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Cultural Characterization of Indigenous Isolates of *Trichoderma* spp. Isolated from Chhattisgarh, India

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ABSTRACT: The study was conducted to culturally characterize 32 native isolates of *Trichoderma* spp. from Chhattisgarh, India. Various experiments were conducted for the characterization of *Trichoderma* isolates. *Trichoderma* isolates' colony colour ranged from white, yellowish-green to green and dark green upon sporulation. 1-2 concentric rings were observed in many isolates. A coconut-like odour was detected in isolates IGKV (T2, T20, T24, T28). A white colour colony and yellow pigmentation were seen in isolate IGKV T18 in the PDA medium. A dark green sporulation centre was observed in several isolates IGKV (T5, T11, T13, T19, T17, T21, T24, T27, T28, T29, T30). Among the three media (*Viz.* PDA, OMA & MEAB) tested for obtaining optimum growth and sporulation, maximum radial growth of 87.10 mm with a growth rate of 29.03 mm/day was observed on PDA by most of the isolates. It was observed that isolates showed variation in colony colour and morphology on different media. Growth and sporulation were observed at different temperatures and pH and it was observed that the most suitable temperature is 25±2°C and pH is 7 for maintaining *Trichoderma* cultures on PDA medium.

Keywords: Cultural characterisation, *Trichoderma* spp., native isolates, sporulation, radial growth.

INTRODUCTION

Food and nutritional security of the growing population amid raised concerns for a safe environment and injudicious use of chemical pesticides is a great challenge for plant protection scientists. The issue has resulted in the need to develop and validate alternative disease management strategies for reducing crop losses due to biotic stresses. Biological control of plant pathogens is one such strategy and involves the use of effective biocontrol agents like *Trichoderma* spp., *Pseudomonas fluorescens*, etc.

The genus *Trichoderma* is a fungal biocontrol agent, with potential for management of soil-borne pathogens like *Rhizoctonia solani*, *Fusarium* spp., *Aspergillus niger* etc. The fungus is cosmopolitan in soils and on decaying wood and organic matter. Species of *Trichoderma* are frequently dominant components of the soil microflora in widely varying habitats. This may be attributable to the diverse metabolic capability of *Trichoderma* species and their aggressive competitive nature. For achieving effective biocontrol activity isolation and characterization of *Trichoderma* spp. from an agro-ecology is required. Cultural characters can be distinctive and may be characteristic of a species; these characters may be used for differentiating between isolates obtained from same agro-ecology. Similarly, *in vitro* growth rates in culture can be useful in distinguishing isolates with higher competitive ability due to vigorous growth. Thus, these descriptive characteristics may be instrumental in identification of the isolates with higher biocontrol and plant growth promotion activity. Furthermore, the native/ local isolate of the *Trichoderma* spp. may have higher ecological adaptability and exhibit higher biocontrol potential. Therefore, the present study was conducted to isolate and characterize native *Trichoderma* spp. from Chhattisgarh, India from the rhizospheres of diverse crops and their cultural characteristics were enumerated.

MATERIAL AND METHODS

Collection of soil samples and isolation of Trichoderma spp. Soil samples were collected from the rhizospheres of various crops (Table 1) from different locations in Chhattisgarh state of India for isolation of the *Trichoderma* spp. The soil samples were brought to the State Biocontrol Laboratory, Indira

Gandhi Krishi Vishwavidyalaya, Barrister Thakur Chhedilal College of Agriculture and Research Station, Chorbhatti, Bilaspur, Chhattisgarh and stored at 4°C till further processing. *Trichoderma* spp. was isolated using soil dilution technique (Ben-David *et al.,* 2014) using serial dilution of the soil sample. 100 µl of fivefold dilution was poured aseptically on the *Trichoderma* Selective Medium (TSM), Himedia, India in three replications and incubated at 27±2°C for 72 hours in BOD incubator, Sanco, India. The colonies were observed after 72 hours and morphologically distinct colonies were purified on Potato Dextrose Agar (PDA), SRL, India and incubated at 27±2°C. The purified colonies were transferred aseptically to PDA slants and preserved at 4°C for further studies. Further studies were conducted at Microbial Biocontrol Laboratory, ICAR-National Research Centre for Integrated Pest Management, New Delhi.

Phenotypic Characterization. The isolates (Table 1) were revived from the slants by transferring aseptically to PDA in Petri plates followed by incubation at 27±2°C. The cultural characteristics of all the isolates under investigation were observed on 20 ml of sterilized Potato Dextrose Agar (PDA) poured aseptically in sterilized 90 mm glass Petri plates. A mycelial disc of approximately 4 mm diameter was transferred aseptically at the centre of each Petri plate in three replicates and incubated at 27±2°C for 5 days in a BOD incubator (SANCO, India). The colony characteristics observed were: - a) Colour of the fungus b) Growth of the fungus; Growth patterns; Appearance: - Ringed/sectoral/uniform/smooth/rough. The cultural characteristics were photographed. Diffusible pigments and characteristic odour were also observed and recorded (Gams and Bissett 1998).

Effect of growth media on *Trichoderma* isolates. The following media were evaluated for maximum growth of *Trichoderma* spp. under in vitro conditions: Potato Dextrose Agar (PDA), Malt Extract Agar Base (MEAB), and Oatmeal Agar (OMA). Radial growth in 'mm' & growth rate (mm/day) was measured after 72 hrs. followed by 120 hrs. of inoculation (Hoyos-Carvajal and Bissett 2011).

