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Molecular Characterization of *Trichodermaviride* Isolated from Rhizospheric Soils of Uttar Pradesh Based on rDNA Markers and Analysis of Their PCR-ISSR Profiles

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Abstract

The objectives of this research were to characterize isolates of *Trichoderma* collected from rhizospheres of chickpea, pigeonpea and lentil crop from different places of Uttar Pradesh, India, using microsatellite-primed polymerase chain reaction (MP-PCR) and ribosomal DNA (rDNA) sequence analysis and to combine these results with morphological characteristics for classification. Thirty isolates of *Trichodermaviride* obtained from rhizosphere soil of plantation crops, and agricultural fields of UP region were studied using ISSR and ITS-PCR. The genetic relatedness among thirty isolates of *T. viride* were analyzed with six microsatellite primers. ISSR profiles showed genetic diversity among the isolates with the formation of eight clusters. Analysis of dendrogram revealed that similarity coefficient ranged from 0.27 to 0.95. ITS-PCR of rDNA region with ITS1 and ITS4 primers produced 600bp products in all isolates. This result indicated the identification patterns of *Trichoderma* isolates.

Keywords: *Trichodermaviride*; Genetic diversity; PCR; Molecular marker; Microsattelite

Introduction

Soil microorganisms influence ecosystems by contributing to plant nutrition [1], plant health [2], soil structure [3] and soil fertility [4]. It has been widely recognized, particularly in the last two decades, that majority of harsh environments are inhabited by surprisingly diverse microbial communities. Bacteria, actinomycetes and fungi are three major groups of soilinhabiting microorganisms. An estimated 1,500,000 species of fungi exist in the world [5]. *Trichoderma*, commonly available in soil and root ecosystems has gained immense importance since last few decades due to its biological control ability against several plant pathogens [6]. Antagonistic microorganisms, such as *Trichoderma,* reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion. In addition, the release of biocontrol agents into the environment has created a demand for the development of methods to monitor their presence or absence in soil. Therefore, monitoring population dynamics in soil is of much importance. Previous methods employed to identify strains of *Trichoderma* spp. in soil samples have included the use of dilution plates on selective media. However, this method does not distinguish between indigenous strains and artificially introduced ones [7]. The *Trichoderma* isolates were differentiated by mycelia growth rate and colony appearance, as well as microscopic morphological features, including phialides and phialospores [8]. These can also be distinguished by randomly amplified polymorphic DNA (ISSR) - PCR, restriction fragment length polymorphisms in mitochondrial DNA and ribosomal DNA and sequence analysis of ribosomal DNA [9-13]. The use of molecular phylogenetic markers has refined *Trichoderma* taxonomy significantly, and phylogenetic analysis of the large number of *Trichoderma* specie is still a field of active ongoing research. Microsatellites, which are also known as short tandem repeats or simple sequence length polymorphisms, are stretches of tandem mono-, di-, tri-, and tetranucleotide repeats of varying lengths Such sequences are widely dispersed in eukaryotic genomes including those of fungi; they are also present but less frequent in prokaryotic genomes.

Materials and Method

Isolation and identification of *Trichoderma*

Trichoderma isolates were originally isolated from soil collected from rhizospheres of chickpea, pigeonpea and lentil crop from different places of Uttar Pradesh, India, and *Trichoderma* isolates were isolated on Potato Dextrose Agar medium by following serial dilution plate technique. They were cultured on PDA 25°C for seven days. After an incubation period, colonies were purified and determined to be *Trichoderma* species and confirmed using *Trichoderma* morphological key. The identity of the purified bioagents was then confirmed by ITCC, Division of Plant Pathology IARI, New Delhi-12. Single-spore isolates of 30 *Trichoderma* isolates were cultured in Erlenmeyer flasks (250 ml) containing 100 ml potato dextrose broth at 25°C for seven days. Mycelia were harvested by filtration through whatman filter paper. Samples were frozen in liquid nitrogen and ground to fine powder using a mortar and pestle.

