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# Molecular Characterization of Trichoderma Mutants with RAPD Marker

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**A B S T R A C T**

*Trichoderma* is an important biocontrol agent of several soil borne plant pathogens. The antagonistic ability of a biocontrol agent was determined by its physiological state so that change in physiological or genetical condition could alter the antagonism. Genetic modification using mutagenesis offers the potential for producing improved bio protection is likely to enhance their biocontrol capabilities against soil borne pathogen. Molecular techniques are important analytical tool to characterized genetic variability and diagnosis of microbial population. Molecular variability of *Trichoderma* mother culture and its mutants was studied by using Random Amplified Polymorphic DNA (RAPD). 20 RAPD primer of OPA series were tested, of which 14 primers produced 72 scorable bands among them 52 bands were polymorphic and level of polymorphism was upto 72%. On the basis of dendrogram, the tested cultures were clearly divided into 2 groups. First group i.e. cluster A included 4 mutants TvM-5, TvM-3, TvM-1 TvM-2 and mother culture TvMC. Second group i.e. cluster B included only one mutant i.e. TvPM-4.

## Introduction

*Trichoderma* is used in different crop like rice, wheat, pulses (black gram, cowpea, and chickpea) vegetable (tomato, brinjal, and chili) against a wide range of plant pathogen. One of the popular species, *Trichoderma viride* is effective against all soil borne fungal pathogen like *Phytophthora, Fusarium, Pythium, Rhizoctonia, Sclerotium. Trichoderma*, reduce growth, survival or infection caused by pathogen by different mechanism. In addition, some species of the genus are economically important producers of industrial enzymes *(Trichoderma reesei, Hypocreajecorina*; and antibiotics (Sivasithamparam and Ghisalberti, 1998). *Trichoderma* is easily identified in culture media, which produces large number of characteristics small, green or white conidia, from phialides present on the profusely or meagrely branched conidiophores. However, the identification of isolates to species level is difficult and confusing due to the complexity and closely related characters of the species. *Trichoderma* isolates were differentiated by mycelial growth rate and colony appearance, as well as microscopic morphological features, including phialides and spores. These can also be distinguished by molecular techniques like DNA sequencing, Random Amplification of Polymorphic DNA (RAPD) analysis, Restriction Fragment Length Polymorphism (RFLP) analysis, internal transcribed

sequences (ITS) of the ribosomal DNA (rDNA) analysis (rDNA-ITS1) and universally primed polymerase chain reaction (UP-PCR) have been used to characterize isolates of *Trichoderma* (Cumagun *et al.,* 1999). Inter simple sequence repeats (ISSR) have been used as another effective method to characterize genetic variability. Since the evolutionary rate within ISSR is considerably higher than other types of DNA, the likelihood of finding polymorphism is greater compared to RAPD. Since the first reports of RAPD markers by (Williams *et al.,* 1990). This method has been widely used for identification of species. This technique has been used in some cases for species identification.

## Materials and Methods

*Trichoderma viride* mother culture and four mutants i.e. ($6^{th}$ generation) irrediatated with gamma rays (Cobalt 60) @41.6 gray/minute at BARC, Trombay, Mumbai were collected from Department of Plant Pathology Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The applied doses level of gamma rays was 75k-rad with time interval of 15, 30, 45 and 60 minutes. One *T.viride* mutant was collected from G.B. Pant University of Agriculture and Technology, Pantnagar. This mutants can be coaded as *Trichoderma viride* mother culture (TvMC), *Trichoderma viride* mutant (*T. Viride*@ 75 k-rad 15 min.TvM-1), *Trichoderma viride* mutant (*T. Viride*@ 75 k-rad 30 min. TvM-2) *Trichoderma viride* mutant (*T. Viride*@ 75 k-rad 45 min. TvM-3) *Trichoderma viride* mutant (Pantnagar TvPM-4), *Trichoderma viride* mutant (*T. Viride*@ 75 k-rad 60 min. TvM-5).

