www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(10): 99-103

www.thepharmajournal.com
Received: 07-07-2022
Accepted: 22-08-2022

Nudrat Sanzida Akhtar
Department of Plant Pathology, Palli Siksha Bhavana, Visva-Bharati Shantiniketan, West Bengal, India

Mohan Kumar Biswas
Department of Plant Pathology, Palli Siksha Bhavana, Visva-Bharati Shantiniketan, West Bengal, India

Dayaram
Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author:
Nudrat Sanzida Akhtar
Department of Plant Pathology, Palli Siksha Bhavana, Visva-Bharati Shantiniketan, West Bengal, India

Characterization of mutant strains developed through Ethyl Methanesulfonate (EMS) and UV-ray mutation

Nudrat Sanzida Akhtar, Mohan Kumar Biswas and Dayaram

Abstract
Chemical and UV-ray mutagenesis of basidiospores of *Lentinula edodes* generated new mushroom strains. The basidospores of parent strains (LE-1501, LE-1503, LE-1504 and LE-1505) were treated with ethyl methanesulfonate, an alkylating agent, to yield 16 mutant monokaryotic mycelia. Eight fast-growing mycelia were selected and mated each other by hyphal fusion. Five (Spore pair 4-1, 7-1, 5-3, 6-5 and 8-6) out of the 28 mattings were successful considered by the formation of clamp connections. For UV mutation, Seven days old culture of parent strains were inoculated then the plates were exposed to UV radiation with the help of G30T8 Germicidal 3FT 30W T8 UVC, Philips, under a laminar air flow hood for four different periods i.e. 15, 30, 45 and 60 minutes. Six UV mutant strains signify UV-1, UV-2, UV-3, UV-4, UV-5 and UV-6 with higher radial growth than parent were selected for further studies. The mutant dikaryons were cultivated to investigate their morphological and cultivation characteristics. Most of the mutant strains observed different shape and size of the sporophore from their parent. Mutant strains SP 7-1, SP 6-5, UV-3 and UV- 5 showed another interesting phenotype i.e. white to light straw coloured pileus which is totally different from parents.

Keywords: Chemical and UV ray mutation, characterization *Lentinula edodes* and sporophore

1. Introduction
Mushrooms are important source of nutraceutical products with multifarious health benefits apart from being taken as food or for spiritual purposes. In modern time, people are becoming more health conscious, mushrooms fit themselves well, to meet the dietary requirements as one of the best low calorie food along with their delicious taste and flavor. Because of their growing relevance in our day to day life, lot of emphasis is being laid on their identification, characterization, domestication and technological improvement for their bulk production (Purkayastha and Chandra, 1985) [6]. In 2018-2019, the total mushroom produced globally was 43 Metric Tonnes (MT) with *Lentinula edodes* (Shiitake) contributing 26%, *Auricularia* spp. (Wood ear) 21%, *Pleurotus ostreatus* (Oyster) 16%, *Agaricus bisporus* (Button) 11%, *Flammulina velutipes* (Enoki) 7%, *Pleurotus eryngii* (king oyster) 5%, *Volvariella volvacea* (paddy straw mushroom) 1% and others 13% (Singh *et al*., 2020) [11], however China produces 40 MT alone accounting 94% of the global mushroom production in 2020 followed by Japan and United States. According to Gautam, (2020) [3] mushroom production in India was 155553 and 201088 MT in the year 2017-18 and 2019-20 respectively, Haryana is the leading state in terms of mushroom production followed by Orissa than Maharashtra. In India, there are five mushroom species *viz*., white button mushroom (*Agaricus bisporus*), oyster (*Pleurotus* spp.), paddy straw (*Volvariella volvacea*), milky (*Calocybe indica*) and shiitake (*Lentinula edodes*) are in commercial cultivation (Sharma *et al*., 2017) [10]. The short duration cultivation technology of shiitake under indoor conditions was standardized at ICAR-DMR, Solan (Sharma *et al.*, 2017) [10], but still this precious mushroom has not been cultivated at commercial scale in India. Few growers of Uttarakhand and Himachal Pradesh successfully cultivated this mushroom using the technology developed by ICAR-DMR, Solan.

