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The Effect of Mutagenesis on the Production of Mycelia Growth of *Auricularia* Species in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Auricularia species is a jelly-like edible mushroom belonging to the class Basidiomycete, Family Auriculariacea. It has many medicinal properties and is widely consumed in Nigeria. Not much research effort appears to have been conducted on its domestication and commercial production in Nigeria. This study evaluated the use of sawdust and rice bran as potential substrates for its cultivation. Two strains of *Auricularia* species were collected from the wild and identified as *Auricularia polytricha* and *Auricularia subglabra* by genetic DNA extraction. Pure cultures of the species were screened for strain improvement by mutagenesis using UV light and ethyl methyl sulfonate mutagens. Spawn of the wild species and the mutants were produced using sorghum

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grain. The produced spawn was used for the production of the mushroom fruit bodies using sawdust supplemented with rice bran. The biological efficiencies of both wild and mutant were 197% and 90% respectively. A combination of sawdust and rice bran supported the growth of both species of *Auricularia*. The Rate of spawn running during spawn production was also negatively affected by mutagenesis in mutants.

Keywords: Mutagenesis; Auricularia polytrichia; Auricularia subglabra; genetic DNA; substrates.

1. INTRODUCTION

The strong demand for mushrooms has stimulated the increase in world commercial production. Interestingly Africa contributes only about 1% of total world production of edible mushrooms [1,2]. Forests and woodlands that are mushrooms natural habitats have been degraded due to human activity like clearing for agriculture, conversion to other land uses, charcoal burning and logging [3]. Edible mushrooms found in many Africa countries have been used in folk medicine [4]. Therefore there is need to protect the mushrooms from being extinct there is need to domesticate as Africans depend on hunting mushrooms in their wild habitat.

The wood ear mushroom (*Auricularia* species) belongs to the Kingdom Fungi and Division Basidiomycota. There are many species of this mushroom and they are widely distributed in the temperate, tropic and semi tropic regions of the world [5,6]. In nature, *Auricularia* species grow in different habitats such as on dead hardwood trees (*Abies balsamea*) [7]. *Sycamore maple* also known as Sycamore [8] on dead or dying branches of trees or decaying log etc. They grow into different sizes, shapes and color in the wet evergreen and forest habitats [9,10].

Auricularia species has a soft, jelly-like texture, with a mild flavor which makes it edible [11]. Conte and [7,3]. The whole fruit body is edible but not eaten raw and must be washed and cooked [12]. The fruit body can be smooth, as is typical of younger specimens or undulating with folds and wrinkles and the colour becomes darker with age [7]. The inner surface is lighter grey-brown in colour and smooth. It is sometimes wrinkled with folds and may have "veins", making it appear even more ear-like when dry [9,10]. The internal characteristic has hyphal stratification and abhymenial hair with medulla in where medulla is surrounded by large zona laxa superioris and narrower zonz laxa inferioris [13]. .*Auricularia* mushroom is the fourth commercially cultivated in the world after *Agaricus, Lentinus,* and *Pleurotus* species [11]. In Nigeria, commercial production of mushroom has been limited to *Pleurotus* species which are only available to the rich and hotel.

The most common method of cultivation of *Auricularia* species is the log or bag culture methods on hardwood chips and/or sawdust (Mcintosh, 2009; Irawati et al. [14]. The mushroom has been used in the treatment of hemorrhoids, hemoptysis, angina, diarrhea, gastrointestinal upsets, healing of colds and fevers in China [15] Harding, [8]. Research reveals that *Auricularia* extract prevents blood clotting (Harding, 2008),[8] stroke, and heart attack [16] and effective in treating diabetes [17].

Mycelia mating, protoplast fusion, induced mutation, cross breeding and molecular genetics transformation has been employed in mushroom breeding [18]. Molecular breeding by gene transformation has been used to produce new strain with specific property [19]. Mutagenesis has been reported to improvement yield and nutritional quality in some mushroom species. Improved colour quality and sporelessness have also been reported in mutant strains of some mushroom species [20,21] has used protoplast fusion to improve desired mushroom traits. This method has been used to produce mushroom hybrids when conventional methods cannot be achieved. Mushroom produces billions of spores into the air, and this causes health problems like allergies and fever attacks [22]. The basidiospore and mycelia of two strains of *Pleurotus florida* and *P. sajor-caju* have been exposed to UV radiation in order to develop low spore strain [23,24]. Mutagenesis of two chemical treatment and UV irradiation has been applied for induction of sporelessness in mutant strains of *Coprinus cinerus, Pleurotus ostreatus* and *Pleurotus pulmonarius* [20].

