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Original Article

Assessment of genome stability of *pfleFB* hybrid generations through molecular DNA markers

Suranjana Sarkar, Neelanjan Bag and Pijush Mallick*

Department of Microbiology, School of Life Sciences, Swami Vivekananda University Barrackpore, Kolkata, West Bengal – 700121, India

**Correspondence E-mail :biology.pijush@gmail.com*

Abstract

Introduction: Using PEG-mediated protoplast fusion, a total of nine *pfle* somatic hybrids were developed between *Pleurotus florida* and *Lentinula edodes*.Only six could developed fruit bodies and the second generation was made of each through tissue culture isolate from their fruit bodies. The first generation were denoted as *pfle1p, pfle1q, pfle1v, pfle 1s, pfle1o* and the second generation were as *pfle1pFB, pfle1qFB, pfle1vFB, pfle 1sFB, pfle1oFB*. The genetic closeness, stability and variance is analyzed compared to their first parental strains using nine RAPD molecular markers. Amplified PCR bands were scored analyzed using SPSS software version 19. Polymorphisms were found calculating a total of 104 bands is 94.23% where the highest polymorphism was observed in RAPD-02, RAPD- 03, RAPD-08 and SS-11. Maximum bands (16) were generated by SRS-06 and minimum in SRS-05, ranged from 330 bp – 3000 bp and 450 bp – 2000 bp, respectively. Jaccards proximity matrix was generated from the scored data followed by dendogram including two generations with parents, using single linkage. The proximity matrix was ranged from 8.00-60.00. Two parental strains *P. florida* and *L. edodes* found to be most distantly related among all, where *pfle 1pFB*, *pfle 1rFB* were very close in genetic distance and another two hybrid lines, *pfle 1vFB*, *pfle 1qFB* were also showed the same. It was observed that the nearest neighbor showed a little variance genetically, but the hybrid lines are mostly similar with their second generation.

Keywords: *Mushroom hybrids, genetic polymorphism, RAPD, dendrogram.*

Introduction

Edible mushrooms known as the meat of the vegetable world and are used extensively in cooking, in many cuisines. Most of the edible mushrooms that are sold in supermarkets have been commercially grown in mushroom farms at large scale commercial purposes. Mushrooms are highly nutritious and environment friendly crops that carry numerous medicinal benefits. *Pleurotus florida*, one of the most commonly growing oyster mushroom, is widely appreciated for its unique flavour, texture, colour, which is highly perishable with moderate shelf life under ambient conditions and poor substrate specificity. In terms of nutritional value, this mushroom has high protein, fibre, vitamin, mineral content, carbohydrate, fibre and essential fatty acids (Crisan and Sands 1978). The most important aspect of this mushroom has been related to the use of its ligninolytic system for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for animal feed and other food products, for the biodegradation of organo-pollutants, xenobiotics and industrial contaminants. Wolter *et al.,* (1997) proposed that *P. florida* is suitable for bioremediation of

contaminated soils because of its ability to degrade highly condensed polycyclic aromatic hydrocarbons (PAH) and its high tolerance of these substrates. Moreover, they are also promising as medicinal mushrooms (Cohen *et al.*, 2004), exhibiting haematological, antiviral, anti-tumour, antibiotic, antibacterial, hypocholesterolic and immuno-modulation activities. *Lentinula edodes* (Shiitake) is an edible mushroom native to East Asia, which is cultivated and consumed in many Asian countries, as well as being dried and exported to many countries around the world.

Due to substrate specificity, the crop yield is somehow limited. To overcome this constrains, breeding had been a prerequisite option where target can be fulfilled to the entrepreneur. Analysis of second generation hybrid genotype of edible mushroom has been reported where genome stability was found unsatisfied and found gene environmental interaction hypothesis (Mallick *et al.,* 2016). A few works have been published on first developed somatic hybrid lines of various mushroom strains. Intergeneric protoplast fusion was done between edible mushroom strains *Volvariella volvacea* and *Pleurotus florida* through polyethylene glycol (Sikdar *et al.,* 2008). Where hybridity was established on the basis of colony morphology, mycelial growth rate, hyphal traits, fruit body morphology, isozyme and RAPD, ISSR, RFLP of rRNA-ITS gene (Mallick & Sikdar 2014, 2016).

However, in this study we worked on the genome stability in pfleFB lines using simple RAPD markers to detect the genetic polymorphism and genetic divergence during fruit body development amongst each other compared to their parental strains.

