European Journal of Agriculture and Forestry Research
Vol.9, No.2, pp. 26-37, 2021
Print ISSN: ISSN 2054-6319 (Print),
Online ISSN: ISSN 2054-6327(online)

ISOLATION AND IDENTIFICATION OF SOME FUNGI AND BACTERIA IN SOILS COLONIZED BY EDIBLE WILD MUSHROOM *AGARICUS SILVATICUS* G. J. KEIZER IN RIVERS STATE, NIGERIA.

Dimkpa, S. O. N. and Orikoha, E. C.
Department of Crop/Soil Science, Rivers State University, Port Harcourt, P.M.B 5080, Rivers State, Nigeria.

ABSTRACT: *This research on the isolated and morphologically identification of fungi and bacteria in soils naturally colonized by wild mushroom Agaricus silverticus was carried out in the crop protection laboratory at the Rivers State University. Soil samples were collected from 4 different locations in Ogwe, Ukwa West Local Government Area, Abia State, Nigeria: 3 sample sites from Obiahia kindred the mushroom colonized soils in its natural habitats and the fourth a control plot from Obiawom kindred where mushroom was not found. The experimental result significantly (P<0.05) revealed the presence of six fungi genera: four mushroom inhibiting microbes namely Penicillium spp., Sclerotium spp., Mucor spp. and Aspergillus spp. in decreasing order from the control sites where Agaricus mushroom was not found and two other benefiting fungi genara: Yeast and Fusarium spp. from the Agaricus Mushroom colonized soils. However, fairly insignificant number (P>0.05) of Penicillium spp. was also recorded in site three of the Agaricus colonized soil habitat though overshadowed by the very high presence of Yeast. Similarly, six bacterial genera (Bacillus spp, Proteus spp, Micrococcus spp, Streptococcus spp, Staphylococcus spp and Pseudomonas spp) were isolated and morphologically identified. It is note-worthy that Pseudomonas and Bacillus spp. which enhances mycelia development and promote primordial differentiation were significantly present in Agaricus colonized soil samples and absent in control soils. This is the first trial report for the microbial assay of Agaricus silvaticus in Rivers State and Nigeria at large and will contribute to the artificial cultivation and management of edible wild mushroom Agaricus spp which is a dietary delicacy in Southern Nigeria and most African Countries.*

KEYWORDS: *Agaricus silvaticus*, fungi and bacteria in soil samples, mycelium, spawnand pin-head.

INTRODUCTION

Agaricus silvaticus (or *Agaricus sylvaticus*) is an edible Mushroom found growing in the humid tropical forests on well decomposed forest litters, soil humus and manure piles (Obe and Mshigeni, 2013). It is found seasonally in the wild around early summer, or September through to November in many parts of the world including Nigeria (Orikoha and Dimkpa, 2021; Wang *et al*., 2003 and 2004). It is a saprophytic fungus, division: *Basidiomycota,* class: *Agaricomycetes*, subclass: *Homobasidiomycetidea*, order: *Agaricales*, and family: *Agaricacea* (Obe and Mshigeni, 2013). *Agaricus* mushroom is also commonly known as the Scaly Wood Mushroom, Blushing Wood Mushroom or Pinewood Mushroom and a major dietary delicacy in Nigeria particularly the

southern Nigeria (Orikoha and Dimkpa, 2021). However, its domestication in Nigeria has faced several challenges not limited to mushroom diseases of fungi and bacteria origin (Umar and Van-Griensven, 2000), but the little knowledge of the configuration of the mushroom holobiont, that is the interaction and interdependence of the mushroom host plus associated microorganisms, the properties of which can have a significant impact on mushroom growth and productivity (Partida-Martinez, 2017). Mushroom growth therefore consists of the development and fructification of different fungal species in soil or selective substrates that provide nutrients and support for the crop (Carrasco and Preston, 2020). The microorganisms present in these habitats strongly influence, and in some cases are required for the growth and fructification of cultivated mushrooms (Carrasco and Preston, 2020).