The goals of mushroom breeding are to produce high yield and good qualities and reduced production cost [21,25] generated cold tolerant strain of *Volvariella volvacea* by random mutagenesis using alkylating mutagen ethyl

methyl sulfonate (EMS). Chemical mutagens are more effective than physical mutagens but chemical mutagens are less suitable due to its disadvantages like uneven penetration into cell wall of mushroom mycelia and target cells, low reproducibility and health risks [26].

To the best of my knowledge and up to date, *Auricularia* species production has not been documented in Nigeria. Africa, particularly Nigeria is still far behind in this regard and cultivation of edible mushroom has experienced unprecedented advancement [27]. There is increasing demand for continuous supply of quality mushrooms, *Auricularia* inclusive, hence the need for this research work.

2. MATERIALS AND METHODS

2.1 Strain Collection

Two strains of *Auricularia* species (EW 001 and EW 002) were collected from the wild in the southern part of Nigeria (University of Benin city) and used for this work. The isolates were identified by a Mycologist (Professor Omon Isikhenen) at University of Benin.

2.2 Isolation of Pure Culture

The collected *Auricularia* species were hydrated, washed several times (six to seven times) with normal saline water, dipped in 70% alcohol for 2 minutes and rinsed with normal saline water. The species were cut into tiny pieces of about 2 mm by 2 mm and inoculated into sterilised potato dextrose agar (PDA). 0.04 g of streptomycin sulphate antibiotic was added to the medium to prevent the growth of bacteria contaminants. Inoculated plates were sealed and incubated at 25°C for mycelia growth. The isolated pure cultures were maintained in PDA slants at 4°C.

2.3 Mutagenesis

2.3.1 Physical mutagen (UV light)

The physical mutagen was carried out using Sharma and Sharma, 2014 method Actively growing cultures (3 days old) of collected *Auricularia* species (EW 001 and EW 002) on PDA plates were exposed to UV light (244nm, Millipore xx63 70000) for 30, 60,90, and 120 minutes intervals to induce mutation. The generated mutants were subculture on PDA plates, incubated at 25 °C for 7 days. The diameter of mycelia growth was measured. The isolated wild species were used as control.

2.3.2 Chemical mutagen (ethyl methyl sulfonate)

The isolated pure cultures of *Auricularia* species (EW 001 and EW 002) were carried out using Sharma and Sharma, 2014 method by inoculating on sterile Potato Dextrose Agar medium that contained different concentrations of ethyl methyl sulfonate (EMS) ranging from 0.001, 0.002, 0.003, 0.004 and 0.005 % and incubated at 25±2ºC for 7 days to generate mutants. The rate of mycelia ramification in the mutants and the wild strains, determined by the diameter of mycelia growth was monitored.

2.3.3 Cultivation of wild and mutant strains of isolated *Auricularia* species

The substrate for the cultivation was composted as follows; sawdust (79%), calcium carbonate (2%) and rice bran (19%) by Onyango *et al.,* 2011 methods. Sawdust and calcium carbonate were mixed in the proportion as stated above and the moisture content adjusted to 60-65%. The properly mixed substrates were piled up in cone-shape heap to about 1meter high for composting. The heap was turned every day to allow evenly distribution of the generated heat to enable lignin decomposition and the heap left to stand for 30 days. After 30 days of composting, rice bran was added in the required proportion and mixed thoroughly. The compounded substrate was packed inside heat resistant polyethylene bags (1000 g/bag) and sterilized at 121 °C for 30 minutes. The sterilized bags were inoculated with the produced spawn and incubated in the dark at 25±3° C, until full mycelia colonization was achieved. Rate of mycelia colonization was monitored. The colonized bags were transferred to fruiting house for fruit body growth. The date of initiation of primordial was noted and biological efficiency calculated.