Genomic DNA extraction from *Trichoderma* isolates

Isolation of fungal genomic DNA was done by growing the fungi for 3-4 days. The mycelia were incubated with lysis buffer containing 250 mMTris-HCl (pH 8.0), 50 mMEDTA (pH8.0), 100 mMNaCl and 2% SDS, for 1 hr at 60°C followed by centrifugation at 12,000 rpm for 15 min. The supernatant was then extracted with equal volume of water saturated phenol and further centrifuged at 12,000 rpm for 10

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min; the aqueous phase was further extracted with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuge at 12,000 rpm for15 min; the aqueous phase was then transferred in a fresh tube and the DNA was precipitated with chilled ehanol (100%). DNA was pelleted by centrifuging at 12000 rpm for 15 min and washed in 70% ethanol by centrifugation. The pellets were air dried and suspended in TE buffer (Tris EDTA buffer) (pH 8.0).

Qualitative and quantitative estimation of DNA

The extraction of total genomic DNA from the *Trichodrma* isolates as per the above procedure was followed by RNAase treatment. Genomic DNA was re suspended in 100 µl 1 X TE buffer and incubated at 37°C for 30 min with RNAse (60 µg). After incubation the sample was re extracted with PCI (Phenol: Chloroform: Isoamylalcohol 25:24:1) solution and RNA free DNA was precipitated with chilled ethanol as described earlier. The quality and quantity of DNA was analyzed both All isolates of *Trichoderma* were taken up for ITS-PCR spectrophotometrically and in 0.8% agarose gel. The DNA from all isolates produced clear sharp bands, indicating good quality of DNA.

PCRamplification of its region of *Trichoderma* isolates

Amplification Genomic DNA was amplified by mixing thetemplate DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10 X Taq pol buffer, 1 µl of 1 U Taq polymerase enzyme, 6 µl 2 mMdNTPs, 1.5 µl of 100 mM reverse and forward primers and 1 µl of 50 ng template DNA. PCR was programmed with an initial denaturing at 94°C for 5 min. followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 70°C for 2 min and the final extension at 72°C for 7 min ina Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading buffer (8 µ1) containing 0.25% bromophenol blue, 40% w/v sucrose in water and then loaded in 2% Agarose gel with 0.1% ethidium bromide for examination with horizontal electrophoresis.

ISSR of *Trichoderma* isolates

For ISSR, six microsatellite primers i.e. A-1; A-2; A-3; A-4; A-5 and A-6 were selected (Table 1). PCR was programmed with an initial denaturing at 94°C for 4 min. followed by 35cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 70°C for 90 s and the final extension at 72°C for 7 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading buffer (8 µ1) containing 0.25% bromophenolblue, 40% w/v sucrose in water and then loaded in 2%Agarose gel with 0.1% ethidium bromide for examination by horizontal electrophoresis [14].

Priner Name	Sequence(5'-3')	Mer	TM	% GC
ITS-Primers pairs				
T/ITS 1	TCTGTAGGTGAACCTGCGG	19	63.9	57
T/ITS4	TCCTCCGCTTATTGATATGC	20	61.5	45
ISSR primers				
A-1	5'YC $(TG)_7$T3'	17	49.77	47
A -2	5'$(GA)_9$AC3'	20	53.70	55
A-3	5'$(GA)_9$T3'	20	58.01	47
A-4	5'$(GA)_8$AC3'	18	56.35	40
A-5	5'$(AG)_8$AC3'	18	60.17	50
A-6	5'$(AG)_8$AT3'	18	60.26	47

Table 1: The nucleotide sequence used for ITS and *Trichoderma* PCR.

