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Genetic diversity and physiological relatedness amongst 30 strains of *Lentinula edodes*

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A total of 35 strains of shiitake mushroom, *Lentinula edodes*, obtained from culture bank of ICAR-Directorate of Mushroom Research, Solan, were identified using ITS 5.8S rDNA sequences. Out of them, 30 were found to be true *L. edodes*. The accessions varied in their morphological characteristics and growth responses (radial growth and downward linear growth) at different temperature and substrates. The differences in growth responses were also used to assess genetic and physiological relatedness of the strains. The *L. edodes* strains showed high heterogeneity. The diversity studies amongst 30 strains were undertaken using ITS 5.8S rDNA sequences and short sequence repeat and inter-retroelement amplified polymorphism markers. A large genetic diversity amongst the test *L. edodes* strains was revealed by analysis of physiological requirements and genetics, which can be tapped for identifying the strains for different climates and substrates. Also, this huge genetic diversity can be used to breed better quality, stress tolerant and high yielding strains.

Keywords: Shiitake mushroom, genetic variability, ITS, SSR, IRAP

Lentinula edodes (Berk.) Pegler, commonly known variously as the black oak mushroom, shiitake or shiang-gu, is a gilled mushroom with polyporaceous affinities (Pegler 1983). It was first cultivated in China more than 800 years ago (Chang & Miles 1987, Zhang & Lai 1993) and today China accounts for about 70 % of the total world production. Shiitake is one of the five most cultivated edible mushrooms in the world (Chang & Miles 2004). These mushrooms grow in the wild in China, Japan, Korea, the Philippines, Papua New Guinea and in the north of Thailand (Campbell & Slee 1987, Mori et al. 1974). This species is mainly cultivated in East Asia (Japan and China) but is now attracting more interest around the world. It is valued for its unique flavour, which derives mainly from its content of the modified amino acid lenthionine and the nucleotide guanine-5-monophosphate (Mizuno 1995, Yang et al. 1998). The fruit bodies are rich in minerals, vitamins, and essential amino acids (especially lysine and leucine) and are high in fibre content but contain less than 10 % crude fat (Mizuno 1995). Apart from its importance as a mushroom crop, it has also been found that *L. edodes* contains medicinal compounds, including lentinan, which has antitumor activity, the hypocholesterolemic eritadenine, and cortinellin, an antibacterial agent (Przybylowicz & Donoghue 1988, Matsuoka et al. 1997). Thus, research on this fungus has increased due to its agricultural and pharmacological properties. As a result cultivation methods of this mushroom are improving rapidly (Sharma et al. 2016). Given the commercial importance of *L. edodes*, tremendous efforts have been devoted to research on various aspects of its biology, especially cultivation, fruiting and breeding (Hashioka et al. 1961, Nakai 1986, Tokimoto & Komatsu 1995, Ishibashi et al. 1996, Ikegaya 1997).

Strain improvement is a continuous exercise in mushroom farming to respond to consumer demand, expand the market, adapt to changing cultivation technology and counteract strain degeneration. Biodiversity in nature is a key element of the genetic resource for breeding programmes and, consequently, hunting for wild *L. edodes* has been practiced for a long time (Mori et al. 1974, Ellor 1992, Zhang & Lai 1993, Pan et al. 1998). Cultivated strains of *L. edodes* collected worldwide reveal genetic heterogeneity (Tokimoto et al. 1973, Mori et al. 1974, Kwan et al. 1992, Shimomura et al. 1992, Chiu et al. 1993, Fox et al. 1994). In contrast to the homogeneity observed in cultivated strains (Chiu et al.

1996), heterogeneity has also been observed in collections of wild isolates made in China (Chiu et al. 1996, 1998a; Hibbett et al. 1998).

The geographical distribution of shiitake in nature is reported to extend beyond Northeast Asia but the exact limits are still uncertain. As per the reports, it extends from northern Japan to Tasmania and New Zeeland in the east to Nepal, Bhutan and India (Koyabasi et al. 1973, Komatsu & Kimura 1968, Pegler 1983, Shimomura et al. 1992). Samgina (1981) reported the occurrence of shiitake from Kazakhstan on coniferous wood, however, shiitake is known generally to occur on logs of broad leafed trees (Tokimoto & Komatsu 1978, Przbylowicz & Donoghue 1988). Moreover, little information is available on the behavior of *L. edodes* strains cultivated in different countries.

This study was carried out on 30 strains of *L. edodes* deposited from different ecological regions of the world to characterize their growth responses to different substrate, pH and temperature conditions, and to analyze their genetic variability using Simple sequence repeats (SSRs), inter-retrotransposon amplified polymorphism (IRAPs) markers and internal transcribed spacer (ITS) 5.8S rDNA sequences. This investigation provides essential information for shiitake strain breeding aiming to better productivity and quality of mushroom.

