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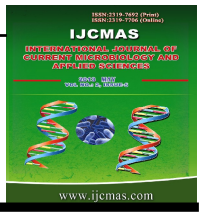


Original Research Article

Effects of Mutagenesis on Mycelial Growth Rate (Mgr), Total Protein, Total Phenolics and Total Sugar Contents of *Lentinus subnudus* Using Ultra-Violet Radiation as Mutagen

O.N.Majolagbe[1, 2*], J.K Oloke[2], H.P. Deka-Boruah[1] and D.A. Aina[3]

[1]Biotechnology Division, North East Institute of Science and Technology, Jorhat-785006, Assam, India
[2]Department of Pure and Applied Biology, Microbiology Unit, Ladoke Akintola University of Technology, P. M. B. - 4000, Ogbomoso, Nigeria
[3]Department of Biosciences and Biotechnology, School of Basic and Applied Sciences, Babcock University Ilisan-Remo, Ogun State PMB 21244, Ikeja, Lagos, Nigeria

***Corresponding author e-mail:** olusola.nat@gmail.com*

Keywords

Ionizing radiations; total protein; total phenolics; total sugar, *Lentinus subnudus.*

A B S T R A C T

Ionizing radiations were applied topically to actively growing mycelial culture of wild strain of *Lentinus subnudus* on fungal agar media. Fifteen mutants were generated from the wild strain of *L. subnudus* over a period of 4 hours at 15 minutes intervals. All the mutants (100%) yielded a remarkable increase in total protein estimated as compared with the wild strain with the highest and lowest protein concentrations recorded in SMT-060 (5187.5 µg/ml) and (833.65 µg/ml) respectively. Eleven (73.33%) of the mutant strains yielded increase in the total phenolics estimated as compared to the wild strain with highest and least phenolic concentrations recorded in SMT-135 (13.14 mg/100g) and SMT-030 (0.86 mg/100g) respectively. About 80% of the mutants gave increase in the total sugar concentrations as compared to the wild, with highest and least sugar compositions recorded in SMT-135 (46.00 µg %) and SMT-060 (18.23 µg%) respectively. In summary, there is a possibility of producing mutants of *L. subnudus* that has higher productivity through ultraviolet radiation treatment.

Introduction

Mutation is applied to edible mushrooms to obtain better quality and productivity. The desired mutant characteristic can be economically beneficial for example resistant to fungicide, higher yield, etc., (Flegg *et al.*, 1985). There are two types of mutation based on the site in nucleus namely point mutation and chromosomal level. Point mutation is the change of a base or several bases in the gene. Point mutation mechanism can be done through substitution, addition or deletion of bases. Substitution can occur between purine or pyrimidine bases (transisition) or between purine and pyrimidine bases (transversion)

(Elliot and Langton, 1981). Mutation can alter the coding region or non coding region of a gene. Mutation at the coding region of a gene will cause the cell to experience silent, missense, frame-shift and nonsense mutation. Silent mutation will not produce any visible phenotype because the base changes yield the same amino acid as the wild-type. Phenotype changes are produced by missense because the base(s) change will yield the different amino acids. Frame-shift mutation produced by deletion or bases substitution that cause reading shift of the triplet codon which will yield different amino acids. On the other hand, nonsense mutation is the formation of codon stop due to a base deletion or substitution which produces a truncated protein. Mutation altering the non coding region will cause different protein production or have no effect on mRNA maturation (Elliot and Langton, 1981). Other types of mutation are null and leaky mutation. These two mutations occur at the active site of a protein. Null mutation occurs if the base(s) change produces an inactive protein and this type of mutation can also be caused by changes in the promoter region. Leaky mutation occurs if the base(s) change only cause a part of protein becomes inactive (Elliot and Langton, 1981). Gamma radiation is an ionizing radiation often applied to eukaryotic organisms.