Materials and method

Parental and hybrid strains:

For these study two parental strains viz; *Pleurotus florida* and *Lentinula edodes* were used. Six hybrid lines with their second generation namely, *pfle1v, pfle1vFB, pfle1q, pfle1qFB, pfle1p, pfle1pFB*, *pfle1r, pfle1rFB, pfle 1s, pfle1sFB, pfle1o* and *pfle1oFB* were used.

Genomic DNA extraction and PCR

Routine maintenance of the strain was carried out in PDA (Potato Dextrose Agar, pH 6.2) medium at 24±1°C. For DNA isolation culture was grown in liquid MYG (10 g/l malt extract, 4 g/l yeast extract and 10 g/l glucose, pH 6.2) medium for two weeks at 24±1°C. Genomic DNA from young fruit bodies or fleshy grown mycelia in liquid MYG media of the parental strains and their hybrid mushroom lines were isolated using the modified CTAB (N-cetyl-NNN-trimethyl ammonium bromide) method (Dellaporta *et al.,* 1983). Frozen materials from -20ºC for short periods or at -80ºCfor longer storage periods should generally be used for extraction of DNA.

DNA purification and quantification

Isolated DNA was treated with RNAse followed by its phenol extraction and ethanol precipitation using sodium acetate and alcohol. The DNA was finally pelleted after precipitation, washed with ethanol to remove the salts and other organic contaminants, dried completely and re-suspended in TE buffer. Quantification was done using Nano-Drop Spectrophotometer (ND- 1000) measuring the UV absorption at 230, 260 and 280 nm wavelength.

PCR and gel run

A total of nine primers were taken in consideration for the present study (table 1). The amplifications were performed as per the protocol of Williams *et al.,* 1990 with little modifications. The total volume of 25 µl reaction mixture, containing 10 ng template DNA, 20 µM of primer, 10X Taq buffer (with KCl), 25 mM $MgCl_2$, 2 mM dNTPs mixture and 5U/l of Taq DNA polymerase. The negative control (without template DNA) was also made in this reaction. The PCR was conducted in a DNA thermal cycler (Applied Bio-systems 2027) by preliminary denaturation of DNA at 94°C for 5 minutes consisting of 30 cycles; DNA template denaturation at 92°C for 60 seconds, primer annealing at 37.5°C for 45

seconds, initial extension at 72°C for 2 min 30 seconds, followed by a final extension at 72°C for 8 min.

Amplified DNA fragments were separated on a 1.8% (w/v) agarose gel pre-stained with Ethidium Bromide solution (0.5 µg / ml) using 1X TAE Buffer (7 V/cm). The gels were run approximately for 2 hours on a horizontal Gel Electrophoresis system (BIORAD) and the amplicons were visualized under a UV Transilluminator (GeNei). The fingerprint profiles were recorded with Image Quant 300(GE HEALTHCARE). The size of the amplified fragments was determined by using Gene Ruler 100 base pair PLUS (MBI FERMENTAS) as a standard molecular weight marker.

