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# MOLECULAR CHARACTERIZATION OF MUTANT STRAINS OF *CALOCYBE INDICA* USING RAPD-PCR

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## ABSTRACT

Tremendous revolution in the mushroom technology in recent years with respect to types and strains of cultivated mushrooms has made *Calocybe indica*, a specialty mushroom, the third most popular and commercially grown mushroom in India. A lot of work has been done as far its cultivation technology is concerned but no systematic work has been undertaken for its genetic improvement. During present investigation improvement of high yielding strain, Ci-3 using mutagenic treatment on protoplasts was conducted. Four mutagenic treatments (Ultraviolet rays, N-methyl-N'-nitro-N-nitrosoguanidine, Ethidium bromide and 5'-Bromouracil) yielded 30 putative mutants. Seven mutants were identified on the basis of growth and enzymes studies. Molecular characterization of the seven selected mutant strains was conducted using RAPD technique. A set of 14 primers were used for molecular characterization of mutants along with the parent. Out of these only 6 primers resulted gave distinct amplification products. Six primers yielded 68 scorable bands ranging from 40bp to 280bp. No two genotypes showed 100% similarity between them. Maximum similarity coefficient of value 0.897 was obtained between CMN-9 and CMB-4. Minimum similarity coefficient (0.617) was found between CMU-5 and CMN-11. Similarity coefficient of Ci-3 ranged from 0.706 for CMN-3 to 0.794 for CMU-2.

## INTRODUCTION

Productivity and quality of widely cultivated mushrooms are mainly dependent on strain make up, therefore, many strains have constantly been produced, aiming at higher yield and improved quality attributes such as accumulation of nutrients of interest, pathogen resistance, adaptability to wide temperature range and sporelessness (Kumara and Edirimann, 2009). For strain improvement, a complete knowledge of the life cycle of species is desirable along with the ability to manipulate it genetically. Genetic manipulation of mushrooms could be achieved in number of ways. The use of protoplast fusion for development of new strains could be traced back to 1980s. The most widely adopted method to produce fusion events between protoplasts has been the use of polyethylene glycol in presence of $CaCl_2$.

Mutagenesis has been attempted using a number of approaches. Chemical mutagenesis may be performed using agents such as N-methyl-N'-nitro-N-nitrosoguanidine, sodium nitrite, 5'-bromouracil and usually results in a single nucleotide substitution in the target genome. Irradiations by gamma rays lead to production of lignocellulolytic mutants in *P. ostreatus* (Lee *et al*., 2000) while in *P. florida,* three putative mutants were developed by Djajnegara and Harsoyo (2008) using gamma rays.

However, newly developed strains are very difficult to discriminate, due to lack of clearly distinguishable characters. Molecular markers of rDNA sequencing, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), microsatellite and mitochondrial genotypes have all been used to discriminate mushroom species and/or strains of *Agaricus* (Castle *et al*., 1987; Sonnenberg *et al*., 1991; Khush *et al*., 1992; Barroso *et al*., 2000; Calvo-Bado *et al*., 2000; Moore *et al*., 2001; Ramirez *et al*., 2001) *Auricularia* (Yan *et al*., 1999), *Ganoderma* (Hseu *et al*., 1996), *Lentinula* (Chiu *et al*., 1996), *Stropharia rugoso-annulata* (*Yan et al*., 2003), and *Volvariella* (Chiu *et al*., 1995). These technologies provide ways to obtain reliable data for mushroom strain identification. Although RAPD analysis was initially developed to detect polymorphism between organisms yet, despite the absence of sequence information, it was used to produce genetic markers and to construct genetic maps (Williams *et al*., 1990).

*Calocybe indica,* a specialty mushroom, belonging to class Basidiomycetes, order Agaricales and family Tricholomataceae was first reported from India by Purkayastha and Chandra in 1974. Twenty species of this genus are known and four species including *C. indica* from India are known to be edible (Pandey, 1998). Till date numerous works have been carried out for improvement on its cultivation technique (Yadav, 2006; Amin *et al*., 2010; Pani, 2010; Senthilnambi *et al*., 2011) but no work has yet been undertaken for its genetic improvement. In present study attempts have been made to genetically modify *Calocybe indica*, specialty mushroom, through mutagenesis. The mutants were then molecularly characterized used RAPD technique.