Materials and methods

Germplasm

A total of 35 strains of *L. edodes* were selected for the study from different regions of the world. All the accessions were taken from culture collection of ICAR-Directorate of Mushroom Research, Solan. The accession numbers and their origin are given in Tab. 1.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from liquid nitrogen dried mycelium grown on malt extract broth medium (malt extract 10 g/l and dextrose 5 g/l) according to the modified method described by Punja & Sun (2001). The DNA concentration was estimated by electrophoresis in 1 % agarose gel containing 0.0 5% ethidium bromide. The DNA concentration was further estimated spectrophotometrically by taking optical density of the DNA solution at 260 and 280 nm. The final working concentration of DNA was standardized to 50 ng μl^{-1}.

The PCR primers ITS-1 and ITS-4 (White et al. 1990) were used to amplify the whole ITS 5.8S rDNA region of ribosomal DNA. Amplifications were done following Singh et al. (2004). The PCR products were visualized on 1.6 % agarose gel in tris-Acetate-EDTA (1× TAE) buffer at 60 V for 100 min. Agarose gels were stained with ethidium bromide and photographed under UV light for amplified ITS products.

The PCR-amplified ITS region was sequenced using the Big Dye Terminator V 3.1 Cycle Sequencing kit (ABI, Foster City, California, USA) and analyzed on an ABI Prism R 3700 DNA Analyzer (ABI).

Diversity analysis using SSRs and IRAPs

A total of 10 IRAPs (Kalendar & Schulman 2007) and 10 SSR (Foulongne-Oriol et al. 2009) primers pairs were used (Tab. 2) to assess diversity and characterization of the 10 test strains selected based on diversity revealed by ITS 5.8S rDNA sequences selecting one from each subclade. The reaction mixture for SSR amplification (17 ml) contained PCR buffer (10 ×) 1.5 ml, dNTPs (25 mM) 2 ml, each primer (forward and reverse) (100 pM) 1 µl, $MgCl_2$ (25 mM) 1.2 ml, glycerol (5 %) 1.2 ml, Taq polymerase (3 U) 0.3 ml and DNA template 50 ng. Reaction conditions maintained were 95 °C for 3 min (initial denaturation), 35 cycles of 94 °C for 60 sec (denaturation), 55–58 °C for 60 sec (annealing), 72 °C for 1 min 20 sec (extension) and final extension at 72 °C for 5 min. While for IRAP amplifications, the reaction mixture (17 ml) contained PCR buffer (10×) 1.5 ml, dNTP (25 mM) 2 ml, each primer (100 pM) 1 ml, $MgCl_2$ (25 mM) 1.2 ml, Glycerol (5 %) 1.2 ml, Taq polymerase (3U) 0.3 ml and DNA template 50 ng. Reaction conditions maintained were the same as for SSR amplifications except for annealing temperature (40–56 °C for 30 sec).

The amplified products were separated with precision on 2.5 % agarose gel containing ethidium bromide. The gels were run at a low voltage of 40 V for 3–5 h for proper separation. The profiles generated were scored for presence and absence of reproducible bands.

Radial mycelial growth rate of 30 shiitake strains on different temperature

All the 30 strains of genus *L. edodes* were grown on three different temperatures (15, 20 and 25 °C) to optimize temperature requirement of the strains; 2 % Malt agar medium was used and the radial growth was measured up to 10 days. The experiment was laid in completely randomized design (CRD) with five replications for each strain.

Tab. 1. List of *Lentinus edodes* strains deposited with ICAR-Directorate of Mushroom Research, Solan (India)

ICAR-DMRO accession no.	Origin	ICAR-DMRO Accession no.	Origin
002	Bhutan	095	USA
007	Philippines	101	Pantnagar (India)
008	Philippines	119	Malaysia
012	USA	276	USA
016	Philippines	297	Japan
018	Philippines	327	Manipur (India)
019	Philippines	328	Manipur (India)
020	Nepal	329	Raipur (India)
022	Nepal	330	Raipur (India)
023	Nepal	331	Raipur (India)
025	Manipur (India)	356	Belgium
026	Udaipur (India)	410	China
030	Manipur (India)	411	China
034	Switzerland	412	Udaipur (India)
035	Japan	430	Manipur (India)
037	Pune (India)	455	Pasighat (India)
040	Coimbatore (India)	623	Kerala (India)
051	Suwon (Korea)		

Radial growth rate of mycelia on substrate extracts

Radial growth of 30 strains of *L. edodes* was studied by using extracts derived from the substrates such as wheat straw, paddy straw, coir pith, sugarcane bagasse and sawdust to establish the strain-substrate compatibility. The extract was prepared by adding 50 g of substrate per liter of water and boiled rigorously. The culture media were prepared by the addition of 15 g of agar powder per liter of the extract. The culture media were sterilized at 121 °C for 20 min at 15 psi. 20 ml of the culture medium was poured in Petri dishes (90 mm diam.). Different strains were inoculated with mycelium disks (5 mm diam.) pre-cultivated on malt extract agar (malt extract 20 g/l, peptone 5 g/l and agar 20 g/l) media. Three replications were prepared for each culture medium and for each strain. Samples were incubated in darkness at 25 °C. Mycelial growth was estimated by measuring the diameter of the mycelia along two perpendicular axes after 10 days of incubation.

