus alhabadii, A. aultaceus, A. candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. oryzae, A. sclerotiorum, Aspergillus sp, A. sulphureus, A. sydowii, A. terrus, Alternaria brassicola, A. citri, Alternaria sp., Alternaria sp., Alternaria sp., A. vitis, Curvularia brachyspora, Exserohilum turcicum, Drechslera australiensis, D. halodes, D. hawaiiensis, Drechslera sp., Humicola insolens, H. brevis, Fusarium acuminatum, Fusarium sp, Rhizopus sp, Torula herbarum and Ulocladium sp.

The isolates from agricultural soils were identified as filamentous fungi belonging to the phyla Ascomycota (7 genera), Deuteromycota (2) and Zygomycota (1). Most of the fungal isolates were identified to the species level. Alternaria, Aspergillus, Drechslera and Fusarium were predominant genera. Curvularia, Exserohilum, Humicola, Rhizopus and Torula were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used incubation conditions. Such strains were designated as Mycelia sterilis. In such soil fungi may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favourable environmental conditions. Some of the species have been reported as common isolates from pesticides polluted soil and used for biodegradation of xenobiotics. In such soil fungi may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favourable environmental conditions. Some of the species have been reported as common isolates from pesticides polluted soil and used for biodegradation of xenobiotics.

The fungal species were able to grow efficiently and appear concurrently which means these indigenous fungi have the capacity to adapt to xenobiobic compounds as novel growth and energy substrate. Therefore, these fungi have a potential to degrade xenobiobic compounds which will be tested in further study for degradation of commonly used pesticides.

References


19. Ottov J.C.G., Rose Bengal as a selective aid in the isolation of fungi and actinomycetes from rational sources, Mycologia, 64 (2), 304-315 (1972)


### Table-1
Physiochemical parameters of soil samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>% Moisture</th>
<th>% Carbon</th>
<th>% Organic Nitrogen</th>
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Figure 1
Flow sheet: Protocol for isolation and characterization of fungal strains

1. Soil sample
2. Dilution plate method employed
3. Culture on CDA / PDA media with Rose Bengal
   - Observation of growth
   - Streaking on CDA/PDA with Rose Bengal
   - CDA/PDA without Rose Bengal
     - Incubated at 25±2 °C for 3-5 days
4. Culture identification
   - Microscopy
   - Culture characteristics
   - Lacto-phenol cotton blue staining