## MATERIALS AND METHODS

Mycelium of high yielding strain of *C. indica*, Ci-3, was subjected to protoplast isolation. The protoplasts thus produced were subjected to mutagenesis using four different treatments which included ultraviolet rays, ethidium bromide, 5'-bromouracil and N-methyl-N'-nitro-N-nitrosoguanidine

treatment. Thirty regenerants (Table 1) were obtained when the treated protoplasts were cultured on regeneration medium. Out of these 30 putative mutants seven mutants (Table 2) were selected on basis of growth and enzyme assay. The seven mutants thus selected were subjected molecular characterization using RAPD (Kaur *et al.*, 2011).

Pure mycelia cultures of *C. indica* mutants and their parent strain were maintained on potato dextrose agar culture media in petri plates and raised in complete yeast extract broth for 4-5 weeks at $30 \pm 2$ºC. The mycelial mat was filtered through Whatman no.1 and used for DNA extraction.

10g of sample was crushed in 15mL of 2X CTAB buffer (Table 3), shifted it to centrifuge tubes and incubated it at 65ºC for 30mins in water bath. Samples were mixed occasionally while maintaining at 65ºC. 15mL of chloroform: isoamyl alcohol (24:1) was added and the tubes were swirled to make an emulsion. The tubes were then placed on rotary shaker for 45 mins at room temperature. The tubes were centrifuged at 8000g for 15 mins at room temperature. The supernatant was transferred to a clean sterile tube and 5$\mu$L RNAase (10mg/mL) was added. The tubes were then incubated at 37ºC for 1h. Later 10mL of potassium acetate was added to each tube and incubated for 20mins in refrigerator. The contents were centrifuged at 8000 rpm for 10mins. About 0.6 volume of isopropyl alcohol was added to the supernatant. The DNA formed white cottony precipitate (good quality DNA floats atop while poor one settles down). The DNA was cleaned 2-3 times with 70% ethanol and was then dissolved in TE buffer.

The extracted DNA was assessed in terms of both quality and quantity using agarose gel electrophoresis. The intensity of florescence of each sample was compared with that of the standard marker (25ng/$\mu$L Lambda cutter) and then the DNA concentration of each sample was ascertained. The quality of DNA samples was judged on the basis of whether the DNA forms a single high molecular weight band (good quality) or a smear (poor quality).

A set of 14 primers (Operon Biotechnologies, Germany) were used for amplification of parent (Ci-3) and mutant strains. Out of these 14 primers 8 resulted in either sub-optimal or non-distinct amplification products. Therefore, these were discarded and remaining which gave clear and reproducible amplification products were used for RAPD analysis. The list of 14 primers along with their sequences is presented in Table 4.

Genomic DNA was amplified through polymerase chain reaction (PCR) using RAPD primers. *In vitro* amplification was performed in a 96 well microtitre plate in Biometra, T3000 thermocycler. Decamer primers were used for RAPD analysis. The reaction mix for PCR amplification and PCR conditions used during this study have been described in details by Singh *et al.*, 2000.

After amplification 3$\mu$L of 6X loading dye was added to each of the amplified product. From this mixture 10$\mu$L of each sample was loaded in 1.5% agarose gel prepared in 0.5X TBE buffer. PCR product was resolved by running gel at 5V/cm for 2-3h. The gels were visualized under UV light and photographed using UV gel documentation system (BIORAD).

The RAPD band size was determined on the position of bands relative to the ladder (1Kb). The amplified bands were recorded as 1 (band present) or 0 (band absent) in a binary matrix.

Computer software programme Numerical Taxonomic and Multivariate Analysis System (NTSYS-PC) VERSION 2.02E (Rholf, 1998) was used for estimation of genetic similarities among the lines using SIMQUAL mode of NTSYS and the similarity matrix value based on Jaccard (1990) coefficient of similarity used to generate dendrogram. Clustering was done by (Unweighted Pair Group Method with Arithmetic Mean)

