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Comparative studies of production of exopolysaccharides from wild type, mutants and hybrid of *Pleurotus pulmonarius* and *Pleurotus ostreatus* in submerged culture

Azuonwu, T.C [1]. , *Aina, D.A[1]., Odutayo, F.O.I.[1] , Majolagbe,O.N.[2] , Ezeamagu, C.O.[1] and Aina, F.O[3]

[1]Department of Microbiology, Babcock University, Ilishan-Remo, Ogun State.
[2].Department of Pure and Applied Biology, Ladoke Akintola University of Technology,Ogbomoso,Oyo State.
[3] Paediatrics Unit, Babcock University Teaching Hospital, Ilishan-Remo, Ogun State.

*Corresponding author: <ainaa@babcock.edu.ng>

Abstract

Background
Some macrofungi have been studied and found to possess antimicrobial and nutritional properties. Mutation and hybridization of the wild types of different species have also been found to improve these qualities.

Methods
Spawn of Pleurotus pulmonarius and Pleurotus ostreatus were obtained and cultured on Potato Dextrose Agar and Malt Extract Agar. Mutants of P. pulmonarius and P. ostreatus were obtained by exposing the cultures to Ultra-violet ray at wavelength of 280nm for 30-60 minutes. The hybrid was obtained by culturing the wild types of both organisms on the same solid medium on plate at a distance of 8mm apart. The point of contact between both organisms was taken as the hybrid and used in submerged fermentation to obtain the mycelia mats and exopolysaccharides (EPS). The antibacterial activity was determined by testing the EPS against Staphylococcus aureus, Pseudomonas sp. Enterobacter sp, Escherichia coli, Salmonella sp, and Yersinia enterocolitica, obtained from environmental samples, using disc diffusion method.

Results
All the EPS produced by these organisms had antibacterial activity except those produced by two of the mutants (PPO_2 and PO_1). Acetone which was used as a control had very little antagonist effect on the isolates. The EPS were also found to contain phenolics. Mutants and hybrid of P. pulmonarius and P. ostreatus produced EPS which had better antibacterial activity against different pathogenic microbes compared with their wild type except PPO_2 and PO_1.

Conclusions
Since exopolysaccharides produced through submerged fermentation of P. pulmonarius and P. ostreatus, their hybrid as well as mutants of both organisms have antimicrobial activities with higher activities recorded from mutants derived from combinations of both organisms, large scale submerged fermentation of these organisms could be carried out to obtain antimicrobials that pathogenic bacteria are not likely to be resistant to, rather than depending solely on synthetic antimicrobial agents.

Keywords: Exopolysaccharides; Antibacterial activity; Macrofungi; Phenolics

Introduction

Polysaccharides produced by submerged cultures of higher fungi including mushrooms have been studied and used for pharmaceutical purposes due to their diverse biological activities (Kim *et al.,* 2003). Exopolysacharides (EPS) are high molecular weight polymers of monosaccharides (>20) and are secreted by microoganisms into the surrounding environments. They have found multi-various applications in various food and pharmaceutical industries.

Species of the genus *Pleurotus* include *P. ostreatus, P. populinus, P. djamor, P. cornucopiae, P. cystidiosus, P. calyptratus, P. dyinus, P. purpureo-olivaceus, P. eryngii, P. sajor-caju* and *P. pulmonarius.* They are characterized by a white spore print, attached to gills, often with an eccentric stip or no stip at all and are popularly called the oyster macrofungi (Miles & Chang, 1997). They grow best within a temperature range of 12-32^{o}C and are widely distributed from the temperate to the tropical regions (Zadrazil, 1978). Their cultivation can be carried out on log, shelf, box, bag and bottle depending on the mushroom variety, market demand and farmer's preference (Won-Sik, 2004). Crude extracts of *P.ostreatus* from fermentation broth has been found to be effective against Gram-positive and Gram-negative bacteria such as *Bacillus* sp., *Escherichia coli, Vibrio cholera, Salmonella typhi* (Periasamy *et al.,* 2005) and *Aspergillus niger* (Gerasimenya *et al*, 2002); Hexane-dichloromethane extract of *P. ostreatus* containing p-anisaldehyde also has been found to have antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger and Fusarium oxysporum* (Okamoto *et al.*, 2002). Erynginan antifungal peptide from *P. eryngii* has effect against *Fusarium osysporum* and *Mycosphaerella arachidicola* (Wang & Ng, 2004); Eryngeolysin- a haemolysin also has effect against *Bacillus* sp. A 12 kDa ribonuclease from *P. sajor-caju* has been studied and found to antimicrobial effect on *Fusarium oxysporum, Mycosphaerella arachidicola, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ngai & Ng, 2006). Compared with their wild type, most mutants of macrofungi are able to produce metabolites with better qualities (Aina *et al.,* 2013; Majolagbe *et al.,* 2013). Improved characteristics such as increased productivity and adaptability to a wide range of temperatures were observed when the mycelia and basidiospores of five strains of *Pleurotus* were subjected to UV irradiation (Beejan and Nowbuth, 2009). Isolated protoplasts from mycelium of a *P. erynjii* strain exposed to UV radiation resulted in the recognition of a mutant dikaryon that was sporeless (Obatake *et al.,* 2003).