## Extraction of DNA

Genomic DNA was isolated from the twenty four selected isolates by the cetyl dimethyl ethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications. For extraction of DNA seven days old mycelial mat was transferred on sterilized blotter paper, air dried to remove moisture. Dried mycelium mat was used for DNA isolation. Approximately, one gram of air dried fungal mat was quickly frozen in liquid nitrogen ($-196^0$C) and crushed into powder form with the help of sterilized mortar and pestle. The powder was immediately homogenized by adding pre-warmed ($65^0$C) extraction buffer and transferred to two ml eppendorf tubes. These tubes were incubated in a water bath at $65^0$C for one hour with gentle shaking at every 15 minutes then add an equal volume of chloroform : isoamyl alcohol (24:1) and centrifuged at 12000 rpm at room temperature for 20 minutes. Then aqueous phase transferred to another tube and equal volume of ice chilled Isopropanol was added. Centrifuged for 10 minutes at 12000 rpm and decanted the supernatant. The pellet was washed with 70 per cent ethanol twice and suspended in $T_{50}E_{10}$ buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA). The DNA solution was treated with RNase at $37^0$C for 1 hr and stored at -20°C until needed.

## RAPD PCR reaction

The PCR cycle was carried out in small reaction tubes, containing a reaction volume typically of 12.5 µl in which10x *Taq* buffer 1.25 µl, $MgCl_2$ (25 mM 1.25 µl), dNTPs (10 mM 0.3 µl), *Taq* polymerase (5 U/µl0.3 µl), Sterile distilled water (5.4 µl), Primer(1.0 µl), Template DNA (37.5 ng 2.0 µl)that was inserted into a thermal cycler (Eppendorf) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. The PCR cycle was as follows initial denaturation at 94°C for 5min, Denaturation at 94°C for 1min, Annealing 35°C for 1min, Extension 72°C for 3min and Final Extension 72°C for 10min. With 35 cycles.20 primers were screened, consisting of RAPD primer of OPA series 1to 20 from

Genaxy Scientific Pvt. Ltd. to evaluate the variability among *Trichoderma viride* mother culture and mutants. The PCR amplified product of each primer was resolved on 1.2%agarose gel electrophoresis and the amplified product was compared with 1kb DNA ladder.

## Results and Discussion

### Internal Transcript Spacer (ITS) analysis

For the confirmation of *Trichoderma viride* mother culture and mutants ITS marker was done with using ITS-1 and ITS-4 primer which gives band size in the range of 500bp to 600bp. the result are in accordance with Chakraborty (2010) who studied the identification and genetic variability of *Trichoderma* isolates which observed amplified DNA fragment approximately 600bp.

### RAPD analysis

The genomic DNA of five *Trichoderma* mutants and one mother culture of *Trichoderma viride* was amplified. Molecular variability of *Trichoderma viride* mother culture and mutants was studied by using 14 RAPD primers of OPA series of which 14 primers produced 72 scorable bands.

Among the 72 scorable bands the 52 bands were polymorphic and level of polymorphism was 72% (Fig. 1 and Table 1).