2. Materials and Methods
2.1 Strains collection and Maintenance of cultures
Pure culture of four strains of *Lentinula edodes viz* LE-1501, LE-1503, LE-1504 and LE-1505 were obtained from Advance Centre of Mushroom Research, Department of Microbiology, Dr. Rajendra Prasad central Agricultural University, Pusa, Samastipur, which was provided by Directorate of Mushroom Research (DMR, Solan). All the strains were maintained on Potato Dextrose Agar (PDA) media slants at 24±2 ºC and sub-cultured monthly.

2.2 EMS (Ethyl methanesulfonate) induced Mutagenesis

2.2.1 Spore print preparation and Quantification of spores

Ethanol disinfected fresh cap were hanging it gills-down on a sterilized bottom of petridish and covered it with a beaker for 10 hours to prepare spore prints. Collected spores were suspended in a known volume of distilled water there after serially diluted until counts of 200 to 300 spores per mL were attained.

2.2.2 Preparation and Screening of monokaryotic cultures

All the parent strains (LE-1501, LE-1503, LE-1504 and LE-1505) treated at variable concentrations (0.25%, 0.50%, 0.75% and 1.00%) of EMS were isolated and cultured on media with their parents. Monokaryotic isolates of different strains were transferred onto PDA media and incubated at 24±2 ^{0}C for 10 days. Out of 16 only eight monokaryotic cultures (at 0.25% and 0.50% EMS conc.) were selected with good cultural characteristics such as fast mycelial growth rate and high density were selected for mating studies. The mycelium was confirmed as a monokaryon with the absence of clamp connections by examination under a microscope at 400X magnification.

2.2.3 Mating between monokaryons of *L. edodes* to develop mutant strains

Each of the eight selected monokaryotic mycelia was mated with the remaining seven monokaryons, resulting in a total of 28 mating. Out of 28 matings, five compatible mating strains (Spore pair 4-1, 7-1, 5-3, 6-5 and 8-6) were generated in which clamp connections is confirmed, after purification these strains were further used for spawn production and cultivation.

2.3 UV induced Mutagenesis

Seven days old culture of four parent strains namely LE-1501, LE-1503, LE-1504 and LE-1505 were inoculated on PDA media at 24±2 ºC then the plates were exposed to UV radiation with the help of G30T8 Germicidal 3FT 30W T8 UVC, Philips, under a laminar air flow hood for four different periods i.e. 15, 30, 45 and 60 minutes. This procedure was carried out in darkness to inhibit photo-reactivation (Chan and Miles, 2004). After UV irradiation, the plates were incubated at 24± 2 ºC for 14 days to allow mycelial growth.

2.3.1. Screening and Cultivation of the mutant dikaryons

The surviving isolates were cultured on PDA and the radial growth of the mycelium was measured. Six UV mutant strains signify UV-1, UV-2, UV-3, UV-4, UV-5 and UV-6 with higher radial growth than parental strains were selected for spawn production.

For the cultivation, the spawn was inoculated onto solid substrate consisting sawdust in a wide-mouth polypropylene bag under aseptic condition. Then the spawned bag was kept into crop house for sporophores development.

3. Results

3.1 Effect of Ethyl methanesulfonate (EMS) on survival of basidiospores

EMS causes lethality in a concentration-dependent manner in spore germination of *Lentinula edodes*. To generate mutant monokaryotic mycelia, basidiospores were treated with four different concentrations varying from 0.25%, 0.50%, 0.75% and 1.0% of EMS. The survival rate decreased with the increase of EMS concentration (Fig. 1). The highest survival rate of basidiospores (92%) was observed at 0.25% followed by 71% at 0.50% of EMS concentration, the rate reached up to 37% at 0.75%. This rate was three times lesser than produced at 0.25% and the lowest survival rate was observed at 1.0% of EMS.