**Table 1: Putative *C. indica* mutants developed**

| S. No. | UV induced mutants | NTG induced mutants | BU induced mutants | $EtBr_2$ induced mutants |
|---|---|---|---|---|
| 1. | CMU-1 | CMN-1 | CMB-1 | CME-1 |
| 2. | CMU-2 | CMN-2 | CMB-2 | CME-2 |
| 3. | CMU-3 | CMN-3 | CMB-3 | CME-3 |
| 4. | CMU-4 | CMN-4 | CMB-4 | CME-4 |
| 5. | CMU-5 | CMN-5 | CMB-5 | CME-5 |
| 6. | CMU-6 | CMN-6 | - | CME-6 |
| 7. | CMU-7 | CMN-7 | - | - |
| 8. | - | CMN-8 | - | - |
| 9. | - | CMN-9 | - | - |
| 10. | - | CMN-10 | - | - |
| 11. | - | CMN-11 | - | - |
| 12. | - | CMN-12 | - | - |

**Table 2: Mutants selected on basis of growth and enzyme study**

| S. No. | Mutants |
|---|---|
| 1. | CMU-2 |
| 2 | CMU-5 |
| 3 | CME-2 |
| 4 | CMN-3 |
| 5 | CMN-9 |
| 6 | CMN-11 |
| 7 | CMB-4 |

**Table 3: Composition of 2X CTAB buffer**

| Salt | 1.0L | 0.5L | Final concentration |
|---|---|---|---|
| NaCl | 81.8g | 40.9g | 1.4M |
| 1M Tris-HCl (pH 8.0) | 100mL | 50mL | 100Mm |
| 0.5M EDTA (disodium) | 40mL | 20mL | 20Mm |
| CTAB | 20g | 10g | 2.0% |
| Sodium bisulphate | 5g | 2.5g | 0.5% |
| Merceptoethanol | 10g | 5g | 1.0% |
| $ddH_2O$ | Q.S. to make 1L | Q.S. to make 0.5L | |

**Table 4: RAPD Primers used to assess genetic diversity**

| S. No. | Primer used | Sequence | Amplification |
|---|---|---|---|
| 1 | Opn 07 | 5'-CAGGCCCTTC-3' | - |
| 2 | Opn 08 | 5'-AATCGGGCTG-3' | + |
| 3 | Opn 09 | 5'-CACCGTATCC-3' | - |
| 4 | Opn 10 | 5'-GTGGGCTGAC-3' | + |
| 5 | Opn 11 | 5'-AAGGGCGAGT-3' | + |
| 6 | Opn 12 | 5'-GGAGTGCCTC-3' | - |
| 7 | Opn 13 | 5'-CCAGCCGAAC-3' | + |
| 8 | Opn 14 | 5'-GGAGCCAAC-3' | + |
| 9 | Opn 15 | 5'-CCAAGCTGCC-3' | - |
| 10 | Opn 16 | 5'-GGGAAGGACA-3' | + |
| 11 | OPAA03 | 5'-TTAGCGCCCC-3' | - |
| 12 | A-2 | 5'-GGAAGCTTGG-3' | - |
| 13 | S-13 | 5'-GTCGTTCCTG-3' | - |
| 14 | OPAA 07 | 5'-CTACGCTCAC-3' | - |

UPGMA using SAHN module of NTSYS version 2.02e.

## RESULTS AND DISCUSSION

Several techniques for molecular studies have been used for analyzing the genetic diversity in basidiomycetes, such as isoenzymes (Lan *et al*., 1998), AFLP (Amplified Fragment Length Polymorfism) (Qi *et al*., 2003), RFLP (Restriction Fragment Length Polymorfism) (Park *et al*., 1996), ITS (Internal Transcribed Spacers) (Kindermann *et al*., 1998) and RAPD (Random Amplified Polymorphic DNA) (Wang *et al*., 2003). Among these techniques, RAPD is still one of the cheapest and quickest methods for accessing the variability at DNA level, being especially useful on intraspecific analysis. These markers have the advantage of amplifying both regions of the genome which may be transcript/translated, and non-coding regions. This is important when the objective is to evaluate the variation along the biggest part of the species genome (Williams *et al*., 1990; Ferreira and Grattapaglia, 1996; Ro *et al*., 2007). Therefore in present study, significant difference in genetic makeup of parent, UV and chemical mutant strains was observed using RAPD analysis.