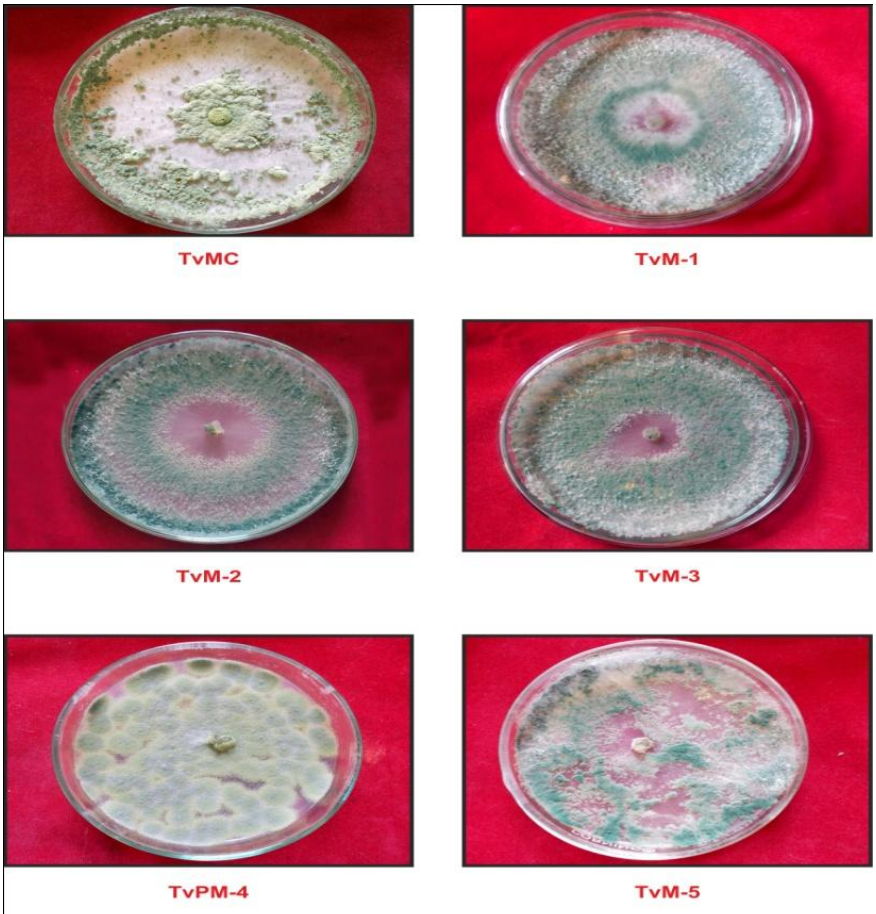



Plate 1. *Trichoderma viride* mother culture and mutants (6th generation) used for morphological and molecular study