Although, *P. ostreatus* and *P. pulmonarius* have been known to produce metabolites that contain bioactive substances that are medicinal and nutritive, the metabolites produced from submerged fermentation of the hybrid and mutants of both organisms have not been studied. Thus, the general objective of this study is to compare the effectiveness of exopolysaccharides from the wild types with that of the mutants and hybrid of both macrofungi.

Materials and methods

The spawns of wild type *Pleurotus pulmonarius* (PP) and *Pleurotus ostreatus* (PO) were obtained from the Mycology Laboratory, University of Ibadan. Both organisms were cultured on Malt Extract Agar (MEA) and incubated at room temperature. The hybrid of both organisms was obtained by culturing both organisms on the same culture plate at a distance of 8mm apart; the intersection of both organisms was taken as the hybrid (Majolagbe *et al*., 2013).

Mutation of the organisms using UV radiation

Mutation of the organisms was carried out by introducing 2 actively growing cultures of each organism to short wavelength ultraviolet radiation (λ=280 nm) at different durations of 30 and 60 minutes respectively, consequently, 2 mutants were obtained for each organism as well as hybrids of the two organisms respectively (PP_1, PP_2, PO_1, PO_2, PPO_1 and PPO_2). Mycelia plugs obtained from these cultures were transferred to fresh MEA plates and incubated at 25^{o}C. A total of 9 organisms (PP, PO, PPO, PP_1, PP_2, PO_1, PO_2, PPO_1 and PPO_2) were obtained and used in this work. These organisms were suspended in MEA slants and stored in the refrigerator as stock cultures which could be used for further reference or study.

Submerged fermentation

Complete mushroom medium was prepared according to the modified method of Nwokoye *et al.* (2010) and this was done by adding $MgSO_4$ (0.5 g), KH_2PO_4 (0.5 g), Peptone (5 g) Maltose (10 g), Ascorbic acid (0.05 g) and Streptomycin (0.1 g). The initial pH of the medium was adjusted to pH 9 by adding 1M NaOH. Using a 7 mm sterile cork borer, equal size of each fungal plug was collected and inoculated onto 100 ml of the mushroom basal medium and incubated under static condition at 28^{o}C for 8 days and observed daily.

The exopolysaccharide (EPS) was extracted from the basal medium by adding acetone in a ratio to the liquid

at a ratio of 2:1 and kept in the refrigerator for 24 hours and then centrifuged (Majolagbe *et al*., 2014). The residue was stored and used as the exopolysaccharide. The total phenolic content was determined using Folin-Ciocalteau colometric method as described by Zovko *et al.* (2010).

The antibacterial activity of each of the EPS was tested on 6 bacteria including *Staphylococcus aureus*, *Pseudomonas* sp., *Enterobacter* sp., *Escherichia coli*, *Salmonella* sp., *and Yersinia enterocolitica*. using microbiological standard method.