Downward linear growth rate on substrate

5 g of grain spawn of different strains were inoculated in the glass tubes containing 200 g of hydrated substrates of wheat straw, paddy straw, coir pith, sugarcane bagasse and sawdust (without N supplementation) till the equal height of 10 cm length and later incubated at 25 °C. The downward

Tab. 2. List of SSRs and IRAPs primers along with their sequence.

SSR primer	Sequence
AbSSR02-F	CGGATCTGTTGAGAAAGACG
AbSSR02-R	CGACAACGACACCAACAATA
AbSSR05-F	ATCAACGACTTGATTGCTGAAG
AbSSR05-R	TGCGCCCACTACTAACTTACAA
AbSSR09-F	ACAAGAAGGGGAGGATTGAG
AbSSR09-R	ATAGTCGCGTAACCCCTCTT
AbSSR14-F	CGTCAAGGAGAACAAGGAGA
AbSSR14-R	CCCGTCCAAATCACATTAAC
AbSSR19-F	TTTAGTTCTGGACAGACCGTTT
AbSSR19-R	CAAACGTATATCAACGACAGGA
AbSSR23-F	TTTGGGATGTGACCAGACTT
AbSSR23-R	AACGTTGGGTTCAATGAAAA
AbSSR33-F	TCGGTAGAAGTTGTGGAAGC
AbSSR33-R	GGCGTTGGACTAGAAGTCAT
AbSSR45-F	CACCTTACACGGCCATTGAT
AbSSR45-R	AAAACTTCGGGCATTTCCTT
AbSSR58-F	ATGTCGAGGAGGAGGAGGAT
AbSSR58-R	AGGGAGAGGGAGAGGGATTT
AbSSR65-F	ACCTCAACGATTCCAACGAC
AbSSR65-R	TCCATAAACACCCCTTCTCG
IRAP primer	Sequence
LTR1L	AAAGTACGCAGCCCCTATCA
LTR1R	GAAGTGGCGGAACCAATAGA
LTR2L	TATAGCCAACCCTCCACAGC
LTR2R	AACTGGACAAGGCAACAAGG
MarY1L	GTGCCCTGTGTGTTTTTCCT
MarY1R	AAGGGGTACTCCGCCTCTAA
RTE-L	CACCGTTGCCTTTTCTGTCT
RTE-R	AGTAAACGAGGGTGGTCACG

linear growth rate of mycelia was measured at every seven days interval.

Bioinformatic analysis

The sequence data obtained from ITS-4 reverse primer were reversed and complemented using Gene doc software and clubbed with sequence data of ITS-1 to obtain complete sequences of amplified ITS product, and nucleotide sequences were compared with database of the National Centre for Biotechnology Information (NCBI) by using BLAST search. Species were identified using lowest e value and maximum identity. The sequences were subjected for further analysis using Mega 6.0 software (Tamura et al. 2013) to maximum likelihood analysis using 1000 bootstrap comparisons. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura & Nei (1993) model. Pairwise distances were estimated using the Maximum Composite Likelihood (MCL) approach. The sequence data was also subjected to Baysian analysis to calculate the posterior predictive distribution to do predictive inference, i.e., to predict the distribution of a new, unobserved data point using Markov Chain Monte Carlo (MCMC) methods (Ronquist & Huelsenbeck 2003). The posterior probability was calculated by back breeding for 100000 generations using MCMC methods. The sample frequency was kept at 10 and the split frequency limit was set to 0.01.

The morphometric data were taken in triplicate and subjected to one-way ANOVA and critical differences were calculated. The data was also subjected to phylogenetic analysis of the isolates used in the study. The data matrix was prepared as quantitative dataset using Winclada version 1.00.08 (copyright K. Nixon 1999–2002). Further maximum parsimony analysis was done using TNT programme for phylogenetic analysis (Goloboff et al. 2008).

The data obtained by scoring the presence (1) or absence (0) of amplified fragments from the SSR and IRAP profiles were collectively subjected to the construction of a similarity matrix using Dice's (1945) and Jaccard's (1901) coefficients of similarity. Among the various similarity indices, those of Jaccard and Dice were chosen as the most appropriate ones for dominant markers, like ISSR and RAPD, since they do not attribute any genetic meaning to the coincidence of band absence. The similarity values were then used for a cluster analysis. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was performed using the unweighted pair group method with arithmetic averages (UPGMA), and the results were summarized as dendrograms. The data analysis was conducted using NTSYSpc software 2.02 (Nei & Li 1979, Kim et al. 1993). To validate the data analyzed using NTSySS, bootstrap analysis was performed using Winboot software keeping 1000 bootstrap comparisons.

Cultivation of shiitake accessions

The shiitake accessions obtained were cultivated on synthetic logs (Royse 1997), where sawdust was the main ingredient supplemented with cereal bran (20 %), calcium carbonate (0.5 %) and calcium sulfate (2.0 %) keeping the moisture content to a level of 60 %. Substrate filled polypropylene bags (2.5 kg) were sterilized and seeded with spawn. The total production cycle on synthetic logs was 3–4 months.