Fungal pathogen have been reported to cause severe damage to mushroom production reducing yield, causing deterioration of commercial value, shortening shell life and generally harmful to the mushroom industry (Aleto, 1990; Osemwegie *et al*., 2006; Rao *et al.,* 2007; Ukoima *et al*., 2009). Some fungi such as *Penicilium spp, Aspergillus spp, Mucor spp* etc are notable soil microbes whose presence in the mushroom mycosphere (the environment within and surrounding the mushroom hyphae) might inhibit the growth and establishment of Mushroom mycelium and are prominent and major fungi isolated from mushroom (In-Young *et al.,* 2010). Mucor mycelium is non-rhizonmorphic and lacks the clamp connections that are characteristics of many mushroom mycelia. Thus are vigorous contaminant and seen at various times in spawn production, inhibiting and overwhelming mushroom mycelium. Mucor infected spawn, when inadvertently inoculated into the mushroom compost, can result in the total contamination of the bed within a few days. Chang (2007) reported *Aspergillus spp* as thermophilous fungi living in various mushroom compost, which is also known as a human and animal pathogen. *Aspergillus spp* is the causal agent of aspergillosis, farmer's lung disease and other related disorders. *Penicilium* competes for preoccupancy with green spores and inhibits the formation of fruiting bodies (In-Young *et al.,* 2010). Yeast in the soil on-like the other harmful fungi, is suitable for the production of bacteria that aids the growth of fruiting bodies (Owaid *et al*., 2014). It is also rich in growth factors for mushroom mycelium such as free amino acids, vitamins, proteins, carbohydrates, some micronutrients and nucleotides which can significantly increase the specific growth rate, simple control of culture process, and good mycelia quality (Zimbro *et al*., 2009; Abdulhadi *et al.*, 2013). Yeast also acts as a front-runner to gobble up fungi mycelium (basidia) and convert them to usable nutrient for the mycelium.

Bacteria have been reported to associate with a variety of fungi including mushroom. Carrasco and Preston (2020) reviewed the interaction between bacteria and cultivated mushrooms and described both positive and negative outcomes for the mushroom, depending on the bacterial isolate and the developmental stage of the mushroom (Frey-Klett *et al.*, 2011). They further reported that the benefits for mushroom forming mutualistic associations with bacteria include the action of helper bacteria in promoting ectomycorrhizal association with plant symbionts, enhanced nutrition through degradation of complex poly-carbohydrates by lignocellulosic enzyme activity, the consumption of volatile organic compounds (VOCs) blocking mushroom fructification by bacteria and secretion of antibiotics to suppress competing fungi or provide protection against crop

parasites (Sbrana *et al*., 2002; Chang and Kim, 2007; Kim *et al*., 2011; Pion *et al*., 2013; Pudelko, 2014; Antunes *et al*., 2016; Vos *et al*., 2017; Pandin *et al*., 2018a,b). Bacteria in turn benefit from enhanced dispersal and growth on mushroom exudates (Warmink and Van Elsas, 2009). However, Mushrooms may also consume bacteria and assimilate bacterial carbon and nitrogen as a nutrient source (Vos *et al*., 2017; Kertesz and Thai, 2018); whereas bacteria can cause significant yield losses by causing a wide range of mushroom diseases (Frey-Klett *et al*., 2011; Gea and Navarro, 2017).

Mushrooms have therefore, been described to modify and select the microbiome associated with the environmental niche where they grow and fructify (Li *et al*., 2017; Zhou *et al*., 2017). Selective processes acting on the microbiota present in substrates and soils determine the composition of the microbiota inhabiting the fruiting bodies or interacting with mushroom hyphae, and both configure the mushroom holobiont (Carrasco and Preston, 2020).Understanding of these complex interactions and the knowledge of this mushroom selected microbiota in its natural habitat would enhance artificial cultivation and domestication of the *Agaricus* Mushroom in Rivers State, Nigeria as to provide all year round commercial production of *Agaricus silvaticus* in Nigeria. Thus this research article documents the first morphological and cultural isolation and identification of fungi and bacteria in soils naturally colonized by wild mushroom *Agaricus silvaticus*.

MATERIALS AND METHODS

Experimental site

This research was conducted in the Crop Protection laboratory, Crop/ Soil Science Department, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Collection of Soil Samples

Soil samples were collected from 4 different locations in Ogwe Ukwa West Local Government Area, Abia State, Nigeria (Plate A1 and A2): 3 from Obiahia kindred the mushroom colonized soils in its natural habitats and the fourth a control plot from Obiawom kindred where mushroom was not found.

