The effect of mutagenesis on the production of mycelia growth of *Auricularia* species in Nigeria

Abstract

Auricularia species is a jelly-like edible mushroom belonging to the class Basidiomycete, Family Auriculariacea. It has many medicinal properties and 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 grain. The produced spawn were used for production of the mushroom fruit bodies using sawdust supplemented with calcium carbonate and 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*. Rate of spawn running during spawn production was also negatively affected by mutagenesis in mutants.

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 (Yongabi and Anchang, 2014; Ofodile and Yusuf, 2010). 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 (Kick *et al.*, 2008; Mukerji and Manoharachary, 2010). In nature, *Auricularia* species grow in different habitats such as on dead hardwood trees (*Abies balsamea*) (Conte and Læssøe, 2008), *Sycamore maple* also known as Sycamore (Harding, 2008), 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 (Sterry and Barry, 2009; Mohanan, 2011).

Auricularia species has a soft, jelly-like texture, with a mild flavor which makes it edible (Priya, *et al.,* 2016; Conte and Læssøe, 2008). *Auricularia* mushroom is the fourth commercially cultivated in the world after *Agaricus*, *Lentinus*, and *Pleurotus* species (Priya *et al.*, 2016). This mushroom is highly consumed in most parts of the world. Mushrooms have antitumor, hypoglycaemic, anticoagulant properties (Ma *et al*., 2018; De Silver *et al.*, 2012a). The mushroom has been used in the treatment of hemorrhoids, hemoptysis, angina, diarrhea, gastrointestinal upsets, healing of colds and fevers in China (Chen *et al*., 2008; Harding, 2008). Research reveals that *Auricularia* extract prevents blood clotting (Harding, 2008), stroke, and heart attack (Elkhaleeb *et al*., 2019) and effective in treating diabetes (Lu *et al.*, 2018b).

Comment [DBAV1]: Of the benefits associated with the species, mention the specific component that provides the benefit for each case.

Mycelia mating, protoplast fusion, induced mutation, cross breeding and molecular genetic transformation has been employed in mushroom breeding (Lee *et al.*, 2011). Molecular breeding by gene transformation has been used to produce new strain with specific property (Lim *et al*., 2009). 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 (Sharma and Sharma, 2014). Prabu *et al.* (2011) has used potoplast 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 (Chakravarty, 2011).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 (Ravishankar *et al.*, 2006; Bahram, *et al.*, 2014). 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* (Sharma and Sharma, 2014).

The goals of mushroom breeding are to produce high yield and good qualities and reduced production cost (Prabu *et al.*, 2011). Liu *et al.* (2011) 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 (Kang *et al.*, 2011).

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 (Osemwegie and Dania, 2016). There is increasing demand for continuous supply of quality mushrooms, *Auricularia* inclusive, hence the need for this research work.

MATERIAL AND METHODS

Strain Collection

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

Comment [DBAV2]: You must make a more specific indication of the collection place.

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Isolation of pure culture:

The collected *Auricularia* species were hydrated, washed several 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.

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Mutagenesis:

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.

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±2 ºC 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.

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.

RESULTS AND DISCUSSION

The isolation of *Auricularia* species.

The isolated species (EW001, EW002) were identified by Abikoye *et al*. (2016) 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. 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. Mutagenesis by physical irradiation, retarded the rate of mycelia running (Figure1a). 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 (Figure 1a). Similar result has been recorded by Ravishanker (2006). This result is also in agreement with Sharma and Sharma (2014) who observed a retardation of mycelia growth of some mushroom species he 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*. (2006) and Sharma and Sharma (2014) 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 (Figure1b). The retardatory effect of the mutagen generally increased with increase in the concentration of the mutagen (Figure1b).

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*. (2012). The sporophores of the wild strains were bigger and wider than the mutants' strain that appears smaller and never opened (Plates 1a and 1b).

Comment [DBAV5]: There is no strict discussion of results, you must base your results with the effects at the biochemical level in the strains studied.
You must give the importance of the results obtained.

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.

Comment [DBAV6]: Conclusions need to be reconsidered

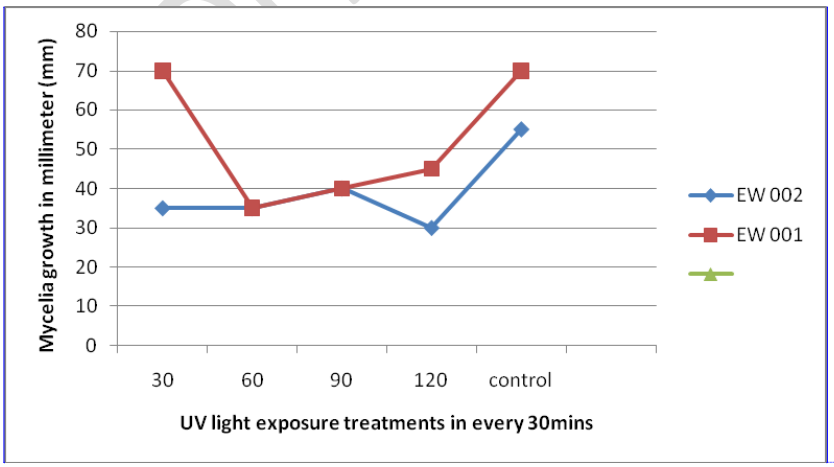

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Figure 1a: The mycelia growth of two *Auricularia* species after exposure to irradiation.

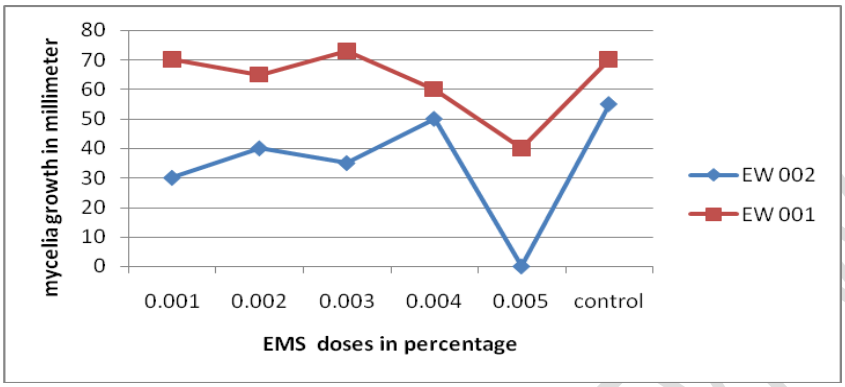

Figure 1b: The mycelia growth of two *Auricularia* species after different treatment with Ethyl Methyl Sulfonate (EMS).

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

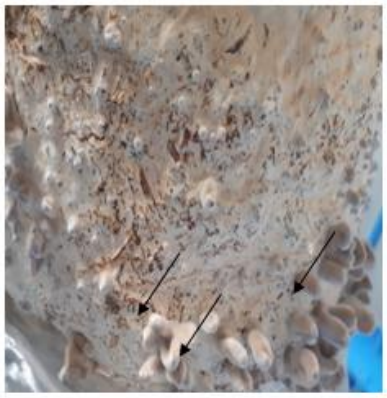

Plate 1b: Matured sporophores from EW1M1.

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Comment [DBAV8]: The references can increase to 30, only it must be updated since there are only 12% of recent references and 88% of references that are more than five years old.

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