Determination of optimum temperature for *in vitro* growth and sporulation. Isolates were inoculated in three replicates on PDA medium and incubated at 15°C, 25°C, and 37°C (Leo *et al.,* 2011). Radial growth, growth rate (mm/day), initiation of sporulation, and sporulation rate after 5 days of inoculation were observed for cultures incubated at 25°C and 37°C, and after 7 days for cultures incubated at 15°C. The sporulation rate was calculated using the Neubauer counting chamber of the Haemocytometer (ROHEM, India). After five days in the case of 25°C and 37°C, and after 7 days in the case of 15°C, the spores were harvested from the plates and diluted following the serial dilution technique. The 50 µl spore suspension was transferred between the cover slip and chamber, and the number of spores in the central square was counted using a compound light microscope (Nikon 80i) at 40X. The number of spores was calculated using the formula (Nirmalkar *et al.,* 2020):

(Area of each square = 1mm, Depth = 0.1mm, Volume = $0.1mm^3$ = 0.1µl occupied by each of the squares. So, 1ml (1000 µl) will have:

$$\frac{\text{Spore} \times 1000}{0.1}$$

Determination of optimum pH for in vitro growth and sporulation. Isolates were assessed for growth on PDA media in three replicates at pH levels 5, 7 & 9. The lower pH was adjusted using 10N HCl acid while the higher pH was maintained by 1N NaOH. Radial growth in 'mm', growth rate (mm/day), initiation of sporulation, and sporulation rate (no. of spore/ml) was measured after 72 hours of inoculation (Omar *et al.,* 2012). The number of spores/ml was calculated using the Neubauer counting chamber of the Haemocytometer (ROHEM, India) as explained earlier after five days of inoculation.

RESULT AND DISCUSSION

The cultural characteristics of all the isolates under study were examined on PDA and are presented in Table 1 & Fig. 1. *Trichoderma* isolates' colony colour ranged from white, yellowish green to green, dark green upon sporulation. 1-2 concentric rings were observed in many isolates. A coconut-like odour was detected in isolates IGKV (T2, T20, T24, T28). A white colour colony and yellow pigmentation were seen in isolate IGKV T18 in the PDA medium. A dark green sporulation centre was observed in several isolates IGKV (T5, T11, T13, T19, T17, T21, T24, T27, T28, T29, T30).

Effect of growth media on *Trichoderma* isolates. Growth and sporulation of *Trichoderma* spp. were studied on three synthetic media PDA, MEAB and OMA after 72 hrs. of inoculation at 27±2° in BOD incubator (Sanco, India) (Table 2, Fig. 2). On MEAB, the maximum radial growth observed was 90 mm in the four isolates IGKV -T4, T5, T6 & T29 with a growth rate of 30.00 mm/day followed by 87.5 mm in IGKV -T2, T11-24, & T31 with a growth rate of 29.17 mm/day. The least radial growth (80 mm) and growth rate (26.67 mm/day) were observed were the isolates IGKV T7 and IGKV T8. On OMA, the maximum radial growth observed was 80 mm in the isolates: IGKV -T11-32; with a growth rate of 26.67 mm/day followed by 75 mm by IGKV -T2 & T3 (growth rate 25.00 of mm/day). The least radial growth observed on OMA was by the isolate IGKV T6 (65mm, growth rate 21.67 mm/day) preceded by IGKV T1 (67.5, growth rate 22.50 mm/day). On PDA, the maximum radial growth observed was 90 mm by all isolates, except IGKV T3, T4, T5, T6, T7, T8, T9 & T10. Least radial growth observed was 67.5 mm, growth rate of 22.50 mm/day in isolate IGKV T4, preceded by IGKV T3 (radial growth 70 mm, growth rate 23.33 mm/day), IGKV T5, T8, T9, T10 (radial growth 80 mm, growth rate 26.67 mm/day) and IGKV T6, T7 (radial growth 85 mm, growth rate 28.83 mm/day).

Amongst the different media under study, PDA supported maximum radial growth with a mean radial growth of 87.10 mm and growth rate of 29.03 mm/day followed by MEAB (86.25 mm, 28.74 mm/day) & OMA (76.73 mm, 25.57 mm/day) (Table 5). The

isolates of *Trichoderma* were slow growing on OMA whereas, on MEAB, the sporulation was very fast as compared to OMA and PDA.

Previously, various authors have studied the cultural and morphological characteristics of *Trichoderma* spp. on different media. Malt extract agar is reported to be appropriate for conidia production and the observation of conidiophore branching, or potato dextrose agar, which is reported useful for observing pigment production (Hoyos-Carvayal and Bissett 2011). *T. harzianum* formed 1-2 concentric rings with green conidial production on PDA while white cottony mycelium with green conidiation was produced on Malt Extract Agar (MEA) (Shah *et al.,* 2012). The authors also reported that density of conidia production is higher at the centre compared to the margins of the colony. On PDA *T. viride* produced granular colonies with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculum in *T. harzianum*, some white pustules were also found growing on the green mat of conidia. *T. pseudokoningii* formed white mycelia with no conidial formation on PDA as well as MEA. The growth of *T. viride* was faster on MEA than the other two species, showing complete growth on 3rd day. Shah *et al*. (2012), uniformly flat and velvety colonies, with aerial mycelium consisting of short hyphae in one lawn over the colony, were observed on the MEAB medium (Carvalho *et al.,* 2018). Colony showed dull green tufts or pustules, colony reverse was discoloured was identified as *Trichoderma aureoviride;* colony showed dark green producing tufts or pustules fringed by sterile white mycelium, colony reverse showed dull yellowish was identified as *Trichoderma harzianum;* colony showed scattered in minute tufts, pale yellowish-green in colour, colony reverse was pale yellowish was identified as *Trichoderma reeseii;* colony showed dull green to bluish green sporulation, colony reverses colourless to pale yellow were identified as *Trichoderma koningii*; colony showed dark green to dark bluish green sporulation, colony reverse was amber or uncoloured were identified as *Trichoderma viride* (Shekhar *et al.,* 2017).