Plate A1: *Agaricus silverticus* in the wild

Plate A2: Soil Samples collection

Preparation of culture media and normal saline

Sabouraud Dextrose Agar, Potato dextrose Agar (PDA) and Nutrient Agar (NA) were used in this research to isolate and identify the fungi and bacteria; and the media was prepared according to manufacturer's instruction. 11g of Sabouraud Dextrose Agar and 2g of Antibiotics was weighed and dissolved into 160ml of distilled water in a conical flask. 5g of Nutrient Agar was weighed and dissolved into 160ml of distilled water. Cotton wool was then placed to cover it and wrapped with aluminium foil. After which the solution was then autoclaved at 121^{o}C for 15 minutes, it was then allowed to cool and dispensed into Petri-dishes. 8.5g of Sodium chloride (NaCl) was weighed and tipped into 100ml of distilled water after which it was sterilized in the autoclave. 1ml of the sample was dropped into 9ml of normal saline and shake vigorously to form a uniform solution of 10^{-1} concentration. The stock was subjected to a decimal dilution using sterile pipette to form 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} concentration (Cheesbrough, 2006; Harrigan, 1998).

Inoculation and Incubation

1ml pipette was employed to drop 0.01ml of the inoculums into the Petri-dishes and evenly spread all over the surface of the agar plate using stirring rod. All plates were incubated immediately after inoculation and placed upside down to prevent drops of condensations from collecting on the inoculated surface. Sabouraud Dextrose Agar plates were incubated for 28^{o}C for 72hrs, after which pure culture was prepared from the distinct fungal isolate observed (Harrigan, 1998).

Characterization and Identification of Isolates

The fungal observed in the pure culture were identified and pure bacteria colonies prepared from the colonies grown on the nutrient agar were later identified on the basis of morphological and biochemical tests as described by CMI (2010). The magnification used in viewing the isolates is X40.

Experimental Design and Statistical Technique

All treatments for the experiment were laid out using a Complete Randomization Design (CRD) having four (4) treatments and replicated five (5). The analysis of variance (ANOVA) was used to determine the treatment effects and means were tested using Turkey means method of grouping at 5% level of probability (Minitab, 2010).

RESULTS

The fungi species identified in this experimental research are Yeast, *Penicillium spp., Sclerotium spp., Mucor spp., Fusarium spp*. and *Aspergillus spp.* (Table 1). Soils collected from control had the highest mean value in *Penicillium spp.* (19.8), *Sclerotium spp.* (10.5) and *Mucor spp.* (2.8). Site three had a significant ($P \leq 0.05$) highest mean value of 29.0 Yeast count compared to Site two (15), site one (13) and completely absent in control soil where no mushroom was found. Except *Fusarium spp*. which was slightly presence in soils of site one, two and three, other identified fungi: *Mucor spp*, *Aspergillus spp*, and *Sclerotium spp.* were significantly absent in mushroom colonized soil samples. Although, *Penicillium spp.* scantly present in site three and one but completely absent in site two (Plate 1).

Table 2 clearly showed that total heterotrophic bacterial count ranges from 5×10^8 to 1.18×10^9. The bacterial count in the various soil samples revealed no significance difference ($P>0.05$) between the different sites but difference in the control. Soil sample from control was significantly lower than the samples from other sites in bacterial count.

The results in Table 3 revealed six bacteria genera: *Bacillus spp., Staphylococcus spp., Pseudomonas spp., Streptococcus spp., Micrococcus spp.* and *Proteus spp.* present in the different soil samples. *Proteus spp., Staphylococcus spp.* and *Bacillus spp.* were present in all soil samples. *Streptococcus spp.* was significantly higher in site three than the other soil samples collected from site one, control and site two. Also, site two had the highest mean value in *Staphylococcus spp., Bacillus spp.* and *Pseudomonas spp.* respectively.