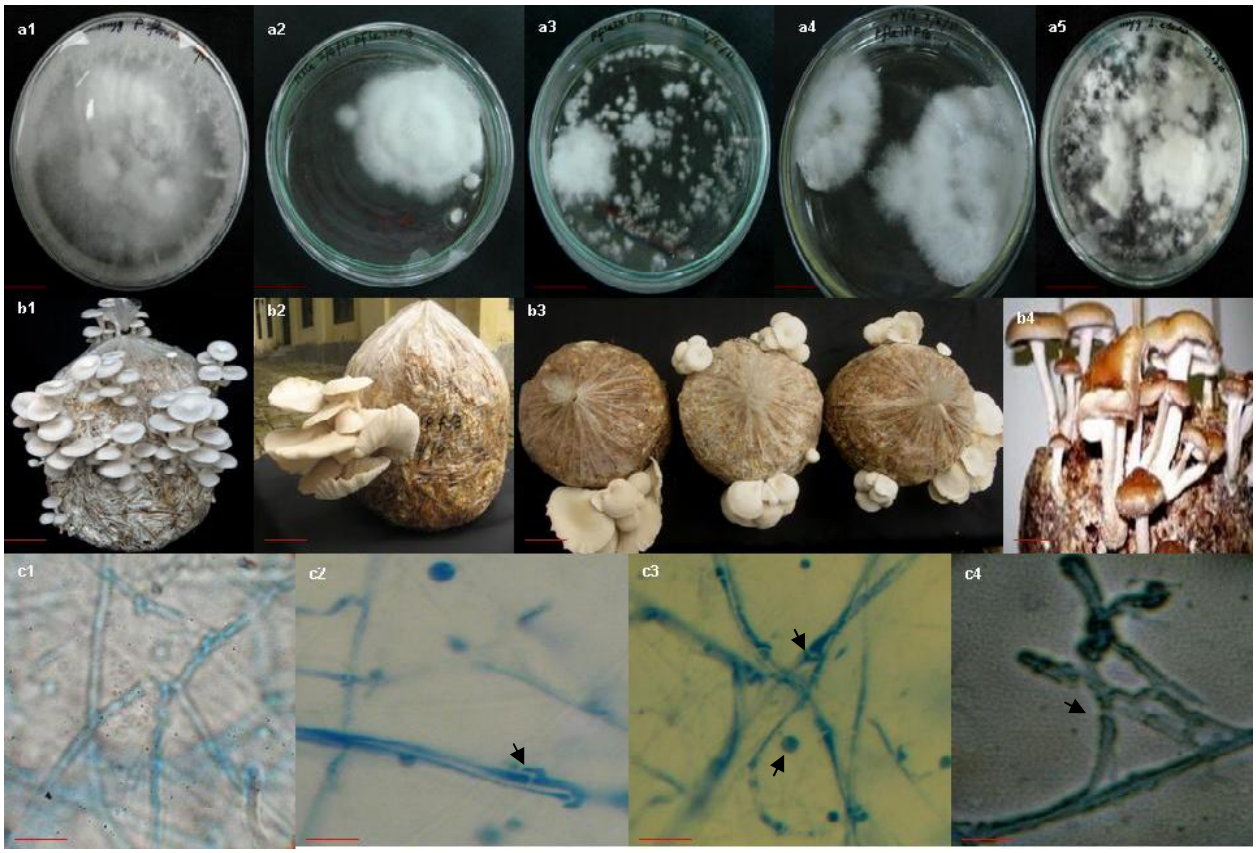

Fig. 1: a1-a5; MYG culture of *P. florida, pfle1vFB, 1rFB, 1pFB* and *L. edodes* respectively., b1-b4; developed basidiocarp of *P. florida, pfle1pFB, 1vFB, 1qFB, 1sFB* and *L. edodes* respectively, c1-c4; Hyphal nature of *P. florida, pfle1pFB, 1qFB* and *L. edodes* respectively. Arrows showing the discrete or friable mycelial cell nature. Bar size; 1cm (a1-a3), 1.2 cm (a4), 0.9 cm (a5), 0.8 cm (b1 & b2), 1.5 cm (b3), 0.7 cm (b4), 10µm (c1-c4).

Scoring and statistical analysis

The DNA profiles generated from PCR were analyzed critically and only the discrete amplicons were scored as either present (1) or absent (0) for each primer cultivar combination. The bivariate (0-1) data were analyzed using the software IBM SPSS STATISTICS VERSION 19 compatible with Windows. Jaccard's proximity matrix was then used to construct a dendrogram employing the Weighted Pair Group Method of Arithmetic Averages algorithm with Average Linkage of nearest neighbor of each hybrid lines and their second generations with parental strains using Squared Euclidean Distance.

Table 1: Primers under study:

Sl No.	Name	Sequence (5'–>3')	Mol. Wt g/mol	Tm °C	GC content (%)
1	RAPD 02	CAATCGCCGT	2988	40.8	60
2	RAPD 03	AGGTGACCGT	3068	31.6	60
3	RAPD 04	GAATGCGACC	3037	34.2	60
4	RAPD 07	TCCCAGCAGA	2997	35.2	60
5	RAPD 08	CACTGGCCA	2973	41.1	70
6	RAPD SS 11	GTGCGCAATG	2972.9	37.9	70
7	RAPD SS 17	ACCCGACCTG	3012.9	37.1	70
8	RAPD SRS 05	CCCGAAGCGA	3022	45.7	70
9	RAPD SRS 06	GTGGCTTGGA	3099	34.7	60

Results

DNA polymorphism and specific genotypic information:

In all the nine RAPD primers a total of one hundred and four discrete amplicons were obtained. The fingerprints obtained for each species against each primer were unique. Reproducible polymorphic amplicons were studied to distinguish between the fingerprints and subsequently the percentage of polymorphism for each primer was calculated. Highest polymorphism was observed in primers RAPD-02, RAPD- 03, RAPD-08 and SS-11. Maximum bands generated primer SRS-06 and minimum in primer SRS-05, ranged from 330 bp- 3 kb and 450 bp- 2 kb, respectively. DNA-typing scored data helped to find out the genetic distance between each hybrid lines with their parents through clustering a dendogram. The proximity matrix ranged from 8.00-60.00. Two parental strains *P. florida* and *L. edodes* found to be most distantly related among all, where *pfle 1pFB*, *pfle 1rFB* were very close in genetic distance and another two hybrid lines, *pfle 1vFB*, *pfle 1qFB* were also showed the same. Amplified new prominent bands generated either in *pfle* hybrid lines or in *pfle FB* lines against several primers. RAPD-02 primer amplified and generated new bands in *pfle 1q* (580 bp) and *pfle1oFB* (1150 bp). Primer RAPD-04 also generated new bands, 700 bp in *pfle 1q* and 1600 bp in *pfle 1oFB*. SRS-06 and SS-17 could generate polymorphic bands in *pfle 1q* (800 bp), *pfle 1oFB* (350 bp) and *pfle 1q* (500 bp), *pfle 1rFB* (700 bp), *pfle 1oFB* (530 bp) respectively.

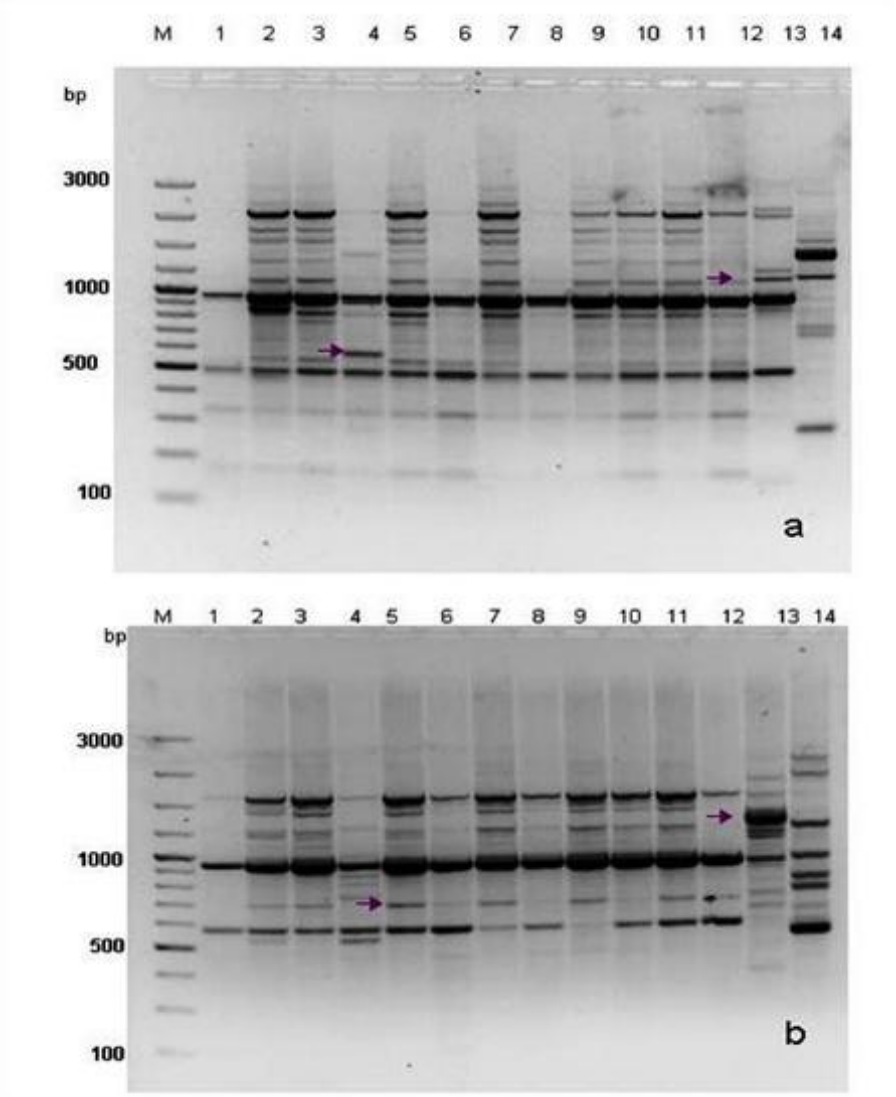

Fig. 2: RAPD marker DNA profiling of hybrid generations and parents using; a) primer RAPD-02; b) primer RAPD-04.