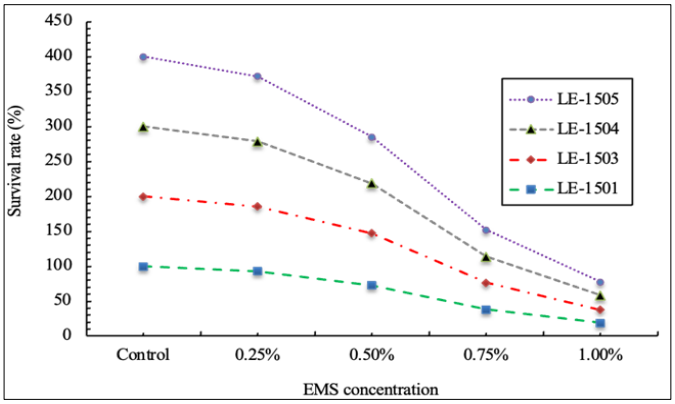

Fig 1: Survival rate of basidiospores at different concentration of EMS.

Table 1: Mating between EMS mutated monokaryons of *Lentinula edodes*

	1	2	3	4	5	6	7	8
1								
2	X							
3	X	X						
4	O	X	X					
5	X	X	O	X				
6	X	X	X	X	O			
7	O	X	X	X	X	X		
8	X	X	X	X	X	O	X	

O, signifies full compatibility in which a fertile and non-restricted dikaryon with true clamp connections is formed i.e. Spore pair 4-1, 7-1, 5-3, 6-5 and 8-6; while X, signifies incompatility, in which an infertile, principally monokaryotic heteyrokaryon without clamps is formed.

3.2 Mating between mutant monokaryotic mycelia

Inbreeding was conducted by the mating of the mutant monokaryons. Each of the eight monokaryotic mycelia was mated with the remaining seven monokaryons, resulting in a total of 28 mating pairs. Five out of 28 mating pairs were observed to make clamp connections presented in Table 1.

3.3 Cultivation of the mutant dikaryon strains

The mutated dikaryotic strains developed through EMS (Spore pair 4-1, 7-1, 5-3, 6-5 and 8-6) and UV-ray mutation (UV-1, UV-2, UV-3, UV-4, UV-5 and UV-6) were cultivated on the solid substrate in polypropylene bags. For the cultivation of the mutant strains, the spawns grown on wheat grain were inoculated into the solid substrate. The inoculated substrates were incubated at 18-22 ^{0}C for the development of fruiting bodies. In contrast, the eleven mutant strains produced fruiting bodies with considerably different morphological characteristic from their parental strains. The morphological characteristics of sporophores for the EMS and UV-ray mutant strains as well as the measurement of their stipe length and pileus width are illustrated in Table 2 and Table 3 respectively.

3.4 Characterization of Mutant strains with abnormal morphologies

Most of the mutant strains exhibited some prominent characteristics in the morphology of fruiting bodies. The morphological characterization of EMS and UV-ray mutated strains were summarized in Table 2 & Table 3 respectively. The Fig. 2 presented that the mutant strains with dark brown sporophore formation (SP 4-1, SP 5-3, SP 8-6, UV-1, UV-2, UV-4 and UV-6) look like parent strains in colour but different in morphology. For example, the Spore pair 4-1 had centrally depressed pileus, spherical wavy cap with dark brown to light brown from center to outside with tiny white spot. The margin is flat during young phase, which later forms wavy when mature. It has fleshy cap with very finely lined gills. Meanwhile, the Spore pair 5-3 produced oblong convex shaped, honey brown with white crust available on the cap, Spore pair 8-6 exhibited big, fleshy, spherical dark brown pileus having white tiny spot with wavy margin.

Table 2: Characterization of mutant strains produced after chemical mutation.

