

Original Research Article

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Cultural and Morphological Characterization of Antagonistic *Trichoderma* Isolates

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ABSTRACT

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In this study, the fifteen isolates of *Trichoderma* were obtained from rhizospheric soil samples of rice, soybean and mungbean from various locations of Pantnagar and Dehradun. These isolates were characterized based on their cultural and morphological characteristics. The cultural characteristics like colony colour, presence or absence of concentric rings, surface topography, pigmentation on the lower side of the plate, time of first appearance of green conidia and their growth at various temperatures were determined on the basal medium Potato Dextrose Agar. The morphological characters like conidiophores, their branching pattern, phialides number, their arrangement, conidial shape and formation of chlamydospores were observed under the microscope.

Introduction

The genus *Trichoderma* is of immense economic importance due to their ability to control a wide array of plant pathogens. They employ several mechanisms like mycoparasitism, antibiosis, competition for space and nutrients, stimulating plant health and inducing plant defenses for managing several harmful plant pathogens. The isolates of *Trichoderma* were differentiated on the

basis of cultural characteristics like colony colour, presence or absence of concentric rings, surface topography and pigmentation on lower side of the plate as well as morphological characteristics including conidiophores, phialides and phialospores (Seaby, 1996). Several workers have classified the isolates of *Trichoderma* on basis of their cultural and morphological characteristics (Rifai and Webster, 1996; Kiffer and Morelet, 2000; Samuels *et al.*,

2002). These cultural and morphological characteristics of *Trichoderma* play a key role in identification of species of *Trichoderma*. This study was carried out to study the variability among the *Trichoderma* isolates on basis of their cultural and morphological characteristics.

Materials and Methods

Sample collection and isolation

The fifteen isolates of *Trichoderma* were obtained according to the method given by Elad *et al.*, (1981) using serial dilution (Krassilnikov, 1950) on *Trichoderma* selective medium (TSM). The *Trichoderma* isolates were obtained from the rhizospheric soils of soybean, rice and mungbean from various locations of Pantnagar and Dehradun (Table 1).

Cultural and morphological characterization of *trichoderma* isolates

The cultural and morphological characteristics of fifteen *Trichoderma* isolates were determined on Potato Dextrose Agar (PDA). A 5 mm disc of each *Trichoderma* isolates was cut from the edge of actively growing fresh colony using sterile cork borer and was transferred to Petri plates containing PDA with mycelium facing downwards. Three replications were maintained for each treatment. The plates were incubated at $28\pm 1^\circ\text{C}$ and the colonies were examined at every 24 hrs interval.

The observations on cultural characters like colony colour, presence or absence of concentric rings, surface topography, pigmentation on lower side of the plate, time of first appearance of green conidia were recorded. For morphological studies microscopic slides were prepared from 3-7 days old culture by taking a thin section of

fresh hyphal tips with help of sterilized inoculating needle in a drop of lactophenol cotton blue solution on to the slide. The hyphae and conidiophores were separated before placing coverslip with the help of needle.

Excess mounting fluid was drained with a clean blotting paper. The cultures were incubated upto 2 weeks at 20°C to observe chlamydospores. The slides were observed under the microscope for the characters like conidiophores, their branching pattern, phialide numbers, their arrangement, conidial shape and formation of chlamydospores.

Results and Discussion

Cultural characteristics and growth rate of *trichoderma* isolates

The cultural characteristics of *Trichoderma* isolates were studied on basal medium PDA. The maximum mycelial growth was recorded at 25°C showing colony diameter of 87 mm to 90 mm after 72 hours followed 20°C and 30°C and abnormal colonies with irregular margins were observed at 35°C . Among the fifteen isolates, 4 were dark green, 2 were light green, 7 were light to dark green and 2 were whitish to light green in colour. Eleven isolates (PT-1, 2, 4, 6, 7, 8, 9, 10, DDNT-1, 2, and 3) showed concentric ring formation.