The RAPD-PCR reaction was setup for *C. indica* mutants along with the parent strain, Ci-3 using the different decamer primers. The amplifications were carried out twice to confirm reproducibility. Occasionally, the intensity of some bands were reduced/increased but the total number of bands obtained with a primer were constant. The analysis of data was based on the absence (0) or presence of bands (1). Among the 14 primers used only 6 confirmed reproducible amplification (Table 4). Work on molecular characterization of *C. indica* strains had been reported to amplify scorable bands. Six random decamer RAPD primers amplified the scorable DNA fragments in 19 accessions of *C. indica* and separated them into seven distinct phylogenetic sub-clades.

DNA amplified with the primers showed varied banding pattern. Primer Opn 14 amplified the lowest number of scorable bands (5) while primer Opn 11 amplified the highest number of scorable bands (17).

The primer Opn 08 produced a total of 12 bands ranging between 77-244 bp, but mutant CMN-11 gave no visible band with this primer. Out of the 12 bands, 8 showed polymorphic and 4 homomorphic banding pattern (Table 5). The table indicated coefficient similarity among different genotypes. The highest similarity coefficient was obtained among CMU-2, CMU-5, CMN-3 and CMB-4 (1.000) while lowest similarity coefficient was obtained between CME-2 and CMN-11 (0.083). Similarity coefficient ranged from 0.417 to 0.667 between parent strain, (Ci-3) and its mutants

Amplification of primer using primer Opn 10 showed 15 bands (59-230 bp). Out of these 7 bands showed homomorphism while 8 bands were polymorphic (Table 6). It was clear from Table 4.4.2.2 that Ci-3 had similarity coefficient ranging from 0.800 to 0.933 with mutants. Similarity coefficient between CMN-9 and CMN-11 was found to be 1.00. Similarity

**Table 5: Matrix of RAPD similarity coefficients (primer Opn 08)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 1.0000000 | 1.0000000 | | | | | | |
| CME-2 | 0.7500000 | 0.7500000 | 1.0000000 | | | | | |
| CMN-3 | 1.0000000 | 1.0000000 | 0.7500000 | 1.0000000 | | | | |
| CMN-9 | 0.9166667 | 0.9166667 | 0.6666667 | 0.9166667 | 1.0000000 | | | |
| CMN-11 | 0.6666667 | 0.666667 | 0.0833333 | 0.1666667 | 0.2500000 | 1.0000000 | | |
| CMB-4 | 1.0000000 | 1.0000000 | 0.7500000 | 1.0000000 | 0.9166667 | 0.1666667 | 1.0000000 | |
| Ci-3 | 0.6666667 | 0.6666667 | 0.4166667 | 0.6666667 | 0.5833333 | 0.5000000 | 0.6666667 | 1.0000000 |

**Table 6: Matrix of RAPD similarity coefficients (primer Opn 10)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 1.8000000 | 1.0000000 | | | | | | |
| CME-2 | 0.7333333 | 0.9333333 | 1.0000000 | | | | | |
| CMN-3 | 0.7333333 | 0.9333333 | 0.8666667 | 1.0000000 | | | | |
| CMN-9 | 0.6666667 | 0.8666667 | 0.8000000 | 0.8000000 | 1.0000000 | | | |
| CMN-11 | 0.6666667 | 0.8666667 | 0.8000000 | 0.8000000 | 1.0000000 | 1.0000000 | | |
| CMB-4 | 0.7333333 | 0.9333333 | 0.8666667 | 0.8666667 | 0.9333333 | 0.9333333 | 1.0000000 | |
| Ci-3 | 0.8666667 | 0.9333333 | 0.8666667 | 0.8666667 | 0.8000000 | 0.8000000 | 0.8666667 | 1.0000000 |

**Table 7: Matrix of RAPD similarity coefficients (primer Opn 11)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 0.6470588 | 1.0000000 | | | | | | |
| CME-2 | 1.0000000 | 0.6470588 | 1.0000000 | | | | | |
| CMN-3 | 0.6470588 | 0.5294118 | 0.6470588 | 1.0000000 | | | | |
| CMN-9 | 1.0000000 | 0.6470588 | 1.0000000 | 0.6470588 | 1.0000000 | | | |
| CMN-11 | 0.8823529 | 0.5294118 | 0.8823529 | 0.5294110 | 0.8823529 | 1.0000000 | | |
| CMB-4 | 0.7058824 | 0.3529412 | 0.7258824 | 0.4705882 | 0.7058824 | 0.7058824 | 1.0000000 | |
| Ci-3 | 0.9411765 | 0.7058824 | 0.9411765 | 0.7058824 | 0.9411765 | 0.8235294 | 0.6470588 | 1.0000000 |

coefficient of CMU-2 with CMN-9 and CMN-11 was found to be the least (0.667).

