OPTIMUM LIQUID CULTURE CONDITIONS FOR MYCELIAL GROWTH AND NUTRACEUTICAL ATTRIBUTES OF *Pleurotus eryngii*

JAKE CARLO A. ESGUERRA

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree of

BACHELOR OF SCIENCE IN BIOLOGY
(Major in Microbiology)

JUNE 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

ABSTRACT

ESGUERRA, JAKE CARLO A., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **MAY 2019. OPTIMUM LIQUID CULTURE CONDITION FOR MYCELIAL GROWTH AND NUTRACEUTICAL ATTRIBUTES OF *Pleurotus eryngii***

Adviser: Sofronio P. Kalaw, Ph. D.

This study was undertaken to evaluate the optimum culture condition for secondary mycelial growth and determine the nutraceutical attributes of *P. eryngii*. Various indigenous media such as potato dextrose broth, coconut water, rice bran broth, corn grit broth and soybean broth were evaluated. The effect of pH, temperature, illumination and agitation were determined. Dry weight of mycelia and volume loss after 10 days of incubation period were assessed to determine the optimum growth condition.

The secondary mycelia grew best on potato dextrose broth at a pH range of 5.0-8.5 incubated in air-conditioned (23°C), alternating light and dark and agitated condition. Based on the mycochemical analysis, the mycelia contain essential oils, triterpenes, coumarins, fatty acids, antraquinones, tannins, steroids, phenols and alkaloids. However, anthrones, phenolic compounds and flavonoids were not detected. It also exhibited radical scavenging activity of 61.19% and contain a total phenolic of 22.29 mg GAE/g of sample. Moreover, the ethanolic extract did not exhibit antibacterial activity against *E. coli* and *S. aureus*.

LITERATURE CITED

Adebayo, E. A., Martinez-Carrera, D., Morales, P., Sobal, M., Escudero, H., Meneses, M., Avila-Nava, A., Castillo, I & Bonilla, M. (2018). Comparative study of antioxidant and antibacterial properties of the edible mushrooms *Pleurotus levis, P. ostreatus, P. pulmonarius* and *P. tuber-regium*. *International Journal of Food Science and Technology*, 53, 1316-1330.

Akyuz, M & Kirbag, S. (2009). Antimicrobial activity of *Pleurotus eryngii* var. ferulae grown on various agro-wastes. *Europe Asian Journal of Biological Sciences*, 3, 58-63.

Akyuz, M., Onganer, A. N., ERECEVIT, P., & Kirbag, S. (2010). Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. *Gazi University Journal of Science*, 23(2), 125-130.

Alves, M. J., Ferreira, I. C., Dias, J., Teixeira, V., Martins, A., & Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta medica*, 78(16), 1707-1718.

Bai, M. S., Wang, C., Zong, S. C., Lei, M., & Gao, J. M. (2013). Antioxidant polyketide phenolic metabolites from the edible mushroom *Cortinarius purpurascens*. *Food Chemistry*, 141(4), 3424-3427.

Barros, L., Baptista, P., Correia, D. M., Casal, S., Oliveira, B., & Ferreira, I. C. (2007). Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chemistry*, 105(1), 140-145.

Barros, L., Ferreira, M. J., Queiros, B., Ferreira, I. C., & Baptista, P. (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, 103(2), 413-419.

Barros, L., Dueñas, M., Ferreira, I. C., Baptista, P., & Santos-Buelga, C. (2009). Phenolic acids determination by HPLC–DAD–ESI/MS in sixteen different Portuguese wild mushrooms species. *Food and Chemical Toxicology*, 47(6), 1076-1079.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *The American Journal of Clinical Pathology*, 36, 4.

Bazała, M. A., Gołda, K., & Bystrzejewska-Piotrowska, G. (2008). Transport of radiocesium in mycelium and its translocation to fruitbodies of a saprophytic macromycete. *Journal of Environmental Radioactivity*, 99(7), 1200-1202.