According to (Djajanegara and Harosoyo, 2001) gamma radiation is an effective ionizing radiation due to its ability to penetrate cell walls of mushroom mycelia. Gamma rays have higher energy which makes this type of radiation penetrates better into the target cells. In several microbiology studies, mutation that produced higher metabolites are mostly obtained by radioisotope mutagenesis (Slater, 2000). Ionizing radiation has been a common practice for extending storage life in mushrooms. Ionizing radiation has also been used to generate mutants in several edible mushrooms. Elliot and Langton (1981) used ultra violet (UV) radiation to produce an *Agaricus bisporus* mutant strain that is resistant to fungicide. Djajanegara and Harsoyo (2000) also had to applied gamma radiation to white oyster mushroom (*Pleurotus floridae*) to obtain genetic variability which has lead to commercially superior white oyster mushroom (*P. floridae*) strains that has higher productivity. Ultraviolet (UV) photon affects the DNA molecules of living organisms in different ways.

Materials and Methods

Organism and culture conditions

L. subnudus^{wt} (wild type) was collected from green vegetation environment specifically from a decaying mango log of wood of six month old and then sub-cultured onto potato dextrose agar PDA. It was maintained on (PDA) slants by sub-culturing in every one month interval. The ambient temperature for culture of the fungus is 25-28^{o}C and it takes a minimum of 72 hours for optimum mycelia elongation. The fully grown fungus was maintained at 4^{0}C until when needed for use.

Production of Mutants

Lentinus subnudus mutant types (SMT**)** were produced by exposing an actively growing culture (5days old) on Mycological agar plate to ultraviolet radiations (ג= 280 nm) over a period of 4 hours at 15 minutes intervals. Equal sized mycelia plugs were obtained from the solid culture with a sterile cork borer and then transferred onto the centre of freshly prepared agar plates, incubated at 25^{o}C.

The growth of the fungus was monitored over a period of 5 days and the morphological characteristics observed were recorded daily.

Linear mycelia growth rate of wild and mutants fungal strains on various basal media

The effects of the ultraviolet induced mutants of *L. subnudus* (SMT) and the wild type (SWT) were investigated by culturing the fungal strains on Potato Dextrose Agar (PDA), Mycological Agar (MA), and Yeast Mannitol Agar (YMA). About 6mm agar discs of the wild and mutants were obtained from culture slants and then inoculated onto the middle of the agar plates. The linear growth rate of the mycelium was taken daily over a period of 3 days.

Inoculation of seed culture

L. subnudus wild and mutants' strains cultured on potato-dextrose-agar (PDA) slants at 4^{o}C were used for seed culture inoculation. The culture was allowed to stand undisturbed for 5 days. All the mutant and wild fungal strains were cultured in an optimized culture conditions containing: glucose 10g/l, yeast extract 10g/l, K_2HPO_4 0.5 g/l, $CaCO_3$ 1.0 g/l, $MgSO_4.7H_2O$ 0.2g/l and NaCl 0.1g/l. The initial pH of the medium was adjusted to 5.5 using 2N NaOH and 2N HCl. Equal size of each fungal agar plugs kept on PDA slants was inoculated into 100 ml substrate volume in a 250 ml Erlenmeyer flask for 5 days at 25^{o}C.

Estimation of total soluble Protein

Protein extracted from the seed culture broths was estimated by Folin-Ciocalteau's method as modified by Lowry *et al*., (1951)**.**

Estimation of total sugar contents of the exopolysaccharides

The total sugar contents of the exopolysaccharides produced from the submerge cultures of wild and mutants fungal strains were estimated using Anthrone method using glucose as standard according to Sadasivam and Manickam (1992).

Determination of total phenolics in the exopolysaccharides

Total phenolic concentrations were determined according to the method of Malick and Sigh (1980) using catechol as standard.

Results and Discussion

A total of fifteen mutants were generated from the wild strain of *L. subnudus* over a period of 4 hours at 15 minutes intervals. It was observed that there were variations in the mycelial growth rate over the three days of incubation when compared with the wild strain (parent strain) in all the three media used as shown in tables 1, 2 and 3. Analysis of variance (ANOVA) showed that there was a significant difference in the linear mycelial growth rate when Mycological agar (MA) and Yeast Mannitol agar (YMA) were used for culturing the wild strain ($P < 0.05$) although no significant differences were noticed in PDA media ($P < 0.05$). All the fifteen mutants generated were further cultured in submerge fermentation and the total protein, total phenolic and total sugar were determined for the exopolysaccharides of each strain as

indicated in Figures 1, 2 and 3 respectively.