**Plate.2** Conformation and identification of Trichoderma mutants by using ITS primer

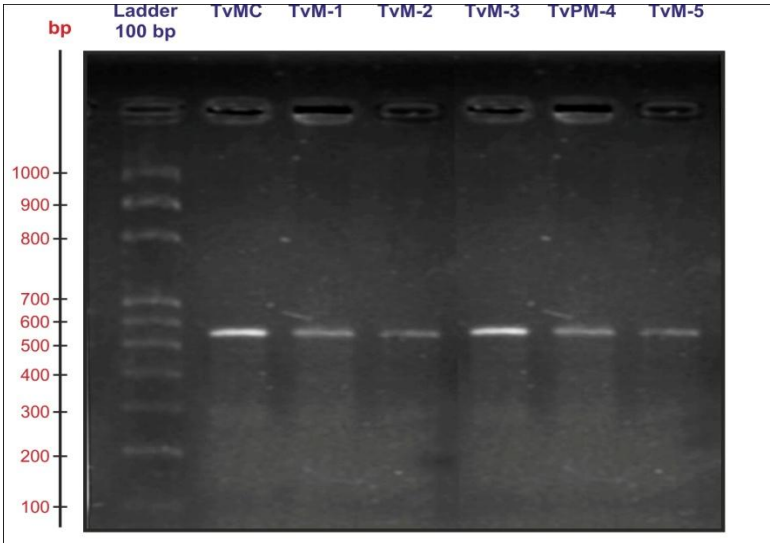



**Plate.3** RAPD analysis of *Trichoderma* mutants using OPA-16 and OPA-4 primers.

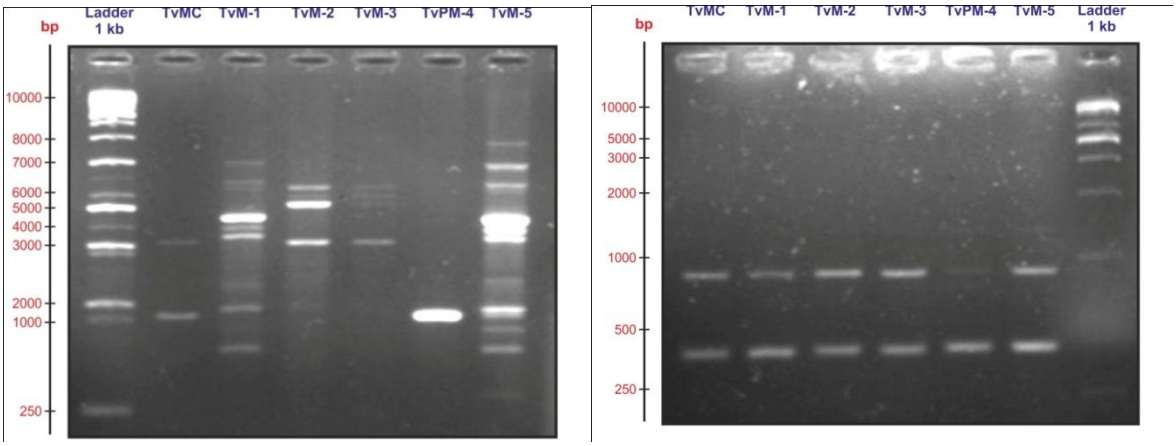



**Plate.4** RAPD analysis of *Trichoderma* mutants using OPA-12 and OPA-13 primers

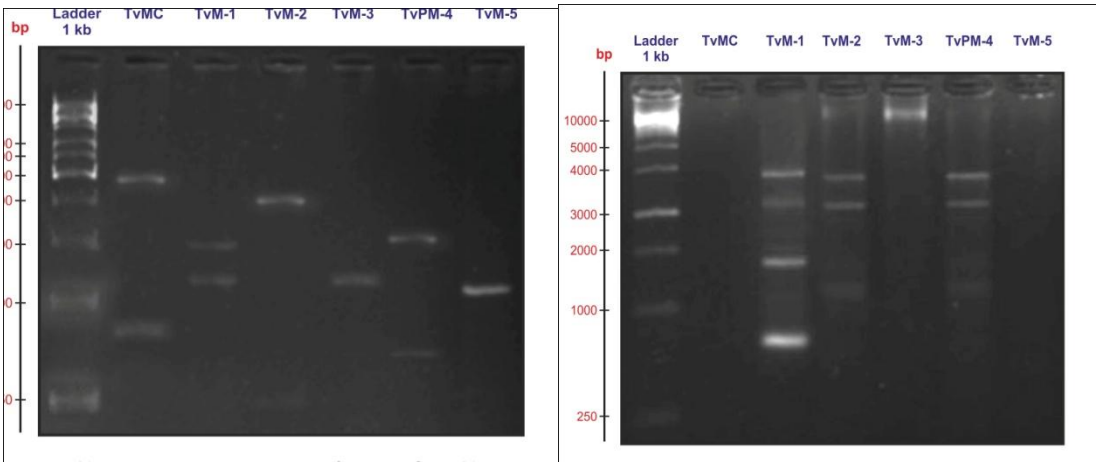
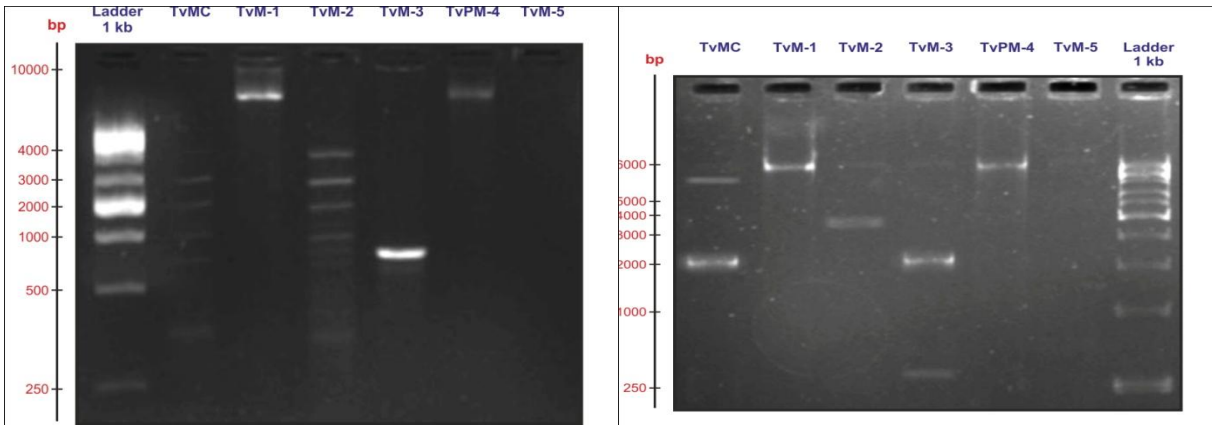