Table 1: Fungi Isolated in the study soil sites

Sample	Yeast	*Penicillium spp.*	*Sclerotium spp.*	*Mucor spp.*	*Fusarium spp.*	*Aspergillus spp.*
Control	0.0^{c}	19.8^{a}	10.5^{a}	2.8^{a}	0.0^{c}	5.0^{a}
Site one	13.0^{b}	1.0^{b}	0.7^{b}	0.0^{b}	0.3^{bc}	0.0^{b}
Site two	15^{c}	0.0^{b}	0.8^{b}	0.0^{b}	0.9^{a}	0.0^{b}
Site three	29.0^{a}	1.5^{b}	0.0^{b}	0.0^{b}	0.7^{ab}	0.0^{b}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

KEY:
Control – Uzopipeline, Site 1 – Obiahia, Site 2 – Obiahia, Site 3 – Obiahia

Table 2: Total Heterotrophic count of the Bacterial Isolates

Samples	THC
Control	5.0×10^{8b}
Site one	1.18×10^{9a}
Site two	1.12×10^{9a}
Site three	1.04×10^{9a}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

KEY:
Control – Uzopipeline, Site 1 – Obiahia, Site 2 – Obiahia, Site 3 – Obiahia

Table 3: Bacteria Isolated in the study soil sites

Sample	*Streptococcus spp.*	*Staphylococcus spp.*	*Micrococcus spp.*	*Bacillus spp.*	*Proteus spp.*	*Pseudomonas spp.*
Control	7.0^{c}	18.0^{a}	7.8^{b}	0.0^{b}	29.3^{a}	0.0^{b}
Site one	20.3^{b}	19.5^{b}	16.4^{a}	11.6^{c}	0.0^{c}	9.0^{b}
Site two	0.0^{c}	28.3^{a}	0.0^{c}	26.8^{a}	17.3^{b}	16.3^{a}
Site three	27.9^{a}	17.2^{b}	12.3^{ab}	17.1^{a}	13.4^{b}	10.0^{b}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

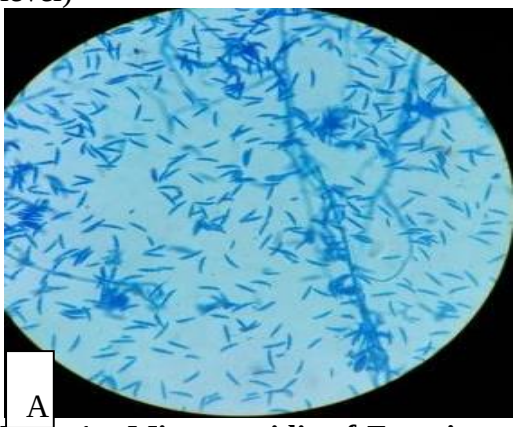
A

Plate 1a: Micro-conidia of *Fusarium spp.*

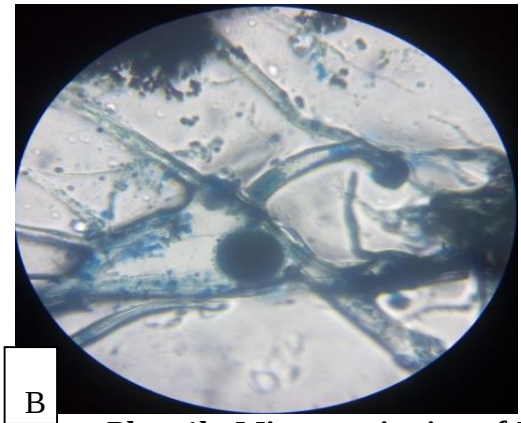
B

Plate 1b: Microscopic view of *Mucor spp.*

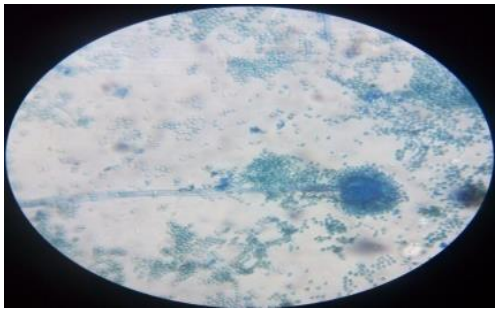

Plate 1c: Microscopic view of *Penicillium spp.* **Plate 1d: Microscopic view of *Aspergillus spp.***

