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Short Communication

Molecular characterization of *Chaetomium* species using URP-PCR

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Abstract

Chaetomium spp. are common colonizers of soil and cellulose-containing substrates. Seventeen isolates of *Chaetomium* spp., which included 15 isolates of *C. globosum* and one each of *C. reflexum* and *C. perlucidum*, were genetically characterized with universal rice primers (URP - primers derived from DNA repeat sequences in the rice genome) using polymerase chain reaction (URP-PCR). Out of the 12 URP's used in the study, nine primers were effective in producing polymorphic fingerprint patterns from DNA of *Chaetomium spp.* Analysis of the entire fingerprint profile using the unweighted pair-group method with arithmetic averages (UPGMA) clearly differentiated *C. globosum* isolates from *C. perlucidum* and *C. reflexum*. One of the primers, URP-2R, produced a uniform DNA band of 1.9 kb in all the isolates of *C. globosum* but not in *C. perlucidum* and *C. reflexum*, which can be used as molecular marker to differentiate *C. globosum* from other species. Our results indicate that URP's are sensitive and give reproducible results for assaying the genetic variability in *Chaetomium* spp.

Key words: Chaetomium globosum, *Chaetomium reflexum, Chaetomium perlucidum*, genetic variability, URP-PCR.

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Chaetomium is a genus belonging to the class Pyrenomycetes (Ascomycotina), order Sordariales and family Chaetomiaceae. It is found extensively on various agricultural commodities. The genus can produce an *Acremonium*-like state (imperfect stage) on culture media and is characterized by superficial flask-shaped perithecia, which are surrounded by dark, stiff hairs. *C. globosum* is one of the commonest species growing saprophytically in the rhizosphere and phyllosphere. It is a common colonizer of soil and cellulose-containing substrates and has been reported to be a potential biocontrol agent. *C. globosum* has been reported effective in minimizing damage caused by seed rot and damping off, due to several seed-borne and soil-borne plant pathogens like *Pythium ultimum, Alternaria raphani, A. brassicol* and *Fusarium* spp. (Harman *et al.*, 1978; Vannacci and Harman, 1987).

The seedling blight caused by *Rhizoctonia solani* has been successfully controlled by seed treatment with *Chaetomium* spp. (Baker, 1968). *C. globosum* has also shown an antagonistic effect against rice blast (*Pyricularia oryzae*) (Soyton and Quimio, 1989). Our recent studies have indicated its bioefficacy in controlling spot blotch of wheat caused by *Cochliobolus sativus* (Aggarwal *et al.*, 2004). Biochemical characterization of the fungus has shown the production of β 1,3-glucanase and xylanase (Ahammed SK, Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, India, 2003; Ahammed *et al.*, 2008). *C. globosum* has a great potential as a biocontrol agent and has been classified based on morphological descriptions of colony growth and perithecia (Millner *et al.*, 1997; Ahammed *et al.*, 2004; 2005a), but this is not sufficient and a significant variation needs to be defined for each strain at the molecular level. *C. reflexum* and *C. perlucidum* are two other species, morphologically very similar to *C. globosum.* Therefore, there is a need for molecular markers to characterize differences at the inter and intraspecific level.

Repeat sequences from Korean weedy rice, originally referred to as universal rice primer (URP), have been used for the fingerprinting of diverse genomes of plants, animals and microbes (Kang *et al.*, 2002), but only very few fungi (Kang *et al.*, 2001; Kang *et al.*, 2002; Jana *et al.*, 2005). *Chaetomium globosum* isolates had been characterized earlier by the PCR-RAPD technique (Ahammed *et al.*, 2005b). However, the use of repetitive sequences derived from plant genomes as molecular markers has not received attention in the fingerprinting of this fungus. The objective of our study was to use URP's for generating DNA fingerprint profiles of different C. *globosum* isolates and compare

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them with *C. reflexum* and *C. perlucidum*, in order to genetically differentiate the species.