Effect of temperature on radial growth and growth rate. Different isolates were inoculated on PDA media and incubated at 15±2°C, 25±2°C, and 37±2°C, observations on RG, GR, initiation of sporulation, sporulation rate were recorded (Table 3, Fig. 3). At 25°C, isolate IGKV T22 exhibited a maximum RG of 90 mm with a GR of 30.00 mm/day followed by IGKV T3 (RG 88 mm, GR 29.33 mm/day) and IGKV-T1, T4, T6, T11, T18, T27 & T31 (RG85 mm, GR 28.33 mm/day). The least growth was observed in the isolates IGKV-T5, T12, T13, T14, T15, T16, T17, T19, T20, T21, T23, T24, T25, T26, T28, T29, T30 & T32 as 80 mm with a GR of 26.67 mm/day. At 37°C, the maximum RG of 80 mm was shown by the isolates IGKV -T24, T27, and T29 with a GR of 26.67 mm/day; while, least RG was recorded as 20 mm in the isolates IGKV -T3, T4, T7, T18, T23 with a GR of 6.67 mm/day. At 15°C, IGKV- T2, T5 & T8 showed a maximum RG of 50 mm with a GR of 16.67 mm/day. The least RG was observed in IGKV -T19, T21, T23, T24, T29 & T30 as 20 mm with a GR of 6.67 mm/day. Interestingly the least RG at 25°C was the same as the maximum RG at 37°C. Amongst the different temperatures, 25±2°C was found to be optimum for in vitro growth of the fungus with a mean value of 82.03 mm followed by 37±2°C (44.68 mm) & 15±2°C (36.75 mm) (Table 5). IGKV T24 & T29 showed similar growth at 25±2°C and 37±2°C (RG 80 mm and GR 26.67 mm/day). No isolates exhibited similar growth rates across the temperature suggesting variation in biocontrol potential of the BCA when exposed to different environmental conditions. At 25±2°C and 37±2°C sporulation started after 72 hours (03 days) of incubation, however, at 15±2°C sporulation was delayed and was observed after 168 hours (Fig. 3).

Mishra (2016) reported *Trichoderma* strain number T1, T4, T7, and T8 was not affected at different temperature *i.e.*, 20°C, 25°C, 30°C, and 35°C whereas in *Trichoderma* strain number T2, T5, T18, T28 less growth was recorded at 20°C and 35°C, suggesting variation in the temperature optima among strains of *Trichoderma* isolated from same agroecology. Tolerance to range of temperature is reported in *T. viride* and *T. harzianum* (20°C to 40°C) & *T. asperellum* and *T. hamatum* (25 °C -35°C) is reported (Ali *et al.,* 2015). However, the author reported slower growth at 15°C. Temperature significantly affected the radial growth and sporulation of *Trichoderma* spp. *T. viride* and *T. harzianum* showed a high range of temperature tolerance. It grew and sporulated well between temperatures 20 to 40°C. *T. asperellum* and *T. hamatum* grew and sporulated well between temperatures (25-35°C). But at low temperature (15°C), the growth was slow and all the *Trichoderma* spp. failed to sporulate even after 7 days of incubation. A similar trend was also observed at high temperatures for all four spp. of *Trichoderma* (Zehra *et al.,* 2017). Three morphologically similar species, *T. harzianum, T. aggressivum*, and *T. atroviride*, can be distinguished by growing them at 35°C. After 96 h, *T. harzianum* grows well and sporulates at 35°C, but the remaining two, *T. aggressivum* and *T. atroviride*, can have a colony radius of more than 5 mm (Gorai *et al.,* 2020). Bastos (2001) studied the effect of temperature (10, 15, 20, 25, 30 & 35 °C), pH (3.5-9.5), and liquid medium composition on the growth and sporulation of *T. stromatium* and observed that a temperature range of 20 and 30°C was optimum for growth and sporulation. No growth occurred at 10 and 35°C.

Effect of pH. Isolates were assessed for growth on amended PDA media in three replicates at pH 5, 7 & 9. RG in 'mm', GR (mm/day), initiation of sporulation, and sporulation rate (no. of spore/ml) were measured after 72 hours of inoculation at 27±2° in BOD incubator, (Table 4, Figure 4). At pH 5, IGKV T3 recorded the max RG of 90.00 mm with a GR of 30.00 mm/day followed by IGKV T6 (RG 86.00 mm, GR 28.67 mm/day). The least RG was noted in isolate IGKV 26 as 50.00 mm with a GR of 16.67 mm/day. The isolates produced greenish cottony colony at pH 5.

Table 1: Details of the *Trichoderma* spp. isolates under study.