Table.1 Linear Mycelial Growth rate of wild and mutants strains of *L. subnudus*^{wt} on Mycological Agar

Fungal Strains	Linear Mycelial Growth 24 hrs (mm^3)	48 hrs (mm^3)	72 hrs (mm^3)
SWT	17.83±0.88^{b}	29.83±1.83^{ab}	39.67±1.42^{a}
SMT030	15.83±0.88^{b}	28.00±1.26^{ab}	41.00±1.76^{a}
SMT045	16.67±1.30^{b}	29.00±1.26^{ab}	41.83±2.19^{a}
SMT060	14.00±1.04^{b}	25.67±0.67^{ab}	37.83±0.17^{bc}
SMT075	14.83±0.17^{b}	27.17±0.83^{ab}	37.50±1.04^{a}
SMT090	15.83±1.09^{b}	29.67±1.30^{ab}	41.67±1.36^{a}
SMT105	13.83±0.60^{b}	30.17±0.60^{ab}	45.17±0.60^{a}
SMT120	13.83±0.83^{b}	29.00±0.50^{ab}	42.33±1.76^{a}
SMT135	15.33±0.17^{b}	30.17±0.73^{ab}	44.17±1.59^{a}
SMT 150	15.33±0.17^{b}	25.83±2.89^{ab}	39.83±3.66^{a}
SMT165	13.67±0.73^{b}	29.00±1.76^{ab}	44.83±3.67^{a}
SMT180	13.00±0.83^{b}	26.00±0.76^{ab}	37.00±1.00^{a}
SMT195	15.17±0.17^{b}	24.83±0.26^{ab}	35.67±3.18^{a}
SMT210	13.67±0.17^{b}	28.83±0.73^{ab}	36.83±0.44^{a}
SMT225	14.67±0.17^{b}	25.67±3.06^{ab}	39.17±4.91^{a}
SMT240	14.67±0.60^{b}	26.50±1.32^{a}	35.50±1.04^{a}

Data are means of three replicates; ± values represents standard deviation of the mean; Mean followed by same letters in the same row are not significantly different ($P<0.05$) from each other according to Turkey's Multiple Comparison Test

Table.2 Linear Mycelial Growth rate of wild and mutants strains of *L. subnudus* [wt] on Potato Dextrose Agar

Fungal Strains	Linear Mycelial Growth 24 hrs (mm^3)	48 hrs (mm^3)	72 hrs (mm^3)
SWT	24.17 ± 0.44^{b}	50.67 ± 1.76^{ab}	74.17 ± 0.67^{a}
SMT030	27.50 ± 0.50^{b}	52.00 ± 0.76^{ab}	74.33 ± 0.67^{a}
SMT045	27.50 ± 0.50^{b}	54.67 ± 0.60^{ab}	78.00 ± 1.52^{a}
SMT060	28.67 ± 0.44^{b}	54.00 ± 1.52^{ab}	76.00 ± 1.00^{a}
SMT075	27.67 ± 1.20^{b}	51.83 ± 1.86^{ab}	73.83 ± 1.33^{a}
SMT090	27.50 ± 0.76^{b}	52.33 ± 1.67^{ab}	76.33 ± 0.17^{a}
SMT105	30.67 ± 0.17^{b}	57.17 ± 0.60^{ab}	79.50 ± 2.25^{a}
SMT120	32.67 ± 0.88^{b}	58.33 ± 1.01^{ab}	79.50 ± 2.25^{a}
SMT135	29.50 ± 0.29^{b}	56.33 ± 0.60^{ab}	78.88 ± 0.73^{a}
SMT150	29.00 ± 1.32^{b}	51.16 ± 1.64^{ab}	73.17 ± 2.46^{a}
SMT165	25.67 ± 0.33^{b}	50.33 ± 0.44^{ab}	74.67 ± 1.09^{a}
SMT180	27.17 ± 0.17^{b}	52.17 ± 0.17^{ab}	75.17 ± 1.20^{a}
SMT195	27.17 ± 1.01^{b}	49.50 ± 0.50^{ab}	74.15 ± 1.59^{a}
SMT210	28.33 ± 0.67^{b}	52.67 ± 0.73^{ab}	74.50 ± 0.87^{a}
SMT225	25.65 ± 1.09^{b}	49.83 ± 0.72^{ab}	73.67 ± 1.97^{a}
SMT240	25.67 ± 0.44^{b}	49.67 ± 0.33^{ab}	72.33 ± 1.01^{a}

