academicJournals

Vol. 8(5), pp. 432-436, 29 January, 2014
DOI: 10.5897/AJMR2013.6410
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Strain improvement in *Pleurotus Ostreatus* using UV light and ethyl methyl sulfonate as mutagens

Rishu Sharma* and B. M. Sharma

Department of Plant Pathology, Centre for Mushroom Research and Training, CSKHPKV, Palampur, Himachal Pradesh, 176062, India.

Accepted 13 January, 2014

Oyster mushroom (*Pleurotus Ostreatus*) is the choicest edible species cultivated in various regions of the world. Strain improvement studies were carried out in three strains of *P. Ostreatus* spp. Three strains of *P. Ostreatus* viz. PO-2, PO-6 and PO-7 were used for strain improvement, emphasizing on lower spore count and colour of the sporophore. It is a gymnocarpous genus of mushroom, which continuously release spores in its close vicinity causing various respiratory allergies. Their spores are highly potent allergens which can also cause exogenous allergic alveolitis. Attempts were made to produce low sporing strains of *P. Ostreatus* through mutagenesis using physical mutagen (UV light) and chemical mutagen (ethyl methyl sulfonate, EMS). Spores of three strains of *P. Ostreatus* spp. were given different treatments with UV light and EMS. Mutants exhibited appressed mycelial growth and showed slower spawn run and creamish white sporophore in PO 7(U4). A lower spore count was also observed in PO-7(E3) mutant as compared to control.

Key words: *Pleurotus*, UV light, ethyl methyl sulfonate, mutagens.

INTRODUCTION

Pleurotus spp. constitute 30% and ranks third among the cultivated mushrooms grown widely in temperate, sub-tropical and tropical regions of the world. The species of *Pleurotus* grow in the forests, attacking both cellulose and lignin components of wood (Zardazil and Kurtzman, 1982). The total world production of mushrooms is 200 thousand lakh tones in 2010. In India, mushroom production has crossed over 1,00,000 tons in 2010 (Singh et al., 2011). In the last few decades, *Pleurotus* cultivation has accelerated in India. It being a predo-minantly agriculture based country, holds a vast potential and stock of lignocellulosic waste, its adaptability to a wide range of subtropical climate temperature (20-30°C), ease of its cultivation, having good culinary and medicinal properties has attracted various farmers, entrepreneurs for its commercial production in the Indian sub continent.

The sporophores of *Pleurotus* are gymnocarpous and continuously release spores in the atmosphere causing of immunologic lung diseases like hay fever and farmer's lung disease among workers (Obatake et al., 2003) The antigens present on the walls of the spores cause the allergy. Also, during cultivation these spores settle on fruit bodies forming a velvety film after germination and thus giving an unpleasant appearance to mushroom (Ravishanker et al., 2006). The importance of fungal spores in causing air borne respiratory allergies has been well established (Hegde et al., 2002). A strict environmental control of Basidiomycetes spores is important to reduce the high risk of sensitization and possible development of various allergic diseases. A reliable spore extraction method has been devised, and a reasonable number of available patients showed positive results with

*Corresponding author. E-mail: rishupath12@gmail.com. Tel: 091-9419295531.

skin test and radioallergosorbent test (RAST).

Strain improvement generally includes higher yield, better nutritional quality, colour and sporelessness. In order to overcome these constraints strain improvement has been carried out using different techniques viz. Protoplast fusion (Das and Mukheerjee, 1995), dikaryon mating (Larraya et al., 2001) and interspecific hybridization (Jaswal et al., 2013). Strain improvement in *P. Ostreatus* was first attempted using two isolates from North America which fructified well between 4-24°C and one German isolate which fructified only below 15°C (Eger et al., 1976). Mutagenesis by chemical treatment and UV irradiation has been applied for induction of sporeless mutants in *Coprinus cinerus*, *Pleurotus ostreatus* and *Pleurotus pulmonarius* (Imbernon and Labarere, 1989), AND*Agrocybe cylindracea* (Murakami, 1993). It has been reported that the mutations responsible for those sporulation defects were recessive or dominant and that most of the sporulation blockages are caused by aberration of the meiotic process or sterigma formation for sexual reproduction. Shekhar and Surjeet (2006) subjected the mycelium of *Pleurotus sajor-caju* to gamma irradiation at 10 different doses and found that with the increase in dose there was a significant decrease in the mycelial growth.

