Cultural and Morphological Characterization of Antagonistic Trichoderma Isolates

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A B S T R A C T

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., 2000).
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±1°C and the colonies were examined at every 24 hrs interval.

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. Chlamydospores 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 | *Trichoderma* isolates | Phialide disposition | Phialide shape | Conidia shape | Conidia colour | Chlamydospores  |
|------|------------------------|----------------------|----------------|---------------|----------------|----------------|
| 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 chlamydospore 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                                    |
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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