Sr. No.	Isolate Code	Place of collection	Crop	Cultural Characterization on Potato Dextrose Agar*		
				No. of Rings	Color	Odour
1.	IGKV T1	Raigarh (A)	Paddy	1	Greenish-yellow	-
2.	IGKV T2	Raigarh (B)	Paddy	0	Dark Green	Coconut
3.	IGKV T3	Raigarh (D)	Paddy	2	Greenish-yellow	-
4.	IGKV T4	Raigarh (E)	Paddy	1	Yellowish-green	-
5.	IGKV T5	Pali, Takhatpur, Bilapur	Ginger	2	Yellowish-green	-
6.	IGKV T6	Kharkena, Takhatpur, Bilapur	Ginger	1	Yellowish-green	-
7.	IGKV T7	Kailashpur, Surajpur, Sarguja	Ginger	1	Yellowish-green	-
8.	IGKV T8	Ramnagar, Surajpur, Sarguja	Ginger	1	Yellowish-green	-
9.	IGKV T9	Saja Bemetara	Soybean	1	Yellowish-green	-
10.	IGKV T10	Saja Bemetara	Pigeon Pea	2	Yellowish-green	-
11.	IGKV T11	Saja Bemetara	Pigeon Pea	2	Dark Green upon sporulation	-
12.	IGKV T12	Setganga	Sugarcane	2	Yellowish-green	-
13.	IGKV T13	Pandaria	Paddy	2	Dark Green upon sporulation	-
14.	IGKV T14	Pandaria	Paddy	2	Dark Green	-
15.	IGKV T15	Setganga	Soybean	2	Dark Green	-
16.	IGKV T16	Setganga	Paddy	2	Yellowish-green	-
17.	IGKV T17	Pandaria	Paddy	2	Dark Green centre	-
18.	IGKV T18	Telai Kachhar, Surajpur, Sarguja	Pigeon Pea	0	White mycelium, yellow pigmentation on the back	-
19.	IGKV T19	Jewran	Soybean	2	Dark Green centre	-
20.	IGKV T20	Sevrankala	Pigeon Pea	1	Yellowish-green	Coconut
21.	IGKV T21	Sonpuri, Tangi	Paddy	2	Yellowish-green	-
22.	IGKV T22	Kharkena, Takhatpur, Bilapur	Paddy	1	Yellowish-green	-
23.	IGKV T23	Kharkena, Takhatpur, Bilapur	Paddy	1	Yellowish-green	-
24.	IGKV T24	Kharkena, Takhatpur, Bilapur	Pigeon Pea	2	Yellowish-green	Coconut
25.	IGKV T25	Sonpuri, Tangi	Paddy	1	Dark Green upon sporulation	-
26.	IGKV T26	Damariya	Soybean	2	Yellowish-green	-
27.	IGKV T27	Kharkena, Takhatpur, Bilapur	Pigeon Pea	1	Yellowish-green	-
28.	IGKV T28	Ambikapur, Sarguja	Pigeon Pea	2	Yellowish-green	Coconut
29.	IGKV T29	Kawardha	Paddy	2	Yellowish-green	-
30.	IGKV T30	Mohtarateli, Lormi, Bilaspur	Paddy	2	Dark Green upon sporulation	-
31.	IGKV T31	Saja Bemetara	Soybean	1	Yellowish-green	-
32.	IGKV T32	Mungeli	Paddy	1	Dark Green upon sporulation	-

- No significant odour detected.

Table 2: Effect of growth media on *Trichoderma* spp.

Isolates	MEAB		OMA		PDA	
	Average RG*	Average GR	Average RG	Average GR	Average RG	Average GR
IGKV T1	85±0.35^{ab}	28.33±2.36^{ab}	67.5±0.18^{ab}	22.50±1.18^{ab}	90±0.00^{c}	30.00±0^{c}
IGKV T2	87.5±0.18^{ab}	29.17±1.18^{ab}	75±0.35^{cd}	25.00±2.36^{cd}	90±0.00^{c}	30.00±0^{c}
IGKV T3	85±0.00^{ab}	28.33±0.00^{ab}	75±0.35^{cd}	25.00±2.36^{cd}	70±0.00^{a}	23.33±0^{a}
IGKV T4	90±0.00^{b}	30.00±0.00^{b}	71±0.07^{bc}	23.67±0.47^{bc}	67.5±0.53^{a}	22.50±3.53^{a}
IGKV T5	90±0.00^{b}	30.00±0.00^{b}	72±0.14^{bc}	24.00±0.94^{bc}	80±0.00^{b}	26.67±0^{b}
IGKV T6	90±0.00^{b}	30.00±0.00^{b}	65±0.35^{a}	21.67±2.36^{a}	85±0.35^{bc}	28.33±2.36^{bc}
IGKV T7	80±0.00^{a}	26.67±0.00^{a}	70±0.00^{abc}	23.33±0.00^{abc}	85±0.35^{bc}	28.33±2.36^{bc}
IGKV T8	80±0.00^{a}	26.67±0.00^{a}	70±0.00^{abc}	23.33±0.00^{abc}	80±0.00^{b}	26.67±0^{b}
IGKV T9	85±0.35^{ab}	28.33±2.36^{ab}	70±0.00^{abc}	23.33±0.00^{abc}	80±0.00^{b}	26.67±0^{b}
IGKV T10	85±0.35^{ab}	28.33±2.36^{ab}	70±0.00^{abc}	23.33±0.00^{abc}	80±0.00^{b}	26.67±0^{b}
IGKV T11	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T12	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T13	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T14	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T15	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T16	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T17	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T18	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T19	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T20	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T21	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T22	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T23	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T24	87.5±0.18^{ab}	29.17±1.18^{ab}	70±0.00^{abc}	23.33±0.00^{abc}	90±0.00^{c}	30.00±0^{c}
IGKV T25	85±0.00^{ab}	28.33±0.00^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T26	82.5±0.18^{ab}	27.50±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T27	82.5±0.18^{ab}	27.50±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T28	82.5±0.18^{ab}	27.50±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T29	90±0.00^{b}	30.00±0.00^{b}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T30	82.5±0.18^{ab}	27.50±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T31	87.5±0.18^{ab}	29.17±1.18^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}
IGKV T32	85±0.35^{ab}	28.33±2.36^{ab}	80±0.00^{d}	26.67±0.00^{d}	90±0.00^{c}	30.00±0^{c}

*RG= radial growth; GR= Growth Rate. The experiment was conducted in three replications. The number followed by ± represents the standard deviation,

In each column, the same alphabet signifies no significant variation in Mean value according to Duncan Mean Range Test (alpha= 0.05), SPSS 20

Table 3: Effect of temperature on radial growth and growth rate on *Trichoderma* spp.