A very high variation in colour of the basidiocarp such as grey, brown or greyish black etc. is one of the limiting factors in marketing of this mushroom. Physical mutagens e.g. UV Light, gamma rays have been used for strain improvement in *P. ostreatus* (Daoping, 1997; Obatake et al., 2003; Ravishanker et al., 2006). Chemical mutagens e.g. EMS, MMS and NTG have been used to induce desirable characters like sporelessness and white colour of the basidiocarp in *P. ostreatus* (Mukherjee and Sengupta, 1986).

However, not much work has been done on the strain improvement study in *P. Ostreatus* through mutagenesis in Indian sub continent. Thus, in the present study, strain improvement in three strains of species *P. ostreatus* was aimed at achieving a lower spore count and a captivating colour of the basidiocarp through mutagenesis using physical (UV light) and chemical (ethyl methyl sulfonate) mutagens. Sporelessness is a desired trait in *P. ostreatus* causing various allergic infections in humans and causes hindrance in the commercial production of this mushroom.

MATERIALS AND METHODS

Strain improvement in *P. ostreatus* was carried out using three cultivated strains viz. PO-2, PO-6 and PO-7 for inducing white colour and sporeless fruit bodies.

UV light (physical mutagen) irradiation treatments and fruting behaviuor of *P. Ostreatus* cultures

Spore prints were made on sterile Petri plate of three selected isolates of *P. ostreatus* viz. PO-2, PO-6 and PO-7 by keeping them covered with a glass jar overnight. A dilute suspension of each spore print was made in sterile distilled water taken in test tubes and exposed to ultraviolet rays giving 8 treatments at two distances (5 and 10 cm) from source for different time of exposure (10,15, 20 and 25 min). Irradiation was given under the dark conditions to avoid the process of photoreactivation. The irradiated spore suspension was poured in sterile Petri plates containing potato dextrose agar medium and incubated at 23±2°C for 8-10 days. The mycelial bits were picked up and placed on potato dextrose agar medium slants for further studies. Mycelial growth characteristics of irradiated cultures were studied on PDA in Petri plates. From these pure cultures, spawn was prepared on wheat grains following standard procedures (Munjal, 1973). The fruiting behaviour of the irradiated cultures was studied under mushroom house conditions using pasteurized wheat straw. The fruiting pattern of irradiated cultures was compared with that of control. Observations were made on number of days for spawn run and fruiting, colour of the sporophore, sporulation. Spore count was calculated using the haemocytometer.

Chemical treatments using EMS and fruiting behavior of *P. Ostreatus* culture

Spore prints were made on sterile Petri plate of three selected isolates of *P. Ostreatus* viz. PO-2, PO-6 and PO-7 by keeping them covered with a glass jar overnight. A dilute suspension of each spore print was made in sterile distilled water taken in test tubes. The spore suspension was poured in sterile Petri plates containing potato dextrose agar medium having different concentration of mutagen ethyl methyl sulfonate (EMS) ranging from 0.001, 0.002, 0.003, 0.004 and 0.005% and incubated at 23±2°C for 8-10 days. The mycelial bits were picked up and placed on PDA slants for further studies. Mycelial growth characteristics of irradiated cultures were studied on PDA in Petri plates. From these pure cultures, spawn was prepared on wheat grains as standard procedures (Munjal, 1973). The fruiting behaviour of the EMS treated cultures was studied under mushroom house conditions using pasteurized wheat straw. The fruiting pattern of EMS treated cultures was compared with that of control. Observations were made on number of days for spawn run and fruiting, colour of the sporophore, biological efficiency and spore count was calculated using haemocytometer.

RESULTS AND DISCUSSION

Three cultivated strains viz. PO-2, PO-6 and PO-7 were used in strain improvement of species *P. ostreatus* and inducing desirable traits such as white colour of the basidiocarp and sporeless fruit bodies. *P. ostreatus* showed Clamp connections in all the three selected strains and basidiospores were oblong, ovate, hyaline in colour and size ranging from 6.5-9.5 x 3.0-4.5 µm (Figure 2).