**Plate.5** RAPD analysis of *Trichoderma* mutants using OPA-10 and OPA-11 primers.



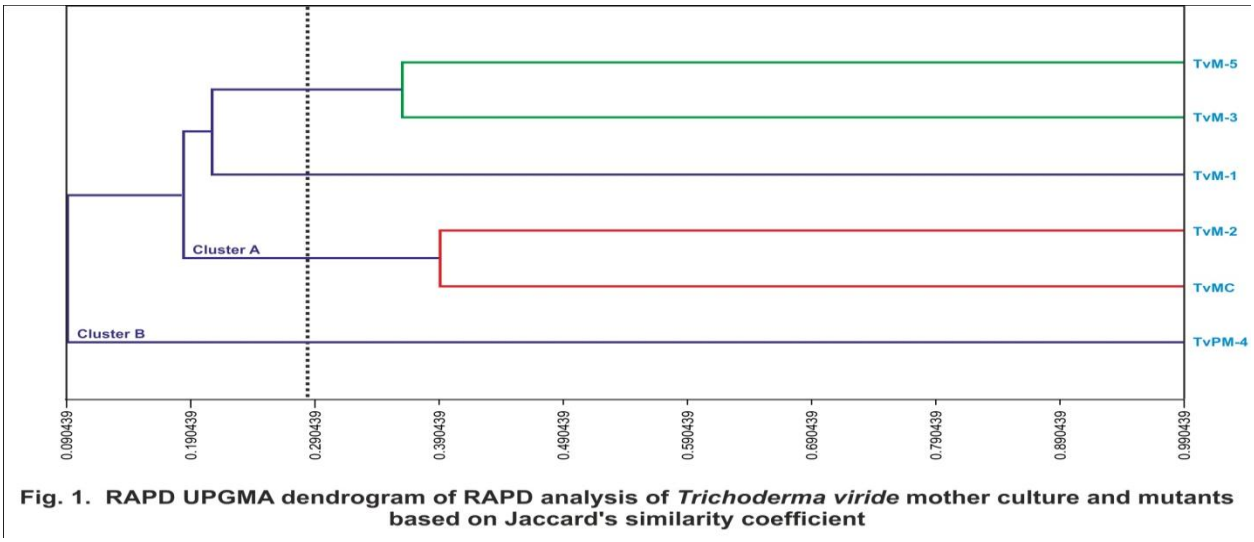




Fig. 1. RAPD UPGMA dendrogram of RAPD analysis of *Trichoderma viride* mother culture and mutants based on Jaccard's similarity coefficient

**Table.1** Per cent polymorphism observed in RAPD primer

| Sr. | Primer | Total bands | Polymorphic bands | % polymorphism |
|---|---|---|---|---|
| 1 | OPA-1 | 6 | 4 | 66.66% |
| 2 | OPA-4 | 7 | 5 | 71.42% |
| 3 | OPA-5 | 3 | 2 | 66.6% |
| 4 | OPA-9 | 6 | 5 | 83.33% |
| 5 | OPA-10 | 7 | 6 | 85.7% |
| 6 | OPA-11 | 5 | 4 | 80% |
| 7 | OPA-12 | 6 | 3 | 50% |
| 8 | OPA-13 | 5 | 3 | 60% |
| 9 | OPA-14 | 2 | 2 | 100% |
| 10 | OPA-15 | 4 | 3 | 75% |
| 11 | OPA-16 | 9 | 6 | 66.6% |
| 12 | OPA-17 | 5 | 4 | 80% |
| 13 | OPA-18 | 4 | 3 | 75% |
| 14 | OPA-19 | 3 | 2 | 66% |
| | TOTAL | 72 | 52 | 72% |