3. RESULTS AND DISCUSSION

3.1 The Isolation of *Auricularia* Species

The isolated species (EW001, EW002) were identified by Abikoye et al. [28]. as *Auricularia polytricha* and *Auricularia subglabra* respectively. Physical mutagenesis (UV light exposure) generated two mutants from each of the identified species; EW1M1and EW1M2, and EW2M1 and EW2M2 from EW001and EW002 respectively. Mutagenesis by physical irradiation, retarded the rate of mycelia running (Fig. 1a). The mycelia growth of mutant and wild of the

Auricularia were observed after incubation for five days at 25-26°C. The *Auricularia* EW001 produced higher mycelia growth than the *Auricularia* EW002 in the physical and chemical mutagens. The four different UV light treatments supported mycelia growth with variations except the 90mm that was not full ramified. EW 001 (30min, 60min and 120 min) showed mycelia growth retarded of 35mm, 35mm and 30mm respectively when compared with the control with the highest mycelia growth of 55mm. EW 002 (60min, 90min and 120min) showed retarded mycelia growth of 35mm, 40mm and 45mm respectively when compared with the control of 70mm mycelia growth (Fig. 1a).

Chemical mutagenesis using ethyl methyl sulfonate (EMS) generated 5 mutants each; E1M1, E1M2, E1M3, E1M4 E1M5 from EW001 and E2M1, E2M2, E2M3, E2M4 E2M5 from EW002. The EW 001 and EW002 were further used because of the better yield of mycelia and faster rate of their mycelia growth. The 90 min and 120 mins of the EW 001 and EW 002 formed the EW1M1, EW1M2, EW2M1 and EW2M2 mutants that were used in this study. Concentrations with 0.001- 0.004% showed mycelia growth for the two selected species and no growth was observed for EW 001 (0.005%) whereas EW 002 (005%) showed slow growth of 40mm. A retarded mycelia growth of 35mm, 65mm and 60mm were observed in EW 001 (0.003%), EW 005 (0.002% and 0.004%) respectively. Both *Auricularia* EW001 and EW002 were further produced into spawn and then cultivated on sawdust.

Variation of exposure time of the isolates to physical mutagen (UV light) did not affect rate of mycelia ramification of the generated mutants of EW001. However, there was an observed increase in the rate of retardation of mycelia running of mutants of EW002 with increase in exposure time (Fig. 1a). Similar result has been recorded by Ravishanker [23]. This result is also in agreement with [20] who observed a retardation of mycelia growth of some mushroom species they subjected to physical mutagenesis using UV light.

Mutagenesis by chemical mutagen also retarded the rate of mycelia running of the mutants. Similar results have been reported by Ravishanker et al. [23] and Sharma and Sharma [20] who also used ethyl methyl sulfonate to induced mutation in some species of *Pleurotus*. The retardation of the mycaelia growth rate was more pronounced in isolate EW002 than in EW001 (Fig. 1b). The retardatory effect of the mutagen generally increased with increase in the concentration of the mutagen (Fig. 1b).

A combination of sawdust and rice bran supported the growth of both species of *Auricularia* and their mutants. Colonization of the production substrates was faster in the wild strains than the mutants. Full colonization was achieved in 30 days in the wild strains, while it took 50 days for the mutants to fully colonize the substrate. The pin head of the wild strains appeared after the 3rd day of exposure while the mutants pin head appeared on the 10th day. Similar result has been reported by Irawati *et al*. [14]. The sporophores of the wild strains were bigger and wider than the mutants' strain that appears smaller and never opened (Plates 1a and 1b).

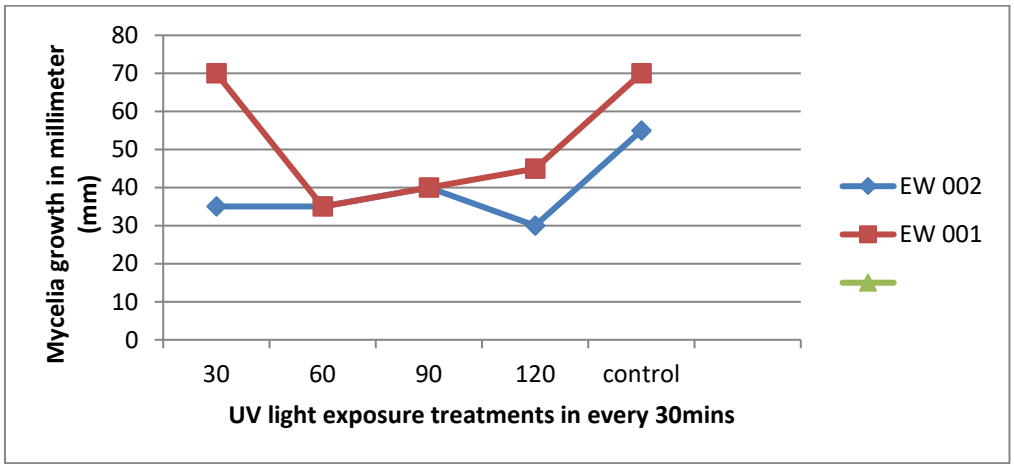

Fig. 1a. The mycelia growth of two *Auricularia* species after exposure to irradiation