Results

Out of 35 DMRO accessions, five, namely, 02, 30, 101, 276 and 455 turned out to be different than *L. edodes* on the basis of ITS sequences while the rest accessions were found to be true *L. edodes* (Tab. 3). In the BLAST analysis, it could also be observed that some of the sequences showed low similarity percent between 88–93 %, however, the e-value was found to be 0.0. This suggested the wide variation in *L. edodes* accessions collected/deposited from various parts of the world.

To confirm the identity of the test strains, all the 30 accessions proven to be true *L. edodes* in the sequence analysis were cultivated on sawdust-based substrate. Out of 30 accessions tested, only 24 accessions of *L. edodes* produced fruiting body. The DMRO-accessions were 007, 018, 019, 022, 023, 025, 028, 034, 035, 040, 051, 297, 327, 328, 329, 330, 331, 356, 356, 410, 411, 412, 430, and 623. The isolates, which did not produce fruit bodies also showed high similarities with *L. edodes,* maybe the strains required a different set of environmental parameters.

The sequences identified as *L. edodes* were subjected to phylogenetic analysis using Maximum parsimony analysis in Mega 6.0 software keep ing bootstrap value as 1000 (Fig. 1A). The phylogram showed a wide genetic variation amongst the analyzed accessions. The analyzed sequences were further subjected to Baysian statistics using Mr. Bayes software for calculation of posterior probability and confirmation of phylogenetic analysis done by Mega 6.0 software (Fig. 1B). When the two phylograms were compared, it could be observed from the tree generated by Mega that DMRO-40 and 329

Tab. 3. Identification of the query sequence in NCBI BLAST search

Strain	Identification	NCBI Accession No.	Strain	Identification	NCBI Accession No.
002	*Flavodon flavus*	MH211817.1	095	*Lentinula edodes*	MK603497
007	*Lentinula edodes*	MK603481	101	*Lentinus sajor-caju*	KT818506.1
008	*Lentinula edodes*	MK603482	119	*Lentinula edodes*	MK603498
012	*Lentinula edodes*	MK603483	276	*Basidiomycota* sp	GQ249873.1
016	*Lentinula edodes*	MK603484	297	*Lentinula edodes*	MK603499
018	*Lentinula edodes*	MK603485	327	*Lentinula edodes*	MK603500
019	*Lentinula edodes*	MK603486	328	*Lentinula edodes*	MK603501
020	*Lentinula edodes*	MK603487	329	*Lentinula edodes*	MK603502
022	Lentinula edodes	MK603488	330	*Lentinula edodes*	MK603503
023	*Lentinula edodes*	MK603489	331	*Lentinula edodes*	MK603504
025	*Lentinula edodes*	MK603490	356	*Lentinula edodes*	MK603505
026	*Lentinula edodes*	MK603491	410	*Lentinula edodes*	MK603506
030	*Trametes hirsuta*	KX028783.1	411	*Lentinula edodes*	MK603507
034	*Lentinula edodes*	MK603492	412	*Lentinula edodes*	MK603508
035	*Lentinula edodes*	MK603493	430	*Lentinula edodes*	MK603509
037	*Lentinula edodes*	MK603494	455	*Panus* sp.	KP686453.1
040	*Lentinula edodes*	MK603495	623	*Lentinula edodes*	MK603510
051	*Lentinula edodes*	MK603496			