Mutant Strains	Basidiocarps		Characteristics*
	Pileus surface (Dorsal)	Lamellae (Ventral)	
Spore pair 4-1			[a] 5.30 ± 0.208 [b] 10.06 ± 0.219 [c] round, wavy [d] dark brown, depressed fleshier
Spore pair 7-1			[a] 6.30 ± 0.252 [b] 11.00 ± 0.252 [c] irregular, wavy [d] dull white, thin wavy
Spore pair 5-3			[a] 5.83 ± 0.376 [b] 11.70 ± 0.252 [c] oblong, convex [d] brown with white crust, fleshier
Spore pair 6-5			[a] 5.43 ± 0.088 [b] 9.20 ± 0.115 [c] round, convex [d] whitish, thin
Spore pair 8-6			[a] 5.30 ± 0.153 [b] 12.73 ± 0.291 [c] round, wavy, downward [d] dark brown, fleshy

* Characteristics of the mutant basidiocarps were signified as [a] stipe length (cm); [b] pileus width (cm); [c] margin of the pileus; [d] colour/texture of the sporophores. Each value is expressed as mean ± standard error (n = 3).

Table 3: Characterization of mutant strains produced after UV-light mutation.

Strains	Stipe length/pileus diameter (cm)		Characteristics	Texture/Aroma
UV-1	5.83 ± 0.318	11.16 ± 0.491	Spherical, centrally depressed, dark brown with white scars uniformly distributed throughout the cap from outside.	Fleshy/strong earthy smell.
UV-2	6.00 ± 0.153	10.96 ± 0.767	Spherical, centrally depressed, dark brown cap with undulated margin and straight stipe.	Fleshy/strong earthy smell.
UV-3	6.26 ± 0.120	11.63 ± 0.418	Spherical, flat, creamy white cap with irregular margin and stumpy stipe.	Fleshy/decrease earthy smell.
UV-4	5.93 ± 0.353	10.20 ± 0.404	Spherical, umbonate blackish brown distorted cap with cylindrical stipe.	Less fleshy/decrease earthy smell.
UV-5	5.96 ± 0.260	11.23 ± 0.536	Hemispherical, light brown cap with white scars distributed from outside having irregular margin.	Fleshy/less earthy smell.
UV-6	5.93 ± 0.186	10.23 ± 0.176	Spherical, centrally depressed, honey brown pileus with white scars uniformly distributed throughout the cap from outside.	Fleshy/earthy smell.

*Each value is expressed as mean ± standard error (n = 3).