Results and discussion

Table 1 : Average weight of mycelia and EPS from each organism after 8 days

Sample code	Weight of mycelia (g)	Weight of EPS (g/100ml)
PO	1.5	0.04
PP	0.5	0.06
PPO	0.4	0.02
PO1	1.2	0.02
PP1	0.2	0.08
PPO1	0.4	0.05
PO2	0.3	0.10
PP2	0.3	0.09
PPO2	0.1	0.02

Table 2: Average zone of inhibition of EPS against bacterial isolates (mm)

Isolates	PO	PP	PPO	PO_1	PP_1	PPO_1	PO_2	PP_2	PPO_2	Acetone
E. coli	18	18	17	7	22	16	17	13	7	7
Yersinia sp.	16	19	14	7	16	16	17	12	7	7
S. aureus	17	18	15	7	20	7	18	13	7	7
Salmonella sp.	7	19	18	7	22	7	30	17	7	7
Pseudomonas sp.	7	7	7	7	7	7	7	7	7	7
Enterobacter sp.	16	20	15	7	17	16	17	7	7	7

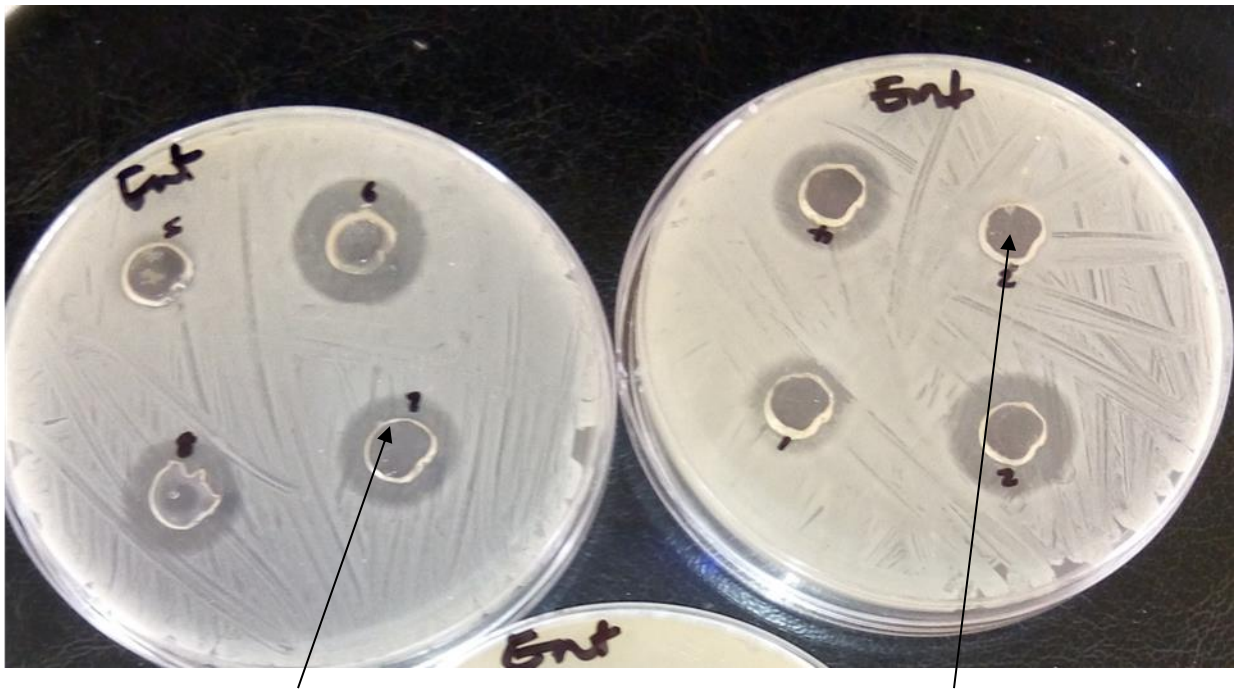

Fig 1: Antibacterial Activity of EPS

Table 3: Total phenolic content of EPS

Sample	Absorbance	Phenolic content
PO	0.030	0.0048
PP	0.021	0.0054
PPO	0.081	0.0012
PO_1	0.087	0.0009
PP_1	0.025	0.0051
PPO_1	0.051	0.0033
PO_2	0.005	0.0065
PP_2	0.020	0.0054
PPO_2	0.048	0.0035