Isolates	15°C		25°C		37°C		Spore Count		
	Average Diameter	Average GR	Average Diameter	Average GR	Average Diameter	Average GR	15°C	25°C	37°C
IGKV T1	40±00	13.33±00	85±0.35^{ab}	28.33±2.36	45±0.35^{ab}	15.00±2.36	2.5×10^7	3.5×10^8	8.8×10^7
IGKV T2	50±00	16.67±00	82±0.14^{ab}	27.33±0.94	40±0.00^{ab}	13.33±0.00	2×10^5	3.6×10^8	2.5×10^7
IGKV T3	40±00	13.33±00	88±0.14^{ab}	29.33±0.94	20±0.00^{a}	6.67±0.00	2.8×10^5	3.7×10^8	6×10^4
IGKV T4	40±00	13.33±00	85±0.35^{ab}	28.33±2.36	20±0.00^{a}	6.67±0.00	4×10^5	3.8×10^8	7×10^4
IGKV T5	50±00	16.67±00	80±0.00^{a}	26.67±0.00	45±0.35^{ab}	15.00±2.36	1.4×10^6	3.6×10^8	8×10^4
IGKV T6	48±00	16.00±00	85±0.35^{ab}	28.33±2.36	45±0.35^{ab}	15.00±2.36	2×10^8	3.7×10^8	1.3×10^7
IGKV T7	40±00	13.33±00	82±0.14^{ab}	27.33±0.94	20±0.00^{a}	6.67±0.00	2×10^7	3.5×10^8	1.0×10^7
IGKV T8	50±000	16.67±00	83±0.21^{ab}	27.67±1.41	40±0.00^{ab}	13.33±0.00	3.2×10^5	3.4×10^8	5.6×10^7
IGKV T9	40±00	13.33±00	81±0.07^{a}	27.00±0.47	30±0.00^{a}	10.00±0.00	2×10^5	3.7×10^8	6.3×10^7
IGKV T10	40±00	13.33±00	84±0.28^{ab}	28.00±1.89	20±0.00^{a}	6.67±0.00	2.6×10^5	3.8×10^8	5×10^4
IGKV T11	40±00	13.33±00	85±0.35^{ab}	28.33±2.36	40±0.00^{ab}	13.33±0.00	1.4×10^6	3.5×10^8	7×10^4
IGKV T12	40±00	13.33±00	80±0.00^{a}	26.67±0.00	50±0.00^{ab}	16.67±0.00	1.0×10^7	3.9×10^8	6.7×10^7
IGKV T13	40±00	13.33±00	80±0.00^{a}	26.67±0.00	40±0.00^{ab}	13.33±0.00	3.2×10^5	2.6×10^8	8.7×10^7
IGKV T14	40±00	13.33±00	80±0.00^{a}	26.67±0.00	40±0.00^{ab}	13.33±0.00	4.0×10^5	2.5×10^8	2.7×10^7
IGKV T15	40±00	13.33±00	80±0.00^{a}	26.67±0.00	50±0.00^{ab}	16.67±0.00	1.0×10^7	2.4×10^8	1.2×10^7
IGKV T16	40±00	13.33±00	80±0.00^{a}	26.67±0.00	40±0.00^{ab}	13.33±0.00	1.0×10^7	2.3×10^8	3.5×10^7
IGKV T17	40±00	13.33±0	80±0.00^{a}	26.67±0.00	55±0.35^{ab}	18.33±2.36	5×10^4	3.4×10^8	1.3×10^8
IGKV T18	40±00	13.33±00	85±0.35^{ab}	28.33±2.36	45±0.35^{ab}	15.00±2.36	2.0×10^5	2×10^5	2.2×10^7
IGKV T19	20±00	6.67±00	80±0.00^{a}	26.67±0.00	45±0.35^{ab}	15.00±2.36	1.5×10^7	2.5×10^8	1.2×10^7
IGKV T20	40±00	13.33±00	80±0.00^{a}	26.67±0.00	55±0.35^{ab}	18.33±2.36	5×10^4	3.6×10^8	3.9×10^7
IGKV T21	20±00	6.67±00	80±0.00^{a}	26.67±0.00	55±0.35^{ab}	18.33±2.36	2.0×10^5	3.5×10^8	1.35×10^8
IGKV T22	40±00	13.33±00	90±0.00^{b}	30.00±0.00	30±0.71^{a}	10.00±4.71	1.5×10^7	2.8×10^8	8×10^7
IGKV T23	20±00	6.67±00	80±0.00^{a}	26.67±0.00	20±0.00^{a}	6.67±0.00	5×10^4	2.7×10^8	1.0×10^7
IGKV T24	20±00	6.67±00	80±0.00^{a}	26.67±0.00	80±0.00^{b}	26.67±0.00	5×10^4	3.5×10^8	3.8×10^8
IGKV T25	44±00	14.67±00	80±0.00^{a}	26.67±0.00	50±2.12^{ab}	16.67±14.14	1.0×10^7	2.7×10^8	3×10^8
IGKV T26	24±00	8.00±00	80±0.00^{a}	26.67±0.00	50±2.12^{ab}	16.67±14.14	5×10^4	2.6×10^8	3×10^8
IGKV T27	40±00	13.33±00	85±0.35^{ab}	28.33±2.36	80±0.00^{b}	26.67±0.00	2.5×10^5	3.6×10^8	7.5×10^8
IGKV T28	40±00	13.33±0	80±0.00^{a}	26.67±0.00	50±2.12^{b}	16.67±14.14	3.2×10^5	2.6×10^8	3.3×10^8
IGKV T29	20±00	6.67±0	80±0.00^{a}	26.67±0.00	80±0.00^{b}	26.67±0.00	3.0×10^5	3.4×10^8	5×10^8
IGKV T30	20±00	6.67±0	80±0.00^{a}	26.67±0.00	50±2.12^{ab}	16.67±14.14	5×10^4	2.5×10^8	5.5×10^8
IGKV T31	40±00	13.33±0	85±0.35^{ab}	28.33±2.36	50±2.12^{ab}	16.67±14.14	4.0×10^5	2.4×10^8	5.6×10^8
IGKV T32	30±00	10.00±0	80±0.00^{a}	26.67±0.00	50±2.12^{ab}	16.67±14.14	5×10^4	2.3×10^8	7×10^8

*RG= radial growth; GR= Growth Rate. The experiment was conducted in three replications. The number followed by ± represents the standard deviation, In each column, the same alphabet signifies no significant variation in Mean value according to Duncan Mean Range Test (alpha= 0.05), SPSS 20

Table 4: Effect of different pH on *Trichoderma* isolates.