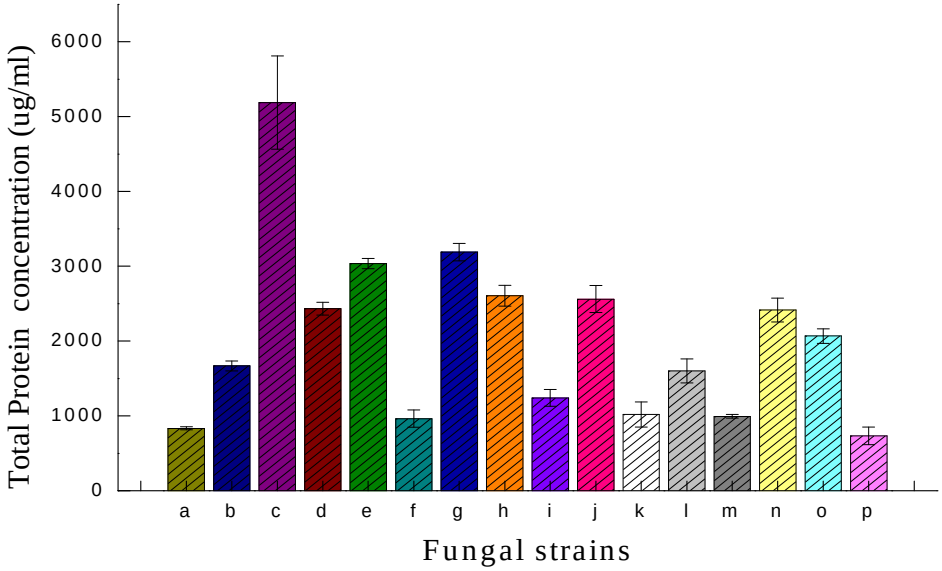
Data are means of three replicates; ± values represents standard deviation of the mean. Mean followed by same letters in the same row are not significantly different ($P<0.05$) from each other according to Turkey's Multiple Comparison Test

Table. 3 Linear Mycelial Growth rate of wild and mutants strains of *L. subnudus*^{wt} on Yeast Mannitol Agar

Fungal Strains	Linear Mycelial Growth 24 hrs (mm^3)	48 hrs (mm^3)	72 hrs (mm^3)
SWT	24.00±3.50^{b}	42.00±0.05^{a}	56.75±2.75^{a}
SMT030	22.75±0.75^{b}	41.25±0.25^{ab}	58.25±1.75^{a}
SMT045	22.00±0.00^{b}	42.00±0.50^{ab}	58.50±1.00^{a}
SMT060	20.75±5.25^{b}	42.50±0.75^{ab}	61.00±9.00^{a}
SMT075	20.50 ±0.00^{b}	40.25±1.25^{ab}	55.50±0.00^{a}
SMT090	21.25±2.25^{b}	43.00±1.50^{ab}	59.50±2.00^{a}
SMT105	23.75±0.25^{b}	46.50±0.50^{ab}	63.25±0.75^{a}
SMT120	24.75±1.75^{b}	43.75±1.25^{ab}	63.75±0.25^{a}
SMT135	21.25±2.25^{b}	42.00±2.00^{ab}	55.75±2.75^{a}
SMT150	23.75±0.25^{b}	43.25±1.25^{ab}	57.75±0.75^{a}
SMT165	20.50±0.50b^{b}	40.00±2.0^{ab}	56.75±0.75^{a}
SMT180	24.00±0.50^{b}	42.75±1.75^{ab}	58.50±0.00^{a}
SMT195	21.50±0.50^{b}	41.75±0.75^{ab}	56.75±0.75^{a}
SMT210	23.25±1.75^{b}	42.00±1.00^{ab}	57.75±0.25^{a}
SMT225	20.25±1.25^{b}	39.75±1.75^{ab}	58.50±1.50^{a}
SMT240	18.25±1.00^{b}	38.00±2.00^{ab}	55.70±0.75^{a}