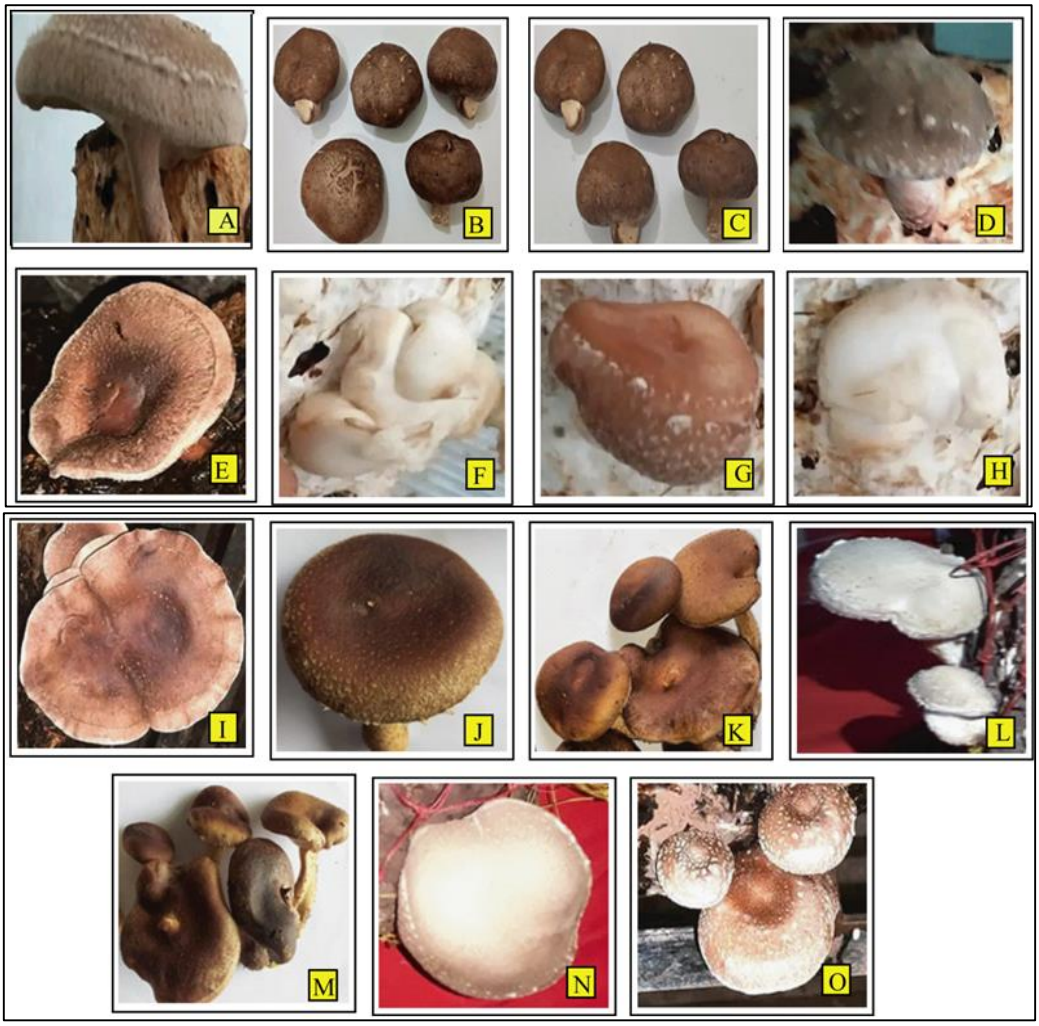

Fig 2: Morphologies of parent and mutant fruiting bodies. A-D, Parental strains LE-1501, LE-1503, LE-1504 and LE-1505 respectively; EMS Mutant strains E, SP 4-1; F, SP 7-1; G, SP 5-3; H, SP 6-5; and I, SP 8-6; UV-ray Mutant strains J, UV-1; K, UV-2; L, UV-3; M, UV-4; N, UV-5 and O, UV-6.

3.5 SP represent spore pair

The Strain UV-1 had spherical, fleshy, centrally depressed, dark brown coloured with white scars uniformly distributed throughout the cap from outside, strain UV-2 were fleshy, spherical, centrally depressed, dark brown cap with undulated margin and straight stipe, while Spherical, umbonate blackish brown distorted cap are the characteristics of UV-4 and honey brown coloured pileus with white scars uniformly distributed throughout the cap from outside are the characteristics of UV-6. The second most frequent phenotype among the mutants was the white to straw coloured pileus morphology. Mutant strains SP 7-1, SP 6-5, UV-3 and UV- 5 showed this phenotype. The Spore pair 7-1 has claw shaped, dull white thin cap with wavy margin, Spore pair 6-5 had big, fleshy, dull white coloured pileus and UV-3 exhibited spherical, flat, creamy white cap with irregular margin and stumpy stipe and Strain UV-5 were hemispherical, fleshy, light brown cap with white scars distributed from outside having irregular margin.

4. Discussion

This is the first report on mutation of *Lentinula edodes* but same research has been done on different mushroom by various scientists and similar results have come in that too. Our results are supported to Lee *et al*. 2011 [4], who worked on Chemical mutagenesis of basidiospores of *Hypsizygus marmoreus* to generate mutant monokaryotic mycelia as well as new mushroom strains treated with different concentrations of MMS. The survival rate decreased with the increase of MMS concentration, the rate reached 12% at 0.65 vol% of MMS. The mutant dikaryons were cultivated to investigate their cultivation and morphological characteristics. Mutant strains No. 3 and No. 5 showed 10% and 6% increase in fruiting body production respectively. Dikaryons generated by mating showed a change in primordial initiation resulting in flat fruiting bodies and reduced yield.