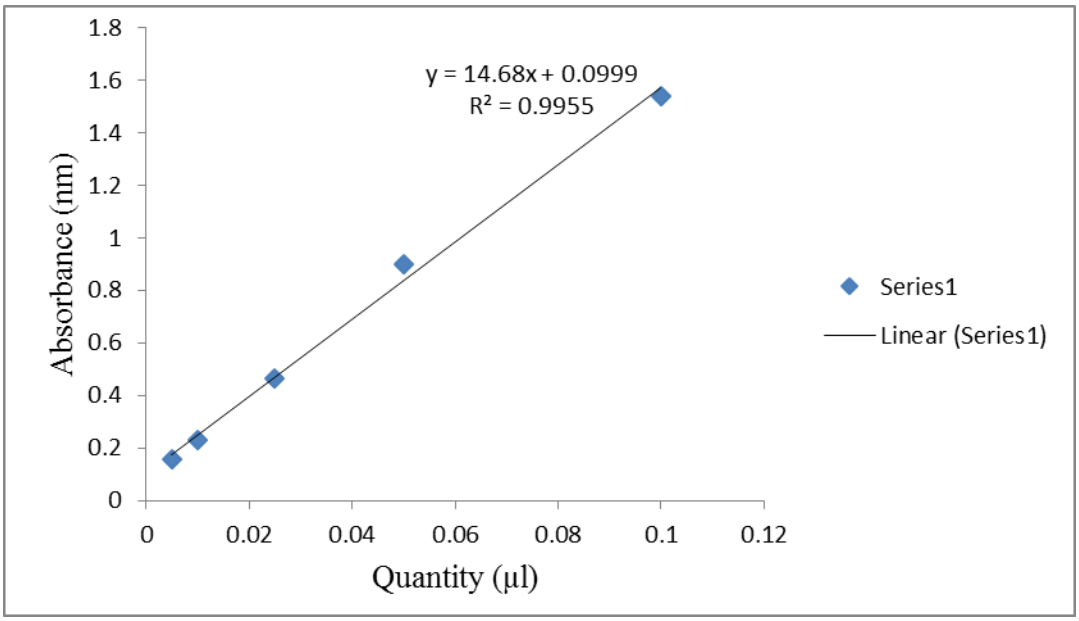

Figure 2: Absorbance of solution A (Gallic acid) at 765nm

The antibacterial activity of the EPS on 6 bacterial isolates indicated that *Pseudomonas* sp. was resistant to all 9 EPS while *Salmonella* sp. had the highest susceptivity (30 mm) when EPS from PO_2 was used against it as seen in Table 2. The high antibiotic resistance of *Pseudomonas* sp. has been reported by Jombo *et al.* (2008). The resistance of *Pseudomonas* sp. to the EPS from PP is in agreement with the study by Adebayo *et al.* (2012) who reported that the metabolite from *P. pulmonarius* had antagonistic effect on all test isolates, except *Pseudomonas* sp.

PP_1 had a better antimicrobial actvity against some of the isolates compared with its wild type PP. This is in agreement with the study by Majolagbe *et al.* (2013) who reported higher productivity from mutants of *Lentinus subnudus* compared with its wild type. The wild type hybrid of both organisms (PPO) showed activity against some of the isolates and was more potent than its mutants. PO_2 showed the highest antimicrobial activity against the test isolates as it showed the highest zone of inhibition when used against *Salmonella* sp. Acetone which was used in the extraction of the EPS showed very little antimicrobial activity against the isolates indicating that antimicrobial effect of the EPS was not as a result of the acetone used in the extraction process.

Total phenolic content

The concentration of phenolics in each of the EPS was determined using gallic acid as a standard. Substituting the absorbance of each of the EPS into the straight line equation y=14.68x+0.0999, R^2 = 0.9955, PO_2 had the highest concentration of phenolics compared with the others, while PO_1 had the least phenolic content. This is in line with the study conducted by Majolagbe *et al.* (2013) who compared the total phenolic concentration of wild type and mutants of *Lentinus subnudus*, and reported that the

mutant which was exposed to UV-light for 60 minutes had the highest phenolic concentration.

PP and PP_2 had high phenolic content compared with PP_1, while PPO_1 and PPO_2 had high phenolic content compared with PPO.

This result showing the presence of phenolics *P. ostreatus* and *P. pulmonarius* agrees with the results of the study done by Obodai *et al* (2014) who analysed the total phenolic content of cultivated *P. ostreatus* and *P. pulmonarius.* In their study, *P. pulmonarius* contained phenol but *P. ostreatus* had the highest total phenolic content.