Isolates	pH 5		pH 7		pH 9		Spore Count		
	AVG RG	AVG GR	AVG RG	AVG GR	AVG RG	AVG GR	pH 5	pH 9	pH 7
IGKV T1	80.00±0	26.67±0	85±0.35	28.33±2.36	80±0	26.67±0	2.4×10^8	5.1×10^8	3.5×10^8
IGKV T2	70.00±0	23.33±0	82±0.14	27.33±0.94	80±0	26.67±0	2.4×10^8	6×10^8	3.6×10^8
IGKV T3	90.00±0	30.00±0	88±0.14	29.33±0.94	80±0	26.67±0	2.1×10^8	5.5×10^8	3.7×10^8
IGKV T4	80.00±0	26.67±0	85±0.35	28.33±2.36	70±0	23.33±0	2.9×10^8	5.9×10^8	3.8×10^8
IGKV T5	70.00±0	23.33±0	80±0.00	26.67±0.00	70±0	23.33±0	2.4×10^8	7×10^8	3.6×10^8
IGKV T6	86.00±0	28.67±0	85±0.35	28.33±2.36	90±0	30.00±0	2.6×10^8	5.4×10^8	3.7×10^8
IGKV T7	80.00±0	26.67±0	82±0.14	27.33±0.94	60±0	20.00±0	2.7×10^8	5.9×10^8	3.5×10^8
IGKV T8	80.00±0	26.67±0	83±0.21	27.67±1.41	90±0	30.00±0	2.3×10^8	5×10^8	3.4×10^8
IGKV T9	70.00±0	23.33±0	81±0.07	27.00±0.47	80±0	26.67±0	2.2×10^8	7×10^8	3.7×10^8
IGKV T10	74.00±0	24.67±0	84±0.28	28.00±1.89	80±0	26.67±0	2.8×10^8	6.2×10^8	3.8×10^8
IGKV T11	70.00±0	23.33±0	85±0.35	28.33±2.36	80±0	26.67±0	2.3×10^8	6×10^8	3.5×10^8
IGKV T12	80.00±0	26.67±0	80±0.00	26.67±0.00	90±0	30.00±0	2.8×10^8	8×10^8	3.9×10^8
IGKV T13	70.00±0	23.33±0	80±0.00	26.67±0.00	80±0	26.67±0	1.5×10^8	4.5×10^8	2.6×10^8
IGKV T14	80.00±0	26.67±0	80±0.00	26.67±0.00	90±0	30.00±0	1.8×10^8	4.9×10^8	2.5×10^8
IGKV T15	70.00±0	23.33±0	80±0.00	26.67±0.00	80±0	26.67±0	1.7×10^8	5.3×10^8	2.4×10^8
IGKV T16	80.00±0	26.67±0	80±0.00	26.67±0.00	90±0	30.00±0	1.4×10^8	3.4×10^8	2.3×10^8
IGKV T17	70.00±0	23.33±0	80±0.00	26.67±0.00	80±0	26.67±0	2.5×10^8	8×10^8	3.4×10^8
IGKV T18	80.00±0	26.67±0	85±0.35	28.33±2.36	90±0	30.00±0	$\mathbf{3.8 \times 10^8}$	$\mathbf{2 \times 10^5}$	$\mathbf{2 \times 10^5}$
IGKV T19	70.00±0	23.33±0	80±0.00	26.67±0.00	80±0	26.67±0	1.4×10^8	4.1×10^8	2.5×10^8
IGKV T20	70.00±0	23.33±0	80±0.00	26.67±0.00	80±0	26.67±0	2.3×10^8	3.9×10^8	3.6×10^8
IGKV T21	60.00±0	20.00±0	80±0.00	26.67±0.00	70±0	23.33±0	2.6×10^8	8×10^8	3.5×10^8
IGKV T22	70.00±0	23.33±0	90±0.00	30.00±0.00	80±0	26.67±0	1.5×10^8	8×10^7	2.8×10^8
IGKV T23	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	1.2×10^8	3.7×10^8	2.7×10^8
IGKV T24	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	2.8×10^8	3.6×10^8	3.5×10^8
IGKV T25	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	1.3×10^8	3.9×10^8	2.7×10^8
IGKV T26	50.00±0	16.67±0	80±0.00	26.67±0.00	80±0	26.67±0	1.5×10^8	4.2×10^8	2.6×10^8
IGKV T27	80.00±0	26.67±0	85±0.35	28.33±2.36	80±0	26.67±0	2.8×10^8	3.9×10^8	3.6×10^8
IGKV T28	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	1.8×10^8	2.9×10^8	2.6×10^8
IGKV T29	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	2.5×10^8	7.5×10^8	3.4×10^8
IGKV T30	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	1.5×10^8	4.4×10^8	2.5×10^8
IGKV T31	70.00±0	23.33±0	85±0.35	28.33±2.36	80±0	26.67±0	1.2×10^8	5.2×10^8	2.4×10^8
IGKV T32	60.00±0	20.00±0	80±0.00	26.67±0.00	80±0	26.67±0	1.6×10^8	7.7×10^8	2.3×10^8

*RG= radial growth; GR= Growth Rate. The experiment was conducted in three replications. The number followed by ± represents the standard deviation. No variation was observed among the isolates, according to Duncan Mean Range Test (alpha= 0.05), SPSS 20

At pH 9, a maximum RG of 90.00 mm was observed in isolates IGKV -T6, T8, T12, T14, T16, and T18 with a GR of 30.00±00 mm/day whereas the least RG of 60.00±00 mm was recorded in isolate IGKV T7. At pH 7, a maximum RG of 90.00 mm with a GR of 30.00 mm/day was shown by isolate- IGKV T22 followed by IGKV T3 (RG 88.00 mm GR 29.33). The minimum RG of 80.00 mm with a GR of 26.67 mm/day was observed in 18 isolates IGKV-T5, T12, T13, T14, T15, T16, T17, T19, T20, T21, T23, T24, T25, T26, T28, T29, T30 & T32. Amongst the different pH levels, pH 7 was found optimum with a mean value of 82.03 mm followed by pH 9 (80.31 mm) & pH 5 (70.93 mm) (Table 5). Higher growth of IGKV T3 and IGKV T6 at different pH levels indicates that these isolates may be useful in highly acidic or alkaline crop fields.