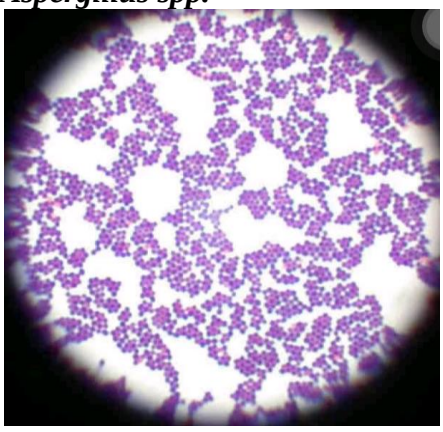

Plate 1e: Microscopic view of *Staphylococcus spp.* after gram staining

DISCUSSION

The result of this experiment most importantly attest to the earlier reports of Deveau *et al*., (2018) and Carrasco and Preston (2020) that Mushrooms natural wide habitat is characterised by microbe-rich soils and substrates where a wide variety of interactions is established among bacteria and fungi ranging from antagonism and competition to mutualism. The experimental result therefore revealed the presence of six fungi genera: four mushroom inhibiting microbes namely *Penicillium spp., Sclerotium spp., Mucor spp.* and *Aspergillus spp*. in decreasing order from the control sites where *Agaricus* mushroom was not found and two other benefiting fungi genara: *Yeast* and *Fusarium spp*. from the *Agaricus* Mushroom colonized soils. However, fairly insignificant number of *Penicillium spp*. was also recorded in site three of the *Agaricus* colonized soil habitat though overshadowed by the very high presence of Yeast. The high present of Yeast and some *Fusarium spp*. and absent of *Penicillium spp., Sclerotium spp., Mucor spp.* and *Aspergillus spp*. in *Agaricus* colonized soils affirmed the reports of Li *et al*. (2017); and Zhou *et al.* (2017) that Mushroom modifies and select the microbiome associated with the natural environmental niche where they grow and fructify.

Owaid *et al*. (2014) described Yeast as a soil beneficial fungi microbe that is suitable for the production of bacteria which aids the growth of mushroom fruiting bodies. It is also rich in growth factors for mushroom mycelium such as free amino acids, vitamins, proteins, carbohydrates, some micronutrients and nucleotides which can significantly increase the specific growth rate, simple control of culture process, and good mycelia quality (Zimbro *et al*., 2009; Abdulhadi *et al.*, 2013). Yeast also acts as a front-runner to gobble up fungi mycelium (basidia) and convert them to usable nutrient for the mycelium. *Fusarium spp*. which is a known root pathogen has been reported to produce indole acetic acid (IAA) that modulate phytohormone levels in plant roots in association with other fungi like *Mortierella*. Thus, can affect colonization of plant roots or fructification by ectomycorrhizal fungi that were found to harbour distinct bacterial communities from those of the bulk soil, with enrichment in α- and γ-Proteobacteria, as reflected by the amplicon pyrosequencing

of PCR-barcoded libraries (Deveau *et al.,* 2016). *Penicilium spp, Sclerotium spp, Mucor spp* and *Aspergillus spp* which were present in control soils importantly confirms the inability of *Agaricus* mushroom to colonized such soils as they negatively affect the growth of mycelium and mushroom production (In-Young *et al.,* 2010). Severe losses have occurred on farms due to contamination of the compost with *Aspergillus spp*. and P*enicillium spp* (Samp, 2007). However, some of the isolated microorganism in this research (*Penicilium spp, Mucor spp, Aspergillus spp*), were among those isolated by In-Young *et al*., (2010).

Similarly, six bacterial genera (*Bacillus Spp, Proteus Spp, Micrococcus Spp, Streptococcus Spp, Staphylococcus Spp and Pseudomonas Spp*) that were isolated and identified in the current experiment were among those isolated by Rainey *et al* (1990, 1991). It is note-worthy that *Pseudomonas* and *Bacillus spp*. which enhances mycelia development and promote primordial differentiation (Liu *et al*., 2017) were present in *Agaricus* colonized soil samples and absent in control soils. Anti-fungal agents produced by some bacteria have been shown to be beneficial to control pathogenic fungi (Chang and Kim 2007; Kim, 2006). Other researchers have reported that most Mushroom bacterial community is dominated by Proteobacteria, Chloroflexi, Actinobacteria and Acidobacteria phyla with high proportions of the genus Pseudomonas during the primordial differentiation stage (Liu *et al*., 2017).