ITCC No. IARI, New Delhi	Culture No	Ref. No.	Source	Fungus identified
ITCC-8305/11	8 CP	1	Kanpur Nagar	*Trichoderma viride*
ITCC-8306/11	11 CP	2	Allahabad	*Trichoderma viride*
ITCC-8307/11	17 CP	3	Kaushambi	*Trichoderma viride*
ITCC-8308/11	33 CP	4	Sitapur	*Trichoderma viride*
ITCC-8309/11	34 CP	5	Hardoi	*Trichoderma viride*
ITCC-8310/11	35 CP	6	Fatehpur	*Trichoderma viride*
ITCC-8311/11	66 CP	7	Auraiya	*Trichoderma viride*
ITCC-8312/11	89 CP	8	Unnao	*Trichoderma viride*
ITCC-8313/11	102 CP	9	Etawah	*Trichoderma viride*
ITCC-8314/11	119 CP	10	Kanpur Dehat	*Trichoderma viride*
ITCC-8315/11	01 PP	11	Hardoi	*Trichoderma viride*
ITCC-8316/11	09 PP	12	Kanpur Nagar	*Trichoderma viride*
ITCC-8317/11	14 PP	13	Barabanki	*Trichoderma viride*
ITCC-8318/11	17 PP	14	Fatehpur	*Trichoderma viride*
ITCC-8319/11	29 PP	15	Unnao	*Trichoderma viride*
ITCC-8320/11	42 PP	16	Bahrich	*Trichoderma viride*
ITCC-8321/11	67 PP	17	Auraiya	*Trichoderma viride*
ITCC-8322/11	74 PP	18	Sitapur	*Trichoderma viride*
ITCC-8323/11	78 PP	19	Etawah	*Trichoderma viride*
ITCC-8324/11	124 PP	20	Kanpur Dehat	*Trichoderma viride*
ITCC-8325/11	13 L	21	Kanpur Nagar	*Trichoderma viride*
ITCC-8326/11	35 L	22	Sitapur	*Trichoderma viride*
ITCC-8327/11	68 L	23	Hardoi	*Trichoderma viride*
ITCC-8328/11	74 L	24	Auriaya	*Trichoderma viride*
ITCC-8329/11	89 L	25	Gonda	*Trichoderma viride*
ITCC-8330/11	100 L	26	Faizabad	*Trichoderma viride*
ITCC-8331/11	104 L	27	Etawah	*Trichoderma viride*
ITCC-8332/11	109 L	28	Allahabad	*Trichoderma viride*
ITCC-8333/11	117 L	29	Sultanpur	*Trichoderma viride*
ITCC-8334/11	119 L	30	Kanpur Dehat	*Trichoderma viride*

Table 2: Isolates of *Trichoderma* spp.

Scoring and data analysis

The image of the gel electrophoresis was documented through Bio-Profil Bio-1D gel documentation system and analysis software. All reproducible polymorphic bands were scored and analysed following UPGMA cluster analysis protocol and computed *In Silico* into similarity matrix using NTSYSpc (Numerical Taxonomy System Biostastiscs, version 2.11W) [15]. The SIMQUAL program was used to calculate the Jaccard's coefficients. The ISSR patterns of each isolate was evaluated, assigning character state "1" to indicate the presence of band in the gel and "0" for its absence in the gel. Thus a data matrix was created which was used to calculate the Jaccard similarity coefficient for each pair wise comparison. Jaccard coefficients were clustered to generate dendograms using the SHAN clustering programme, selecting the Unweighted Pair-Group Methods with Arithmetic Average (UPGMA) algorithm in NTSYSpc.

Results and Discussion

Thirty isolates were obtained using the *Trichoderma* selective medium from the rhizospheres soil (Table 2). The ribosomal RNA genes (rDNA) possess characteristics that are suitable for the identification of fungal isolates at the species level. These rDNA are highly stable and exhibit a mosaic of conserved and diverse regions within the genome [16]. They also occur in multiple copies with up to 200 copies per haploid genome [17,18] arranged in tandem repeats with each repeat consisting of the 18S small subunit (SSU), the 5.8S and the 28S large subunit

(LSU) genes. Internal Transcribed Spacer (ITS) regions have been used successfully to generate specific primers capable of differentiating closely related fungal species [19]. In the broader context, taxon-selective amplification of IT Sregions is likely to become a common approach in molecular identification strategies, In the present study, we focused on the ITS regions of ribosomal genes for the construction of primers that can be used to identify *Trichoderma* spp. ITS region of rDNA was amplified using genus specific ITS-1 and ITS4 primers amplified the primers (Figure 1-3). The results are in accordance with variability of the *Trichoderm*a isolates. These results are also in accordance with several workers who observed the amplified rDNA fragment of approximately 500 to 600 bp by ITS-PCR in *Trichoderma* [20-24]. The ITS PCR has helped to detect polymorphism at ITS region of rDNA among the *Trichoderma* isolates. Products of size in the range of 600bp was produced by Mukherjee [20] who studied the identification and the genetic relatedness among thirty isolates of *Trichodermaviride* and were analyzed by six microsattelite primers A-1; A-2; A-3; A-4; A-5 and A-6 to generate reproducible polymorphisms. All amplified products with the primers had shown polymorphic and distinguishable banding patterns which indicate the genetic diversity of *Trichoderma* isolates. A total of 53 reproducible and scorable polymorphic bands ranging from approximately 100bp to 2000bp were generated with six primers among the thirty *Trichoderma* isolates (Table 3). ISSR profiles showed that primer A-1 and A4 scored highest bands which ranged between 100bp to 2000bp. Relationships among the isolates was evaluated by cluster analysis of the data based on the similarity matrix. The Dendogram was generated by unweighted pair-group methods with arithmetic mean (UPGMA) using NTSYSpc software (Figure 4). Based on the results obtained all the thirty isolates can be grouped into two main clusters. One cluster represents *T.viride*. Again the *T. viride* cluster is also sub grouped into two. First subgroup with 18 isolates and second one is with 12 isolates of two sub clusters. The cluster of *T. viride* divided into two different cluster contains different isolates.