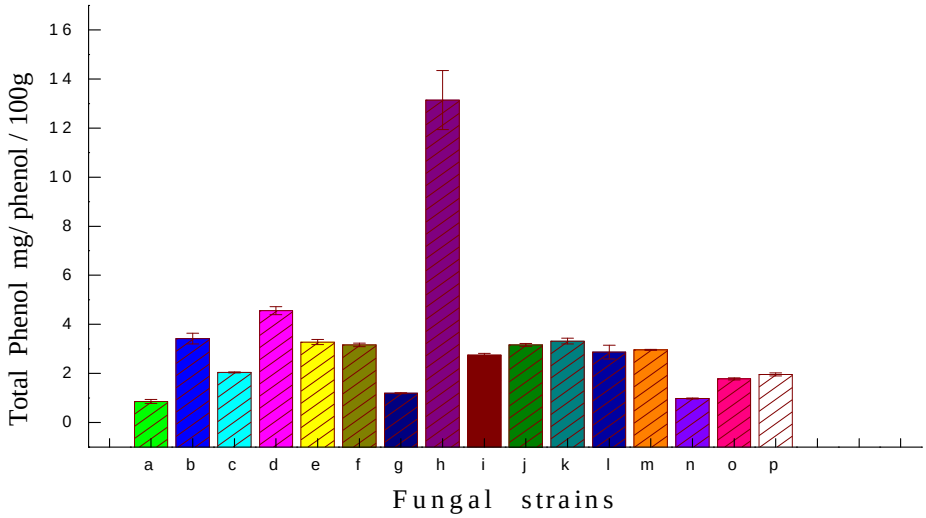
Data are means of three replicates; ± values represents standard deviation of the mean. Mean followed by same letters in the same row are not significantly different ($P<0.05$) from each other according to Turkey's Multiple Comparison Test

Figure.1 Comparing total protein concentration in the wild and mutants fungal strains



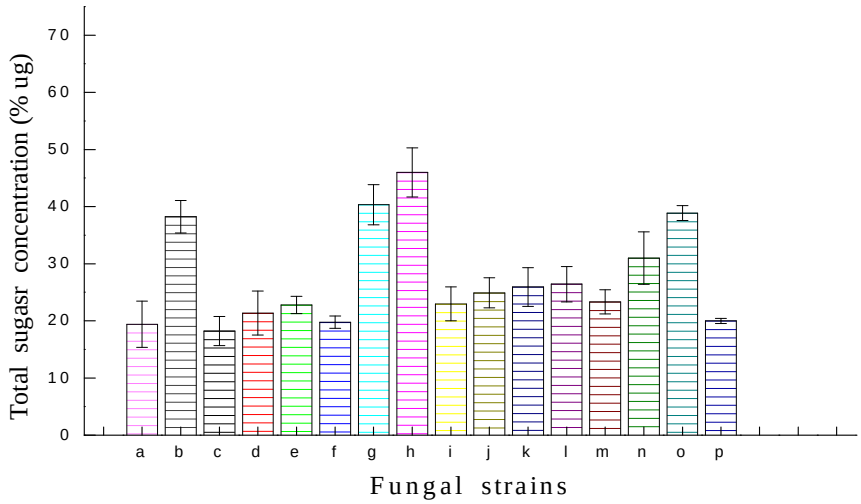
a=SMT030; b=SMT045; c=SMT060; d=SMT075; e=SMT090; f=SMT105; g=SMT120; h=SMT135; i=SMT150; j=SMT165; k=SMT180; l=SMT195; m=SMT210; n=SMT225; o=SMT240; p=SWT