Table 5: Repeated measure analysis of Physiological Characterisation.

Treatment		Mean	Standard Deviation
Media (Radial Growth)	MEAB	86.25	3.78
	OMA	76.73	5.10
	PDA	87.10	6.16
Media (Growth Rate)	MEAB	28.74	1.25
	OMA	25.57	1.70
	PDA	29.03	2.05
Temperature	15±2°C	36.75	9.42
	25±2°C	82.03	3.79
	37±2°C	44.68	20.77
pH	5	70.93	9.33
	9	80.31	6.41
	7	82.03	3.79

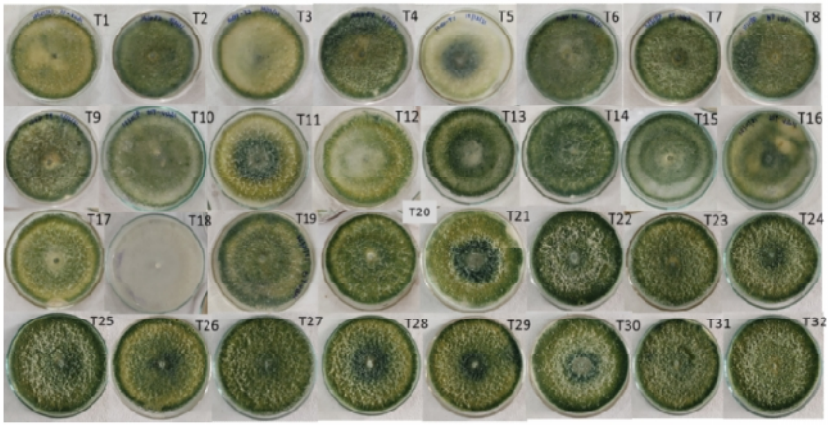

Fig. 1. Cultural Characteristics of *Trichoderma* Isolates from Chhattisgarh, India.

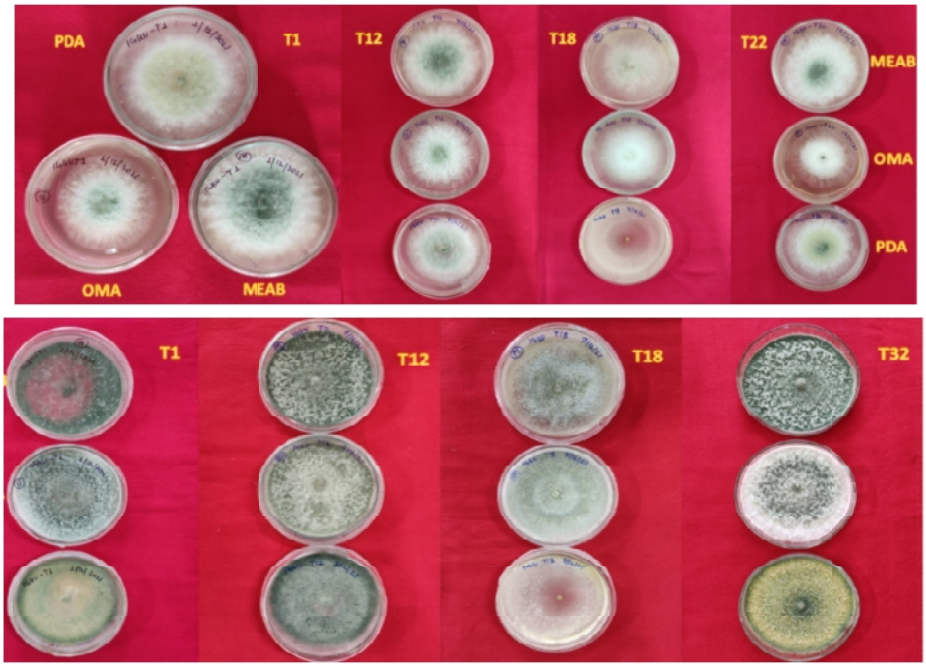

Fig. 2. Effect of different media on radial growth & cultural characteristics of *Trichoderma* after 72 hours & 120 hours.