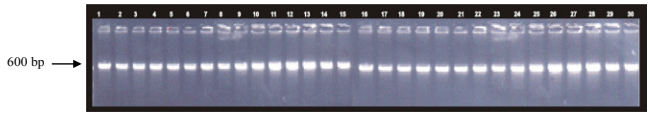

Figure 1: PCR amplification of ITS region of *Trichoderma* isolates. *T. viride* (Lane 1-30) Lane M: Low range DNA Marker, Lane 1- ITCC-8305/11, Lane2- ITCC-8306/11, Lane3- ITCC-8307/11, Lane4- ITCC-8308/11, Lane5- ITCC-8309/11, Lane6- ITCC-8310/11, Lane7- ITCC-8311/11, Lane8- ITCC-8312/11, Lane9- ITCC-8313/11, Lane10- ITCC-8314/11, Lane11- ITCC-8315/11, Lane12- ITCC-8316/11, Lane13- ITCC-8317/11, Lane14- ITCC-8318/11, Lane15- ITCC-8319/11, Lane16- ITCC-8320/11, Lane17- ITCC-8321/11, Lane18- ITCC-8322/11, Lan19- ITCC-8323/11, Lan20- ITCC-8324/11, Lane 21- ITCC-8325/11, Lane 22- ITCC-8326/11, Lane 23- ITCC-8327/11, Lane24- ITCC-8328/11, Lane 25 - ITCC-8329/11, Lane 26 - ITCC-8330/11, Lane 27 - ITCC-8331/11, Lane 28- ITCC-8332/11, Lane 29 - ITCC-8333/11 and Lane 30 - ITCC-8334/11.

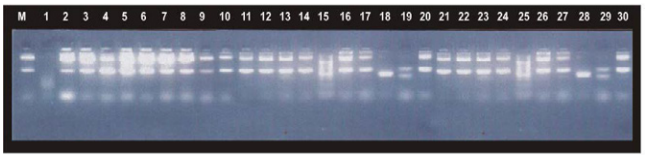

Figure 2: PCR amplification of ISSR (A1 marker) of *Trichoderma* isolates. *T. viride* (Lane 1-30) Lane M: Low range DNA (1 Kilobasepair) Marker, Lane 1- ITCC-8305/11, Lane2- ITCC-8306/11, Lane3- ITCC-8307/11, Lane4- ITCC-8308/11, Lane5- ITCC-8309/11, Lane6- ITCC-8310/11, Lane7- ITCC-8311/11, Lane8- ITCC-8312/11, Lane9- ITCC-8313/11, Lane10- ITCC-8314/11, Lane11- ITCC-8315/11, Lane12- ITCC-8316/11, Lane13- ITCC-8317/11, Lane14- ITCC-8318/11, Lane15- ITCC-8319/11, Lane16- ITCC-8320/11, Lane17- ITCC-8321/11, Lane18- ITCC-8322/11, Lan19- ITCC-8323/11, Lan20- ITCC-8324/11, Lane 21- ITCC-8325/11, Lane 22- ITCC-8326/11, Lane 23- ITCC-8327/11, Lane24- ITCC-8328/11, Lane 25 - ITCC-8329/11, Lane 26 - ITCC-8330/11, Lane 27 - ITCC-8331/11, Lane 28- ITCC-8332/11, Lane 29 - ITCC-8333/11 and Lane 30 - ITCC-8334/11.