P. ostreatus cultures irradiated with UV light

The selected isolates of *P. ostreatus* were irradiated with eight different treatments of UV light viz. T1, T2, T3, T4, T5, T6, T7 and T8. Out of which only four treatments viz

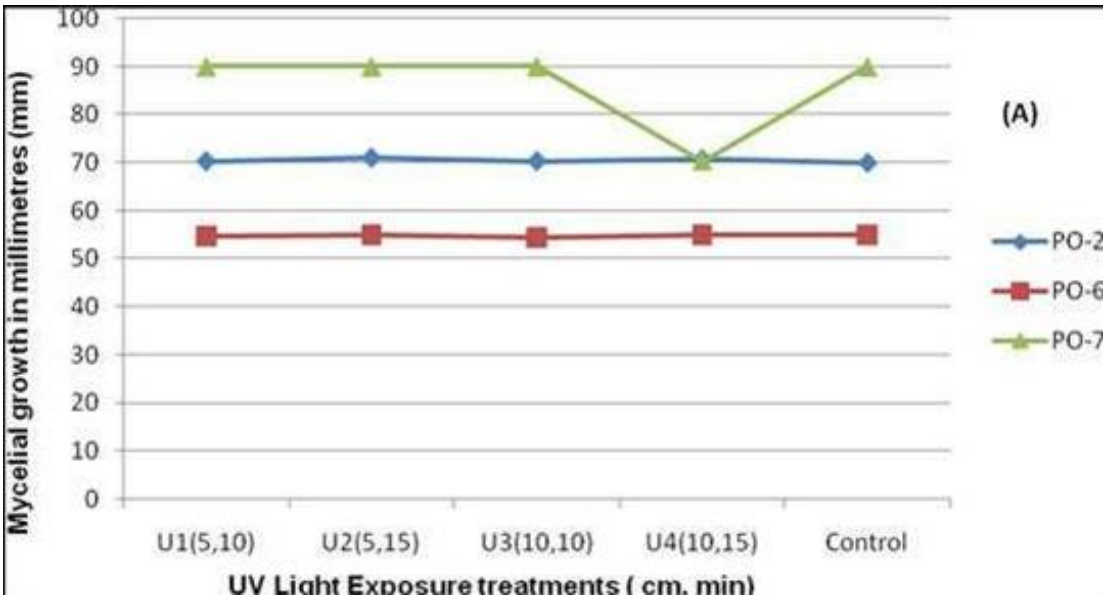

Figure 1A. Mycelial growth of three selected strains after UV light irradiation.

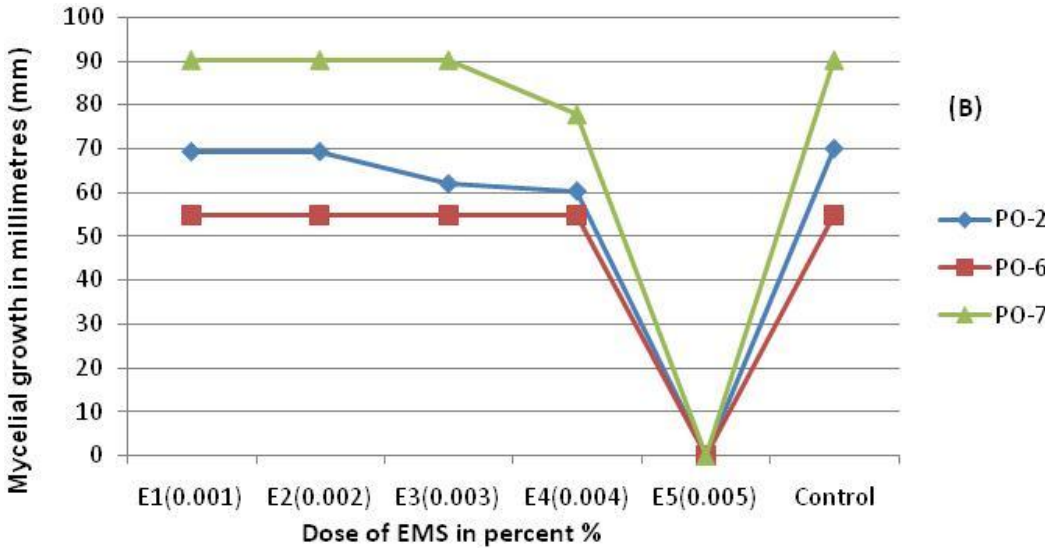

Figure 1B. Mycelial growth of three selected strains after different doses of EMS.

T1, T2, T5 and T6 supported mycelial growth in all the isolates. The irradiated isolates PO-2 and PO-6 showed no variation in mycelial growth (Figure 1A) whereas isolate PO-7(U4) showed a retarded, cottony fluffy mycelial growth 70.33 mm as compared to control which has an appressed strandy mycelial growth of 90.00 mm.

Our results are in congruence to Ravishanker et al. (2006), who also observed that with the increase in duration of exposure, the growth of mycelium retards. The mutant PO-7(U4) was further used in fruiting experiments.