Among the postulated roles of the most abundant bacteria in morel substrates, members from the genus *Pseudomonas* are posited to be involved in morel *Morchella spp.* fructification (Liu *et al*., 2017; Noble *et al.,* 2009). Other dominant genera from the phyla Proteobacteria, Geobacter and Rhodoplanes, could help to degrade organic compounds into carbon sources available for the morels (Berlemont and Martiny, 2015) and enhance the availability of metal ions such as iron and manganese for fungi and plants through metal-reducing activity and siderophore production (Jin *et al*., 2013; Kügler *et al.,* 2019) or simply they are denitrifying bacteria that thrive in the presence of fungal exudates (Zhang *et al*., 2016). Species from the phylum Acidobacteria have been described to play an important role in maintaining the acidic pH value of the soil and therefore, also influence the uptake of trace elements from substrates, whereas bacteria belonging to the phylum Bacteroidetes have been reported to have the capacity to degrade cellulose and chitin (Liu *et al*., 2017).

It is worthy to note that, wild mushroom colonized soil is based on a series of solid fermentation stages under controlled environmental conditions in which bacteria and fungi have major roles in processing raw materials, minimizing fungal competitors and inducing fructification (Kertesz and Thai, 2018; McGee *et al*., 2018; Vieira and Pecchia, 2018). While further research is ongoing to molecularly elucidate the identities of these microbes associated with mushroom growing environment to species level. The experimental result gives an insight to the complex microbial interaction that could enhance the artificial cultivation of *Agaricus* mushroom in Nigeria to substitute its seasonal growth in the wild.

CONCLUSION

The experimental result therefore revealed the presence of six fungi genera: four mushroom inhibiting microbes namely *Penicillium spp., Sclerotium spp., Mucor spp.* and *Aspergillus spp*. in decreasing order from the control sites where *Agaricus* mushroom was not found and two other benefiting fungi genara: *Yeast* and *Fusarium spp*. from the *Agaricus* Mushroom colonized soils. However, fairly insignificant number of *Penicillium spp*. was also recorded in site three of the *Agaricus* colonized soil habitat though overshadowed by the very high presence of Yeast. Similarly, six bacterial genera (*Bacillus spp, Proteus spp, Micrococcus spp, Streptococcus spp, Staphylococcus spp and Pseudomonas spp*) were isolated and morphologically identified. It is note-worthy that *Pseudomonas* and *Bacillus spp*. which enhances mycelia development and promote primordial differentiation (Liu *et al.*, 2017) were present in *Agaricus* colonized soil samples and absent in control soils. Therefore understanding of these microorganisms associated with the growth of *Agaricus* could contribute to preparation of artificial habitats for the domestication and cultivation of edible *A. silvaticus* in Rivers State, Nigeria.

REFERENCES

Abdulhadi, A.M., Abdul-Qader, Z. M., Rustum, A.N. and Hammoodi, J.K. (2013). "Use of yeast extract to improve yield and cold storage of oyster mushroom," The Iraqi Journal of Agricultural Sciences, Vol. 44(1):89-96.

Aletor, V. A. (1990). Anti-nutritional factors in some Nigeria feedstuffs, herbage plants. A Monograph Prepared on Alternative Formulation of Livestock Feed Products Development, Quality, Evaluation and Health Implications, Cabinet Office, Lagos, Nigeria. Conference on Environmental Science and Technology, 8-9thSeptember, 2002, Greenshore 43-59.

Antunes, L.P., Martins, L.F., Pereira, R.V., Thomas, A.M., Barbosa, D., Lemos, L.N., *et al*. (2016). Microbial community structure and dynamics in thermophilic composting viewed through metagenomics and metatranscriptomics. *Sci Rep.*, 6: 38915.

Berlemont, R., and Martiny, A.C. (2015). Genomic potential for polysaccharide deconstruction in bacteria. *Applied Environmental Microbiology*, 81: 1513–1519.

Carrasco, J. and Preston, G.M. (2020). Growing edible mushrooms: a conversation between bacteria and fungi, *Environmental Microbiology*, 22(3):858–872.

Chang, I. & Kim, J. D. (2007). Inhibition of aflatoxin production of *Aspergillus flavus* by *Lactobacillus casei*. *Mycobiology*, 35: 76–81.