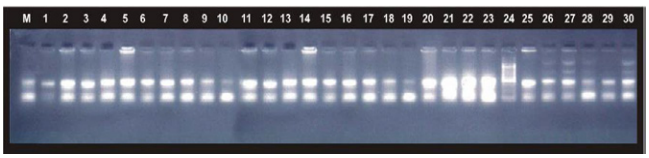

Figure 3: PCR amplification of ISSR (A4 marker) of *Trichoderma* isolates. *T. viride* (Lane 1-30) Lane M: Low range DNA (1 Kilobasepair) Marker, Lane 1- ITCC-8305/11, Lane2- ITCC-8306/11, Lane3- ITCC-8307/11, Lane4- ITCC-8308/11, Lane5- ITCC-8309/11, Lane6- ITCC-8310/11, Lane7- ITCC-8311/11, Lane8- ITCC-8312/11, Lane9- ITCC-8313/11, Lane10- ITCC-8314/11, Lane11- ITCC-8315/11, Lane12- ITCC-8316/11, Lane13- ITCC-8317/11, Lane14- ITCC-8318/11, Lane15- ITCC-8319/11, Lane16- ITCC-8320/11, Lane17- ITCC-8321/11, Lane18- ITCC-8322/11, Lan19- ITCC-8323/11, Lan20- ITCC-8324/11, Lane 21- ITCC-8325/11, Lane 22- ITCC-8326/11, Lane 23- ITCC-8327/11, Lane24- ITCC-8328/11, Lane 25 - ITCC-8329/11, Lane 26 - ITCC-8330/11, Lane 27 - ITCC-8331/11, Lane 28- ITCC-8332/11, Lane 29 - ITCC-8333/11 and Lane 30 - ITCC-8334/11.

Sl.No.	Primers	Total loci	Polymorphic loci	Polymorphic %age
A-1	5'YC $(TG)_7$T3'	6	6	100
A -2	5'$(GA)_8$AC3'	9	7	77
A-3	5'$(GA)_9$T3'	9	5	55
A-4	5'$(GA)_8$AC3'	9	9	100
A-5	5'$(AG)_8$AC3'	10	6	60
A-6	5'$(AG)_8$AT3'	10	8	80
Total		**53**	**41**	**77**

Table 3: Analysis of the polymorphism obtained with ISSR markers in 30 *Trichoderma* isolates.

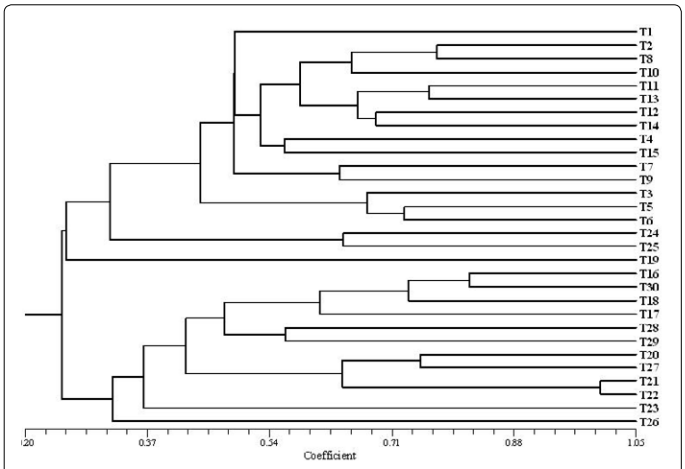

Figure 4: Dendrogram showing the genetic relationships among 30 *Trichoderma* fungal isolates based on ISSR analysis.

Conclusion

Preliminary studies indicates that the *Trichoderma* isolates had very good diversity and there are strong possibility to get the isolate specific primers that will be utilized for identifying the particular *Trichoderma* isolates with good biological potential from the field isolates without going the cumbersome bioassay again.