P. ostreatus cultures treated with chemical mutagen (EMS)

Three selected isolates of *P. ostreatus* were 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% (Figure 1B). Among all the treatments showing mycelial growth, a retarded mycelial growth, 62.00 and 60.33 mm was observed in PO-2(E3) and PO-2(E4), respectively (Figure 1B). A similar pattern

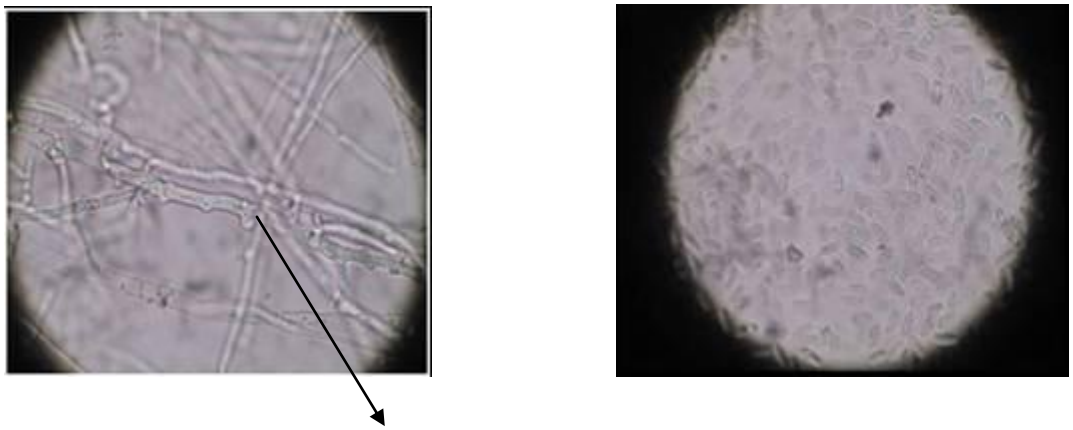

Figure 2. (A) Characteristic clamp connection and (B) basidiospores of *P. ostreatus* spp.

Figure 3. The observed change in the colour of the sporophore in mutant PO 7(U4).

of retarded mycelial growth was also observed in isolate PO-7(E4) with a 77.66 mm radial mycelial growth.

Fruiting behavior of UV and EMS treated cultures

The mutants exhibiting variations in terms of mycelial growth viz PO-7(U4), PO-2(E3), PO-2(E4) and PO-7(E4) were selected for spawn preparation. In fruiting trials of the selected mutants, data were recorded in terms of number of days for spawn run and fruiting, colour of the sporophore and sporulation. Among all the mutants, only PO-7(U4) showed creamish white colour of the sporophore in comparison with creamish brown of the control (Figure 3). However, no other significant variation was recorded in terms of biological efficiency in any of the mutants (Table 1). Lee et al. (2011) observed that basidiospores treated with chemical mutagens showed a change in primordial initiation resulting in reduced yield. Strain improvement in three isolates of *P. ostreatus* viz. PO-2, PO-6 and PO-7 using physical (UV light) and chemical mutagens (EMS) resulted in mutants showing retarded mycelial growth in PO-7(U4), PO-2(E3) and PO-2(E4). Another mutant PO-7(U4) exhibited desirable white colour of the sporophore as compared to the control. Also, a lower sporulation count was observed in PO-7(E3) while no other strains with any of the treatments showed a significant decrease in spore count.

Conclusions

Exposure of spores to UV light resulted in retarded cottony growth of mycelium 70.33 mm in mutant PO-7(U4) without causing any aberration in days for spawn run, biological efficiency and on the spore count. But mutant PO-7(U4) exhibited an attractive creamish white colour of the basidiocarp which was exposed to UV light for 15 min at 10 cm distance from the source. Similarly, spores of three strains treated at different doses of EMS,

Table 1. Fruiting behavior of selected mutants under mushroom house conditions.

Isolate	Days for spawn run (days)[a]	Colour of the sporophore	Biological efficiency[b] (%)	Effect on spore count* (cm^3/ml)
PO-2(E3)	16.66 ±1.23	Light brown	62.66 ±0.58	5.3×10^9
PO-2(E4)	16.66±1.53	Light brown	63.33 ±0.58	5.4×10^9
Control	15.55 ±0.58	Light brown	63.33 ±1.15	5.4×10^9
PO-7(U4)	14.66 ±0.58	Creamish white	62.66 ±0.58	3.7×10^9
PO-7(E3)	14.86 ±0.58	Light brown	60.33 ±0.58	2.9×10^9
Control	13.65 ±1.15	Light brown	60.66± 0.58	3.8×10^9

[a]Days for spawn run: average mean of three replications in RBD ± S.E (standard error); [b]Biological efficiency = fresh weight of mushroom / dry weight of substrate x 100% using average mean of 3 replications with RBD ± S.E (standard error); *Spore count = number of cells per ml : Number of cells/Number of squares counted x volume of a square ($1x10^{-9}$). RBD- random block design.

mutant PO-7(E4) exhibited a retarded mycelial growth of 77.66 mm at 0.004%. A lower spore count was found in mutant PO-7(U4) after conducting the fruiting trials of all the mutants. The observed results were found to be stable for more than five generations during the stipulated time of the research duration. Although, if time permits we would test the stability of the improved character for more than ten generations.