Eleven isolates exhibited rough, spongy and raised colony, two showed smooth and flat colony, one isolate was having smooth and flat colony with dense sporulation at border and one showed spongy and fluffy growth. One isolate produced light brown pigmentation, 5 showed whitish creamy, 3 yellowish, 5 yellowish green and 1 pinkish pigmentation on the reverse side of the plate. Seven isolates produced conidia at 48 hrs, three at 48-72 hrs and one at 72 hrs (Plate 1 and Table 2).

Morphological characterization of *trichoderma* isolates

All the isolates were branched at right angle, the branching pattern was difficult to define. Primarily two types of phialides were observed i.e. lageniform and ampulliform. Conidia were globose, subglobose and globose to subglobose, globose to ellipsoidal and green in colour. Chlamydo spores were either terminal or intercalary or both in some isolates as presented in Table 3 and Plate 2. On the basis of morphological characteristics as presented in Table 3 the *Trichoderma* isolates were grouped into four categories (Table 4).

Colony of *Trichoderma* usually grow rapidly,

initially smooth surface, almost translucent or watery and later became floccose or compactly tufted, in various shades of pure white or green. *Trichoderma* colonies secreted pigments into the medium or reverse side of plates (Mukherjee, 1991). Cultural characters such as growth rate, colour and appearance of the colonies were considered as taxonomically important characters (Samuels *et al.*, 2002). Sharma (2009) characterized the *Trichoderma* isolates of Uttarakhand based on culture colour, pigmentation, conidiation and growth rate. Erayya (2014) also observed the characters like colour of the colony, formation of concentric rings, surface topography and pigmentation on the reverse side of the plate to characterize the *Trichoderma* isolates from Uttarakhand.

Table.1 Soil samples collected from different locations

S. No.	Location	Site	Crop
1.	Pantnagar	Agronomy block, CRC	Soybean
2.	Pantnagar	Agronomy block, CRC	Rice
3.	Pantnagar	Plant Pathology block, CRC	Soybean
4.	Pantnagar	Plant Pathology block, CRC	Rice
5.	Pantnagar	Plant Pathology block, CRC	Mungbean
6.	Pantnagar	Breeding block, CRC	Soybean
7.	Pantnagar	Entomology block, CRC	Soybean
8.	Pantnagar	Agrometeorology block, CRC	Soybean
9.	Pantnagar	Breeding block, CRC	Rice
10.	Pantnagar	Entomology block, CRC	Rice
11.	Dehradun	Premnagar	Soybean
12.	Dehradun	Bhaniawala	Soybean
13.	Dehradun	Raipur	Soybean
14.	Dehradun	Mehuwala	Soybean
15.	Dehradun	Doiwala	Soybean

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Table.2 Cultural characteristics of *Trichoderma* isolates

S.No .	Isolate code	Colony colour	Concentric rings	Colony morphology	Pigmentation on lower side of the plate	Sporulation initiate After (hrs)
1.	PT-1	Light to dark green	Present	Rough, spongy and raised	Whitish creamy	48-72
2.	PT-2	Light green	Present	Rough, spongy and raised	Yellowish green	48-72
3.	PT-3	Light to dark green	Absent	Smooth, flat and dense sporulation at border	Pinkish	48-72
4.	PT-4	Light to dark green	Present	Rough, spongy and raised	Yellowish green	48
5.	PT-5	Dark green	Absent	Spongy and fluffy	Yellowish	48
6.	PT-6	Whitish to light green	Present	Rough, spongy and raised	Whitish creamy	48-72
7.	PT-7	Light to dark green	Present	Rough, spongy and raised	Yellowish green	48
8.	PT-8	Dark green	Present	Rough, spongy and raised	Yellowish	48
9.	PT-9	Light to dark green	Present	Rough, spongy and raised	Whitish creamy	48
10.	PT-10	Dark green	Present	Rough, spongy and raised	Whitish creamy	48-72
11.	DDNT-1	Light to dark green	Present	Rough, spongy and raised	Yellowish green	72
12.	DDNT-2	Light to dark green	Present	Rough, spongy and raised	Whitish creamy	48-72
13.	DDNT-3	Dark green	Present	Rough, spongy and raised	Yellowish green	48-72
14.	DDNT-4	Whitish to light green	Absent	Smooth and flat	Light brown	48
15.	DDNT-5	Light green	Absent	Smooth and flat	Yellowish	48