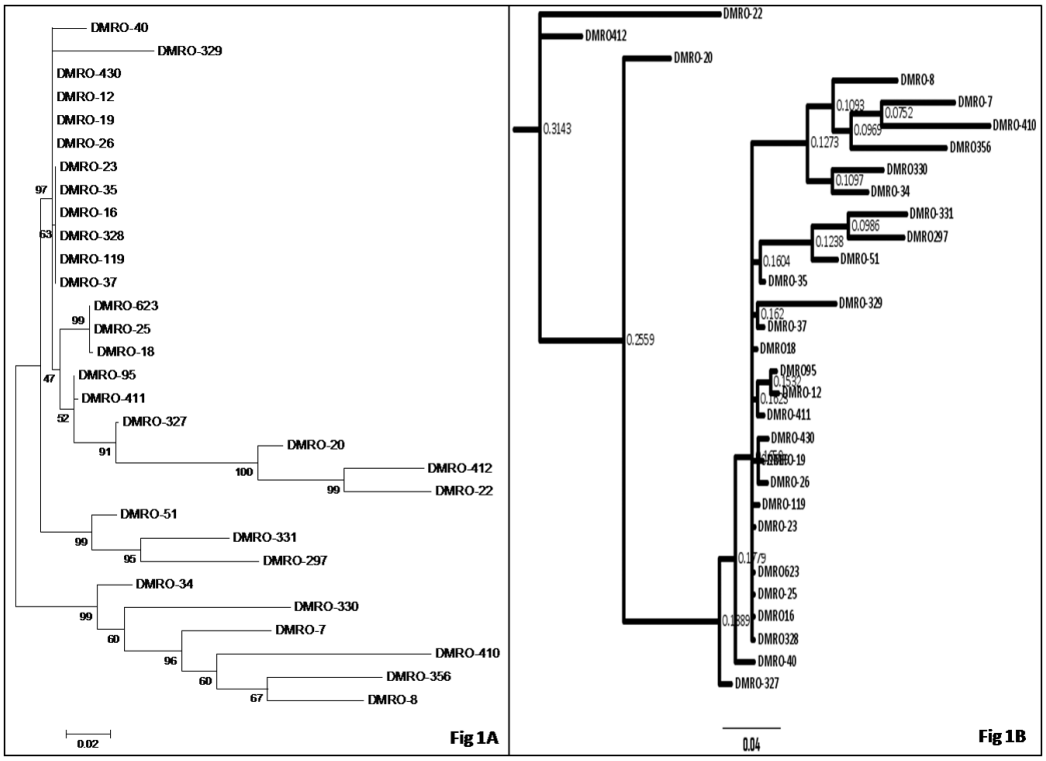

Fig. 1. A. Phylogram of the genetic diversity amongst 30 accessions of *L. edodes* based on the ITS 5.8S rDNA sequences. Evolutionary history inferred using the Maximum Parsimony method in Mega 6.0. **B.** Phylogram generated by Mr. Bayes The phylogram validated maximum parsimony analysis by Mega 6.

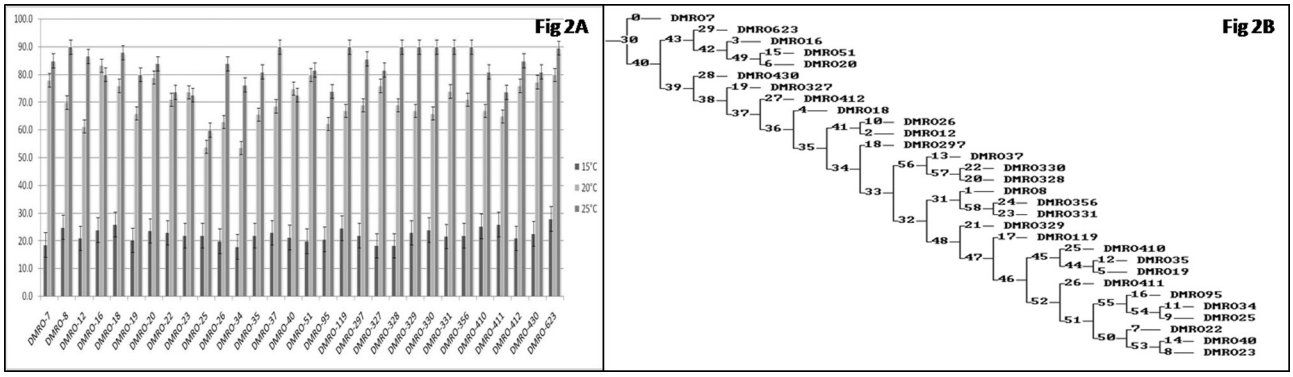

Fig. 2. A. Radial growth of 30 strains of *L. edodes* under three different temperatures (15, 20 and 25 °C) showing their optimum growth on 25 °C except six strains showing statistically equal growth at 20 and 25 °C. **B.** Phylogenetic tree drawn using radial growth data on different temperature (15, 20 and 25 °C). Data matrix prepared as quantitative dataset using Winclada version 1.00.08 (copyright K. Nixon 1999–2002). Maximum parsimony analysis performed with TNT programme for phylogenetic analysis (Goloboff et al. 2008) using 1000 bootstrap comparisons.

formed the outgroup, whereas 20, 412 and 22 showed the maximum genetic difference from all the other strains. In the posterior probability calculation by Baysian statistics, the results were supported. The accessions DMRO-20, 412 and 22 have made an outgroup and showed maximum similarity whereas 40 and 329 were clubbed together. For the Baysian analysis a total of 288011 trees was sampled where 281861 trees showed 99 % credibility.

All the 30 *L. edodes* accessions were initially evaluated for their temperature requirement at 15, 20 and 25 °C for mycelial growth on malt agar plates. The results obtained indicated that almost all the cultures of *L. edodes* showed maximum