Pure cultures of 15 *Chaetomium globosum* isolates (Cg1-Cg15) and one isolate each of *C. reflexum* (Cr) and *C. perlucidum* (Cp) collected from various locations of India were established. For DNA isolation, the cultures were grown in potato dextrose broth (PDB; pH 5.5) for 7 days at 28 ± 1 °C in a shaker incubator. Mycelia were filtered through filter paper (Whatman n. 1) and DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The mycelium was ground in liquid nitrogen, transferred to DNA extraction buffer (0.1 M Tris, 1.5 M NaCl, 0.01 M EDTA) and kept at 65 °C for one hour with occasional stirring. Equal volumes of chloroform:isoamyl alcohol (24:1) were added to all tubes, followed by centrifugation. The upper aqueous phase obtained by precipitation with 0.6^{th} volume of ice-cold isopropanol was again centrifuged. The pellet was washed with 70% ethanol and dried at room temperature. Finally, the nucleic acid was dissolved in TE and stored at -20 °C.

URP's are primers with 20 oligonucleotides each, originally obtained from repeat elements of weedy rice by Kang *et al.* (2002). There are 12 URP primers which were synthesized by Genuine Chemical Corporation (GCC), India. PCR was performed in a Temperature Gradient Thermal Cycler (BioRAD, USA). Concentrations of DNA template, primer and deoxynucleotide triphosphates (dNTPs) and the optimum annealing temperature were standardized for each primer in preliminary trials to obtain DNA fingerprint profiles (Table 1). Each PCR reaction contained 50-100 ng of genomic DNA, 200 µM of each dNTP (dATP, dGTP, dCTP and dTTP), 0.2 µM primer, 1.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase, and 1X Taq buffer in a total reaction volume of 25 µL. Thermal cycling conditions were: initial denaturation at 94 °C for 4 min, followed by 35 denaturation cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. A final extension step at 72 °C for 7 min was also performed.

The URP - PCR products were electrophoresed on a 1.2% agarose gel in TBE buffer, visualized by staining with ethidium bromide and photographed using a Gene Genius Gel Documentation System (Syngene Inc, Cambridge, UK).

Relatedness among the 17 isolates of *Chaetomium* spp. was estimated by means of scorable DNA bands amplified from different URP markers. Each band was considered as character and was scored as either present (coded as 1) or absent (coded as 0). Cluster analysis with the unweighted pair group method with an arithmetic average (UPGMA) algorithm was performed using NTSYS-PC (v. 2.01; Rohlf, 1998) to produce a dendrogram. The experiments were repeated three times, and each time identical results were obtained.

Twelve oligonucleotide primers (URP's) were used for the molecular analysis of 15 isolates of *Chaetomium globosum* and one isolate each of *C. reflexum* and *C. perlucidum*. These isolates were used as templates to assess the wide distribution of the URP nucleotide motif sequences in the genome of *Chaetomium* spp. Nine URP's out of 12 gave good amplification in all the *C. globosum, C. reflexum* and *C. perlucidum* isolates used in this study. Different levels of polymorphism were obtained with these primers (Table 1). Amplification products for all primers were polymorphic, except for URP-30F, which produced a monomorphic band. The amplified DNA bands ranged from 250 bp to 3000 bp for each isolate.

Amplification with primer URP-2R produced a uniform DNA band of 1.9 kb in all the isolates of *C. globosum*, but not in *C. reflexum* and *C. perlucidum* (Figure 1a). The data on banding pattern with this primer was analyzed, and the dendrogram obtained showed the formation of two major clusters with *C. perlucidum* separating out singly, showing only 33% similarity with the *C. globosum* and *C. reflexum* isolates. In cluster I, there was 100% similarity

Table 1 - Sequences of Universal Rice Primers (URP's) and polymorphism obtained in *Chaetomium* spp.