Figure.2 Comparing total phenolic concentration in the wild and mutants fungal strains



a=SMT030; b=SMT045; c=SMT060; d=SMT075; e=SMT090; f=SMT105; g=SMT120; h=SMT135; i=SMT150; j=SMT165; k=SMT180; l=SMT195; m=SMT210; n=SMT225; o=SMT240; p=SWT

Figure.3 Comparing total sugar concentration in the wild and mutants fungal strains



a=SMT030; b=SMT045; c=SMT060; d=SMT075; e=SMT090; f=SMT105; g=SMT120; h=SMT135; i=SMT150; j=SMT165; k=SMT180; l=SMT195; m=SMT210; n=SMT225; o=SMT240; p=SWT

All the mutants (100%) yielded a remarkable increase in total protein estimated among all the mutant strains when compared with the wild strain with the highest and lowest concentrations recorded in SMT-060 (5187.5 µg/ml) and (833.65 µg/ml) respectively as indicated in Figure 1. Total phenolics was also estimated among all the mutant strains and then compared with *L. subnudus* wild strain. Eleven (73.33%) of the mutant strains yielded increase in the total phenolics estimated as compared to the wild strain with highest and least phenolic concentrations recorded in SMT-135 (13.14 mg/100g) and SMT-030 (0.86 mg/100g) respectively as shown in Figure 2. Figure 3 compares the total sugar concentrations among the mutants and the wild. About 80% of the mutants had increase in the total sugar concentrations as compared to the wild, with highest and least sugar compositions recorded in SMT-135 (46.00µg%) and SMT-060 (18.23µg%) respectively.

In this work, ionizing radiations were applied topically to actively growing mycelial culture of the wild strain of the *Lentinus subnudus* on fungal agar as already discussed. This method is in line with the statement of Esser (1971) who reported that the effective method for radiating mushroom is by applying the mutagenic agents to mycelia grown on agar media. However, Djajanegara and Harsoyo (2000) already indicated that one way to introduce genetic variability in organisms is through mutation using chemical agents or ionizing radiations. By using this method, the radiating ions are more evenly distributed and more easily reach the cells of the mushroom. The application of ionizing radiation in this

research is a way of strain improvement which may induce one or more changes in characteristic of the mutated organisms. The presence of phenolics is an indication that these mutants strains are promising source of antioxidant which are of natural origin as already implicated in medicinal mushroom such as *Inonotus obliquus* (In *et al*., 2007), *Pleurotus pulmonarius* (Badole *et al*., 2008) and Kale plants (Imtiyaz et al. 2005). According to Shahidi and Wanasundara (1992), phenolic compounds are known as powerful chain breaking antioxidants and are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). The phenolic compounds may contribute directly to anti-oxidative action (Duh *et al*., 1999).

It is suggested that polyphenolic compounds have inhibitory effects on carcinogenesis in humans, when ingested up to 1g daily from a diet rich in fruits and vegetables (Tanaka *et al*., 1998). There is considerable evidence that these antioxidants present in these strains could help to prevent various diseases including myocardial and cerebral ischemia, diabetes, rheumatoid arthritis, inflammation, and cancer-initiation as well as in aging process (Coyle 1993; Margaill *et al*., 2005) because they have the capacity to quench free radicals. These natural antioxidants have advantage over some synthetic antioxidants such as butylated hydroxyanisola (BHA) and butylated hydroxytoluene (BHT), which have been reported to exerts toxicological effects as compared to natural antioxidants (Saito *et al.,* 2003; Stefanidou *et al*., 2003). From these results obtained, the desired mutant characteristic is economically beneficial.

This result is in alignment with that of Elliot and Langton (1981) that used ultra violet (UV) radiation to produce an *Agaricus bisporus* mutant strain that is resistant to fungicide. In line with this, there is a possibility of producing mutants of *L. subnudus* that has higher productivity through ultraviolet radiation treatment.

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