Bellettini, M. B., Assumpc a˜ o Fiorda, F., Maieves, H. A., Teixeira, G. L., A´ vila, S., Hornung, P. S., Ju´ nior, A. M. & Ribani, R. H. (2016). Factors affecting mushroom *Pleurotus spp. Saudi Journal of Biological Sciences*, 1319-562.

Carrasco-Gonzalez, J. A., Serna-Saldivar, S. O., & Gutierrez-Uribe, J. A. (2017). Mycochemical changes induced by selenium enrichment in *P. ostreatus* fruiting bodies. *Journal of Agricultural and Food Chemistry*, 65(20), 4074-4082.

Devi, G. S., Prasad, M. H., Saraswathi, I., Raghu, D., Rao, D. N., & Reddy, P. P. (2000). Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. *Clinica Chimica Acta*, 293(1-2), 53-62.

Dimou, D. M., Georgala, A., Komaitis, M., & Aggelis, G. (2002). Mycelial fatty acid composition of *Pleurotus spp.* and its application in the intrageneric differentiation. *Mycological Research*, 106(8), 925-929.

Dulay, R. M. R., Kalaw, S. P., Reyes, R. G., Cabrera, E. C., & Alfonso, N. F. (2012). Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., a new record of domesticated wild edible mushroom in the Philippines. *Philippine Agricultural Scientist*, 95(3), 278-285.

Dulay, R. M. R., Cabrera, E. C., Kalaw, S. P., & Reyes, R. G. (2012). Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus* (Bull.) Fr. *Mycosphere*, 3(6), 926-933.

Dulay, R. M. R., Miranda, L. A., Malasaga, J. S., Kalaw, S. P., Reyes, R. G., & Hou, C. T. (2017). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*. *Biocatalysis and Agricultural Biotechnology*, 9, 141-144.

Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., & Sarek, J. (2006). Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural Product Reports*, 23(3), 394-411.

Günç Ergönül, P., Akata, I., Kalyoncu, F., & Ergönül, B. (2013). Fatty acid compositions of six wild edible mushroom species. *The Scientific World Journal*, 2013.

Faridur, R. M., Rezaul, K. M., Farhadul, I. M., Rowshanul, H. M., & Tofazzal, H. M. (2010). Phytochemical and cytotoxic investigation on oyster mushroom (*Pleurotus ostreatus*). *International Research Journal of Pharmacy*, 1(1), 342-345.

Feng, Y., Li, W., Wu, X., He, L & Ma, S. (2010). Rapid and efficient microwave-assisted sulfate modification of lentinan and its antioxidant and antiproliferative activities *in vitro*. *Carbohydrate Polymers*, 82, 605-612.

Ferreira, I. C., Barros, L., & Abreu, R. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, 16(12), 1543-1560.

Gbolagade, J. S., Fasidi, I. O., Ajayi, E. J., & Sobowale, A. A. (2006). Effect of physico-chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. *Food Chemistry*, 99(4), 742-747.

Gbolagade, J., Sobowale, A., & Adejoye, D. (2006). Optimization of sub-merged culture conditions for biomass production in *Pleurotus florida* (mont.) Singer, a Nigerian edible fungus. *African Journal of Biotechnology*, 5(16).

Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. *International Journal of Molecular Sciences*, 8(9), 950-988.

Hearst, R., Nelson, D., McCollum, G., Millar, B. C., Maeda, Y., Goldsmith, C. E., Rooney, P. J., Loughrey A., Rao, J. R. % Moore, J. E. (2009). An examination of antibacterial and antifungal properties of constituents of Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. *Complementary Therapies in Clinical Practice*, 15, 5-7.

Hsu, T. H., Shiao, L. H., Hsieh, C., & Chang, D. M. (2002). A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chemistry*, 78(4), 463-469.

Ikewuchi, C. C., & Ikewuchi, J. C. (2008). Chemical profile of *Pleurotus tuber regium* (Fr) Sing's sclerotia. *The Pacific Journal of Science and Technology*, 10, 295-9.