	Primer Sequence (5' - 3')	GC content (%)	Temp. (°C)	Total n. of bands	Polymorphic bands	Monomorphic bands	% polymorphism
1.	URP-6R GGCAAGCTGGTGGGAGGTAC	50	65	13	13	-	100
2.	URP-4R AGGACTCGATAACAGGCTCC	50	66	8	8	-	100
3.	URP-30F GGACAAGAAGAGGATGTGGA	50	65	8	7	1	87.5
4.	URP-25F GATGTGTTCTTGGAGCCTGT	50	65	8	8	-	100
5.	URP-1F ATCCAAGGTCCGAGACAACC	50	65	8	8	-	100
6.	URP-2F GTGTGCGATCAGTTGCTGGG	50	67	7	7	-	100
7.	URP-9F ATGTGTGCGATCAGTTGCTG	50	67	7	7	-	100
8.	URP-2R CCCAGCAACTGATCGCACAC	50	65	10	10	-	100
9.	URP-17R AATGTGGGCAAGCTGGTGGT	55	74	10	10	-	100
	Total			79	78	1	98.61

between isolates Cg2 and Cg 8, Cg12 and Cg 13, and Cg 10 and Cg 11, while in cluster II, Cg4, Cg5, Cg6 and Cg7 exhibited 100% similarity. The *C. reflexum* isolate separated out in this cluster, showing 82% similarity with the above isolates and with the *C. globosum* isolate Cg3 (Figure 1b). A DNA band of 1300 bp was specifically amplified by primer URP-6R only in *C. reflexum* and not in any of the *C. globosum* and *C. perlucidum* isolates (Results not shown). On the other hand, URP-4R amplified a band of 1200 bp specific to *C. perlucidum* and not present in any isolate of *C. globosum* and *C. reflexum* (Results not shown). The analysis based on individual URP primers (URP-2R, URP-4R and URP-6R) indicated a high level of genetic similarity between isolates Cg4, Cg6, Cg7 and Cg9 in one cluster and Cg10, Cg11, Cg12, Cg13, Cg14 and Cg15 in a separate cluster, in spite of the fact that they were obtained from different geographical regions of India.

Phylogenetic analysis of a combined data set obtained from nine URP's showed formation of two main clusters with only 65.3% similarity between them (Figure 2). The dendrogram showed high genetic similarity among different isolates of *C. globosum* obtained from different sources. Cluster I consisted of isolates Cg1, Cg9, Cg4, Cg5, Cg6, Cg7 and *C. perlucidum* and *C. reflexum*. Bootstrap analysis indicated that, within this cluster, isolates Cg4 and Cg6 showed 53.9% similarity, and these two isolates showed 92.4% similarity with Cg5. Isolate Cg9 and *C. reflexum* formed a separate group within cluster I, separating out at a bootstrap value of 21 from the other isolates and *C. perlucidum*. Within this cluster, isolates Cg4 and Cg5 showed 93% similarity. The other *C. globosum* isolates were grouped into cluster II, in which Cg2 and Cg3 showed a high bootstrap value (98.2%), forming a separate subgroup within this cluster. This group showed > 63% genetic similarity among the isolates. Isolate Cg8 separated out from this other subgroup within this cluster at the bootstrap value of 54.6%, having the rest of the isolates viz., Cg10, Cg11, Cg12, Cg13, Cg14 and Cg15. In our earlier studies on molecular characterization of *C. globosum* using RAPD primers, nine isolates were grouped into two distinct clusters, with isolates Cg2, Cg3 and Cg4 in one cluster and the remaining isolates in another (Ahammed *et al.*, 2005b). Genetic diversity using AFLP markers in this fungus was also explored earlier (Aggarwal *et al.*, 2003), showing that five *C. globosum* isolates formed two distinct clusters, one comprising isolates Cg6, Cg7 and Cg8, and the other encompassing isolates Cg1 and Cg5. The use of URP markers in the present study enabled us to distinguish 15 different *C. globosum* isolates collected from different sources, and also from *C. reflexum* and *C. perlucidum*. Previous studies have indicated that these rice repeat sequences based on which the URP primers were designed are conserved in fungi too, enabling us to detect the variations at the interspecific and intraspecific levels. There is one previous report on the use of URP's for the genetic differentiation of a fungus, *Macrophomina phaseolina* (Jana *et al.*, 2005). Our analysis revealed that 75% of the URP's could amplify the scorable and reproducible bands with genomic DNA of *Chaetomium globosum*, *C. perlucidum* and *C. reflexum* isolates. Primers URP-6R, URP-4R and URP-2R produced distinct amplification profiles among the *Chaetomium* spp. isolates used in the present study and were therefore considered the best markers. All URP primers, except URP-30F, revealed more polymorphism in our study, although