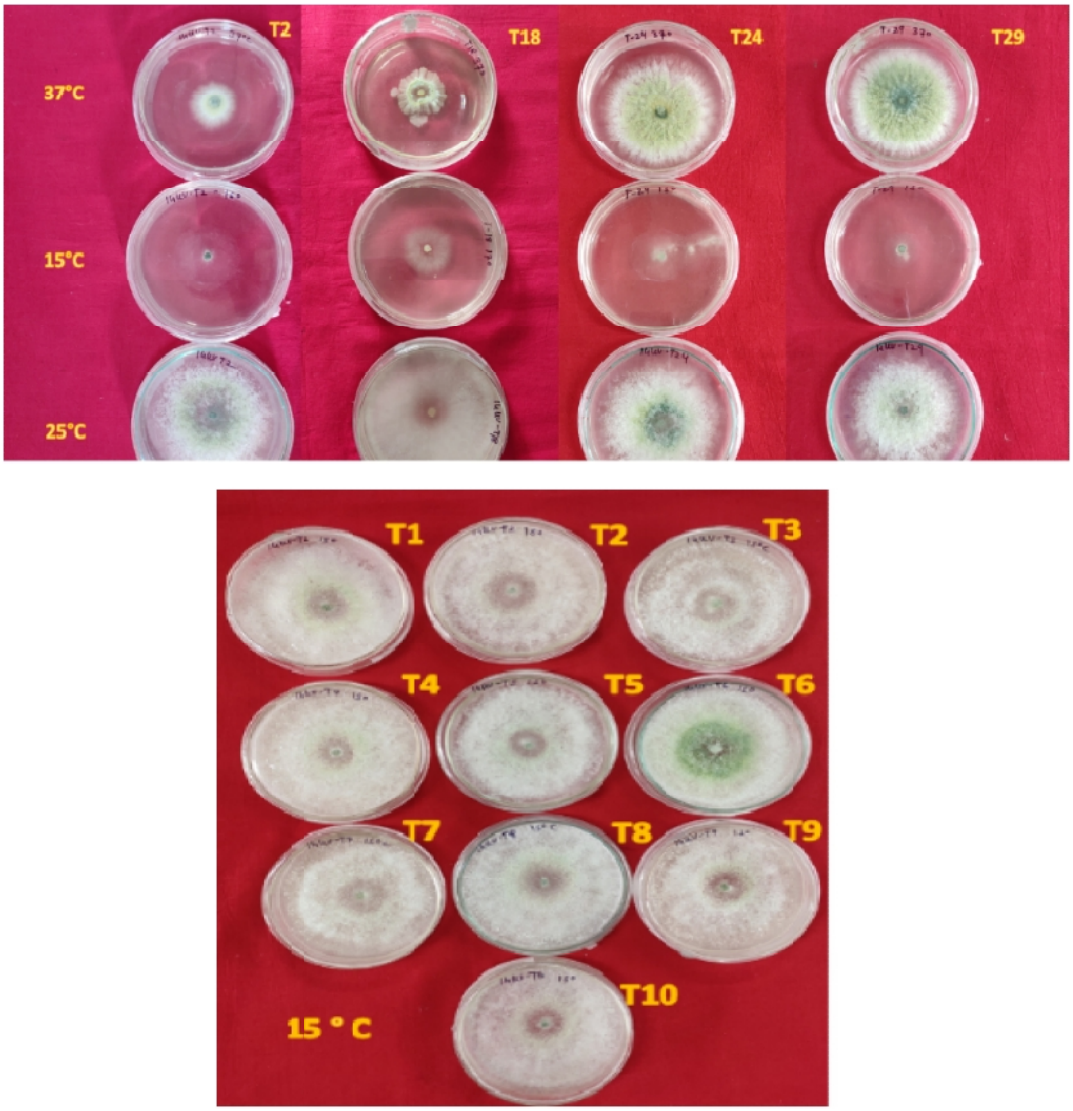

Fig. 3. Effect of temperature on radial growth of *Trichoderma* spp. after 72 hours & radial growth at 15°C After 168 hours.

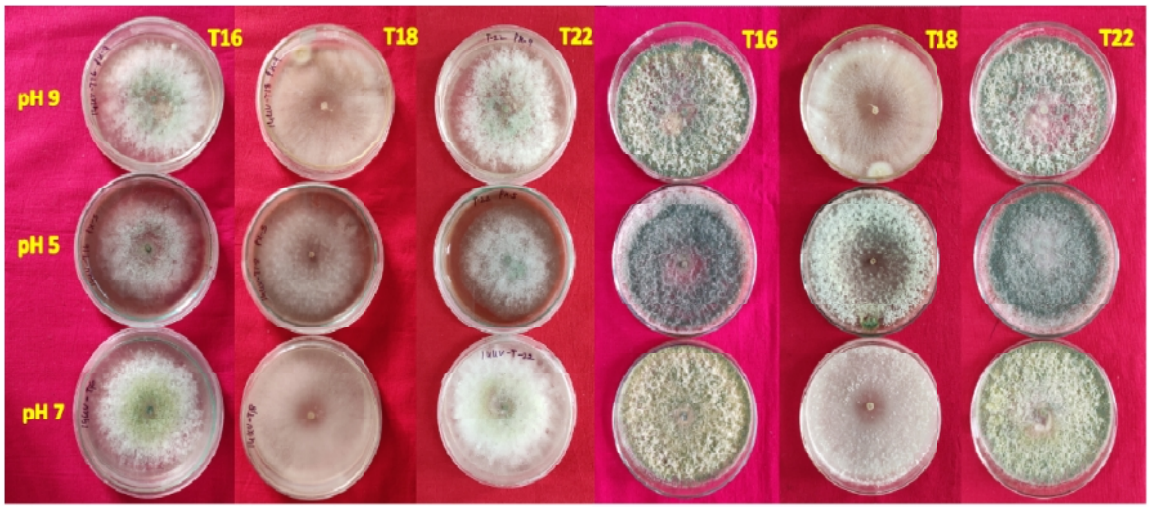

Fig. 4. Effect of pH on *Trichoderma* isolates after 72 hours and 120 hours.

Optimum growth and sporulation of four species of *Trichoderma* were recorded between pH 4.1 to 8.6. Growth and sporulation of all the isolates decreased significantly with either decrease or increase in the pH below 4.6 or above 7.6. At pH 4.6, the growth was still good but sporulation was poor. At pH 8.6, the growth completely ceased (Zehra *et al.,* 2017). Lejeune *et al.* (1995) using glucose-limited continuous culture showed that between pH 4 and 5 the specific growth rate, the maximum tip extension rate, and the branching frequency were highest, whereas the average hyphal diameter was smallest (2.1 µm vs. 2.5–2.7). Their data are in contrast to similar studies by Brown and Halsted (1988), who reported that the maximal specific growth rate of *T. reesei* under glucose limitation increased with increasing H^+ concentration, whereas the biomass yield Y remained constant at 0.4.

CONCLUSION

From the study, it can be concluded that
(i) Colony characteristics are overlapping among the isolates limiting their application in identification of *Trichoderma* spp., however polyphasic approach combining data sets can be done.
(ii) PDA is the suitable media for culturing *Trichoderma* spp.
(iii) The *Trichoderma* isolates under study showed temperature and pH preference.
(iv) 25±2°C is the optimum temperature for most of the isolates.
(v) The optimum pH level is 7 for most of the isolates.

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Conflict of Interest. None.

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