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References

Aina, D.A., Oloke, J.K., Omomowo, I.O., Alatise, F.A., Akeredolu, A.A. & Aina, F. O. (2013). The influence of metabolites from wild type and mutant strains of *Schizophyllum commune* on *Escherichia coli* induced changes in Albino rats. *Sch. Acad. J. Biosci*.1(4):136-143.

Beejan, P.H.F. and Nowbuth, R. (2009). Use of irradiation for the induction of mutations in oyster mushrooms for improvement of strains. Induced plant mutations in the genomics era. *F. A. O. Rome.* 289-292.

Gerasimenya, V.P., Efremenkova, O.V., Kamzolkina, O.V., Bogush, T.A., Tolstych, I.V. & Zenkova, V.A. (2002). Antimicrobial and antitoxical action of edible and medicinal mushroom *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. Extracts. *International Journal of Medical Mushrooms. 4:* 48–54.

Jumbo, G.T.A., Jonah, P. & Ayeni, J.A. (2008). Multidrug resistance *Pseudomonas aeruginosa* isolated from the urine of patients with urinary tract infection. *Nigerian Journal of Physiological Science*. 23(1-2): 105-109.

Majolagbe, O.N., Oloke, J.K., Deka-Boruah, H.P. & Aina, D.A. (2013). Extraction of Pyranose 2-oxidase from submerge culture of wild, mutants and Hybrid strain of *Lentinus subnudus* and determination of its optimum reaction conditions. *International Journal of Basic and applied sciences.3(2): 80-90.*

Majolagbe, O.N., Oloke, J.K., Deka-Boruah, H.P. & Aina, D.A. (2013). 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. *International Journal of Current Microbiology and Applied Science.*

Miles, P.G. & Chang, S.T. (1997). Mushroom Biology: Concise Basics and Current Development. *World Scientific Publishing Company, New York.*

Ngai, P.H. & Ng, T.B. (2006). A haemolysin from the mushroom *Pleurotus eryngii. Appl Microbiol Biot.* 72: 1185-1191.

Nwokoye, A.I., Kuforiji, O.O. & Oni, P.I. (2010). Studies on Mycelia Growth Requirements of *Pleurotus ostreatus* (Fr.) *Singer. International Journal of Basic and Applied Sciences.* 10(2): 47-53.

Obatake, Y., Murakami, S., Matsumoto, T. & Fukumasai-Nakai, Y. (2003). Isolation and characterization of a sporeless mutant in *Pleurotus eryngii*. *Mycoscience*. 44: 33-40.

Obodai, M., Owusu, E., Schiwenger, G.O., Asante, I.K. & Dzomeku, M. (2014). Phytochemical and Mineral Analysis of 12 Cultivated Oyster Mushrooms (*Pleurotus* Species). *Advances in Life Science and Technology*. 26.

Okamoto, K., Narayama, S., Katsuo, A.,Shigematsu, I. & Yanase, H. (2002). Biosynthesis of *p* anisaldehyde by the white-rot basidiomycete *Pleurotus ostreatus*. *Journal of Bioscience and Bioengineering.* 93: 207–210.

Periasamy, K. (2005). Novel antibacterial compounds obtained from some edible mushrooms. *International Journal of Medicinal Mushrooms.* 7: 443– 444.

Wang, H. & Ng, T.B (2004). Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii*. *Peptides.* 25: 1–5.

Won-Sik, K. (2004). Descriptions of commercially important *Pleurotus* species. *Mushroom Growers Handbook*. Pg 54

Zadrazil, F. (1978). Cultivation of *Pleurotus*. In: The Biology and Cultivation of Edible Mushroom Ed. Chang S.T. and Hayes W.A. Academic Press, New York, pp. 521-554

Zovko, M.K., Kremer, D., Gruz, J., Strnad, M., Bisevac, G., Kosalec, I., Samec, D., Piljac, J.Z. & Karlovic, K. (2010). Antioxidant and antimicrobial properties of *Moltkia petraea* (Tratt) Griseb. Flower, leaf and stem infusions. Food and Chemical Toxicology. 48(6): 1537-1542