Primer Opn 11 gave the highest number of polymorphic bands (15) while only 2 bands showed homomorphic pattern. The band size varied from minimum 40bp to maximum 280bp (Table 7). The similarity coefficient ranged from 0.353 to 1.000 with mean value of 0.647. 100% similarity was observed among CMU-2, CMN-9 and CME-2. The least similarity coefficient was obtained between CMU-5 and CMB-4. Ci-3 was found to be more closely related to CMU-2, CME-2 and CMN-9 (similarity coefficient 0.941). Ten bands (68 to 240bp) were obtained with primer Opn13 (Table 8). Six bands showed polymorphism. 100% similarity was obtained for CMN-3 with CMN-9, CMN-11 and CMB-4, CMN-9 with CMN-11 and CMB-4. Similarity coefficient of 1.000 was observed between CMU-2 and CMU-5 and also between CMN-11 and CMB-4. In this case, Ci-3 was found to be more close to CME-2 (similarity coefficient = 0.900) as in the case with primer Opn11.

Five homomorphic amplified bands were also obtained with primer Opn 14 with size 53, 72, 123, 141 and 186 bp respectively (Table 9). As this primer showed no polymorphism it was not found to be useful for the present study.

In case of primer Opn 16, 1 to 9 bands appeared in each genotype ranging from 81 to 278 bp, but only one band (278 bp) appeared in case of CMN-3 (Table 10). 100% similarity was obtained between CMU-2 and CMN-3, CMN-9 and CMB-4 and also between CMU-2 and CMN-11. 100% similarity has also been observed between CMU-2 and CMN-3 in case of primer Opn-08 and between CMN-11 and CMU-2 in case of Opn 10. CME-2 and CMN-9 were found to be 100% dissimilar. Mutant, CME-2 gave least value of coefficient of similarity (0.333) with parent strain.

In total 6 primers yielded 68 scorable bands ranging from 40 bp to 280bp for all the genotypes. The combined effect of all the primers on all the genotypes indicated that there was no 100% similarity between any 2 genotypes. Maximum similarity coefficient of value 0.897 was obtained between CMN-9 and CMB-4. Similarity coefficient of Ci-3 with mutants ranged from 0.706 for CMN-3 and CMN-11 to 0.794 for CMU-2. Minimum

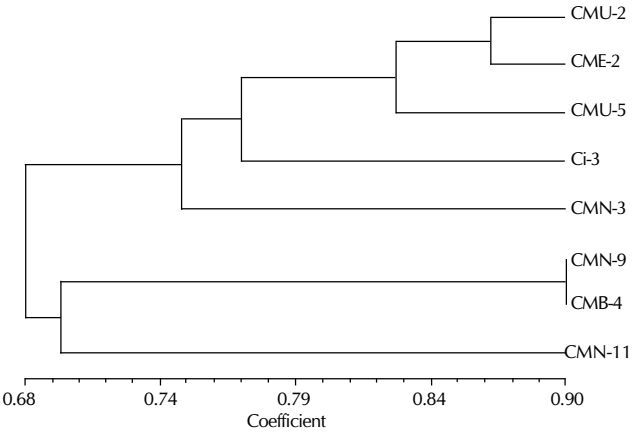


**Figure 1: Dendrogram (tree plot) of mutants of *C. indica***

**Table 8: Matrix of RAPD similarity coefficients (primer Opn 13)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 1.0000000 | 1.0000000 | | | | | | |
| CME-2 | 0.9000000 | 0.9000000 | 1.0000000 | | | | | |
| CMN-3 | 0.4000000 | 0.4000000 | 0.5000000 | 1.0000000 | | | | |
| CMN-9 | 0.4000000 | 0.4000000 | 0.5000000 | 1.0000000 | 1.0000000 | | | |
| CMN-11 | 0.4000000 | 0.4000000 | 0.5000000 | 1.0000000 | 1.0000000 | 1.0000000 | | |
| CMB-4 | 0.4000000 | 0.4000000 | 0.5000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | |
| Ci-3 | 0.8000000 | 0.8000000 | 0.9000000 | 0.6000000 | 0.6000000 | 0.6000000 | 0.6000000 | 1.0000000 |