Table.3 Morphological characteristics of *Trichoderma* isolates

S.No	<i>Trichoderma</i> isolates	Phialide disposition	Phialide shape	Conidia shape	Conidia colour	Chlamydo spores
1.	PT-1	2-4	Lageniform	Globose to subglobose	Green	Terminal
2.	PT-2	2-4	Ampulliform	Globose to subglobose	Green	Terminal and intercalary
3.	PT-3	2-3	Lageniform	Globose	Green	Terminal and intercalary
4.	PT-4	2-4	Ampulliform	Globose to subglobose	Green	Terminal
5.	PT-5	2-3	Ampulliform	Globose to subglobose	Green	No chlamydo spore formation
6.	PT-6	2-4	Lageniform	Globose to subglobose	Green	Terminal and intercalary
7.	PT-7	2-3	Ampulliform	Globose to subglobose	Green	Intercalary
8.	PT-8	2-3	Lageniform	Globose to subglobose	Green	Terminal and intercalary
9.	PT-9	2-3	Lageniform	Globose	Green	Intercalary
10.	PT-10	2-3	Ampulliform	Globose to subglobose	Green	Intercalary
11.	DDNT-1	2-4	Ampulliform	Globose to subglobose	Green	Terminal and intercalary
12.	DDNT-2	2-3	Lageniform	Globose to ellipsoidal	Green	Intercalary
13.	DDNT-3	2-3	Lageniform	Globose to subglobose	Green	Terminal and intercalary
14.	DDNT-4	2-3	Ampulliform	Globose to subglobose	Green	Terminal
15.	DDNT-5	2-3	Ampulliform	Globose to subglobose	Green	Terminal and intercalary

Table.4 Grouping of *Trichoderma* isolates based on the morphological characters

S.No.	Group	Isolates	Characteristics features
1.	Group I	PT-2, PT-4, PT-5, PT-7, PT-10, DDNT-1, DDNT-4 and DDNT-5	Phialides: Ampulliform Conidia: Globose to subglobose
2.	Group II	PT-3 and PT-9	Phialides: Lageniform Conidia: Globose
3.	Group III	PT-1, PT-6, PT-8 and DDNT-3	Phialides: Lageniform Conidia: Globose to subglobose
4.	Group IV	DDNT-2	Phialides: Lageniform Conidia: Globose to ellipsoidal