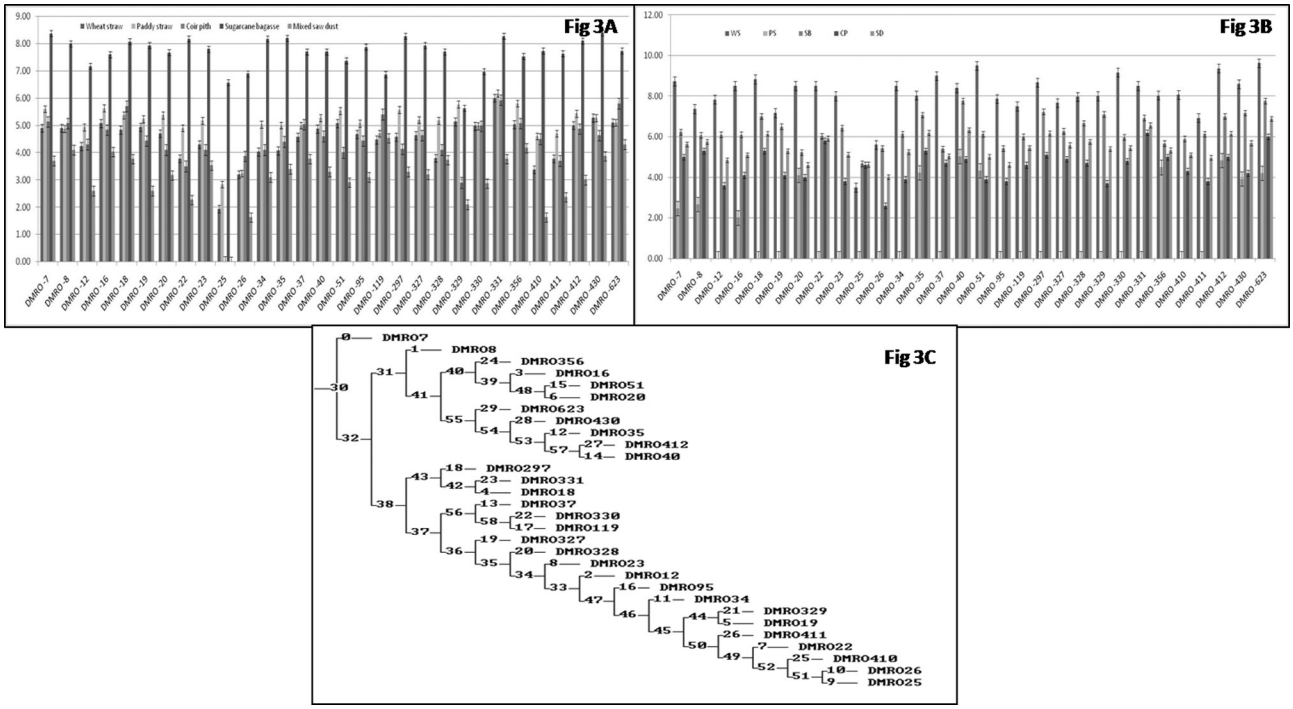

Fig. 3. A. Radial growth of 30 strains of *Lentinula edodes* on extract of different substrate at 25 °C temperature showing maximum growth on sugarcane bagasse extract. **B.** Downward linear growth of 30 strains of *L. edodes* on different substrate at 25 °C temperatures showing optimum growth on wheat straw substrate. **C.** Phylogenetic tree drawn using radial growth data on different media supplemented with extracts of different substrates and downward linear growth data on the same substrates. Data matrix prepared as quantitative dataset using Winclada version 1.00.08 (copyright K. Nixon 1999–2002). Maximum parsimony analysis performed with TNT programme for phylogenetic analysis (Goloboff et al. 2008) using 1000 bootstrap comparisons.

growth invariably at 25 °C (Fig. 2A). However, some of the accessions, namely DMRO-16, 22, 23, 40, 51, and 430 showed wide adaptation for temperature and grew equally at temperatures 20 and 25 °C. This gave us hints that these accessions can be grown at a temperature between 20 to 25 °C. It was interesting to note that the accession showing diverse temperature requirements were submitted from tropical / subtropical climates of Philippines, Nepal, Korea and India.

The radial growth data on different temperatures was also used for the phylogenetic analysis using maximum parsimony analysis of the strains using TNT software (Goloboff et al. 2008). The phylogenetic analysis showed high diversity with respect to growth response to different temperature with accession DMRO-7 forming the outgroup (Fig. 2B). All the accessions showed a wide diversity of temperature requirements. The accession forming the outgroup, DMRO-07, was collected from the Philippines, where the temperature ranges between 25–28 °C. The accessions showing statistically similar growth at 20 and 25 °C temperature are placed in two separate clades A and C. This analysis showed that even the optimum temperature requirements of the accessions were the same but growth responses of these accessions at different temperatures varied. Also these accessions showed diversity within the clade.

To standardize the most favored substrate by these strains, all the strains were evaluated for their radial mycelial growth on different substrates, including wheat straw, paddy straw, coir pith, sugarcane bagasse and mixed saw dust. It was observed that all the strains showed their maximum radial mycelial growth on sugarcane bagasse extract. Although, almost all the strains showed good radial mycelia growth on all the substrates except DMRO-25, which failed to grow on coir pith and mixed saw dust (Fig. 3A). Furthermore, the test strains were evaluated for downward linear growth on the known quantity of substrates filled in the thick test tubes and inoculated with known quantity of spawn. The linear growth of the mycelium was