Imtiaj, A., Jayasinghe, C., Lee, G. W., & Lee, T. S. (2009). Comparative study of environmental and nutritional factors on the mycelial growth of edible mushrooms. *Journal of Culture Collections*, 6(1), 97-105.

Islam, M. R., Omar, M., PK, M. M. U., & Mia, M. R. (2015). Phytochemicals and antibacterial activity screening of three edible mushrooms *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* accessible in Bangladesh. *American Journal of Biology and Life Sciences*, 3(2), 31.

Jacob, J. K. S., Kalaw, S. P., & Reyes, R. G. (2015). Mycelial Growth Performance of Three Species of *Pleurotus* on Coconut Water Gelatin. *Current Research in Environmental & Applied Mycology*, 5(3), 263-268.

Jonathan, S. G., and I. O. Fasidi. (2003). Studies on *Psathyerella atroumbonata* (Pegler), a Nigerian edible fungus. *Food Chemistry*, 81, 481-484.

Kalaw, S. P., Alfonso, D. O., Dular, R. M. R., De Leon, A. M., Undan, J. Q. & Reyes, R. G. (2016). Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. *Current Research in Environmental & Applied Mycology*, 6(4), 277-287.

Kalaw, S., & Albinto, R. F. (2014). Functional activities of Philippine wild strain of *Coprinus comatus* (OF Müll.: Fr.) Pers and *Pleurotus cystidiosus* OK Miller grown on rice straw based substrate formulation. *Mycosphere*, 5(5), 646-55.

Kavishree, S., Hemavathy, J., Lokesh, B. R., Shashirekha, M. N., & Rajarathnam, S. (2008). Fat and fatty acids of Indian edible mushrooms. *Food Chemistry*, 106(2), 597-602.

Keypour, S., Riahi, H., Moradali, M. F., & Rafati°, H. (2008). Investigation of the Antibacterial Activity of a Chloroform Extract of Ling Zhi or Reishi Medicinal. *International Journal of Medicinal Mushrooms*, 10(4), 345-349.

Kim, H. O., Lim, J. M., Joo, J. H., Kim, S. W., Hwang, H. J., Choi, J. W., & Yun, J. W. (2005). Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybe cylindracea*. *Bioresource Technology*, 96(10), 1175-1182.

Kim, K. H., Noh, H. J., Choi, S. U., & Lee, K. R. (2012). Isohericenone, a new cytotoxic isoindolinone alkaloid from *Hericium erinaceum*. *The Journal of Antibiotics*, 65(11), 575.

Klaus, A., & Niksic, M. (2007). Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. *Zbornik Matice Srpske Za Prirodne Nauke*, 113(219), 26.

Kumar, V., Mishra, S. K. & Kaur, M. (2019). Effect of different media, temperature and pH on radial mycelial growth of *Lentinula edodes* strain Le-17-04. *Journal of Pharmacognosy and Phytochemistry*, 8, 345-348.

Kumari, D & Achal, V. (2008). Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (Oyster mushroom). *Life Science Journal*, 5, 3.

Kwon, J. S., Lee, J. S., Shin, W. C., Lee, K. E., & Hong, E. K. (2009). Optimization of culture conditions and medium components for the production of mycelial biomass and exo-polysaccharides with *Cordyceps militaris* in liquid culture. *Biotechnology and Bioprocess Engineering*, 14(6), 756-762.

Lalitha, M. K. (2004). Manual on antimicrobial susceptibility. *Twelfth Informational Supplement*, 24-31.

Lee, B. C., Bae, J. T., Pyo, H. B., Choe, T. B., Kim, S. W., Hwang, H. J., & Yun, J. W. (2004). Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. *Enzyme and Microbial Technology*, 35(5), 369-376.