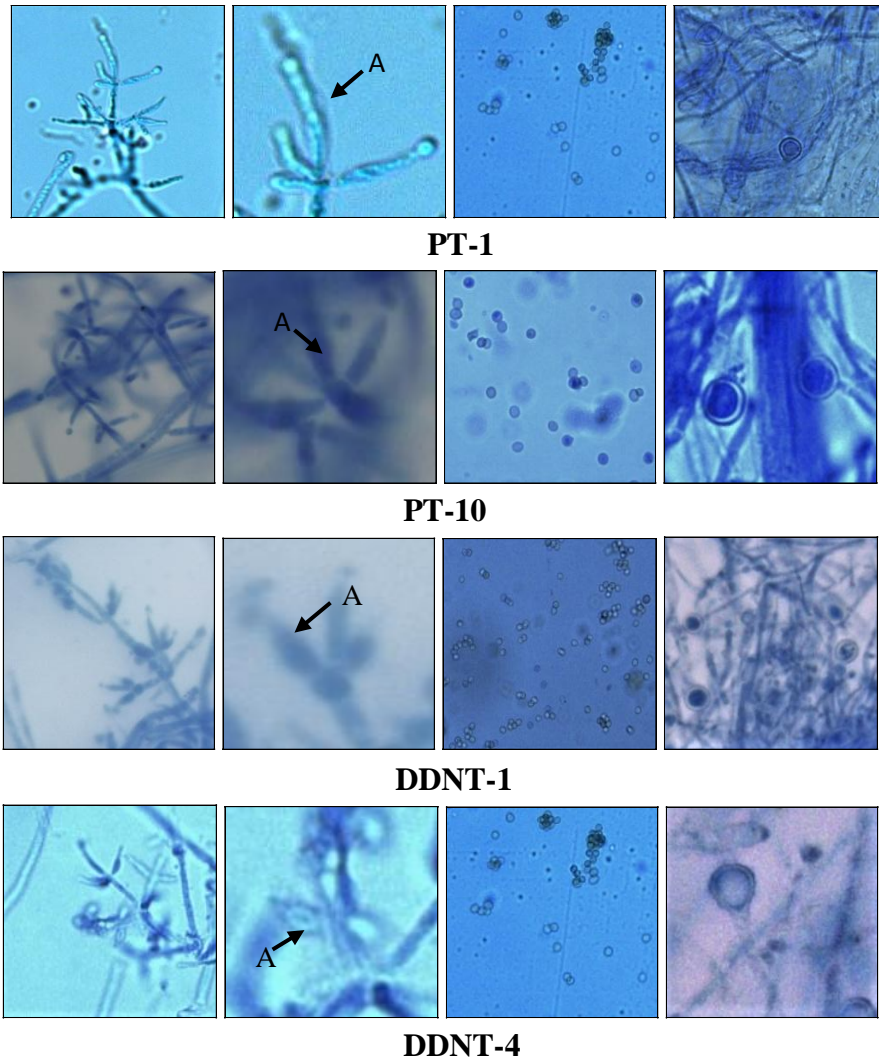


Fig.1 Conidiophores Phialides Conidia Chlamydospores

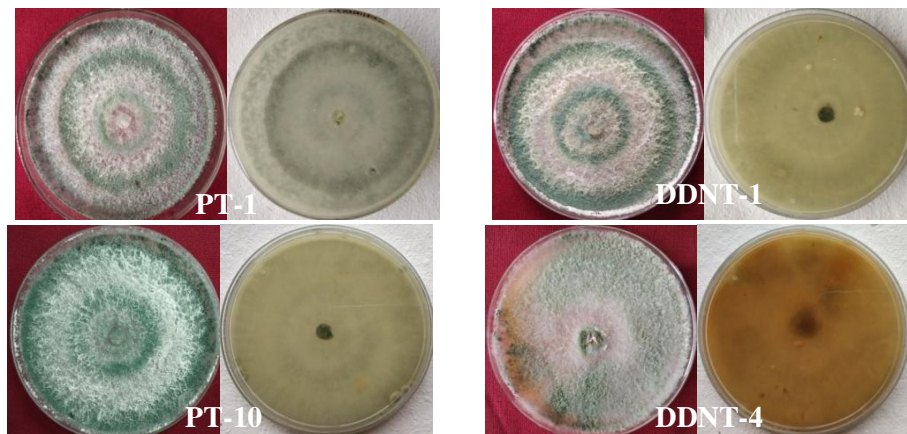


Plate.1 Cultural characteristics of *Trichoderma* isolates

Trichoderma was divided into nine species aggregate which was based on morphological characteristics (Rifai and Webster, 1996) and morphological characterization was done based on key provided by Bissett (1991 a and b) which included characters like colony colour, colony morphology and spore pattern. Similarly, Kiffer and Morelet (2000) grouped *Trichoderma* isolates on colony colour, pigmentation, conidiophores and phialide characters.

The morphological characteristics like branching pattern of conidiophores, shape and size of phialides, conidia and formation of chlamydospores are frequently observed for identification of *Trichoderma* (Gams and Bissett 2002; Samuels *et al.*, 2002). Isolates of *Trichoderma* were identified up to species level based on morphological characteristics such as size and shape of conidiophores, conidia and phialides (Srivastava *et al.*, 2014).

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