Cheesebrough, M. (2006). District laboratory practice in Tropical Countries. Cambridge University Press, 434.

CMI (2010). Commonwealth Mycological Institute Description of Pathogenic Fungal and Bacteria. Kew Survey England. 451-460.

Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., et al. (2018) Bacterial-fungal interactions: ecology, mechanisms and challenges. FEMS Microbiol Rev 42: 335-352.

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol Mol Biol Rev.,* 75: 583–609.

Gea, F.J., and Navarro, M.J. (2017). Mushroom diseases and control. In Edible and Medicinal Mushrooms: Technology and Applications, Cunha, D., and PardoGimenez, A. (eds). Hoboken, NJ: John Wiley & Sons, pp. 239–259.

Harrigan, W. F. (1998). Laboratory methods in Food Microbiology, 3rd edn. Academic Press, London UK.

In-Young Choi, Jang-Nam Choi, and Wang-Hyu Lee (2010). Isolation and identification of mushroom pathogens from *Agrocybeaegerita.*

Jin, C.W., Ye, Y.Q., and Zheng, S.J. (2013). An underground tale: contribution of microbial activity to plant iron acquisition via ecological processes. *Ann Bot.*,113: 7-18.

Kertesz, M.A., and Thai, M. (2018). Compost bacteria and fungi that influence growth and development of Agaricus bisporus and other commercial mushrooms. Applied Microbiology and Biotechnology, 102: 1639-1650.

Kim, M.H., Park, S.W., and Kim, Y.K. (2011). Bacteriophages of Pseudomonas tolaasii for the biological control of brown blotch disease. *J Korean Soc Appl Biol Chem* 54: 99-104.

Kügler, S., Cooper, R.E., Wegner, C.E., Mohr, J.F., Wichard, T., and Küsel, K. (2019). Iron-organic matter complexes accelerate microbial iron cycling in an iron-rich fen. Sci Total Environ 646: 972-988.

Li, Q., Zhao, J., Xiong, C., Li, X., Chen, Z., Li, P., and Huang, W. (2017). *Tuber indicum* shapes the microbial communities of ectomycorhizosphere soil and ectomycorrhizae of an indigenous tree (*Pinus armanidi*). PLoS One 12: e0175720.

Liu, Q., Liu, H., Chen, C., Wang, J., Han, Y., and Long, Z. (2017). Effects of element complexes containing Fe, Zn and Mn on artificial morel's biological characteristics and soil bacterial community structures. PLoS One 12: e0174618.

McGee, C.F. (2018). Microbial ecology of the Agaricus bisporus mushroom cropping process. Appl Microbiol Biotechnol 102:1075-1083.

Minitab 17 statistical software (2010). Computer software. State college, P.A: Minitab, Inc. (www.minitab.com).

Noble, R., Dobrovin-Pennington, A., Hobbs, P.J., Pederby, J., and Rodger, A. (2009). Volatile C8 compounds and pseudomonads influence primordium formation of Agaricus bisporus. *Mycologia* 101: 583–591.

Obe, S. C. and Mshigeni, K. E. (2013). Mushroom Farming: Life-changing Humble Creatures Scholars Press, pp132.

Orikoha, E. C. and Dimkpa, S. O. N. (2021). Possible Domestication of Edible Wild Mushroom *Agaricus silvaticus* G. J. Keizer In Rivers State, Nigeria, European Journal of Botany, Plant Sciences and Phytology..(Accepted).

Osemwegie, O. O. Ericaremu, E. G. & Abdulmalik, J. (2006). A survey of macro fungi in Edo/Delta region of Nigeria heir morphology and uses. *Global Journal of Pure and Applied Science,* 12(2): 149 – 157.

Owaid, N. M., Al-Ani, B.M.A. and Muslat, M. M. (2014). Effect of *Saccharomyces cerevisiae* Extract on Mycelium Growth and Primordia Formation of *Pleurotus salmoneostramineus*

and *Pleurotus cornucopiae* (In Vitro), *Journal of Life Sciences and Technologies* Vol. 2(1):28-31.