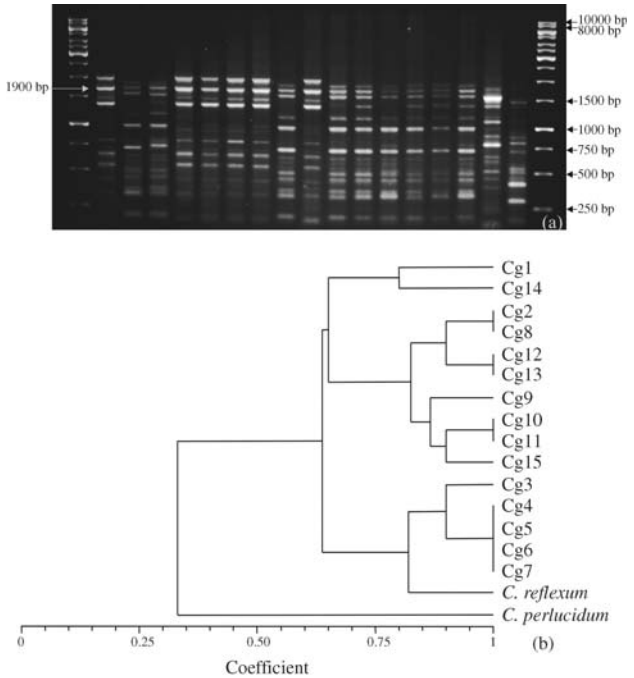

Figure 1 - (a) DNA fingerprint profile of different *Chaetomium* spp. isolates obtained with primer URP-2R. M is the 1 kb DNA ladder (MBI, Fermenats), Lanes 1-15, isolates of *C. globosum*, Cr - *C reflexum*, Cp - *C. perlucidum.* (b) Dendrogram obtained from 17 isolates of *Chaetomium* spp. with UPGMA-based similarity coefficient, using primer URP-2R.

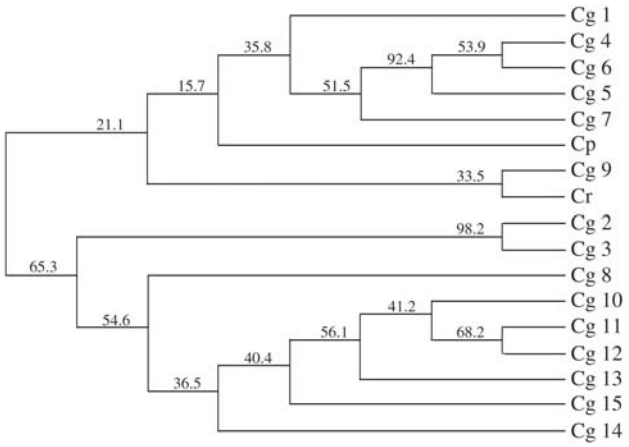

Figure 2 - Dendrogram obtained after combined bootstrap analysis. The numbers at the forks show the percentage of times the group consisting of the species which are to the right of that fork occurred. Cg = *Chaetomium globosum*; Cr = *C. reflexum*; Cp = *C. perlucidum*.

the results of Kang *et al.* (2001, 2002) and Jana *et al.* (2005) showed more polymorphism with primers having a GC content > 50%. Primers URP-13 R, URP-32F and URP-38F (Kang *et al.*, 2002) did not amplify the genomic DNA of *Chaetomium* spp. in this study. The genetic differentiation of *C. globosum* isolates has a great significance, considering that our earlier findings have indicated the potentiality of this fungus as a biocontrol agent (Aggarwal *et al.*, 2004).