Ravishanker *et al.* (2006) [7], who also observed that with the increase in duration of exposure of UV light, the growth of mycelium retards. The mutant PO-7(U4) was further used in fruiting experiments. Sharma and Sharma (2014) [9] worked on chemical and physical mutation of *P. ostreatus.* Three selected isolates were irradiated with eight different treatments of UV light *viz*. T1, T2, T3, T4, T5, T6, T7 and T8. Only four treatments *viz* T1, T2, T5 and T6 supported mycelial growth in all the isolates. Selected isolates of *P. ostreatus* were also treated with EMS with five treatments. Treatments with concentrations ranging from 0.001-0.004% showed mycelial growth whereas no mycelial growth was observed at a concentration of 0.005. The mutants exhibiting variations in terms of fruiting bodies, mutant strain PO-7(U4) exhibited creamish white sporophore as compared to the control i.e. creamish brown.

Bangyeekhun *et al.* (2020) [1] reported that UV radiation

causes lethality in a time-dependent manner in paddy straw mushroom mycelia. The mycelial growth remains same with UV exposure times of less than 20 min. The survival rate was over 75% at 25–40 min of exposure, After 45 min of UV exposure, the survival rate drastically decreased to less than 25.

Mutagenesis induced by chemical and physical agents, such as UV light, gamma rays, ethyl methanesulfonate, and methyl methanesulfonate, is commonly used in mushrooms. Several high-yielding mutant strains with morphological and physiological changes caused by mutagenesis have been reported (Djajanegara & Hardoyo, 2009; Lee *et al*., 2011; Liu *et al*., 2011 [5]; Sermkiattipong & Charoen, 2014 [8]; Sharma & Sharma, 2014) [2, 4, 9].

5. Conclusion

This work displayed that some mutant dikaryons generated by the chemical and UV-ray mutagenesis exceed the parental sporophore characteristics and this was worthy of further investigation. The various irregular fruiting bodies generated by the both type of mutagenesis will be useful resources for molecular genetic studies.

6. Acknowledgements

Authors are grateful to Advance Centre of Mushroom Research, Department of Microbiology, Dr. Rajendra Prasad central Agricultural University, Pusa, Samastipur, Bihar for continues help and support.

7. References

1. Bangyeekhun E, Sawetsuwannakul K, Romrue U. UV-induced mutagenesis in *Volvariella volvacea* to improve mushroom yield, Songklanakarin J Sci. Technol. 2020;42(4):910-916.
2. Djajanegara I Harsoyo. Mutation study on white oyster mushroom (*Pleurotus floridae*) using gamma (60CO) irradiation. Journal of Chemical and Natural Resources Engineering. 2009;4(1):12-21.
3. Gautam Y. Present mushroom production scenario of India and the World. International Journal of Research in Engineering and Applied Sciences, 2020, 10(02).
4. Lee J, Kan HW, Kim SW, Lee CY, Ro, HY. Breeding of New Strains of Mushroom by Basidiospore Chemical Mutagenesis, Microbiology. 2011;39(4):272-277.
5. Liu *et al*., 2011.
6. Purkayastha RP Chandra. A New species of edible mushroom from India. Trans. Br. Mycol. Soc. 1974;62:415-418.
7. Ravishanker S, Pandey M, Tewari RP, Krishna V. Development of sporeless/low sporing strains of *Pleurotus* through mutation. World J Microbiol. Biotechnol. 2006;22:1021-1025.
8. Sermkiattipong N, Charoen S. Development of straw mushroom strain for high yield by gamma radiation. Journal of Agricultural Technology. 2014;10(5):1151-1164.
9. Sharma R, Sharma BM. Strain improvement in *Pleurotus Ostreatus* using UV light and ethyl methyl sulfonate as mutagens, African Journal of Microbiology Research. 2014;8(5):432-436.
10. Sharma VP, Annepu SK, Gautam Y, Singh M, Kamal S. Status of Mushroom Production in India. Mushroom Research. 2017;26(2):111-120.
11. Singh M, Kamal S, Sharma VP. Status and Trends in World Mushroom Production-III World Production of Different Mushroom Species in 21st Century. Mushroom Research. 2020;29(2):75-111.