A binary similarity matrix of combined data from 14 RAPD primers of *Trichoderma viride* mother culture and mutants were prepared by scoring presence or absence of band. The same molecular weight was assumed to be identical. On the basis of calculated similarity matrix the similarity between genotypes can be predicted. The genotypes showing similarity index "1" are presumed to be 100% similar while that of "0" are 100% genetically dissimilar. In present study the similarity coefficient value ranged from 0.370 to 0.000 across *Trichoderma viride* mother culture and mutants indicating high degree of polymorphism in respect to genetic similarity. Genetic similarity estimate (Jaccard's coefficient) based on RAPD banding pattern was used for cluster analysis to present genetic relationship in the form of dendrogram. In this Dendrogram, higher value of similarity coefficient (0.370) whereas 0.000 was found to have lower value of similarity coefficient. Two major clusters were obtained on the basis of analysis. First group is named as cluster –A, includes TvM-5, TvM-3, TvM-1, TvM-2, TvMC. Second group is named as cluster –B which include TvPM-4. The TvMC was found to have a higher similarity index with TvM-2. The TvPM-4 with TvM-3 and TvPM-4 with TvMC was found to have a lower similarity index

## References


Agrawal, T. and A.S. Kotasthane. 2009: Assessment of diversity in isolates of *Trichoderma* species from soils of Chhattisgarh region in Central India using RAPD markers. *J. Mycol. Plant Pathology*. 139(3): 484-489.

Chakraborty, B.N., U. Chakraborty, A. Saha, P.L. Dey and K. Surnar. 2010: Molecular Characterization of *Trichoderma viride* and *Trichoderma hazarium* Isolated from soil from north Bengal based on rDNA marker and analysis of their PCR-RAPD profiles. *Global J. Biotech. and Biochem*. 5(1): 55-61.

Cumagun, C.J., J. Hockenhull and M. Lubeck. 1999. Identification characterization of *Trichoderma* isolates from Philippine rice yields by UP-PCR and rDNA ITSI analysis. Res. Program. Plant Protect. Plant Nutrition. pp 37-47.

Hermosa, M.R., I. Grondona, E.A. Iturriaga., J.M. Diaz-Minguez., C. Castro and E. Monte. 2001: Molecular characterization and identification of biocontrol isolates of *Trichoderma* species. *Appl. Environ. Microbiol*. 66: 1890-1898

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA Cambridge Institution of Washington, Department of Plant Biology, Stanford, CA 94305, USA, Volume 8.

Muthumeenaksh, S., A.E. Brown and P.R. Mills. 1998: Genetic comparison of the aggressive weed mould strains of *Trichoderma harzianum* from mushroom compost in North America and the British Isles. *Mycol. Res*. 102: 385-390.

Naher, L., U. Yusuf, A. Ismail and K. Hossain, 2014: *Trichoderma Spp*.: A Biocontrol Agent For Sustainable Management Of Plant Diseases. *Pak. J. Bot.* 46(4): 1489-1493.

Persoon, C.H. 1794: Disposition methodicafungorum, Romersneues Mag. *Bot*. 1: 8-128.

Qian, Y.E., Y.X. Qiu., J.X. Yan-qi., S. Zhao and F. Cheng-xin. 2006: J. Zhejiang Univ. Sci., 7(11): 868-872.

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Mycological Papers. 116:1-56.

Shafique, S. and R. Bajwa. 2011: Strain improvement in *Trichoderma viride*

through mutation for over expression of cellulase and characterization of mutants using random amplified polymorphic DNA (RAPD) *African Journal of Biotechnology*, 10(84) : 19590-19597.

Shahbazi, S., H.A. Fallah., H. Askari and M.A. Ebrahimi. 2017: Molecular marker based diagnosis of genetic diversity in gamma mutated *Trichoderma* spices. *International Journal of Agricultural and Environmental Research*. 3(1): 56-71.

Siameto, E.N., S. Okoth., N.O. Amugune and N. Chege. 2011: Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from Embu district, Kenya. Trop. Subtrop. *Agroecosys*. 13:81-90.

Sivasithamparam, K. and F.L. Ghisalberti. 1998: Secondary metabolism *Trichoderma* and *Gliocladium*. Volume I. Taylor and Francis Ltd. London. pp 139-191.

Williams, J.G.K., A.R. Kubelik., K.L. Livak., J.A. Rafalski and S.V. Tingey. 1990: DNA polymorphisms amplified by arbitrary primers are used as genetic marker, *Nucleic Acids Res.*, 18:6531-6535.

Zimand, G., L. Valinsky., Y. Elad., I. Chet and S. Manulis. 1994: Use of RAPD procedure for the identification of *Trichoderma* strains, *Mycol. Res.* 98: 531–534.

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