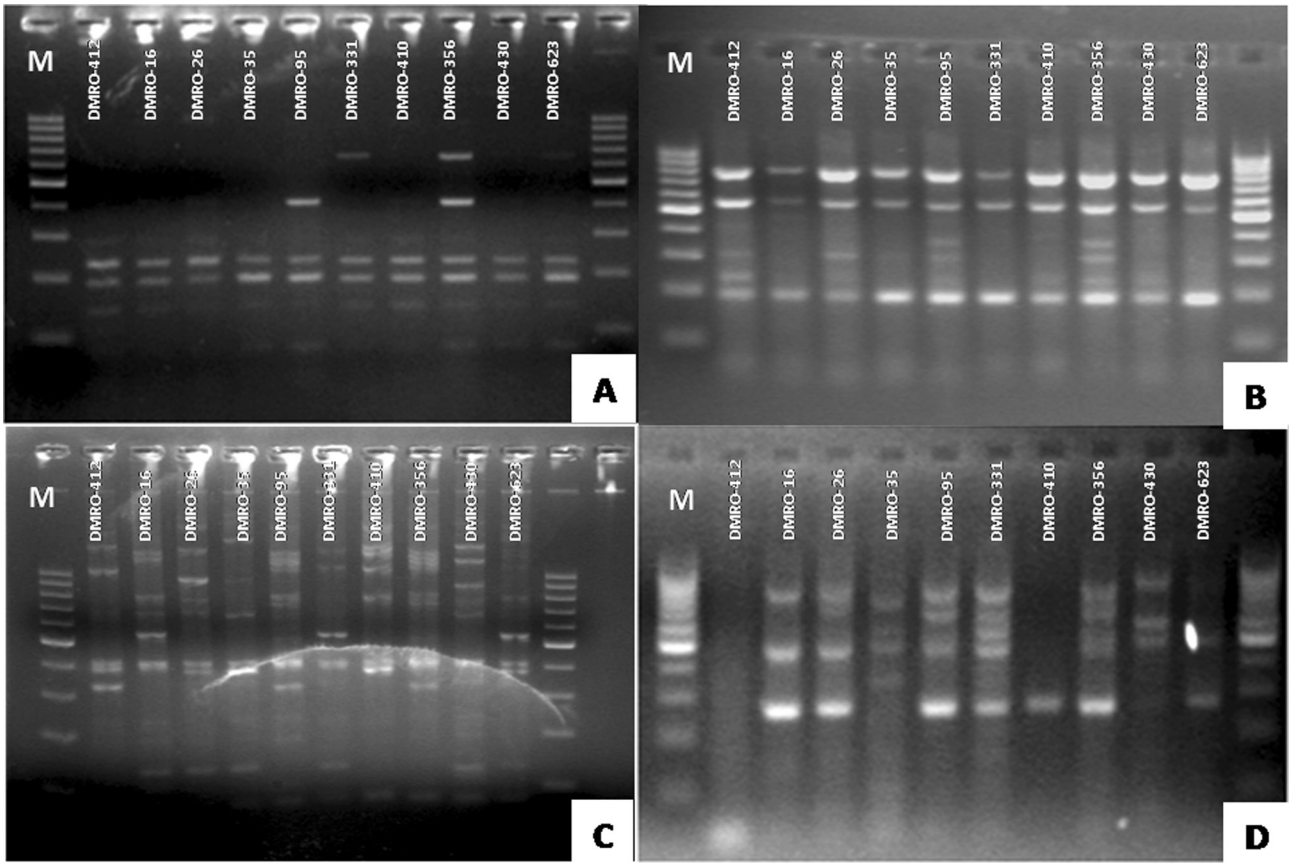

Fig. 4. DNA profile generated using SSR primers ABSSR-19 (A); ABSSR-02 (B) and IRAP primers LTR2L+LTR1R (C); LTR2L+RTER (D)

measured periodically (Fig. 3B). The results showed that all the strains grew better on wheat straw substrate, while Paddy straw did not support the downward linear growth of many of the test strains. Overall, good growth was recorded on sugarcane bagasse, coir pith and saw dust substrates by all the strains.

The data obtained from radial mycelial growth on substrate extract and downward linear growth was subjected to phylogenetic analysis using maximum parsimony analysis using TNT software (Goloboff et al. 2008). The results obtained showed two major clades and the two accessions DMRO-2 and 119 making outgroups (Fig. 3C). The results indicated that the accessions can be used to grow on different substrates according to their preferences and not only on saw-dust-based substrate. This will ease the undue pressure on saw dust and ultimately on trees. This phylogenetic analysis based on morphometric data was also supported by variation in the ITS sequences of the accessions.

A total of 10 combinations of IRAPs and 10 sets of SSR primers were used to assess diversity and characterization of the 10 test strains selected based on diversity revealed by ITS 5.8S rDNA sequences selecting one from each group (Fig. 4). The analysis resulted in high genetic diversity of 48 % using the two marker system. The dendrogram generated by NTSyS was bootstrapped 1000 times to validate the analysis and a very high bootstrap value of 96 % was observed and validated the analysis results (Fig. 5). The diversity analysis showed that the collection of *L. edodes* strains at ICAR-Directorate of Mushroom Research, Solan (India) possess a wide gene pool for breeding purpose and each accession can be effectively used for breeding new *L. edodes* strains after proper characterization for each important trait.