The present investigation shows that URP-PCR fingerprinting is a valuable tool for rapid identification and differentiation of fungal strains. PCR fingerprints have shown high DNA polymorphism between strains of the same species and even between different *Chaetomium* spp. Moreover, our purpose of using URP markers was to differentiate the strains of *C. globosum* and also to develop species-specific markers. Amplification with URP-2R produced a uniform band of 1.9 kb in all the *C. globosum* isolates, but not in *C. perlucidum* and *C reflexum*. Further work on cloning and sequencing of this amplicon to develop a SCAR marker is in progress.

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References

Aggarwal R, Srinivas P, Malathi VG and Renu (2003) Evaluation of genetic diversity of *Chaetomium globosum*, a potential biocontrol agent by amplified fragment length polymorphism (AFLP). Indian Phytopath 56:313 (Abstr.).

Aggarwal R, Tiwari AK, Srivastava KD and Singh DV (2004) Role of antibiosis in the biological control of spot blotch (*Cochliobolus sativus*) of wheat by *Chaetomium globosum.* Mycopathologia 157:369-377.

Ahammed SK, Aggarwal R and Srivastava KD (2004) Colony characteristics and bioefficacy of different isolates of *Chaetomium globosum* Krunze ex Fr. against *Bipolaris sorokiniana* (Sacc.) Shoem. J Biol Control 18:167-171.

Ahammed SK, Aggarwal R and Singh DV (2005a) Morphological variability in different isolates of *Chaetomium globosum*. Indian Phytopath 58:71-74.

Ahammed SK, Aggarwal R and Renu (2005b) Use of PCR based RAPD technique for characterization of *Chaetomium globosum* isolates. Acta Phytopath Entomol Hungarica 40:303-314.

Ahammed SK, Aggarwal R and Kapoor HC (2008) Production, partial purification and characterization of extracellular xylanase from *Chaetomium globosum.* J Plant Biochem Biotechnol 17:95-98.

Baker R (1968) Mechanism of biological control of soil borne pathogens. Annu Rev Phytopath 6:263-294.

Harman GE, Eckenrode CJ and Webb DR (1978) Alteration of spermosphere ecosystems affecting oviposition by the bean seed fly and attack by soil borne fungi on germinating seeds. Ann Appl Biol 90:1-6.

Jana TK, Singh NK, Koundal KR and Sharma TR (2005) Genetic differentiation of charcoal rot pathogen, *Macrophomina phaseolina*, into specific groups using URP-PCR. Can J Microbiol 51:159-164.

Kang HW, Park DS, Go SJ and Eun MY (2001) Genomic differentiation among oyster mushrooms (*Pleurotus* spp.) cultivars released in Korea by URP-PCR. Mycobiology 29:85-89.

Kang HW, Park DS, Park YJ, You CH, Lee BM, Eun MY and Go SJ (2002) Fingerprinting of diverse genomes using PCR with universal rice primers generated from repetitive sequence of Korean weedy rice. Mol Cells 13:281-7.

Millner PD, Motta JJ and Lentz PL (1997) Ascospores, germpore, ultrastructure and thermophilism of *Chaetomium*. Mycologia 69:722-733.

Murray MG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321-4326.

Rohlf FJ (1998) NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System, v. 2.02. Exeter Software, Setauket.

Soytong K and Quimio TH (1989) Antagonism of *Chaetomium globosum* to rice blast pathogen *Pyricularia oryzae.* Kasetsart J Nat Sci 23:198-203.

Vannacci G and Harman GF (1987) Biocontrol of seed borne *Alternaria raphani* and *A. brassicicola.* Can J Microbiol 33:850-856.

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