Li, S & Shah, N. P. (2014). Antioxidant and antibacterial activities of sulphated polysaccharides from *Pleurotus eryngii* and *Streptococcus thermophilus* ASCC 1275. *Food Chemistry*, 165, 262-270.

Liby, K. T., Yore, M. M., & Sporn, M. B. (2007). Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nature Reviews Cancer*, 7(5), 357.

Luximon-Ramma, A., Bahorun, T., Crozier, A., Zbarsky, V., Datla, K. P., Dexter, D. T., & Aruoma, O. I. (2005). Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas. *Food Research International*, 38(4), 357-367.

Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture*, 83(5), 496-502.

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, 73(3), 321-325.

Maurya, Sunita & Singh, D. (2010). In vitro callus culture of *Adhatoda vasica*: a medicinal plant. *Annals of Biological Research*, 1, 57-60.

Moradali, M. F., Mostafavi, H., Hejaroude, G. A., Tehrani, A. S., Abbasi, M., & Ghods, S. (2006). Investigation of potential antibacterial properties of methanol extracts from fungus *Ganoderma applanatum*. *Chemotherapy*, 52(5), 241-244.

Nattoh, G. I., Gatebe, G. E., Maina, J. M., & Musieba, F. (2015). Radical scavenging properties, total polyphenols and flavonoids of two coloured basidiomycetes from four pre-determined phases.

Obatake, Y., Murakami, S., Matsumoto, T & Fukumasa, Y. (2003). Isolation and characterization of a sporeless mutant in *Pleurotus eryngii*. *The Mycological Society of Japan and Springer*, 44, 33-40.

Orhan, I., & Üstün, O. (2011). Determination of total phenol content, antioxidant activity and acetylcholinesterase inhibition in selected mushrooms from Turkey. *Journal of Food Composition and Analysis*, 24(3), 386-390.

Rajewska, J., & Bałasińska, B. (2004). Biologically active compounds of edible mushrooms and their beneficial impact on health. *Postepy Higieny i Medycyny Doswiadczalnej*, 58, 352-357.

Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology*, 50(5), 1201-1207.

Reyes, R. G., Lopez, L. L. M. A., Kumakura, K., Kalaw, S. P., Kikukawa, T., & Eguchi, F. (2009). *Coprinus comatus*, a newly domesticated wild nutriceutical mushroom in the Philippines. *Journal of Agricultural Technology*, 5(2), 299-316.

Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Letters*, 285(2), 109-115.

Shah, P., & Modi, H. A. (2018). Optimization of Culture Conditions for Biomass Production of *Ganoderma lucidum*. *International Journal Current Microbiological Applied Science*, 7(2), 1882-1889.

Shah, Z. A, Ashraf, M. & Ishtiaq, M. (2004). Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (Wheat straw, leaves, saw dust). *Pakistan Journal of Nutrition*, 3, 158-160.

Shah, N. P. & Li, S. (2016). Characterization, antioxidative and bifidogenic effects of polysaccharides from *Pleurotus eryngii* after heat treatments. *Food Chemistry*, 197, 240-249.

Shamaki, B. U., Sandabe, U. K., Fanna, I. A., Adamu, O. O., Geidam, Y. A., Umar, I. I., & Adamu, M. S. (2012). Proximate composition, phytochemical and elemental analysis of some organic solvent extract of the wild mushroom *Ganoderma lucidum*. *Journal of Natural Sciences Research*, 2(4), 24-35.

Shon, Y. H., & Nam, K. S. (2001). Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. *Journal of Ethnopharmacology*, 77, 103–109.

Shu, C. H., & Lung, M. Y. (2004). Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. *Process Biochemistry*, 39(8), 931-937.

Soković, M., & van Griensven, L. J. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. *European Journal of Plant Pathology*, 116(3), 211-224.

Soković, M., Tzakou, O., Pitarokili, D., & Couladis, M. (2002). Antifungal activities of selected aromatic plants growing wild in Greece. *Food Research*, 46(5), 317-320.