Lane M – DNA ladder (100 bp Plus), 1 – P. florida., 2 – pfle1v, 3 – pfle1vFB, 4 – pfle1q, 5 – pfle1qFB, 6 – pfle1p, 7 – pfle1pFB, 8 – pfle1r, 9 – pfle1rFB, 10 – pfle1s, 11 – pfle1sFB, 12 – pfle1o, 13 – pfle1oFB, and 14 – L. edodes.

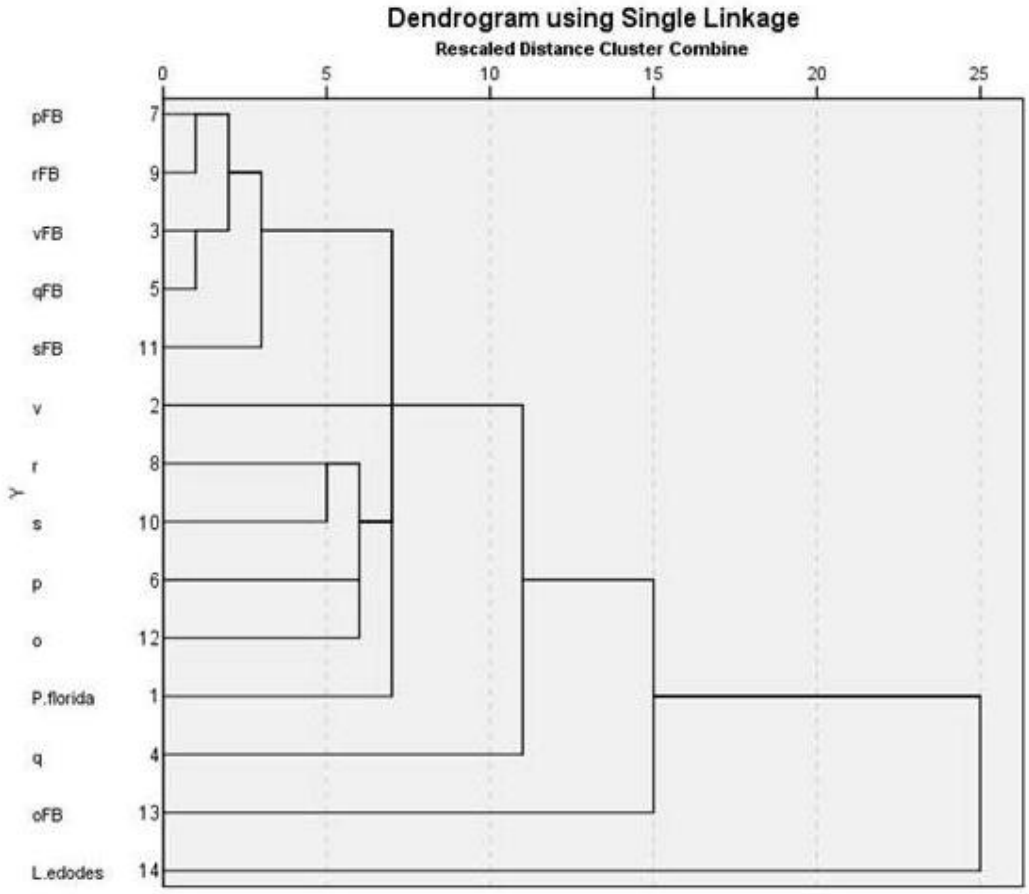

Fig.3: Dendrogram on the basis of data generated from RAPD analysis:

Table 2: Proximity matrix generated from RAPD scored data

	P. Florida	pfle1v	pfle1vFB	pfle1q	pfle1qFB	pfle1p	pfle1pFB	pfle1r	pfle1rFB	pfle1s	pfle1sFB	pfle1o	pfle1oFB	L. edodes
P. Florida	.000													
pfle1v	41.000	.000												
pfle1vFB	40.000	19.000	.000											
pfle1q	34.000	51.000	48.000	.000										
pfle1qFB	44.000	23.000	8.000	44.000	.000									
pfle1p	19.000	38.000	35.000	25.000	35.000	.000								
pfle1pFB	47.000	20.000	17.000	45.000	11.000	40.000	.000							
pfle1r	24.000	29.000	26.000	32.000	26.000	17.000	27.000	.000						
pfle1rFB	49.000	26.000	21.000	47.000	15.000	38.000	8.000	29.000	.000					
pfle1s	33.000	26.000	21.000	39.000	19.000	26.000	20.000	15.000	20.000	.000				
pfle1sFB	45.000	26.000	19.000	43.000	17.000	36.000	12.000	25.000	14.000	20.000	.000			
pfle1o	28.000	29.000	26.000	28.000	24.000	17.000	31.000	20.000	33.000	21.000	29.000	.000		
pfle1oFB	52.000	43.000	44.000	50.000	42.000	47.000	37.000	44.000	33.000	43.000	39.000	42.000	.000	
L. edodes	60.000	53.000	54.000	52.000	52.000	55.000	51.000	50.000	59.000	55.000	51.000	56.000	56.000	.000