Pandin, C., Le Coq, D., Deschamps, J., Védie, R., Rousseau, T., Aymerich, S., et al. (2018a). Complete genome sequence of Bacillus velezensis QST713: a biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. J Biotechnol 278: 10–19.

Pandin, C., Védie, R., Rousseau, T., Le Coq, D., Aymerich, S., and Briandet, R. (2018b). Dynamics of compost microbiota during the cultivation of Agaricus bisporus in the presence of Bacillus velezensis QST713 as biocontrol agent against Trichoderma aggressivum. *Biol Control* 127: 39-54.

Partida-Martínez, L.P. (2017). The fungal holobiont: evidence from early diverging fungi. *Environ Microbiol* 19: 2919-2923.

Pion, M., Spangenberg, J.E., Simon, A., Bindschedler, S., Flury, C., Chatelain, A., *et al*. (2013). Bacterial farming by the fungus Morchella crassipes. *Proc R Soc Lond B Biol Sci* 280: 2013-2242.

Pudełko, K. (2014). Effect of forced ventilation during composting on Agaricus bisporus substrate selectivity. *Int Biodeter Biodegr* 93: 153-161.

Rainey, P.B. (1991). Effect of Pseudomonas putida on hyphal growth of *Agaricus bisporus*. *Mycol Res* 95: 699-704.

Rainey, P.B., Cole, A.L.J., Fermor, T.R., and Wood, D.A. (1990). A model system for examining involvement of bacteria in basidiome initiation of *Agaricus bisporus*. *Mycol Res* 94: 191–195.

Samp, R . (2007). The development of *Agaricus* mushroom compost processing systems. In: Sanchez JE *et al* (eds) Cultivation, marketing and food safety of *Agaricus bisporus*, Mexico City.

Sbrana, C., Agnolucci, M., Bedini, S., Lepera, A., Toffanin, A., Giovannetti, M., and Nuti, M.P. (2002). Diversity of culturable bacterial populations associated to Tuber borchii ectomycorrhizas and their activity on *T. borchii* mycelial growth. FEMS *Microbiol Lett* 211: 195-201.

Ukoima, H. N., Ogbonnaya, L. O., Arikpo, G. E. & Pepple, G. A. (2009a). Nutritional organoteptic and palatability studies of selected mushrooms in Nigeria. *World Applied Science Journal*, 7(4): 470-484.

Umar, M. H., and Van-Griensven, L.J. (2000). Gross- and microscopic anatomy of Agaricus bisporus in health and disease. *Mushroom Science* Vol.15:121-127.

Vieira, F.R., and Pecchia, J.A. (2018). An exploration into the bacterial community under different pasteurization conditions during substrate preparation (compostingphase II) for Agaricus bisporus cultivation. Microb Ecology, 75: 318-330.

Vos, A.M., Heijboer, A., Boschker, H.T.S., Bonnet, B., Lugones, L.G., and Wösten, H.A.B. (2017). Microbial biomass in compost during colonization of Agaricus bisporus. AMB Express 7: 12.

Wang, Z. S., Chen, L. F. and Chen, M. Y. (2004). Thermotolerance to related genes in *Agaricus bisporus*. Mushroom Science, 16:133-137.

Wang, Z. S., Chen, L. F. and Chen, M.Y. (2003). Study on the thermotolerance related genes of *Agaricus bisporus*. *Mycosystema*, 22:325-328.

Warmink, J.A., and Van Elsas, J.D. (2009). Migratory response of soil bacteria to Lyophyllum sp. strain Karsten in soil microcosms. *Appl Environ Microbiol* 75: 2820-2830.

Zhang, S., Pang, S., Wang, P., Wang, C., Guo, C., Addo, F. G., and Li, Y. (2016). Responses of bacterial community structure and denitrifying bacteria in biofilm to submerged macrophytes and nitrate. *Sci Rep* 6:36178.

Zhou, J., Bai, X., and Zhao, R. (2017). Microbial communities in the native habitats of Agaricus sinodeliciosus from Xinjiang Province revealed by amplicon sequencing. *Sci Rep* 7: 15719.

Zimbro, M.J., Power, D.A., Miller, S.M., Wilson, G.E. and Johnson, J.A. (2009). Difco & BBL Manual, Manual of Microbiological Culture Media, 2nd ed. US: BD Diagnostics, pp. 686.