Suberu, H. A., Lateef, A. A., Bello, I. M., & Daudu, O. A. Y. (2013). Mycelia biomass yield of *Ganoderma lucidum* mushroom by submerged culture. *Nigerian Journal of Technological Research*, 8(2), 64-67.

Sun, Y., Liang, H., Cai, G., Guan, S., Tong, H., Yang, X., & Liu, J. (2009). Sulfated modification of the water-soluble polysaccharides from *Polyporus albicans* mycelia and its potential biological activities. *International Journal of Biological Macromolecules*, 44(1), 14-17.

Synytsya, A., Mic˘kova, K., Synytsya, A., Jablonsky, I., Spe˘vac˘ek, J., Erban, V., Kovar˘ikova, E. & C˘opikova J. (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, 76, 548-556.

Tsai, S-Y., Tsai, H-L. & Mau, J-L. (2008). Non-volatile taste components of *Agaricus blazei, Agrocybe cylindracea* and *Boletus edulis*. *Food Chemistry*, 107, 977-983.

Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*, 2015.

Wandati, T. W., Kenji, G. M., & Onguso, J. M. (2013). Phytochemicals in edible wild mushrooms from selected areas in Kenya. *Journal of Food Research*, 2(3), 137.

Wang, H. X., Ng, T. B. & Ooi, V. E. C. (2000). A protein with inhibitory activity on cell-free translation from cultured mycelia of the edible mushroom *Tricholoma lobayense*. *Comparative Biochemistry and Physiology*, 125, 247–253.

Wani, B. A., Bodha, R. H., & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, 4(24), 2598-2604.

Xu, C. P., Sinha, J., Bae, J. T., Kim, S. W., & Yun, J. W. (2006). Optimization of physical parameters for exo–biopolymer production in submerged mycelial cultures of two entomopathogenic fungi *Paecilomyces japonica* and *Paecilomyces tenuipes*. *Letters in Applied Microbiology*, 42(5), 501-506.

Xue, Z., Li, J., Cheng, A., Yu, W., Zhang, Z., Kou, X., & Zhou, F. (2015). Structure identification of triterpene from the mushroom *Pleurotus eryngii* with inhibitory effects against breast cancer. *Plant Foods for Human Nutrition*, 70(3), 291-296.

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3.

Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. *Mycologia*, 95(4), 584-589.

Yıldız, S., Yılmaz, A., Can, Z., Kılıç, C., & Yıldız, Ü. C. (2017). Total phenolic, flavonoid, tannin contents and antioxidant properties of *Pleurotus ostreatus and Pleurotus citrinopileatus* cultivated on various sawdust. *The Journal of Food*, 42(3), 315-323.

Yilmaz, N., Solmaz, M., Türkekul, İ., & Elmastaş, M. (2006). Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. *Food Chemistry*, 99(1), 168-174.

Yuan, Y., Yi, R., Sun, Yi., Zeng, J., Li, J., Hu, J., Zhao, Y., Sun, W., Zhao, C., Yang, Li. & Zhao, C. (2018). Facile Fabrication of Porous Carbon Materials Derived from *Pleurotus Eryngii* for Supercapacitor Electrodes, 8487098.

Zhang, R., Li, X. & Fadel, J.G. (2002). Oyster mushroom cultivation with rice and wheat straw. *Bio-Resource Technology*, 82, 277-284.

Zhao, C., Sun, H., Tong, X. & Yipeng, QI. (2003). An antitumour lectin from the edible mushroom *Agrocybe aegerita*. *Biochemistry Journal*, 374, 321-327.

Zhou, Y., Han, L. R., He, H. W., Sang, B., Yu, D. L., Feng, J. T., & Zhang, X. (2018). Effects of Agitation, Aeration and Temperature on Production of a Novel Glycoprotein GP-1 by *Streptomyces kanasenisi* ZX01 and Scale-Up Based on Volumetric Oxygen Transfer Coefficient. *Molecules*, 23(1), 125.