**Table 9: Matrix of RAPD similarity coefficients (primer Opn 14)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 1.0000000 | 1.0000000 | | | | | | |
| CME-2 | 1.0000000 | 1.0000000 | 1.0000000 | | | | | |
| CMN-3 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | | | | |
| CMN-9 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | | | |
| CMN-11 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | | |
| CMB-4 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | |
| Ci-3 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 | 1.0000000 |

**Table 10: Matrix of RAPD similarity coefficients (primer Opn 13)**

| Rows/cols | CMU-2 | CMU-5 | CME-2 | CMN-3 | CMN-9 | CMN-11 | CMB-4 | Ci-3 |
|---|---|---|---|---|---|---|---|---|
| CMU-2 | 1.0000000 | | | | | | | |
| CMU-5 | 0.8888889 | 1.0000000 | | | | | | |
| CME-2 | 0.8888889 | 0.7777778 | 1.0000000 | | | | | |
| CMN-3 | 1.0000000 | 0.8888889 | 0.8888889 | 1.0000000 | | | | |
| CMN-9 | 0.1111111 | 0.2222222 | 0.0000000 | 0.1111111 | 1.0000000 | | | |
| CMN-11 | 0.8888889 | 1.0000000 | 0.7777778 | 0.8888889 | 0.2222222 | 1.0000000 | | |
| CMB-4 | 0.1111111 | 0.2222222 | 0.0000000 | 0.1111111 | 1.0000000 | 0.2222222 | 1.0000000 | |
| Ci-3 | 0.4444444 | 0.5555556 | 0.3333333 | 0.4444444 | 0.6666667 | 0.5555556 | 0.6666667 | 1.0000000 |

similarity coefficient (0.617) was found between CMU-5 and CMN-11 (Fig. 1).

Genetic variation among different strains can be documented by using different molecular markers (Ali, 2003; Castano and Becerril, 2004). The method of RAPD has been proved a valuable tool to distinguish different genotypes in edible mushrooms such as *Ganoderma lucidum* (Hseu *et al*., 1996), *Lentinula edodes* (Zhang and Molina, 1995), *Agaricus bisporus* (Khush *et al*., 1992) and *Calocybe indica* and to evaluate genetic similarities. Lee *et al* (2000) induced mutations in *Pleutotus ostreatus* and reported 10 times higher extra cellular enzyme activities of isolated mutant strains by RAPD-PCR and accounted 64.4 to 93.3% genetic similarities of mutants and wild strains.

Nineteen cultures of *Calocybe indica* were molecularly identified and characterized using RAPD profiles. The RAPD profiles of *C. Indica* group of accessions generated with six primers exhibited significant polymorphism in scorable banding patterns. All the six random decamer RAPD primers amplified the scorable DNA fragments in all the 19 accessions of *C. indica* and separated them into seven distinct phylogenetic sub-clades (Saranya *et al*., 2011). Intra-specific genetic variation in *C. indica* accessions was also obtained by Mahesh and Yadav (2006). According to experiment conducted by Kaur (2010) percentage similarity among various strains of *C. indica* ranged between 91.2 to 96.8 giving an overall combined similarity up to 93.1%.

Shafique *et al*. (2009) obtained hyper-active α-amylase producing mutants of *Alternaria tenuissima* FCBP-252 with UV irradiation and ethyl methanesulfonate (EMS). In order to have a comprehensive idea of variability amongst *A. Tenuissima* FCBP-252 and its mutant derivatives, RAPD analysis was carried out with 20 decamers. Percentage polymorphism in these genotypes ranged from 40-77.3%.

The random and genomic wide nature of the RAPD technique is better able to indicate over all genetic relatedness/ dissimilarity (Achenbach *et al*., 1996). The assay is rapid, independent of gene expression and is proving beneficial for relatedness of the mutants with the parent strain.

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