Discussion

Analysis of second generation of somatic hybrids shows a little, but significant polymorphism at phenotypic and genotypic level. Data reveals that more variations explore during fruit body development on a specific substrate at in-vivo condition, where nuclear genome organization showed slight different for all lines. In this case, hybrids were generated through protoplast fusion between *Pleurotus florida* and *Lentinula edodes*. Inter-generic somatic hybrids of this edible mushroom strains

produced fruit bodies at field level. Second generations were isolated from tissue culture of first hybrid's fruit body lines. Followed the same procedure of fruit body development, all the second generations were subjected and produced fruit bodies. Mycellial cultures of two generations were maintained in PDA medium.

Nine somatic hybrid lines were raised through polyethylene glycol-mediated inter-generic protoplast fusion between *Pleurotus florida* and *Lentinula edodes* using a double selection method. From them, six could develop fruit body. The genetic changes of these hybrid lines under the environment were analyzed by RAPD markers. Molecular markers are significant tools to substantiate genotypic and phenotypic characters as they are numerous and are not affected by external variants as climate, temperature, soil, environment or care. Tools like molecular markers work at DNA level and reveals polymorphisms, thus being useful for characterization and genetic diversity estimation.

Conclusion

Data reveals that more variations explore during fruit body development on a specific substrate at in-vivo condition, where nuclear genome organization showed slight different for all lines so it was concluded that the nearest neighbor showed a little variance genetically, but the hybrid lines are mostly similar with their second generation.

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References

Cohen R, Persky L, Hadar Y (2004) Biotechnological applications and potential of wood-degrading mushrooms of the genus Pleurotus. *Appl Microbiol Biotechnol* 58(5):582–594. https://doi.org/10.1007/s00253-002-0930-y

Crisan EV, Sands A (1978) Nutritional value. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushrooms. *New York Academic Press., New York*, pp 137–165.

Kwan Hoi-Shan and Xu Hai-Lou (2002) Construction of a Genetic Linkage Map of Shiitake Mushroom *Lentinula edodes* Strain L-54 *Journal of Biochemistry and Molecular Biology*, 35(5): 465-471. https://doi.org/10.5483/BMBRep.2002.35.5.465

Chakraborty U and Sikdar SR (2008) Production and characterization of somatic hybrids raised through protoplast fusion between edible mushroom strains *Volvariella volvacea* and *Pleurotus florida* World Journal of Microbiology and Biotechnology, 24(8):1481-1492. https://doi.org/10.1007/s11274-007-9630-1.

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreprationt: version II. Plant Mol Biol Rep 1:19-21. https://doi.org/10.1007/BF02712670

Mallick P and Sikdar SR (2016) Restriction fragment length polymorphism and sequence analysis of rRNA-ITS region of somatic hybrids produced between *Pleurotusflorida* and *Lentinulaedodes*. Annals of Microbiology 66: 389-395. https://doi.org/10.1007/s13213-015-1121-2

Mallick P and Sikdar SR (2014) Production and molecular characterization of somatic hybrids between *Pleurotusflorida* and *Lentinula edodes*. World Journal of Microbiology and Biotechnology 30(8):2283-2293. https://doi.org/10.1007/s11274-014-1652-x

Widholm JM (1972) The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. Stain Technol 47:189–194. https://doi.org/10.3109/10520297209116483

Williams JGK, Kueelik AR, Lccak KJ, Ralskt JA & Ting SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535. https://doi.org/10.1093/nar/18.22.6531

Wolter M, Zadrazil R, Martens Bahadir M (1997) Degradates of eight highly condensed polycyclic aromatic hydrocarbons by Pleurotus sp. florida in solid wheat substrates. Appl Microbial Biotechnol 48:398–404. https://doi.org/10.1007/s002530051070.