ACKNOWLEDGEMENTS

Authors are highly thankful to National Horticulture Board, India for providing funds under the mushroom production and training project. We are also grateful to CSK HP KV University for timely help and cooperation.

REFERENCES

Daoping XXX (1997). A Study on Mutation Breeding of High Yield and Sporless *Pleurotus ostreatus* by UV Radiation of Protoplast. Acta Edulis Fungi 4:13-17.

Das N, Mukheerjee M (1995). Conditions for isolation of regenerating protoplasts from *Pleurotus sajor caju.* J. Basic Microbiol. 35:157-161.

Eger G, Eden G, Wissing E (1976). *Pleurotus Ostreatus* - breeding potential of a new cultivated mushroom. Theor. Appl. Genet. 47:155-163.

Hegde VL, Das JR, Venkatesh YP (2002). Anaphylaxis caused by the ingestion of cultivated mushroom (*Agaricus bisporus*): Identification of allergen as mannitol. Allergol. Int. 51:121-129.

Imbernon M, Labarere J (1989). Selection of sporeless or poorly spored induced mutants from *P. pulmonarius* and selective breeding. In: Mushroom science XII (part I): proceedings of the 12th international congress on the science and cultivation of edible fungi. pp. 109-123.

Jaswal RK, Sodhi HS, Kapoor S, Khanna PK (2013).Development of high yielding morphologically improved strains of *Pleurotus* through interspecific hybridization. Indian J. Agric. Sci. 83:374-379.

Larraya L, Rez G, Iribarren A, Blanco JA, Alfonso M, Pisabarro AG and Rez L (2001). Relationship between monokaryotic growth rate and mating type in the edible basidiomycete *Pleurotus ostreatus.* Appl. Environ. Microbiol. 67:3385-3390.

Lee J, Kang HW, Kim SW, Lee CY, RO HS (2011). Breeding of new strains of mushroom by basidiospores chemical mutagenesis. Mycobiology 39:272-277.

Mukherjee M, Sengupta, R (1986). Mutagenesis of *Pleurotus* and regeneration of mycelium in the mushroom *Volvariella volvacea*. Appl. Environ. Microbiol. 52:1412-1414.

Munjal RL (1973). Production of quality spawn of *Agaricus bisporus* and Volvariella spp. Indian J. Mushroom 1:1-4.

Murakami S (1993). Genetics and breeding of spore deficient strains in *Agrocybe cylindracea* and *Lentinus edodes.* In: Chang ST, Buswell AB, Chiu SW (eds) Mushroom biology and mushroom products. The Chinese University Press, Hong Kong. pp. 63-69.

Obatake Y, Murakami S, Matsumoto T, Fukumasa Y (2003). Isolation and characterization of a sporeless mutant in *Pleurotus eryngii. Mycoscience* 44:33- Lee YH, Park YH, Yoo YB and Min KH. 1986. Isolation of auxotropic mutants from basidiospores of *Pleurotus cornucopiae.* Korean J. Mycol. 14:185-188.

Ravishanker S, Pandey M, Tewari RP, Krishna V (2006). Development of sporeless/low sporing strains of *Pleurotus* through mutation. World J. Microbiol. Biotechnol. 22:1021-1025.

Shekhar VC, Surjeet S (2006). Strain improvement in oyster mushroom (*Pleurotus Sajor-caju*) through mutation breeding. Mushroom Res. 15:19-22.

Singh M, Bhuvnesh V, Shwet K, Wakachaure GC (2011). Mushrooms: Agribusiness Activity: *In Mushrooms Cultivation, Marketing and Consumption*. Publisher, Directorate of Mushroom Research, ICAR, Solan. pp. 1-20.

Zardazil F, Kurtzman RH (1982). The biology of Pleurotus cultivation in the tropic tropical mushrooms. In Chang S, Quimio TH(eds). The Chinese University Press, Hong Kong. pp. 227-298.