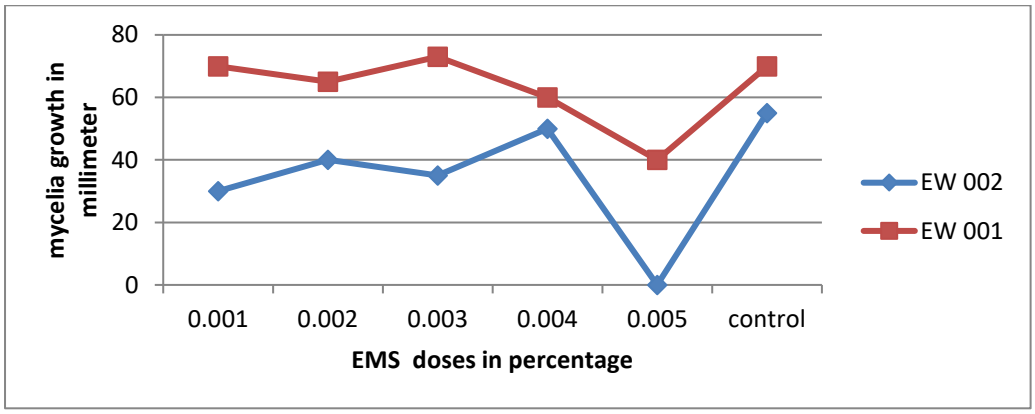

Fig. 1b. The mycelia growth of two *Auricularia* species after different treatment with Ethyl Methyl Sulfonate (EMS)

Plate 1a. Matured sporophores from the EW01 grown on sawdust and rice bran

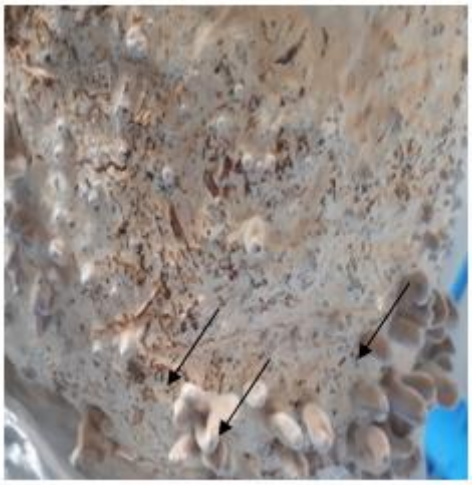

Plate 1b. Matured sporophores from EW1M1.

In this report, the *Auricularia* species fruit bodies were very low in both wild and mutant strains when grown on sawdust and rice bran substrate. The biological efficiencies of wild is 197% and mutant species 90%. Though both wild species and mutant strains ultilises the nutrients in the substrates, the wild species gene was not altered this makes it easier to ultise the nutrient in the substrate unlike the mutant strain genes that has been altered by mutagens. Biological efficiencies are highly affected by the mutagens. [29] reported that sawdust mixed with oil palm front and sawdust mixed with empty fruit bunch and spent grain gave the highest yield of sporophore with 288.9% and 260.7% biological efficiencies almost three times the control (sawdust) when *Auricularia polytricha* was grown on these substrates. This is an indication that *Auricularia* species uses the chemical components of the wood especially cellulose and lignin for mycelia growth and produce fruiting body. [30] observed wheat straw and maize cob to be the best substrates for *Auricularia* species with higher yield of mushroom compared to the other substrates they used [31,32,33].

4. CONCLUSION

A combination of sawdust and rice bran in the right proportion could be a suitable substrate for the cultivation of *Auricularia* species. Mutagenesis, either by physical means (UV light) or chemical means (EMS) negatively affects the mycelia growth of this mushroom and by extension its production. The negative effect of mutagenesis on the mycelia of the *Auricularia* species (mutant strains) may have resulted in aberrant, impaired or loss of function for the gene responsible for yield and the accumulation of the

mutation may have lead to the sporophore folding that refused to open.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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