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References

1. George E, H Marochner, Jakobson I (1995) Role of arbuscular mycorrhizal Fungi in uptake of Phosphors and nitrogen from soil. Crit Rev Botechnol 15: 257-270.
2. Smith KP, Goodman RM (1999) HOST VARIATION FOR INTERACTIONS WITH BENEFICIAL PLANT-ASSOCIATED MICROBES. Annu Rev Phytopathol 37: 473-491.
3. Wright SF, Upadhyaya A (1998) A survey of soil for aggregate stability and glomalin, a glycoprotein produced by hyphae of mycorrhizal fungi. Plant Soil 198: 97-107.
4. Yao H, He Z, Wilson MJ, Campbell CD (2000) Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use. Microb Ecol 40: 223-237.
5. Giller KE, Beare MH, Cavelle P, Izac AMN, Swift MJ (1997) Agricultural infestation, soil biodiversity and agroecosystem function. Appl Soil Ecol 6: 3-16.
6. Kubicek CP, Harman GE (1998) Trichoderma and Gliocladium,Vol.I and II. Taylor and Francies Ltd. London, UK.
7. Knudsen IMB, Jensen B, Jensen DF, Hockenhull J (1996) Occurrence of Gliocladium roseum on barley roots in sand and field soil. Kluwer Academic Publishers, Netherlands.
8. Seaby DA (1996) Differentiation of Trichoderma taxa associated with mushroom production. Plant Pathol 45: 905-912.
9. Castle A, Speranzini D, Rghei N, Alm G, Rinker D, et al. (1998) Morphological and molecular identification of Trichoderma isolates on North American mushroom farms. Appl Environ Microbiol 64: 133-137.
10. Muthumeenakshi S, Mills PR, Brown AE, Seaby DA (1994) Intraspecific molecular variation among Trichoderma harzianum isolates colonizing mushroom compost in the British Isles Microbiol 140: 769-777.
11. Muthumeenakshi S, Mills PR (1995) Detection and differentiation of fungal pathogens of Agaricus bisporus. Mushroom Sci 14: 603-610.
12. Ospina-Giraldo MD, Royse DJ, Thon MR, Chen X, Romaine CP (1998) Phylogenetic relationships of Trichoderma harzianum causing mushroom green mold in Europe and North America to other species of Trichoderma from world-wide sources. Mycologia 90: 76-81.
13. Ospina-Giraldo MD, Royse DJ, Chen X, Romaine CP (1999) Molecular Phylogenetic Analyses of Biological Control Strains of Trichoderma harzianum and Other Biotypes of Trichoderma spp. Associated with Mushroom Green Mold. Phytopathology 89: 308-313.
14. Elad Y, Chet I (1983) Improved selective media for isolation of Trichoderma spp. or Fusarium spp. Phytoparasitica 11: 55-58.
15. Rohlf FJ (1993) NTSYSpc: Numerical Taxonomy and Multivariate analysis system. Ver 2.1. Exeter Software: Setauket, U.S.A.
16. Hibbett DS (1992) Ribosomal RNA and fungal systematics. Trans Mycol Soc 33: 533-556.
17. Bruns TD, White TJ, JW Talyor (1991) Fungal molecular systematics. Annu Rev Ecol Sys 22: 525-564.
18. Yao C, Frederiksen RA, Magill CW (1992) Length heterogeneity in ITS 2 and the methylation status of CCGG and GCGC sites in the rRNA genes of the genus Peronosclerospora. Curr Genet 22: 415-420.
19. Bryan GT, Daniels MJ, Osbourn AE (1995) Comparison of fungi within the Gaeumannomyces-Phialophora complex by analysis of ribosomal DNA sequences. Appl Environ Microbiol 61: 681-689.
20. Mukherjee PK, Verma A, Latha J (2002) PCR fingerprinting of some Trichoderma isolates from two Indian type culture collection-a need for re-identification of these economically importance fungi. Science Correspondence 83: 372-374.
21. Ospina-Giraldo MD, Royse DJ, Chen X, Romaine CP (1999) Molecular Phylogenetic Analyses of Biological Control Strains of Trichoderma harzianum and Other Biotypes of Trichoderma spp. Associated with Mushroom Green Mold. Phytopathology 89: 308-313.
22. Muthumeenakshi S, Mills PR, Brown AE, Seaby DA (1994) Intraspecific molecular variation among Trichoderma harzianum isolates colonizing mushroom compost in the British Isles. Microbiology 140 : 769-777.
23. Lieckfeldt E, Samuels GJ, Nirenberg HI, Petrini O (1999) A morphological and molecular perspective of Trichoderma viride: is it one or two species? Appl Environ Microbiol 65: 2418-2428.
24. Venkateswarlu R, Reddi KM, Reddy ENP, P Sudhakar (2008) Molecular characterization of Trichoderma spp. Used against Fusarium wilt with PCR based RAPD and ITS-PCR. J Mycol Plant Pathol 38: 569-563.