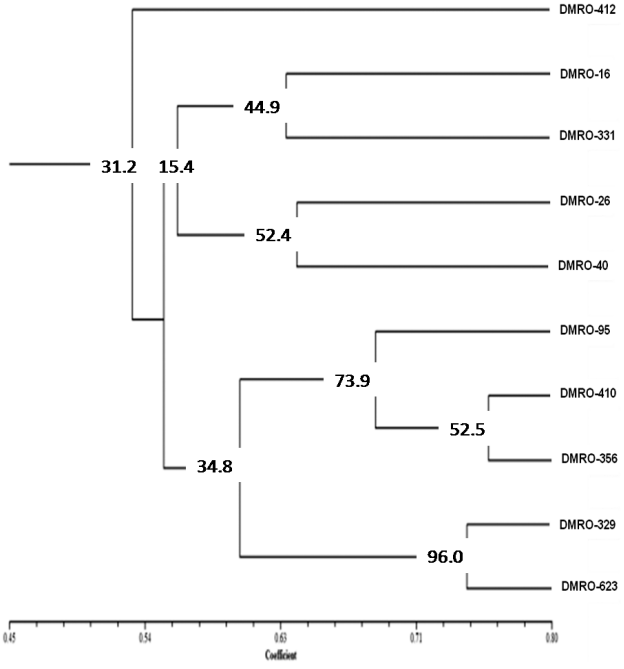

Fig. 5. Dendrogram generated by NTSyS ver 2.02 using 10 SSR and 10 IRAP profiles of 10 strains of *Lentinula edodes* showing a maximum genetic diversity of 48 % amongst the ten isolates with strong bootstrapping values generated by winboot software.

Discussion

Strain improvement is a continuous process in mushroom cultivation to achieve higher yields, better quality, satisfy consumer preferences, expand the market, adapt to changing cultivation technology and counteract strain degeneration. Natural biodiversity is a key element of the genetic resource for any breeding programme. As a result, hunting for wild *L. edodes* has been practiced for a long time (Mori et al. 1974, Ellor 1992, Zhang & Lai 1993, Pan et al. 1998). Cultivated strains of *L. edodes* collected worldwide reveal genetic diversity (Tokimoto et al. 1973, Mori et al. 1974, Kwan et al. 1992, Shimomura et al. 1992, Chiu et al. 1993, Fox et al. 1994) in response to ecological conditions and temperature profile of the area. Low genetic diversity was reported in cultivated strains in China by Chiu et al. (1996), while heterogeneity has also been reported in collections of wild isolates in China (Chiu et al. 1996, 1998; Hibbett et al. 1998). During the present study, we have tried to assess the genetic diversity present in *Lentinula edodes* strains available at ICAR-Directorate of Mushroom Research culture collection, which has been deposited by various workers from throughout the world. The purpose of assessing the genetic diversity amongst the strains of *L. edodes* is to assess the gene pool available and to use them in different breeding programme so as to develop a high yielding, better quality and stress tolerant variety of shiitake mushroom.

Due to economic importance of shiitake, strain typing and natural biodiversity assessment have become essential and for this various methods have been assessed. Some of the methods used were isozymes (Ohmasa & Furukawa 1986, Royse & May 1987) and DNA markers such as restriction fragment length polymorphisms (RFLPs) (Loftus et al. 1988, Kulkarni 1991, Fukuda et al. 1994), polymerase chain reaction (PCR) based techniques (RafaIski & Tingey 1993), arbitrarily-primed polymerase

chain reactions (AP-PCR) (Kwan et al. 1992, Chiu et al. 1993), and random amplified polymorphic DNA markers (Khush et al. 1992, Zhang & Molina 1995). In the present study, we have used nucleotide sequences of ITS 5.8S rDNA region to confirm the identity of the accession and genetic diversity. Also microsatellite and inter retroelement amplified polymorphism markers were used to assess the diversity in 30 *Lentinula edodes* strains.

Little information is available on the behavior of *L. edodes* strains cultivated in tropical countries. This study was carried out on 30 strains of *L. edodes* to characterize their developmental responses to temperature, radial and downward linear growth on different substrates. A similar study was also conducted in Brazil, where 34 strains of *L. edodes* were screened for essential physiological requirement of genetic diversity assessment based on RAPD markers (Maki et al. 2001). However, the data on physiological requirements of 34 strains were not used for biodiversity assessment. During the present study, we have not only used radial growth, temperature and also downward linear growth rate for generating essential information about the strains, but also the data generated was used for phylogenetic analysis and genetic diversity assessment. Retroelements-based markers particularly, use of outward facing primers to amplify around pairs of insertion sites, the IRAP technique, first developed by Schulman et al. (2004), has been found useful to provide multi-locus anonymous markers. The markers are now used in several studies to observe biodiversity and phylogenetic relations of the species and to investigate the retrotransposons.

Downward linear growth on compost has been reported as an important selection criterion for high yielding single spore isolates in *Agaricus bisporus* (Singh & Kamal 2011). They reported that downward linear growth has a dominant direct effect on yield with a high correlation coefficient of 0.618. During the present study, we have also used the downward linear growth of *L. edodes* strains on different substrate as selection criteria of high yielding strain on particular substrate.

In conclusion, we showed a high degree of genetic diversity in 30 strains of *L. edodes*. The accessions also varied in their temperature and substrate requirements. This study will be highly helpful in selecting a strain with high yield potential on particular substrate and for different agro-climatic regions with different temperatures. Moreover, for breeding better yielding and stress tolerant strains, the available genetic diversity can very well be used.

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